

Sougata Jana
Subrata Jana *Editors*

Alginate Biomaterial

Drug Delivery Strategies and Biomedical
Engineering

 Springer

Alginate Biomaterial

Sougata Jana • Subrata Jana
Editors

Alginate Biomaterial

Drug Delivery Strategies and Biomedical
Engineering

Editors

Sougata Jana
Department of Health & Family Welfare
Directorate of Health Services
Kolkata, West Bengal, India

Subrata Jana
Department of Chemistry
Indira Gandhi National Tribal University
Amarkantak, Madhya Pradesh, India

ISBN 978-981-19-6936-2

ISBN 978-981-19-6937-9 (eBook)

<https://doi.org/10.1007/978-981-19-6937-9>

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2023

This work is subject to copyright. All rights are solely and exclusively licensed by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.

The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Contents

Alginate Based Matrix Tablet for Drug Delivery	1
Ali Mujtaba, Arshiya Parveen, Nawaf M. Alotaibi, Mohammad Daud Ali, and Munfis Patel	
Alginate Based Micro Particulate Systems for Drug Delivery	19
Jyosna Doniparthi, Suryaprakash Reddy Chappidi, and E. Bhargav	
Alginate Based Nanocarriers for Controlled Drug Delivery Applications	61
Deepa Thomas and M. S. Latha	
Alginate Based Carriers for Topical Drug Delivery	85
Gourav Parmar, Manish Kumar, Abhishek Jha, and Brahmeshwar Mishra	
Alginate Based Hydrogel in Drug Delivery and Biomedical Applications	109
Suchita Dattatray Shinde, Neeraj Kulkarni, Govinda Shivaji Jadhav, Bhaskar Dewangan, Stephin Baby, Salil Pophali, and Bichismita Sahu	
Alginate Based Interpenetrating Polymer Network (IPN) in Drug Delivery and Biomedical Applications	135
Pooja Mittal, Ramit Kapoor, and Brahmeshwar Mishra	
Alginate Based Micelle in Biomedical Applications	155
P. R. Sarika and Nirmala Rachel James	
Alginate Based Polyelectrolyte Complexes for Drug Delivery and Biomedical Applications	179
Parneet Kaur Deol, Amritpal Kaur, Jasleen Kaur Kooner, Amoljit Singh Gill, Mandeep Singh, and Indu Pal Kaur	
Alginate-Based Inhalable Particles for Controlled Pulmonary Drug Delivery	207
Hao-Ying Li	

Biomedical Applications of Alginate in the Delivery System for Natural Products	241
Jia Wang and Haixia Chen	
Alginate in Cancer Therapy	267
Vikas, Abhishesh Kumar Mehata, Chandrasekhar Singh, Ankit Kumar Malik, Aseem Setia, and Madaswamy S. Muthu	
Alginate Carriers in Wound Healing Applications	297
Lisette Agüero and Marcos L. Dias	
Alginate as Support Material in Enzyme Immobilization	327
Zahra Ashkan, Sahar Zahirinejad, Roohullah Hemmati, and Ali Dinari	
Alginate in Gene and Vaccine Delivery	361
Hani Nasser Abdelhamid	
Alginate Based Scaffolds in Tissue Engineering and Regenerative Medicine	389
Debleena Ghosh, Taposi Trishna Neog, Rishik Patra, Kritideepa Nath, and Kishor Sarkar	

About the Editors

Sougata Jana has completed his Ph.D. in pharmaceutical technology from Maulana Abul Kalam Azad University of Technology (MAKAUT), West Bengal (Formerly Known as WBUT), India. He has spent 14 years in pharmacy, including teaching, research, and health services. Sougata has published over 30 research and review articles in different national and international peer-reviewed journals. He has also edited 12 books and published more than 45 book chapters in edited books by international publishers. He is a reviewer for peer-reviewed international journals. Sougata is a life member of the Association of Pharmaceutical Teachers of India (APTI) and holds an associateship with the Institution of Chemists (AIC), India. He successfully guided 17 postgraduate students in their research projects. Sougata is working on drug delivery science and technology, including modification of synthetic and natural biopolymers, microparticles, nanoparticles, and semisolids and interpenetrating network (IPN) system for controlled drug delivery.

Subrata Jana is a Professor in the Department of Chemistry, Indira Gandhi National Tribal University (Central University), Amarkantak, Madhya Pradesh, India. His current research focuses on the design and synthesis of artificial receptors for the recognition of anions, cations, and biomolecules along with biodegradable polymeric-based carrier systems for the delivery of drug molecules. So far, he has published around 40 research papers in peer-reviewed international journals and contributed more than 20 book chapters in edited books published by internationally renowned publishers. He is currently serving as executive editor of *Mekal Insights*, the official research journal of IGNTU. He also served as a reviewer for international journals. Subrata has obtained his Ph.D. in organic chemistry from the Indian Institute of Engineering Science and Technology (IIST), Shibpur, India. Then he worked with Professor (Dr.) Fraser Hof at the University of Victoria, Canada, and Dr. Kenneth J. Woycechowsky at the University of Utah, USA, as a postdoc. Overall, he has extensively studied the supramolecular behavior of the host–guest interaction and synthesis of different heterocyclic moieties.

Alginate Based Matrix Tablet for Drug Delivery



Ali Mujtaba, Arshiya Parveen, Nawaf M. Alotaibi, Mohammad Daud Ali, and Munfis Patel

Abstract The polymer-based matrix tablets are mostly used to manufacture orally prolonged released dosage forms due to the cost savings and relative ease of design process and scale-up production. Alginates are a kind of biopolymer that has been employed in various applications. Sodium alginate offers a lot of potential in drug delivery. Alginate is a biodegradable and biocompatible controlled-release polymer that is widely employed in pharmaceutical research. The drug delivery systems based on alginate offer the potential to address the disadvantages of conventional drug delivery. As a result, alginate has gotten a lot of interest from researchers in the recent years. This precise polymer is available in both neutral and charged forms, making it appropriate for a variety of products. Because of the potential to make two different types of gel relying on the medium's pH, the physicochemical properties vary greatly. Other scholars have looked into the industrial aspects of making alginate matrix. When developing modified-release dosage forms, careful selection of alginate grade is critical.

Keywords Alginate · Matrix tablets · Viscosity · Sustained release · Release profile

A. Mujtaba (✉)

Department of Pharmaceutics, Faculty of Pharmacy, Northern Border University, Rafha, Saudi Arabia

e-mail: m.mujtaba@nbu.edu.sa

A. Parveen

Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, Delhi, India

N. M. Alotaibi

Department of Clinical Pharmacy, Faculty of Pharmacy, Northern Border University, Rafha, Saudi Arabia

M. D. Ali

Department of Pharmacy, Mohammed Al-Mana College for Medical Sciences, Dammam, Saudi Arabia

M. Patel

Foundation Year Department, Mohammed Al-Mana College for Medical Sciences, Dammam, Saudi Arabia

1 Introduction

Alginates are metallic salts (sodium, potassium, calcium, magnesium etc.) of alginic acid and are edible polysaccharides derived from naturally occurring brown algae-seaweed. Different species of seaweed produce different types of alginate gels. They are hydrophilic in nature and form viscous gel when hydrated with aqueous medium. In addition to that some of the bacterial species also produce variety of alginate gels. They are non-toxic, relatively low cost, biocompatible, and mild gelation property used in biosynthesis as well as in production of micro- or nanostructures suitable for medical applications. Their biological function is based on their chemical structure and has a wide prospective in drug design hence their use and demand in pharmaceutical area is increasing day by day. They can be used to make products that provide protective, therapeutic, or wellness medicinal characteristics, owing to the availability of phenolics, terpenoids, and alkaloids functional groups, that are the main ingredients of various pharmacologically active constituents (Lee and Mooney 2012).

Drug delivery through gastrointestinal, intravenous, respiratory, and topical routes has been intensively studied with alginates. It's a biodegradable polymer with a low toxicity level (Ching et al. 2017). These are the major characteristics that distinguish alginate as one of the natural polymers with the broadest biological applications (Hariyadi and Islam 2020; Sosnik 2014). Alginates can be tailored to fulfill the requirements of biomedical and pharmaceutical industries. This has many properties that used as a formulation excipient which makes it as an important component in polymeric-controlled delivery. Biopolymers, notably alginates, have piqued the interest of the pharmaceutical and biotechnology industries in recent years (Shilpa et al. 2003). From long time, the naturally present alginate polymers have been applied as a thickening, gel-forming, and dispersion stabilizing ingredient in the foodservice industry. They're also employed in tablet manufacturing as binders and disintegrants.

Alginate has various properties which make it viable polymer for the development of sustained-release delivery systems along with the commonly applicable food additive (Liew et al. 2006). Oral dosage is currently the most recurrent use of alginate in therapeutic applications, while usage of alginate forms hydrogels for biomedical application is rising. Oral dose forms such as tablets and capsules are the most used. The products are typically manufactured in an immediate-release form, which allows for fast absorption of the drug. The process of coating over the drug dosage form can reproduced as sustained-release formulation. Sustained release systems are intended to provide drug in a consistent and kinetically predictable manner. Alginates can be used in pharmaceutical dosage formulations as type of release (Tønnesen and Karlsen 2002). Sodium alginate is typically utilized as a tablet binding agent, whereas, in compressed tablets engineered for quick drug release (Nahar et al. 2017).

2 Physicochemical Properties of Alginate

Marine algae including *Laminaria hyperborea*, *Ascophyllum nodosum*, and *Macrocystis pyrifera* are used to make commercial alginates made of the linear unbranched polysaccharides of alginates with different quantities of D-mannuronic acid (M) and -L-guluronic acid (G) (Wee and Gombotz 1998). The glycosidic 1–4 linkages connect the M and G monomers, generating MM or GG homopolymeric blocks which are interleaved with MG or GM heteropolymeric blocks. The sources of marine algae and the tissue from which alginates are derived, and the crop harvesting season all influence the molecular variability of this polymer. In the leaves of *Laminaria hyperborean* have a more concentration of mannuronic acid, whereas the outer cortex and stipe have a huge concentration of guluronic acid. In the same examples of *Ascophyllum nodosum* fruits hold more mannuronic acid than old tissue that rich in guluronic acid (Galus and Lenart 2013). The structural arrangement of the M, G, and MG blocks is established using ^1H -NMR and ^{13}C -NMR techniques. The content, order of polymer blocks, and molecular weight of alginates are all factors that influence the gel's physicochemical qualities.

Alginates rich in guluronic acid blocks produce significantly stronger gels than alginates abundant in mannuronate, since the G content have a larger attraction with divalent ions in compared to M content (Tønnesen and Karlsen 2002). The M/G ratio has a considerable impact in respect to transmittance, swelling, and viscoelasticity of gel forming alginate membranes (Sanchez-Ballester et al. 2021). When compared to neutralized macromolecules, alginate's capacity to generate two forms of gels depends on an acidic gel, pH and an ionotropic gel provides special features of the polymer. The type of gel generated will determine the physicochemical features of the polymeric matrix as well as the swelling procedure used to stimulate drug release. Alginic acid and its salts are considered non-toxic and biocompatible (Szekalska et al. 2016). These materials are available in pharma market in more than 200 form of alginate, as well as alginic acid and several related salts, are produced. In the food, cosmetic and pharma industries, alginates are frequently used (Jain and Bar-Shalom 2014). Alginates have carboxyl groups that are chargeable at pH values more than 3–4, making them soluble in neutral and alkaline circumstances, allowing them to be widely used. Alginate is a preferred polymer for some medications that require higher protection and favorable consumption in the digestive tract or even other parameters such as altered drug release. Alginate is an excellent biomaterial for drug delivery systems because of its solubility and pH sensitivity (Jain and Bar-Shalom 2014).

Sodium alginate (SA) has been the most common kind of alginate utilized in the pharma industry, and it can be used to prolong drug release. SA with the various features impacting the release of drug from matrices such as, particle size distributions, viscosities, and elemental composition are employed to manufacture matrix tablets of different doses. The release of drugs from one of these matrices is influenced by particle size. It has an impact on the amount of the burst release; increased viscosity of alginate retards the release of drug in the buffer media but

increase in the acidic media; the impact of increasing alginate amount is greater with bigger alginate particles. Following that, SA is separated into smaller particle size by the process of cryogenically milled as needed (Tuğcu-Demiröz et al. 2007). The molecular structure and viscosity of polymer are essential factors in influencing their swelling and gelling properties; the initial structure is frequently denoted from alginate's FG value, which is really proportion of guluronic acid content in the alginate (Holte et al. 2003).

The type and amount of crosslinker used are also crucial. Ionic strength influences the solubility of alginate, which is a polyanion. The pKa of mannuronic acid and guluronic acid are 3.38 and 3.65 respectively, and the solubility of alginate is similarly influenced by its main component and pH (Haug et al. 1967). Moreover, alginate is a very interesting polymer in numerous therapeutic plants because of its ability to connect differently with diverse chemical environments (Tønnesen and Karlsen 2002). Alginates gel at mild chemical circumstances and are usually well tolerated and non-toxic (Kikuchi et al. 1999; King 1983). It can be used to encapsulate cells and deliver a variety of bioactive components, including macromolecules with essential secondary or tertiary structures. They have a variety of drug delivery applications.

3 Oral Drug Delivery of Alginate-Based Matrix Tablet

The oral administration of drug is the most preferred route for wide range of drug because of its better patient compliance with safety parameters. Tablets are the most commonly used dosage form because of its simple handling and preparation procedure. The formulation of matrix tablet involved simple mechanical mixing of all ingredients followed by direct compression (Mujtaba and Kohli 2016). This method of preparation avoids initial procedure such as granulation or coating. The preparation of tablet from alginate polymers carried following procedure including; dry or wet granulation, direct compression and different coating methods (Holte et al. 2003).

The polymer-based matrix tablets where specially alginate polymers are used to design the matrix tablet for oral delivery that provide controlled drug release for extended periods. These matrix tablets are very economical with low-cost production and simple development procedure enhanced their scale-up production. Now from few years, alginate has been mostly utilized to fabricate the hydrophilic based matrix tablets. The general mechanism for drugs released from hydrophilic matrix are either dispersed or dissolved in aqueous medium through hydration, swelling and dissolving of alginate matrix (Tiwari et al. 2011). The hydrophilic alginate-based matrices able to provide appropriate release profiles for broad categories of drugs. The summary of matrix tablet preparation, advantages and limitation are presented in Table 1.

Table 1 Development of sodium alginate matrix tablets formulations; their advantages and limitations

<i>Developmental path for matrix tablet formulations</i>
- Active drug/New Chemical entity is homogeneously dispersed (embedded) in an inert material - Polymers could be an ideal matrix material that can control the rate of diffusion - Many matrix materials consist of hydrophilic or hydrophobic polymers - Sodium alginate has been employed as a hydrophilic matrix as it can quickly hydrate and swell in water to produce a gel layer - The particle size and mannuronate/guluronate (M/G) content ratio can influence drug release in sodium alginate - In terms of the release rate and subsequent absorption of drugs, diffusional path length will affect their release out of the tablet - Factors such as diffusion, permeation, and dissolution affect the material properties that affect drug release - Formulation of matrix tablets is based on clinical/commercial triggers
<i>Advantages of matrix tablets formulations</i>
<i>Clinical advantages</i>
- Most commonly used means of controlled release system because of their compatibility - Patients comply better with the dosage by administering the medication less often, the drug levels are more stable, maximum benefit is obtained, and the drug has a higher safety margin - Better control of therapeutic drug concentration which ultimately reduce drug toxicity - Enhanced drug stability in GI milieu - Minimize the local and systemic side effects
<i>Commercial/industrial advantages matrix tablet formulations</i>
- Cost effective - Easy to fabricate - Drug could be protected from hydrolysis or other changes in GIT, so enhanced stability - Compounds with high molecular weight could be formulated - Reproduce to a desirable drug profile - Less dose of drug is required
<i>Limitation of matrix tablet formulations</i>
- Limited control of mechanical properties - Possibility of dose dumping if not formulated well - Greater dependence on GI transit time of tablets - Not all drugs can be formulated as matrix tablets

3.1 Types of Matrix-Based Tablets

Matrix tablets are an effective option for establishing prolonged drug therapy because they are the most cost-effective solid dosage forms for sustained and controlled release. Matrix tablets also seem to be an oral dosage forms wherein the active pharmaceutical ingredient or drug is uniformly distributed throughout hydrophobic or hydrophilic matrices. These processes use dissolution- and

diffusion-controlled mechanisms to continuously release the drug. Alginate based matrix tablets is of following types:

3.1.1 Immediate Release Matrix Tablets

In compressed tablets intended for quick release of drug, SA is traditionally seen in the form of tablet binding agent, however alginic acid is often utilized as a tablet disintegrator (Onsøyen 1996). The influence of SA on the durability's of tablet is related to the proportion used in the designed dosage form while in some studies their disintegration can enhance with the alginate salt. SA is available in different grades that showed different properties. These factors influencing the drug release in preparation of matrix tablets. The size, viscosity, and concentration of alginate particles have been shown to influence not only the rate but also the mechanism of drug release. Moreover, for a hydrophilic drug in the availability of hydrophilic excipient, SA formulations can retain drug release until at least 8 h (Liew et al. 2006). Moreover, easy water solubility, significant swelling and quick erosion of SA based matrix tablets are little to downsides that prevent it from being a perfect matrix material. Chemical modification of SA could be a viable option for forming a stable network and changing its physico-chemical properties (Yang et al. 2011).

3.1.2 Controlled Released Matrix Tablets

SA, that can constitute viscous solutions whenever it comes into contact with water, is often used to make matrices for extended-drug release (ER), including such beads, microspheres, and tablets (Liew et al. 2006; Fu et al. 2014). Direct compression can be used to make SA matrix tablets, which is recommended in the industry because it is less expensive (Holte et al. 2003). In peroral controlled drug delivery systems, hydrophilic polymer matrices have been broadly used. The formulation and production of polymer-based matrix tablets is relatively simple and inexpensive (Mujtaba et al. 2014).

Many authors have reported the development of alginate-based bioadhesive formulations or dosage form with prolong residence time in gastric that could result in sustained release delivery from the dosage form (Miyazaki et al. 1995; Yong et al. 2001; Murata et al. 2000). Direct compression was used to make matrix tablets with SA as release retarding agent (Moroni and Drefko 2002; Holte et al. 2003). These kinds of works have shown that alginate matrix tablets can be manufactured in a commercial setting. Alginate matrices can be manufactured by direct compression as well as compacting alginate granules (Timmins et al. 1992; Holte et al. 2003). However, there is still a scarcity of research on alginate matrix tablets. Commercially available SA comes in a variety of particle sizes, molecular weights, and chemical compositions. These differences could have an impact on how drugs are released. Liew et al. prepared matrix tablets with 17 grades of SA and found that viscosity, particle size of alginate and quantity impact the release rate of drug as well as

mechanism of drug release. This study also demonstrated the existence of a water-miscible ingredients, drug release can be sustained for at least 8 h (Liew et al. 2006).

Biopolymers and their derivatives are efficient matrix content for the preparation of oral extended-release tablet. It's used to make various drug matrix tablets. The aqueous viscous layer that forms surrounding the tablet when it encounters with water serves as a barrier against water penetration and drug diffusion. These features have been used to create a drug delivery system with a sustained release effect (Mandal et al. 2009).

Two different types of SA in different amounts were used to produce matrix tablets. According to Tuğcu-Demiröz et al. (2007) findings, the kind and quantity of alginate in the formulations had impact in basic medium. The alginate matrix tablet of mesalazine formulations release with low viscosity alginate was calculated and the results were nearly equivalent to the marketed formulation in acidic and basic medium. The medication can be given to both the small and large intestines. As a result, the alginate-based matrix tablet could be an effective treatment option that affects the whole intestine in case of Crohn's disease.

According to Fu et al. (2014) identify the inter-grade and inter-batch fluctuation of SA used during preparation of tablets matrix. To make matrix tablets, four distinct grades with three sets of each grade of SA have been used. Different viscosity grades showed a significant difference of swelling and erosion activity of matrix tablets. Although different sets of the same grade have significant variances in their matrix tablet swelling and erosion pattern. Some of the erosion nature of SA matrix tablets can be elaborated with its rheological qualities (both apparent viscosity and visco-elasticity) in aqueous medium. A summary of alginate based hydrophilic matrix tablets system is presented in Table 2.

4 Preparation of Alginate-Based Matrix Tablets with Co-polymer

4.1 *Grafted Co-polymers of Polyacrylamide-Sodium Alginate (PAam-g-SA) Based Matrix Tablet*

One of the studies performed by Mandal et al. (2010) in which they prepared the matrix tablets of diltiazem hydrochloride (DTZ) who have used grafted copolymer of polyacrylamide-sodium alginate (PAam-g-SA) with various grafting proportion and their own limited hydrolyzed molecules in order to attain prolonged release of the hydrophilic drug. DTZ enloaded tablets was designed with wet granulation techniques of PAam-g-SA co-polymers or limited hydrolyzed PAam-g-SA co-polymers. In simulated gastrointestinal fluid, the impacts of, drug loading, process of grafting, and presence of calcium gluconate (CG), which was considered were investigated as ingredients. Even as tablets produced with co-polymers with higher grafting percentages presented rapid release of drug (100% in 5.5 h)

Table 2 Alginate based hydrophilic matrix system used in pharmaceutical tablets

Various alginate based hydrophilic matrix tablets system	References
Carbopol [®] 971P and low-viscosity sodium alginate for pH-independent controlled drug release matrix tablets	Al-Zoubi et al. (2011)
Mechanistic study on hydration and drug release behavior of sodium alginate matrix tablets	Chan et al. (2007)
Modifying matrix micro-environmental pH to achieve sustained drug release from highly laminating alginate matrices	Ching et al. (2008)
Effect of erosion and swelling on the dissolution of theophylline from low and high viscosity sodium alginate matrices	Efentakis and Buckton (2002)
Inter-grade and inter-batch variability of sodium alginate used in alginate-based matrix tablets	Fu et al. (2014)
Alginate compressed matrices as prolonged drug delivery systems	Giunchedi et al. (2000)
Sustained release of water-soluble drug from directly compressed alginate tablets	Holte et al. (2003)
Effect of formulation variables on in vitro release of a water-soluble drug from chitosan–sodium alginate matrix tablets	Li et al. (2015)
Evaluation of sodium alginate as drug release modifier in matrix tablets	Liew et al. (2006)
Sustained release of a water-soluble drug from alginate matrix tablets prepared by wet granulation method	Mandal et al. (2009)
Matrix tablet prepared with polyacrylamide-g-sodium alginate co-polymers and their partially hydrolyzed co-polymers for sustained release of diltiazem hydrochloride	Mandal et al. (2010)
Drug release from oral mucosal adhesive tablets of chitosan and sodium alginate	Miyazaki et al. (1995)
Formulations of zero-order, pH-dependent, sustained release matrix systems by ionotropic gelation of alginate-containing mixtures	Moroni et al. (2011)
In vitro/in vivo evaluation of HPMC/alginate based extended-release matrix tablets of cefpodoxime proxetil	Mujtaba and Kohli (2016)
Controlled delivery of drugs from alginate matrix	Shilpa et al. (2003)
Swelling, erosion and release behavior of alginate-based matrix tablets	Sriamornsak et al. (2007)
Spray-dried composite particles of lactose and sodium alginate for direct tableting and controlled releasing	Takeuchi et al. (1998)
Evaluation of alginate based mesalazine tablets for intestinal drug delivery	Tuğcu-Demiröz et al. (2007)

whenever tablets produced from correlating of hydrolyzed grafted co-polymer revealed slow drug release (71% in 12 h). The needed scale respect to viscosity and the copolymer's swelling capability appeared to be controlling this release behavior. Furthermore, an increase in drug load reduced release of drug across all kinds of tablets, whereas a rise in CG enhanced drug release. The findings suggest that slightly hydrolyzed PAam-g-SA co-polymer showed a decent matrix content for preparing DTZ tablets with sustained release.

In another study by Mandal et al. (2009) traditional wet granulation method was used to produce a DTZ loaded matrix tablet with SA and CG for extended drug

release. The tablets designed with a 2:1 w/w ratio of CG/SA developed the controlled drug release, enduring up to 13.5 h. The drug was released faster at this higher and lower ratio. The drug load and tablet hardness resulted in very little deviation in release of drug. However, the inclusion of acidic or alkaline micro environmental remarked really does not prolong the release; rather, these excipients caused diltiazem to be released more quickly. This research found that choosing the right SA/CG ratio is critical for producing alginate-based matrix tablets using the wet granulation techniques for long-term DTZ release.

4.2 Hydroxypropyl Methylcellulose (HPMC) with Sodium Alginate-Based Matrix Tablets

Because of excellent biocompatibility and simplicity of gelation, SA hydrogels are showing promise for oral medication administration. For example, a combination of HPMC and SA in verapamil hydrochloride prolonged-release matrices produces a better drug release profile *in vivo*. SA is pH sensitive owing to the availability of carboxylate groups that could receive or donate protons in responding with pH change. The soluble form of sodium salt is changed to insoluble form of alginic acid for the pH lower than the pKa of M (3.38) and G (3.65) content. After then the pH sensitivity of SA in matrix tablets may have an impact on the diffusion barrier's properties and, as a consequent, drug release (Lee and Mooney 2012).

Giunchedi et al. (2000) studied the application of SA in the design of hydrophilic matrix tablets of ketoprofen for prolonged release. This study demonstrated the use of SA to change release kinetics in hydrophilic matrix tablets produced by direct compression. The results revealed SA matrices and mixed matrices of SA and HPMC had the best sustained release effects and shown zero-order release rate. The presence of CG causes the change in release rate of matrices as the calcium ions, cause an ionic interaction, which cause a rise in ketoprofen release from the tablet matrices. Furthermore, only formulations with the highest amount of HPMC are capable of providing a prolonged drug release.

Mujtaba and Kohli (2016) used a combination of biodegradable polysaccharides as matrix material, including HPMC and SA, to reach pH-independent extended release. *In vitro* study revealed that the release of drug was in a sustained manner over a 24-h period. The drug release pattern accompanied Higuchi kinetics because the obtained plots had the maximum linearity, but a significant linearity had also observed with zero-order models, indicating that the drug release method was non-Fickian in nature. *In vitro* release of drug was also applied in antimicrobial assay, which indicated that release of drug over a 24-h period was maximum than the MIC reported. *In vivo* studies performed on rabbits revealed that cefpodoxime from matrix tablets has an ER pharmacokinetic profile. There was a good linkage between *in-vitro* release of drug and *in-vivo* absorption of drug. The obtained results indicated

the cefpodoxime matrix tablets under investigative process showed more potential for controlled release dosage forms.

4.3 Methyl Cellulose Polymers with Sodium Alginate-Based Matrix Tablets

Reddy et al. (2003) designed the nicorandil loaded sustained-release matrix tablets with ethylcellulose (EC), Eudragit RS-100, Eudragit RL-100 and polyvinylpyrrolidone as granulating agents and hydrophilic polymer such as sodium carboxymethyl cellulose, HPMC and SA using a wet granulation method. Dissolution studies revealed that a formulation (drug-to-HPMC ratio of 1:4; with granulating agent ethanol) might prolonged release of drug for 24 h. In the subsequent dosage design process, the most effective preparation of the investigation (drug-HPMC, 1:4; 4% w/v of granulating agent EC) demonstrated excellent drug release in initial hours, and the overall release profile was mostly like the reported value.

4.4 Chitosan with Sodium Alginate-Based Matrix Tablets

By changing formulation variables, Li et al. (2015) examine the possibility of matrix tablets by considering chitosan-sodium alginate (CS-SA) combination in prolonged-release of hydrophilic drugs trimetazidine hydrochloride (TH). In this study, if the content of CS-SA increased, the release rate of drug decreased. The CS:SA ratio had only a minor impact on drug release, and there were not showed the effect related to types of SA on release of drug. These findings revealed that CS able to cover the amount of SA in gastric fluid accompanied by intestinal fluid through a synergic interaction with SA, allowing for longer release of drug. The quantity of CS-SA had the greater impact on kinetics of drug release. The CS:SA ratio, on the other hand, had only a minor impact on release of drug, while different form of SA had no impact on TH release rate.

4.5 Carbopol with Sodium Alginate-Based Matrix Tablets

Al-Zoubi et al. (2011) designed the matrix tablets with various proportion of Carbopol (CP) with less-viscosity SA to obtained pH-independent sustained drug release. Prior to actually compaction, two methodologies including physical mixing (PM) and spray-drying (SD) were used to compare the drug release from respective matrices. Three different categories of drugs (verapamil HCl, paracetamol and salicylic acid) with two dissolution media (0.1 N HCl or phosphate buffer,

pH = 6.8) were used to examine the release of drug parameters from SA-CP PM matrixes. The results revealed that the method of processing (SD or PM) had a considerable influence on the drug release process from CP-SA matrices. The matrix tablets with CP:SA ratio can be adjusted to control the rate of drug release and eliminate dependencies on pH. Matrix tablets made of CP and SA are a possibly beneficial efficient for pH-independent sustained release which could be made using the SD methodology or much easily with direct compressing PM.

5 Release Pattern from Alginate-Based Matrix Tablets

Alginate and other hydrocolloids can be useful in the development of a sustained release formulation. Moreover, alginate moiety would be hydrated instantly resulting in a hydrocolloidal layer with a more viscosity. This creates a diffusion layer, preventing small molecules from migrating such as drugs. Alginate is most commonly employed in diffusion-based systems. The drug is diffused uniformly through polymer matrix in the rate-controlled way. Diffusion across matrix swelling and dissolution/erosion through the matrix region modulates drug release when these complexes are subjected to the dissolution medium (Takka et al. 1998). The “swelling–dissolution–erosion” cycle is a complicated one. The osmotic pressure gradient which persists around the alginate gel and surroundings media is a crucial component in the swelling process in systems that depend on the cross-linking of SA with calcium chloride.

The hydration features including swelling or erosion of selected polymer and the physicochemical parameters for the subsequent layer of polymer gel was developed surrounding the matrices control the drug release through water soluble polymer matrices (Efentakis and Buckton 2002; Sriamornsak et al. 2007). Drugs soluble in water are released initially through diffusion of solubilized drug entity through the layer of polymer gel, while hydrophobic drugs are primarily released through polymer erosion from layer of polymer gel-solution interface (Liew et al. 2006). Slower erosion and higher swelling rates are observed in SA revealed relatively high viscoelasticity and apparent viscosity in aqueous medium. The release of drugs is slower in compacts made with grades or formulation batches have maximum viscoelasticity and viscosity. The apparent viscosities of SA aqueous medium at small concentrations singly are not capable of predicting SA functionality in tablets matrix for grades or batches with equivalent apparent viscosities. The viscoelastic features of SA aqueous medium at a single maximum concentration, which corresponds to the state of polymer gel, could be useful indicators of SA matrix tablet prolonged-release behavior. Varies in different grades of SA show a significant difference in erosion and swelling actions of SA tablets matrix. Also, different batches from same grade of SA have considerable differences in swelling and erosion nature of matrix tablet.

The swelling and erosion activity of SA can be used to better understand SA's capabilities in ER matrix tablets. Several groups have investigated the differences in

swelling and erosion patterns in matrix tablets designed from different grades of SA with distinct molecular weights and chemical compositions.

5.1 Release Pattern Affected with Different Viscosity Grades of Alginate

The significant differences observed in erosion pattern of matrix tablets formulated from different SA grades could explain the differences obtained in swelling behavior, that could be understood with the rheological properties (both apparent viscosity and viscoelasticity) of SA solution medium. The less erosion and high swelling rates are observed in SA with elevated apparent viscosity and viscoelasticity in aqueous phase. Drug release is slower in formulations made with grades or batches that have higher viscoelasticity and viscosity. The use of SA with various chemical features and degrees of viscosity was used to slow the ibuprofen release through press-coated tablets (Cardoso et al. 2016). Alginate carboxyl radicals are protonated in acidic conditions, restricting drug release. When the pH is above 6, alginate could crosslink to Ca^{2+} ions via ionotropic gelation. Ba^{2+} and Zn^{2+} are also used as crosslinkers (Mirtič et al. 2018; Agulhon et al. 2012).

Holte et al. (2003) prepared compressed tablets of acetyl salicylic acid prepared with four different grades of alginate. In various formulations, the effect of the type and quantity of alginate on the release process of drugs was investigated. In this study there was no discernible variation in the drug release patterns of the tablets produced by the various grades of alginate. SA in conjunction with calcium dihydrogen phosphate provided controlled drug release for up to 16 h.

According to Efentakis and Buckton (2002) research on SA based matrix tablets made from two grades of SA determined that the alginate with high viscosity grade developed a more considerable layer of gel and degraded with slower rate in aqueous medium compared with low viscosity. The measuring viscosity of two grades of SA was 14 and 0.2 Pa s (measured at 25 °C in a 2% solution).

Sriamornsak et al. (2007) studied the swelling and erosion patterns of SA matrix tablets made with three different viscosity grades 0.3 Pas (high percent guluronic acid), 0.3 Pas (low percent guluronic acid) and 0.035 Pas. Their findings showed that the swelling and erosion pattern of such three grades of SA does not differ considerably in an acidic solution and did differ considerably in a phosphate buffer. In case of phosphate buffer, high viscous grades swelled with greater extent and eroded with lesser extent than low viscosity grades. The swelling and erosion features of two grades of similar viscosity but different %G value was considerably not different. Chan et al. (2007) also tried to compare the swelling and erosion properties of two different SA grades (kinematic viscosity of 3 and 108 mm²/s respectively). Their findings revealed that the dissolution patterns of alginate composites with various viscosities were equivalent in acid medium but changed as the pH increased because

the effect of viscosity of alginate on uptake of water, erosion, and significant swelling at pH close to neutral.

5.2 Release Pattern of Alginate in Different pH Media

Cryogenic electron microscopy demonstrated that the SA matrices under simulated gastric fluid (SGF) formed hydrated surface layer appeared as particulate form and porous, causing formation of crack or lamination on the SA tablet matrix and resulting in burst release of drug in selected gastric environment. These weakened the diffusion barrier of drug, resulting in deficiency of controllable release. However, in simulated intestinal fluid (SIF), a prominent uniform hydrous swollen layer was developed (Hodsdon et al. 1995). Because of swelling and erosion in SIF, SA designed matrix tablets never could usually prolonged release of drug above 12 h (Sriamornsak et al. 2007; Liew et al. 2006). To address this insufficiency, some novel approaches to modifying SA matrices for better drug release control have been tried, including the addition of pH-modifiers (Ching et al. 2008), the inclusion of crosslinking agents (Lee and Mooney 2012), and the combination with some other hydrophilic matrices (Al-Zoubi et al. 2011). SA in combination with CS was found to be particularly effective at limiting drug release.

5.3 Release Pattern of Sodium Alginate-Chitosan Matrix Tablets

CS is a positively charge polysaccharide made of repeated D-glucosamine and N-acetyl-D-glucosamine linked with glycosidic (1–4) bonds and procured by deacetylation process of crustacean shells chitin (Ding et al. 2012). It has been revealed that CS-SA polymer complexes can be utilized as orally prolonged-release matrix formulation. As a result of the interaction with CS, the unification of SA matrices was boosted, and the encapsulated drugs were preserved for prolonged period. All the while, due to gelling, CS showed the ability to control drug release (George and Abraham 2006). SA was used to try to sustained the release of highly water miscible drugs like chlorpheniramine maleate (Ching et al. 2008), diltiazem hydrochloride (Mandal et al. 2009), and verapamil hydrochloride (Al-Zoubi et al. 2011), but it has some limitations. As a result, CS-SA based tablets containing a hydrophilic drug elicit more interest because they are simple and inexpensive using standard procedures to make the tablet.

5.4 Release Pattern Affected with Physiochemical Properties of Alginate

Sriamornsak et al. (2007) investigated the effect of several factors on release of drug through alginate-based matrix tablets, including drug particle size, additive used, and medium pH. The alginate-based matrix tablets swelled or eroded when contact with the water, generating a persistent gel layer and encountering the hybrid of swelling and erosion. In this study swelling response of alginate matrices is governed by the hydration process in the medium. In an acidic medium, various grade of alginate had no effect at matrix swelling; however, in a neutral medium, they had a significant impact. In an acidic environment, the inclusion of calcium or ammonium salts caused the tablets to disintegrate. Moreover, inclusion of sodium bicarbonate and calcium acetate could change the swelling of the tablet around acidic environment. The kinetics and release of drug mechanism through alginate-based matrix tablets were understand with extent of matrix swelling, erosion and diffusion of the drug. In acidic medium, the majority data of release fit well into the Korsmeyer-Peppas equation, but in neutral medium, the zero-order release model fit well.

5.5 Release Pattern of Different Nature of Drug from Alginate Matrix

The creation of a solubilized viscous film surrounds the tablet control the drug release by resisting water permeation and movement of suspended particles through the matrix tablet, regulates the process of drug release across the alginate matrix tablets (Bamba et al. 1979). Water miscible drugs are released primarily through the diffusion of solubilized therapeutic agents across the gel layer, however water-insoluble drugs are primarily released through erosion processes. Drug solubility, as well as the mechanical and physical features of the gel shield those develops surrounding the tablet, influence the participation of every release process to the cumulative drug release parameters (Hodsdon et al. 1995). The physical appearance of the hydrated gel layer, as well as the polymer's hydration properties, may have a characteristic impact on drug release (Melia 1991), as well as any changes in the features of the aqueous surface layer produced by pH fluctuations are mostly affect the properties of water-soluble polymers as a controlled release carrier.

Lipophilic drugs are released initially from mechanisms of erosion, however diffusion of drug entity across the gel is the primary techniques through that hydrophilic drug are released. Drugs with a high solubility in water are released considerably quicker in gastric medium than in intestinal medium in a formulation prepared with direct compression of drug–alginate mixture, whereas drugs with a low water miscibility are significantly released slower in gastric medium than in intestinal medium (Hodsdon et al. 1995) This is explained by the fact that the two media have different hydration kinetics.

Due to the obvious negative charge of the matrix, cationic drug molecules (e.g., lidocaine HCl) appear to release more slowly than anionic drug molecules (e.g., sodium salicylate) (Park et al. 1998). The pH-independent gelling agent such as cellulose polymers can be used in a tablet of a basic drug to provide the pH-independent release rate (Howard and Timmins 1988). The release of acetaminophen was obtained to be extremely slow in an acidic medium and faster in a neutral medium from tablet formulated by spray-dried lactose–alginate particles (Takeuchi et al. 1998).

The pharmaceutical industry has made extensive use of sodium salt of alginate ability which is quickly develop viscous gels solutions in contact with aqueous medium, in the form of water-soluble matrix prolonged the release of oral formulation. Many drugs were strongly prolonged with the use of matrices containing salts of alginate or combined effect of alginate with different polymers. A few of these studies have revealed that different alginate grades have different effects on the qualities of drug release (Efentakis and Buckton 2002; Liew et al. 2006).

5.6 Release Pattern from Alginate-Containing Mixtures

The combination of the ionic gum SA with the other gel-forming gums including propylene glycol alginate (PGA), xanthan, or HPMC for the development of controlled dosage forms containing three medicines of varied solubility and ionic character was studied by Moroni et al. Moroni et al. (2011). The mixture was compacted into tablets and evaluated for stability and transit through GI tract at various pHs to imitate in the context of Ca^{2+} . The findings revealed that mixtures could prolong drug release lasting up to 12 h during movement from acidic to alkaline pHs to replicate GI tract travel and even in the presence of Ca^{2+} . The release rate was regulated by choosing the suitable SA/other gum mixture in the right ratio.

Gutsche et al. (2008) used sustained release preparation based on the SA to produce pH-independent releasing of weakly basic medicines. The pH-dependent dissolution of a weakly basic molecule verapamil hydrochloride was examined using three different methodologies. To begin, matrix tablets were made by compressing the drug component directly with several forms of SA. In other way, pH-modifying agents were introduced into the drug/alginate matrix composites. Next of it, they made press-coated tablets with an internal pH-changer tablets core and an exterior coating of drug or SA. Matrix tablets containing solely specified alginates and pharmacological material were used to obtained pH-independent release of drug. Higher pH improves alginates' solubility. As a result, they can compensate for the less miscibility of weakly basic compounds at higher pH since the tablet matrix quickly dissolves. This method worked well with alginates, which showed rapid hydration and disintegration at high pH. This strategy failed with alginates with minimum degradation at higher pH. When fumaric acid was added to drug or alginate-based tablet, the pH inside the tablets was reduced, boosting the solubility

of the weakly basic drug at higher pH. As a result, regardless of the various form of alginate utilized, pH-independent release of drug was obtained.

6 Conclusion

Alginates are natural polymers that could be utilized to develop many drug dosage formulations. This type of polymer has several properties that make it helpful as a formulation aid as a traditional ingredient and, more crucially, as just a device in controlled delivery system. It was discovered that the viscosity, molecule size and concentration of alginate influence the rate as well as drug release mechanism. A unique property of SA that can be used in dosage form design is its sensitivity to pH and capability to create gel layer at acidic and near-neutral environments. The amount and type of crosslinker used are also crucial. In developing modified-release dosage forms, proper use of different grades of alginate is crucial.

References

- Agulhon P, Robitzer M, David L, Quignard F (2012) Structural regime identification in ionotropic alginate gels: influence of the cation nature and alginate structure. *Biomacromolecules* 13(1): 215–220
- Al-Zoubi NM, AlKhatib HS, Obeidat WM (2011) Evaluation of hydrophilic matrix tablets based on Carbopol® 971P and low-viscosity sodium alginate for pH-independent controlled drug release. *Drug Dev Ind Pharm* 37(7):798–808
- Bamba M, Puisieux F, Marty JP, Carstensen JT (1979) Release mechanisms in gel forming sustained release preparations. *Int J Pharm* 2:307–315
- Cardoso MJ, Costa RR, Mano JF (2016) Marine origin polysaccharides in drug delivery systems. *Mar Drugs* 14(2):34
- Chan LW, Ching AL, Liew CV, Heng PW (2007) Mechanistic study on hydration and drug release behavior of sodium alginate compacts. *Drug Dev Ind Pharm* 33(6):667–676
- Ching AL, Liew CV, Chan LW, Heng PW (2008) Modifying matrix micro-environmental pH to achieve sustained drug release from highly laminating alginate matrices. *Eur J Pharm Sci* 33(4–5):361–370
- Ching SH, Bansal N, Bhandari B (2017) Alginate gel particles-A review of production techniques and physical properties. *Crit Rev Food Sci Nutr* 57(6):1133–1152
- Ding J, Na L, Mao S (2012) Chitosan and its derivatives as the carrier for intranasal drug delivery. *Asian J Pharm Sci* 7(6):349–361
- Efentakis M, Buckton G (2002) The effect of erosion and swelling on the dissolution of theophylline from low and high viscosity sodium alginate matrices. *Pharm Dev Technol* 7(1):69–77
- Fu S, Buckner IS, Block LH (2014) Inter-grade and inter-batch variability of sodium alginate used in alginate-based matrix tablets. *AAPS PharmSciTech* 15(5):1228–1237
- Galus S, Lenart A (2013) Development and characterization of composite edible films based on sodium alginate and pectin. *J Food Eng* 115(4):459–465
- George M, Abraham TE (2006) Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan—a review. *J Control Release* 114(1):1–14

- Giunchedi P, Gavini E, Moretti MDL, Pirisino G (2000) Evaluation of alginate compressed matrices as prolonged drug delivery systems. *AAPS PharmSciTech* 1(3):31–36
- Gutsche S, Krause M, Kranz H (2008) Strategies to overcome pH-dependent solubility of weakly basic drugs by using different types of alginates. *Drug Dev Ind Pharm* 34(12):1277–1284
- Hariyadi DM, Islam N (2020) Current status of alginate in drug delivery. *Adv Pharmacol Pharm Sci* 2020:8886095
- Haug A, Mykkestad S, Larsen B, Smidsrod O, Eriksson G, Blinc R, Pausak S, Ehrenberg L, Dumanović J (1967) Correlation between chemical structure and physical properties of alginate. *Acta Chem Scand* 21(3):768–778
- Hodsdon AC, Mitchell JR, Davies MC, Melia CD (1995) Structure and behaviour in hydrophilic matrix sustained release dosage forms: 3. The influence of pH on the sustained-release performance and internal gel structure of sodium alginate matrices. *J Control Release* 33(1):143–152
- Holte Ø, Onsøyen E, Myrvold R, Karlsen J (2003) Sustained release of water-soluble drug from directly compressed alginate tablets. *Eur J Pharm Sci* 20(4–5):403–407
- Howard JR, Timmins P (1988) Controlled release formulation. US Patent 4,792,452
- Jain D, Bar-Shalom D (2014) Alginate drug delivery systems: application in context of pharmaceutical and biomedical research. *Drug Dev Ind Pharm* 40(12):1576–1584
- Kikuchi A, Kawabuchi M, Watanabe A, Sugihara M, Sakurai Y, Okano T (1999) Effect of Ca^{2+} – alginate gel dissolution on release of dextran with different molecular weights. *J Control Release* 58(1):21–28
- King AH (1983) Brown seaweed extracts (Alginates). In: Glicksham M (ed) *Food hydrocolloids*, vol 2. CRC Press, Boca Raton, FL, pp 116–154
- Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. *Prog Polym Sci* 37(1):106–126
- Li L, Li J, Si S, Wang L, Shi C, Sun Y, Liang Z, Mao S (2015) Effect of formulation variables on in vitro release of a water-soluble drug from chitosan–sodium alginate matrix tablets. *Asian J Pharm Sci* 10(4):314–321
- Liew CV, Chan LW, Ching AL, Heng PW (2006) Evaluation of sodium alginate as drug release modifier in matrix tablets. *Int J Pharm* 309(1–2):25–37
- Mandal S, Basu SK, Sa B (2009) Sustained release of a water-soluble drug from alginate matrix tablets prepared by wet granulation method. *AAPS PharmSciTech* 10(4):1348–1356
- Mandal S, Ray R, Basu SK, Sa B (2010) Evaluation of a matrix tablet prepared with polyacrylamide-g-sodium alginate co-polymers and their partially hydrolyzed co-polymers for sustained release of diltiazem hydrochloride. *J Biomater Sci Polym Ed* 21(13):1799–1814
- Melia CD (1991) Hydrophilic matrix sustained release systems based on polysaccharide carriers. *Crit Rev Ther Drug Carrier Syst* 8(4):395–421
- Mirtič J, Ilaš J, Kristl J (2018) Influence of different classes of crosslinkers on alginate polyelectrolyte nanoparticle formation, thermodynamics and characteristics. *Carbohydr Polym* 181:93–102
- Miyazaki S, Nakayama A, Oda M, Takada M, Attwood D (1995) Drug release from oral mucosal adhesive tablets of chitosan and sodium alginate. *Int J Pharm* 118(2):257–263
- Moroni A, Drefko W (2002). pH-dependent sustained release. US Patent US 6,465,014 B1
- Moroni A, Drefko W, Thone G (2011) Formulations of zero-order, pH-dependent, sustained release matrix systems by ionotropic gelation of alginate-containing mixtures. *Drug Dev Ind Pharm* 37(2):216–224
- Mujtaba A, Kohli K (2016) In vitro/in vivo evaluation of HPMC/alginate based extended-release matrix tablets of cefpodoxime proxetil. *Int J Biol Macromol* 89:434–441
- Mujtaba A, Ali M, Kohli K (2014) Statistical optimization and characterization of pH-independent extended-release drug delivery of cefpodoxime proxetil using Box–Behnken design. *Chem Eng Res Des* 92(1):156–165
- Murata Y, Sasaki N, Miyamoto E, Kawashima S (2000) Use of floating alginate gel beads for stomach-specific drug delivery. *Eur J Pharm Biopharm* 50(2):221–226

- Nahar K, Hossain MK, Khan TA (2017) Alginate and its versatile application in drug delivery. *J Pharm Sci Res* 9(5):606–617
- Onsøyen E (1996) Commercial applications of alginates. *Carbohydr Europe* 14:26–31
- Park HY, Choi CR, Kim JH, Kim WS (1998) Effect of pH on drug release from polysaccharide tablets. *Drug Deliv* 5(1):13–18
- Reddy KR, Mutalik S, Reddy S (2003) Once-daily sustained-release matrix tablets of nicorandil: formulation and in vitro evaluation. *AAPS PharmSciTech* 4(4):480–488
- Sanchez-Ballester NM, Bataille B, Soulairel I (2021) Sodium alginate and alginic acid as pharmaceutical excipients for tablet formulation: structure-function relationship. *Carbohydr Polym* 270:118399
- Shilpa A, Agrawal SS, Ray AR (2003) Controlled delivery of drugs from alginate matrix. *J Macromol Sci Polym Rev* 43:187–221
- Sosnik A (2014) Alginate particles as platform for drug delivery by the oral route: state-of-the-art. *ISRN Pharm* 2014:926157. <https://doi.org/10.1155/2014/926157>
- Sriamornsak P, Thirawong N, Korkerd K (2007) Swelling, erosion and release behavior of alginate-based matrix tablets. *Eur J Pharm Biopharm* 66(3):435–450
- Szekalska M, Puciłowska A, Szymańska E, Ciosek P, Winnicka K (2016) Alginate: current use and future perspectives in pharmaceutical and biomedical applications. *Int J Polym Sci* 2016: 7697031. <https://doi.org/10.1155/2016/7697031>
- Takeuchi H, Yasuji T, Hino T, Yamamoto H, Kawashima Y (1998) Spray-dried composite particles of lactose and sodium alginate for direct tableting and controlled releasing. *Int J Pharm* 174(1–2):91–100
- Takka S, Ocak OH, Acartürk F (1998) Formulation and investigation of nicardipine HCl-alginate gel beads with factorial design-based studies. *Eur J Pharm Sci* 6(3):241–246
- Timmins P, Delargy AM, Minchom CM, Howard JR (1992) Influence of some process variables on product properties for a hydrophilic matrix controlled release tablet. *Eur J Pharm Biopharm* 38(3):113–118
- Tiwari SB, DiNunzio J, Rajabi-Siahboomi A (2011) Drug–polymer matrices for extended release. In: Wilson CG, Crowley PJ (eds) *Controlled release in oral drug delivery*. Springer, Boston, MA, pp 131–159
- Tønnesen HH, Karlsen J (2002) Alginate in drug delivery systems. *Drug Dev Ind Pharm* 28(6): 621–630
- Tuğcu-Demiröz F, Acartürk F, Takka S, Konuş-Boyunağa O (2007) Evaluation of alginate based mesalazine tablets for intestinal drug delivery. *Eur J Pharm Biopharm* 67(2):491–497
- Wee S, Gombotz WR (1998) Protein release from alginate matrices. *Adv Drug Deliv Rev* 31(3): 267–285
- Yang JS, Xie YJ, He W (2011) Research progress on chemical modification of alginate: a review. *Carbohydr Polym* 84(1):33–39
- Yong CS, Jung JH, Rhee JD, Kim CK, Choi HG (2001) Physicochemical characterization and evaluation of buccal adhesive tablets containing omeprazole. *Drug Dev Ind Pharm* 27(5): 447–455

Alginate Based Micro Particulate Systems for Drug Delivery



Jyosna Doniparthi, Suryaprakash Reddy Chappidi, and E. Bhargav

Abstract Alginate (ALG) as a platform for development of micro particulate drug delivery systems, has resulted in great interest in scientific community which has given place for continuous rise in the literature from past decade. Alginate based micro particulates (AMP) have established as one of the most extensively searched drug delivery systems due to its intrinsic features such as biocompatibility and biodegradability. To create optimal AMP for particular drug delivery systems, a well-established fabrication method is necessary. Reports on AMP fabrication techniques have advanced during the previous decade. A number of processing parameters improve the characteristics of AMP. Hence, this chapter focuses on the work done on the fabrication procedures of AMP systems for drug delivery systems and the affect of process parameters on the morphology, characteristics, drug content, drug entrapment efficiency, and drug release. The applications and limitations of AMP systems are given special attention. Before learning about such systems for any drug delivery system, it is essential to understand the structure and characteristics of alginate, which are also discussed in detail.

Keywords Alginate · Micro particulate systems · Drug delivery · Properties · Fabrication methods

J. Doniparthi (✉)

Department of Pharmaceutics, Sri Krishnadevaraya University College of Pharmaceutical Sciences, SK University, Anantapur, Andhra Pradesh, India

S. R. Chappidi

Department of Pharmaceutics, Annamacharya College of Pharmacy, Rajampet, YSR Kadapa, Andhra Pradesh, India

e-mail: drcspreddy.ancp@annamacharyagroup.org

E. Bhargav

RERDS-CPR, Raghavendra Institute of Pharmaceutical Education and Research Campus, Anantapur, Andhra Pradesh, India

1 Introduction

1.1 *Micro Particulate Systems*

The process of encapsulating active pharmaceutical ingredients (API) in the form of micro particulate systems, itself protecting them from the harsh environment, is an excellent breakthrough in material science and pharmaceutical technology (Pavan Kumar et al. 2011; Bale et al. 2016; Flammini et al. 2021; Mandracchia and Tripodo 2020). Micro particulate (MP) systems are a type of drug delivery system where solids, liquids, or gases are entrapped in size ranging from 1 to 1000 μm in inert polymeric shells, irrespective of precise interior/exterior surface. Microspheres and microcapsules fall under the category of MP systems. “Microspheres” are spherical micro particles, and microcapsules have a core surrounded by a distinctly different material from the core (Bale et al. 2016; Karmakar et al. 2022; Plamen et al. 2015). As small particles, embedding active therapeutic agents with different polymers of varying thickness and degree of permeation can able to provide modified delivery and manage to ship medication to long periods, represent a prominent class of delivery systems (Gupta et al. 2012; Karmakar et al. 2022). Designing MP systems is considered one of the most attractive research areas as an encapsulation of active polymeric materials delivers drugs to the right place, at appropriate times, and at the correct dose (Hazra et al. 2015; Jurić et al. 2021).

MP systems have been proved to increase APIs’ bioavailability, which is challenging to deliver due to solubility (Karmakar et al. 2022; Madhav and Kala 2011). MP systems are particularly valuable alternative approach of delivering drugs in overcoming the challenges associated with traditional dosage forms, such as protection of pharmaceuticals against enzymatic degradation, high drug loading, decreased variation in GIT time, decreased inter subject variability, decrease of side effects and repeated doses, increased patient comfort, compliance, and drug release control. Different methods of administration are possible, as well as hydrophilic and hydrophobic drug loading (Cetin and Sahin 2016; Desai et al. 2001; Parida et al. 2013; Qurrat-ul-Ain et al. 2003). MP systems deliver potent drugs precisely, minimize drug concentrations outside of the target tissue, and act as excellent delivery vehicles for insoluble (or sparingly water soluble) substances. They also provide enhanced relative bioavailability of drugs with immediate release, such as Nimusleide and also exhibits taste masking feature (Cetin and Sahin 2016; Parida et al. 2013; Rafiee and Abdul Rasool 2022; Solanki 2018).

As a result, MP systems have been discovered to be excellent modified release dosage forms, and they represent significant pharmaceutical advancements in the field of drug delivery technology, with interdisciplinary advances for the treatment of variety of ailments. The utilization of MP technology as an innovative approach has blossomed, and biocompatible materials have gained economic interest (Butte et al. 1988; Solanki 2018).

1.2 Biopolymeric Based MP Systems for Drug Delivery

Natural polymers are materials found in nature, have a long history as biomaterials that are obtained from a variety of sources, including plants, animals, and microorganisms. Traditionally biomaterials are designed to be inert and should not interact with host's biological systems and are defined as a material proposed to interface with biological systems in order to evaluate, treat, augment, or replace any tissue, organ, or function of the body. They were used in biological applications like as wound healing and prosthetics, and were finally overtaken by synthetic polymers and ceramics in the past century. Benefits of biomaterials have been reported upon in pharmaceuticals, tissue regeneration scaffolds, drug administration, and imaging agents. As a result of their many uses, natural materials have recently regained the attention of material science and engineering.

Conventional drug delivery systems that use biopolymeric materials to give weight, volume, and flowability are being phased out by pharmaceutical companies. Towards development of novel drug delivery systems, biopolymers are used as drug performance enhancer to improve drug release and bioavailability. Owing their inherent value, biopolymers, especially polysaccharide-based polymers might be viewed as “key formulation ingredients” (Guastaferrero et al. 2021; Gupta et al. 2012; Hazra et al. 2015; Sreekumar and Bindhu 2019; Zou et al. 2020). Polysaccharide matrices, such as ALG, the most often used encapsulating component, have the ability to improve drug delivery efficiency (Adinarayana et al. 2005; Janes et al. 2001; Kim et al. 2005).

Alginate (ALG) is a renewable material, marine-derived polysaccharide, with its exceptional biodegradability, low toxicity, chemical diversity, crosslinking capacity, and pH sensitivity has significant promise in food industry and biomedical applications. Despite, of its wide range of applications in food industry as an additive, in recent times, ALG has been explored for its biomedical applications, in terms of tissue generation, wound healing, three dimensional (3D)- printing and also exhibits its significance in delivery of bioactive compounds as “functional” foods. Improved knowledge and performance of ALG will have a significant influence on its competitiveness against synthetic polymers. Due to the inherent properties of ALG it can be useful in development of ALG based MP for drug delivery (Kim et al. 2005; Murata et al. 2004). Lim and Sun (1980) pioneered cell encapsulation by creating ALG-poly(Lysine) microcapsules (Lim and Sun 1980).

2 Alginate

2.1 Source and Structure

The US Food and Drug Administration (US FDA) classifies ALG as a “generally recognised as safe” (GRAS) ingredient that is utilized in the pharmaceutical and

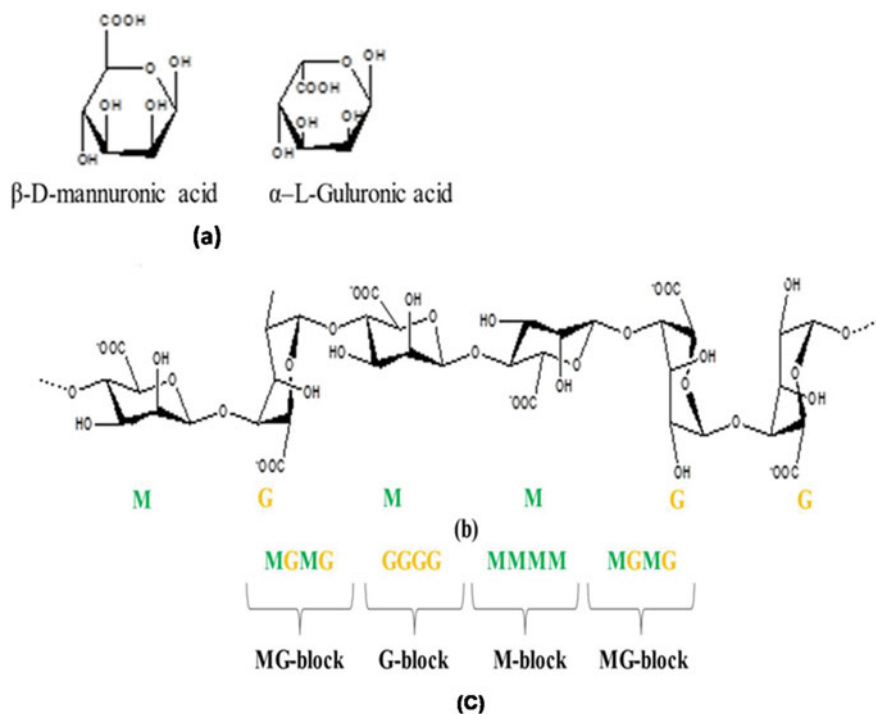


Fig. 1 (a) β -D-mannuronic acid and α -L-guluronic acid units (b) chain conformation (c) block distribution

cosmetic industries (Sosnik 2014; Wawrzyńska and Kubies 2018). ALG, is a generic name, commonly used to refer alginic acid and its salts, but it may also apply to all alginic acid derivatives. It is considered as the world's second most common biopolymer and the most abundant spontaneously formed biopolymer from a marine source (Hasnain et al. 2020; Sosnik 2014; Szekalska et al. 2016). ALGs are anionic and hydrophilic polysaccharides occur as major component in the cell walls of marine brown seaweeds confer for strength and flexibility (Phaeophyceae) such as *Laminaria hyperborea*, *Laminaria digitata*, *Laminaria japonica*, *Ascophyllum nodosum*, and *Macrocystis pyrifera*. It also present in capsular polysaccharide in several bacterial strains such as *Azotobacter vinelandii*, *Pseudomonas* spp) as protection from desiccation and mechanical stress. Acid extraction from algal tissue is followed by alkali neutralization and precipitation with calcium chloride or mineral acid to produce alginic acid. Alkali treatment is used to turn it back into sodium ALG (Abasalizadeh et al. 2020; Comaposada et al. 2015; Draget and Taylor 2011; Guarino et al. 2015; Hariyadi and Islam 2020; Nahar et al. 2017; Smidsrød and Skjåk-Braek 1990; Wawrzyńska and Kubies 2018).

β -D-mannopyranuronate and α -L-gulopyranuronate are the two monomer units of alginic acid. Chemically, alginates are copolymers composed of β -D-mannuronic acid (M) and α -L-guluronic acid (G) units depicted in Fig. 1a joined by a 1 $\rightarrow$ 4

linkage in an irregular block wise configuration. The M units have a flexible and linear conformation and are due to steric hindrance around the carboxyl groups in the ring structure, there is no spatial interaction between groups, thus β -D-mannopyranuronate in alginic acid adopt a stable $4C_1$ chair conformation, results in more stable form of M units. $1C_4$ conformation in α -L-gulopyranuronate in alginic acid has spatial interaction between the axially situated COOH - group at C_5 and OH - group at C_3 positions results in less stable form, leads to rigidity and folded structural conformations by G units. Monomer chair conformations (Fig. 1b) results in four possible glycosidic linkages, thus repeating G units (GG), M units (MM) and alternating M and G units (GM/MG) appear as homogenous and heterogenous blocks (Fig. 1c) in polymer. The content and sequencing of G- and M- blocks in commercially available ALG may differ based on the originating source material ("Alginate-Based Biomaterials" [n.d.](#); Bhattarai et al. 2011; Comaposada et al. 2015; He et al. 2020; Lazar 2018; Manev et al. [n.d.](#); Sosnik 2014; Sreekanth Reddy et al. 2019; Szekalska et al. 2016; Wawrzyńska and Kubies 2018).

2.2 ALG Properties

2.2.1 Molecular Weight, Solubility and Viscosity

The molecular weight of sodium ALG ranges from 32,000 to 400,000 g/mol (Lee and Mooney 2012). Over 200 ALG variants have been commercialized due to differences in molecular weight, relative G/M compositions, and chain configurations dependent on the source of extraction and algae age. Furthermore, the range of molecular weight present in ALG is conditioned with G/M ratio, composition and pH of the medium affects the viscosity, gelation and drug release kinetics (Neves et al. 2020; Sosnik 2014; Tønnesen and Karlsen 2002).

Pure alginic acid is difficult to dissolve in cold water, but it swells 200–300 times in it. In comparison to its original form, ALG salt is extremely soluble in water. ALG dissolves easily in hot water and gels when exposed to divalent/multivalent ions and monovalent metal ions due to acidification. Because ALG possesses carboxyl groups that are charged at pH values more than 3–4, it is soluble under neutral and alkaline environments, promoting the widespread usage of ALGs. pH, ionic strength and existence of gelling ions in solvent modifies ALG solubility (Hasnain et al. 2020; Manev et al. [n.d.](#); Pina et al. 2015). As a virtue of its solubility and pH sensitivity, ALG is considered as an excellent biomaterial for drug-delivery systems (Hariyadi and Islam 2020).

The viscosity of ALG solutions rises when carboxylate groups in the ALG backbone become protonated and form hydrogen bonds, with maximum viscosity attained at pH range of 3–3.5. Monovalent salt forms of ALG (especially sodium form) and alginates with high M generate viscous solution upon dissolution, resulting in a characteristic thickening property (related to the semirigid

conformation of this copolymer) that has found important applications (Beata Łabowska et al. 2019; Rinaudo 2014).

2.2.2 Molecular Rigidity, Flexibility

ALG has different degrees of stiffness or flexibility because of the strong or weak hindrance of sugar rings, electrostatic repulsion between the charged groups present on the polymer chain, and restricted rotation around glycosidic links. ALG rigidity or flexibility depends on the arrangement of homogeneous (GG/MM) and heterogeneous (GM/MG) block units on the polymer chain. If the polymer chain is mostly made up of GG blocks, it will be stiffer, or have an extended chain conformation, than those made up of MM blocks, which are stiffer than MG or GM blocks, and therefore the relative flexibility increases as the MG, MM, and GG block units sequence (“Alginate-Based Biomaterials” [n.d.](#))

2.2.3 Gel Formation

ALG has excellent gelling properties in safe and moderate conditions, ideal for thermosensitive molecules (Flamminii et al. 2021). These are recognized as biosorbents of metal ions because of their ability to bind metal ions in aqueous solutions via an ion-exchange mechanism. Cd(II) ions sorbed by *Sargassum* spp. of ALG, Pb(II), Cu(II), Cd(II), and Zn(II) ions sorbed by *Sargassum filipendula* species of ALG, and Cu(II) and Pb(II) ions sorbed by *Laminaria digitata* species of ALG was evidenced by several authors (Beata Łabowska et al. 2019). In the presence of an abundance of monovalent salts or complexing agents (EDTA, sodium oxalate, sodium citrate), Ba, Sr, and Ca ions bind strongly with ALG to form a reversible gel (Rinaudo 2014). Depending on pH, ALG can able to form acid gel and ionotropic gels with distinct characteristics in comparison with neutral macromolecules. The physicochemical parameters of the polymer system and the swelling mechanism trigger drug release will be influenced by the type of gel generated (Tønnesen and Karlsen 2002). Molecular weight, M/G ratio and manufacturing parameters (type of gelation, type of cation and concentration) of ALGs can affect the porosity, permeability of ALG gel particles as well as molecule diffusion kinetics (Flamminii et al. 2021).

The ability to form micro particles, hydrogels, fibers/films is primarily by divalent calcium ions, that binding between GG block rich zone in ALG polymer chain, can form three-dimensional “egg-box model” (Fig. 2a, b) that can hold the gel (Flamminii et al. 2021; Mollah et al. 2021; Rinaudo 2014). When calcium ions are present, the G-enriched sample can create a hard and brittle hydrogel, whereas the M-enriched sample forms soft and elastic gels (Ea 2020; Mollah et al. 2021). The intermolecular cross-linking of ALG by divalent cations is primarily responsible for the creation of hydrogels. The affinity of alkaline multivalent cations is $Mg^{2+} \ll Ca^{2+} < Sr^{2+} < Ba^{2+}$, rises preferentially with increasing-L-gulonate residue

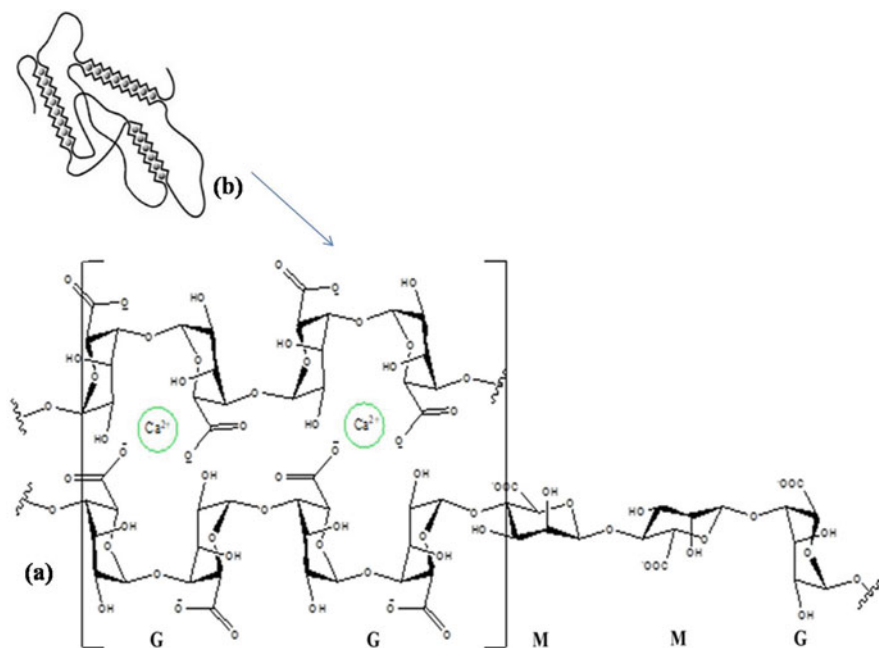


Fig. 2 (a) Schematic representation of calcium ions, that binding between GG block rich zone in ALG polymer chain, (b) Three-dimensional “egg-box model”

concentration and is independent of poly-mannuronate blocks. Specific gels based on the H-bond network are produced by ALGs in acidic media (Hasnain et al. 2020; Hasnain and Nayak 2019; Jain and Bar-Shalom 2014; Rinaudo 2014). The capacity of ALG to hydrate and form gels allows sustained or prolonged release from at the administration site. As a result, alginates been widely researched for modified release drug delivery systems (Szekalska et al. 2016).

2.2.4 Mucoadhesion

ALG exhibits excellent mucoadhesive properties due to the availability of free carboxyl groups, which allow the polymer to interface with mucin via hydrogen and electrostatic bonding. As ionized carboxyl groups alone may interact with mucosal tissue, micro environmental pH has a significant influence on ALG solubility and, as a result, mucoadhesiveness. Additionally, soluble ALG allows penetration of solvent into the ALG matrix, resulting in formation of a more viscous and cohesive gel structure, that is capable for strengthening the mucoadhesive bonds. On the other hand, high hydration of polymer matrix in physiological fluids may diminish mucoadhesiveness by reducing the number of ALG functional groups accessible for interactions with mucosal tissue. MP drug delivery systems containing sodium ALG are also being investigated as gastroretentive drug carriers for drugs

that are unstable at intestinal pH, owing to their wide surface area, that might promote intimate contact between the polymer and mucin (Szekalska et al. 2016).

2.2.5 Biocompatibility and Biodegradability

ALG materials are biocompatible, nonimmunogenic, and nontoxic (Nikhil et al. 2009). Alginase/lyase enzymes responsible for degradation of ALG by depolymerization via β -elimination is exclusively found in prokaryotic and eukaryotic microorganisms, except in humans, still it dissolves due to elution of cross-linking calcium ions (Hasnain and Nayak 2019; Hutcheson et al. 2011). It should be noted that the renal clearance threshold eliminates only small ALG molecules. The partial oxidation of the polymer backbone aids in complete elimination of ALG from the body without affecting ALG gel formed by divalent ions (Broderick et al. 2006; Forster et al. 2010; Hutcheson et al. 2011). The chair conformational changes to open-chain adduct, which accelerates degradation of polymer back bone by partial oxidation. The pH, degree of oxidation, temperature of the medium, all has a significant impact on the degradation of ALG gels (Kristiansen et al. 2010). The biocompatibility of alginic acid has been carefully investigated in-vitro and in-vivo at various levels of purity. The FDA has approved few ALG salts (calcium, sodium, ammonium, and potassium) and a propylene glycol ALG derivative for oral administration as GRAS (generally recognized as safe) (US FDA 2020). In animal investigations on rats, calcium salts of ALG were injected into the kidneys of rats to determine biocompatibility, and the results were fairly good (Becker et al. 2001).

Multiple studies have found that the structure of ALG may influence the immunogenicity of polymers. Otterlei M et al., reported that ALG with high M monomer has been demonstrated to be more immunogenic and 10 times more effective in inducing cytokine synthesis than ALG with low G monomers (Lee and Mooney 2012), although some reported very little or no immune response throughout ALG implants (Zimmermann et al. 1992). A high level of quality must be maintained throughout ALG processing and extraction since several contaminants (heavy metals, endotoxins, proteins, and phenolic chemicals) identified in ALG may provoke an immunogenic response (Greco et al. 2014; Ménard et al. 2010).

3 Methods for Fabricating AMP Systems

ALG is a good choice for drug delivery, because it gels well with divalent cations at moderate environment. Numerous ALG based micro/nanoparticles have been developed for effective delivery of active ingredients by chemical synthesis (He et al. 2020). MP systems offer numerous advantages in respect with its structural and functional abilities and their application in various routes of administration and convenient for its drug delivery. Thanks to MP systems for being advantageous over nanoparticles as they don't cross the interstitium beyond the particle size of

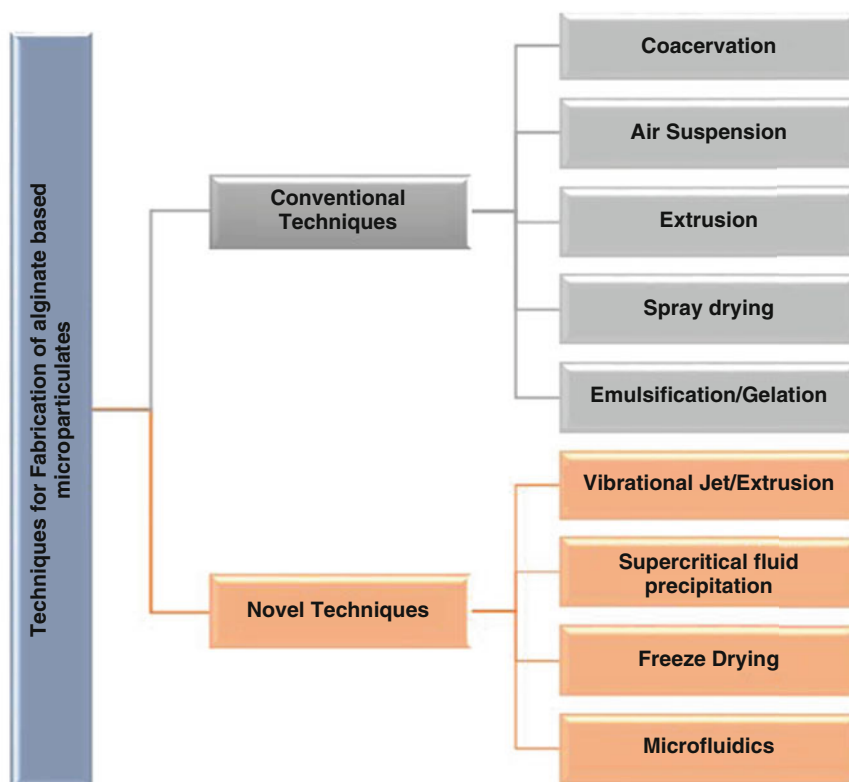


Fig. 3 Techniques for fabrication of alginate based micro particulates

100 nm delivered by lymph hence function locally. Optimized drug encapsulation efficiency and drug release can be achieved by chemical modification or by different fabrication techniques depicted in Fig. 3.

3.1 Coacervation

The term coacervation is derived from the latin *acervus*, which means aggregation, and the prefix *co*, which denotes the previous union of colloidal particles (Sateesh and Shivani 2011). This was the first approach disclosed that was adapted for industrial production of MPs (microcapsules) (Pavan Kumar et al. 2011). This approach is favored for challenging the encapsulation of hydrophilic pharmaceuticals into biopolymers. The API and biopolymer must be water insoluble, and the polymer requires a water immiscible solvent (Sateesh and Shivani 2011). The API is deposited in the coated polymer solution during coacervation, and at a specified environmental impact (ionic, pH, or heat change), phase separation occur, whereas

the core material is encased by coating polymer. There are basically two approaches for coacervation: simple and complex. Polymer incompatibilities results in simple coacervation, commonly occurs with ALG and divalent or trivalent cations like Ca^{2+} or Ba^{2+} results in salting out. If, calcium is used as a crosslinker, it forms an “egg-box” structure by linking the G and M units of ALG polymer with calcium ions. The formed stable microparticles with such chemical bonds are stable. Viscosity, surface tension and stirring element arrangement will affect the particle size of the microparticles in this method (Chuang et al. 2017; Nemethova 2015). Polyelectrolyte polymers possessing opposing charges create an adduct and enclose the API in complex coacervation process in which, pH is important parameter. Generally coacervation is commonly used to encapsulate hydrophobic components, in case of water soluble drugs, double emulsion method followed by coacervation is beneficial (Santos et al. 2015).

3.2 Air Suspension Method

In 1959, Wurster effectively used fluidization in microencapsulation of solid particles, whereas Hinkes et al., used it to envelop MPs in the size range of 74–250 μm (Sanjoy Kumar et al. 2011; Wurster et al. 2007). Pressurized air fluidizes the solid drug particles, and coating material solution is sprayed on the particles from the bottom/top of the fluidization chamber parallel to the air stream. The coating solution droplets travel just a short distance before touching the solid particles when the spraying nozzle is submerged in the airflow and sprays the coating materials concurrently into the fluidized particles. As a result, the particles received a homogeneous coatings. The air stream lifts the coated particles, dries the coat as it carries the particles away from the nozzle. The particles ascend through the air, settle, and then restart the cycle (Kinam and Yoon 2002).

3.3 Extrusion/Ionic Gelation Technique

Extrusion through a nozzle is the most basic and widely used method for producing ALG gel-based MPs (Uyen et al. 2020). This approach is known as ionic gelation, and it includes a simple diffusion mechanism using Ca^{2+} crosslinking (Anusree et al. 2018; Heng et al. 2003). In brief, the core medication is disseminated in the ALG solution before being dripped into a gelling bath containing calcium chloride using a simple dripping instrument. The Na^+ ions in the ALG framework are replaced by Ca^{2+} ions, which stiffen the membrane and create particles. Effectiveness of formed ALG gel-based MP rely on composition of calcium chloride, which can bind with –COOH groups of ALG and results in formation of thermo-stable gel (Uyen et al. 2020). Lin et al. effectively formulated ALG gel-based MP in the form of microbeads by encapsulating 90% astaxanthin into ALG by varying composition

of calcium chloride, stirring rate and duration, and needle size. It was discovered that the involvement of calcium ions enhances the drug stability in ALG matrix, more than 90% of the actual quantity of astaxanthin remained after 21 days of storage at 25 °C in their investigation (Lin et al. 2016).

3.4 *Spray Drying*

Samuel Percy was the first to patent a spray drying technology in 1872, and is a typical process for producing MPs (Choukaife et al. 2020). It is a one-step technology, which atomizes the liquid feed material to dry particles (Plamen et al. 2015). Operation principle involves four fundamental steps: (i) preparation of drug-ALG solution by emulsification or dispersion. (ii) drug-ALG solution was atomized by a nozzle. (iii) As drying gas dried the droplets, dried microparticles were created. (iv) Finally, dried microparticles were isolated and collected from the drying gas (Choukaife et al. 2020; Lengyel et al. 2019). Patil et al. created innovative spray-dried ALG microspheres containing β - cyclodextrin that provided prolonged drug delivery. This study found that encapsulated spray-dried microspheres had a higher therapeutic potential in rats when administered via the pulmonary route (Patil et al. 2015).

Takeuchi et al. created spray-dried lactose composite particles containing an ALG-chitosan complex by spray drying technique and compacted them as a dry tablet. The prepared composite particles had strong compacting properties as well as a longer induction time due to the creation of an insoluble ion complex between sodium ALG and chitosan, which resulted in the production of a hard gel structure on the tablet surface (Takeuchi et al. 2000).

3.5 *Emulsification/Gelation Technique*

The emulsification/gelation process is relatively cost effective and simple technique, capable of MP's with less than 100 μm size. It carries out by producing stable polymer droplets in the emulsion and hardening them (Mishra 2016; Vinod et al. 2013). Gelation happens in this approach when the ALG solution collides with the cross-linker ions. It could be internal and external gelation. External gelation method is the popular approach for encapsulating both hydrophilic and hydrophobic drugs. It refers to the extrusion technique, where ions from external source migrate into the ALG droplets results in formation of ALG based MPs by surface gelation rather than core gelation. Particles formed were found to possess rigid outer exterior and soft core. Internal gelation, on the other hand, is moderate, resulting in soft particles with considerable agglomeration, which leads to reduced encapsulation efficiency and a quicker release profile (Uyen et al. 2020).

Duanquan Lin et al. investigated the efficacy of external/internal/O/W/O emulsion-gelation techniques for the fabrication of ALG-based emulsion micro-gel particles. When size, structure, rheological characteristics, and in-vitro digestion were evaluated, emulsion micro-gel particles generated by external gelation approach had a modest size (100 μm) compared to other methods. Furthermore, external gelation particles increased the Young's modulus of gel particles, slowed structural collapse, and so delayed the release of encapsulated lycopene during intestinal digestion (Lin et al. 2021). The challenge in employing dispersion/external gelation procedures with ionic polysaccharide was that the calcium chloride is insoluble in the oil phase. Internal gelation of the distributed ALG droplets may be triggered as an alternative by releasing calcium from an insoluble compound (calcium salt) via pH decrease. M. M. Ahmed et al., successfully prepared diclofenac loaded sodium-alginate MPs by emulsification/internal gelation method, and revealed that stirring speed is the critical process parameter for optimizing the drug loaded alginate based micro particulates (AMP) droplet size in the continuous phase at 200 rpm. They concluded that there was no impact of surfactant (span 80), and at high levels of drug:polymer ratio (1:3), drug release followed zero order kinetics, which is optimal for regulated drug delivery (Ahmed et al. 2013).

Principles, advantages and disadvantages of conventional methods for fabrication of AMP systems are shown in Table 1.

3.6 Novel Techniques

Various innovative procedures accomplish fabrication of AMP via extrusion: vibrating jet technique (a variable frequency vibration divides the laminar jet into beads). The jet is exposed to an electric field at the electrostatic extrusion, where particles are formed after reaching a particular voltage (5–6 kV); with repulsive forces preventing their agglomeration. Cell death is not caused by the applied high electric potential (Lengyel et al. 2019).

Despite the fact that supercritical fluid precipitation produces very homogenous particles, it is most generally utilized in nanoencapsulation. Supercritical fluids are highly compressed gases. If the API is insoluble in gas but soluble in liquid, this method is advantageous. The drug substance to be encapsulated must be dissolved in a polymer solution containing a suitable solvent. Anti solvent usage decreases the solubility of the dissolved component in solution, resulting in the formation of MP beads that must be rebuilt. Rapid supercritical solution expansion, gas anti-solvent technique, and particles from gas-saturated solution are all frequently used methods for producing MP with supercritical fluids (Pavan Kumar et al. 2011; Sateesh and Shivani 2011).

The freeze-drying process has been demonstrated to be successful for microencapsulating protein API's. Steps in the process include freezing, sublimation, primary drying, and secondary drying. Lyoprotectants or cryoprotectants (trehalose, dextran) can stabilize drug molecules during the process by replacing

Table 1 Principle, advantages and disadvantages of different fabrication methods of AMP systems

Fabrication method	Principle of the process	Advantages	Disadvantages	Critical process parameters	References
Simple coacervation	Increasing the polymer's solubility by introducing a second solvent in which the polymer is insoluble. A phase separation then occurs	Effective for fast release preparations due to high porosity	Drawback for sustained release formulations	Viscosity, surface tension of alginate and stirrer setup	Pavan Kumar et al. (2011), Lengyel et al. (2019), Plamen et al. (2015), Poncelet (2006), and Whelehan and Marison (2011)
Double coacervation	Two polymers with opposite charges interact. As a result of this interaction, insoluble complexes develop and phase separation occurs	Effective for heat sensible drugs if freeze dryer is used for drying	Expensive in case of lyophilization process	pH and isoelectric point of polymers	
Air suspension	Drug particles are suspended in air stream, coating solution was sprayed on the drug particles where uniform coating occurs on drug particles relative to one another, then dried within the coating chamber	Optimized particle size is achieved	Expensive process	Concentration and quantity of coating material, inlet, outlet air temperature, and spray settings	
Extrusion	Simple diffusion process, using cross linking agent	Simplest method for the formation alginate gel microparticles	Viscosity of the polymer blocks the nozzle	Concentration, feeding rate, and surface tension of the polymer	

(continued)

Table 1 (continued)

Fabrication method	Principle of the process	Advantages	Disadvantages	Critical process parameters	References
				solution, solvent, temperature, and nozzle diameter	
Spray drying	Liquid feed is transferred into dry particles by atomizing the feed into hot medium	Optimized particle size, all steps carried out in apparatus, use of organic solvents, ability to encapsulate heat-labile drug substances and high encapsulation efficiency	High loss, less yield, expensive, maintenance is needed	Concentration and quantity of coating material, inlet, outlet air temperature, and spray settings	

water, forming a glassy matrix, and restricting molecular mobility by creating hydrogen or vander waals linkages between the molecules. Despite its high price, it is advantageous for heat-sensitive chemicals. Solidification is accomplished by freeze-drying, which allows particles to be reconstituted (Lengyel et al. 2019).

Microfluidics is a technology for manipulating fluids in micrometric-sized channels. By connecting the channels with connectors, fluids are mixed. Droplet-based microfluidics occurs when immiscible or partially miscible fluids are combined in the junction. The basic idea is similar to that of traditional emulsification, which involves combining two immiscible liquids. Droplet-based microfluidics has the benefit of droplet monodispersity and repeatability owing to precise control over experimental parameters such as channel shape, flow rates, fluid viscosities, and so on. Moreover, unlike traditional emulsification, monodisperse droplets can be generated without the need of a surfactant. Droplets created in droplet-based microfluidics can be used as microreactors to perform physical, chemical, or biological processes. Because they are tiny (nL to μ L in volume), they require a little amount of reactants. And similar composition of droplet composition, several identical tests may be undertaken, allowing for a valid statistical approach to data (Zhang et al. 2021).

It is possible to select the best manufacturing procedure for an API based on its physicochemical properties. However, because the use of organic solvents in the development of drug-delivery systems is a significant issue, organic solvents used in any approach were classified using the International Conference on Harmonization classification wherever appropriate. As a consequence, solvents are classified as solvents 1 (to be avoided), solvents 2 (to be regulated), and solvents 3 (low

hazardous potential), based on their potential for harm to human health. This classification, together with efficiency characteristics like particle size, drug content, entrapment efficiency (EE), swelling and drug loaded with its release pattern will be crucial for evaluating the most often used procedures and assessing if a certain method applies to a specific pharmaceutical application (Lopes et al. 2017). Certain examples of drug loaded AMP systems are clearly mentioned for a better understanding in Table 2.

4 Characterization of AMP

AMP systems should be evaluated after fabrication in order to have a better understanding of their characteristics, which may influence their further applications. This section discuss about general characterization of AMPs which includes particle size, surface area and morphological characters, percentage yield, EE, swelling index and in-vitro drug release studies.

One of the most essential aspects of AMPs is their size. In drug delivery, for example, micro particle size and size distribution influence drug release kinetics. Size can be evaluated using optical or light-scattering (sub-micrometer range) microscopy or microgrippers (Zhang et al. 2021). The surface area can be estimated by BET techniques. The morphology of the AMP systems can be studied by Scanning electron microscopy, Atomic force microscopy and Transmission electron microscopic techniques. The percentage yield of AMP systems can be calculated by weight of the MPs obtained to the weight of drug with excipients added for formulation of MPs. Drug EE can be evaluated by ultrasonication of the MP systems at specified pH, followed by centrifugation at specified RPM then collecting the supernatant and estimating the untrapped content by UV visible spectrophotometric or HPLC techniques. Swelling index can be determined by placing the drug loaded MP systems in to various buffer medium of suitable pH. Then weighing the MP systems initially and continuing the study until constant weight is achieved.

The release of drug from AMP systems can be studied by transferring the MP systems containing specified equivalent weight of the drug in to USP dissolution apparatus II containing suitable dissolution medium. The apparatus maintained at 37 ± 0.5 °C with specified RPM. The samples are to be collected at specified time intervals and replacing with buffer at each sample collection and analyzing the drug release by suitable UV visible spectrophotometric, HPLC or GC techniques (Gupta et al. 2012; Hazra et al. 2015; Lengyel et al. 2019).

Table 2 Examples of drug loaded alginate micro particulates using different fabrication techniques (Cui et al. 2001; Feng et al. 2014; Hazra et al. 2015; Hussein et al. 2020; Lucinda-Silva and Evangelista 2003; Mandal et al. 2010; Rajesh et al. 2003; Ramya Krishna and Eliyas Kadi 2016; Patil et al. 2015; Saravanan and Rao 2010; Silva et al. 2006; Sreekanth Reddy et al. 2021; Szekalska et al. 2015, 2018)

Method	Drugs encapsulated	Ingredients (composition)	Mean diameter (µm)	Yield (%)	Drug content (%)	Entrapment efficiency (%)	Swelling (%)	Drug release (%)
Coacervation (Soheila et al. 2009; Feng et al. 2014; Saravanan and Rao 2010)	Micronized prednisolone	Drug (2–4%w/w)	140–165	–	95.92–98.97	–	66.09–77.54	Suitable release characteristics
		Alginate (2% w/v)						
		Chitosan (0.5–1.5% w/v)						
		CaCl ₂ (0.5–1.5%w/v)						
	Doxorubicin hydrochloride	Drug (1%)	2.78–4.59	–	–	94.93–97.21	NMT 12.6	NMT 12.6% in simulated duodenum fluid that attributed to sustained release ability of multilayer sodium alginate beads
		Sodium alginate (1.3or 5%w/v)						
		CaCl ₂ (1–3%w/v)						
	Metranidazole (MH)	Na alginate/pectin (Polymer) (1:1)					–	Slow release in gastric medium due to hydrophilic drugs (DS&IM) and once reaches alkaline medium burst release followed by sustained release over 16 h
	Diclofenac sodium (DS)	Drug:polymer ratio						
Air suspension (Cui et al. 2001)	Indomethacin (IM)	MH (2:1)	41.34	0.96	6.51	6.25		
		DS (1:1)	82.93	2.86	59.91	85.66		
		IM (1:1)	110.64	3.07	62.73	110.64		
		Na alginate (1.2–2.0% w/v)	89.6–128.5	60–75%	–	61–83	–	96% in 20 min
		Poly-L-lysine (0–0.04%w/v)						
		Gelatin (1.5%)						
	Ketoconazole, Acetaminophen vitamin C, and Bifidobacterium							

Extrusion/ionic gelation (Mandal et al. 2010; Rajesh et al. 2003; Sreekanth Reddy et al. 2021; Hazra et al. 2015; Ramya Krishna and Eliyas Kadi 2016)	Trimetazidine dihydrochloride	PEG 6000 (0.75%)								
		Drug (2–3%w/v)	1005–	–	45.21–	53–62	–	–	30–40% (2 h)	
		Sodium alginate (2–2.5%w/v)	1075		62.15				82–89% (9 h)	
		Con of CaCl ₂ (1–3% w/v)								
	Ketoprofen	Ketoprofen (30%)	616.46–703.55	–	–	97.52–100.02	–		16.92–22.67 (t _{50%})	
		Alginate (2%w/v)	1162.5–1511.12			97.03–100			21.67–36.01 (t _{50%})	
		Acrycoat (0.03, 0.06 and 0.12% dry acrycoat of total solids)	1144–1192.83			99.15–100			55–88 (t _{50%})	
		CaCl ₂ (1–8%w/v)								
	Curcumin	Curcumin (50 mg)	800–	–	–	55.40	Approx 70% pH (1.2)	Approx 30% pH (7.4)	Calcium alginate beads shows low drug release rates	
		Alginate (200 mg)	1100							
		Montmorillonite (200 mg) CaCl ₂ /MgCl ₂ /BaCl ₂ /AlCl ₃ (2.5–5%w/v)/(2.5% w/v)/(2.5%w/v)/(2.5%w/v)								
		Drug:Chitosan:sodium alginate: 2:5:30	200–500	–	–	70 ± 3.4	pH 1.2 (150.5–195)		87.2 ± 1.9	
	Quercetin	2:10:30				81.59 ± 4.8			78.45 ± 2.0	

(continued)

Table 2 (continued)

Method	Drugs encapsulated	Ingredients (composition)	Mean diameter (μm)	Yield (%)	Drug content (%)	Entrapment efficiency (%)	Swelling (%)	Drug release (%)
Spray drying (Szekalska et al. 2015; Patil et al. 2015; Hussein et al. 2020; Szekalska et al. 2015)							pH 6.8 (620.1–715.2)	
		2:15:30				80.33 $\pm$ 2.9	pH (715.4–850.5)	72.15 $\pm$ 2.7 in pH 7.4
		Alginate (2%w/v) CaCl ₂ (10%w/v)	950			5.7 mg per 100 mg	–	Slow release and spread over a period of 22 h
	Piroxicam	Sodium alginate (ALG) (1–3%)						
		RNT:ALG mass ratio in 1%ALG						
		1:1	0.9 $\pm$ 0.1	67.3 $\pm$ 1.7	32.4 $\pm$ 1.8	95.6 $\pm$ 2.1	23–30 (swelling ratio)	Sustained upto 4 h
		1:2	1.7 $\pm$ 0.9	61.3 $\pm$ 2.4	49.2 $\pm$ 3.5	90.8 $\pm$ 2.7		
		2:1	2.2 $\pm$ 0.1	40.7 $\pm$ 1.0	66.2 $\pm$ 1.7	81.9 $\pm$ 3.2		
	Rifampicin	Drug: β -cyclodextrin: Sodium alginate (1:1:1)	6.234 to 6.634	Upto 67%	62.1–71.2		Nebulisation efficiency nearly 75%	% of drug permeated per cm ² of skin at 6 h (87.36 $\pm$ 0.98)
	Ropinirole hydrochloride	Sodium alginate (0.5% w/v)	2.35–2.58	58.87–77.2%	101.55–106.99		101.99 (maximum)	(> 95%) within 1 min from the optimized formulation
		Alginate (2%w/v)	3.0–3.5	56.1–61.7	73.9–77.1	91.5–113.4	3–12 (swelling ratio)	MF was released in the first 2 h, and

	Metformin hydrochloride (MF)	CaCl ₂ (0.05–0.1%w/v)							maintained for 12 h, reaching 97.5 ± 2.7%
Emulsification (Lucinda-Silva and Evangelista 2003; Silva et al. 2006)	Isoniazid	Sodium alginate (0.5% w/v)	2–7	–	35–75	–			50% in gastric juice (5 h)
		Sorbiton monooleate (0.5%w/v)							65% in enteric juice
		Chitosan contains 1% w/v CaCl ₂ solution							
		Sodium alginate (2% w/v)	48.8–61.9		3.61–4.18	65.3–75.8			Within 5 min, there was quick and complete insulin release, reaching 75%, followed by a slow release, culminating in a total cumulative release of 80%. The microspheres dissolved swiftly and completely once the intestinal pH was adjusted, allowing for fast total insulin release
	Recombinant human insulin	Actrapid formulation in water (1:2 v/v)							
		CaCO ₃ (5%w/v)							
		Paraffin oil (30% internal phase ratio, v/v)							
		Span 80 (1%v/v)							

5 Factors Affecting AMP Systems

Particle size, size distribution, EE, and drug release are few assessment characteristics that may impact drug's injectability, distribution, degradation, release kinetics and therapeutic efficiency regarding patient safety throughout its administration. Indeed, larger particles have been shown to block tiny blood arteries, resulting in embolism, and drug release has been discovered to be reliant on the cross-linking agent. To achieve the desired results, drug encapsulation into AMP systems through any of the manufacturing processes necessitates a vast number of formulation parameters and operational conditions. As a result, it is vital to have a complete understanding of the influence of synthesis variables on the morphologies, properties, and encapsulation efficiency of AMP. In this context, we outline current work on parameters impacting the characteristics of manufactured AMP systems (Uyen et al. 2020).

5.1 ALG Concentration

ALG concentration is recognized as the most critical element influencing ALG based microparticles features like as shape, particle size, and drug EE. It can be stated that the mean particle size and drug EE of the ALG micro particles increase when ALG concentration increases. M. Saravanan and K. P. Rao have emphasized the influence of ALG concentration on the particle size distribution prepared by complex coacervation. Results revealed that average diameters of diclofenac sodium loaded pectin–gelatin and ALG–gelatin microcapsules were 94.6 and 82.3 μm , respectively. The average diameters of indomethacin-loaded pectin–gelatin and ALG–gelatin microcapsules were 120.94 and 110.64 μm , respectively. It was concluded that ALG was more suited than pectin in terms of reduced aggregation, small/uniform particle size, facile dispersion in water, and free flowing (Saravanan and Rao 2010).

Cui et al. investigated the influence of ALG on the size distribution and surface morphology of ALG poly-l-lysine MPs using an air atomization approach. The geometric mean diameter of particles reduced from 128.5, 112.9 and 89.6 μm respectively, with increase in the concentration of sodium ALG (1.2–2.0%). Thus an inverse relationship was discovered between ALG concentration and size of MP. The shape was found to be irregular spherical or elliptical. At low concentration of sodium ALG, the surface structure was wrinkled and concave, and however the surface of the MPs become dense and tight as the concentration of ALG solution increased (Cui et al. 2001).

As per the results proposed by Xiguang Chen et al., the particle size was related to the initial drop size, the concentrations of CaCl_2 and ALG, and the duration of solidification. The optimal concentration of ALG must be kept within 1–3%. Low ALG concentration (1%) causes dilute gelling, resulting in no layering out of the core bead. On the other hand, high ALG concentration (>3%) causes high viscosity,

which hinders the creation of core beads (Feng et al. 2014). Patil et al., noticed that increase in ALG concentration with constant amount of drug (Rifampicin), may led to highly viscous solution that reduces drug solubility and hence drug loading. Spray-dried ALG microspheres had an average particle size of 6.634–6.234 μm respectively (Patil et al. 2015).

5.2 Influence of Surfactant Concentration

By adding surfactant during the manufacturing process, the attributes of MP's, such as size, shape, loading efficiency, surface properties, and colloidal stability, may be enhanced (Stachowiak et al. 2021). Hariyadi et al. observed increase in particle size of glutathione ALG microspheres produced utilizing surfactants tween 80 and span 80 by ionotropic gelation technique by aerolization. It was clear that the addition of surfactants (tween 80 and span 80) results in high viscous solutions, which increase droplet size and particle size from $1.89 \pm 0.03 \mu\text{m}$ to $2.42 \pm 0.08 \mu\text{m}$. The combination of surfactant and polymer concentration significantly affected drug loading from 5.72% to 6.23% and entrapment efficiency from 34.74% to 56.63% (Dewi et al. 2019).

L. S. C. Wan et al. used an emulsification technique to evaluate the effect of surfactants on the generation of calcium ALG microspheres. Interestingly, HLB had no effect on the drug content of ALG microspheres generated with surfactants having 20 ethylene oxide units, while there was a notable variation with different HLB of surfactants containing five ethylene oxide units. Surfactants with longer fatty acid chains also result in microspheres with increased drug content. In microspheres produced from surfactants with lower HLB values, medication release was delayed. Smaller microspheres are produced by surfactants with longer polyoxyethylene chains (Wan et al. 1994).

Mohammad Alnaief et al. prepared hybrid chitosan – ALG aerogel MP by emulsion gelation method and demonstrated that particle size can be affected by emulsifier selection, with span 85 enabling small ALG micro particles with particle size (1–35 μm) when compared to span 60 (5–70 μm) (Alnaief et al. 2020). According to H. Kaygusuz et al., the Young's modulus of ALG beads varies depending on the kind of surfactant, which might alter the mechanical characteristics of ALG-based MP. They concluded that the addition of sodium dodecyl sulphate enhances the Young's modulus, and so the rise in sodium dodecyl concentration was more evident for barium ALG beads than calcium beads, which are more stiff (Kaygusuz et al. 2016).

5.3 *CaCl₂ Concentration*

Marta Szekalska et al. used spray drying to fabricate calcium chloride modified AMPs. The produced CaCl₂ modified AMPs marginally increase the mean particle diameter (3.0–3.5 μm), resulting in a decrease in percentage yield. Furthermore, calcium chloride cross-linking of ALG resulted in a little drop in encapsulation efficiency from 113.4% to 91.5%. These MP also displayed lower swelling ratio values than the non-modified formulation, with a linear increase up to 300 min and an improved mucoadhesive property. The reduced swelling of cross-linked MPs is caused by the formation of insoluble calcium ALG upon contact with the acidic medium, resulting in a lower water input into the matrix. The drug release behavior of Ca²⁺ cross-linked formulations was more stable, with $60.1 \pm 3.8\%$ released in the first 2 h and sustained for 12 h, reaching $97.5 \pm 2.7\%$ (Szekalska et al. 2018).

Hazel Peniche et al., reported calcium ions have been shown to react extraordinarily quickly with ALG, occupying all accessible binding sites on the ALG molecule and causing a gel state. Swelling of this calcium–ALG matrix is pH dependent and is restricted to acid pH (below ALG pKa). At pH 7.4, on the other hand, ALG swells due to the exchange of cross-linking calcium ions with non-gelling Na⁺ or K⁺ ions (components of phosphate saline buffer). Furthermore, at pH 7.4 most of the carboxylate groups are deprotonated, resulting in a more stretched polymer shape due to repulsive electrostatic interactions. Because of the influence of pH on the polymeric matrix, it has been discovered that ketoprofen release from spray-dried ALG microcapsules cross linked using calcium chloride is greater in basic media than in acidic media (Hazel et al. 2019).

Sanat Kumar Basu et al., developed calcium ALG beads using two distinct techniques for drug incorporation by ionotropic gelation technique. In the sequential technique, drug entrapment rises when CaCl₂ and polymer concentrations increase, but decreases as drug concentration increases. And also mentioned that increase in CaCl₂ concentration slows the drug release in the sequential approach, but in the simultaneous method, drug release is sustained up to a specific level of CaCl₂ (Mandal et al. 2010).

5.4 *Stirring Speed*

According to the Nguyenn Thi Thanh Uyen et al., the features of AMPs such as particle size, size distribution, form, and agglomeration are largely dependent on stirring speed during production. The impact of stirring speed on the manufacture and characterization of ALG microspheres by emulsification or gelation technique was investigated in the study, and it was discovered that at low stirring speed, the ALG microspheres were irregular in form with a rough surface. The particle size of microspheres is found to be in the range of 28.5 to 69.6 μm with a smooth surface as stirring speed rises. As a result, they proposed that ALG microspheres be produced

by gelation or emulsification, and that particle size may be controlled by varying the stirring speed (Nguyen et al. 2022).

Boon- beng Lee et al., examined the influence of stirring speed and natural polymer type on the size and shape of chitosan-ALG capsules generated by extrusion-dripping method. At low stirring rates (400 and 500 rpm), the capsules were deformed/elongated, causing the capsule diameter to deviate and become larger than that of spherical capsules. At high stirring speeds, the centrifugal force induced by the whirlpool cavity in the gelation bath was strong enough to allow the droplets to enter the gelation bath, resulting in spherical particles. They concluded that the ALG type influenced the diameter and membrane thickness of chitosan-ALG capsules, whereas the stirring speed and ALG type influenced the capsule shape (Lim et al. 2013).

In another study, Y. P. Lemos et al., have prepared buriti oil microcapsules by gelatin-sodium ALG complex coacervation. By varying the hydrodynamic conditions using five various stirring speeds (600, 950, 1350, 1650 and 2000 rpm) and with two various concentrations of polymers during coacervation process results in decreased particle size with increase in stirring speed which was confirmed by decreased values of volume mean diameter. Thus, it was clear that stirring speed had strong influence on the size of the microcapsules, and Reynolds number higher than 70,000 resulted in particle size lower than 200 μm .

5.5 pH

Another adjustable parameter in the ALG gelation process is pH, which is closely connected to interfacial tension. Surprisingly, there have been few reports on the shape tailoring of aspheric ALG particles by pH-induced interfacial tension. Jui-Jung Chung et al. shown experimentally those ALG droplets in low pH gelation solutions prefer to form oblate ALG particles. It happened at low ALG and Ca^{2+} concentrations, where a low pH value has a bigger impact on particle formation by decreasing interfacial tension, resulting in an oblate particle with a higher aspect ratio. With a bigger surface area, an oblate particle provided a faster release rate than a spheric one (Chuang et al. 2017).

Lianzhou Jiang et al. investigated the impact of pH on the characteristics of soybean protein isolate/sodium ALG (SPI/SA) micro particles. SPI/SA micro particles with uniform distribution and smooth surface were created by adding and adjusting the pH value, i.e., when the pH is 9.0. When compared to SPI micro particles, results in increased apparent viscosity, protein solubility, foaming, and emulsifying capabilities (Cao et al. 2022).

5.6 Cross-Linking Time

In general, cross-linking duration had little effect on AMPs morphology in both extrusion and emulsification/gelation methods. However, when cross-linking time increased, the particle size of AMPs increased. This might be related to the degree of crosslinking between Ca^{2+} ions and the guluronic acid units of sodium ALG, which results in the creation of bigger ALG particles. Ziyaur Rahman et al., found that increasing the cross-linking period from 10 to 30 min reduces EE from 84.31% to 46.52%. It is due to the more gelation time allowed Ca^{2+} ions to permeate into the microspheres, and displace the drug, lowering drug EE (Rahman et al. 2006). Lin et al., utilized extrusion technique to develop astaxanthin encapsulated ALG beads. An increase in particle size of beads was observed from 1029.2 to 1112.9 μm as there is extended crosslinking time from 15 to 60 min (Lin et al. 2016). Some reports concluded that the cross-linking time had no significant influence on the size of beads when they evaluated the effect of cross-linking time on the properties of *Lactobacillus acidophilus* loaded ALG beads using the extrusion method (Lotfipour et al. 2012).

Rajinikanth et al. used an emulsification/gelation process to successfully formulated bioadhesive sodium ALG microspheres of Metoprolol tartrate. From the results it was evident that increase in mean particle size from 55.3 to 74.5 μm is due to enhanced cross-linking time from 5 to 25 min. This may be explained by the degree of cross-linking between Ca^{2+} ions and the COO^- functional groups of sodium ALG by enhanced the cross-linking time. As a result, the viscosity of the ALG solution rises, resulting in the development of massive microspheres. With increased cross-linking time, the drug EE demonstrated an opposite behavior, where it falls from 77.3% to 49.9% (Rajinikanth et al. 2003).

5.7 Model Drug and Drug Content

Highly water soluble drugs, obtaining a homogenous dispersion of the drug in ALG solution prior to emulsification is easy. Furthermore, highly water-soluble pharmaceuticals reduce drug diffusion from the ALG phase to the oil phase during preparation, increasing drug EE. Some moderately hydrophilic drugs, like curcumin, have been observed to be loaded in ALG micro particles with high EE (Hu et al. 2017).

In general, another major element influencing drug EE, is the drug content where increase in drug content leads to enhanced drug EE. In few cases, a large amount of drug content yields in diminished drug EE. This may be due to the fact that when the drug amount exceeds the loading limit of the MP systems, the overloaded drugs shift to the oil phase. It is proven by Shukla et al., where he found that increase in diloxanide furoate concentration from 100 to 200 mg reduces the entrapment effectiveness of diloxanide furoate loaded ALG microspheres from 73.8% to 51.4% utilizing emulsification/gelation method (Shukla et al. 2014). By utilizing the same

technique Salunkhe et al. illustrated the effect of granisetron hydrochloride -loaded ALG microspheres on the morphology of microspheres, transforming a smooth microsphere into a rough surface with the addition of drug (Salunkhe et al. 2014).

6 Applications of AMP Systems for Drug Delivery

The applications of AMPs in drug delivery systems are highly intriguing due to prerequisites of ALG demonstrated in Fig. 4, since the ALG matrix may shield the drug from hostile circumstances. Because of its mucoadhesive properties, it may increase the contact length between MPs and absorptive sites, resulting in increased encapsulated drug absorption. The release kinetics of biodegradable AMP may vary. ALG is a safe matrix due to its low toxicity and immunogenicity. It is well known that ALG is readily available and reasonably priced (Ahmed et al. 2013).

Because of the increasing use of ALG delivery systems, the literature has a wide range of applications few were summarized in Table 3 (Lopes et al. 2017; Nahar et al. 2017). In this context, AMP systems have grown increasingly appealing, which leads to increased applicability in drug delivery systems shown in Fig. 5. These can increase drug stability, control drug release, lengthen the duration of therapeutic

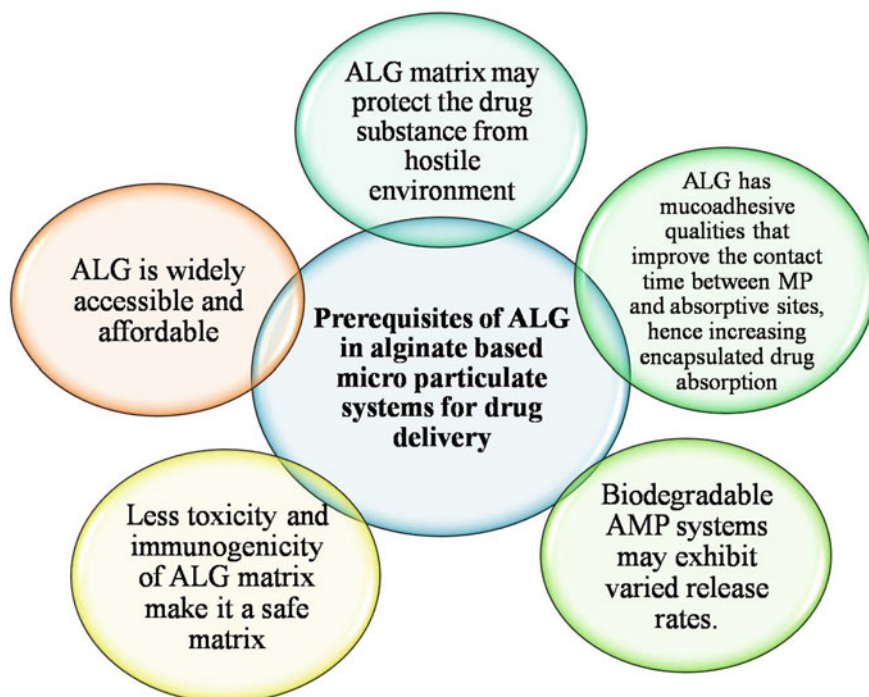


Fig. 4 Prerequisites of ALG in alginate based micro particulate systems for drug delivery

Table 3 Examples of functionalization of AMP systems in drug delivery

AMP systems (composition)	Particle diameter	Material of functionalization/ Drug	Therapeutic goal	Outcome	References
Microparticles (sodium alginate-chitosan)	251.5 ± 40.2 µm	Cyclophosphane and 5-fluorouracil	Eye cancer	In comparison to free drug, the duration of anticancer action for drug-containing alginate microparticles rises by 5–8 times	Batyrbekov et al. (2009)
Microparticles (Super-Paramagnetic ionic oxide (SPIO)/alginate)	200 µm	Disulfiram	Ovarian cancer	Cross-linked disulfiram/SPIO/ALG microparticles lower suppression tumor volume and so function as possible drug carriers for ovarian cancer therapy	Bai et al. (2021)
Mucoadhesive microparticles (sodium alginate/guar gum/carbopol 940)	21.10 ± 0.18 µm	Methotrexate	Colorectal cancer	Because of their adhesiveness to the colonic mucosa, microparticles distribute the methotrexate to the target areas with a sustained release profile	Deshmukh et al. (2021)
Microspheres (portulaca mucilage/sodium alginate/epichlorohydrin/Ca ²⁺)	930–977 µm	5-fluorouracil	Colon cancer	Sustained release of 5-fluorouracil in the colonic area upto 16 h and improved the antitumor effect of the drug	Asnani and Kokare (2018)
Microparticles (alginate-chitosan)	1 µm (mean diameter of particle)	BSA	Oral administration of antigens	ALG coated chitosan microparticles may modify the release pattern of BSA from microparticles and successfully prevent model protein from degradation against acidic medium in-vitro for at least 2 h	Li et al. (2008)
Microspheres (milk/alginate)	Micron level	Enterococcus faecalis HZNU P2	Stability of encapsulated Enterococcus faecalis HZNU P2 highly improved	Encapsulated cells showed good tolerance to simulated gastric fluid and bile	Shi et al. (2016)
Hydrogel microparticles (alginate)	90–100 µm	Isoniazid, rifampicin and pyrazinamide	Enhanced antimycobacterial therapy	Alginate loaded drugs showed nine-fold increase in relative bioavailability in comparison with free drugs	Qurrat-ul-Ain et al. (2003)

Microspheres (chitosan/alginate/CaCl ₂)	740–810 µm	Bacteriophage Felix O1	Oral administration of bacteriophage	Microencapsulated bacteriophage Felix O1 remains reactive in acidic conditions (simulated GIT environment), implying that microspheres may aid in therapeutic phage delivery to the gut	Ma et al. (2008)

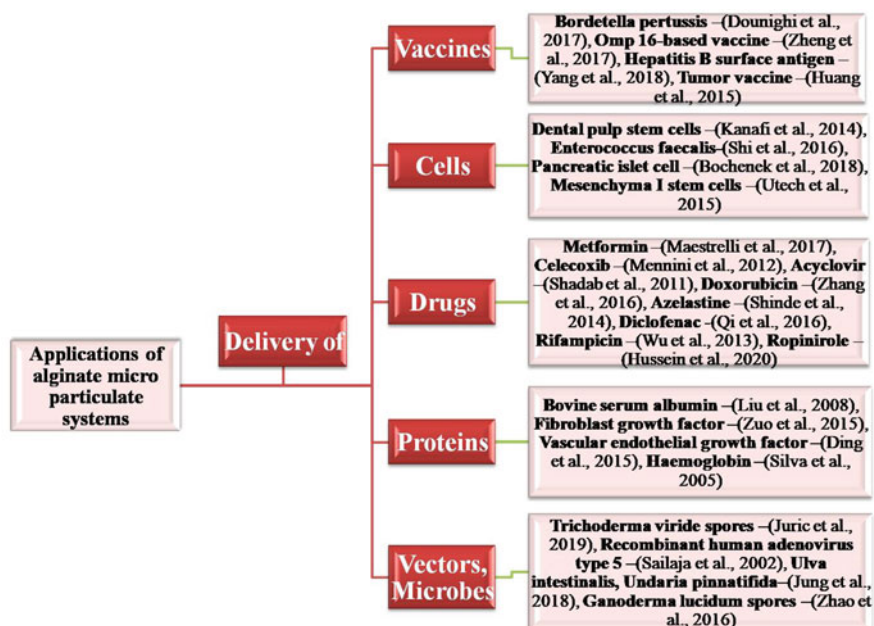


Fig. 5 Applications of alginate based micro particulate systems for drug delivery

effect, and allow for enteral or parenteral administration, which may avoid or minimize drug degradation and metabolism.

6.1 Cancer Targeting

Because ALG may concentrate at the tumor site and distinguish minor changes between healthy and malignant cells, targeted DDSs for cancer therapy are predicted to improve therapeutic effectiveness while minimizing adverse effects (He et al. 2020). Yerkesh O. Batyrbekov et al., utilized the MP delivery approach for the controlled release of two antitumor drugs (Cyclophosphane and 5-Fluorouracil). The smooth texture and spherical shape of the MPs obtained from calcium ALG gel aided the immobilization of the antitumor drugs, thus enhancing the effectiveness of treatment of eye tumor. Anticancer activity was checked by determining the quality of tumor clones from 120 rats and has been injected with malignant Rhabdomyoma strain. The AMP of antitumor drugs were successful in showing anticancer activity, which was further sustained by using chitosan coating (40–50 min for uncoated and 80–120 min for coated) (Batyrbekov et al. 2009).

6.2 Vaccine Delivery

A vaccine is a product or substance which is administered with the primary intention of enhancing the antibody production by the immune system as a specific response to a certain condition, either for prevention against that condition or curative therapy of the same. They can be used to train the immune system against some immunologic response or can also be used to terminate any unwanted response. A large number of contagious diseases like rubella, measles, and mumps are the main reason to bring the commercial spotlight on vaccines. The parenteral route is the most commonly used route for vaccines, but certain issues related to patient compliance and scientific rationale are forcing us to devise other routes too. In the case of an antigen whose primary target is the lungs or a certain area of the GIT, intranasal delivery and oral delivery respectively would be far more effective in targeting the root cause and curing it. Mucoadhesive property and protection from gastric media make ALG microspheres the most desired approach for oral or intranasal delivery of vaccines. Several studies supporting the role of AMP systems in vaccine delivery have also been carried out (Dhamecha et al. 2019).

Hariyadi et al., carried out a study to check the release pattern of a model antigen (BSA) from ALG microspheres, at the same time, the formulation was evaluated for the integrity of encapsulated material on free drying. The study reported ALG microspheres of size range 22–65 μm which showed 7% release in 2 h and 90% release in 10 h in simulated gastric and intestinal fluid respectively (Hariyadi et al. 2014). Another study carried out by Kim et al., found that ALG microspheres encapsulating rotavirus enhanced the immunological response to enteric and oral immunization in domestic animals (Kim et al. 2002). Dounighi et al., also utilized the sustained release capability of ALG micro particles in the delivery of *Bordetella pertussis* (Dounighi et al. 2017).

6.3 Cell Delivery

Immobilization and immunologic response are the major hurdles in the delivery of cells. These problems can be dealt with by encapsulating the desired cells within a polymeric membrane. ALG has proved to be a better suit for this purpose. The gel-like semi-permeable environment provided by ALG enables movement of the essentials (Oxygen, Nutrients, Proteins) and at the same time restricts the entry of substances with molecular weight higher than 150 kDa (Antibodies, Immunologic substances) thus acting as a shield from environmental stress. In addition to this, the gel ensures the viability of the cells. Many studies have been performed to evaluate the cell delivery capability of ALG microspheres (Dhamecha et al. 2019).

Yu et al., in their study successfully evaluated the capability of angiogenesis induction and remodeling on intramyocardial delivery of human mesenchymal cells, which were encapsulated into ALG microspheres modified with

arginylglycylaspartic acid peptide. The study concluded an enhanced cell growth and attachment and better expression of angiogenic factor. A large number of studies were focused on the delivery of pancreatic islet cells, with the purpose of treating diabetes (Yu et al. 2010). Schneider et al., carried out a study in which concentric layers of polymers namely polythelenimine, polyacryladid, or carboxymethylcellulose in combination with ALG were used to form a multilayer microcapsule containing pancreatic islets. The study concluded that the procedure of encapsulation or the membrane formation had no effect on the expected response of the islet cells.

Another study performed by Qi et al., was aimed at comparing the glycemic levels and mean graft survival time on intraperitoneal administration of ALG encapsulated islet cells as well as non-encapsulated islet cells. The study concluded that the microencapsulation of the pancreatic islet cells in $\text{Ca}^{2+}/\text{Ba}^{2+}$ crosslinked ALG, not only managed to overcome graft rejection but also maintained desired glycemic levels. On the other hand, the non-encapsulated islet cells faced xenogeneic rejection after 2 to 7 days of transplantation (Qi et al. 2012). Another study performed by Cappai et al., aimed at harnessing the power of ALG microspheres in overcoming the immune rejection of transplanted cells. The study constituted of encapsulating islets of Langerhans with the help of a barium-ALG polymer combination. To the researchers' content, the microencapsulated islets after intraperitoneal administration successfully managed the hypoglycemic condition without any major xenograft rejection (Cappai et al. 1995).

6.4 Protein Delivery

Proteins have been in the limelight of treatment options for a very long period, and so has been the ordeal pertaining to the delivery of proteins. Proteins hold a position of high importance in biomedical research due to their capability to regenerate bones or tissues at the same time their action as therapeutic agents. The susceptibility to denaturation due to changes in pH, temperature, or unfavorable conditions makes the delivery of proteins like enzymes, hormones, and growth factors a herculean task to accomplish. In order to overcome this problem many techniques have been tried, the use of the AMPs is also one of them. The properties of AMPs like prevention from the harsh environment, bioadhesion, site targeting, and controlled release have gained attention as a method for protein delivery.

Quinlan et al., used the combination of ALG and poly lactic-co-glycolic acid in order to encapsulate recombinant bone morphogenic protein-2 of humans for aiding in cell growth. The resultant spheres were of a size range of 1–10 μm and showed a positive potential for the delivery of proteins (Quinlan et al. 2015). Another study carried out by Zhu et al., focused on encapsulated BMP-2 in ALG microspheres, which was further site targeted with the help of the thermosensitive property of dextran-poly(lactide/chitosan)/glycerophosphate hydrogel in order to induce osteogenesis (Zhu et al. 2016). Cetn et al., also focused their study on the formulation and evaluation of ALG microspheres containing bovine serum albumin (BSA). The

resultant microspheres had a mean size of $11.96 \pm 0.043 \mu\text{m}$. The study concluded that ALG microspheres modulated the release of the encapsulated BSA, at the same time; it maintained the structural integrity of the BSA (Cetin et al. 2007). Büyüköz et al., used a combination of the nanofibrous scaffold of gelatin along with ALG microspheres in order to encapsulate nerve growth factors. Apart from these studies, ALG microspheres have also been used to encapsulate proteins with the aim of regenerative medicine and tissue engineering (Büyüköz et al. 2018).

6.5 *Microbes, Vectors, and Bacteria Delivery*

Not all microbes are harmful to the body, some of them are most required for the maintenance of normal physiology. The presence of certain microbes and bacteriophages is very much important for the normal functioning of GIT. As microbes, bacteriophages, and probiotics are susceptible to the harsh acidic environment of the stomach, ALG microspheres can be utilized to ensure acid stability, and prevent degradation at the same time ensuring a controlled release. The property of ALG to shrink in an acidic medium and exert a buffering action protects the encapsulated material from drastic changes in temperature. Apart from this, ALG microspheres also provide protection from the cation of bile and enzymatic degradation. A large number of studies have been carried out in this genre too.

Lamas et al., compared the germination time of encapsulated cells of *Bacillus subtilis* and fungal spores to that of the free cells and fungal spores. The study concluded that the encapsulated cells had a longer germination time at the same time they demonstrated a similar growth to the free cells (Lamas et al. 2001). Another study carried out by Ma et al., focused on enhancing the protective property of ALG microspheres, for this a combination of chitosan and ALG was used. This combination was used to encapsulate bacteriophages. The study was considered successful with the reported result of enhanced protection from the acidic environment of gastric media (Ma et al. 2008). In one more study carried out by Chen et al., *Lactobacillus bulgaricus* was encapsulated with the help of a combinational complex of protein and ALG microsphere which served as a carrier for probiotics. The study concluded that the diameter of the microsphere was the determining factor for efficacy (Chen et al. 2014). Few examples regarding functionalization of ALG based micro particles with different applications are summarized in Table 3.

In this context, AMP systems have grown increasingly appealing, which leads to increased applicability in drug delivery systems shown in Fig. 5. These can increase drug stability, control drug release, lengthen the duration of therapeutic effect, and allow for enteral or parenteral administration, which may avoid or minimize drug degradation and metabolism.

7 ALG Limitations in MP Systems for Drug Delivery

Despite of its widespread applications in drug delivery, ALG's main drawback is difficulty in developing reproducible manufacturing processes due to their complex structure, which frequently challenges its modification and purification (Nela and David 1999). Furthermore, large batch-to-batch changes and wide range of molecular weight occur as a result of their 'biopreparation' in live organisms. Some authors reported the instability of ALG based particulates at micro environmental pH and leads to drug leakage owing to its low drug encapsulation (Hasnain and Nayak 2019; Lee and Mooney 2012). In some circumstances, extensive leaching of drug from ALG-based particles to the surrounding medium, is due to the hydrophilic nature of ALG results in swelling and faster release (burst effect) especially in case of water-soluble API (Kamaly et al. 2016). As a result, ALG utilization is limited, making it less spectacular in comparison to synthetic polymers with superior repeatability and adaptability (Ige et al. 2012). ALGs are commercially available in a wide range of grades and purities, depending on the manufacturer and source. Any difference in the M/G block unit ratio in ALG can eventually activate the innate immune system due to the existence of cytokines in connection to high M block concentration. It induces an immunological reaction at the site of administration (Choukaife et al. 2020). Fabrication conditions like usage of organic solvents, high processing temperatures, lengthy manufacture time, low percentage yield, and challenging purifying conditions for ALG based microparticulates, have also hampered the application of ALG in drug delivery (Lopes et al. 2017).

However, ALG has a variety of limitations owing to its qualities (molecular weight, mannuronic acid/guluronic acid ratio) and manufacturing variables (type of cation, gelation and composition). So, ALG gel based particles exhibit varying porosity and permeability resulting in different molecules diffusion kinetics and potential drawbacks including instability or low mechanical and barrier capabilities, incompatibility with heavy metals, and heat treatment instability (López-Córdoba et al. 2014). These qualities can be enhanced by blending ALG with other biopolymers, particularly protein-based ones, or with synthetic polymers, that can function as wall material or by changing or combining the fabrication procedure (Belščak-Cvitanović et al. 2015; Gheorghita Puscaselu et al. 2020). Such an idea can latter work by strengthening the hydrogel structure via interactions between drugs, ALG, and co-structurants found that encapsulating olive leaf phenolic extracts with ALG combined with pectin, sodium caseinate, or whey protein isolate by emulsification internal inotropic gelation was an effective procedure (Bušić et al. 2018; Flamminii et al. 2020; López-Córdoba et al. 2014; Pacheco et al. 2018). F. Flamminii et al., findings demonstrated that ALG/pectin microbeads had superior encapsulation effectiveness with more regular structures, and DSC findings established a higher degree of physicochemical interactions and thermal stability of olive leaf phenolic extracts in conjunction with ALG/pectin polymers (Flamminii et al. 2021).

Several such ways have been implemented to address restrictions of ALG use, but one of the most effective strategies was to chemically modify ALG to increase its

hydrophobicity, resulting in increased encapsulation efficiency of microparticulates (Hasnain et al. 2020). Despite safety concerns, chemical cross-linking may improve the mechanical properties of ALG based microparticles (Nitta and Numata 2013). Marta Szekalska et al. suggested a unique approach for optimizing microparticulate manufacturing with a fast, continuous, reproducible, and customizable spray drying methodology. By using a one-step crosslinking approach with calcium chloride, a highly water-soluble drug (metformin hydrochloride) was efficiently encased as ALG microparticles. ALG-based microparticles containing metformin hydrochloride, made by 2% ALG crosslinked with 1% calcium chloride, exhibited a prolonged dissolution rate, better mucoadhesion, and a lower swelling ratio (Szekalska et al. 2018).

8 Conclusion and Future Perspectives

Natural polymer-based MP systems will try to fascinate drug delivery experts. Because of its intrinsic characteristics and FDA clearance as a food additive, ALG is a prominent pharmaceutical excipient for the development of novel drug delivery systems. Some of the most notable aspects of ALG include its biocompatibility, ease of gelation, and availability of open functional groups for facile modification in order to manufacture alginate derivatives with enhanced desired properties. The scientific community's great interest in alginate corresponds with the therapeutic revolution driven by MP systems. Thus ALG, along with its incredible flexibility in conducting minor adjustments customized certain qualities by combining with other natural and synthetic polymers, has aided in the continual advancement of study at the interface of ALG and micro particles. In this study, we outlined various approaches for AMP systems, also addressed the process factors that govern the drug delivery and release efficiency of drugs. Encapsulation inside AMPs is appealing for GIT protection, high drug entrapment and loading, and drug administration via a biocompatible and mucoadhesive carrier. Looking forward, AMP systems utilized in medicine are anticipated to change significantly in controlled release and targeted drug delivery of numerous drugs including antibiotics, anticancer, vaccines, and genes.

By combining various other strategies, AMP systems will play an important role in novel drug delivery, particularly in diagnostics, diseased cell sorting, gene & genetic materials, targeted, safe, effective, and specific in-vitro delivery & supplements as minute versions of diseased tissues & organs within the body. Increasing our understanding of alginate's inherent properties, various novel kinds of cell and tissue-interactive alginate gel based MP systems may pave the way for future improvements in biomedical research and engineering. Because of their capacity to avoid antigen reaction and so protect the cells from the host immune response, ALG microspheres are good formulations for the microencapsulation and administration of islet cells. Advanced clinical studies are now underway in which procaine islet cells loaded alginate microcapsules are being evaluated in human volunteers for type 1 diabetes without the need of an immunosuppressive drug and the results

revealed a long-term hypoglycemic effect. This clearly shows that AMP systems have a tremendous economic potential as cell carriers in drug delivery. The growth in research papers on alginate-based cell microencapsulation methods in recent years suggests that considerable progress in this study field employing AMP systems may be expected in the future.

References

- Abasalizadeh F, Moghaddam SV, Alizadeh E, Akbari E, Kashani E, Fazljou SMB, Torbati M, Akbarzadeh A (2020) Alginate-based hydrogels as drug delivery vehicles in cancer treatment and their applications in wound dressing and 3D bioprinting. *J Biol Eng* 14:8. <https://doi.org/10.1186/s13036-020-0227-7>
- Adinarayana K, Jyothi B, Ellaiah P (2005) Production of alkaline protease with immobilized cells of *Bacillus subtilis* PE-11 in various matrices by entrapment technique. *AAPS PharmSciTech* 6: E391–E397. <https://doi.org/10.1208/pt060348>
- Ahmed MM, El-Rasoul SA, Auda SH, Ibrahim MA (2013) Emulsification/internal gelation as a method for preparation of diclofenac sodium–sodium alginate microparticles. *Saudi Pharm J* 21: 61–69. <https://doi.org/10.1016/j.jsps.2011.08.004>
- Alginate-based biomaterials (n.d.). <https://encyclopedia.pub/entry/10217>
- Alnaief M, Obaidat RM, Alsmadi MM (2020) Preparation of hybrid alginate-chitosan aerogel as potential carriers for pulmonary drug delivery. *Polymers* 12:2223. <https://doi.org/10.3390/polym12102223>
- Anusree R, Shreya B, Prosenjit M, Monit P, Anindya B (2018) Design and characterization of ibuprofen loaded alginate microspheres prepared by ionic gelation method. *Int J Pharma Res Health Sci* 6:2713–2716
- Asnani GP, Kokare CR (2018) In vitro and in vivo evaluation of colon cancer targeted epichlorohydrin crosslinked Portulaca-alginate beads. *Biomol Concepts* 9:190–199. <https://doi.org/10.1515/bmc-2018-0019>
- Bai M-Y, Yu M-H, Wang T-T, Chen S-H, Wang Y-C (2021) Plate-like alginate microparticles with disulfiram–SPIO–coencapsulation: an in vivo study for combined therapy on ovarian cancer. *Pharmaceutics* 13:1348. <https://doi.org/10.3390/pharmaceutics13091348>
- Bale S, Khurana A, Reddy ASS, Singh M, Godugu C (2016) Overview on therapeutic applications of microparticulate drug delivery systems. *Crit Rev Ther Drug Carrier Syst* 33:309–361. <https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2016015798>
- Batyrbekov YO, Rakhimbaeva D, Musabekov K, Zhubanov B (2009) Alginate based microparticle drug delivery systems for the treatment of eye cancer. *Mater Res Soc Symp Proc* 1209:304. <https://doi.org/10.1557/PROC-1209-YY03-04>
- Beata Łabowska M, Michalak I, Detyna J (2019) Methods of extraction, physicochemical properties of alginates and their applications in biomedical field—a review. *Open Chem* 17:738–762. <https://doi.org/10.1515/chem-2019-0077>
- Becker TA, Kipke DR, Brandon T (2001) Calcium alginate gel: a biocompatible and mechanically stable polymer for endovascular embolization. *J Biomed Mater Res* 54:76–86. [https://doi.org/10.1002/1097-4636\(200101\)54:1<76::AID-JBM9>3.0.CO;2-V](https://doi.org/10.1002/1097-4636(200101)54:1<76::AID-JBM9>3.0.CO;2-V)
- Belščak-Cvitanović A, Đorđević V, Karlović S, Pavlović V, Komes D, Ježek D, Bugarski B, Nedović V (2015) Protein-reinforced and chitosan-pectin coated alginate microparticles for delivery of flavan-3-ol antioxidants and caffeine from green tea extract. *Food Hydrocoll* 51: 361–374. <https://doi.org/10.1016/j.foodhyd.2015.05.039>
- Bhattarai R, Shrestha A, Dhandapani N (2011) Drug delivery using alginate and chitosan beads: an overview. *Chron Young Sci* 2:192. <https://doi.org/10.4103/2229-5186.93023>

- Broderick E, Lyons H, Pembroke T, Byrne H, Murray B, Hall M (2006) The characterisation of a novel, covalently modified, amphiphilic alginate derivative, which retains gelling and non-toxic properties. *J Colloid Interface Sci* 298:154–161. <https://doi.org/10.1016/j.jcis.2005.12.026>
- Bušić A, Belščak-Cvitanović A, Vojvodić Cebin A, Karlović S, Kovač V, Špoljarić I, Mršić G, Komes D (2018) Structuring new alginate network aimed for delivery of dandelion (*Taraxacum officinale* L.) polyphenols using ionic gelation and new filler materials. *Food Res Int* 111:244–255. <https://doi.org/10.1016/j.foodres.2018.05.034>
- Butte NF, Wong WW, Patterson BW, Garza C, Klein PD (1988) Human-milk intake measured by administration of deuterium oxide to the mother: a comparison with the test-weighing technique. *Am J Clin Nutr* 47:815–821. <https://doi.org/10.1093/ajcn/47.5.815>
- Büyükcök M, Erdal E, Alsoy Altinkaya S (2018) Nanofibrous gelatine scaffolds integrated with nerve growth factor-loaded alginate microspheres for brain tissue engineering. *J Tissue Eng Regen Med* 12:e707–e719. <https://doi.org/10.1002/term.2353>
- Cao J, Tong X, Wang M, Tian T, Yang S, Sun M, Lyu B, Cao X, Wang H, Jiang L (2022) Soy protein isolate/sodium alginate microparticles under different pH conditions: formation mechanism and physicochemical properties. *Foods* 11:790. <https://doi.org/10.3390/foods11060790>
- Cappai A, Petruzzo P, Rui G, Congiu T, Dessy E, De Seta W, Santa Cruz G, Brotzu G (1995) Evaluation of new small barium alginate microcapsules. *Int J Artif Organs* 18:96–102
- Cetin M, Sahin S (2016) Microparticulate and nanoparticulate drug delivery systems for metformin hydrochloride. *Drug Deliv* 23:2796–2805. <https://doi.org/10.3109/10717544.2015.1089957>
- Cetin M, Vural I, Capaz Y, Hincal A (2007) Preparation and characterization of BSA-loaded alginate microspheres. *FABAD J Pharm Sci* 32:103–107
- Chen M-Y, Zheng W, Dong Q-Y, Li Z-H, Shi L-E, Tang Z-X (2014) Activity of encapsulated *Lactobacillus bulgaricus* in alginate-whey protein microspheres. *Braz Arch Biol Technol* 57: 736–741. <https://doi.org/10.1590/S1516-8913201402377>
- Choukaife H, Doolaanea AA, Alfatama M (2020) Alginate nanoformulation: influence of process and selected variables. *Pharmaceuticals* 13:335. <https://doi.org/10.3390/ph13110335>
- Chuang J-J, Huang Y-Y, Lo S-H, Hsu T-F, Huang W-Y, Huang S-L, Lin Y-S (2017) Effects of pH on the shape of alginate particles and its release behavior. *Int J Polym Sci* 2017:1–9. <https://doi.org/10.1155/2017/3902704>
- Comaposada J, Gou P, Marcos B, Arnau J (2015) Physical properties of sodium alginate solutions and edible wet calcium alginate coatings. *LWT Food Sci Technol* 64:212–219. <https://doi.org/10.1016/j.lwt.2015.05.043>
- Cui J-H, Goh J-S, Park S-Y, Kim P-H, Lee B-J (2001) Preparation and physical characterization of alginate microparticles using air atomization method. *Drug Dev Ind Pharm* 27:309–319. <https://doi.org/10.1081/DDC-100103730>
- Desai J, Patil J, Kulkarni R, Marapur S, Dalavi V (2001) Alginate-based microparticulate oral drug delivery system for rifampicin. *Res J Pharm Tech* 2:301–303
- Deshmukh R, Harwansh RK, Rahman MA (2021) Sodium alginate-guar gum and carbopol based methotrexate loaded mucoadhesive microparticles for colon delivery: an in vitro evaluation. *Braz J Pharm Sci* 57:e19147. <https://doi.org/10.1590/s2175-97902020000419147>
- Dewi MH, Noorma R, Asti R (2019) Design, optimization and characterization of glutathione loadedalginate microspheres for topical antiaging. *J Pharm Pharmacogn Res* 7:223–233
- Dhamecha D, Movsas R, Sano U, Menon JU (2019) Applications of alginate microspheres in therapeutics delivery and cell culture: past, present and future. *Int J Pharm* 569:118627. <https://doi.org/10.1016/j.ijpharm.2019.118627>
- Dounighi NM, Shahcheraghi F, Razzaghi-Abyaneh M, Nofeli M, Zolfagharian H (2017) A new vaccine delivery vehicle and adjuvant candidate: Bordetella pertussis inactivated whole cells entrapped in alginate microspheres. *Curr Pharm Des* 23:2665–2672. <https://doi.org/10.2174/1381612823666170112124303>
- Dragnet KI, Taylor C (2011) Chemical, physical and biological properties of alginates and their biomedical implications. *Food Hydrocoll* 25:251–256. <https://doi.org/10.1016/j.foodhyd.2009.10.007>

- Ea P (2020) Advanced applications of biomaterials based on alginic acid. *Am J Biomed Sci Res* 9: 47–53. <https://doi.org/10.34297/AJBSR.2020.09.001350>
- Feng C, Song R, Sun G, Kong M, Bao Z, Li Y, Cheng X, Cha D, Park H, Chen X (2014) Immobilization of coacervate microcapsules in multilayer sodium alginate beads for efficient oral anticancer drug delivery. *Biomacromolecules* 15:985–996. <https://doi.org/10.1021/bm401890x>
- Flamminii F, Di Mattia CD, Nardella M, Chiarini M, Valbonetti L, Neri L, Difonzo G, Pittia P (2020) Structuring alginate beads with different biopolymers for the development of functional ingredients loaded with olive leaves phenolic extract. *Food Hydrocoll* 108:105849. <https://doi.org/10.1016/j.foodhyd.2020.105849>
- Flamminii F, Paciulli M, Di Michele A, Littardi P, Carini E, Chiavaro E, Pittia P, Di Mattia CD (2021) Alginate-based microparticles structured with different biopolymers and enriched with a phenolic-rich olive leaves extract: a physico-chemical characterization. *Curr Res Food Sci* 4: 698–706. <https://doi.org/10.1016/j.crfs.2021.10.001>
- Forster REJ, Thürmer F, Wallrapp C, Lloyd AW, Macfarlane W, Phillips GJ, Boutrand J-P, Lewis AL (2010) Characterisation of physico-mechanical properties and degradation potential of calcium alginate beads for use in embolisation. *J Mater Sci Mater Med* 21:2243–2251. <https://doi.org/10.1007/s10856-010-4080-y>
- Gheorghita Puscaselu R, Lobiuc A, Dimian M, Covasa M (2020) Alginate: from food industry to biomedical applications and management of metabolic disorders. *Polymers (Basel)* 12:E2417. <https://doi.org/10.3390/polym12102417>
- Greco M, Sáez CA, Brown MT, Bitonti MB (2014) A simple and effective method for high quality co-extraction of genomic DNA and total RNA from low biomass ectocarpus piliculosus, the model brown alga. *PLoS One* 9:e96470. <https://doi.org/10.1371/journal.pone.0096470>
- Guarino V, Caputo T, Altobelli R, Ambrosio L (2015) Degradation properties and metabolic activity of alginate and chitosan polyelectrolytes for drug delivery and tissue engineering applications. *AIMS Mater Sci* 2:497–502. <https://doi.org/10.3934/matricsci.2015.4.497>
- Guastaferrero M, Reverchon E, Baldino L (2021) Agarose, alginate and chitosan nanostructured aerogels for pharmaceutical applications: a short review. *Front Bioeng Biotechnol* 9:688477. <https://doi.org/10.3389/fbioe.2021.688477>
- Gupta MK, Prakash D, Mishra B (2012) Biodegradable microparticulate drug delivery system of diltiazem HCl. *Braz J Pharm Sci* 48:699–709. <https://doi.org/10.1590/S1984-82502012000400014>
- Hariyadi DM, Islam N (2020) Current status of alginate in drug delivery. *Adv Pharmacol Pharm Sci* 2020:8886095. <https://doi.org/10.1155/2020/8886095>
- Hariyadi DM, Ma Y, Wang Y, Bostrom T, Malouf J, Turner MS, Bhandari B, Coombes AGA (2014) The potential for production of freeze-dried oral vaccines using alginate hydrogel microspheres as protein carriers. *J Drug Deliv Sci Technol* 24:178–184. [https://doi.org/10.1016/S1773-2247\(14\)50029-9](https://doi.org/10.1016/S1773-2247(14)50029-9)
- Hasnain MS, Nayak AK (eds) (2019) *Alginates: versatile polymers in biomedical applications and therapeutics*. Apple Academic Press, Oakville, ON
- Hasnain MS, Nayak AK, Kurakula M, Hoda MN (2020) Alginate nanoparticles in drug delivery. In: Nayak AK, Hasnain MS (eds) *Alginates in drug delivery*. Elsevier, London, pp 129–152. <https://doi.org/10.1016/B978-0-12-817640-5.00006-6>
- Hazel P, Niuris A, Inmaculada A, Lorenzo G, Nancy B, Angeles H, Carlos P (2019) Calcium cross-linked carboxymethylchitosan/alginate microparticles as a system for the prolonged release of ketoprofen. *Revista Cubana de Química* 31(1):37–57
- Hazra M, Dasgupta Mandal D, Mandal T, Bhuniya S, Ghosh M (2015) Designing polymeric microparticulate drug delivery system for hydrophobic drug quercetin. *Saudi Pharm J* 23: 429–436. <https://doi.org/10.1016/j.jsps.2015.01.007>
- He L, Shang Z, Liu H, Yuan Z (2020) Alginate-based platforms for cancer-targeted drug delivery. *Biomed Res Int* 2020:1–17. <https://doi.org/10.1155/2020/1487259>

- Heng PWS, Chan LW, Wong TW (2003) Formation of alginate microspheres produced using emulsification technique. *J Microencapsul* 20:401–413. <https://doi.org/10.3109/02652040309178078>
- Hu L, Kong D, Hu Q, Yang X, Xu H (2017) Preparation and optimization of a novel microbead formulation to improve solubility and stability of curcumin. *Part Sci Technol* 35:448–454. <https://doi.org/10.1080/02726351.2016.1165322>
- Hussein N, Omer H, Ismael A, Albed Alhnan M, Elhissi A, Ahmed W (2020) Spray-dried alginate microparticles for potential intranasal delivery of ropinirole hydrochloride: development, characterization and histopathological evaluation. *Pharm Dev Technol* 25:290–299. <https://doi.org/10.1080/10837450.2019.1567762>
- Hutcheson SW, Zhang H, Suvorov M (2011) Carbohydrase systems of saccharophagusdegradans degrading marine complex polysaccharides. *Mar Drugs* 9:645–665. <https://doi.org/10.3390/md9040645>
- Ige OO, Umoru LE, Aribio S (2012) Natural products: a minefield of biomaterials. *ISRN Mater Sci* 2012:1–20. <https://doi.org/10.5402/2012/983062>
- Jain D, Bar-Shalom D (2014) Alginate drug delivery systems: application in context of pharmaceutical and biomedical research. *Drug Dev Ind Pharm* 40:1576–1584. <https://doi.org/10.3109/03639045.2014.917657>
- Janes KA, Calvo P, Alonso MJ (2001) Polysaccharide colloidal particles as delivery systems for macromolecules. *Adv Drug Deliv Rev* 47:83–97. [https://doi.org/10.1016/s0169-409x\(00\)00123-x](https://doi.org/10.1016/s0169-409x(00)00123-x)
- Jurić S, Jurić M, Režek Jambrak A, Vinceković M (2021) Tailoring alginate/chitosan microparticles loaded with chemical and biological agents for agricultural application and production of value-added foods. *Appl Sci* 11:4061. <https://doi.org/10.3390/app11094061>
- Kamaly N, Yameen B, Wu J, Farokhzad OC (2016) Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release. *Chem Rev* 116:2602–2663. <https://doi.org/10.1021/acs.chemrev.5b00346>
- Karmakar A, Dev Maity S, Barua S, Das R (2022) Microsphere: a wander around drug delivery. *Revis Bionatura* 7:1–11. <https://doi.org/10.21931/RB/2022.07.01.13>
- Kaygusuz H, Evingür GA, Pekcan Ö, von Klitzing R, Erim FB (2016) Surfactant and metal ion effects on the mechanical properties of alginate hydrogels. *Int J Biol Macromol* 92:220–224. <https://doi.org/10.1016/j.ijbiomac.2016.07.004>
- Kim B, Bowersock T, Griebel P, Kidane A, Babiuk LA, Sanchez M, Attah-Poku S, Kaushik RS, Mutwiri GK (2002) Mucosal immune responses following oral immunization with rotavirus antigens encapsulated in alginate microspheres. *J Control Release* 85:191–202. [https://doi.org/10.1016/s0168-3659\(02\)00280-8](https://doi.org/10.1016/s0168-3659(02)00280-8)
- Kim YJ, Park HG, Yang YL, Yoon Y, Kim S, Oh E (2005) Multifunctional drug delivery system using starch-alginate beads for controlled release. *Biol Pharm Bull* 28:394–397. <https://doi.org/10.1248/bpb.28.394>
- Kinam P, Yoon Y (2002) Microencapsulation technology encyclopedia of pharmaceutical technology. *GPET* 6:67–85
- Kristiansen KA, Potthast A, Christensen BE (2010) Periodate oxidation of polysaccharides for modification of chemical and physical properties. *Carbohydr Res* 345:1264–1271. <https://doi.org/10.1016/j.carres.2010.02.011>
- Lamas MC, Bregni C, Daquino M, Degrossi J, Firenstein R (2001) Calcium alginate microspheres of *Bacillus subtilis*. *Drug Dev Ind Pharm* 27:825–829. <https://doi.org/10.1081/ddc-100107246>
- Lazar NK (2018) Alginate: application in the modern world. *Chemia Naissensis* 1:187–194
- Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. *Prog Polym Sci* 37:106–126. <https://doi.org/10.1016/j.progpolymsci.2011.06.003>
- Lengyel M, Kállai-Szabó N, Antal V, Laki AJ, Antal I (2019) Microparticles, microspheres, and microcapsules for advanced drug delivery. *Sci Pharm* 87:20. <https://doi.org/10.3390/scipharm87030020>

- Li X, Kong X, Shi S, Zheng X, Guo G, Wei Y, Qian Z (2008) Preparation of alginate coated chitosan microparticles for vaccine delivery. *BMC Biotechnol* 8:89. <https://doi.org/10.1186/1472-6750-8-89>
- Lim F, Sun AM (1980) Microencapsulated islets as bioartificial endocrine pancreas. *Science* 210: 908–910. <https://doi.org/10.1126/science.6776628>
- Lim G-P, Ong YT, Lee B-B, Ahmad MS, Singh H, Ravindra P (2013) Formation of chitosan-alginate capsules using extrusion-dripping method: effect of stirring speed and biopolymers types. *Aust J Basic Appl Sci* 7(5):84–90
- Lin D, Kelly AL, Miao S (2021) Alginate-based emulsion micro-gel particles produced by an external/internal O/W/O emulsion-gelation method: Formation, suspension rheology, digestion, and application to gel-in-gel beads. *Food Hydrocoll* 120:106926. <https://doi.org/10.1016/j.foodhyd.2021.106926>
- Lin S-F, Chen Y-C, Chen R-N, Chen L-C, Ho H-O, Tsung Y-H, Sheu M-T, Liu D-Z (2016) Improving the stability of astaxanthin by microencapsulation in calcium alginate beads. *PLoS One* 11:e0153685. <https://doi.org/10.1371/journal.pone.0153685>
- Lopes M, Abraham B, Veiga F, Seica R, Cabral LM, Arnaud P, Andrade JC, Ribeiro AJ (2017) Preparation methods and applications behind alginate-based particles. *Expert Opin Drug Deliv* 14:769–782. <https://doi.org/10.1080/17425247.2016.1214564>
- López-Córdoba A, Deladino L, Martino M (2014) Release of yerba mate antioxidants from corn starch–alginate capsules as affected by structure. *Carbohydr Polym* 99:150–157. <https://doi.org/10.1016/j.carbpol.2013.08.026>
- Lotfipour F, Mirzaeei S, Maghsoodi M (2012) Evaluation of the effect of CaCl₂ and alginate concentrations and hardening time on the characteristics of *Lactobacillus acidophilus* loaded alginate beads using response surface analysis. *Adv Pharm Bull* 2:71–78. <https://doi.org/10.5681/apb.2012.010>
- Lucinda-Silva RM, Evangelista RC (2003) Microspheres of alginate-chitosan containing isoniazid. *J Microencapsul* 20:145–152
- Ma Y, Pacan JC, Wang Q, Xu Y, Huang X, Korenevsky A, Sabour PM (2008) Microencapsulation of bacteriophage *felix O1* into chitosan-alginate microspheres for oral delivery. *Appl Environ Microbiol* 74:4799–4805. <https://doi.org/10.1128/AEM.00246-08>
- Madhav NV, Kala S (2011) Review on microparticulate drug delivery system. *Int J PharmTech Res* 3:1242–1254
- Mandal S, Kumar SS, Krishnamoorthy B, Basu SK (2010) Development and evaluation of calcium alginate beads prepared by sequential and simultaneous methods. *Braz J Pharm Sci* 46:785–793. <https://doi.org/10.1590/S1984-82502010000400021>
- Mandracchia D, Tripodo G (2020) Micro and nano-drug delivery systems. In: Bari E, Perteghella S, Torre ML (eds) *Silk-based drug delivery systems*. Royal Society of Chemistry, Cambridge, pp 1–24. <https://doi.org/10.1039/9781839162664-00001>
- Manev Z, Benev T, Zsivanovits G, Ludneva D (n.d.) Structural mechanical and gelling properties of alginate beads. *Bulg J Agric Sci* 19:770–774
- Ménard M, Dusseault J, Langlois G, Baille WE, Tam SK, Yahia L, Zhu XX, Hallé J-P (2010) Role of protein contaminants in the immunogenicity of alginates. *J Biomed Mater Res* 93B:333–340. <https://doi.org/10.1002/jbm.b.31570>
- Mishra MK (ed) (2016) *Handbook of encapsulation and controlled release*. CRC Press, Boca Raton
- Mollah MZI, Zahid HM, Mahal Z, Faruque MRI, Khandaker MU (2021) The usages and potential uses of alginate for healthcare applications. *Front Mol Biosci* 8:719972. <https://doi.org/10.3389/fmolb.2021.719972>
- Murata Y, Jinno D, Kofuji K, Kawashima S (2004) Properties of calcium-induced gel beads prepared with alginate and hydrolysates. *Pharm Bull* 52:605–607
- Nahar K, Hossain MK, Khan TA (2017) Alginate and its versatile application in drug delivery. *J Pharm Sci Res* 9(5):606–617
- Nela A, David H (1999) Rationalizing the design of polymeric biomaterials. *Trends Biotechnol* 17: 409–421

- Nemethova V (2015) Vibration technology for microencapsulation: the restrictive role of viscosity. *J Bioprocess Biotech* 5:199. <https://doi.org/10.4172/2155-9821.1000199>
- Neves MI, Moroni L, Barrias CC (2020) Modulating alginate hydrogels for improved biological performance as cellular 3D microenvironments. *Front Bioeng Biotechnol* 8:665. <https://doi.org/10.3389/fbioe.2020.00665>
- Nguyen TTU, Hamid ZAA, Nurazreena A (2022) Effect of stirring speed on the formation and characteristic of alginate microspheres via gelation/emulsification method. *KEM* 908:171–176. <https://doi.org/10.4028/p-4c2w46>
- Nikhil KS, Seema P, Antesh J, Bhattacharya A (2009) Sodium alginate: the wonder polymer for controlled drug delivery. *J Pharm Res* 2:1191–1199
- Nitta SK, Numata K (2013) Biopolymer-based nanoparticles for drug/gene delivery and tissue engineering. *Int J Mol Sci* 14:1629–1654. <https://doi.org/10.3390/ijms14011629>
- Pacheco C, González E, Robert P, Parada J (2018) Retention and pre-colon bioaccessibility of oleuropein in starchy food matrices, and the effect of microencapsulation by using inulin. *J Funct Foods* 41:112–117. <https://doi.org/10.1016/j.jff.2017.12.037>
- Parida KR, Panda SK, Ravanan P, Roy H, Talwar P (2013) Microparticles based drug delivery systems: preparation and application in cancer therapeutics. *Int Arch App Sci Technol* 4(3): 68–75
- Patil JS, Devi K, Devi K, Suresh S (2015) Formulation and evaluation of novel spray-dried alginate microspheres as pulmonary delivery systems of rifampicin in rats. *Indian J Pharm Educ Res* 49: 320–328. <https://doi.org/10.5530/ijper.49.4.9>
- Pavan Kumar B, Sarath Chandiran I, Bhavya B, Sindhuri M (2011) Microparticulate drug delivery system: a review. *Indian J Pharm Sci Res* 1:19–37
- Pina S, Oliveira JM, Reis RL (2015) Natural-based nanocomposites for bone tissue engineering and regenerative medicine: a review. *Adv Mater* 27:1143–1169. <https://doi.org/10.1002/adma.201403354>
- Plamen K, Vissera P, Margarita K (2015) Preparation techniques of microparticles for drug delivery (review). *Pharm Dental Med* 18:285–289
- Poncelet D (2006) Microencapsulation: fundamentals, methods and applications. In: Blitz JP, Gun'ko VM (eds) *Surface chemistry in biomedical and environmental science*. Springer, Cham, pp 23–34. https://doi.org/10.1007/1-4020-4741-X_3
- Qi M, Mørch Y, Lacík I, Formo K, Marchese E, Wang Y, Danielson KK, Kinzer K, Wang S, Barbaro B, Kolláriková G, Chorvát D, Hunkeler D, Skjåk-Braek G, Oberholzer J, Strand BL (2012) Survival of human islets in microbeads containing high guluronic acid alginate crosslinked with Ca^{2+} and Ba^{2+} . *Xenotransplantation* 19:355–364. <https://doi.org/10.1111/xen.12009>
- Quinlan E, López-Noriega A, Thompson E, Kelly HM, Cryan SA, O'Brien FJ (2015) Development of collagen-hydroxyapatite scaffolds incorporating PLGA and alginate microparticles for the controlled delivery of rhBMP-2 for bone tissue engineering. *J Control Release* 198:71–79. <https://doi.org/10.1016/j.jconrel.2014.11.021>
- Qurrat-ul-Ain, Sharma S, Khuller GK, Garg SK (2003) Alginate-based oral drug delivery system for tuberculosis: pharmacokinetics and therapeutic effects. *J Antimicrob Chemother* 51:931–938. <https://doi.org/10.1093/jac/dkg165>
- Rafiee MH, Abdul Rasool BK (2022) An overview of microparticulate drug delivery system and its extensive therapeutic applications in diabetes. *Adv Pharm Bull* 12(4):730–746. <https://doi.org/10.34172/apb.2022.075>
- Rahman Z, Kohli K, Khar RK, Ali M, Charoo NA, Shamsheer AAA (2006) Characterization of 5-fluorouracil microspheres for colonic delivery. *AAPS PharmSciTech* 7:E113–E121. <https://doi.org/10.1208/pt070247>
- Rajesh K, Khanrah A, Biswanth S (2003) Release of Ketoprofen from alginate microparticles containing film forming polymers. *J Sci Ind Res* 27(10):965–989

- Rajinikanth PS, Sankar C, Mishra B (2003) Sodium alginate microspheres of metoprolol tartrate for intranasal systemic delivery: development and evaluation. *Drug Deliv* 10:21–28. <https://doi.org/10.1080/713840323>
- Ramya Krishna S, Eliyas Kadi A (2016) Preparation and evaluation of alginate microspheres of piroxicam for controlled release. *Eur J Ther* 22:27–32
- Rinaudo M (2014) Biomaterials based on a natural polysaccharide: alginate. *TIP* 17:92–96. [https://doi.org/10.1016/S1405-888X\(14\)70322-5](https://doi.org/10.1016/S1405-888X(14)70322-5)
- Salunkhe NH, Jadhav NR, Mali KK, Dias RJ, Ghorpade VS, Yadav AV (2014) Mucoadhesive microsphere based suppository containing granisetron hydrochloride for management of emesis in chemotherapy. *J Pharm Investig* 44:253–263. <https://doi.org/10.1007/s40005-014-0123-6>
- Sanjoy Kumar D, Sheba Rani ND, Rajan R, Hirak Kumar M, Tripti H, Mohanraj P, Jasmina K (2011) Microencapsulation techniques and its practices. *Int J PharmSciTech* 6:1
- Santos MG, Bozza FT, Thomazini M, Favaro-Trindade CS (2015) Microencapsulation of xylitol by double emulsion followed by complex coacervation. *Food Chem* 171:32–39. <https://doi.org/10.1016/j.foodchem.2014.08.093>
- Saravanan M, Rao KP (2010) Pectin–gelatin and alginate–gelatin complex coacervation for controlled drug delivery: Influence of anionic polysaccharides and drugs being encapsulated on physicochemical properties of microcapsules. *Carbohydr Polym* 80:808–816. <https://doi.org/10.1016/j.carbpol.2009.12.036>
- Sateesh MN, Shivani K (2011) Review on microparticulate drug delivery system. *Int J PharmTech Res* 3:1242–1254
- Shi L-E, Zheng W, Zhang Y, Tang Z-X (2016) Milk-alginate microspheres: protection and delivery of *Enterococcus faecalis* HZNU P2. *LWT Food Sci Technol* 65:840–844. <https://doi.org/10.1016/j.lwt.2015.08.071>
- Shukla S, Jain D, Verma K (2014) Formulation and in vitro characterization of alginate microspheres loaded with diloxanide furoate for colon-specific drug delivery. *Asian J Pharm* 4. <https://doi.org/10.22377/ajp.v4i4.229>
- Silva CM, Ribeiro AJ, Figueiredo IV, Gonçalves AR, Veiga F (2006) Alginate microspheres prepared by internal gelation: development and effect on insulin stability. *Int J Pharm* 311:1–10. <https://doi.org/10.1016/j.ijpharm.2005.10.050>
- Smidsrød O, Skjåk-Braek G (1990) Alginate as immobilization matrix for cells. *Trends Biotechnol* 8:71–78. [https://doi.org/10.1016/0167-7799\(90\)90139-o](https://doi.org/10.1016/0167-7799(90)90139-o)
- Soheila H, Maryam M, Karami M (2009) The effect of chitosan molecular weight on the properties of alginate/chitosan microparticles containing prednisolone. *Trop J Pharm Res* 8:53–61
- Solanki N (2018) Microspheres an innovative approach in drug delivery system. *MOJ Bioequiv Availab* 5(1):56–58. <https://doi.org/10.15406/mojbb.2018.05.00083>
- Sosnik A (2014) Alginate particles as platform for drug delivery by the oral route: state-of-the-art. *ISRN Pharm* 2014:1–17. <https://doi.org/10.1155/2014/926157>
- Sreekanth Reddy O, Subha MCS, Jithendra T, Madhavi C, Chowdoji Rao K (2021) Curcumin encapsulated dual cross linked sodium alginate/montmorillonite polymeric composite beads for controlled drug delivery. *J Pharm Anal* 11:191–199. <https://doi.org/10.1016/j.jpha.2020.07.002>
- Sreekanth Reddy O, Subha N, Jithendra P, Madhavi C (2019) Emerging novel DRUG delivery system for control release of curcumin through sodium alginate/poly(ethylene glycol) semi IPN microbeads-intercalated with kaolin nanoclay. *J Drug Deliv Therap* 9:324–333
- Sreekumar K, Bindhu B (2019) A comprehensive review on alginates. *Int J Recent Technol Eng* 8: 1095–1097
- Stachowiak N, Kowalonek J, Kozłowska J (2021) Freeze-dried matrices composed of degradable polymers with surfactant-loaded microparticles based on pectin and sodium alginate. *Materials (Basel)* 14:3044. <https://doi.org/10.3390/ma14113044>
- Szekalska M, Amelian A, Winnicka K (2015) Alginate microspheres obtained by the spray drying technique as mucoadhesive carriers of ranitidine. *Acta Pharma* 65:15–27. <https://doi.org/10.1515/acph-2015-0008>

- Szekalska M, Puciłowska A, Szymańska E, Ciosek P, Winnicka K (2016) Alginate: current use and future perspectives in pharmaceutical and biomedical applications. *Int J Polym Sci* 2016:1–17. <https://doi.org/10.1155/2016/7697031>
- Szekalska M, Sosnowska K, Czajkowska-Kośnik A, Winnicka K (2018) Calcium chloride modified alginate microparticles formulated by the spray drying process: a strategy to prolong the release of freely soluble drugs. *Materials (Basel)* 11:E1522. <https://doi.org/10.3390/ma11091522>
- Takeuchi H, Yasuji T, Yamamoto H, Kawashima Y (2000) Spray-dried lactose composite particles containing an ion complex of alginate-chitosan for designing a dry-coated tablet having a time-controlled releasing function. *Pharm Res* 17:94–99. <https://doi.org/10.1023/a:1007530927887>
- Tønnesen HH, Karlsen J (2002) Alginate in drug delivery systems. *Drug Dev Ind Pharm* 28:621–630. <https://doi.org/10.1081/DDC-120003853>
- US Food and Drug Administration (2020). Generally recognized as safe (GRAS). FDA. <https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras>
- Uyen NTT, Hamid ZAA, Tram NXT, Ahmad N (2020) Fabrication of alginate microspheres for drug delivery: a review. *Int J Biol Macromol* 153:1035–1046. <https://doi.org/10.1016/j.ijbiomac.2019.10.233>
- Vinod M, Jitendra N, Gayatri K, Gokul K (2013) Formulation and development of diltiazem hydrochloride sustained release alginate beads by ionotropic external gelation technique. *Adv Pharmacol Pharm* 1(3):139–143. <https://doi.org/10.13189/app.2013.010304>
- Wan L, Heng P, Chan L (1994) Surfactant effects on alginate microspheres. *Int J Pharm* 103:267–275. [https://doi.org/10.1016/0378-5173\(94\)90177-5](https://doi.org/10.1016/0378-5173(94)90177-5)
- Wawrzyńska E, Kubies D (2018) Alginate matrices for protein delivery—a short review. *Physiol Res* 67:S319–S334. <https://doi.org/10.33549/physiolres.933980>
- Whelehan M, Marison IW (2011) Microencapsulation using vibrating technology. *J Microencapsul* 28:669–688. <https://doi.org/10.3109/02652048.2011.586068>
- Wurster DE, Bhattacharjya S, Flanagan DR (2007) Effect of curing on water diffusivities in acrylate free films as measured via a sorption technique. *AAPS PharmSciTech* 8:E71. <https://doi.org/10.1208/pt0803071>
- Yu J, Du KT, Fang Q, Gu Y, Mihardja SS, Sievers RE, Wu JC, Lee RJ (2010) The use of human mesenchymal stem cells encapsulated in RGD modified alginate microspheres in the repair of myocardial infarction in the rat. *Biomaterials* 31:7012–7020. <https://doi.org/10.1016/j.biomaterials.2010.05.078>
- Zhang C, Grossier R, Candoni N, Veessler S (2021) Preparation of alginate hydrogel microparticles by gelation introducing cross-linkers using droplet-based microfluidics: a review of methods. *Biomater Res* 25:41. <https://doi.org/10.1186/s40824-021-00243-5>
- Zhu Y, Wang J, Wu J, Zhang J, Wan Y, Wu H (2016) Injectable hydrogels embedded with alginate microspheres for controlled delivery of bone morphogenetic protein-2. *Biomed Mater* 11: 025010. <https://doi.org/10.1088/1748-6041/11/2/025010>
- Zimmermann U, Klöck G, Federlin K, Hannig K, Kowalski M, Bretzel RG, Horcher A, Entenmann H, Sieber U, Zekorn T (1992) Production of mitogen-contamination free alginates with variable ratios of mannuronic acid to guluronic acid by free flow electrophoresis. *Electrophoresis* 13:269–274. <https://doi.org/10.1002/elps.1150130156>
- Zou Z, Zhang B, Nie X, Cheng Y, Hu Z, Liao M, Li S (2020) A sodium alginate-based sustained-release IPN hydrogel and its applications. *RSC Adv* 10:39722–39730. <https://doi.org/10.1039/D0RA04316H>

Alginate Based Nanocarriers for Controlled Drug Delivery Applications



Deepa Thomas and M. S. Latha

Abstract Alginate is an anionic copolymer of guluronic and mannuronic acids that occurs naturally. Alginate's superior biocompatibility, non-toxicity, and drug-carrying capabilities have drawn a lot of interest in drug delivery applications. Its pendent carboxyl groups can release or receive protons as the pH changes, allowing pH-controlled drug release. Furthermore, it is an efficient polymer for the encapsulation and oral delivery of physiologically active molecules including medicines and proteins due to its ease of gelation with multivalent cations and tuneable swelling features.

Keywords Alginate · Biopolymer · Nanocarriers · Drug delivery · Controlled drug release

1 Introduction

The use of biocompatible and biodegradable natural polymers in a wide range of healthcare applications is on the upsurge, and they are accepted as valuable biopolymers. Biopolymers degrade in the body, are inexpensive, and are deemed harmless, as they do not disrupt the cellular osmotic balance. Incorporating the drug within polymeric matrix can enhance the physiologically active compound's protection from degradation, manage its release profile, and improve its absorption, resulting in a greater therapeutic impact and lower dosage frequency. Apart from biodegradability, biopolymers provide a lot of versatility in terms of size and surface charge control through chemical changes.

D. Thomas

Research and Post Graduate Department of Chemistry, Bishop Moore College, Mavelikara, Kerala, India

M. S. Latha (✉)

Department of Chemistry, Sree Narayana College, Chathannur, Kerala, India

Department of Chemistry, Sree Narayana College, Kollam, Kerala, India

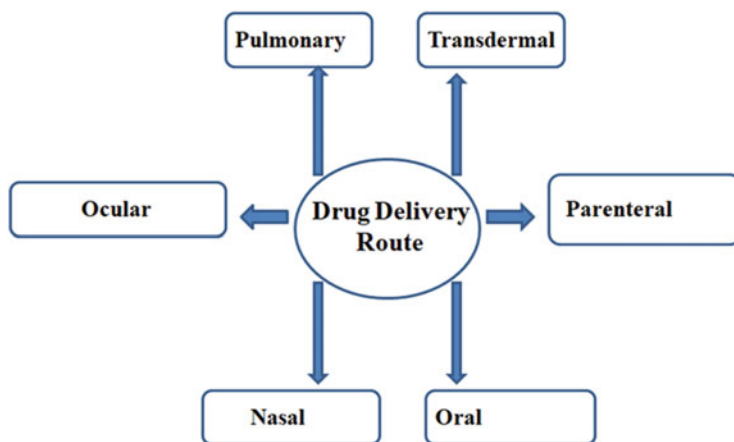


Fig. 1 Classification of systemic drug delivery routes

Alginates (ALG) are natural bio polysaccharide groups that are primarily produced in large quantities from brown sea algae. ALG is an anionic polysaccharide. Calcium alginate and sodium alginate are the most extensively used commercial alginates. ALG used in the design and creation of many biopolymeric systems for use in numerous biomedical and medical applications, including drug delivery, oil encapsulation, drug targeting, cell and enzyme encapsulation, protein delivery, wound dressing, and so on, for the past few decades. ALG has a number of appealing biopharmaceutical features, including pH sensitivity, biocompatibility, biodegradability, mucoadhesiveness, lack of toxicity, and lack of immunogenicity, making it an appealing candidate for modified drug release. For drug delivery, ALG can be converted into hydrogels, micro- and nanoparticles, and porous scaffolds. ALG also feature key functional groups including hydroxyl and carboxyl that could be easily changed to provide suitable characteristics for drug transport.

Nanomaterials as pharmaceutical carriers have received a lot of attention recently. Nanotechnology allows pharmaceuticals to be delivered at the nanoscale level while also reducing side effects. Nanotechnology has the potential to improve pharmacological treatment efficacy. Absorption, distribution, metabolism, and excretion (ADME) of nanodelivery systems are influenced by the physicochemical features of nanoparticles. As a result, nanoparticles offer various benefits for drug administration, ranging from greater stability to better control of bioactive release and targeting for improved functioning. The drug entrapped in drug delivery system can be introduced into the body by several anatomic routes.

The choice of suitable administration route is very important for the effective treatment. The factors such as drug properties, the disease to be treated and the desired therapeutic time must be considered during the selection of drug administration route. The major systemic drug delivery routes were mentioned in Fig. 1.

2 Source, Structure and Composition of Alginate

ALG is a structural component that serves as the seaweed's main skeletal compound that has a carboxylic group in each constituent residue by nature. ALG come from two main sources: algal and bacterial sources. These are mostly derived from various brown sea algae types (Lee and Mooney 2012). *Laminaria hyperborean*, *Sargassum* spp, *Laminaria digitata*, *Macrocystis pyrifera*, *Ascophyllum nodosum*, *Ecklonia maxima*, *Laminaria japonica* and *Lessonia nigrescens* are the principal algal sources of alginate (Liu et al. 2019). ALG are extracted from algal resources in a multi-step process (Elnahtawy et al. 2022). *Pseudomonas* sp. and *Azotobacter* sp. are two well-known bacterial sources for alginate extraction (Kirti 2022). For the biosynthesis of ALG, these two bacteria use very similar processes which comprise production of precursor substrate, polymerization and cytoplasmic membrane transfer, periplasmic transfer and modification, and export across the outer membrane (Hay et al. 2013).

ALG, a linear anionic polysaccharide contains 1,4'-linked- β -D-Mannuronic acid (M) and α -L-Guluronic acid (G) residues, which is organized in homopolymeric blocks of MM or GG or in heteropolymeric blocks of MG (Fig. 2a, b).

The geometrical structure of these blocks varies significantly depending on the monomer's shape and bonding style. The M blocks are long ribbon, while the G blocks are buckled together. The molecular weight of ALG can range from 10 to 1000 kDa, depending on relative G/M ratios, chain configurations, and the source of extraction, production process and age of the algae. ALG produced from diverse sources varies in terms of the contents of M and G, as well as the length of each block. The G content influences the physical and mechanical stability of ALG gels;

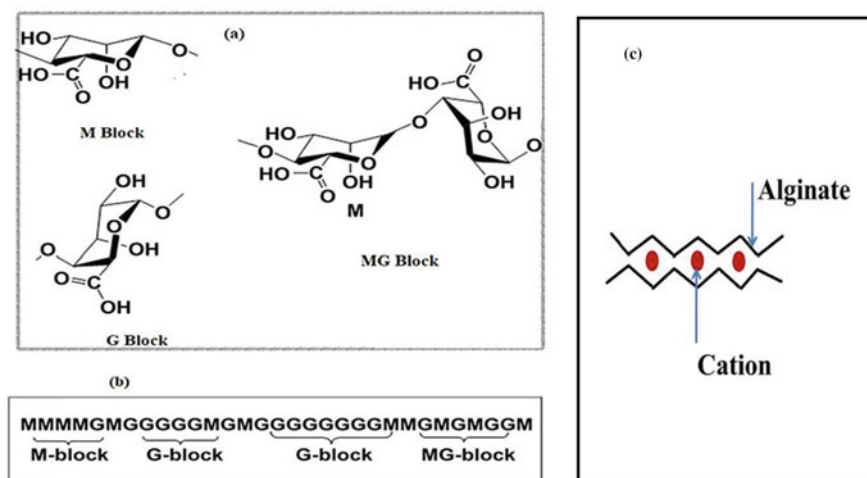


Fig. 2 Representative structure of ALG (a) chemical blocks (b) block distribution (c) schematic representation of egg box structure

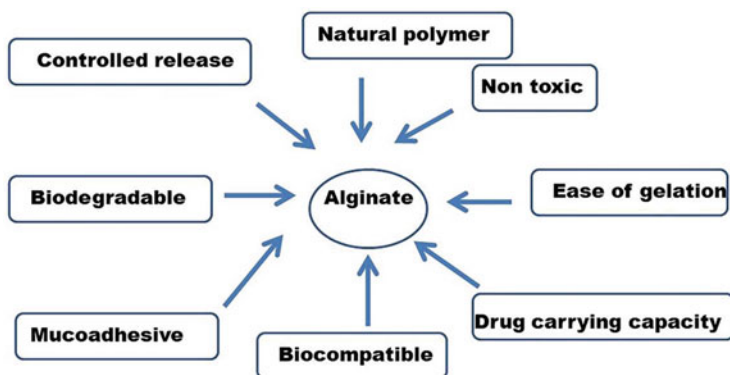


Fig. 3 Properties of alginate make it suitable for drug delivery applications

higher the amount of G content, it is stiffer and delicate the matrix. Furthermore, the pH of the medium, molecular weight, G/M ratio and concentration all influence the rheological and drug delivery effectiveness of ALG (Pereira and Cotas 2020). The molecular weight of ALG is usually calculated using intrinsic viscosity and light scattering measurements. Figure 3 shows the properties of ALG make it suitable for biomedical applications.

3 Physiochemical Characteristics

3.1 Solubility

ALG's solubility is determined by the pH of the solvent, its ionic concentration, the nature of gelling divalent ions used and polymer structure. Sodium ALG is insoluble in organic solvents such as chloroform, alcohol, ether as well as hydro alcoholic solutions with 30% by weight alcohol concentration. In contrast, Calcium ALG is insoluble in water and organic solvents. On the other hand, it is soluble in sodium citrate. The tetrabutylammonium (TBA) salt of ALG dissolves in ethylene glycol, water, and polar aprotic solvent combinations such as DMSO/TBAF, DMF/TBAF, DMI/TBAF and DMAc/TBAF. However, it is insoluble in DMF, DMAc, DMAc/LiCl and DMSO (Chaturvedi et al. 2019).

3.2 Viscosity

The molecular weight, stiffness, and chain elongation of the linear polymer ALG affect its viscosity. ALG gets more viscous as its molecular weight increases. As the concentration of a strong electrolyte like NaCl is increased to 100 mM, the viscosity

of sodium ALG solution decreases due to a change in polymer conformation (Sehgal et al. 2019).

3.3 Cross-Linking and Gel Formation

The unique gel formation capacity of ALG in aqueous media under mild condition in presence of multivalent cations makes it suitable for drug delivery applications and immobilization of cells. ALG matrix can be formed by crosslinking the polymer chains in a physical and/or chemical way. The ionic crosslinking creates a three-dimensional network primarily by interaction between the carbonyl groups of guluronate monomers and multivalent cations, resulting in the well-known “egg-box” conformation (Fig. 2c). The interaction strength of alginate crosslinking cations follows the order: trivalent cations > Pb^{2+} > Cu^{2+} > Cd^{2+} > Ba^{2+} > Sr^{2+} > Ca^{2+} . While Ca^{2+} ion does not display the maximum level of interaction, it is the most commonly used (Chan et al. 2002; Sikorski et al. 2007; Harper et al. 2014). This choice may be correlated with sufficient network of calcium ALG gel and human organism’s acceptability of calcium due to its function as a key element of the skeletal system and also controlling many physiological processes. Sr^{2+} and Ba^{2+} cations are also known to be cross-linking agents but the toxicity limits the use of Pb^{2+} , Cu^{2+} and Cd^{2+} . Calcium chloride is ideal for bio encapsulation purposes for calcium crosslinking, while calcium carbonate and sulphate are suitable for tissue-engineering scaffolds as it holds slower gelation, leading to a homogeneous network.

3.4 pH Sensitivity

The pH sensitivity of ALG has been exploited by many research groups for the development of oral drug delivery device. Among the ALG’s excellently-known features, pH sensitivity remains an enticing choice to use it for drug delivery applications. This important feature is beneficial for improving the potential for treatment of inflammatory bowel diseases, which frequently involve multi-drug administration over a long period of time. The drugs loaded in sodium alginate hydrogel-based drug delivery systems remain intact in the acidic pH of stomach, and is released at a regulated rate in the slightly alkaline pH of intestinal tract, which helps to maintain blood drug concentration, increase drug performance, and reduce side effects. Its pKa value can be used to explain this pH-dependent swelling. At pH 1.2, ALG shows a minimal swelling response. The majority of the COOH groups are shown to become protonated and form physical crosslinks as a result of hydrogen bonding interactions between the nearby $-\text{COOH}$ groups when the pH value is less than the polymer’s pKa value. ALG has a 3.5 pKa value. The carboxylate groups of ALG are therefore protonated and changed into insoluble alginic acid in acidic media (pH 1.2). As a result of these potent hydrogen bond interactions, a dense

and impenetrable polymer network structure is created. In an alkaline media, there is a significant percentage of ALG swelling (pH7.4). Since this pH is higher than the polymer's pKa value, most of ALG's carboxylate groups get ionised, increasing the repulsive forces between the ions. Additionally, an ion-exchange mechanism between the multivalent ions in the "egg-box" cavity of the poly guluronate blocks of ALG and the Na⁺ ions of the simulated intestinal fluid occurs, increasing the repulsions among negatively charged carboxyl groups. Both of these effects improve polymer hydrophilicity, which promotes chain relaxation and increases swelling. They also increase anionic density. The water molecule is encouraged to enter the network structure by this relaxation. These findings demonstrated that ALG swells less in the stomach (pH1.2) and substantially when it enters the upper intestine (pH7.4) (Yan et al. 2021; Fernando et al. 2022).

3.5 Mucoadhesivity

Mucoadhesion is a substance's capability to bind to the mucus layer by making use of various forces including hydrogen bonds, hydrophobic interactions, electrostatic interactions and popular ligand-receptor interactions. The adhesion characteristics of a polymer matrix thus rely on the polar chemical groups present in its structure and are allowing contact with the surface of the mucus. It is significant to mention that the mucus layer contains about 95% water by mass and is composed of lipids, glycoproteins and inorganic ions. The carboxyl and hydroxyl groups of ALG can make non covalent interactions with the mucin which is responsible for ALG's mucoadhesive property (Saqib Hasnain et al. 2018). In physiological conditions, the repulsive electrostatic forces between ALG and mucin exist because of major negative charges as the effect of sialic acid and sulphate residues on the mucous networks and anionic carboxylic groups present in ALG. Such facts indicate that hydrogen bonding is the predominant association between the mucin and ALG during the adhesion process. The hydrophilic group present in ALG allows multipoint associations for the intra- and inter-molecular hydrogen bond, may lead to an enhanced adhesion. This helps to prolong the contact and residence time of delivery system at the mucous membrane and can be used to deliver the drugs for prolonged period, thereby increasing the absorption of drugs through the mucosa. This mucoadhesive nature of ALG makes it versatile for colon specific drug delivery applications (Bernkop-Schnürch et al. 2001; Xing et al. 2003; Wittaya-Areekul et al. 2006; Davidovich-Pinhas et al. 2009; Kesavan et al. 2010; Davidovich-Pinhas and Bianco-peled 2011; Jelvehgari et al. 2014; Thomas et al. 2018; Gorshkova et al. 2019; Dhamecha et al. 2019).

3.6 *Biocompatibility*

Biocompatibility of ALG is another factor which is important in biomedical applications. Numerous studies are reported based on the investigation of its biocompatibility. The various contaminants exist in raw ALG that can cause inflammatory reaction, but the multi - stage extraction process helps to eliminate endotoxins heavy metals and polyphenolic compounds and to ensure a better grade ALG for clinical uses (Spasojevic et al. 2014). ALG purification dramatically reduced polyphenols, endotoxins, and protein levels. Furthermore, it is found that when ALG are implanted in mice, the residual contaminants do not cause an antibody response (Orive et al. 2002). ALG purification processes results in the significant reduction of contaminants such as proteins, polyphenols and endotoxins (Dusseault et al. 2006). Removal of residual protein contamination may help to decrease the immunogenicity of certain ALG preparations. The measurement of proteins could be used as a screening method for evaluating ALG formulations. Gels formed from ALG were considered to be non-toxic to cells and its administration via various routes is considered safe for in vivo applications (Becker et al. 2001; Kim et al. 2020).

4 Alginate Nanoparticles Preparation Methods

4.1 *Spray Drying Technique*

Spray drying is an important method for turning nanoparticle solution into dry powder, which is significant in the pharmaceutical sector, particularly for storage and transportation. This method resulted in spherical nanoparticles with high drug entrapment efficiency. It is reliable, can be done with appropriate production procedures and can easily be scaled up. It is used as a simple one-step method for making nanodimension drug carriers based on ALG (Mishra et al. 2021).

4.2 *Emulsification/Gelation Technique*

Emulsification/gelation is the process of forming nanoparticles from emulsion droplets containing an ALG solution spread in an oil phase. This is a low-cost and easy procedure. It consists of the fabrication of an alginate-in-oil emulsion followed by the gelation of the alginate emulsion droplets using ionic or covalent cross linker. External and Internal gelation are the two most common methods for alginate gelation. Cross linkers like CaCl_2 , which react as well with carboxyl group of -L-guluronic acid, migrate into the internal structure of ALG emulsion droplets from the outer phase during external gelation. Alginate emulsion droplets' inner cores must

release cations for internal gelation to occur, whereas external gelation creates nanospheres with a hard outside matrix and a soft interior (Choukaife et al. 2020).

4.3 Emulsification-Solvent Displacement Technique

This comprises the rapid displacement of solvents with quick evaporation by vacuum and/or heating followed by the emulsification of an organic polymer and drug solution in an aqueous medium containing a stabilizer using standard stirrers. It involves both the diffusion of the organic solvent into an aqueous medium with or without the presence of a surfactant, as well as the precipitation of polymer from an organic solution. This approach relies on the polymer and drug aggregation by fast solvent diffusion from the internal to the exterior phase (Mendoza-Muñoz et al. 2016).

4.4 Polyelectrolyte Complexation Technique

Polyelectrolytes are macromolecular compounds with a large number of ionizable functional groups with different molecular weights and chemical compositions. These are completely or partially dissociated in aqueous solutions to create charge on macromolecules. They are made by combining multiple charged polyelectrolyte solutions simultaneously. Intramolecular and/or intermolecular hydrophobic interactions can cause amphiphilic polysaccharides to bind together in aqueous solutions to form polyelectrolyte complex nanoparticles. If a positive-charged electrolyte is present in a polyelectrolyte solution, it may also be accompanied by negatively charged small ions, which means that charges on repetitive polyelectrolyte units are neutralized by smaller counter ions, which are appropriate for maintaining electro neutrality (Lankalapalli and Kolapalli 2009).

4.5 Self-assembling Technique

Self-assembly is a typical approach for nanoparticle preparation. It's an aqueous method that doesn't have any unfavorable reaction consequences. It entails the spontaneous development of well-organized and orderly structures. It employs amphiphilic polymers, which are made by covalently bonding hydrophobic molecules to the hydrophilic backbone. Amphiphilic polymers have the ability to self-assemble nanostructures with distinct rheological properties, such as particles, hydrogels, and micelles, when hydrophobic segments are added to the hydrophilic macromolecular backbone (Myrick et al. 2014).

5 Alginate Nanoparticles in Drug Delivery

ALG nanoparticles have been used in a variety of ways, including pulmonary, oral, nasal, intravenous, vaginal, and ophthalmic administration. These particles have also been used to deliver a variety of drugs.

5.1 Alginate Nanoparticles for Cancer Drug Delivery

As carriers for oral drug administration, ALG-Chitosan nanoparticles offer a wide range of applications. For ulcerative colitis inflammation-targeted therapy, Oshi et al. produced oral core-shell nanoparticles with curcumin nanocrystals in the core and chitosan/alginate multilayers in the shell. In a dextran sodium sulphate (DSS)-induced colitis mouse model, this formulation successfully reduced inflammation-related symptoms. With this oral drug delivery approach, they hoped to precisely deliver curcumin nanocrystals to the colon while shielding the medicines from the gastrointestinal tract, resulting in a significant therapeutic advantage. By interacting with negatively charged mucins in the mucosa, pH-induced surface charge inversion of ALG-Chitosan nanoparticles improves the attachment and accumulation of curcumin nanocrystals in inflamed colonic tissue during ulcerative colitis treatment (Oshi et al. 2020). Sorasitthyanukarn et al. utilized chitosan-alginate-based nanocarriers for the encapsulation of curcumin diethyl diglutarate and curcumin diglutaric acid. They discovered that encapsulating medicines in polysaccharide particles enhanced bio availability and in vitro digestibility under simulated gastrointestinal conditions, provided regulated drug release, improved cancer cell cellular absorption, and enhanced drug anticancer activity (Sorasitthyanukarn et al. 2018).

Anirudhan et al. designed a folic acid-polyethylene glycol-graft-polyethylene imine-curcumin-loaded ALG nanoparticle and used for the targeted oral delivery of curcumin. Folic acid served as a targeting ligand, allowing cancer cell lines with overexpressed folate receptors to be targeted specifically. The cytocompatibility of polyethyleneimine was improved by combining polyethylene glycol and folic acid, and the stability of alginate nanoparticles was improved by combining folic acid-polyethylene glycol-graft-polyethyleneimine. The carrier's targeting effect was also improved by the folic acid functionalization. The selective destruction of tumor cell lines is confirmed by in vitro cytotoxicity tests and fluorescence imaging of HeLa and H9c2 cell lines (Anirudhan et al. 2017). Curcumin-loaded casein nanoparticles coated with ALG and chitosan as a double polysaccharide layer were generated in another investigation for curcumin oral administration. The nanoformulation had a higher curcumin encapsulation efficiency and regulated release in gastric and intestinal environments, as well as improved in vivo pharmacokinetic parameters than free curcumin. The in vivo results, which included tumor inhibitory rate, genotoxicity, and tumor histopathology, showed that multiple oral administration

of nanoformulation in mice had a higher therapeutic efficacy against Ehrlich carcinoma than Curfree treatment and was capable of achieving a high therapeutic index in cancer treatment (Elbially and Mohamed 2022). Another study developed -cyclo-dextrin/alginate nanoparticles for the oral delivery of 5-fluorouracil, one of the most commonly used cytotoxic chemotherapy medicines in cancer treatment. The developed nanocomposites were stable and had a high loading and encapsulation efficiency. According to the cytotoxicity experiment, the nanocomposite is an efficient delivery vehicle for 5-FU, with high antiproliferative activity against MCF-7 cells and negligible impacts on normal healthy cells (Nguyen et al. 2022). Rosch et al. created ALG/chitosan nanoparticles loaded with doxorubicin. The 4T1 murine breast cancer cells quickly absorbed the nanoparticles, leading in a higher concentration sufficient to exert a therapeutic impact in vitro (Rosch et al. 2019). Baghbani et al. used a nanoemulsion technique to make doxorubicin/curcumin-loaded ALG-shelled ultrasound-responsive phase-shift perfluorocarbon nanodroplets. The perfluorocarbon nanodroplets that can deliver drugs locally in the target tissue under the action of ultrasound and ALG coating stabilize them. The effective drug release mechanism was found to be substantially associated with the sonication frequency, with low-frequency sonication resulting in increased acoustic cavitation and, as a result, stronger ultrasound-induced drug release. In vitro results proved that sonication at a frequency of 28 kHz greatly improved the cytotoxicity of nanodroplets on A2780 human ovarian cancer cells. The use of nanodroplets in combination with ultrasonic irradiation to treat ovarian cancer in vivo resulted in tumor regression (Baghbani and Moztarzadeh 2017).

Manatunga et al. created iron oxide nanoparticles with hydroxyapatite bicoating and curcumin and 6-gingerol incorporated pH-sensitive sodium ALG for anticancer therapy (Manatunga et al. 2017). Zhang et al. examined doxorubicin-loaded ALG nanoparticles modified with glyceric acid. Glyceric acid was used as an active targeting agent with a high affinity for hepatocyte membrane receptors. The produced nanoparticles have the potential to be useful in treating of liver cancer since they can be specifically deposited in the liver. Anticancer drug regimens, which are known for their severe toxicity, require increased selectivity and concentrated therapy. In a mouse model, the investigated formulation was contrasted with a standard doxorubicin solution and ALG nanoparticles devoid of glyceric acid. It was discovered that the tested formulation significantly increased the amount of medicine that reached the liver. Additionally, histological examinations indicated tumor necrosis, a frequent doxorubicin side effect, absent any signs of cardiotoxicity (Jadach et al. 2022). For doxorubicin encapsulation, Sahatsapan et al. used maleimide-bearing chitosan and catechol-bearing ALG to generate nanoparticles with dual mucoadhesive moieties. They found that the developed nanoparticles had substantial inhibitory action against the mouse bladder carcinoma cell line MB49 with remarkable sustained doxorubicin release and improved cell uptake, showing that they could be useful as bladder cancer delivery vehicles (Sahatsapan et al. 2021).

By layering sodium ALG as a polyanion and chitosan as a polycation on top of each other, Jardim et al. developed magneto-responsive MnFe_2O_4 nanoparticles.

Curcumin-loaded platforms with uniformly implanted MnFe_2O_4 nanoparticles were produced. Encapsulated curcumin could be delivered remotely and its release could be directed to specific regions using an oscillating magnetic field, according to in vitro cytotoxicity testing on human breast cancer cells. The layer-by-layer deposition technique was used in this study because it makes suitable for the generation of stimuli-responsive ALG and chitosan. Drugs can also be loaded across layers to deliver a wide range drug release profiles, with the drugs being released by magnetic stimuli (Jardim et al. 2018).

The oral chemotherapeutic drug exemestane was used to treat breast cancer. It was discovered that encapsulating exemestane in ALG nanoparticles reduces negative effects and controls exemestane release (Jayapal and Dhanaraj 2017). Saralkar et al. used an emulsification and cross-linking procedure to make curcumin and resveratrol-loaded ALG nanoparticles. It was shown that the ALG nanoformulation is secure for intravenous use and to have cytotoxic effects on DU145 cells (Saralkar and Dash 2017). Sorasithiyanukarn et al. used an emulsion/reticulation approach to create curcumin diglutaric acid loaded ALG-chitosan nanoparticles and shown their potential for efficient targeting. When compared to free drug, the drug-loaded nanoparticles showed good stability in UV and GI conditions, as well as better cellular absorption. It has the ability to break through bio-barriers and physically destroy tumor tissues (Sorasithiyanukarn et al. 2018). Ciofani et al. used an emulsion/reticulation process to create ALG magnetic nanoparticles and shown their promise as a drug delivery system with efficient targeting. The nanoformulation is capable of physically disrupting tumor tissues and overcoming bio-barriers (Ciofani et al. 2009). Mirrahiimi et al. used an ALG hydrogel that was co-loaded with cisplatin and gold nanoparticles to create a multi nanocomplex. The nanocomplex dramatically improved drug transport efficiency and suppressed tumor development more effectively than free cisplatin. The researchers discovered that incorporating gold nanoparticles and cisplatin into an ALG hydrogel network is a fantastic way to combine chemotherapy, radiation, and photothermal therapy for a locally synergistic cancer treatment (Mirrahiimi et al. 2020).

5.2 Alginate Nanoparticles for Antibiotics Delivery

Infections caused by *Staphylococcus aureus* in the skin and soft tissues are a major public health hazard that has yet to be addressed. The key clinical problem is medication penetration into the infected site. When a pathogen infects a wound on the skin, it weakens biological response pathways, resulting in a chronic infection. Antibiotics loaded with alginate nanoparticles have been routinely used to treat infections caused by *Staphylococcus aureus*. George et al. developed an injectable nanoparticulate system based on ALG and polyvinyl alcohol as a vancomycin carrier. Ex-vivo permeation experiments and imaging demonstrated that when nanoformulation is administered, it penetrates deep into the infected crevices, fully killing *Staphylococcus aureus* and delivering continuous drug release via diffusion

with the highest biocompatibility to L929 cells (George et al. 2017). Costa et al. developed mucoadhesive chitosan-coated ALG nanoparticles as a delivery vehicle for daptomycin penetration through the ocular epithelia, with the potential to heal bacterial endophthalmitis. The study demonstrated that encapsulating daptomycin into nanoparticles had no effect on its antibacterial activity against major pathogens that cause bacterial endophthalmitis. The encapsulation enhances epithelial retention of the drug (Costa et al. 2015). The bactericidal action of rifampicin-ascorbic acid co-loaded ALG-chitosan nanoparticles against *Staphylococcus aureus* strains was significant, than those seen with the free antibiotic rifampicin. This formulation could be an effective technique for administering antibiotics to the lungs, with great prospects for treating pulmonary intracellular infections with known drugs that are losing potency due to antimicrobial resistance issues (Scolari et al. 2020).

ALG nanoparticles are also used as carrier for antibiotics against *Pseudomonas aeruginosa*. Inhaled antibiotics for *Pseudomonas aeruginosa* pulmonary infections, such as tobramycin, have been linked to an increase in life expectancy in cystic fibrosis patients in recent years. However, the capacity of this aminoglycoside to penetrate the thick DNA-rich mucus in these patients' lungs limits its efficiency, resulting in poor antibiotic exposure to resident bacteria. Deacon et al. presented ALG-chitosan polymeric nanoparticle delivery devices to address these difficulties. This study shows how to make efficient antibacterial NPs that could be used as mucus-penetrating tobramycin delivery vehicles in the clinic. This nano-antibiotic is a technique for breaking through the mucus barrier, increasing local medication concentrations, avoiding systemic side effects, and improving pulmonary infection outcomes in cystic fibrosis patients (Deacon et al. 2015).

Doxycycline-loaded chitosan-ALG nanoparticles are more effective against gram-negative and positive bacteria than native doxycycline. The antibacterial activity and drug delivery efficiency of the drug-loaded nanoparticles were improved (Kadhum and Zaidan 2020). Polymyxins are polypeptide antibiotics that have a broad spectrum of antibacterial activity against gram-negative bacteria. Because less hazardous medications were created between 1970 and 1980, they were virtually entirely phased out during that time. Interest in polymyxins has recently returned owing to the increase of multiresistant gram-negative bacteria, notably in ICU patients, and the absence of new antimicrobials to treat these infections. Initial solid lipid nanoparticle syntheses of polymyxin B sulphate (PLX) were cross-linked with sodium ALG. By mixing alginate ALG with PLX in a 1:1 ratio, the difficulty of loading the hydrophilic drug in lipid matrices was overcome. The gel-like dynamic behavior of the formulation was demonstrated to be non-toxic in HaCat and NIH/3T3 cell lines and the polymer-drug combination improved the minimum inhibitory concentrations in *Pseudomonas aeruginosa* strains (Severino et al. 2015).

Kumar et al. efficaciously incorporated rifaximin into ALG-chitosan nanoparticles, resulting in nanoparticulate antibacterial substance with exceptional antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus haynesii* (Kumar et al. 2021). Furthermore, when loading camptothecin into calcium alginate-chitosan nanocomposites, Al-Getahami and Al-Qasbi obtained significant results. The authors discovered increased inhibitory action against

Gram-negative bacteria, which can be accounted by the generation of genetic effects. The nanopatform leads to somatic mutations in treated bacteria, affecting metabolic pathways that produce DNA and proteins (Al-Gethami and Al-Qasmi 2021).

5.3 *Alginate Nanoparticles for Protein Delivery*

ALG can crosslink with proteins, decreasing diffusion or inactivating proteins, owing to its positively charged nature. The intranasal delivery of venlafaxine-loaded ALG nanoparticles for the treatment of depression demonstrated the ability of ALG nanoparticles for brain medication delivery. Behavioral tests on albino Wistar rats found that intranasal ALG nanoparticles enhanced behavioral characteristics such as swimming, climbing, and immobility when compared to venlafaxine pills given orally. Furthermore, as compared to intramuscular or oral venlafaxine dosage formulations, intranasal ALG nanoparticles boosted locomotor activity. Following the therapy, a confocal laser scanning fluorescence microscopy evaluation of isolated rat organs revealed that ALG nanoparticles were superior for direct intranasal delivery to the brain (Haque et al. 2014).

Due to its limited bioavailability and enzymatic breakdown, the most common route of insulin therapy is subcutaneous injection. Chai et al. designed ALG nanoparticles with glucose oxidase immobilized for improved glucose-triggered insulin administration. The subcutaneous injection of insulin/glucose oxidase-loaded nanoparticles had a considerable hypoglycemic impact in diabetic mice. Cytotoxicity assay, hemolysis investigation, and histopathology examination revealed that the nanoparticles were biocompatible (Chai et al. 2020).

Oral insulin administration has been demonstrated to be a better option than subcutaneous injection in terms of reducing pain and increasing patient compliance. Li et al. studied the encapsulation of insulin in octaarginine-modified ALG nanoparticles for oral administration. The nanoformulation displayed a profound stability in the simulated gastrointestinal fluids and had a controlled insulin release (Li et al. 2021). In another study, quaternized chitosan/ALG nanoparticles were used for the oral delivery of protein (Li et al. 2007). Mukhopadhyay et al. used alginate as the core and chitosan as the coating to create core-shell nanoparticles with an average size of 100–200 nm and an insulin encapsulation efficiency of 85%. In an in vitro release assay, the nanoparticles kept roughly 80% of the encapsulated insulin in SGF and released it slowly in SIF. The developed nanoparticles were shown to be safe after being given an oral dose of 150 mg/kg body weight per day (Mukhopadhyay et al. 2015). For oral insulin delivery, Zhang et al. developed chitosan-deoxycholic acid/ALG nanoparticles. The nanoparticles had pH-triggered release properties, could partially shield wrapped insulin from enzymatic degradation, and could increase Caco-2 cell permeability and FITC-labeled insulin intracellular absorption. The hypoglycemia test revealed that the produced nanoparticles extended insulin release and had a significant hypoglycemic effect on diabetic rats, with 15% oral bioavailability (Zhang et al. 2021).

Amani et al. used ALG based self-assembled polyelectrolyte complex nanoparticles for BSA delivery (Amani et al. 2019). To improve efficacy, Li et al. developed a pH-responsive nano-sized complex based on ALG and chitosan as an oral carrier for bursting release of BSA at the target site. The complex's greatest effective dose indicates that it has the potential to be used to give protein-based medicines orally (Li et al. 2022). Thomas et al. used starch-modified ALG nanoparticles for BSA delivery (Thomas et al. 2021).

5.4 Alginate Nanoparticles for Vaccine Delivery

Pathogen transmission is a major concern to human health around the world. Vaccines are critical in the event of a pandemic breakout. It is the most efficacious, cost-effective, and long-term technique for preventing and eliminating infectious diseases, as well as preventing their mortality. Although new vaccines have been created in recent years with improved stability and longer duration, as well as fewer hazardous side effects, they still have limitations such as poor immunogenicity and expression levels. The use of an effective vaccine delivery technology opens up new possibilities for increasing vaccination rates. Carrier molecules or adjuvants are suitable for optimum immune response generation. Adjuvants boost the impact of the vaccine and reduce the amount of antigen and required vaccination frequency for protective immunity by enhancing the immunogenicity of weaker immunogens. Natural polymers, such as alginate, have a lot of potential as adjuvants or delivery routes for vaccine formulation and device design. It can limit antigen degradation, improve antigen stability, and produce delayed release, all of which improve immunogenicity. Nanotechnologies have the potential to speed up the development of vaccinations for newly developing infectious viruses. Delivery systems based on ALG nanoparticles have been investigated as promising nanoplatforams for delivering antigens to immune cells. ALG nanoparticles have adjuvant properties due to their hydrophilic carrier nature, which slows antigen release and improves immunogenicity over conventional vaccine carriers/adjuvants.

In a study by Yu et al. ALG-chitosan coated layered double hydroxide nanoparticles produced and utilized oral protein carrier for vaccine delivery. Protein release properties of layered double hydroxide nanoparticles with and without polymer coating revealed that ALG-chitosan protects protein release under acidic conditions. According to the cellular absorption efficiency of protein given by ALG-chitosan -layered double hydroxide nanoparticles for intestinal cells and macrophages, the polymer coating considerably increased protein internalization at CaCO_2 and macrophage cells (Yu et al. 2019).

Diphtheria is a bacterial disease caused by *Corynebacterium diphtheria*, which is infectious and severe. The release of strong diphtheria toxin from lysogenized strains bacteria causes the majority of the disease's clinical symptoms. Diphtheria vaccines are currently made with diphtheria toxoid, a harmless but antigenic toxoid that is usually mixed with alum or $\text{Al}(\text{OH})_3$ as an adjuvant. Alum has been linked to some

side effects, including muscle discomfort and the occurrence of a fever. Alum can also cause granulomas at the injection site, which is a concern for vaccines that require regular boosters. When alum-based vaccinations are given subcutaneously or intradermal, the risk of granulomas increases dramatically (Eldridge et al. 1991). Using an ionic gelation process, Sarei et al. produce a vaccine formulation based on ALG nanoparticles encapsulating diphtheria toxoid. The nanoparticles had great loading capacities during the loading trials, and following release investigations revealed a longer profile. The antigenic integrity and activity of toxoid were tested, and it was shown that the encapsulating procedure had no effect on them. Guinea pigs inoculated with diphtheria toxoid-loaded ALG nanoparticles had the strongest humoral immune response compared as those who received a standard vaccine. The diphtheria toxoid released from the nanoparticles was found to be effective in active state, and the encapsulation had no negative impact on the toxoid's integrity or activity (Sarei et al. 2013).

Biswas et al. designed ALG coated chitosan nanoparticles as carriers for oral vaccination administration. Measles antigen was encapsulated in ALG-coated chitosan nanoparticles in this work. The findings demonstrated that an alginate coating may effectively protect antigen for at least 2 h in an acidic environment. The immunological responses of mice were assessed after the formulations were given orally to them, and the results showed that measles antigen-loaded nanoparticles elicited a significant immune response. The created system appeared to be a viable platform for oral vaccine delivery due to the antigen's capacity to defend itself in the gastric environment, sustained release rates, mucosal immune responses, and lower toxicity of the ALG coated nanoparticles (Biswas et al. 2015).

ALG-coated chitosan nanoparticles are an appropriate system for designing a mucoadhesive vaccine delivery strategy due to their high mucoadhesive capabilities. Amin et al. created ALG-modified chitosan nanoparticles for delivery of vaccines to the oral mucosa. In vitro studies revealed that ALG-modified nanoparticles increased mucin binding significantly. In simulated gastric and intestinal fluids, surface modification of chitosan nanoparticles by ALG enhanced particle stability and mucoadhesive properties, indicating that it might be employed as a nanocarrier platform for mucosal protein vaccine administration (Amin and Boateng 2022).

The use of the nasal route for vaccine delivery has drawn the interest of numerous pharmaceutical companies in recent decades due to the possibility of receiving both local and systemic immune responses (Illum 2007). Due to the fact that the nasal mucosa is frequently the very first site for inhaled antigen contact, intranasal vaccination has become the most efficient way to trigger mucosal immune responses in the respiratory tract, where main bacterial and viral infections are frequent. Many studies in animal models have indicated that nanoscale carriers can improve nasal vaccination administration when compared to traditional medication solution formulations (Fukuyama et al. 2015). ALG or ALG mannose-coated chitosan nanogels carrying recombinant NcPDI antigen were used to immunize mice against infection with Neosporacanimtachyzoites, and % of the challenged animals were protected. These nanosized carriers are suitable for the uptake of extracellular chemicals by cells that phagocytose them. An examination of humoral and cytokine immune

responses pre- and post-challenge suggested that the interaction of this antigen with nanogel may influence both the antibody isotype response and the cytokine pattern in challenged animals. In terms of lowering parasite load in the brain, these nanogels had the most noticeable effect (Debache et al. 2011). Another study used humoral mucosal immune responses to demonstrate the adjuvant impact of ALG coated-chitosan nanoparticles coupled with recombinant hepatitis B surface antigen. This delivery method was able to efficiently encapsulate hepatitis B antigen and was proven to be ingested by NALT cells. The findings imply that ALG coating can be employed to alter antigen release from chitosan nanoparticles, as well as to shield antigen from enzymatic destruction as it passes through the mucosal surface of the nasal mucosa (Borges et al. 2008).

Another study looked into the feasibility of ALG nanoparticles like recombinant LTB from an Enterotoxigenic *Escherichia coli* (ETEC) carrier. The ion cross-linking approach was used to efficiently entrap the LTB protein in ALG polymers and produce LTB-containing ALG nanoparticles. The sera of inoculated mice could suppress the LT attachment to the GM1 ganglioside by up to 70% in an in vitro toxin neutralization experiment when they were immunized using nanoparticle formulation. The sera of immunized mice were also able to neutralize the toxin in an in vivo toxin neutralization assay, and fluid accumulation in the gut of rabbits was dramatically reduced when compared to the placebo group (Kordbacheh et al. 2018).

Yuan et al. used an ionotropic complexation approach to make self-assembled ϵ -polylysine-sodium ALG nanoparticles as vaccine carriers with a mean particle diameter of 133.2 ± 0.5 nm and a negatively charged surface. In vitro tests shown that the nanosuspension prevented burst release of the loaded model antigen, bovine serum albumin, and exhibited sustained-release behavior. The bio-assessment employing macrophage cells revealed no cytotoxicity, and considerably greater absorption was detected when compared to the free bovine serum albumin solution. ϵ -polylysine - sodium ALG nanoparticles were discovered to be a promising choice for vaccine administration (Yuan et al. 2018). *Vibrio cholerae* lipopolysaccharide (LPS) is important in generating primary protection and immunological responses. The activation of inflammatory cytokines has reduced the transport of LPS. Bakshi et al. described the manufacture and effectiveness of an ALG-chitosan-LPS-selenium nanoparticle composite in regulating immunological responses and shielding LPS from acidic stomach media. An ionic cross-linking/in situ reduction approach was used to make the ALG-chitosan-LPS-selenium the ALG-chitosan complex resulted in high entrapment efficiency and effectively protected LPS from the acidic GIT medium. The ALG-chitosan nanoparticles composite. The encapsulation of LPS-loaded selenium nanoparticles in -LPS-selenium nanoparticles composite was found to dramatically boost anti-inflammatory cytokines while reducing the production of pro-inflammatory cytokines (Bagheri-Josheghani and Bakhshi 2022).

5.5 Alginate Nanoparticles for Other Drugs

The nanospray drying process was used to create composite alginate-gelatine NPs loaded with metformin hydrochloride for diabetic patients. In vitro, the produced NPs successfully demonstrated a sustained drug release profile, while in vivo rat model studies revealed a substantial reduction in blood glucose levels over 24 h (Shehata and Ibrahima 2019). Due to their beneficial qualities, including as enhanced bioadhesion, biocompatibility, ability to prolong drug's residency period, permeability-enhancing capabilities and capacity for sustained drug release, ALG is a promising substrate for ocular drug delivery vehicles. Kianersi et al. utilized ALG nanoparticles for the ocular delivery of betamethasone sodium phosphate. The study indicated that using an ALG-based nanoparticle as an ocular drug carrier reduces medication administration frequency by one-third and reduces the requirement for duplicate therapy by less than the commercial drop, paving the way for improving the quality of life of patients with eye illnesses (Kianersi et al. 2021).

6 Limitations for Use of Alginate Nanoparticles in Pharmaceutical Applications

Despite the fact that ALG has been broadly explored as a nanocarrier, its application is inadequate due to batch-to-batch inconsistency and wide molecular weight ranges, making it less apparent in comparison to synthetic polymers with higher consistency and adaptability (Ige et al. 2012). ALG's hydrophilicity also resulted in nanoparticle instability at biological pH, as well as inadequate drug encapsulation due to drug leakage. The hydrophilicity of ALG leads to swelling and faster release than synthetic polymers in many situations due to uncontrolled leaching of drug from ALG nanoparticles to the surrounding fluid during preparation (Kamaly et al. 2016).

Several ways have been used to address these restrictions. The most successful method was to chemically modify ALG to increase its hydrophobicity, which resulted in increased encapsulation efficiency of these nanoparticles (Hasnain and Nayak 2019). Despite concerns about safety, chemical cross-linking may improve the mechanical strength of ALG nanoparticles. Chemical conjugation of ALG with cross-linking chemicals is one method of covalent cross-linking (Nesamony et al. 2012).

7 Conclusions and Future Research Perceptive

Nanotechnology is well renowned for revolutionizing drug delivery techniques. Advances in nanotechnology have resulted in a plethora of nanomedicines in the market with desirable properties such as reduced toxicity, enhanced patient

compliance, and improved clinical outcomes. The combined applications of nanoparticles in drug delivery systems in preformulation work speeds up the development of novel therapeutic moieties and reduces attrition of new molecular entities due to undesirable biopharmaceutical and pharmacokinetic features. In order to improve bioavailability and stability of bioactives, nanodelivery devices have a number of physical and chemical advantages. The qualities of the bioactive as well as the objective of administration determine which type of nanoparticle should be used. The chemical, physical, and morphological features of the system will regulate the nano-bio interaction and ADME profile. It is feasible to adjust the location of bioactive release by using nanomaterials with unique surface chemistry that facilitates their release at the target.

ALG is a biocompatible natural polymer that is commonly used in a variety of commercial and scientific applications. It has been utilized as an attractive excipient in many different drug delivery systems due to its versatility, nontoxicity, and low cost. The most significant characteristics of ALG include its ability to form gels, mucoadhesive capacity, and ability to interact with di- and trivalent cations found in physiological fluids. This feature is noteworthy because it could be used in the development of *in situ* gelling drug delivery systems for specific drug release control, which is particularly beneficial in innovative drug development. It can be chemically altered to dramatically change its initial properties and tailor them to the desired shape. Its properties as a pharmaceutical excipient can also be altered by combining it with other natural or synthetic components.

In targeted therapy, a popular strategy in emerging anticancer therapies, chemical alterations can potentially result in novel drug carriers. ALG is perfect for transporting stem cells and creating injection implants for bone and cartilage regeneration because it is non-toxic and has great biocompatibility. ALG hydrogels can also be utilized as wound dressings since they help facilitate the healing of ulcers and damaged skin. Despite the fact that ALG seems to be an established polymer of naturally derived, there are many potential uses for this substance, and recent years have seen a growth in the study fields that involve it. The utilization of ALG nanoparticles as nonviral gene delivery vehicles promises to be a crucial area in future research among all potential breakthroughs in polymeric nanocarriers.

As a result, it is widely believed that the application of nanotechnology to medication delivery would alter the pharmaceutical and biotechnology sectors in the near future. Many areas of research, including target-specific medication therapy and approaches for early identification of pathologies, can benefit from nanotechnology. A careful examination of the present state of knowledge on ALG nanoparticles reveals that natural polymer studies continue to attract a lot of research attention.

Designing and manufacturing nanoparticulate delivery systems at an industrial scale with nominal control over surface characteristics, particle size, and the release of active therapeutic agents is still difficult and little understood. In order to achieve population scale, more effort is needed to reduce nanoparticle fabrication costs. In summary, despite enormous progress, as evidenced by investigators' tireless efforts, there are still a number of difficulties that must be addressed.

References

- Al-Gethami W, Al-Qasbi N (2021) Antimicrobial activity of Ca-Alginate/chitosan nanocomposite loaded with camptothecin. *Polymers (Basel)* 13(20):3559. <https://doi.org/10.3390/polym13203559>
- Amani S, Mohamadnia Z, Ahmadi E et al (2019) Self-assembled polyelectrolyte complex nanoparticles as a potential carrier in protein delivery systems. *J Drug Deliv Sci Technol* 54: 101250. <https://doi.org/10.1016/j.jddst.2019.101250>
- Amin MK, Boateng JS (2022) Enhancing stability and mucoadhesive properties of chitosan nanoparticles by surface modification with sodium alginate and polyethylene glycol for potential oral mucosa vaccine delivery. *Mar Drugs* 20:156. <https://doi.org/10.3390/md20030156>
- Anirudhan TS, Anila MM, Franklin S (2017) Synthesis characterization and biological evaluation of alginate nanoparticle for the targeted delivery of curcumin. *Mater Sci Eng C* 78:1125–1134. <https://doi.org/10.1016/j.msec.2017.04.116>
- Baghban F, Moztarzadeh F (2017) Bypassing multidrug resistant ovarian cancer using ultrasound responsive doxorubicin/curcumin co-deliver alginate nanodroplets. *Colloids Surf B Biointerfaces* 153:132–140. <https://doi.org/10.1016/j.colsurfb.2017.01.051>
- Bagheri-Josheghani S, Bakhshi B (2022) Formulation of selenium nanoparticles encapsulated by alginate-chitosan for controlled delivery of *Vibrio Cholerae* LPS: a novel delivery system candidate for nanovaccine. *Int J Biol Macromol* 208:494–508. <https://doi.org/10.1016/j.ijbiomac.2022.03.087>
- Becker TA, Kipke DR, Brandon T (2001) Calcium alginate gel: a biocompatible and mechanically stable polymer for endovascular embolization. *J Biomed Mater Res* 54:76–86. [https://doi.org/10.1002/1097-4636\(200101\)54:1<76::AID-JBM9>3.0.CO;2-V](https://doi.org/10.1002/1097-4636(200101)54:1<76::AID-JBM9>3.0.CO;2-V)
- Bernkop-Schnürch A, Kast CE, Richter MF (2001) Improvement in the mucoadhesive properties of alginate by the covalent attachment of cysteine. *J Control Release* 71:277–285. [https://doi.org/10.1016/S0168-3659\(01\)00227-9](https://doi.org/10.1016/S0168-3659(01)00227-9)
- Biswas S, Chattopadhyay M, Sen KK, Saha MK (2015) Development and characterization of alginate coated low molecular weight chitosan nanoparticles as new carriers for oral vaccine delivery in mice. *Carbohydr Polym* 121:403–410. <https://doi.org/10.1016/j.carbpol.2014.12.044>
- Borges O, Cordeiro-da-Silva A, Tavares J et al (2008) Immune response by nasal delivery of hepatitis B surface antigen and codelivery of a CpG ODN in alginate coated chitosan nanoparticles. *Eur J Pharm Biopharm* 69:405–416. <https://doi.org/10.1016/j.ejpb.2008.01.019>
- Chai Z, Dong H, Sun X et al (2020) Development of glucose oxidase-immobilized alginate nanoparticles for enhanced glucose-triggered insulin delivery in diabetic mice. *Int J Biol Macromol* 159:640–647. <https://doi.org/10.1016/j.ijbiomac.2020.05.097>
- Chan LW, Jin Y, Heng PWS (2002) Cross-linking mechanisms of calcium and zinc in production of alginate microspheres. *Int J Pharm* 242:255–258. [https://doi.org/10.1016/S0378-5173\(02\)00169-2](https://doi.org/10.1016/S0378-5173(02)00169-2)
- Chaturvedi K, Ganguly K, More UA et al (2019) Sodium alginate in drug delivery and biomedical areas. In: Nayak AK, Hasnain MS (eds) *Natural polysaccharides in drug delivery and biomedical applications*. Elsevier, London, pp 59–100
- Choukaife H, Doolaanea AA, Alfatama M (2020) Alginate nanoformulation: influence of process and selected variables. *Pharmaceuticals* 13:1–35
- Ciofani G, Riggio C, Raffa V et al (2009) A bi-modal approach against cancer: magnetic alginate nanoparticles for combined chemotherapy and hyperthermia. *Med Hypotheses* 73:80–82. <https://doi.org/10.1016/j.mehy.2009.01.031>
- Costa JR, Silva NC, Sarmento B, Pintado M (2015) Potential chitosan-coated alginate nanoparticles for ocular delivery of daptomycin. *Eur J Clin Microbiol Infect Dis* 34:1255–1262. <https://doi.org/10.1007/s10096-015-2344-7>
- Davidovich-Pinhas M, Bianco-peled H (2011) Alginate—PEGAc: a new mucoadhesive polymer. *Acta Biomater* 7:625–633. <https://doi.org/10.1016/j.actbio.2010.09.021>

- Davidovich-Pinhas M, Harari O, Bianco-Peled H (2009) Evaluating the mucoadhesive properties of drug delivery systems based on hydrated thiolated alginate. *J Control Release* 136:38–44. <https://doi.org/10.1016/j.jconrel.2009.01.029>
- Deacon J, Abdelghany SM, Quinn DJ et al (2015) Antimicrobial efficacy of tobramycin polymeric nanoparticles for *Pseudomonas aeruginosa* infections in cystic fibrosis: formulation, characterisation and functionalisation with dornase alfa (DNase). *J Control Release* 198:55–61. <https://doi.org/10.1016/j.jconrel.2014.11.022>
- Debache K, Kropf C, Schütz CA et al (2011) Vaccination of mice with chitosan nanogel-associated recombinant NcPDI against challenge infection with *Neospora caninum* tachyzoites. *Parasite Immunol* 33:81–94. <https://doi.org/10.1111/j.1365-3024.2010.01255.x>
- Dhamecha D, Movsas R, Sano U, Menon JU (2019) Applications of alginate microspheres in therapeutics delivery and cell culture: past, present and future. *Int J Pharm* 569:118627
- Dusseault J, Tam SK, Ménard M et al (2006) Evaluation of alginate purification methods: effect on polyphenol, endotoxin, and protein contamination. *J Biomed Mater Res A* 76:243–251. <https://doi.org/10.1002/jbm.a.30541>
- Elbially NS, Mohamed N (2022) Fabrication of the quaternary nanocomplex curcumin-casein-alginate-chitosan as a potential oral delivery system for cancer nutraceutical therapy. *J Drug Deliv Sci Technol* 70:103226. <https://doi.org/10.1016/j.jddst.2022.103226>
- Eldridge JH, Staas JK, Meulbroek JA et al (1991) Biodegradable and biocompatible poly (DL-lactide-co-glycolide) microspheres as an adjuvant for staphylococcal enterotoxin B toxoid which enhances the level of toxin-neutralizing antibodies. *Infect Immun* 59:2978–2986. <https://doi.org/10.1128/iai.59.9.2978-2986.1991>
- Elnahtawy AI, Elshafei NS, Elzoghby AO (2022) Marine polymer-based nano-carriers for drug delivery applications. In: Jana S, Jana S (eds) *Marine biomaterials*. Springer, Singapore, pp 15–59
- Fernando IPS, Kirindage KGIS, Jeon HN et al (2022) Preparation of microspheres by alginate purified from *Sargassum horneri* and study of pH-responsive behavior and drug release. *Int J Biol Macromol* 202:681–690. <https://doi.org/10.1016/j.ijbiomac.2022.01.171>
- Fukuyama Y, Yuki Y, Katakai Y et al (2015) Nanogel-based pneumococcal surface protein A nasal vaccine induces microRNA-associated Th17 cell responses with neutralizing antibodies against *Streptococcus pneumoniae* in macaques. *Mucosal Immunol* 8:1144–1153. <https://doi.org/10.1038/mi.2015.5>
- George L, Bavya MC, Rohan KV, Srivastava R (2017) A therapeutic polyelectrolyte–vitamin C nanoparticulate system in polyvinyl alcohol–alginate hydrogel: an approach to treat skin and soft tissue infections caused by *Staphylococcus aureus*. *Colloids Surf B Biointerfaces* 160:315–324. <https://doi.org/10.1016/j.colsurfb.2017.09.030>
- Gorshkova MY, Volkova IF, Vanchugova LV et al (2019) Sodium alginate based mucoadhesive hydrogels. *Appl Biochem Microbiol* 55:371–374. <https://doi.org/10.1134/S0003683819040045>
- Haque S, Md S, Sahni JK et al (2014) Development and evaluation of brain targeted intranasal alginate nanoparticles for treatment of depression. *J Psychiatr Res* 48:1–12. <https://doi.org/10.1016/j.jpsychires.2013.10.011>
- Harper BA, Barbut S, Lim LT, Marcone MF (2014) Effect of various gelling cations on the physical properties of “wet” alginate films. *J Food Sci* 79:E562–E567. <https://doi.org/10.1111/1750-3841.12376>
- Hasnain MS, Nayak AK (2019) *Alginates: versatile, versatile polymers in biomedical applications and therapeutics*. Apple Academic Press, Cambridge
- Hay ID, Rehman ZU, Moradali MF et al (2013) Microbial alginate production, modification and its applications. *Microb Biotechnol* 6:637–650
- Ige OO, Umoru LE, Aribo S (2012) Natural products: a minefield of biomaterials. *ISRN Mater Sci* 2012:1–20. <https://doi.org/10.5402/2012/983062>
- Illum L (2007) Nanoparticulate systems for nasal delivery of drugs: a real improvement over simple systems? *J Pharm Sci* 96:473–483

- Jadach B, Świetlik W, Froelich A (2022) Sodium alginate as a pharmaceutical excipient: novel applications of a well-known polymer. *J Pharm Sci* 111(5):1250–1261
- Jardim KV, Palomec-Garfias AF, Andrade BYG et al (2018) Novel magneto-responsive nanoplatforms based on MnFe_2O_4 nanoparticles layer-by-layer functionalized with chitosan and sodium alginate for magnetic controlled release of curcumin. *Mater Sci Eng C* 92:184–195. <https://doi.org/10.1016/j.msec.2018.06.039>
- Jayapal JJ, Dhanaraj S (2017) Exemestane loaded alginate nanoparticles for cancer treatment: formulation and in vitro evaluation. *Int J Biol Macromol* 105:416–421. <https://doi.org/10.1016/j.ijbiomac.2017.07.064>
- Jelvehgari M, Mobaraki V, Montazam SH (2014) Preparation and evaluation of mucoadhesive beads/discs of alginate and algino-pectinate of piroxicam for colon-specific drug delivery via oral route. *Jundishapur J Nat Pharm Prod* 9:e16576
- Kadhun WN, Zaidan IA (2020) The synergistic effects of chitosan-alginate nanoparticles loaded with doxycycline antibiotic against multidrug resistant proteus mirabilis, Escherichia coli and enterococcus faecalis. *Iraqi J Sci* 61:3187–3199. <https://doi.org/10.24996/ijcs.2020.61.12.6>
- Kamaly N, Yameen B, Wu J, Farokhzad OC (2016) Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release. *Chem Rev* 116:2602–2663
- Kesavan K, Nath G, Pandit JK (2010) Sodium alginate based mucoadhesive system for gatifloxacin and its in vitro antibacterial activity. *Sci Pharm* 78:941–957. <https://doi.org/10.3797/scipharm.1004-24>
- Kianersi S, Solouk A, Saber-Samandari S et al (2021) Alginate nanoparticles as ocular drug delivery carriers. *J Drug Deliv Sci Technol* 66:102889. <https://doi.org/10.1016/j.jddst.2021.102889>
- Kim SH, Thambi T, Giang Phan VH, Lee DS (2020) Modularly engineered alginate bioconjugate hydrogel as biocompatible injectable scaffold for in situ biomineralization. *Carbohydr Polym* 233:115832. <https://doi.org/10.1016/j.carbpol.2020.115832>
- Kirti KSS (2022) Alginate: a promising biopolymer in drug delivery system. In: *Marine biomaterials*. Springer, Singapore, pp 61–95
- Kordbacheh E, Nazarian S, Hajizadeh A, Sadeghi D (2018) Entrapment of LTB protein in alginate nanoparticles protects against Enterotoxigenic Escherichia coli. *APMIS* 126:320–328. <https://doi.org/10.1111/apm.12815>
- Kumar D, Kumar S, Kumar S et al (2021) Synthesis of rifaximin loaded chitosan-alginate core-shell nanoparticles (Rif@CS/Alg-NPs) for antibacterial applications. *Int J Biol Macromol* 183:962–971. <https://doi.org/10.1016/j.ijbiomac.2021.05.022>
- Lankalapalli S, Kolapalli VRM (2009) Polyelectrolyte complexes: a review of their applicability in drug delivery technology. *Indian J Pharm Sci* 71:481–487
- Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. *Prog Polym Sci* 37:106–126
- Li T, Shi XW, Du YM, Tang YF (2007) Quaternized chitosan/alginate nanoparticles for protein delivery. *J Biomed Mater Res A* 83:383–390. <https://doi.org/10.1002/jbm.a.31322>
- Li M, Sun Y, Ma C et al (2021) Design and investigation of penetrating mechanism of octaarginine-modified alginate nanoparticles for improving intestinal insulin delivery. *J Pharm Sci* 110:268–279. <https://doi.org/10.1016/j.xphs.2020.07.004>
- Li J, Jin H, Razzak MA et al (2022) Crosslinker-free bovine serum albumin-loaded chitosan/alginate nanocomplex for pH-responsive bursting release of oral-administered protein. *Biotechnol Bioprocess Eng* 27:40–50. <https://doi.org/10.1007/s12257-021-0243-6>
- Liu J, Yang S, Li X et al (2019) Alginate oligosaccharides: production, biological activities, and potential applications. *Compr Rev Food Sci Food Saf* 18:1859–1881
- Manatunga DC, de Silva RM, de Silva KMN et al (2017) pH responsive controlled release of anti-cancer hydrophobic drugs from sodium alginate and hydroxyapatite bi-coated iron oxide nanoparticles. *Eur J Pharm Biopharm* 117:29–38. <https://doi.org/10.1016/j.ejpb.2017.03.014>
- Mendoza-Muñoz N, Alcalá-Alcalá S, Quintanar-Guerrero D (2016) Preparation of polymer nanoparticles by the emulsification-solvent evaporation method: from Vanderhoff's pioneer

- approach to recent adaptations. In: Vauthier C, Ponchel G (eds) *Polymer nanoparticles for nanomedicines*. Springer International Publishing, Cham, pp 87–121
- Mirrahimi M, Beik J, Mirrahimi M et al (2020) Triple combination of heat, drug and radiation using alginate hydrogel co-loaded with gold nanoparticles and cisplatin for locally synergistic cancer therapy. *Int J Biol Macromol* 158:617–626. <https://doi.org/10.1016/j.ijbiomac.2020.04.272>
- Mishra A, Pandey VK, Shankar BS, Melo JS (2021) Spray drying as an efficient route for synthesis of silica nanoparticles-sodium alginate biohybrid drug carrier of doxorubicin. *Colloids Surf B Biointerfaces* 197:111445. <https://doi.org/10.1016/j.colsurfb.2020.111445>
- Mukhopadhyay P, Chakraborty S, Bhattacharya S et al (2015) pH-sensitive chitosan/alginate core-shell nanoparticles for efficient and safe oral insulin delivery. *Int J Biol Macromol* 72:640–648. <https://doi.org/10.1016/j.ijbiomac.2014.08.040>
- Myrick JM, Vendra VK, Krishnan S (2014) Self-assembled polysaccharide nanostructures for controlled-release applications. *Nanotechnol Rev* 3:319–346
- Nesamony J, Singh PR, Nada SE et al (2012) Calcium alginate nanoparticles synthesized through a novel interfacial cross-linking method as a potential protein drug delivery system. *J Pharm Sci* 101:2177–2184. <https://doi.org/10.1002/jps.23104>
- Nguyen CH, Banh KS, Dang CH et al (2022) β -cyclodextrin/alginate nanoparticles encapsulated 5-fluorouracil as an effective and safe anticancer drug delivery system. *Arab J Chem* 15:103814. <https://doi.org/10.1016/j.arabjc.2022.103814>
- Orive G, Ponce S, Hernández RM et al (2002) Biocompatibility of microcapsules for cell immobilization elaborated with different type of alginates. *Biomaterials* 23:3825–3831. [https://doi.org/10.1016/S0142-9612\(02\)00118-7](https://doi.org/10.1016/S0142-9612(02)00118-7)
- Oshi MA, Lee J, Naeem M et al (2020) Curcumin nanocrystal/pH-responsive polyelectrolyte multilayer core-shell nanoparticles for inflammation-targeted alleviation of ulcerative colitis. *Biomacromolecules* 21:3571–3581. <https://doi.org/10.1021/acs.biomac.0c00589>
- Pereira L, Cotas J (2020) Introductory chapter: alginates—a general overview. In: Pereira L (ed) *Alginates: recent uses of this natural polymer*. Intech Open, London
- Rosch JG, Winter H, DuRoss AN et al (2019) Inverse-micelle synthesis of doxorubicin-loaded alginate/chitosan nanoparticles and in vitro assessment of breast cancer cytotoxicity. *Colloids Interface Sci Commun* 28:69–74. <https://doi.org/10.1016/j.colcom.2018.12.002>
- Sahatsapan N, Rojanarata T, Ngawhirunpat T et al (2021) Doxorubicin-loaded chitosan-alginate nanoparticles with dual mucoadhesive functionalities for intravesical chemotherapy. *J Drug Deliv Sci Technol* 63:102481. <https://doi.org/10.1016/j.jddst.2021.102481>
- Saqib Hasnain M, Rishishwar P, Rishishwar S et al (2018) Isolation and characterization of Linum usitatissimum polysaccharide to prepare mucoadhesive beads of diclofenac sodium. *Int J Biol Macromol* 116:162–172. <https://doi.org/10.1016/j.ijbiomac.2018.04.151>
- Saralkar P, Dash AK (2017) Alginate nanoparticles containing curcumin and resveratrol: preparation, characterization, and in vitro evaluation against DU145 prostate cancer cell line. *AAPS PharmSciTech* 18:2814–2823. <https://doi.org/10.1208/s12249-017-0772-7>
- Sarei F, Dounighi N, Zolfagharian H et al (2013) Alginate nanoparticles as a promising adjuvant and vaccine delivery system. *Indian J Pharm Sci* 75:442–449. <https://doi.org/10.4103/0250-474X.119829>
- Scolari IR, Páez PL, Musri MM et al (2020) Rifampicin loaded in alginate/chitosan nanoparticles as a promising pulmonary carrier against staphylococcus aureus. *Drug Deliv Transl Res* 10:1403–1417. <https://doi.org/10.1007/s13346-019-00705-3>
- Sehgal R, Mehta A, Gupta R (2019) Alginates: general introduction and properties. In: Ahmed S (ed) *Alginates: applications in the biomedical and food industries*. Wiley, London, pp 1–20
- Severino P, Chaud MV, Shimojo A et al (2015) Sodium alginate-cross-linked polymyxin B sulphate-loaded solid lipid nanoparticles: antibiotic resistance tests and HaCat and NIH/3T3 cell viability studies. *Colloids Surf B Biointerfaces* 129:191–197. <https://doi.org/10.1016/j.colsurfb.2015.03.049>

- Shehata TM, Ibrahima MM (2019) BÜCHI nano spray dryer B-90: a promising technology for the production of metformin hydrochloride-loaded alginate–gelatin nanoparticles. *Drug Dev Ind Pharm* 45:1907–1914. <https://doi.org/10.1080/03639045.2019.1680992>
- Sikorski P, Mo F, Skjåk-Bræk G, Stokke BT (2007) Evidence for egg-box-compatible interactions in calcium–alginate gels from fiber x-ray diffraction. *Biomacromolecules* 8:2098–2103. <https://doi.org/10.1021/bm0701503>
- Sorasithiyankarn FN, Muangnoi C, Ratnatilaka Na Bhuket P et al (2018) Chitosan/alginate nanoparticles as a promising approach for oral delivery of curcumin diglutamic acid for cancer treatment. *Mater Sci Eng C* 93:178–190. <https://doi.org/10.1016/j.msec.2018.07.069>
- Spasojevic M, Bhujbal S, Paredes G et al (2014) Considerations in binding diblock copolymers on hydrophilic alginate beads for providing an immunoprotective membrane. *J Biomed Mater Res A* 102:1887–1896. <https://doi.org/10.1002/jbm.a.34863>
- Thomas D, Latha MS, Thomas KK (2018) Synthesis and in vitro evaluation of alginate-cellulose nanocrystal hybrid nanoparticles for the controlled oral delivery of rifampicin. *J Drug Deliv Sci Technol* 46:392–399. <https://doi.org/10.1016/j.jddst.2018.06.004>
- Thomas D, Mathew N, Nath MS (2021) Starch modified alginate nanoparticles for drug delivery application. *Int J Biol Macromol* 173:277–284. <https://doi.org/10.1016/j.ijbiomac.2020.12.227>
- Wittaya-Areekul S, Kruenate J, Prahsarn C (2006) Preparation and in vitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone. *Int J Pharm* 312:113–118. <https://doi.org/10.1016/j.ijpharm.2006.01.003>
- Xing L, Dawei C, Liping X, Rongqing Z (2003) Oral colon-specific drug delivery for bee venom peptide: development of a coated calcium alginate gel beads-entrapped liposome. *J Control Release* 93:293–300. <https://doi.org/10.1016/j.jconrel.2003.08.019>
- Yan M, Chen T, Zhang S et al (2021) A core-shell structured alginate hydrogel beads with tunable thickness of carboxymethyl cellulose coating for pH responsive drug delivery. *J Biomater Sci Polym Ed* 32:763–778. <https://doi.org/10.1080/09205063.2020.1866350>
- Yu X, Wen T, Cao P et al (2019) Alginate-chitosan coated layered double hydroxide nanocomposites for enhanced oral vaccine delivery. *J Colloid Interface Sci* 556:258–265. <https://doi.org/10.1016/j.jcis.2019.08.027>
- Yuan J, Guo L, Wang S et al (2018) Preparation of self-assembled nanoparticles of ϵ -polylysine-sodium alginate: a sustained-release carrier for antigen delivery. *Colloids Surf B Biointerfaces* 171:406–412. <https://doi.org/10.1016/j.colsurfb.2018.07.058>
- Zhang YW, Tu LL, Tang Z et al (2021) pH-sensitive chitosan-deoxycholic acid/alginate nanoparticles for oral insulin delivery. *Pharm Dev Technol* 26:943–952. <https://doi.org/10.1080/10837450.2021.1966036>

Alginate Based Carriers for Topical Drug Delivery



Gourav Parmar , Manish Kumar , Abhishek Jha ,
and Brahmeshwar Mishra

Abstract Topical drug delivery is a promising approach for drug susceptible to degradation on oral administration or drug undergoing first pass effect. Topical delivery allows the drug to reach target site in effective concentration without drug lose, minimizing drug dose, frequency and cost load. In addition, topical delivery also offers localized drug delivery for treatment of various health disorders like skin infections, chronic wound, skin carcinoma etc. Biopolymers use in topical formulation is a promising strategy for design of safe and efficient drug delivery systems (DDS). Alginate is such a potent biopolymer with diverse advantages over synthetic polymers, including natural abundance, economic cost, biodegradability, biocompatibility, cyto-compatibility, no toxicity, and hydrophilicity. Alginate also has intrinsic bioactivity like anti-inflammatory and antimicrobial properties that may give synergistic effect with drug. Furthermore, due to its structural diversity, alginate allows for surface functionalization to give versatility in biomedical and pharmaceutical applications. Alginate can be effectively employed for the formulation of various DDS such as gels, dermal patches, films, microneedles, nanofibrous scaffolds, nanoparticles, and microparticles, for topical applications.

Keywords Alginate biomaterials · Drug delivery · Topical formulations · Nanocarriers

1 Introduction

Topical route of administration can be used for delivery of therapeutic molecules either for localized or systemic action. It owes numerous advantages including; elimination of first pass effect, avoid systemic toxicity, confer sustained drug delivery, lower plasma drug concentration fluctuation, minimize dose, ease use,

G. Parmar · M. Kumar · A. Jha · B. Mishra (✉)

Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (BHU), Varanasi, Uttar Pradesh, India

e-mail: bmishra.phe@itbhu.ac.in

and improve patient-compliance. However, topical delivery of drug is limited by various barriers which includes; stratum corneum, skin moisture, skin diseases, and aqueous solubility and permeability of drug (Bouwstra and Ponc 2006; Kumar et al. 2019). Therefore, there is an unmet need for design of novel formulations for drug delivery to or across skin. Recently, number of biopolymer-based formulations has been utilized for drug delivery to or across skin. Biopolymers are of natural origin, due to which are safe, economic, biocompatible, biodegradable, hydrophilic, and non-toxic (Kumar et al. 2022). Alginate is such a biopolymer derived from marine sources that can be utilized for design of safe and acceptable DDS for topical drug delivery. Alginate owe characteristics properties like easy processing, biodegradability, biocompatibility, low cytotoxicity, hydrophilicity, high payload potential, good gelation property, mucoadhesion, and stimuli responsiveness (Aguero et al. 2021). Alginate drug carriers aids in drug delivery to or across skin by improving the drug permeation across stratum corneum, thereby increasing the optimal concentration required for therapeutic effect in desired time period. Alginate carriers owe superior properties like high mechanical strength, flexibility, and remarkable physico-chemical properties (Lee and Mooney 2012). Alginate can be employed for design of drug carriers like hydrogel (Eldeeb et al. 2022), film (Karimi-Khorrami et al. 2022), patches (Weber et al. 2006), microneedles (Zhou et al. 2022), nanofibrous scaffolds (Muthulakshmi et al. 2022), microparticles (Uyen et al. 2020) etc., for various biomedical and pharmaceutical applications. These features render alginate an ideal biomaterial for encapsulation of therapeutic moiety apart from its implications in the fabrication of biomimetic regenerative and wound dressing materials. Topical drug delivery systems have been presented with new technologies for controlled dosing, enhanced skin penetration, and site-specific delivery, thereby extending the range of applications of therapeutic compounds *via* the skin (Benson et al. 2019). This chapter addresses the sources, extraction methods, and properties of alginate. Furthermore, role of alginate in the formulation of topical DDS for treatment of various health disorders is reported.

2 Alginate: Source, Isolation, Extraction and Purification

Alginate is a brown seaweed-derived linear, unbranched anionic biopolymer composed of -D-mannuronic acid residue (M-block) and -L-guluronic acid residue (G-block). Alginate's backbone is made up of consecutive G residues (GGGGGG), consecutive M residues (MMMMMM), and alternating M and G residues (GMGMGM) (Lee and Mooney 2012). The alginate backbone presents number of free hydroxyl and carboxyl groups which gives it a hydrophilic property and functionalization potential. Since bacterial alginates have a lower G/M ratio than algal alginates, the proportion of M and G content in alginates extracted from different sources varies. Alginate G-blocks participate in intermolecular cross-linking with divalent cations (for example, Ca^{2+}) to form hydrogels. Increasing the length of the G-block and the molecular weight of alginate gels typically improves

their mechanical properties. Alginate rich in G-blocks produces highly rigid and porous gels that are less prone to erosion and shrinkage, and show no swelling once dried, whereas M-blocks or G/M-blocks give flexibility to gel networks making them low porous, softer, and highly elastic.

Alginates are primarily derived from two sources: algal sources and bacterial sources. Brown seaweeds (also known as macroalgae) are common in coastal areas and are the major source of polysaccharides alginate. Alginate is found in the cell-wall and intercellular matrix of macroalgae Phaeophyceae, which exist as divalent alginic acid salts and form an intercellular gel matrix (Severino et al. 2019). It provides flexibility and mechanical strength to macroalgae for surviving in water reservoirs. Alginic acid gels are usually found in the intercellular matrix of microalgae and are made up of divalent cation (such as Na^+ and Mg^{2+}) salts of alginic acid. The type, harvesting season, as well as age of the tissues; all influence the quality and quantity of alginate obtained. Alginates are obtained commercially from various Phaeophyceae species such as *Laminaria*, *Macrocystis*, *Sargassum*, *Ascophyllum*, *Lessonia*, *Eclonia*, and *Durvillea* (Singha et al. 2021). “Alginophytes, “which are alginate-producing seaweeds of wild strain, can also be obtained through artificially cultivated algae, such as *Saccharina japonica* (Severino et al. 2019). Alginate is also produced by bacterial strains such as *Azotobacter* and *Pseudomonas* that are abundant in vegetative growing cells (Kivilcimdan Moral and Yıldız 2016). Alginates derived from algae have a varying molecular weight, whereas those derived from bacteria have high molar masses as well as a high degree of polymerization.

Alginate can be produced biotechnologically by members of two heterotrophic bacteria families, *Pseudomonas* and *Azotobacter*. The formation of a spherically dormant cyst distinguishes *Azotobacter vinelandii* from the Azotobacteriaceae family. Alginate is a necessary component of the cyst that protects it from desiccation and adverse conditions. According to Hay et al. (2013), *A. vinelandii* produces relatively rigid alginates due to the presence of high number of guluronate residues (G) (Hay et al. 2013). Alginate is an important component of the biofilm of *P. aeruginosa* and other pseudomonads. This bacterium’s alginate formation is primarily governed by oxygen, which is also responsible for the bacterium’s mucoid appearance. Aeration favors *P. aeruginosa* mucoid strains, whereas non-mucoid strains are oxygen sensitive. Mucoid strains of *P. aeruginosa* have been shown to biosynthesize a significant amount of bacterial alginates (Valentine et al. 2020).

The pathway for bacterial alginates biosynthesis includes (1) precursor substrate synthesis in cytoplasm, (2) polymerization and cytoplasmic membrane transfer, (3) periplasmic modification and (4) export through the outer membrane (Remminghorst and Rehm 2006). Valentine et al. (2020) created a non-pathogenic strain of *P. aeruginosa*. The marker free strain PGN5 was created by sequentially deleting five genes from the chromosomes using a homologous recombination strategy. The systemic virulence of PGN5 was greatly reduced showing 0% mortality on intraperitoneal injection compared to wild-type *P. aeruginosa* which resulted in 95% mortality. Importantly, when MucE, an activator of alginate biosynthesis, is overexpressed, a high alginate yield is provided by PGN5. PGN5 alginate is

structurally identical to wild-type alginate, suggesting the active alginate biosynthetic pathway in modified strain (Valentine et al. 2020).

2.1 Extraction of Alginate

Alginate extraction from marine algae may comprises of multiple steps including; (1) seaweeds acidification (2) alkaline extraction (Na_2CO_3), (3) phase separation, (4) precipitation and (5) drying. Firstly, raw algae are collected and desiccated with an exceptional of *M. pyrifera* that follow wet process for alginates production. The raw algae are processed with mineral acids to eliminate the counter-ions by the proton-exchange mechanism. Then, alginate extraction is done by treating with alkali agents like sodium hydroxide and/or sodium carbonate to solubilize the alginic-acid fractions by neutralization process. The separation techniques used can involve centrifugation/floatation or shifting followed by filtration. The obtained precipitates are then treated with mineral acids or alcohols to extract sodium alginate, and also to eliminate the potential impurities like homopolysaccharides; laminarin and fucoidin. Th extracted alginates are further purified by suitable methods like electrophoresis to remove possible cytotoxic impurities. An alternative low cost and less time-consuming process can be employed for alginate extraction that involve use of Ba^{2+} ions with higher affinity towards alginate molecules. The barium-alginate gels are stable at all pH and free from impurities and mitogens. The pure-alginate is obtained by treating barium-alginate gels with alkali followed by ethyl alcohol.

2.2 Novel Extraction Techniques of Alginates from Brown Seaweeds

Recently, eco-friendly techniques are employed for efficient extraction of alginate with high yield value compared to conventional process. One of novel techniques involves use of Ultrasound for extraction which limits the energy consumption and reduces the process time. Youssouf et al. (2017) reported the extraction of alginates from *Sargassum binderi* using ultrasound. Ultrasound result in time efficient extraction compared to conventional extraction; without affecting the chemical structure and molar mass distribution of alginates (Youssouf et al. 2017). Another novel extraction technique is microwave-assisted extraction (MAE) which can overcome the major drawbacks accompanied by conventional extraction methods like thermal and/or solvent based techniques. The MAE method uses microwave radiation to speed up the extraction process by faster and efficient heating of solvent. Microwave heating is most effective at a frequency of 2450 MHz with a power level of 600–700 W. Microwave radiation's heat can heat and evaporate the water in the

sample cells due to which the pressure on the cell wall rises, cell expands (swells), and the pressure within cells pushes against the cell wall, stretching and breaking it. The breakdown of plant cells allows target substances to escape and be extracted more easily (Jain et al. 2009). Ruslan et al. (2019) extracted sodium alginate from *Sargassum* sp. using MAE method. The optimum power level and extraction time observed with commercial microwave was at levels 70, 80, 90 and 100, and 15, 16, 17 and 18 min, respectively (Ruslan et al. 2019).

2.3 Properties of Alginates

The alginates have varying molecular weight (MW) ranging between 32,000–400,000 g/mol. The viscosity and gelation property of alginate depends on MW of alginate. High MW tends to form a gel with high viscosity (LeRoux et al. 1999). Alginate is an aqueous soluble polysaccharide, commercially available as its sodium salt, which is more soluble and has large water retention capacity (Lee and Mooney 2012). The alginate properties heavily depend on M/G ratio. Alginate with predominant guluronic-acid (G)-residues display strong gelling behavior while those having predominant mannuronic acid (M)-residues exhibits high flexibility. If G-residues predominate, it gives harder and fibrous gel with slow gelation, in contrast to alginates with predominant M-residues that result in softer and flexible gel with quick gelation. Alginate being anionic polymer form hydrogel instantly with monovalent, divalent or trivalent cations via well-known gelation mechanism; “egg-box model” (Wee and Gombotz 1998) (Hu et al. 2021). Alginate resembles the extracellular matrix and possess ability to absorb and retain water and fluids; making it an ideal candidate for wound dressing. Alginate hydrogel responsive to different pH are also available which releases the cargo in a smart manner, i.e., environmental stimuli responsiveness (Roquero and Katz 2022). Alginate owing to its mucoadhesion property improves the bioavailability of drug due to intimate contact with the mucosal surface (Swain et al. 2012). Alginates are safe, biodegradable, non-toxic, and low immunogenic (Lee and Mooney 2012). Alginates have a versatile structure that can be modified for obtaining desired properties (Banks et al. 2019).

3 Alginate Role in Design of Topical Drug Delivery Systems

3.1 Alginate-Hydrogel

Lopedota et al. (2018) reported use of Alginate for hydrogel preparation and encapsulation of Minoxidil inclusion complex with Hydroxypropyl- β -Cyclodextrin; for the treatment of Topical Alopecia. The prepared system eliminated the side effects associated with cosolvents propylene-glycol and ethanol in commercial product. The inclusion complex loaded hydrogel improved the bioavailability of

drug, where hydrogel aided the application, modulated the release and thus played key role in improving drug permeation (Lopedota et al. 2018).

Alginate can be used with other polymer to prepare blend or composite hydrogel with improved physicochemical properties. Chou et al. (2020) used thermosensitive pluronic F127 (PF127) for regulating the conformation of Ca^{2+} -crosslinked alginate and form a reversible and smart, interpenetrating polymeric-network (IPNs) for wounds. The study showed that hydrogels consisting of a rigid calcium alginate network promote payload (vascular endothelial growth factor) stability and control the release. Additionally, the hydrogel can be removed with ease only by rinsing it with cold PBS due to gel-sol conversion (Chou et al. 2020). Peng et al. (2012) used blend of sodium alginate and gelatin for preparation of astragaloside IV loaded hydrogel. The study showed localized effect on wound healing upon topical application. Prepared gel matrix promoted the collagen synthesis and stimulated the TGF- β 1 secretion in skin wounds. The gelatin and sodium alginate enhanced the healing process by initiating hemostasis, maintaining a moist environment, initiating wound healing by upregulating inflammatory markers (TNF- α and IL-6), and improving skin elasticity. The hydrogel also sustained the release of the astragaloside IV at wound site and showed good biocompatibility and wound healing (Peng et al. 2012). Ataide et al. (2017) prepared hydrogel by using blend of Alginate and Arabic Gum, for loading and topical delivery of Bromelain. The hydrogel found to have higher rehydration capability when alginate and Arabic gum were used at higher concentration. Prepared hydrogel had a high swelling ratio of 227%, good payload potential and supported the debridement of burns or wounds (Ataide et al. 2017).

Kim et al. (2008) reported Polyvinyl alcohol (PVA) and sodium alginate (SA) based hydrogel for design of nitrofurazone (NFZ) containing wound dressing. High concentration of SA was responsible for higher swelling ability, elasticity and thermal stability of hydrogel film (Kim et al. 2008). Kaur et al. (2019) also prepared PVA-SA hydrogel for delivery of bacteriophages and minocycline, against burn wound infections. The hybrid hydrogel membrane was novel wound dressing with unique properties such as high swelling, self-adherence, high gel fraction, high protein absorption, hemocompatibility, biocompatibility and antibacterial action. The MRSA-infected murine burn wound model revealed significant reduction in bacterial infection, contraction of wound and reduction in inflammation, compared to untreated or control group (Kaur et al. 2019).

3.2 Alginate Film

Ng et al. (2016) prepared Alginate based bilayer films loaded with 5% and 10% Hidrox-6. The film increased the permeation of HT across rat skin in a controlled manner. The film found to be non-irritant, and was more effective in RA treatment (Ng et al. 2016). Shahzad et al. (2019) formulated cefazolin nanoparticles incorporated ion-crosslinked films for the topical delivery. The prepared alginate/

pectin-based film exhibited higher stability and improved mechanical strength, even upon exposure to wound fluid. The films showed better results in terms of breaking elongation, water absorption, water vapor transmission, wetting ratio and release kinetics (Shahzad et al. 2019). A bioadhesive film was reported by Pagano et al. (2021) consisting of xanthan gum combined with alginic acid. The film revealed remarkable mechanical attributes like high surface deformability that ease the application on site. The film had easy skin adhesion, good exudates absorption, controlled release of payload, and was active against various bacterial strains; *S. pyogenes*, *S. aureus* and *E. faecalis*. The film allowed complete protection against injured area and promoted regeneration by triggering the growth of keratinocytes (Pagano et al. 2021). Chiaoprakobkij et al. (2020) reported a composite film consisting of Bacterial Cellulose/Alginate/Gelatin and loaded with curcumin. The film exhibited uniform structure, acceptable mechanical strength and good flexibility for wound dressings. The film found to adhere firmly onto the skin on hydration and the mucoadhesion time was in desired range. The prepared film demonstrated potent anti-microbial activity towards *E. coli* and *S. aureus* infection, and had no-toxic effects on normal cells. The films can be used as dressing materials for wound care, periodontitis and oral cancer (Chiaoprakobkij et al. 2020). Rezvanian et al. (2016) developed a composite film for simvastatin delivery and wound care. The composite films consist of pectin or gelatin blended with sodium alginate. The alginate/pectin based composite film demonstrated superior mechanical properties and controlled drug release. The film was safe, non-toxic and potent wound dressing material (Rezvanian et al. 2016).

3.3 Alginate Nanoparticles

Costa et al. (2015) reported chitosan-alginate nanoparticles for encapsulation of daptomycin and ocular drug delivery. The results showed suitable size, high encapsulation, good permeability, extended drug release, and potent antimicrobial activity (Costa et al. 2015). Sarheed et al. (2021) prepared Lidocaine loaded alginate-nanoemulsion for dermal delivery. Prepared nano-emulsions had higher encapsulation due to formation of bonds between the amine and hydroxyl groups of lidocaine and Tween 80/alginate, respectively. The nanoemulsions extended the release of lidocaine, attributed to alginate role. The nanoemulsion demonstrated capability to maintain drug permeation through (Sarheed et al. 2021). Khampieng et al. (2015) developed alginate nanoparticles (Alg-NPs) loaded with Silk sericin. Prepared Alg-NPs were loaded further into the hydrogel. It provided a high initial release for quick therapeutic effect followed with slow controlled release profile for the prolonged therapeutic effect on transdermal application. The Alg-NPs provided better stability to encapsulated silk sericin (Khampieng et al. 2015).

In a study by Esentürk et al. (2020), voriconazole loaded nanofibers consisting of polyvinyl alcohol and sodium alginate (SA) was prepared for topical antifungal treatment. SA was difficult to electrospun due to its hydrophilic properties, thus

blended with PVA for good electro-spinnability behavior. To avoid burst release of drug, the nanofibers were crosslinked with glutaraldehyde (GTA). The nanofibers had high drug loading potential, and found to control the drug release. The prepared nanofibers enhanced the penetration and deposition of payload in deeper layers of the skin and showed potent activity against *C. albicans* (Esentürk et al. 2020). In another study by Kotroni et al. (2019), sodium alginate based electrospun micro/nanofibrous patches was fabricated for loading of aqueous extract of *Pinus halepensis* bark (PHBE). The prepared patch showed high wettability, maintained the moist environment of wound and provided high diffusion of loaded extract to skin layers. The patches significantly attenuated the inflammation damage in UV-inflamed skin and significantly reduced the healing period. The alginate was found to further enhanced the anti-inflammatory action of patches (Kotroni et al. 2019). Kyzioł et al. (2017) also developed electrospun alginate-nanofibers for encapsulation of ciprofloxacin hydrochloride. Developed nanofibers were stable, uniform, and nanosized (average diameter of 109 and 161 nm for unloaded and loaded fibers, respectively). The fibers mimic structure similar to skin and thus favor attachment and proliferation of cells (Kyzioł et al. 2017).

3.4 Alginate Microparticles

Uyen et al. (2020) described several methods including spray-drying, extrusion, and emulsification/gelation techniques, for development of alginate-based microspheres for drug delivery applications (Uyen et al. 2020). Elmowafy et al. (2019) reported use of alginate for preparation of Soy isoflavone loaded microspheres for wound-healing applications. The result showed biphasic drug release involving an initial rapid release (approximately 40% within 1 h) followed by a slow and controlled release. The release was due to swell of alginate (hydrophilic polymer) and was determined by pH. Approximately, 67%, 74% and 82% of drug release was observed at pH 5.8, 6.3 and 7.4, respectively. This can be due to the charge and degree of ionization of alginate. Alginate found to improve the adhesion, cell proliferation, and thus rate of healing and cellular activity. It also mimics feature of native extracellular matrix responsible for promoting the aggregation of neutrophils and macrophages at the wound site and show significant wound-healing in terms of mature-collagen synthesis, re-epithelization, and pro-angiogenesis (Elmowafy et al. 2019). Faragò et al. (2016) reported use of alginate to improve the hydrophilicity of fibroin. The result showed that the alginate addition improved the microparticle yields and morphology. The microparticles were spherical, slightly rough surface, economic and easy scale-up possible. The study revealed fibroin and sodium alginate as a promising combination for formulation of various topical DDS (Faragò et al. 2016). Shinde et al. (2014) developed chitosan and alginate-based microspheres by ionotropic gelation technique. The microspheres were capable of encapsulation of azelastine hydrochloride and prolonged drug presence in cul-de-sac. Prepared microspheres were stable when and suitable for sterilized by γ radiation (Shinde et al.

2014). Dhat et al. (2009) reported Vitamin E encapsulated pectin/alginate microspheres. The result showed that sodium alginate addition changed the shape of disc-microcapsules to spherical shape, and exhibited good encapsulation efficiency, and high yield value (Dhat et al. 2009).

3.5 *Microneedles*

Demir et al. (2013) developed sodium alginate-based microneedle arrays (MNs) for delivery of bovine serum albumin across human skin. The MNs rapidly dissolved to release the payload within minutes and was capable of significantly delivering the payload across the skin, increasing the permeation by about 15-fold than needle-free patch (Demir et al. 2013). Zhang et al. (2018) developed Microneedles composed of alginate and maltose for transdermal delivery of insulin on diabetic rats. The prepared microneedles exhibited a strong mechanical strength with highest failure force around 0.41 N/needle. The study showed excellent cytocompatibility, excellent swelling and dissolution properties, and high pharmacological activity (sustained hypoglycemic effect) compared to same subcutaneous injected insulin dose (Zhang et al. 2018). Weijiang et al. (2017) prepared alginate and hyaluronate based microneedles for insulin delivery via skin. The MNs presented a strong mechanical strength, sufficient to penetrate layers of skin where solubilize to release the insulin (Weijiang et al. 2017).

3.6 *Polyelectrolyte Complex*

Chitosan and alginate tend to form polyionic complexes through ionic gelation which involves interactions between the carboxyl groups of alginates and the amine groups of chitosan. Both polymers are bioadhesive in nature, providing prolonged contact to the skin surface. The complex was biocompatible, biodegradable, protected the encapsulant, high swelling capacity and controls the release of payload more effectively than either alginate or chitosan alone. The complex provided enhanced penetration of the drug into the skin. Developed complex was suggested for preparation of inserts or tablets for vaginal and transdermal drug delivery. The metronidazole loaded complex had enhanced entrapment efficiency, and mucoadhesion compared to the reference commercial gel formulation (Cirri et al. 2021). Alshhab and Yilmaz (2020) reported a polyelectrolyte multilayer films composed of sodium alginate (anionic) and poly(4-vinylpyridine) (cationic). Prepared film was suitable for encapsulation of ciprofloxacin HCl and resulted in a hydrophilic film with controlled physicochemical properties and drug release behavior. The film was thin, transparent, and stable, with a smooth, hydrophilic surface. The film had optimum swelling capacity, high drug loading capacity, and released drug via diffusion mechanism, suitable for transdermal delivery (Alshhab and

Yilmaz 2020). Prajapati et al. (2007) prepared a polyelectrolyte complex film using alginate combined with chitosan, for topical delivery of clotrimazole. The film had maximum swelling and sustained release of clotrimazole (Prajapati et al. 2007).

4 Alginate Role in Drug Delivery

4.1 Dermal Drug Delivery

Abebe et al. (2020) prepared a self-adhesive hydrogel for transdermal delivery of caffeine. In this study, alginate and gallic acid mixture was used for preparation of interpenetrating adhesive hydrogel due to dynamic hydrogen bond formation between gallic acid, alginate, and polyacrylic acid. This offered good mechanical characteristics to hydrogel like high elasticity and good tensile strength. Gallic acid further improved the mechanical property due to induction of various non-covalent interactions. The study showed excellent release kinetics from prepared adhesive patches, showing a faster release of about 87% when strain is applied. The release was increased by twofold compared to release observed in absence of strain (Abebe et al. 2020). Chen et al. (2011) designed a temperature-sensitive systems using blend or graft copolymer of alginate and PF127, serving as topical DDS for selegiline. The graft copolymer retained the temperature sensitivity due to PF127 and the sol–gel transition was observed only when applied on skin, avoiding solution form prior to application. Prepared thermogels emerged as an effective carrier for topical delivery of selegiline in sustained manner (Chen et al. 2011).

4.2 Mucosal Drug Delivery

El Moussaoui et al. (2021) reported sodium-alginate based hydrogel loaded with ketorolac tromethamine for pain relief. Because of the alginate, KT-hydrogel demonstrated good extensibility, ease of application, and a high swelling property. Permeation results revealed penetration and retention of loaded drug in buccal and sublingual mucosae. This resulted in a localized analgesic and anti-inflammatory effect, as-required in surgical and/or ablative operations like treatment of certain oral carcinomas, dental extractions, etc. (El Moussaoui et al. 2021). Camargo et al. (2021) produced a chitosan-alginate film loaded with imiquimod (IQ) for treatment of oral-cancer. Due to the polysaccharide matrix, films were capable for effective penetration and retention the drug. The film was discovered to be 132 m thick, thin, transparent, and mucoadhesive. Film was capable to absorb large amount of artificial saliva at a higher rate and was stable. Furthermore, the films provided controlled drug release with no burst effect (Camargo et al. 2021).

4.3 Vaginal Drug Delivery

Urogenital infections are a major health concern for women. Urogenital infections, such as bacterial vaginosis, urinary tract infections (UTIs), and yeast vaginitis, are thought to affect one billion women worldwide. In order to treat these diseases, vaginal administration of drug has several advantages over oral administration, including localized intervention, low dose requirement, bypassing first pass effect or harsh gut conditions, and avoiding side effects in non-target organs/areas. However, a few challenges that limit drug administration via this route include short residence times, which necessitate frequent applications to achieve similar effective drug levels. This can be overcome by developing formulations designed to stay at the vagina for an extended period of time, allowing for intimate contact at the application site and sustained drug release for localized drug delivery. Because of its unique properties, alginate has been investigated for the design of such formulations. El Moussaoui et al. (2021) prepared mucoadhesive alginate-based hydrogel for encapsulation and vaginal delivery of Ketorolac. The drug release study showed 73% drug release from gel in less than 6 h. The permeation study demonstrated that application of hydrogel on vaginal mucosa significantly elevated the permeation by sevenfold, as compared to permeation observed when applied on skin. In addition, ketorolac was also retained for longer period where vaginal application retained of about 20-fold of drug within vaginal mucosa than in human skin. The hydrogel thus showed mucoadhesive property and was well tolerated without any visible irritation (El Moussaoui et al. 2021).

Cirri et al. (2021) prepared a mucoadhesive MTZ (metronidazole) vaginal formulation consisting of alginate and chitosan at ratio of 2:1 w/w, respectively. The study showed 70% drug entrapment efficiency, good mucoadhesion on vaginal mucosa compared to commercial gel, and potent antibacterial effect than formulation with chitosan alone (Cirri et al. 2021). Maestrelli et al. (2018) also prepared microspheres for cefixime vaginal administration using alginate and chitosan. The study showed good mucoadhesion, huge water-uptake capability, high entrapment efficiency and potent localized action against urogenital infection (Maestrelli et al. 2018). De Bastiani et al. (2020) described amphotericin B and miltefosine-loaded alginate nanocarriers for vaginal candidiasis. Alginate nanoparticles had comparable levels of fungal infection control as vaginal cream formulations. A single dose was as effective as microemulsion (with three times dose) and cream (with six times dose) in reducing fungal burden. Nanocarriers found to prolong the antifungal action when administered via intra-vaginal route and also decreased the dosing frequency (de Bastiani et al. 2020).

4.4 Ocular Drug Delivery

The most commonly employed method for the treatment of ocular diseases is through topical drug administration. The main drawbacks are rapid drug clearance due to nasolacrimal duct drainage and tears. As a result, only a small fraction of administered dose penetrates the cornea to reach intraocular tissues. To address these shortcomings, DDS with muco-adhesion and prolonged ocular-residence are proposed, which improve the drug retention at site and avoid drug drainage, thereby improving drug permeation. Gilhotra et al. (2011) created an ocular-insert for azithromycin delivery. Alginate and hydroxypropyl methylcellulose (HPMC) were used to make the insert. The alginate films had higher bioadhesion, tensile strength (TS), elasticity, and swelling or water uptake. HPMC content in alginate films improved TS, elasticity, and water uptake. The prepared films were non-irritant and had a long release time (Gilhotra et al. 2011). Wang et al. (2018) prepared Retinoic acid-loaded alginate microspheres for intravitreal treatment. The prepared microspheres were smooth, round, homogeneous, and biocompatible, with an average diameter of about 90 μm . Retinoic acid-loaded alginate microspheres showed sustained-release in the vitreous cavity (Wang et al. 2018). Yoncheva et al. (2011) created pilocarpine-loaded poly (lactic-co-glycolic acid) nanoparticles coated with sodium alginate particles (Alg2) that possessed highest pilocarpine loading and the least burst effect. After local ocular application, the coated nanoparticles provide a longer residence time for the nanoparticles (Yoncheva et al. 2011).

Ibrahim et al. (2015) developed brimonidine-loaded bioadhesive chitosan (cationic)/alginate (anionic) nanoparticles (CA-NPs) for glaucoma management. Brimonidine release from nanoparticles was significantly slower than in the control preparation, demonstrating sustained release without any burst effect (Ibrahim et al. 2015). Ibrahim et al. (2013) also reported celecoxib-loaded CA-NPs for topical ophthalmic use. The findings revealed that CA-NPs had spherical shapes, pH and viscosity values compatible with the eye, sustained release without burst effects, and were non-toxic (Ibrahim et al. 2013). Motwani et al. (2008) also developed mucoadhesive CA-NPs for the prolonged ophthalmic delivery of gatifloxacin. The nanoparticles' encapsulation efficiency ranged between 61% and 82%. The prepared nanoparticles provide extended drug release. The nanoparticle provided prolonged ocular drug delivery due to the mucoadhesive property of alginate and chitosan blend (Motwani et al. 2008).

5 Applications

Alginate has been widely used for dermal and transdermal drug delivery, as demonstrated above. Table 1 illustrates the role of alginate-based carriers in topical delivery and health disorder management. Alginate carriers can be used effectively to treat chronic wounds (such as burn wounds and diabetic foot ulcers), bacterial and

Table 1 Alginate based formulation for topical drug delivery

Formulation	Role of alginate	Application	Observations	References
Alginate hydrogel	For encapsulation of drug loaded nanovesicles and sustained delivery of drug	Bioactive wound dressing	Pitavastatin nanovesicles loaded nanocomposite hydrogel were prepared, possessed high water uptake, sustained drug release for 7 days, and promoted wound healing	Eldeeb et al. (2022)
Alginate hydrogel	For encapsulation of S-nitroso-mercapto-succinic acid as NO donor, and for AgNPs synthesis	For topical application	Hydrogel controlled the release of NO and AgNPs and increased their concentration in target area	Urzedo et al. (2020)
Alginate film	Form porous film with acceptable mechanical property	Drug delivery and sustained antimicrobial activity	Thymol loaded NLCs and NEs were incorporated into film for sustained drug release	Karimi-Khorrami et al. (2022)
Bi-layered hydrogel films of zinc oxide and sodium alginate	High swelling capacity	For wound-dressings	The prepared films had porous network, high hydrophilicity and good swelling capacity	Wang et al. (2019)
Alginate film	Alginate showed mucoadhesive property and enhanced residence at the site	For treatment of mucosal candidiasis	Showed accelerated drug release than Candid-V6 [®]	Mishra et al. (2016)
Alginate film	Alginate film showed good bio-adhesion, high residence and controlled release kinetics	For treatment of oral candidiasis	The alginate disks loaded with fluconazole showed high swelling and adhesion characteristics. It prolonged the residence and drug release	Yehia et al. (2009)
Sodium alginate and polyvinyl alcohol bio-adhesive film,	Sodium alginate responsible for bio-adhesion in prepared film	For antifungal activity	Bio-adhesive films acts as a better alternative for administration of FLZ with a low dosage requirement	Patel et al. (2015)
Multi-layered alginate–PCL membrane patch	Promote tissue regeneration by promoting cell	For wound healing	Accelerated the wound healing process	Dodero et al. (2020)

(continued)

Table 1 (continued)

Formulation	Role of alginate	Application	Observations	References
incorporating ZnO-NPs	viability, and exudate and gas exchange			
Nanoparticles	Alginate-chitosan complex enhance the stability and drug encapsulation efficiency	For ocular drug delivery	Sustained the release of encapsulated 5-FU compared to its solution, showed high mucoadhesiveness and thus bioavailability compared nanoparticles without chitosan	Nagarwal et al. (2012)
Electrospun nanofibers	Form nanofibrous scaffold and encapsulate bioactive compounds	Drug delivery	Form <i>Terminalia catappa</i> extract loaded nanofibers for anticancer activity against skin cancer.	Muthulakshmi et al. (2022)
Microparticles	Form microparticles and promote wound healing	For wound healing	Prepared microparticles were biocompatible and biodegradable	Shi et al. (2019)
Alginate microneedles	Form swellable microneedle via Ca^{2+} induced cross-linking of alginate	Dermal drug delivery	Possessed good mechanical properties, biocompatibility, improved transdermal delivery of drug	Zhou et al. (2022)
β -Cyclodextrin-functionalized chitosan/alginate compact poly-electrolyte complexes	Alginate-chitosan shower enhanced anti-inflammatory property	For anti-inflammatory action	Were non-cytotoxic, inhibited NO and proinflammatory cytokine, TNF- α production which favor wound healing	Hardy et al. (2018)
Calcium alginate (CA) wafer	Maintain moist wound environment	For diabetic foot ulcers (DFUs)	Porous and uniform morphology results in an ideal dressing material for exudate and gaseous exchange	Ahmed et al. (2018)

fungal infections, skin regeneration, oral disorders, and dentistry; also discussed below with few examples.

5.1 Alginate Dressing in Wound Healing

Traditional wound dressing materials have the following limitations: poor maintenance of moist environment, poor wound-exudates absorption capacity, slow healing process, poor gas exchange between wound and environment, poor shielding against pathogens, and difficulty in wound dressing removal. Alginate is a potential material for the design of suitable wound-dressings, owing to its unique characteristics such as biocompatibility, biodegradability, non-immunogenicity, non-toxicity, easy wash-out potential, high drug encapsulation, high wound exudates absorption capacity, active wound healing action, antibacterial activity, high water content to provide moist interface, and haemostasis promotion (Abasalizadeh et al. 2020). Alginate-based wound dressings come in a variety of forms, including hydrogels, films, foams, nanofibers, membranes, and sponges.

Pagano et al. (2021) used xanthan gum and alginic acid to develop Pycnogenol (PYC) loaded bioadhesive films. Because of the presence of alginate, the film demonstrated suitable mechanical properties and high deformability to the surface. A high alginate content improved the tensile properties of the film. The film demonstrated the ability to absorb wound exudates, promote healing by stimulating keratinocyte growth, and be easily removed from the skin. The film demonstrated higher adhesion due to alginate and showed activity against *S. pyogenes*, *S. aureus*, and *E. faecalis*. The formulation found to sustain the release of PYC which ensure complete protection with a single application per day (Pagano et al. 2021). Abou-okeil et al. (2018) developed a crosslinked topical bioactive hyaluronic acid/Na-alginate film. As a bioactive agent, sulfadiazine was used alone or in combination with silver nanoparticles (AgNPs) in the film. The findings demonstrated potent antibacterial activity and played an important role in restoring skin tissue homeostasis, suggesting the role of prepared film as novel bioactive wound dressings (Abou-okeil et al. 2018).

Rezvanian et al. (2016) developed a simvastatin-loaded alginate-based composite film for wound dressings. The wound dressing properties of the alginate/pectin composite film were favorable, as were the mechanical properties. The water vapor transmission rates suggest that an alginate-based composite film can keep the wound site moist without causing excessive dehydration in exudative wounds. A controlled release drug profile was produced by the alginate/pectin film, and a cell viability assay revealed that the film was non-toxic (Rezvanian et al. 2016). Kim et al. (2008) prepared nitrofurazone-loaded wound dressing with polyvinyl alcohol (PVP) and sodium alginate (SA). Increased SA concentration improved the swelling ability, elasticity, and thermal stability of the hydrogel film. The results revealed accelerated healing of wound in rats. Nitrofurazone-loaded wound dressing with PVP and SA was more swollen, flexible, and elastic than PVA alone (Kim et al. 2008).

Ng et al. (2017) produced alginate-beads loaded with monoolein and adenosine for wet-wound, so that excess exudate could be absorbed and wound healing could be promoted. In comparison to the control beads, the monoolein-alginate beads

demonstrated improved drug encapsulation. The addition of 20% monoolein: almond-oil (2:1, w/w) found to increase the swelling capability of beads while controlling the release of adenosine. When compared to intact tissue, the adenosine transport into viable layers and across damaged tissue was increased (Ng et al. 2017). Chen et al. (2019) proposed RapidClot, a novel biomaterial made of hyaluronic acid (HA) and a sodium/calcium alginate composite. RapidClot outperformed Celox (378.7 s) and WoundSeal (705.3 s) in terms of water absorption efficiency and blood clotting time (132.7 s). Furthermore, RapidClot dressing degraded at the same rate as Celox in the presence of hyaluronidase and lysozyme, whereas WoundSeal showed negligible degradation. RapidClot improved cell proliferation and migration and thus has a high potential for use as an effective healing dressing with haemorrhage control (Chen et al. 2019).

5.2 Alginate Dressings for Healing Diabetic Foot Ulcers

Diabetic foot ulcers (DFUs) are one of the leading causes of morbidity in diabetic patients, with a high risk of lower extremity amputation. DFUs can be effectively treated via use of alginate-based dressing materials encapsulated with wound healing agent. Maatouk et al. (2021) produced heparin-mimetic alginate sulfate/polycaprolactone double emulsion nanoparticles (NPs) to deliver CTGF and IGF-I to treat DFUs. Due to the heparin-mimetic properties of AlgSulff, cells treated with NPs closed wounds faster than control and the synthesis of fibronectin was maintained. NPs allowed for controlled delivery while also protecting growth factors from degradation. It also preserved FN (fibronectin), which accelerated the healing processes. Even after 72 h of treatment, FN expression was up-regulated that highlighted the Alg and AlgSulf role in GFs protection (Maatouk et al. 2021). Jeong et al. (2020) prepared Gelatin and Alginate Coacervates for entrapment of epidermal growth factor (EGF) for diabetic wound healing. Coacervates controlled the release, increased keratinocyte migration, granulation, and re-epithelialization, and thus accelerated the healing of wound in diabetic mice. Coacervates were more effective than EGF-PM at protecting the encapsulated EGF from trypsin digestion (Jeong et al. 2020).

5.3 Alginate in Burn Injury

Nuutila et al. (2020) developed an alginate hydrogel loaded with gentamicin, minocycline, and vancomycin. The prepared hydrogel was shown to have high water uptake, a good bacterial barrier, high stability, and good drug release properties. By rinsing with saline, the hydrogel was easily removed from the wound surface. In comparison to controls, alginate hydrogel reduced the number of bacteria in the injured tissue and the burn depth (Nuutila et al. 2020). Pereira et al. (2014)

developed bioadhesive alginate films containing Aloe vera and vitamin E to treat burn wounds. The films were thin, flexible, and resistant, making them ideal for use on burn wounds. The films displayed high flexibility and mechanical properties, making them ideal for use in burn-wound. Compared to conventional formulation, the release from film was regulated showing biphasic release kinetics for over 12 h and facilitated accumulation of vitamin E acetate across stratum corneum to deeper layers (Pereira et al. 2014).

5.4 Alginate in Dentistry and Treatment of Oral Disorders

Farid and Wen (2017) developed topical bilayer buccal mucoadhesive films from sodium alginate and gellan gum. The bilayer films were loaded with prednisolone sodium phosphate and were thin, flexible, with good water uptake and mechanical properties. The film provided sustained drug release and demonstrated complete ulcer healing in 4–5 days by once daily application with no indication of inflammation on treatment sites compared to inflamed tissue on control site (Farid and Wen 2017). Watanabe et al. (2013) prepared sodium alginate–chitosan films loaded with royal jelly to treat 5-fluorouracil (5-FU)-induced oral mucositis in hamsters. Alginate sustained the release of RJ from the film and improved the healing effect of RJ on oral mucositis as a result of their drug-eluting properties (Watanabe et al. 2013).

Kim et al. (2018) developed alginate microparticles to regulate nystatin release. The nystatin-loaded spherical microparticles were uniformly distributed throughout the tissue conditioner matrix after being incorporated into it. Over a 14-day period, the release of nystatin was controlled by microparticles and demonstrated good antifungal activity against *Candida albicans* (Kim et al. 2018). Okeke and Boateng (2016) formed buccal films and composite wafers for nicotine replacement therapy using HPMC and sodium alginate. In comparison to nonporous composite films, wafers had a porous structure with high muco-adhesiveness, swelling index and payload efficiency. By increasing the swelling index and muco-adhesion, sodium alginate improved the performance of HPMC (Okeke and Boateng 2016).

6 Conclusion

Alginate based drug delivery systems are one of the widely acceptable systems for topical drug delivery. Alginate based carriers including hydrogels, film, patches, nanoparticles, microparticles, microneedles etc., have gained widespread popularity for their use in topical drug delivery. These carriers effectively deliver the drug across the biological barriers to obtain desired drug concentration at target site. In addition, the carriers are biocompatible, non-irritant, non-immunogenic and easily washable or removable when required. The topical carriers are also able to control the drug release for prolonged drug delivery at therapeutic range for efficient

therapeutic action. The alginate nanocarriers can also be used for design of nanocomposite systems due to its capability to encapsulate drug loaded nanocarriers or microparticles. These nano/microparticles loaded alginate nanocomposite systems are advanced DDS with better performance like enhanced payload, high penetration efficiency and sustained drug delivery. Such systems can emerge as potent topical DDS for effective treatment of skin disorders like skin melanoma, skin infections, chronic wounds, tissue regeneration etc., In conclusion, alginate as biopolymer can be effectively used in design of safe and potent topical drug delivery systems.

References

- Abasalizadeh F, Moghaddam SV, Alizadeh E, Akbari E, Kashani E, Fazljou SMB, Torbati M, Akbarzadeh A (2020) Alginate-based hydrogels as drug delivery vehicles in cancer treatment and their applications in wound dressing and 3D bioprinting. *J Biol Eng* 14:8. <https://doi.org/10.1186/s13036-020-0227-7>
- Abebe MW, Appiah-Ntiamoah R, Kim H (2020) Gallic acid modified alginate self-adhesive hydrogel for strain responsive transdermal delivery. *Int J Biol Macromol* 163:147–155. <https://doi.org/10.1016/j.ijbiomac.2020.06.257>
- Abou-okeil A, Fahmy HM, El-Bisis MK, Farid O (2018) Hyaluronic acid/Na-alginate films. As topical bioactive wound dressings. *Eur Polym J* 109:101–109. <https://doi.org/10.1016/j.eurpolymj.2018.09.003>
- Agüero L, Alpdagtas S, İlhan E, Zaldivar-Silva D, Gunduz O (2021) Functional role of crosslinking in alginate scaffold for drug delivery and tissue engineering: a review. *Eur Polym J* 160:110807. <https://doi.org/10.1016/j.eurpolymj.2021.110807>
- Ahmed A, Getti G, Boateng J (2018) Ciprofloxacin-loaded calcium alginate wafers prepared by freeze-drying technique for potential healing of chronic diabetic foot ulcers. *Drug Deliv Transl Res* 8:1751–1768. <https://doi.org/10.1007/s13346-017-0445-9>
- Alshhab A, Yilmaz E (2020) Sodium alginate/poly(4-vinylpyridine) polyelectrolyte multilayer films: preparation, characterization and ciprofloxacin HCl release. *Int J Biol Macromol* 147: 809–820. <https://doi.org/10.1016/j.ijbiomac.2019.10.058>
- Ataide J, Cefali L, Rebelo M, Spir L, Tambourgi E, Jozala A, Chaud M, Silveira E, Gu X, Gava Mazzola P (2017) Bromelain loading and release from a hydrogel formulated using alginate and Arabic gum. *Planta Med* 83:870–876. <https://doi.org/10.1055/s-0043-102948>
- Banks SR, Enck K, Wright M, Opara EC, Welker ME (2019) Chemical modification of alginate for controlled oral drug delivery. *J Agric Food Chem* 67:10481–10488. <https://doi.org/10.1021/acs.jafc.9b01911>
- Benson HAE, Grice JE, Mohammed Y, Namjoshi S, Roberts MS (2019) Topical and transdermal drug delivery: from simple potions to smart technologies. *Curr Drug Deliv* 16:444–460. <https://doi.org/10.2174/1567201816666190201143457>
- Bouwstra JA, Ponc M (2006) The skin barrier in healthy and diseased state. *Biochim Biophys Acta* 1758:2080–2095. <https://doi.org/10.1016/j.bbamem.2006.06.021>
- Camargo LG, de Freitas Rosa Remiro P, Rezende GS, Di Carla Santos S, Franz-Montan M, Moraes ÂM (2021) Development of bioadhesive polysaccharide-based films for topical release of the immunomodulatory agent imiquimod on oral mucosa lesions. *Eur Polym J* 151:110422. <https://doi.org/10.1016/j.eurpolymj.2021.110422>
- Chen C-C, Fang C-L, Al-Suwayeh S, Leu Y-L, Fang J-Y (2011) Transdermal delivery of selegiline from alginate-pluronic composite thermogels. *Int J Pharm* 415:119–128. <https://doi.org/10.1016/j.ijpharm.2011.05.060>

- Chen Y, Lu C, Shen M, Lin S, Chen C, Chuang C, Ho C (2019) In vitro evaluation of the hyaluronic acid/alginate composite powder for topical haemostasis and wound healing. *Int Wound J* 17: 394–404. <https://doi.org/10.1111/iwj.13285>
- Chiaoprakobkij N, Suwanmajo T, Sanchavanakit N, Phisalaphong M (2020) Curcumin-loaded bacterial cellulose/alginate/gelatin as a multifunctional biopolymer composite film. *Molecules* 25:3800. <https://doi.org/10.3390/molecules25173800>
- Chou H-Y, Weng C-C, Lai J-Y, Lin S-Y, Tsai H-C (2020) Design of an interpenetrating polymeric network hydrogel made of calcium-alginate from a thermos-sensitive pluronic template as a thermal-ionic reversible wound dressing. *Polymers* 12:2138. <https://doi.org/10.3390/polym12092138>
- Cirri M, Maestrelli F, Scuota S, Bazzucchi V, Mura P (2021) Development and microbiological evaluation of chitosan and chitosan-alginate microspheres for vaginal administration of metronidazole. *Int J Pharm* 598:120375. <https://doi.org/10.1016/j.ijpharm.2021.120375>
- Costa JR, Silva NC, Sarmiento B, Pintado M (2015) Potential chitosan-coated alginate nanoparticles for ocular delivery of daptomycin. *Eur J Clin Microbiol Infect Dis* 34:1255–1262. <https://doi.org/10.1007/s10096-015-2344-7>
- de Bastiani FWMS, de Castro Spadari C, de Matos JKR, Salata GC, Lopes LB, Ishida K (2020) Nanocarriers provide sustained antifungal activity for amphotericin B and miltefosine in the topical treatment of murine vaginal candidiasis. *Front Microbiol* 10:2976
- Demir YK, Akan Z, Kerimoglu O (2013) Sodium alginate microneedle arrays mediate the transdermal delivery of bovine serum albumin. *PLoS One* 8:e63819. <https://doi.org/10.1371/journal.pone.0063819>
- Dhat S, Naik SR, Agharkar A, Parimal K (2009) Vitamin E loaded pectin alginate microspheres for cosmetic application. *J Pharm Res* 2:1098–1102
- Dodero A, Alloisio M, Castellano M, Vicini S (2020) Multilayer alginate–polycaprolactone electrospun membranes as skin wound patches with drug delivery abilities. *ACS Appl Mater Interfaces* 12:31162–31171. <https://doi.org/10.1021/acsami.0c07352>
- El Moussaoui S, Fernández-Campos F, Alonso C, Limón D, Halbaut L, Garduño-Ramírez ML, Calpena AC, Mallandrich M (2021) Topical mucoadhesive alginate-based hydrogel loading ketorolac for pain management after pharmacotherapy, ablation, or surgical removal in condyloma acuminata. *Gels* 7:8. <https://doi.org/10.3390/gels7010008>
- Eldeeb AE, Salah S, Amer MS, Elkasabgy NA (2022) 3D nanocomposite alginate hydrogel loaded with pitavastatin nanovesicles as a functional wound dressing with controlled drug release; preparation, in-vitro and in-vivo evaluation. *J Drug Deliv Sci Technol* 71:103292. <https://doi.org/10.1016/j.jddst.2022.103292>
- Elmowafy M, Shalaby K, Salama A, Soliman GM, Alruwaili NK, Mostafa EM, Mohammed EF, Moustafa AEGA, Zafar A (2019) Soy isoflavone-loaded alginate microspheres in thermosensitive gel base: attempts to improve wound-healing efficacy. *J Pharm Pharmacol* 71:774–787. <https://doi.org/10.1111/jphp.13066>
- Esentürk İ, Balkan T, Özhan G, Döşler S, Güngör S, Erdal MS, Sarac AS (2020) Voriconazole incorporated nanofiber formulations for topical application: preparation, characterization and antifungal activity studies against *Candida* species. *Pharm Dev Technol* 25:440–453. <https://doi.org/10.1080/10837450.2019.1706563>
- Faragò S, Lucconi G, Perteghella S, Viganì B, Tripodo G, Sorrenti M, Catenacci L, Boschi A, Faustini M, Vigo D, Chlapanidas T, Marazzi M, Torre ML (2016) A dry powder formulation from silk fibroin microspheres as a topical auto-gelling device. *Pharm Dev Technol* 21:453–462. <https://doi.org/10.3109/10837450.2015.1022784>
- Farid MR, Wen MM (2017) Promote recurrent aphthous ulcer healing with low dose prednisolone bilayer mucoadhesive buccal film. *Curr Drug Deliv* 14:123–135
- Gilhotra RM, Nagpal K, Mishra DN (2011) Azithromycin novel drug delivery system for ocular application. *Int J Pharm Investig* 1:22–28. <https://doi.org/10.4103/2230-973X.76725>
- Hardy A, Seguin C, Brion A, Lavalle P, Schaaf P, Fournel S, Bourel-Bonnet L, Frisch B, De Giorgi M (2018) β -cyclodextrin-functionalized chitosan/alginate compact polyelectrolyte complexes

- (CoPECs) as functional biomaterials with anti-inflammatory properties. *ACS Appl Mater Interfaces* 10:29347–29356. <https://doi.org/10.1021/acsami.8b09733>
- Hay I, Rehman Z, Moradali M, Wang Y, Rehm B (2013) Microbial alginate production, modification and its applications. *Microb Biotechnol* 6:637–650. <https://doi.org/10.1111/1751-7915.12076>
- Hu C, Lu W, Mata A, Nishinari K, Fang Y (2021) Ions-induced gelation of alginate: mechanisms and applications. *Int J Biol Macromol* 177:578–588. <https://doi.org/10.1016/j.ijbiomac.2021.02.086>
- Ibrahim MM, Abd-Elgawad A-EH, Soliman OA-E, Jablonski MM (2013) Natural bioadhesive biodegradable nanoparticles-based topical ophthalmic formulations for sustained celecoxib release: in vitro study. *J Pharm Technol Drug Res* 2:7. <https://doi.org/10.7243/2050-120X-2-7>
- Ibrahim MM, Abd-Elgawad A-EH, Soliman OA-E, Jablonski MM (2015) Natural bioadhesive biodegradable nanoparticle-based topical ophthalmic formulations for management of glaucoma. *Transl Vis Sci Technol* 4:12. <https://doi.org/10.1167/tvst.4.3.12>
- Jain T, Jain V, Pandey R, Vyas A, Shukla SS (2009) Microwave assisted extraction for phytoconstituents—an overview. *Asian J Res Chem* 2:19–25
- Jeong S, Kim B, Park M, Ban E, Lee S-H, Kim A (2020) Improved diabetic wound healing by EGF encapsulation in gelatin-alginate coacervates. *Pharmaceutics* 12:E334. <https://doi.org/10.3390/pharmaceutics12040334>
- Karimi-Khorrami N, Radi M, Amiri S, Abedi E, McClements DJ (2022) Fabrication, characterization, and performance of antimicrobial alginate-based films containing thymol-loaded lipid nanoparticles: comparison of nanoemulsions and nanostructured lipid carriers. *Int J Biol Macromol* 207:801–812. <https://doi.org/10.1016/j.ijbiomac.2022.03.149>
- Kaur P, Gondil VS, Chhibber S (2019) A novel wound dressing consisting of PVA-SA hybrid hydrogel membrane for topical delivery of bacteriophages and antibiotics. *Int J Pharm* 572: 118779. <https://doi.org/10.1016/j.ijpharm.2019.118779>
- Khampieng T, Aramwit P, Supaphol P (2015) Silk sericin loaded alginate nanoparticles: preparation and anti-inflammatory efficacy. *Int J Biol Macromol* 80:636–643. <https://doi.org/10.1016/j.ijbiomac.2015.07.018>
- Kim JO, Park JK, Kim JH, Jin SG, Yong CS, Li DX, Choi JY, Woo JS, Yoo BK, Lyoo WS, Kim J-A, Choi H-G (2008) Development of polyvinyl alcohol–sodium alginate gel-matrix-based wound dressing system containing nitrofurazone. *Int J Pharm* 359:79–86. <https://doi.org/10.1016/j.ijpharm.2008.03.021>
- Kim H-J, Son JS, Kwon T-Y (2018) Antifungal effect of a dental tissue conditioner containing nystatin-loaded alginate microparticles. *J Nanosci Nanotechnol* 18:848–852. <https://doi.org/10.1166/jnn.2018.14866>
- Kıvılcımdan Moral Ç, Yıldız M (2016) Alginate production from alternative carbon sources and use of polymer based adsorbent in heavy metal removal. *Int J Polym Sci* 2016:1–8. <https://doi.org/10.1155/2016/7109825>
- Kotroni E, Simirioti E, Kikionis S, Sfniadakis I, Siamidi A, Karalis V, Vitsos A, Vlachou M, Ioannou E, Roussis V, Rallis M (2019) In vivo evaluation of the anti-inflammatory activity of electrospun micro/nanofibrous patches loaded with *Pinus halepensis* bark extract on hairless mice skin. *Materials (Basel)* 12:2596. <https://doi.org/10.3390/ma12162596>
- Kumar M, Shanthi N, Mahato AK (2019) Pharmaceutical drug nanocrystals: role in dermal delivery. *Nanosci Nanotechnol Asia* 9:300–310
- Kumar M, Jha A, Mishra B (2022) Marine biopolymers for transdermal drug delivery. In: Jana S, Jana S (eds) *Marine biomaterials: drug delivery and therapeutic applications*. Springer Nature, Singapore, pp 157–207. https://doi.org/10.1007/978-981-16-4787-1_6
- Kyzioł A, Michna J, Moreno I, Gamez E, Irusta S (2017) Preparation and characterization of electrospun alginate nanofibers loaded with ciprofloxacin hydrochloride. *Eur Polym J* 96:350–360. <https://doi.org/10.1016/j.eurpolymj.2017.09.020>
- Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. *Prog Polym Sci* 37: 106–126. <https://doi.org/10.1016/j.progpolymsci.2011.06.003>

- LeRoux MA, Guilak F, Setton LA (1999) Compressive and shear properties of alginate gel: effects of sodium ions and alginate concentration. *J Biomed Mater Res* 47:46–53. [https://doi.org/10.1002/\(SICI\)1097-4636\(199910\)47:1<46::AID-JBM6>3.0.CO;2-N](https://doi.org/10.1002/(SICI)1097-4636(199910)47:1<46::AID-JBM6>3.0.CO;2-N)
- Lopodota A, Denora N, Laquintana V, Cutrignelli A, Lopalco A, Tricarico D, Maquod F, Curci A, Mastrodonato M, la Forgia F, Fontana S, Franco M (2018) Alginate-based hydrogel containing minoxidil/hydroxypropyl- β -cyclodextrin inclusion complex for topical alopecia treatment. *J Pharm Sci* 107:1046–1054. <https://doi.org/10.1016/j.xphs.2017.11.016>
- Maatouk B, Jaffa MA, Karam M, Fahs D, Nour-Eldine W, Hasan A, Jaffa AA, Mhanna R (2021) Sulfated alginate/polycaprolactone double-emulsion nanoparticles for enhanced delivery of heparin-binding growth factors in wound healing applications. *Colloids Surf B Biointerfaces* 208:112105. <https://doi.org/10.1016/j.colsurfb.2021.112105>
- Maestrelli F, Jug M, Cirri M, Kosalec I, Mura P (2018) Characterization and microbiological evaluation of chitosan-alginate microspheres for cefixime vaginal administration. *Carbohydr Polym* 192:176–183. <https://doi.org/10.1016/j.carbpol.2018.03.054>
- Mishra R, Joshi P, Mehta T (2016) Formulation, development and characterization of mucoadhesive film for treatment of vaginal candidiasis. *Int J Pharm Investig* 6:47–55. <https://doi.org/10.4103/2230-973X.176487>
- Motwani SK, Chopra S, Talegaonkar S, Kohli K, Ahmad FJ, Khar RK (2008) Chitosan-sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: formulation, optimisation and in vitro characterisation. *Eur J Pharm Biopharm* 68:513–525. <https://doi.org/10.1016/j.ejpb.2007.09.009>
- Muthalakshmi L, Prabakaran S, Ramalingam V, Rajulu AV, Rajan M, Ramakrishna S, Luo H (2022) Sodium alginate nanofibers loaded Terminalia catappa scaffold regulates intrinsic apoptosis signaling in skin melanoma cancer. *Process Biochem* 118:92–102. <https://doi.org/10.1016/j.procbio.2022.04.004>
- Nagarwal RC, Kumar R, Pandit JK (2012) Chitosan coated sodium alginate–chitosan nanoparticles loaded with 5-FU for ocular delivery: in vitro characterization and in vivo study in rabbit eye. *Eur J Pharm Sci* 47:678–685. <https://doi.org/10.1016/j.ejps.2012.08.008>
- Ng S-F, Tan L-S, Buang F (2016) Transdermal anti-inflammatory activity of bilayer film containing olive compound hydroxytyrosol: physical assessment, in vivo dermal safety and efficacy study in Freund's adjuvant-induced arthritic rat model. *Drug Dev Ind Pharm* 43:1–46. <https://doi.org/10.1080/03639045.2016.1224893>
- Ng W, Migotto A, Ferreira T, Lopes L (2017) Monoolein-alginate beads as a platform to promote adenosine cutaneous localization and wound healing. *Int J Biol Macromol* 102:1104–1111. <https://doi.org/10.1016/j.ijbiomac.2017.04.094>
- Nuutila K, Grolman J, Yang L, Broomhead M, Lipsitz S, Onderdonk A, Mooney D, Eriksson E (2020) Immediate treatment of burn wounds with high concentrations of topical antibiotics in an alginate hydrogel using a platform wound device. *Adv Wound Care (New Rochelle)* 9:48–60. <https://doi.org/10.1089/wound.2019.1018>
- Okeke OC, Boateng JS (2016) Composite HPMC and sodium alginate based buccal formulations for nicotine replacement therapy. *Int J Biol Macromol* 91:31–44. <https://doi.org/10.1016/j.ijbiomac.2016.05.079>
- Pagano C, Puglia D, Luzi F, Michele AD, Scuota S, Primavilla S, Ceccarini MR, Beccari T, Iborra CAV, Ramella D, Ricci M, Peroli L (2021) Development and characterization of xanthan gum and alginate based bioadhesive film for pycnogenol topical use in wound treatment. *Pharmaceutics* 13:324. <https://doi.org/10.3390/pharmaceutics13030324>
- Patel SK, Shah DR, Tiwari S (2015) Bioadhesive films containing fluconazole for mucocutaneous candidiasis. *Indian J Pharm Sci* 77:55–61
- Peng L-H, Chen X, Chen L, Li A, Liang W, Gao J-Q (2012) Topical astragaloside iv-releasing hydrogel improves healing of skin wounds in vivo. *Biol Pharm Bull* 35:881–888. <https://doi.org/10.1248/bpb.35.881>

- Pereira GG, Guterres SS, Balducci AG, Colombo P, Sonvico F (2014) Polymeric films loaded with vitamin E and aloe vera for topical application in the treatment of burn wounds. *Bio Med Res Int* 2014:e641590. <https://doi.org/10.1155/2014/641590>
- Prajapati B, Raichandani Y, Patel R, Sawant K (2007) Chitosan alginate film for topical drug delivery of antimicrobial agents. *Curr Pharma Res J* 1(03):66–74
- Remminghorst U, Rehm BHA (2006) In vitro alginate polymerization and the functional role of Alg8 in alginate production by *Pseudomonas aeruginosa*. *Appl Environ Microbiol* 72:298–305. <https://doi.org/10.1128/AEM.72.1.298-305.2006>
- Rezvani M, Amin MCIM, Ng S-F (2016) Development and physicochemical characterization of alginate composite film loaded with simvastatin as a potential wound dressing. *Carbohydr Polym* 137:295–304. <https://doi.org/10.1016/j.carbpol.2015.10.091>
- Roquero DM, Katz E (2022) “Smart” alginate hydrogels in biosensing, bioactuation and biocomputing: state-of-the-art and perspectives. *Sens Actuators Rep* 4:100095. <https://doi.org/10.1016/j.snrs.2022.100095>
- Ruslan R, Amir A, Wiraningtyas A (2019) Extraction of sodium alginate from sargassum sp. using microwave-assisted extraction (MAE). *J Pure Appl Chem Res* 8:23–30
- Sarheed O, Dibi M, Ramesh KVRNS, Drechsler M (2021) Fabrication of alginate-based O/W nanoemulsions for transdermal drug delivery of lidocaine: influence of the oil phase and surfactant. *Molecules* 26:2556. <https://doi.org/10.3390/molecules26092556>
- Severino P, da Silva CF, Andrade LN, de Lima Oliveira D, Campos J, Souto EB (2019) Alginate nanoparticles for drug delivery and targeting. *Curr Pharm Des* 25:1312–1334. <https://doi.org/10.2174/1381612825666190425163424>
- Shahzad A, Khan A, Afzal Z, Umer MF, Khan J, Khan GM (2019) Formulation development and characterization of cefazolin nanoparticles-loaded cross-linked films of sodium alginate and pectin as wound dressings. *Int J Biol Macromol* 124:255–269. <https://doi.org/10.1016/j.ijbiomac.2018.11.090>
- Shi M, Zhang H, Song T, Liu X, Gao Y, Zhou J, Li Y (2019) Sustainable dual release of antibiotic and growth factor from pH-responsive uniform alginate composite microparticles to enhance wound healing. *ACS Appl Mater Interfaces* 11:22730–22744
- Shinde UA, Shete JN, Nair HA, Singh KH (2014) Design and characterization of chitosan-alginate microspheres for ocular delivery of azelastine. *Pharm Dev Technol* 19:813–823. <https://doi.org/10.3109/10837450.2013.836217>
- Singha K, Pandit P, Maity S (2021) Applications of alginate-based bionanocomposites in drug delivery. In: Ahmed S, Annu (eds) *Bionanocomposites in tissue engineering and regenerative medicine*, Woodhead publishing series in biomaterials. Woodhead Publishing, Duxford, pp 399–416. <https://doi.org/10.1016/B978-0-12-821280-6.00015-5>
- Swain S, Behera A, Beg S, Patra CN, Dinda SC, Sruti J, Rao MEB (2012) Modified alginate beads for mucoadhesive drug delivery system: an updated review of patents. *Recent Pat Drug Deliv Formul* 6:259–277. <https://doi.org/10.2174/187221112802652697>
- Urzedo AL, Gonçalves MC, Nascimento MHM, Lombello CB, Nakazato G, Seabra AB (2020) Cytotoxicity and antibacterial activity of alginate hydrogel containing nitric oxide donor and silver nanoparticles for topical applications. *ACS Biomater Sci Eng* 6:2117–2134. <https://doi.org/10.1021/acsbomaterials.9b01685>
- Uyen NTT, Hamid ZAA, Tram NXT, Ahmad N (2020) Fabrication of alginate microspheres for drug delivery: a review. *Int J Biol Macromol* 153:1035–1046. <https://doi.org/10.1016/j.ijbiomac.2019.10.233>
- Valentine ME, Kirby BD, Withers TR, Johnson SL, Long TE, Hao Y, Lam JS, Niles RM, Yu HD (2020) Generation of a highly attenuated strain of *Pseudomonas aeruginosa* for commercial production of alginate. *Microb Biotechnol* 13:162–175. <https://doi.org/10.1111/1751-7915.13411>
- Wang F, He S, Chen B (2018) Retinoic acid-loaded alginate microspheres as a slow release drug delivery carrier for intravitreal treatment. *Biomed Pharmacother* 97:722–728. <https://doi.org/10.1016/j.biopha.2017.10.109>

- Wang T, Wang J, Wang R, Yuan P, Fan Z, Yang S (2019) Preparation and properties of ZnO/sodium alginate bi-layered hydrogel films as novel wound dressings. *New J Chem* 43: 8684–8693. <https://doi.org/10.1039/C9NJ00402E>
- Watanabe S, Suemaru K, Takechi K, Kaji H, Imai K, Araki H (2013) Oral mucosal adhesive films containing royal jelly accelerate recovery from 5-fluorouracil-induced oral mucositis. *J Pharmacol Sci* 121(2):110–118. <https://doi.org/10.1254/jphs.12181FP>
- Weber DE, Semaan MT, Wasman JK, Beane R, Bonassar LJ, Megerian CA (2006) Tissue-engineered calcium alginate patches in the repair of chronic chinchilla tympanic membrane perforations. *Laryngoscope* 116:700–704. <https://doi.org/10.1097/01.mlg.0000208549.44462.fa>
- Wee S, Gombotz WR (1998) Protein release from alginate matrices. *Adv Drug Deliv Rev* 31:267–285. [https://doi.org/10.1016/s0169-409x\(97\)00124-5](https://doi.org/10.1016/s0169-409x(97)00124-5)
- Weijiang Y, Jiang G, Zhang Y, Liu D, Xu B, Zhou J (2017) Polymer microneedles fabricated from alginate and hyaluronate for transdermal delivery of insulin. *Mater Sci Eng C* 80:187–196. <https://doi.org/10.1016/j.msec.2017.05.143>
- Yehia SA, El-Gazayerly ON, Basalious EB (2009) Fluconazole mucoadhesive buccal films: in vitro/in vivo performance. *Curr Drug Deliv* 6:17–27. <https://doi.org/10.2174/156720109787048195>
- Yoncheva K, Vandervoort J, Ludwig A (2011) Development of mucoadhesive poly(lactide-co-glycolide) nanoparticles for ocular application. *Pharm Dev Technol* 16:29–35. <https://doi.org/10.3109/10837450903479954>
- Youssef L, Lallemand L, Giraud P, Soulé F, Bhaw-Luximon A, Meilhac O, D’Hellencourt CL, Jhurry D, Couprie J (2017) Ultrasound-assisted extraction and structural characterization by NMR of alginates and carrageenans from seaweeds. *Carbohydr Polym* 166:55–63. <https://doi.org/10.1016/j.carbpol.2017.01.041>
- Zhang Y, Jiang G, Yu W, Liu D, Xu B (2018) Microneedles fabricated from alginate and maltose for transdermal delivery of insulin on diabetic rats. *Mater Sci Eng C* 85:18–26. <https://doi.org/10.1016/j.msec.2017.12.006>
- Zhou Z, Xing M, Zhang S, Yang G, Gao Y (2022) Process optimization of Ca²⁺ cross-linked alginate-based swellable microneedles for enhanced transdermal permeability: more applicable to acidic drugs. *Int J Pharm* 618:121669. <https://doi.org/10.1016/j.ijpharm.2022.121669>

Alginate Based Hydrogel in Drug Delivery and Biomedical Applications



Suchita Dattatray Shinde, Neeraj Kulkarni, Govinda Shivaji Jadhav, Bhaskar Dewangan, Stephin Baby, Salil Pophali, and Bichismita Sahu

Abstract Alginates are polysaccharides resourced from natural sources such as brown algae and bacteria. Alginate is a natural, biodegradable, biocompatible and non-toxic polymer possessing numerous specific physicochemical properties. These properties are responsible for its wide applications in the emerging area of biomedical sciences. Alginates undergo crosslinking with di- or tri-valent metal ions at room temperature to form uniform, transparent, thermo-irreversible gels which are insoluble in water. These properties also help in the preparation of various formulations of Alginate. Formulation of Alginate hydrogel, can be accomplished by employing physical or chemical cross-linking strategies. Alginate hydrogels owing to their inherent properties encompass tremendous potential and will have wide applications in Drug Delivery as well as Biomedical sciences. Therefore, in view of the vast literature support, we have discussed the applications of alginate hydrogels in drug delivery as well as biomedical sciences. Various properties of alginates, their hydrogels along with the various techniques employed for fabricating alginate hydrogels have been reviewed.

Keywords Alginate · Hydrogel · Chemical and physical cross-linking · Drug delivery

1 Introduction

Alginates are polysaccharides obtained from marine sources like brown algae (*Ascophyllum nodosum*, *Laminaria Hyperborean*, *Macrocystis pyrifera*) and bacteria (*Azotobacter vinelandii*, *Pseudomonas spp.*) in which alginates account for up to 40% of its dry weight (Zhang et al. 2020a). Alginates are biocompatible and biodegradable as well as rich in sources, non-toxic, gelable and abundant natural

S. D. Shinde · N. Kulkarni · G. S. Jadhav · B. Dewangan · S. Baby · S. Pophali · B. Sahu (✉)
Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gujarat, India
e-mail: bichismita@niperahm.res.in

polymer (Hernández-González et al. 2020). Alginates have an ability to form various drug delivery forms like hydrogel, fibers, sponges, microspheres and composite porous scaffolds (Zhang et al. 2021; Uyen et al. 2020). All these properties of alginates make it a very promising polymer in the area of pharmaceutical biomaterials and biomedical applications. In this book chapter these two applications with only hydrogel form of alginates have been covered.

1.1 Extraction of Alginates

The term alginates are usually used for the all alginic acid derivatives and its salt forms. Acidic condition attained using dilute HCl is employed for the extraction of alginates from algae and bacteria. NaCl, CaCl₂ or MgCl₂ salts are further added to the isolated extracts which results in precipitation of sodium alginate, calcium alginate or magnesium alginate. Free acid can be obtained by treating salts with dilute acid followed by its purification and residual solvent evaporation by lyophilization (Rinaudo 2008; Fenoradosoa et al. 2010; Zhang et al. 2021).

1.2 Chemical Compositions and Structures of Alginate

Alginates essentially consist of two monomers viz., β -D-mannuronic acid (M) and α -L-guluronic acid (G), which are linked through 1,4-glycosidic bond. Depending upon natural sources of alginates, its proportion, occurrence and distribution varies resulting in homopolymeric blocks like GG or MM which are interspersed with heteropolymeric blocks like GM or MG (Zhong et al. 2020) (Fig. 1).

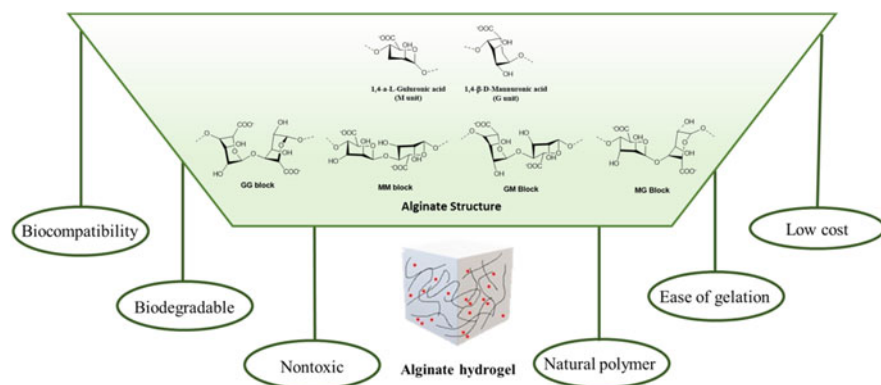


Fig. 1 Structure of block distribution of alginates with its monomer and Properties of Alginate

1.3 Physicochemical Properties of Alginates

Alginate has varying molecular weight, with its commercial values ranging between 33,000–400,000 g/mol. Increase in molecular weight of alginates affects its physical properties like gelation, viscosity and solubility. High molecular weight alginates are comparatively more viscous than low molecular weight alginates (Zhang et al. 2021). Viscosity of alginate depends upon the pH of the solution in which alginate dissolves. As the pH of alginate solution decreases, its viscosity increases and vice versa. The carboxylate groups play a chief role in governing the viscosity of alginate due to its protonation ability. Solubility of polymer is a very important physiochemical property. Monovalent salts of alginates are soluble in water and organic solvents while alginic acid and esters are insoluble. Alginates are used in various pharmaceutical and food industries as a stabilizer, thickening agent in suspension and emulsions and a viscosity enhancer due to its ability to transition from sol to gel (Abasalizadeh et al. 2020). Alginates form hydrogel with metal ions. Alginate gel or precipitates are formed with divalent and multivalent metal ions whereas mono valent ions form salts with it which are water soluble. α -L-guluronic acid (G) residues show a stronger affinity towards divalent ions than β -D-mannuronic acid (M) thus the gel of G blocks possesses higher mechanical strength than the M block (Giri et al. 2012).

1.4 Various Formulations of Alginates

There are different forms of alginates for drug delivery and biomedical applications. Here, we are discussing about few like hydrogel, microsphere and fiber.

1.4.1 Hydrogels

Hydrogel is the three-dimensional structure of hydrophilic material crosslinked in an aqueous medium. The alginate hydrogel finds numerous biomedical applications in drug delivery, wound healing, and tissue engineering (Reakasame and Boccaccini 2018; Zhang and Zhao 2020). Methods employed for preparing alginate hydrogels include ionic interactions, covalent crosslinking and thermal gelation (Lee and Mooney 2012). Most common method employed for gelation is ionic crosslinking under mild condition with multiple cations such as Ca^{2+} and Sr^{2+} (Zhang et al. 2020b). Another method for hydrogel formation is covalent crosslinking through which polycondensation reaction or copolymerization reaction gels were formed by using crosslinking agents, which improves the physical properties of hydrogels (Eiselt et al. 1999). Cell-crosslinking includes the specific receptor-ligand interactions for the hydrogel formation. As this method includes the crosslinking with cells to form gel some anchoring and adhesion molecules like RGD (Arg-Gly-Asp)

tripeptide are coupled with alginate by coupling reactions. When the solution of such alginates is prepared and cells are suspended in to it then by receptor-ligand interaction gels are formed which exhibit excellent bioactivity in tissue engineering approaches (Yan et al. 2016; Lee et al. 2003). Thermal gelation method is used to form thermosensitive hydrogels for drug delivery purpose. Poly (N-isopropylacrylamide) (PNIPAAm) is a thermosensitive polymer, incorporation of such materials into alginate results in formation of thermosensitive alginate hydrogels (Wang et al. 2009; Rzaev et al. 2007).

1.4.2 Microsphere

Alginate microspheres find their use as delivery agent for various therapeutic agents such as, drugs, growth factors and cells (Basmanav et al. 2008; Supramaniam et al. 2018). Alginate microspheres are of two types, gel microsphere and solid microsphere. Ionic crosslinking in an aqueous media produces alginate gel-microspheres, which is useful for entrapment of biologically active agents such as cells, growth factors and protein (Man et al. 2012; Chen and Subirade 2006). Whereas the preparation of alginate solid microspheres involves the emulsion solvent evaporation techniques and are used for drug entrapment. This technique is further divided into two, external and internal emulsification method (Babu et al. 2007; Chuah et al. 2009).

1.4.3 Fiber

Alginate fibers find their use mainly as medical dressing, due to high oxygen permeability, hygroscopicity, better biodegradability and biocompatibility. Generally, the raw material is obtained from seaweed (Aderibigbe and Buyana 2018). Fibers can be prepared by wet spinning method and the alginate nanofibers are fabricated by electrospinning method. These nanofibers possess high surface area with large porosity which mimics the extracellular matrix and promotes epithelial cell growth and proliferation thereby aiding tissue regeneration (Abrigo et al. 2014). Apart from this it also promotes hemostasis of damaged tissues, high gas penetration, cell penetration, enhance liquid absorption, and aids drug delivery thereby preventing bacterial infections (Homaeigohar and Boccaccini 2020).

2 Alginate Based Hydrogels

Alginate hydrogels can be produced by utilizing two approaches: noncovalent (physical cross-linking) (Hennink and van Nostrum 2012) and covalent (chemical cross-linking) (Patil et al. 1997) (Fig. 2). The physicochemical properties of the hydrogels are governed by the type of structure, its composition and molecular

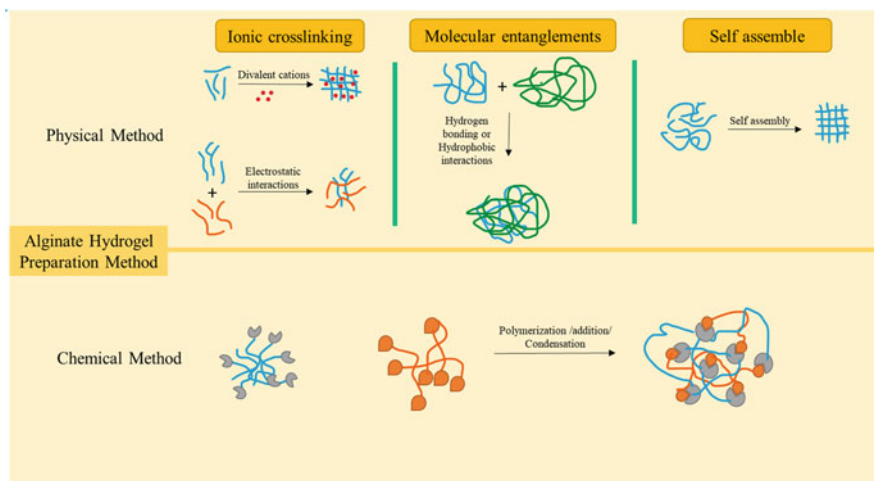


Fig. 2 Methods of Preparation of Alginate Hydrogels

weight. In case of physically cross-linked hydrogels, there is a physical interaction between the polymeric chains which in turn prevents the dissolution of hydrogel matrix. Whereas, in the case of chemically cross-linked hydrogels, there is a generation of covalent linkages among the polymeric chains. Various chemical reagents are employed as cross-linking agents for this purpose (Hasnain and Nayak 2019). Here, different preparation methods/strategies/techniques for cross-linked alginate hydrogels and their subsequent effects on the properties of hydrogel pertinent to biomedical applications have been summarized.

2.1 Physical Hydrogels

Physical or Non-covalent crosslinked alginate hydrogels fabrication is normally achieved by freeze-thawing, lower pH, anionic and cationic polymers, and the fabrication methods of such hydrogels are gentle and straightforward (Dimatteo et al. 2018). The interactions between the polymeric chains comprise of the relatively weak electrostatic interaction, hydrogen bonding, and hydrophobic interaction. In this case, the strength of the hydrogel network mainly depends on the pH, temperature, or organic solvent that is being used. Chemical crosslinking agents are not or rarely employed for the synthesis (Hasnain and Nayak 2019).

2.1.1 By Ionic Cross-linking

Ionic cross-linked alginate hydrogel formation involves the creation of ionic crosslinking bridges within the polymer network. The ionic cross-linking involves

addition of various divalent metal cations (Ba^{2+} , Ca^{2+} and Sr^{2+}) or trivalent metal cations (Al^{3+} and Fe^{3+}) to the aqueous alginate solution resulting in formation of physical cross-linkages (Racoviță et al. 2009). Thus, the ionic interactions of these divalent or trivalent metal cations with alginate solution are responsible for the formation of alginate hydrogels. The intramolecular ionic interactions between the carboxylic acid functionality ($-\text{COOH}$) chiefly present in the guluronate blocks and di/trivalent metal cations affords the formation of an insoluble gel network (Gaumann et al. 2000).

Apart from the G blocks, M and MG blocks are also involved in the formation of weak junctions. The divalent Ca^{2+} cations can bind to G block and MG blocks, Ba^{2+} cations bind to G blocks and M blocks, whereas the Sr^{2+} ions bind with the G blocks. The trivalent lanthanide ions (e.g., La^{3+}) are capable of binding with GG and MM blocks (DeRamos et al. 1997). Highly toxic metal cations (e.g., Pb^{2+} , Cu^{2+} , and Cd^{2+}) are rarely employed whereas the slightly toxic ions, such as Sr^{2+} and Ba^{2+} , are generally employed at low concentrations for cellular immobilizations. The M/G ratio can be varied to modulate the mechanical strength and gelling capability of alginate hydrogels (Sun et al. 2016). Electrostatic interactions allow alginate to crosslink with other ionic compounds. Since the ionic crosslinking is reversible, the swelling of ionically crosslinked hydrogels is pH-dependent. The green fabrication imparts more biocompatibility to the hydrogel, but may impact the long-term stability of the hydrogels (Kuo and Ma 2001).

The electrostatic interactions between the divalent cations and the G blocks of alginate structure afford the formation of ionic gels of alginate. Interlinking of guluronate from one block with another guluronate block from adjacent polymer chains occurs through the ionic interaction between guluronic acid groups (Fundueanu et al. 1999). The vander Waals forces between the alginate segments results in a 3D ionically gelled network because of the creation of an electronegative cavity with hydrophilic group, which binds Ca^{2+} cations through the coordination using more oxygen atoms than the carboxyl ($-\text{COO}^-$) groups. Such tightly bound polymeric configuration controls the formation of an “egg-box” model, which ultimately results in a gel structure. Internal or external gelations, as well as cooling, are commonly used to generate ionic cross-linking of alginate. The methodologies differ in the mannerism of interaction of cross-linking ions with the anionic site of the alginate molecules (Chan et al. 2006).

2.1.2 By Hydrogen Bonding

Hydrogen bonds are formed between two polar groups, usually a hydrogen atom and a highly electronegative atom, such as nitrogen, oxygen, and halogens (Hasnain and Nayak 2019). The hydrogen bonds are generally weak relative to covalent bonding, ionic bonding. Hydrogen bonding interactions contribute towards the improvement of the bonding strength resulting in formation of hydrogel. In this case, generally, alginates are mixed with other polymers like Polyvinyl Alcohol (PVA), Polyvinylpyrrolidone (PVP) or Carboxymethyl Cellulose (CMC) leading to the

molecular entanglement and hydrogel bonding between these polymers which ultimately leads to the formation of hydrogel (Zhang and Zhao 2020).

2.1.3 By Polyelectrolyte Complexation

Alginate gels can also be fabricated by utilizing the alginate electrostatic complex formation capacity. Polycations like chitosan, poly-L-lysine, and albumin have already been exploited for the complexation. This method is devoid of organic precursors, catalysts, or reactive agents which eliminates the possibility of cross-reactions with a therapeutic entity to be delivered. Sarmento et al. mixed a low content solution of CaCl_2 (18 mM) with a solution containing precalculated amount of sodium alginate with constant agitation to afford the synthesis of network of alginate-based polyelectrolyte complexes. The addition of calcium chloride solution (18 mM) initiated the cross-linking of the sodium alginate, whereas the mechanical agitation caused breakdown of pregelled alginate into smaller aggregates thereby preventing formation of mass gel. After 1 h, the chitosan solution was added slowly by constant stirring in the pre-gellated alginate solution. The addition of chitosan (cationic natured) resulted in the formation a polyelectrolyte complex with alginate (anionic natured) and thus, stabilized the pre-gelled microgel in single sponge-like nanoparticles (Sarmento et al. 2006).

2.1.4 By Hydrophobic Interaction

Nonpolar hydrophobic groups commonly exhibit hydrophobic bonds, which are reversible noncovalent interactions. The purpose of the hydrophobic alteration of alginates is to impart amphiphilic or hydrophobic properties into the hydrophilic molecule. By covalently attaching hydrophobic components such as long alkyl chains or aromatic groups to the hydrophilic alginate skeleton, an amphiphilic polymer can be created (Pawar and Edgar 2012). Hydrophobic pharmaceuticals could potentially be carried in the newly generated hydrophobic cavities. The hydrophilic parts will impart aqueous solubility to the polymer, whereas the hydrophobic domains aggregate in the aqueous solutions resulting in the development of a various structures having hydrophilic and hydrophobic regions (Kashyap et al. 2005). The amide coupling reactions are generally employed for fabrication of hydrophobically modified alginate with different degree of substitution. Because of the hydrophobic contacts, these amphiphilic polymers form robust networks in the form of gel in water. The dodecyl or octadecyl chains are covalently attached to the alginate scaffold to afford hydrophobically modified alginate derivatives (Leonard et al. 2004).

2.1.5 By Self Assembly

For the construction of physical networks, spontaneous self-assembled physical interactions are commonly used. Peptides, thermally responsive polymers, and guest-host polymers are few of the compounds with capability of forming network structures. These polymer smart-blocks can self-assemble or recognize molecules in response to dynamic environment, causing their copolymer to gel in aqueous solution (Hoang Thi et al. 2020).

2.2 Chemical Hydrogels

Chemical hydrogels are formed by Covalent crosslinking and comprise a common way of hydrogel fabrication. Since the linkages formed in this are irreversible, the covalent crosslinking imparts hydrogels with high mechanical strength. The covalent linkages do not compromise the mechanical integrity which aids in the maintenance of water and drugs within the network (Berger et al. 2004). The functional groups present in alginate such as -COOH or -OH, participate in the covalent interactions and therefore, can be utilized for the formation of covalent hydrogels. The synthesis of covalently crosslinked alginate hydrogels can be afforded using various reactions viz., polymerization, addition, click chemistry, or high energy irradiations (gamma-ray or electron beam). The elimination of hydrogels implants in human body requires the intervention of chemical degradation or other strategies (Sarker et al. 2015).

2.2.1 Cross-linking by Aldehydes

Chan et al. demonstrated the development of pH-sensitive dynamically regulated alginate networking gels. To achieve the equilibrium state, thermodynamic alginate-based gels were made by aiding the cross-linking reaction. Other different cross-linkers have been utilized to allow glutaraldehyde concentrations to be toxic and limit cell development (e.g., adipic acid dihydrazide) (Chan et al. 2009). Riyajan and Sakdapipanich, developed glutaraldehyde cross-linked alginate-based gel for delivery of Neem Azadirachtin-A (Aza-A) in controlled manner. Aza-A is highly unstable an insecticide, it is easily degradable or isomerized, when exposed to light. The findings of this study revealed that Aza-A degradation was frequently inhibited by the cross-linked alginate gel's microencapsulation. The efficiency of Aza-A encapsulation within the cross-linked alginate gel was found to be a function of cross-linking duration, with efficiency decreasing as time went on (Riyajan and Sakdapipanich 2009). Poly (guluronate aldehyde), produced by oxidizing sodium periodate alginate, was converted into a hydrogel by cross-linking with proteins

(such as gelatin) in the presence of low quantities of borax (sodium tetraborate) (Bouhadir et al. 1999).

2.2.2 Cross-linking by Condensation Reactions

Condensation reactions between hydroxyl (-OH) or amino (-NH₂) groups with carboxylic acids or derivatives can be employed to afford the synthesis of polymers such as polyesters and polyamides. Condensation reactions can also be employed for the fabrication of hydrogels. Bu et al. employed a multicomponent Ugi condensation reaction wherein an aldehyde or a ketone or an amine or a carboxylic acid, and an isocyanide forms a bis-amide, to afford the synthesis of hydrophobically modified alginate-based hydrogel (Bu et al. 2004).

Lee et al. crosslinked diamine polyethylene glycol (PEG), N, N-(3-dimethylaminopropyl)-N-alginate, and ethyl carbodiimide to fabricate a covalently cross-linked alginate hydrogel. To control the properties of these alginate hydrogels, multifunctional molecules were crosslinked, allowing for a wider range and better control of degradation rates as well as mechanical strength (Lee et al. 2000). Xu et al. employed water-soluble carbodiimide chemistry to synthesize cross-linked alginate hydrogels. A covalently cross-linked matrix is formed as a result of reaction between the carboxylic acid functionalities in the alginate molecule and the hydroxyl groups (Xu et al. 2003).

2.2.3 Cross-linking by Polymerization

The hydrogel preparation is based on graft-copolymerization, which involves polymerization of a monomer in a preformed polymer scaffold. The chief advantage of process is the inculcation of different attributes/characteristics into the hydrogel due to the addition of macromers which are then/further subjected to copolymerization (Hasnain and Nayak 2019). Kulkarni et al. employed an electrical response to fabricate alginate-based hydrogels by acrylamide grafting and further subjecting the amide groups to hydrolysis (Kulkarni et al. 2010).

In recent years, UV light has also been employed to induce polymerization for the preparation of hydrogels. Under physiological conditions, a brief exposure to the ultraviolet light induced in situ photo scaling of alginate. This was carried out in direct contact with the cells under mild environment. Carbodiimide chemistry was employed to modify the alginate with 2-aminoethyl methacrylate. The resulting methacrylate alginates were exposed to ultraviolet light under the impact of a photoinitiator or for cross-linking (Jeon et al. 2011).

2.3 Alginate Hybrid Hydrogels

Apart from the above mentioned physical (non-covalently cross-linked) and chemical (covalently crosslinked) hydrogels, different/various polymeric materials can be employed to fabricate hybrid hydrogels by utilizing a blend of chemical and physical cross-linking techniques. The fabricated hybrid hydrogel can exhibit novel functions relative to the conventional hydrogels along with attributes/characteristics imparted from multiple cross-linking techniques, such as, improved mechanical and biological attributes to mimic the cellular microenvironment and tissue reorganization. Thus, hybrid hydrogels are gaining increased research prospect. Few of the main types of these hydrogels are Covalent and Non-covalent hydrogels, Ionic and photo-cross linked hydrogels and 3D printed hydrogels (Lau and Kiick 2015).

Apart from the above-mentioned methods, Enzymatic cross-linking has also been employed for the fabrication of alginate hydrogel, due to the catalytic effect, high efficiency and specificity of enzymes which results in shorter time and reduced by-products. Peroxidases are generally used in enzymatic cross-linking reactions in which horseradish peroxidase class is commonly used (Nezhad-Mokhtari et al. 2019).

3 Alginate Based Hydrogels in Drug Delivery

Drug delivery systems based on the alginate hydrogel can be broadly categorized into injectable and non-injectable. This biodegradable gel is generally nanoporous in nature and employs for encapsulate small-molecule drugs to macromolecules such as proteins, peptides, peptide hormones and polysaccharides (Ray et al. 2020). Alginate-based hydrogels are possessing attractive characteristics for a drug delivery system in comparison with the traditional drug delivery like encapsulation efficiency, low toxicity and excellent patient compliance (Kurakula et al. 2020). The alginate hydrogel gained favorable attention as drug carrier due to its unique biological and physicochemical properties. Drug release from the 3-D matrix of alginate hydrogel is attained through different mechanisms in a system and is controlled by diffusion, swelling, chemical or environmental stimuli.

3.1 Oral Drug Delivery

pH-sensitive hydrogels are useful in the oral drug delivery due to the ability of site specific and controlled release of drug (Liu et al. 2017a). Chitosan-g-poly (acrylic acid)/attapulgit (CTS-g-PAA/APT) composite hydrogel beads dispersed in the sodium alginates (SA) was a pH sensitive carrier of diclofenac sodium (DS). The cumulative release ratio of those hydrogel beads was less than 5% at pH 2.1 after

24 h whereas at the pH 6.8 cumulative release ratio could reach 100% after 24 h. Composition of APT along with polymeric network of SA could control the burst effect of CTS-g-PAA hydrogel and prolong the release of drug (Wang et al. 2008). Another pH sensitive hydrogel based on the alginate micro particles encapsulated with silymarin-loaded poly (D, L-lactic-co-glycolic) acid (PLGA) Nanoparticles (NPs) was produced. Silymarin is poorly water soluble and has low oral bioavailability. Hydrogel micro particles could achieve sustained release and enhance the dissolution of silymarin. NPs were synthesized through a modified single emulsion-solvent evaporation method followed by suspending in the SA aqueous medium. The percent release of silymarin from the hydrogel micro particles was greater in simulated intestinal fluid (SIF) as compared to simulated gastric fluid (SGF) and alginate content had a definite role of on the percent of silymarin release in the SIF (El-Sherbiny et al. 2011). Similar to the silymarin, oral hypoglycemic drug gliclazide also has poor dissolution and slow absorption rate. Entrapment of gliclazide in the gel matrix could control the release of drug through a swelling and release mechanism at various pH conditions. Swelling behavior of gliclazide loaded beads were depend on the alginate concentration and pH of the medium. At an alkaline pH of 7.2, the release rate of drug was enhanced by the swelling and erosion of ionically cross-linked beads (Al-Kassas et al. 2007). The backbone chain of SA was covalently linked by the polyacrylamide (PAAm) to produce graft PAAm-g-SA co-polymer. Graft co-polymers were synthesized by radical polymerization reactions followed by alkaline hydrolysis of PAAm amide group to acid group produced pH responsive co-polymer. Further, ketoprofen was loaded into the co-polymer matrices and cross-linked to hydrogel beads. These pH sensitive beads could increase the release of the ketoprofen in the alkaline medium (Kulkarni and Sa 2009). Even though alginate-based hydrogels were served as good drug delivery systems, there are some bottlenecks in the development carrier systems based on the alginate. The excessive porosity of Ca^{2+} or other divalent cation cross-linked alginate hydrogels rise a problem of leaching. This rapid diffusion of drugs or proteins depends on the molecular size and charge. Small molecule drugs and peptides are difficult to entrap in the gel matrix of alginate due the faster disintegration of hydrogels in the intestinal pH and considerably higher diffusion rate (Roquero et al. 2021). Alginate-g-poly (ε-caprolactone) (PCL)/ Ca^{2+} amphiphilic hydrogel beads were employed as the depots for the poorly water-soluble drug theophylline. In contrast to the above cation cross linked hydrogels, alginate-g-PCL/ Ca^{2+} showed lower swelling rate than the alginate/ Ca^{2+} beads in the intestinal fluid. However, these beads could protect drug from acidic medium and released 100% of the drug in the intestinal environment (SIF, pH of 6.8) within 80 min (Colinet et al. 2009). Cross linked poly (aldehydes guluronate) hydrogel was developed for the sustained and controlled release of daunomycin, an anti-neoplastic drug. These hydrogels exhibited a sustained release of daunomycin from 2 days to 6 weeks (Bouhadir et al. 2000). Agar/alginate hydrogel beads (AALB) were synthesized to improve the mechanical performance and prolong the drug release using agar as modifying agent. Incorporation of a natural polymer like agar could modify the swelling and release properties without affecting the pH sensitivity (Yin et al. 2018). Alginate hydrogels were successfully

utilized as a drug carrier for the oral drug delivery system for many other drugs including atenolol (Rigo et al. 2006), aminophylline (Gao et al. 2012), carbamazepine (Liu et al. 2010), Dipyridamol (Jamstorp et al. 2010), indomethacin (Xin et al. 2010) etc.

3.2 Protein Drug Delivery

Advancements in the field of biological sciences and biotechnology provided the momentum for the economical and large-scale production of protein-based drugs into market (Lagassé et al. 2017). Protein therapeutics received an unprecedented clinical acceptance in recent years and these come up with a serious of challenges in the protein-based drug delivery systems such as chemical stability, poor membrane permeability, rapid plasma clearance and immunogenicity (Laszlo and Wade 2014). Alginate based hydrogels produced from the ionic cross linking are stable at lower pH of gastric environment and this can protect the protein drugs from the denaturation. Indeed, the ability of pH responsiveness of alginate hydrogels can be exploited for the controlled delivery of protein and peptide drugs to the intestinal pH environment. Hydrogels of Alginate and CMCs grafted sodium acrylate coated with chitosan-grafted- poly (ethylene glycol) employed to develop pharmaceutical formulation for the release of protein-based drugs to the alkaline pH of intestine (El-Sherbiny 2010). A dual cross-linked hydrogel of AL and N-guluronic acid chitosan (GAC) was investigated for the controlled release of *Bovine serum albumin* (BSA) in SIF and Simulated colonic fluid (SCF). AL/GAC was cross linked by adding the aqueous solution of AL/GAC into the calcium chloride salt solution and these hydrogels beads were suspended in a sodium sulfate solution. GAC has a positive effect in the entrapment efficiency of BSA, the concentration of GAC was correlated with the loading of BSA inside the beads. The reasonable explanation of the enhanced loading efficiency was the charge interaction between positively charged GAC and negatively charged amino acids of BSA. GAC also directly influenced the swelling ratio of beads and it was 11 times higher in the SIF in comparison with SGF. BSA was 100% released in the SIF with in 3 h whereas only 14–18% of drug release into SGF in 75 min (Gong et al. 2011). Charge and size of the proteins were the two parameters determining the protein diffusion. Rapid release of negatively charged protein was observed from the gel matrix and the high porosity of alginate hydrogels inherently limits the loading of small peptides. The instability of alginate at higher pH conditions and porosity can be modulated by incorporation of other polymers by modifications with other natural or synthetic polymers.

3.3 Ocular Drug Delivery

In ocular delivery of xenobiotic, crossing the ocular barriers to improve the bio-availability and control the drug release is a hurdle in the development of ocular drug delivery system. In order to address the various static and dynamic barriers of drug delivery, ideal drug delivery systems are required over conventional formulations such as topical eye drops and intravitreal injections (Gote et al. 2019). Sustaining the therapeutic range of drug concentration of drug is a challenge with topical eye drops. Hydrogel ophthalmic drug depots could promise increased contact time and increased bioavailability. Alginate microspheres loaded with BSA was designed for ocular drug deliver and a sustained delivery for 11 days could achieve in natural phosphate buffer (Liu et al. 2008). SA based ophthalmic drug delivery system that formed in situ was developed for the pilocarpine, a drug for the management of xerostomia and glaucoma. This ocular delivery system obtained a sustained in vitro release of pilocarpine for more than 24 h by a gel diffusion mechanism (Cohen et al. 1997). Similarly, in situ gel formation in lachrymal fluid was reported for the alginic acid based novel ophthalmic formulation (Schoyo et al. 2000). A combination of alginate and pluronic solutions was investigated as in situ gel forming vehicle for the ocular drug delivery. An optimum concentration (2% (w/w) of alginate and 14% (w/w) of pluronic solution) of these two solutions could retain the pilocarpine inside the gel and release in a sustainable fashion (Lin et al. 2004).

3.4 Vaccine Delivery

Oral delivery of vaccines is an extensively explored area due the advantages of this route of administration. Oral delivery is being successful in various human diseases including, cholera, tetanus, influenza, and HIV in animal models. However, the pace in the development of oral vaccines lagged by the lack of delivery systems. Other than cholera toxin (CT) and its nontoxic B subunit pentamer moiety, most of the oral vaccines suffer from the degradation at gastrointestinal tract (GIT). Alginate gel matrix was introduced as a carrier candidate and adjuvant for the vaccine-relevant antigens. Alginate gel was delivered in the form of micro particles as the required amount of antigen was dispersed. A macroporous and biodegradable alginate-based gel was synthesized by crosslinkg the *N*-succinyl chitosan (S-CS) and oxidized alginate (O-Alg) by schiff-base reaction. This lyophilized alginate-chitosan scaffold was loaded with mRNA lipoplexes and this converted into a gel state from dry state by the rehydration-loading process. mRNA lipoplexes can be released by either diffusion or degradation mechanism and further into the cells by endocytosis (Yan et al. 2019).

3.5 *Injectable Delivery*

Injectable hydrogels have gained a lot of interest as a medication delivery strategy because of their ease of administration without invasive surgical and implantation operations and patient convenience (Maxwell et al. 2022). “Smart” hydrogels that can perceive and respond to environmental stimuli such as temperature, pH, light, and electric fields, have received a lot of attention (Liu et al. 2017b). The thermoresponsive hydrogel, which response to temperature change, is one of the most popular and well-studied varieties of stimulus-responsive hydrogels. Thermoresponsive hydrogels are made of poly(N-isopropylacrylamide) (PNIPAAm), which can undergo a reversible phase transition in response to temperature change. Sodium alginate is a good choice for forming thermoresponsive hydrogels with PNIPAAm because it is biocompatible, biodegradable, non-toxic, chelatable, and chemically modifiable (Cheaburu-Yilmaz et al. 2019; Wu et al. 2017). The pure alginate-g-PNIPAAm copolymers don’t show cytotoxicity for in-vitro studies. As a result, injectable thermoresponsive hydrogels show a lot of promise for delivering anti-cancer medications over time and overcoming multidrug resistance in cancer treatment (Mignon et al. 2019; Tang et al. 2021).

The photodynamic efficacy of Methylene blue-loaded Aerosol OT (AOT) and sodium alginate nanoparticles synthesized via a multiple emulsion cross-linking technique was developed. This encapsulated methylene blue acts as a photosensitizer, when a photosensitizer is exposed to the light of a certain wavelength, it produces lethal singlet oxygen species ($^1\text{O}_2$) and reactive oxygen species (ROS). As a result, photodynamic treatment can be used to kill tumor cells in a selective manner (Khdaier et al. 2008).

3.6 *Wound Dressing*

Wound dressings are crucial for wound care as they serve as a physical barrier between the wound and the outside world, preventing infection and additional damage. When compared to standard wound dressings, alginate wound dressings have a promising future in the management of exudative wounds as well as the treatment of acute and chronic wounds (Lozeau et al. 2020; Abasalizadeh et al. 2020). Alginate wound dressings maintain a moist environment in situ, retain wound exudates, promote fibroblast proliferation and keratinocyte migration, aid wound healing, and reduce bacterial infection at the site of skin tissue damage. Alginate is a tempting material for wound dressings because of its hydrophilicity, superior biocompatibility, and large liquid-absorbing capacity (Bari et al. 2020).

Hydrogels, films, foams, nanofibers, membranes, and sponges are all examples of alginate-based wound dressings. Alginate wound dressings are generally produced by ionic cross-linking (which results in the formation of a gel), freeze-drying (which results in the formation of foam), and fibrous non-woven dressings. Alginate

hydrogel has biodegradability, moisture permeability, and exudate absorption in addition to the hydrophilic properties of the hydrogel (Wenshan et al. 2018). These wound dressings can serve several functions. For example, the dry form absorbs wound fluid to re-gel, and the gels can then deliver water to a dry wound, maintaining the physiological environment's moisture and reducing in-situ bacterial infection. These features can also promote the production of granulation tissue as well as epithelialization and healing (Fahmy et al. 2021). In comparison to typical medical dressings, alginate hydrogels are non-toxic, have a high-water absorption rate, and can build a hydrogel network on the wound's surface to retain its moist environment. Most importantly, alginate hydrogels do not cling to wound tissues, and their removal does not result in additional wound surface harm (Nurzynska et al. 2021).

3.7 Cell Delivery and Implants

Alginate is the most commonly used biopolymer for cell encapsulation (Ching et al. 2017). It functions as a semipermeable membrane that facilitates bidirectional molecular movement. Nutrients and oxygen are allowed in, but cellular waste is expelled. Nutrient supply and gas exchange are promoted because of the high surface/volume ratio (Gonzalez-Pujana et al. 2018). Encapsulated cells offer constant synthesis of active compounds once they are introduced into an organism. Not only does this allow for a longer and more consistent supply of therapeutic chemicals in cell therapy, but it also allows for more complex release profiles. Small molecules and proteins can pass through the alginate layer, allowing therapeutic medicines to pass through (Gandhi et al. 2013). Ionotropic gelation of alginate hydrogels provides further benefits. The integrity and viability of cells are preserved when hydrogels are made under moderate circumstances. It ensures cellular functionality and the administration of therapeutic factors. Furthermore, quick alginate gelation cuts down on cell manipulation time. Alginate hydrogels offer cells a suitable habitat, like the natural extracellular matrix, due to their biological and mechanical properties, as well as their high-water content. Furthermore, Physico-chemical properties can be modified to meet the needs of specific cell types (Reig-Vano et al. 2021).

As a cell delivery technique for bone TE, Zhou et al. developed OA/fibrin microbeads. OA/fibrin microbeads, as well as alginate and OA microbeads, were used to encapsulate human umbilical cord mesenchymal stem cells (hUCMSCs). Microbeads were mixed with calcium phosphate cement to create scaffolds for cell delivery. When compared to alginate and OA microbeads, the OA/fibrin microbeads displayed a higher rate of degradation and cell release. On day 21, the number of cells detected in OA/fibrin samples was significantly higher than in OA and alginate samples. Furthermore, the cells liberated from the microbeads exhibited a high level of bone marker genes, indicating that osteogenic differentiation had occurred (Zhou and Xu 2011).

For contrast, Saenz del Burgo et al. used an alginate-poly-L-lysine matrix to encapsulate genetically engineered human HEK-293 cells. Anti-CEA (carcinoembryonic antigen) x anti-CD3 recombinant bispecific antibodies (bsAbs) were produced by cultured cells. These bsAbs bind tumor-associated CEA while also activating lymphocytes in the peripheral blood. The ability of this method to eliminate CEA-expressing tumor cells through local T-cell activation was demonstrated by the results (Saenz del Burgo et al. 2015). Johansson et al. encapsulated genetically altered human glioblastoma BHK cells within alginate for in vivo research. The extracellular component of the protein Lrig1 (sLrig1), a tumor suppressor that causes EGFR downregulation and degradation, was overexpressed in the cells. In glioblastoma cancer cells, the EGFR gene is frequently amplified and altered. As a result, the beads were implanted into the brains of glioma-bearing mice. Lrig1 inhibited tumor growth and improved mouse survival, according to the findings (Johansson et al. 2013).

For encapsulation of bacterial cells, a different approach was used, Alginate was employed by Funaro et al. to immobilize recombinant *E. coli*. Cytosine deaminase (CD), which catalyzes the conversion of 5-fluorocytosine, a non-toxic prodrug, to 5-FU, was synthesized in genetically engineered cells. In vitro tests on rat 9, L glioma cells revealed that it has an anti-cancer effect similar to free 5-FU. This technique, on the other hand, would allow for targeted 5-FU action, avoiding systemic toxicity (Funaro et al. 2016).

4 Alginate in Biomedical Applications

There has been a remarkable growth in the usage of alginates as scaffolds in the field of tissue engineering and regenerative medicine since their initial identification. Hydrogels are the most promising scaffolds for tissue engineering among the current alternatives. Non-injectable or injectable alginate-based hydrogels for biomedical applications are available. Prior to in vivo implantation, a non-injectable alginate hydrogel is pre-formed. Injectable hydrogel, on the other hand, has a longer gelation window before it assumes its final shape. This allows for the filling of irregularly shaped and sized defects with a minimally invasive approach (Bidarra et al. 2014). Both types of hydrogels are frequently combined with foreign objects, whether in the form of particles or fibers, to create an alginate composite hydrogel with increased overall structural integrity and desirable qualities.

When pure alginate is employed as a scaffold material, cell clustering is prevalent. This is to be expected, as alginate lacks cell adhesion moieties and thus does not support cell attachment. To increase cell adhesion and imitate the native cell microenvironment, an alginate composite hydrogel including ECM such as gelatin, bioactive agents such as bioglass, and cell sticky peptides with the RGD sequence (Arg-Gly-Asp) is created (Shachar et al. 2011).

4.1 Wound Healing

Hydrogels can aid speed wound healing by donating water to the injured area and maintaining a moist atmosphere for cell migration (Yoshii et al. 1999). The hydrogels can indeed assist to cool the wound and relieve pain, which is beneficial for burns and other painful wounds (Weller and Team 2019). Along with efficiency (e.g., targeted at wounds), such a targeted delivery route also reduces drug toxicity or adverse effects. Arian E. et al. used glutaraldehyde and calcium chlorides to develop a vitamin E-loaded alginate-based hydrogel that integrated the benefits of alginate, chitosan, and Vitamin E in wound healing. Because of the antioxidant properties of vitamin E, the composite hydrogel demonstrated a strong proliferation of cells in vitro. The growth of neotissue and granulation tissue can also be shown in an in vivo experiment (Ehterami et al. 2019). Hydrogels made from CMC-ALG showed a wide range of mechanical properties when they were dual crosslinked. To mediate wound closure, Epidermal Growth Factor (EGF) was infused into the porous dual crosslinked hydrogel. In vivo, this hydrogel increased cell proliferation and accelerated wound healing (Hu et al. 2018). By homogenising SA, gelatin, and silver nanoparticles (AgNPs), a hydrogel was created. To prevent the wound from microbial contamination, the AgNPs-containing hydrogel served as both a mechanical and a biomechanical barrier. AgNPs-loaded hydrogels were found to speed up tissue development and increase the early emergence of primary collagen scars in in vivo investigations. Using the solvent casting process, a poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymer (F127) was introduced to the PVA/alginate hydrogel composites to improve chain entanglement. Alginate stimulated macrophages, causing them to release TNF- α , which triggered the inflammatory phase of wound healing. Then there's the chain of events that leads to the remodelling stage, like epithelialization, where a protective layer is made (Abbasi et al. 2020). Recently Huifeng D et al., developed alginate based polycationic hydrogel. Hydrogel was functionalized using solvent displacement and in situ polymerization method to improve water retention, antifreezing, environmental adaptability, as well as the photothermal and adhesion property. Hydrogel is very effective against both *Escherichia coli* and *Staphylococcus aureus*. It dramatically promotes the healing of infected wounds in a rat model of full-thickness wound infection, with a remarkable healing rate of 96.49% (Dong et al. 2022).

4.2 Tissue Engineering (Repair and Regeneration)

Tissue engineering (TE) uses a blend of biological field and materials field technologies to promote tissue regeneration and repair. Tissue-specific cells, scaffold materials, and signals to guide cell phenotypic are the three fundamental components of TE. Scaffolds provide temporary support for cells, allowing them to adhere, develop, proliferate, and regenerate new tissue in order to regain functionality

(Wang et al. 2010; Reakasame and Boccaccini 2018). Hydrogels are an important type of scaffold material. They are hydrophilic polymeric networks that can hold enormous quantities of water and other biological liquids. Hydrogels have attracted attention for TE applications as an injectable scaffold that allows cells to be delivered into the body with minim invasion, as well as the development of matrices for cell encapsulation and bio fabrication of 3D scaffolds (Malda et al. 2013), because to their soft and rubbery consistency, which is identical to biological tissue. Since alginate-based hydrogels share many characteristics with human tissues' extracellular matrix, they have found extensive application in tissue engineering (TE).

To meet the demand for biodegradable, electroactive conduits that speed up neuron regeneration, researchers developed a citric acid functionalized graphite nanofilament-enhanced alginate hydrogel. The nanofilaments are spread out evenly, so the mechanical stability of the nanocomposite hydrogel is up to three times that of the plain one. The nanofilaments also allow for electrical contact and intercellular signaling, which boosts biological activity. In vitro investigations showed that the nanocomposite hydrogel is biocompatible, since PC12 cells grow and disseminate readily (Homaeigohar et al. 2019). Lueckgen et al. crafted a photocrosslinkable alginate-based system with degradable crosslinkers incorporating VPMSMRGG sequences. In study UV-initiated thiol-ene chemistry was used to crosslink norbornene modified alginate with peptide crosslinkers vulnerable to cleavage by matrix metalloproteinases. Non-degradable hydrogels made from non-enzymatically cleavable variations of this peptide with identical mechanical strengths, on the other hand, indicated no susceptibility to this collagenase, confirming the bioactivity of the protease-sensitive sequences. Finally, the addition of degradable crosslinkers facilitated the spreading of implanted fibroblasts in these hydrogels, whereas non-degradable analogue remained essentially circular even after 14 days. In compared to non-degradable hydrogels, degradable hydrogels showed increased tissue and cell penetration in vivo (Lueckgen et al. 2019). Bidarra et al., evaluated the potential of injectable RGD- Alginate hydrogel to deliver the endothelial cells. Cells immobilized in RGD- Alginate hydrogel were able to grow and retain 80% viability for at most 48 h. Entrapped cells also developed 3D cellular networks, and when they came into contact with matrigel, they migrated out of the hydrogel matrix (Bidarra et al. 2011). Tong and colleagues developed a biodegradable and injectable hydrogel made of glycyrrhizin (GL), alginate (Alg), and calcium (Ca) for three-dimensional (3D) cell culture. HepG2 cells from a human hepatoma were grown in hydrogels and showed good morphology. In comparison to the control group, cells in hydrogels had better biocompatibility and were able to retain viability, proliferation, and liver function for longer duration. In addition, the hydrogel enhanced the mRNA expression of cytochrome P450, a critical enzyme in hepatocyte metabolism (Tong et al. 2018).

4.3 Bone Regeneration and Cartilage Repair

Biomimicry of natural cartilage structures, functions, and biology has led to cartilage tissue engineering as the preferred method for repairing defects in the cartilage. In 2020 for cartilage healing, a hydroxyapatite nanowires composite with dual-network bovine serum albumin/sodium alginate hydrogel was created. This hydrogel has high porosity, mechanical attributes, swelling ratio and also exceptional biological activity to enhance the human bone marrow derived mesenchymal stem cells (hBMSCs) differentiation and proliferation (Yuan et al. 2020). Synovial-derived MSCs are a promising source for cartilage tissue engineering due to their similarity to cartilage parenchyma cells or stem cells. In 2018, scientists investigated vitality and differentiation of Mesenchymal stem cells produced from synovial membrane (MSCSM) encased in alginate hydrogel. Chondrogenic differentiation was detected after 21 days. The authors stated that this approach could be beneficial for horse cartilage defects (Zhang et al. 2016). For facilitating bone defect repair, strontium (Sr) alginate hydrogels containing chondroitin sulphate (CS) were created by Fenbo et al., In vitro findings imply that Sr-CS/alginate hydrogels with a greater CS ratio promote osteoblast growth. Furthermore, the effectiveness of as-fabricated Sr-CS/alginate hydrogels in bone regeneration was assessed using a rabbit model to repair femoral cylindrical lesions. According to the results of the animal investigation, Sr-CS/alginate hydrogel might greatly aid in the repair of bone defects and hence could be effective for osteochondral tissue engineering (Fenbo et al. 2020).

Xu et al., crafted Metformin Hydrochloride Encapsulated Alginate Strontium Hydrogel a new hydrogel that combines metformin, a well-known drug for inhibiting senescence and strontium a potent anti-inflammatory substance for cartilage tissue creation. The hydrogel has a strong inhibitory effect on the expression of senescent, apoptotic, inflammatory and oxidative genes, according to an RT-PCR test. Histological tests show that the Alg/MH-Sr hydrogel expedited cartilage healing and dramatically reduced chondrocyte senescence (Xu et al. 2021). 3D scaffolds, such as hydrogels, can be used as cell carriers for in vitro or in vivo colonization and have become a hot topic in the field of tissue replacement. A novel composite hydrogel was effectively created using sodium alginate (SA) and platelet-rich plasma (PRP) in various blending ratios, cross-linked with calcium ions, and released from calcium carbonate-D-Glucono-d-lactone (CaCO_3 -GDL). The physical properties and biological performance of the composite hydrogels were found to be greatly affected by the addition of PRP, which was dependent on the blending ratio. The addition of PRP to alginate hydrogels considerably lowered the gelation rate and swelling ratio, resulting in a more uniform gel structure. This composite hydrogel enhanced bone marrow-derived mesenchymal stem cell growth and chondrogenesis by up-regulating Sox9 and Aggrecan (Gao et al. 2019).

5 Conclusion

Alginate, owing to its wide functionalities, is one of the prominently studied biopolymers in pharmaceutical and biomedical fields. Alginate or its derivatives have tendency of gel formation via cross-linking either physically or chemically. Peculiar physiochemical properties of alginate make it a versatile biopolymer. The swelling property, gel-forming ability and mucoadhesive nature of alginate make it easily employable for the development of numerous hydrogel-based drug delivery systems. In recent years, many alginate-based hydrogels have found their use in the controlled as well as targeted delivery of the drugs and proteins. Apart from this, Alginate based hydrogels have also made their way in biomedical applications like tissue engineering, tissue regeneration, wound dressings, etc.

References

- Abasalizadeh F, Moghaddam SV, Alizadeh E, Kashani E, Fazljou SMB, Torbati M, Akbarzadeh A (2020) Alginate-based hydrogels as drug delivery vehicles in cancer treatment and their applications in wound dressing and 3D bioprinting. *J Biol Eng* 14:1–22
- Abbasi AR, Sohail M, Minhas MU, Khaliq T, Kousar M, Khan S, Hussain Z, Munir A (2020) Bioinspired sodium alginate based thermosensitive hydrogel membranes for accelerated wound healing. *Int J Biol Macromol* 155:751–765
- Abrigo M, McArthur SL, Kingshott P (2014) Electrospun nanofibers as dressings for chronic wound care: advances, challenges, and future prospects. *Macromol Biosci* 14:772–792
- Aderibigbe BA, Buyana B (2018) Alginate in wound dressings. *Pharmaceutics* 10:42
- Al-Kassas RS, Al-Gohary OM, Al-Faadhel MM (2007) Controlling of systemic absorption of gliclazide through incorporation into alginate beads. *Int J Pharm* 341(1–2):230–237
- Babu VR, Sairam M, Hosamani KM, Aminabhavi TM (2007) Preparation of sodium alginate–methylcellulose blend microspheres for controlled release of nifedipine. *Carbohydr Polym* 69: 241–250
- Bari E, Di Silvestre D, Mastracci L, Grillo F, Grisoli P, Marrubini G, Nardini M, Mastrogiacomio M, Sorlini M, Rossi R, Torre ML (2020) GMP-compliant sponge-like dressing containing MSC lyo-secretome: proteomic network of healing in a murine wound model. *Eur J Pharm Biopharm* 155:37–48
- Basmanav FB, Kose GT, Hasirci V (2008) Sequential growth factor delivery from complexed microspheres for bone tissue engineering. *Biomaterials* 29:4195–4204
- Berger J, Reist M, Mayer JM, Felt O, Peppas NA, Gurny R (2004) Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. *Eur J Pharm Biopharm* 57:19–34
- Bidarra SJ, Barrias CC, Fonseca KB, Barbosa MA, Soares RA, Granja PL (2011) Injectable in situ crosslinkable RGD-modified alginate matrix for endothelial cells delivery. *Biomaterials* 32: 7897–7904
- Bidarra SJ, Barrias CC, Granja PL (2014) Injectable alginate hydrogels for cell delivery in tissue engineering. *Acta Biomater* 10:1646–1662
- Bouhadir KH, Hausman DS, Mooney DJ (1999) Synthesis of cross-linked poly (aldehyde guluronate) hydrogels. *Polymer* 40:3575–3584

- Bouhadir KH, Kruger GM, Lee KY, Mooney DJ (2000) Sustained and controlled release of daunomycin from cross-linked poly (aldehyde guluronate) hydrogels. *J Pharm Sci* 89:910–919
- Bu H, Kjøniksen AL, Knudsen KD, Nyström B (2004) Rheological and structural properties of aqueous alginate during gelation via the Ugi multicomponent condensation reaction. *Biomacromolecules* 5:1470–1479
- Chan AW, Whitney RA, Neufeld RJ (2009) Semisynthesis of a controlled stimuli-responsive alginate hydrogel. *Biomacromolecules* 10:609–616
- Chan LW, Lee HY, Heng PW (2006) Mechanisms of external and internal gelation and their impact on the functions of alginate as a coat and delivery system. *Carbohydr Polym* 63:176–187
- Cheaburu-Yilmaz CN, Lupuşoru CE, Vasile C (2019) New alginate/PNIPAAm matrices for drug delivery. *Polymers* 11:366
- Chen L, Subirade M (2006) Alginate–whey protein granular microspheres as oral delivery vehicles for bioactive compounds. *Biomaterials* 27:4646–4654
- Ching SH, Bansal N, Bhandari B (2017) Alginate gel particles—a review of production techniques and physical properties. *Crit Rev Food Sci Nutr* 57:1133–1152
- Chuah AM, Kuroiwa T, Kobayashi I, Zhang X, Nakajima M (2009) Preparation of uniformly sized alginate microspheres using the novel combined methods of microchannel emulsification and external gelation. *Colloids Surf A: Physicochem Eng Asp* 351:9–17
- Cohen S, Lobel E, Trevoda A, Peled Y (1997) A novel in-situ forming ophthalmic drug delivery system from Alginate undergoing gelation in the eye. *J Control Release* 44:201–208
- Colinet I, Dulong V, Mocanu G, Picton L, Le Cerf D (2009) New amphiphilic and pH-sensitive hydrogel for controlled release of a model poorly water-soluble drug. *Eur J Pharm Biopharm* 73: 345–350
- DeRamos CM, Irwin AE, Nauss JL, Stout BE (1997) ¹³C NMR and molecular modeling studies of alginic acid binding with alkaline earth and lanthanide metal ions. *Inorg Chim Acta* 256:69–75
- Dimatteo R, Darling NJ, Segura T (2018) In situ forming injectable hydrogels for drug delivery and wound repair. *Adv Drug Deliv Rev* 127:167–184
- Dong H, Wang L, Du L, Wang X, Li Q, Wang X, Zhang J, Nie J, Ma G (2022) Smart polycationic hydrogel dressing for dynamic wound healing. *Small* 18:2201620
- Ehterami A, Salehi M, Farzamfar S, Samadian H, Vaez A, Ghorbani S, Ai J, Sahraeyima H (2019) Chitosan/alginate hydrogels containing alpha-tocopherol for wound healing in rat model. *J Drug Deliv Sci Technol* 51:204–213
- Eiselt P, Lee KY, Mooney DJ (1999) Rigidity of two-component hydrogels prepared from alginate and poly (ethylene glycol)—diamines. *Macromolecules* 32:5561–5566
- El-Sherbiny IM (2010) Enhanced pH- responsive carrier system based on Alginate and chemically modified carboxymethyl chitosan for oral delivery of protein drugs: preparation and in-vitro assessment. *Carbohydr Polym* 80:1125–1136
- El-Sherbiny IM, Abdel-Mogib M, Dawidar AAM, Elsayed A, Smyth HD (2011) Biodegradable pH-responsive alginate-poly (lactic-co-glycolic acid) nano/micro hydrogel matrices for oral delivery of silymarin. *Carbohydr Polym* 83:1345–1354
- Fahmy HM, Aly AA, Sayed SM, Abou-Okeil A (2021) K-carrageenan/Na-alginate wound dressing with sustainable drug delivery properties. *Polym Adv Technol* 32:1793–1801
- Fenbo M, Sijing L, Ruiz-Ortega LI, Yuanjun Z, Lei X, Kui W, Lijun L, Bin T (2020) Effects of alginate/chondroitin sulfate-based hydrogels on bone defects healing. *Mater Sci Eng C* 116: 111217
- Fenoradosa TA, Ali G, Delattre C, Laroche C, Petit E, Wadouachi A, Michaud P (2010) Extraction and characterization of an alginate from the brown seaweed *Sargassum turbinarioides* Grunow. *J Appl Phycol* 22:131–137
- Funaro MG, Nemani KV, Chen Z, Bhujwalla ZM, Griswold KE, Gimi B (2016) Effect of alginate microencapsulation on the catalytic efficiency and in vitro enzyme-prodrug therapeutic efficacy of cytosine deaminase and of recombinant *E. coli* expressing cytosine deaminase. *J Microencapsul* 33:64–70
- Fundueanu G, Nastruzzi C, Carpov A, Desbrieres J, Rinaudo M (1999) Physico-chemical characterization of Ca-alginate microparticles produced with different methods. *Biomaterials* 20: 1427–1435

- Gandhi JK, Opara EC, Brey EM (2013) Alginate-based strategies for therapeutic vascularization. *Ther Deliv* 4:327–341
- Gao C, Liu M, Chen J, Chen C (2012) pH- and Temperature-Responsive P(DMAEMAGMA)-Alginate Semi-IPN Hydrogels formed by radical and ring-opening polymerization for aminophylline. *J Biomater Sci Polym* 23:1039–1054
- Gao X, Gao L, Groth T, Liu T, He D, Wang M, Gong F, Chu J, Zhao M (2019) Fabrication and properties of an injectable sodium alginate/PRP composite hydrogel as a potential cell carrier for cartilage repair. *J Biomed Mater Res A* 107:2076–2087
- Gaumann A, Laudes M, Jacob B, Pommersheim R, Laue C, Vogt W, Schrezenmeier J (2000) Effect of media composition on long-term in vitro stability of barium alginate and polyacrylic acid multilayer microcapsules. *Biomaterials* 21:1911–1917
- Giri TK, Thakur D, Alexander A, Badwaik H, Krishna Tripathi D (2012) Alginate based hydrogel as a potential biopolymeric carrier for drug delivery and cell delivery systems: present status and applications. *Curr Drug Deliv* 9:539–555
- Gong R, Li C, Zhu S, Zhan Y, Du Y, Jiang J (2011) A novel pH sensitive hydrogel based on dual cross-linked Alginate/N-glutaric acid chitosan for oral delivery of protein. *Carbohydr Polym* 85: 869–874
- Gonzalez-Pujana A, Orive G, Pedraz JL, Santos-Vizcaino E, Hernandez RM (2018) Alginate microcapsules for drug delivery. In: Rehm BHA, Moradali MF (eds) *Alginates and their biomedical applications*. Springer, Singapore, pp 67–100
- Gote V, Sikder S, Sicotte J, Pal D (2019) Ocular drug delivery: present innovations and future challenges. *J Pharmacol Exp Ther* 370:602–624
- Hasnain MS, Nayak AK (2019) *Alginates: versatile polymers in biomedical applications and therapeutics*. CRC Press, Oakville
- Hennink WE, van Nostrum CF (2012) Novel crosslinking methods to design hydrogels. *Adv Drug Deliv Rev* 64:223–236
- Hernández-González AC, Téllez-Jurado L, Rodríguez-Lorenzo LM (2020) Alginate hydrogels for bone tissue engineering, from injectables to bioprinting: a review. *Carbohydr Polym* 229: 115514
- Hoang Thi TT, Sinh LH, Huynh DP, Nguyen DH, Huynh C (2020) Self-assemblable polymer smart-blocks for temperature-induced injectable hydrogel in biomedical applications. *Front Chem* 8:19
- Homaeigohar S, Boccaccini AR (2020) Antibacterial biohybrid nanofibers for wound dressings. *Acta Biomater* 107:25–49
- Homaeigohar S, Tsai TY, Young TH, Yang HJ, Ji YR (2019) An electroactive alginate hydrogel nanocomposite reinforced by functionalized graphite nanofilaments for neural tissue engineering. *Carbohydr Polym* 224:115112
- Hu Y, Zhang Z, Li Y, Ding X, Li D, Shen C, Xu FJ (2018) Dual-crosslinked amorphous polysaccharide hydrogels based on chitosan/alginate for wound healing applications. *Macromol Rapid Commun* 39:1800069
- Jamstorp E, Bodin A, Gatenholm P, Jeppsson A, Stromme M (2010) Release of antithrombotic drugs from AL gel beads. *Curr Drug Deliv* 7:297–302
- Jeon O, Powell C, Solorio LD, Krebs MD, Alsberg E (2011) Affinity-based growth factor delivery using biodegradable, photocrosslinked heparin-alginate hydrogels. *J Control Release* 154:258–266
- Johansson M, Oudin A, Tiemann K, Bernard A, Golebiewska A, Keunen O, Fack F, Stieber D, Wang B, Hedman H, Niclou SP (2013) The soluble form of the tumor suppressor Lrig 1 potently inhibits in vivo glioma growth irrespective of EGF receptor status. *Neuro-Oncology* 15:1200–1211
- Kashyap N, Kumar N, Kumar MR (2005) Hydrogels for pharmaceutical and biomedical applications. *Crit Rev Ther Drug Carrier Syst* 22:107–149
- Khdair A, Gerard B, Handa H, Mao G, Shekhar MP, Panyam J (2008) Surfactant–polymer nanoparticles enhance the effectiveness of anticancer photodynamic therapy. *Mol Pharm* 5: 795–807

- Kulkarni RV, Sa B (2009) Polyacrylamide-grafted-alginate-based pH-sensitive hydrogel beads for delivery of ketoprofen to the intestine: in vitro and in vivo evaluation. *J Biomater Sci Polym Ed* 20:235–251
- Kulkarni RV, Setty CM, Sa B (2010) Polyacrylamide-g-alginate-based electrically responsive hydrogel for drug delivery application: synthesis, characterization, and formulation development. *J Appl Polym Sci* 115:1180–1188
- Kuo CK, Ma PX (2001) Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: part 1. Structure, gelation rate and mechanical properties. *Biomaterials* 22:511–521
- Kurakula M et al (2020) Alginate-based hydrogel systems for drug releasing in wound healing. In: Nayak AK, Hasnain MS (eds) *Alginates in drug delivery*. Academic Press, London, pp 323–358
- Lagassé HD, Alexaki A, Simhadri VL, Katagiri NH, Jankowski W, Sauna ZE, Kimchi-Sarfaty C (2017) Recent advances in (therapeutic protein) drug development. *F1000Res* 6:113
- Laszlo O, Wade JD (2014) Current challenges in peptide-based drug discovery. *Front Chem* 2:62
- Lau HK, Kiick KL (2015) Opportunities for multicomponent hybrid hydrogels in biomedical applications. *Biomacromolecules* 16:28–42
- Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. *Prog Polym Sci* 37:106–126
- Lee KY, Kong HJ, Larson RG, Mooney DJ (2003) Hydrogel formation via cell crosslinking. *Adv Mater* 15:1828–1832
- Lee KY, Rowley JA, Eiselt P, Moy EM, Bouhadir KH, Mooney DJ (2000) Controlling mechanical and swelling properties of alginate hydrogels independently by cross-linker type and cross-linking density. *Macromolecules* 33:4291–4294
- Leonard MM, de Boisseson R, Hubert P, Dellacherie E (2004) Production of microspheres based on hydrophobically associating alginate derivatives by dispersion/gelation in aqueous sodium chloride solutions. *J Biomed Mater Res A* 68:335–342
- Lin HR, Sung KC, Vong WJ (2004) In situ gelling of AL/Pluronic solutions for ophthalmic delivery of pilocarpine. *Biomacromolecules* 2004(5):2358–2365
- Liu HJ, Li P, Wei Q (2010) Magnetic N-succinyl chitosan/AL beads for carbamazepine delivery. *Drug Dev Ind Pharm* 36:1286–1294
- Liu L, Yao W, Rao Y, Lu X, Gao J (2017a) pH-Responsive carriers for oral drug delivery: challenges and opportunities of current platforms. *Drug Deliv* 24:569–581
- Liu M, Song X, Wen Y, Zhu JL, Li J (2017b) Injectable thermoresponsive hydrogel formed by alginate-g-poly (N-isopropylacrylamide) that releases doxorubicin-encapsulated micelles as a smart drug delivery system. *ACS Appl Mater Interfaces* 9:35673–35682
- Liu W, Griffith M, Li F (2008) Alginate microsphere-collagen composite hydrogel for ocular drug delivery and implantation. *J Mater Sci Mater Med* 19:3365–3371
- Lozeau LD, Grosha J, Smith IM, Stewart EJ, Camesano TA, Rolle MW (2020) Alginate affects bioactivity of chimeric collagen-binding LL37 antimicrobial peptides adsorbed to collagen–alginate wound dressings. *ACS Biomater Sci Eng* 6:3398–3410
- Lueckgen A, Garske DS, Ellinghaus A, Mooney DJ, Duda GN, Cipitria A (2019) Enzymatically-degradable alginate hydrogels promote cell spreading and in vivo tissue infiltration. *Biomaterials* 217:119294
- Malda J, Visser J, Melchels FP, Jüngst T, Hennink WE, Dhert WJ, Groll J, Huttmacher DW (2013) 25th anniversary article: engineering hydrogels for biofabrication. *Adv Mater* 25:5011–5028
- Man Y, Wang P, Guo Y, Xiang L, Yang Y, Qu Y, Gong P, Deng L (2012) Angiogenic and osteogenic potential of platelet-rich plasma and adipose-derived stem cell laden alginate microspheres. *Biomaterials* 33:8802–8811
- Maxwell CJ, Soltisz AM, Rich WW, Choi A, Reilly MA, Swindle-Reilly KE (2022) Tunable alginate hydrogels as injectable drug delivery vehicles for optic neuropathy. *J Biomed Mater Res A* 110(10):1621–1635. <https://doi.org/10.1002/jbm.a.37412>
- Mignon A, De Belie N, Dubruel P, Van Vlierberghe S (2019) Superabsorbent polymers: a review on the characteristics and applications of synthetic, polysaccharide-based, semi-synthetic and ‘smart’ derivatives. *Eur Polym J* 117:165–178

- Nezhad-Mokhtari P, Ghorbani M, Roshangar L, Rad JS (2019) Chemical gelling of hydrogels-based biological macromolecules for tissue engineering: photo-and enzymatic crosslinking methods. *Int J Biol Macromol* 139:760–772
- Nurzynska A, Klimek K, Palka K, Szajnecki Ł, Ginalska G (2021) Curdlan-based hydrogels for potential application as dressings for promotion of skin wound healing—preliminary in vitro studies. *Materials* 14:2344
- Patil NS, Li Y, Rethwisch DG, Dordick JS (1997) Sucrose diacrylate: a unique chemically and biologically degradable crosslinker for polymeric hydrogels. *J Polym Sci A Polym Chem* 35: 2221–2229
- Pawar SN, Edgar KJ (2012) Alginate derivatization: a review of chemistry, properties and applications. *Biomaterials* 33:3279–3305
- Racoviță S, Vasiliu S, Popa M, Luca C (2009) Polysaccharides based on micro- and nanoparticles obtained by ionic gelation and their applications as drug delivery systems. *Rev Roum Chim* 54: 709–718
- Ray P et al (2020) Alginate-based hydrogels for drug delivery applications. In: Nayak AK, Hasnain MS (eds) *Alginates in drug delivery*. Academic Press, London, pp 41–70
- Reakasame S, Boccaccini AR (2018) Oxidized alginate-based hydrogels for tissue engineering applications: a review. *Biomacromolecules* 19:3–21
- Reig-Vano B, Tylkowski B, Montané X, Giamberini M (2021) Alginate-based hydrogels for cancer therapy and research. *Int J Biol Macromol* 170:424–436
- Rigo MV, Allemandi DA, Manzo RH (2006) Swellable drug polyelectrolyte matrices (SDPM) of alginic acid characterization and delivery properties. *Int J Pharm* 322:36–43
- Rinaudo M (2008) Main properties and current applications of some polysaccharides as biomaterials. *Polym Int* 57:397–430
- Riyajan SA, Sakdapipanch JT (2009) Development of a controlled release neem capsule with a sodium alginate matrix, crosslinked by glutaraldehyde and coated with natural rubber. *Polym Bull* 63:609–622
- Roquero DM, Bollella P, Katz E, Melman A (2021) Controlling porosity of calcium alginate hydrogels by interpenetrating polyvinyl alcohol–diboronate polymer network. *ACS Appl Polym Mater* 3:1499–1507
- Rzaev ZM, Dincer S, Pişkin E (2007) Functional copolymers of N-isopropylacrylamide for bioengineering applications. *Prog Polym Sci* 32:534–595
- Saenz del Burgo L, Compte M, Aceves M, Hernández RM, Sanz L, Alvarez-Vallina L, Pedraz JL (2015) Microencapsulation of therapeutic bispecific antibodies producing cells: immunotherapeutic organoids for cancer management. *J Drug Target* 23:170–179
- Sarmento B, Ferreira D, Veiga F, Ribeiro A (2006) Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies. *Carbohydr Polym* 66:1–7
- Sarker B, Rompf J, Silva R, Lang N, Detsch R, Kaschta J, Fabry B, Boccaccini AR (2015) Alginate-based hydrogels with improved adhesive properties for cell encapsulation. *Int J Biol Macromol* 78:72–78
- Schoyo O, Tissie G, Sebastian C, Maurin F, Drioti JY, Trinquand C (2000) A new long acting ophthalmic formulation of carteolol containing alginic acid. *Int J Pharm* 207:109–116
- Shachar M, Tsur-Gang O, Dvir T, Leor J, Cohen S (2011) The effect of immobilized RGD peptide in alginate scaffolds on cardiac tissue engineering. *Acta Biomater* 7:152–162
- Sun W, Han Y, Li Z, Ge K, Zhang J (2016) Bone-targeted mesoporous silica nanocarrier anchored by zoledronate for cancer bone metastasis. *Langmuir* 32:9237–9244
- Supramaniam J, Adnan R, Kaus NHM, Bushra R (2018) Magnetic nanocellulose alginate hydrogel beads as potential drug delivery system. *Int J Biol Macromol* 118:640–648
- Tang L, Wang L, Yang X, Feng Y, Li Y, Feng W (2021) Poly(N-isopropylacrylamide)-based smart hydrogels: design, properties and applications. *Prog Mater Sci* 115:100702
- Tong XF, Zhao FQ, Ren YZ, Zhang Y, Cui YL, Wang QS (2018) Injectable hydrogels based on glycyrrhizin, alginate, and calcium for three-dimensional cell culture in liver tissue engineering. *J Biomed Mater Res A* 106:3292–3302

- Uyen NTT, Hamid ZAA, Tram NXT, Ahmad N (2020) Fabrication of alginate microspheres for drug delivery: a review. *Int J Biol Macromol* 153:1035–1046
- Wang Q, Zhang J, Wang A (2008) Preparation and characterization of a novel pH- sensitive chitosan-g-poly (acrylic acid)/attapulgate/SA composite hydrogel bead for controlled release of diclofenac sodium. *Carbohydr Polym* 78:731–737
- Wang J, Chen L, Zhao Y, Guo G, Zhang R (2009) Cell adhesion and accelerated detachment on the surface of temperature-sensitive chitosan and poly (Nisopropylacrylamide) hydrogels. *J Mater Sci Mater Med* 20:583–590
- Wang J, Fu W, Zhang D, Yu X, Li J, Wan C (2010) Evaluation of novel alginate dialdehyde cross-linked chitosan/calcium polyphosphate composite scaffolds for meniscus tissue engineering. *Carbohydr Polym* 79:705–710
- Weller C, Team V (2019) Interactive dressings and their role in moist wound management. In: Rajendran S (ed) *Advanced textiles for wound care*. Woodhead Publishing, Duxford, pp 105–134
- Wenshan S, Yuankun D, Bafang L (2018) The research progress of alginate wound dressing. *Gongneng Cailiao/J Funct Mater* 49:9043–9049
- Wu J, Wu Z, Sun X, Yuan S, Zhang R, Lu Q, Yu Y (2017) Effect of sodium alginate on the properties of thermosensitive hydrogels. *J Chin Chem Soc* 64:231–238
- Xin J, Guo Z, Chen X, Jiang W, Li J, Li M (2010) Study of branched cationic beta-cyclodextrin polymer/indomethacin complex and its release profile from Alginate hydrogel. *Int J Pharm* 386: 221–228
- Xu JB, Bartley JP, Johnson RA (2003) Preparation and characterization of alginate hydrogel membranes crosslinked using a water-soluble carbodiimide. *J Appl Polym Sci* 90:747–753
- Xu L, Ma F, Huang J, Frankie Leung KL, Qin C, Lu WW, Guo XE, Tang B (2021) Metformin hydrochloride encapsulation by alginate strontium hydrogel for cartilage regeneration by relieving cellular senescence. *Biomacromolecules* 22:671–680
- Yan J, Chen F, Amsden BG (2016) Cell sheets prepared via gel–sol transition of calcium RGD–alginate. *Acta Biomater* 30:277–284
- Yan J et al (2019) Injectable biodegradable chitosan-alginate 3D porous gel scaffold for mRNA vaccine delivery. *Macromol Biosci* 19:e1800242
- Yin ZC, Wang YL, Wang K (2018) A pH-responsive composite hydrogel beads based on agar and alginate for oral drug delivery. *J Drug Deliv Sci Technol* 43:12–18
- Yoshii F, Zhanshan Y, Isobe K, Shinozaki K, Makuuchi K (1999) Electron beam crosslinked PEO and PEO/PVA hydrogels for wound dressing. *Radiat Phys Chem* 55:133–138
- Yuan H, Zheng X, Liu W, Zhang H, Shao J, Yao J, Mao C, Hui J, Fan D (2020) A novel bovine serum albumin and sodium alginate hydrogel scaffold doped with hydroxyapatite nanowires for cartilage defects repair. *Colloids Surf B: Biointerfaces* 192:111041
- Zhang C, Wang W, Zhao X, Wang H, Yin H (2020a) Preparation of alginate oligosaccharides and their biological activities in plants: a review. *Carbohydr Res* 494:108056
- Zhang H, Cheng J, Ao Q (2021) Preparation of alginate-based biomaterials and their applications in biomedicine. *Mar Drugs* 19:264
- Zhang X, Wang L, Weng L, Deng B (2020b) Strontium ion substituted alginate-based hydrogel fibers and its coordination binding model. *J Appl Polym Sci* 137:48571
- Zhang M, Zhao X (2020) Alginate hydrogel dressings for advanced wound management. *Int J Biol Macromol* 162:1414–1428
- Zhang Z, Zhang R, Zou L, McClements DJ (2016) Protein encapsulation in alginate hydrogel beads: effect of pH on microgel stability, protein retention and protein release. *Food Hydrocoll* 58:308–315
- Zhong H, Gao X, Cheng C, Liu C, Wang Q, Han X (2020) The structural characteristics of seaweed polysaccharides and their application in gel drug delivery systems. *Mar Drugs* 18:658
- Zhou H, Xu HH (2011) The fast release of stem cells from alginate-fibrin microbeads in injectable scaffolds for bone tissue engineering. *Biomaterials* 32:7503–7513

Alginate Based Interpenetrating Polymer Network (IPN) in Drug Delivery and Biomedical Applications



Pooja Mittal, Ramit Kapoor, and Brahmeshwar Mishra

Abstract Interpenetrating polymer is the type of hydrogels which has gained consideration in the last decade due to their enormous biomedical applications. IPN has proven to be great delivery system for providing sustains release drug delivery. This chapter aims to discuss the basics of IPN, their synthesis procedure, biomedical applications of IPN based on the nature of the network. To provide the controlled release to the formulation, IPN systems are constructed. A number of polymers can be used for the creation of IPN networks, like alginate, chitosen, starch and polysaccharides, protein based polymers. Semi synthetic and synthetic polymers can also be employed for the formulation of the same. IPN posses the properties like biodegradability, biocompatibility and bio erodibility. Apart from being used in drug delivery for sustained release properties, they are also used for the formulation of Scaffolds, implants, burn dressings and wound healing bandages etc. Although the alginates and other polymers have found to be very effective in the synthesis of IPNs, modifications of their bonds can improve their properties like cross linking, erodibility and time for the synthesis of IPNs etc. This chapter summarizes the biomedical applications, advantages of IPNs and different dosage forms based on IPNs.

Keywords IPNs · Biomedical · Controlled release · Drug delivery · Alginate

P. Mittal

Chitkara College of Pharmacy, Chitkara University, Rajpura, Punjab, India

R. Kapoor

Clarivate Analytics, Noida, Uttar Pradesh, India

B. Mishra (✉)

Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (BHU), Varanasi, Uttar Pradesh, India

e-mail: bmishrabhu@itbhu.ac.in

1 Introduction to Interpenetrating Polymeric Networks (IPNs)

Since there is already a large market for drug delivery, numerous controlled releasing systems are being created to enable efficient therapeutic applications to treat diverse ailments. After taking their prescribed amounts, medicines with a lower molecular weight are instantly released. However, in the majority of cases, small molecular medicines cannot produce the necessary extended activities or the benefits of target specificity when taken in normal dosages. Thus, new drug delivery technologies enable a number of significant advantages, enhanced solubility of the drug, extension of drug release, reduction of systemic side effects, and tailored drug administration. The prolonged drug releasing formulations are the main focus of the design of innovative drug delivery systems. There are several different new drug delivery dosage forms that can be used to administer medications at regulated or sustained release rates (Boppana et al. 2015; Das et al. 2019; Hasnain et al. 2020; Hoare and Kohane 2008; Matricardi et al. 2013). Recently, a variety of pharmaceutical drug delivery formulations has been established using a variety of biocompatible polymers to manage sustained drug delivery. Restricting the release of tiny compounds by biopolymeric carrier matrices is very difficult. Drugs are typically dispersed or incorporated within various carriers based on biopolymers, which occasionally results in rapid drug release via drug diffusion through matrix pores. Therefore, in order to decrease the number of doses needed, further study is especially needed for medications with short biological half-lives and high water solubilities. The nature of the drug carriers and the type of polymer (s) utilized determine how the drug will release from the polymeric matrix. To achieve the sustained release of the drugs, a variety of formulations has been tried which includes nanoparticles, nanosponges, quantum dots, nano micelles, nano crystals, IPNs and hydrogels etc. The creation of several innovative drug delivery systems has already attracted significant interest for the usage of naturally generated biopolymers. To achieve high drug concentrations at the desired site and extended drug release, numerous natural polymers have been studied. In comparison to synthetic and semi-synthetic polymers, natural polymers are also less cytotoxic. However, several naturally occurring polymers, such protein and polysaccharides, show signs of possible issues like microbial contamination, uncontrolled hydration, and a decrease in viscosity after extended storage. Numerous polymer modification or functionalization techniques can be used to address these issues with the use of naturally derived polymers, including polymer blending, polymer grafting, cross-linking, carboxy-methylation, esterification, thiolation, formation of poly-electrolyte complexes, interpenetrating polymeric networking (IPNs), and others (Nayak and Hasnain 2020; Qi et al. 2015). The restrictions provided by natural polymers can be reduced and their uses can be enhanced by implementing these modifications/functionalization. Since natural polymers are unable to satisfy a variety of demands regarding polymer characteristics and behaviors, the development of IPNs is one of the best and simplest methods for quickly changing or functionalizing the functional characteristics of many natural polymers.

The natural polysaccharide based polymers that were used previously for research includes chitosan, alginates, gelatine gum, carrageenan, sterculia gum, tamarind gum and pectin etc. to create a variety of IPN systems for the sustained release of various medications. IPNs and their various classes have been discussed first. The sources, basic characteristics, and requirement for alginate changes have since been studied (Kumar Giri et al. 2012; Matricardi et al. 2013; Nayak and Hasnain 2020).

2 Design, Synthesis and Characterization of IPNs

A vast no. of methods and precursors of hydrophilic polymers are being utilized for the synthesis of hydrogels. Various natural polymers and their derivatives, synthetic and semi synthetic polymers having various hydrophilic groups like $-\text{COOH}$, $-\text{OH}$ and amines are being utilized for the synthesis of IPNs (Qi et al. 2015; Rastegari et al. 2020; Reddy et al. 2014). Basically, three schemes are used most commonly for the synthesis of IPNs by the combination of various polymers (Boppana et al. 2015; Das et al. 2019; Matricardi et al. 2008, 2013; Reddy et al. 2014). The various schemes employed in the synthesis of IPNs are represented in Fig. 1.

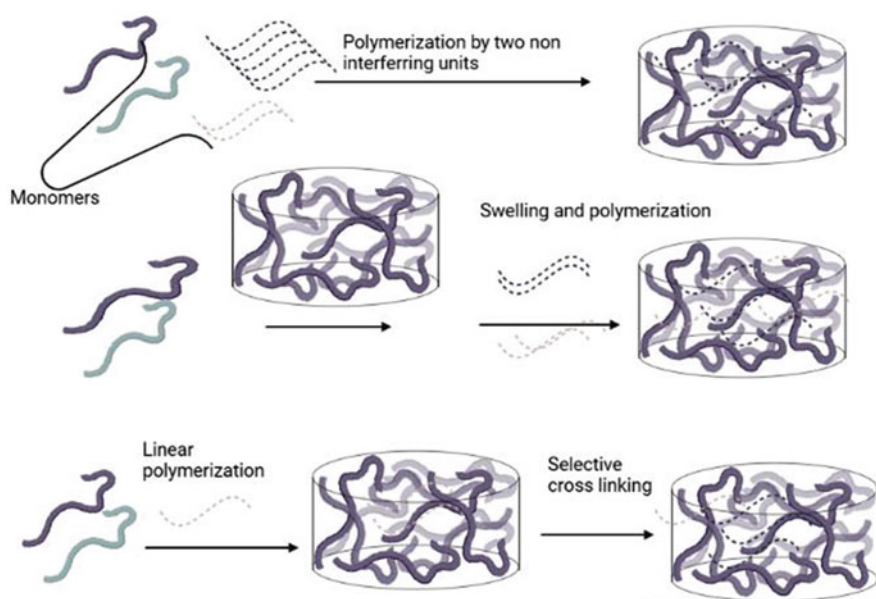


Fig. 1 Methods for the synthesis of IPNs

3 Polymers Used for the Synthesis of IPNs

A large no. of polymers can be used for the synthesis of IPNs, either of natural or synthetic/semi synthetic origin. Some of the most commonly used polymers are mentioned in detail in this section (Boppana et al. 2015; Cui et al. 2014; Das et al. 2019).

3.1 Natural Polymers

3.1.1 Chitosan (CS)

Chitosen (CS), which is a linear compound with cationic nature, exhibits the exceptional behavior like biodegradation, biocompatibility, antibacterial in nature etc. chemically the CS is composed of b-(1,4)-2-amino-2-deoxy-D-glucopyranose and b-(1,4)-2-acetamido-2-deoxy-D-glucopyranose units that are randomly distributed along with its polymeric chain. Due to its exceptional properties and biosorbant, CS has attracted the interest of formulation scientists. Due to its biosorbant action, it has the potential to absorb the proteins, dyes and various metal ions because of the presence of higher content of amino and hydroxyl groups in it. It had been used for the synthesis of hydrogels from last 10 years by utilizing the properties of CS alone or by combining the other polysaccharides from the natural origin or sometimes along with the synthetic or semi synthetic polysaccharides (Eswaramma et al. 2017; Lohani et al. 2014; Mallikarjuna Reddy et al. 2008; Matricardi et al. 2013). The maximum combination of polymers that can be used for the synthesis of IPNs can be shown in Fig. 2.

Yang et al. had created the hydrogels which were made up of PEG grafted on carboxymethyl chitosan and alginate (Alg) which were able to release the protein at pH 7.4 which indicates its effectiveness for the delivery of protein and related compounds to the intestine effectively. In IPN formulations, GA is used as a cross linker very frequently, because the $-NH_2$ groups of CS and aldehyde groups of GA rapidly formulate a Schiff base. However, its use as crosslinker is restricted because of its higher toxicity. Genipin, a new natural cross-linking agent, has recently been used to successfully create hydrogels based on CS (Khurma and Nand 2008; Yang et al. 2013).

3.1.2 Alginates

Sodium alginate (SA) which is a linear polysaccharide and is originated from the marine algae, made up of 1-4-linked b-D-mannuronic acid (M) and a-L-guluronic acid (G), which are arranged in either homo polymeric groups such as MM or GG or they are arranged in altered groups like MG or GM in different polymeric ratios.

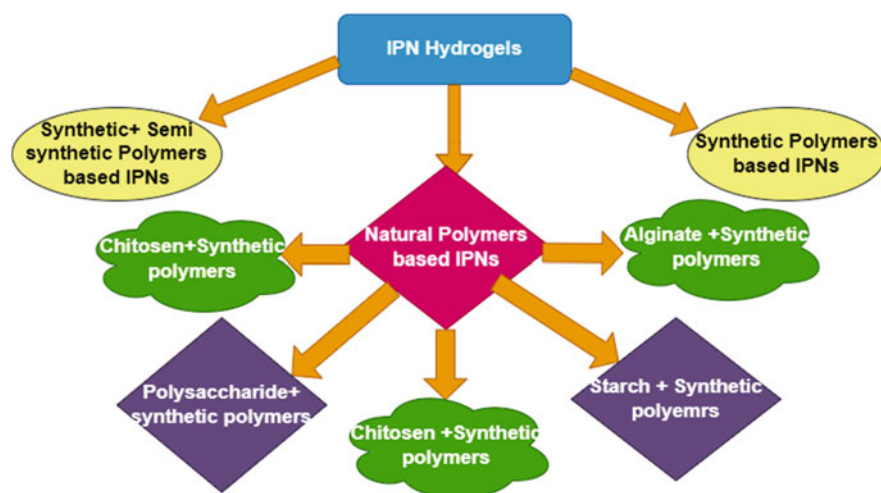


Fig. 2 Schematic representation of the maximum used combination of polymers for the synthesis of IPN

Gluconic acid is mainly used for its crosslinking as the Ca^{2+} ions present in that, can easily cross link it and hydrogelation process will help in binding the same with the glucuronic residues. These properties has increased the widespread usage of SA in various drug delivery systems and in other sectors as well like conditioning of foods and fabrics (Hasnain et al. 2020; Kumar Giri et al. 2012; Mallikarjuna Reddy et al. 2008).

To formulate IPN hydrogens, SA should be mixed with various other synthetic polymers. Kim et al. (2004) has created the IPN hydrogels by SA along with poly (diallyldimethylammonium chloride) (PDADMAC) which were temperature as well as pH sensitive in nature. The swelling index of these hydrogels gets increased with increased pH values and attains a maximum value at pH4, and start dropping down with a range of 4–6 pH.

Carboxylic acid groups take the form of COOH when their pK_a values are lower than the threshold. The $-\text{COOH}$ were ionized to $-\text{COO}^-$ when the pH of the solution rose, and the electrostatic repulsion that resulted in this process causes the hydrogels to swell. The $-\text{COO}^-$ in Alg and the ammonium sites in PDADMAC, on the other hand, coexisted in this pH range and generated polyelectrolyte complexes, due to which the swelling ratios of the IPNs starts decreasing. By using this technique, the IPN hydrogels with new features like super-porous, sensitivity to electricals, controlled release of the formulation etc. were generated. These hydrogels are made of SA and synthetic polymers having carboxylic groups.

The super porous, IPN hydrogels were created by Yin et al. by using sequential cross-linking and fast cross crosslink of sodium acrylate and AAM through polymerization reactions. Here SA was used for the entrapment and sodium bicarbonate as blowing agent. Similarly, in semi IPN hydrogels, the CaCl_2 was utilized as

crosslinking agent. Due to their higher porosity, the higher swelling ratio as well as the high swelling index will be observed in the IPNs created in the above mentioned way. The IPNs also had higher biocompatibility as well as higher mechanical gel strength (Yin et al. 2007).

SA IPNs had received much of industrial attention as it can be used as seal material for muscles and actuators. The seal material must be having polymeric gels with prompt response to electric currents and strong mechanical strength as well. The hydrogels made up of PMAA and SA was shown to have the quick bending in response to electric field in HCl solution. Therefore, this gel was thought to have significant application in preparation of artificial organ components like muscles like contractile structures, electric current modulated drug delivery systems and electric sensors, etc. (Shaikh et al. 2021; Zou et al. 2020). To improve the porosity of these gels, the approach which is being used by most of the researchers is synthesis of IPN composite hydrogels based on the SA and PNIPAAm, by which the researchers were able to create the hydrogels with high reaction rate which is required for the release of the drugs from the delivery systems. Other compounds such as PAAm, PEG, PVA and PVP were also reported to form the hydrogels with SA which were synthesized with the motive to control the release of the drugs from the formulations (Gallagher et al. 2014; Kim et al. 2004; Reddy et al. 2014; Yin et al. 2007).

3.1.3 Starch and Their Derivatives

After cellulose, starch is the second most abundant natural polymer which bids a number of applications in the era of drug delivery. It is biodegradable, bioerrodable and bioactive substance.

- Original starch granules are insoluble in water and contain two components majorly. One is the amylose component which makes about 20–30% of the total starch, is made up of linear chains of α -(1-4-linked-D-glucose) units, while another component is amylopectin which is present as 70 to 80 percent of the granules and is made up of branching chains of α -(1-4-linked-D-glucose) units interconnected by α -(1-6-linked-D-glucose) links. To make the starch, water soluble, a need of modification of the starch is needed. Certain modifications were done previously to make it hydrophilic in nature so that it can be used biomedically. Bothe naïve and modified starch are being utilized as basic material biomedically. However, multicomponent hydrogels were also created by using starch which exhibit excellent mechanical properties, quick response and better solute dispersion which posses variety of applications biomedically (Boppana et al. 2015; Cao et al. 2006; Ganguly et al. 2017; Li et al. 2009).

Semi IPNs which were amphoteric in nature were created by using graft copolymerization by using AA and cationic starch in presence of either PDADMAC or poly (methacryloyloxyethyl-lammonium chloride) (PDMC).

FT-IR spectroscopy has provided evidence of salt connections between the carboxyl groups in the cationic starch-g-AA network and the quaternary ammonium groups on the PDMC chains.

Also, it was revealed from the swelling index investigation, that the semi IPN hydrogels possess high swelling index in distilled water with extraordinary pH behavior. These hydrogels which possess higher PDMC, shows less sensitivity in the basic media which enhance their potential for their use in agriculture as well (Md Nasir et al. 2020; Wang et al. 2021).

3.1.4 Other polysaccharides Based Polymers

A vast number of other polymers such as cellulose, carboxy methyl cellulose (CMC) Hyaluronic acid (HA), Xanthan, Guar gum, chondroitin sulfate are being used for the synthesis of IPNs and semi IPNs. Semi IPNs were created by Bajpai and Mishra which utilizes the cross linked PAA and CMC with average molecular weight between cross link and cross linking density (Bajpai and Mishra 2004; Choudhary et al. 2011; Dragan 2014; Zoratto and Matricardi 2018).

Also, Wang et al. synthesized unique super-absorbent semi-IPN composite hydrogels by grafting NaAA in the presence of linear PVP and a cross-linker to further boost the hydrophilic nature of CMC (Wang et al. 2011). For the final assessment, various parameters of gel like network parameters, structure, pH responsiveness etc. had been checked. Also, the PVP was added additionally to improve swelling index, swelling kinetics, salt resistance so that it can be made strong candidate for its usage as drug delivery system.

Hyaluronic acid (HA), due to its status as a polyanion, HA is also known as hyaluronan and is a linear polysaccharide with a high molecular weight that is made up of two disaccharide units of 1,4-D-glucuronic acid and 1,3-N-acetyl-D-glucosamine that alternate. HA is most widely utilized material for tissue engineering, scaffold synthesis and implant formation. It is highly hydrophilic and known to influence a variety of cellular processes such as migration, adhesion and proliferation etc. is involved in the regulation of water balance, also helps in shielding the surface of cartilage and a free radical scavenger. Excellent biocompatibility, biodegradability, and adaptability are all advantages of HA-based materials when developing materials for tissue engineering scaffolds. It suffers from one major drawback which is because of its high water solubility, due to which the stability of the constructs is very low. To improve the stability, a number of techniques have been developed such as synthesis of semi IPN hydrogels composed of HA in combination with semi synthetic or synthetic polymer (Dragan 2014; Dromel et al. 2021; Guo et al. 2022; Lee and Kurisawa 2013; Lou et al. 2018; Wang et al. 2011).

3.1.5 Protein Based IPNs

During the formulation of IPNs or semi IPNs, proteins are being combined with synthetic or natural polymers. The objective of combining the polymers with the protein is to enhance the bio compatibility, structural activity, mechanical strength and biological activity of the IPN hydrogels and also to boost up the stability of the formulations. In the most recent instance, poly ethylene glycol diacrylate (PEG-DA) was utilized as a cross-linker because, in contrast to other cross-linkers that interact directly with gelatin, PEG-DA undergoes free radical cross-linking polymerization without reaction with the functional groups of GE, creating a matrix that enhanced the structural stability of the semi-IPN scaffold in aqueous solutions (Chang et al. 2007; Dragan 2014; Liu et al. 2009; Zou et al. 2020).

Another protein being employed very frequently is silk fibrin for the synthesis of IPNs. Is fibrous in nature and separated from silk which have heavy and light chains in it. It is regenerated and fibrous in nature. This property makes it an excellent candidate due to its higher biocompatibility and higher mechanical strength which can prevent the protein from the degradation by various enzymatic activities. The IPN synthesized by using silk fibroin are the area of interest for tissue engineering and regenerative medicine due to its good mechanical strength and biocompatibility (Goczkowski et al. 2019; Park et al. 2019; Xiao et al. 2011).

Another vastly using protein is silk sericin (SS) which is water soluble, globular protein and is derived from the silk worm. Other less commonly used proteins are fibrin and PEG, collagen and HA combination and fibrin and HA combinations (Yang et al. 2017; Zhang et al. 2015a, b).

3.2 Synthetic Polymers for IPN Hydrogels

There are multiple ways by which we can combine the polymers (natural/semi synthetic) with the synthetic one and a wide variety of IPN gels are there in literature where the synthetic polymers were combined along with the natural one. In contrast to the biopolymers, whose structures depends upon the nature and natural conditions, the synthetic polymers can be created with a properties according to the need. (Chen et al. 2010; Rabiee et al. 2014) Two basic classes of synthetic polymers can be proposed:

1. IPN hydrogels formulated by using nonionic synthetic polymers like PVA, PAAm and PEG. They are thought to be the most widely used polymers for the synthesis of semi IPNs or IPN hydrogels.
2. IPN hydrogels based on the ionic polymers like anionic and cationic polymers. Only a single, i.e., polyampholyte IPN has been reported in this category (Mohan and Geckeler 2007).

3. These type of hydrogel, with or without modifications, serve in a very good way for the delivery of drugs due to their higher crosslinking index and high mechanical strength.

4 Alginate Based Hydrogels

Alginates are the anionic natural polysaccharides compounds which have been isolated from the cell wall of *Ascophyllum nodosum*, *Laminaria hyperborea*, and *Macrocystis pyrifera*, which are the well-known brown marine algae. Also, they can be isolated from some of the bacterial strains such as *Azotobacter* sp., *Pseudomonas* sp., and others. To recover the alginates from the seaweed, the multistep process is being utilized commercially, which starts with treatment of the dried material with dilute mineral acids, then purification of the material and finally isolated alginic acid is converted into sodium alginate in the presence of calcium carbonate. Sodium alginate is water soluble form of alginic acid. Although, commercially other forms of alginic acid are also available, which are the fermentation products of various microbial extractions, marketable alginates are fully obtained from the algae sources. Alginates are typically linear macromolecules made up of 1,4-linked D-mannuronic acid (M) and 1,4-linked guluronic acid (G) residues that have been organized in heterogeneous (MG) or inhomogeneous (poly-G, poly-M) block-like patterns. Commercially, they differ in the composition and sequencing of G and M blocks (Kumar and Singh 2010; Mukhopadhyay et al. 2013; Shaikh et al. 2021).

The distinct physicochemical properties of alginates influence their usefulness and their quality related parameters. For purchasing the alginates, we have to check the various classes of the same for different parameters like molecular weight, distribution pattern of M and G blocks, rheology, water uptake capacity etc. the average molecular weight of alginates differ from 33,000 to 400,000 g/M which depends upon the average number of molecules present in the structure. The amount of G block in the alginate structure is responsible for the formulation of different type of gel by using alginates. It had been observed that, originally, alginic acids are water insoluble while the monovalent salt and esters of the same like sodium alginate is having comparatively the good solubility and the formulations possess excellent stability as well. The solubility behavior can be controlled by the pH of the solvent, concentration of gelling ions present and the ionic strength of the system. As the alginates have the ability to undergo sol-gel transitions, many semi solid and solid structures can be easily created. It has been found that calcium chloride is mainly used as the cross linking agent for alginates. The pace of gelation is a crucial factor in controlling alginate's ionic gelation.

Alginates generate uniform gels as a result of the delayed gelation. It is possible to lower the gel generation rates by using phosphate buffers (e.g., sodium hexametaphosphate). The reaction between carboxylate (COO_2) groups of alginates and phosphate groups that occur in buffer solutions slows the process of alginate gelation because they compete with the divalent calcium cations. The configuration

of alginate gels is improved by calcium salts with lower water solubility, such as calcium sulphate and calcium carbonate. The temperature affects the pace of gelation because a low temperature reduces the reactivity of calcium ions. The usage of a freeze-thaw method is examined as a highly developed controlled approach for the manufacture of alginate hydrogels. The quality of the hydrogels' formation is linked to the molecular structure of alginate and the proportions of M blocks, G blocks, and M-G blocks (Anwar et al. 2017; Banerjee et al. 2010a; Fitzgerald et al. 2015; Mallikarjuna Reddy et al. 2008).

5 Need of Modifications of Alginates

For the improvement of physicochemical as well as biological properties of alginates, a little modifications are needed sometimes. These modifications can be processed via chemical or the physical crosslinking pathways. The crosslinking pathways can be phase transition process, click reaction process or free radical polymerization process. Through the enzymatic epimerization mediated by mannuronan C-5 epimerases, the proportion of M-blocks to G-blocks may be changed, as well as the alginate structure in M-blocks, G-blocks, or MG-blocks can be intensified. From the alginate backbone, one can separate oligosaccharides, which are polymer fragments with 310 straightforward monosaccharides. Enzymatic depolymerization and acid hydrolysis processes can both be used to prepare alginate oligosaccharides. Acetylation, oxidation, sulfation, and phosphorylation grafted copolymerization are among the most common chemical modifications of hydroxyl (OH) groups seen in alginate structures. Esterification and amidation are two processes that can change the carboxyl group. By adding longer alkyl chains or aromatic groups via covalent cross-linking to the alginate structure, hydroxyl (OH) groups (in the C2 and C3 positions) or carboxyl (COO₂) groups (in the position of C6) can alter the solubility of the alginates. Also, by enhancing the hydrophobicity of alginates, we can decrease the polymer dissolution and therefore its erosion as well. This modification can be a good approach in the formulation of pace controlled/sustained release/delayed release type of formulations (Anwar et al. 2017; Hasnain et al. 2020; Khalid et al. 2018; Mukhopadhyay et al. 2013).

6 Role of IPNs in Drug Delivery

To create a drug delivery system which is efficient in delivering the drugs at a controlled pace, is an exciting as well as difficult for any drug development industry. A number of initiatives were taken using the numerous drug delivering technologies. IPN based technology is novel in all cases for providing the sustained and controlled release to the drugs. Also, IPN can be used to target the medicines to their particular targets. IPNs can be formulated as tablets, pellets, hydrogels, capsules,

microspheres, beads, spheroids, nanoparticles and microparticles etc. in this section, the significantly used formulation of IPN has been elaborated (Madolia 2013; Raina et al. 2020).

IPN based Films: IPN-based films, which are not mosaic membranes, are employed as piezodialysis membrane. The uralkyd/poly(glycidylmethacrylate) based film, which exhibits greater mechanical and tensile strength, is an essential application of the IPN delivery system. Films or matrices made of biodegradable collagen have been used as scaffolds to help transfected fibroblasts survive (Silan et al. 2012).

IPN-based films that are made by combining collagen and polyvinyl alcohol and cross-linking them with vaporized glutaraldehyde demonstrate depot formulation for recombinant human growth hormones. After the transplantation of transfected cells, a long-term expression of the foreign gene has not been established in several animal models. Suh et al. investigated the type I atelocollagen graft copolymerization onto the surface of polyurethane (PU) films treated with ozone. They have been demonstrated to be able to increase fibroblast attachment, proliferation, and cell growth (Park et al. 2018).

IPN based hydrogels: hydrogels are the three dimensional drug delivery systems which can be formulated by a variety of polymers. They can hold water in their structure in lieu of their hydrophilic functional groups.

The most important achievement of IPN based drug delivery systems is the formulation of smart drug delivery systems (SDDS) and also they can be known as stimuli responsive/sensitive drug delivery systems. Moreover, in IPN systems, hydrogels are conveniently used as medication carriers which owes to their ability of self application and ease of creation. Hydrogels are known to be very stable and durable in nature. The stimuli responsive hydrogels are very sensitive to the change in external environment such as temperature, pH or chemical environment. In response to this change, they increase or decrease their volumes and thereby can be used for the creation of stimuli responsive biomaterials. These materials have attracted various biomedical aspects of drug delivery (Park et al. 2019; Qi et al. 2015).

Interpenetrating polymer network PVA/GE hydrogels were created by Eltahir Hago et al. who used the mix of enzymatic and physical cross linking process, a freezing-thawing procedure, and in situ gelatin/mTG synthesis in PVA solution. The prepared IPN were subjected to scanning electron microscopy (SEM) techniques for the evaluation of their structure. Additionally, the proliferation of fibroblast-like L929 cell culture was tested in vitro in order to understand the origin of fibroblasts' behavior (Hago and Li 2013).

Microsphere based IPNs: Microspheres are the most recent and widely used class of formulations. Their use in IPN technology lies on the fact that they can also be prepared for IPNs. They are generally tiny, solid and small particles and composed of natural or synthetic polymers, possess size range of 1 to 1000 nm in diameter. They are the carrier linked drug delivery systems which possess the polymer coat at the top and the medicine is entrapped at center. The microsphere based IPNs seem to be a versatile carrier drug delivery system as they have the ability

to encapsulate a variety of drug molecules and showed the improved release behavior, biocompatibility and biodegradability (Prajapati et al. 2015; Raina et al. 2020; Rastegari et al. 2020).

Ray et al. utilized the emulsion cross linking method by employing glutaraldehyde as a cross linker and formulated the IPN based microspheres of sodium alginate and poly vinyl alcohol (PVA). This formulation was used to regulate the release of diclofenac. Also, the sustained release drug delivery can also be achieved from anticancer medicines (Banerjee et al. 2010b).

However, sometimes, the mucoadhesive microspheres were also employed for IPN formulations. The main idea behind the creation of mucoadhesive microsphere based IPN is that it will provide the localized and targeted drug delivery and enhancement of bioavailability will also be achieved. To distribute the anti-inflammatory medication, IPN microspheres based on Xanthan gum and Polyvinyl alcohol were created by using an emulsion cross-linked technique. Cross-linker glutaraldehyde is utilized in this formulation (Chowdary and Rao 2004).

Sheet based IPNs: Sheet formulation for the drug delivery via IPN is relatively a new approach in the drug delivery era. Basically, this technology is being utilized for the treatment of scars and also for wound dressings. An epoxy resin mixture which consists of 10–90 weight percent of aliphatic or cycloaliphatic epoxide and 90–10 weight percent of polyol/anhydrous is used to create an IPN made of polymeric material such as polyol (allyl carbonate), such as nouryset[®] 200, and epoxy resin (Gao and Zhang 2001; Patri et al. 2007).

IPN based sponges: In the recent era, sponges based IPNs are also being utilized effectively for the delivery of medicines. They are also being used for the delivery of antiseptics to treat skin scars and wound dressings and are also beneficial for the treatment of burns. They are made up of collagen protein as the collagen possesses the ability to absorb huge amount of tissue exudates and can preserve the moist environment and also protect the wound from bacterial attack. Because of their structure, a new granular tissue like structure is formed on the epithelium of the wound. However, 3D sponges can also be formulated to provide the support to the cell cultured skin components (Bhardwaj et al. 2012; Huang et al. 2020).

Capsules based IPNs: IPN-based capsules are one of the crucial methods for medication delivery. IPN capsules are also employed as a means of delivering medications for sustained drug release. Polyacrylamide and polyvinyl alcohol are the main ingredients in interpenetrating polymer networks (IPNs) hydrogel capsules, which provide prolonged medication release. By radical polymerizing the interior phase to create hollow supracolloidal structures with a raspberry core-shell morphology, micro-sized colloidosomes of poly(methyl methacrylate/vinyl benzene) micro gels were employed as the scaffold in the supracolloidal IPN reinforced capsules (Hasnain et al. 2020; Ramaraj and Radhakrishnan 1994).

7 Biomedical Applications of Alginate Based IPNs

The role and biomedical applications of alginate based IPNs in drug delivery can be summarized in Table 1.

Table 1 Biomedical applications of alginate based IPNs in drug delivery

Sr. no.	Drug/drugs incorporated	IPN system	Application	References
1.	Ketoprofen	Sodium alginate and PAA grafted gum ghatti based IPN microbeads through dual (ionic and covalent bond)crosslinking process	Sustained release drug delivery over the period of 12 h	Mishra et al. (2020)
2	Zidovudine	Dual responsive IPN hydrogel microbeads based on sodium alginate and guar gum were synthesized via chemical (covalent) crosslinking method	Controlled release profile of the drug	Eswaramma and Rao (2017)
3	Cefadroxil	Two forms of alginate based IPN were synthesized. These were the sodium alginate along with protein based polymers like gelatin and egg albumin via covalent crosslinking method	Sustained release of the drug	Kulkarni et al. (2001)
4	Diltiazem HCl	Alginate and tamarind seed polysaccharide based IPN synthesized by dual crosslinking viz ionic and covalent crosslinking	Better entrapment and better bio-availability profile of the drug	Kulkarni et al. (2012) and Nayak and Pal (2017)
5	5- Fluorouracil	Sodium alginate based semi IPN were synthesized by using N-propyl isoacrylamide along with sodium alginate	Sustained and delayed release of the drug over 12 h was obtained	Mallikarjuna Reddy et al. (2008)
6	Ofloxacin	IPN beads were synthesized by dual crosslinking (ionic and covalent) using sodium CMC and sodium alginate	Prolonged release over 10 h was obtained	Yazdi et al. (2021)
7	Chlorpheniramine maleate	Sodium alginate grafted with PMMA, IPN beads were formulated via covalent cross linking method	Controlled release over 10 h was developed	Lakshmi Narayana Reddy et al. (2010)
8	Diclofenacpottacium	IPN composite matrices synthesized for grafted copolymers of sodium alginate and acrylic acid via free radical polymerization	To obtain the pH responsive drug release of diclofenac pottacium	Jalil et al. (2017)

(continued)

Table 1 (continued)

Sr. no.	Drug/drugs incorporated	IPN system	Application	References
9	Ibuprofen	IPN beads with sodium alginates and sodium carboxymethyl xanthan	Sustained release of the drug	Bulut (2021)
10.	Flutamide	IPN hydrogel systems by employing Gum karaya and sodium alginate	Sustained release of the system	Reddy et al. (2021)
11	Diclofenac sodium	(Polyethylene glycol)methacrylate and N-isopropylacrylamide and methacrylated alginate based injectable IPN hydrogels	Sustained release	Zhao et al. (2014)
12	Acetaminophen	Sodium alginate with PAA semi IPN hydrogels	Sustained release of the formulation	Samanta and Ray (2014)
13	Tramadol	Alginate and gelatin based IPN microgels	Sustained release of the formulation	Anwar et al. (2017)
14	Prazosin HCl	Sodium alginate and PVA based IPN membranes	Sustained release of the formulation	Kulkarni et al. (2010)
15	Isoxsuprine HCl	Microbeads of zinc alginate with cashew gum	Sustained release of the formulation	Das et al. (2014)

8 Conclusion and Future Prospective

The comprehensive literature review leads to the conclusion that IPN-based systems are widely used in pharmaceuticals and medical fields. Drug, protein/peptide, hormone, and medicinal active agent release behavior can be considerably altered by polymeric materials based on IPN. Understanding serious diseases like acquired immune deficiency syndrome (AIDS), cancer, and cardiac conditions as well as inflammatory disorders like rheumatoid arthritis, osteoarthritis, and meningitis, among others, may be helped by research on IPN as a medication delivery mechanism. IPN is primarily utilized as a carrier system for medicines with brief biological half-lives. IPN provides a number of benefits, including exceptional swelling capacity, specificity, and mechanical strength, all of which are crucial for regulated and targeted drug administration. They can act as potential candidates for tissue engineering, regenerative medicines and sustained drug delivery and also as scaffolds. They are expected to become a most commonly used matrix systems for therapeutic applications in future.

References

- Anwar H, Ahmad M, Minhas MU, Rehmani S (2017) Alginate-polyvinyl alcohol based interpenetrating polymer network for prolonged drug therapy, optimization and in-vitro characterization. *Carbohydr Polym* 166:183–194
- Bajpai A, Mishra A (2004) Ionizable interpenetrating polymer networks of carboxymethyl cellulose and polyacrylic acid: evaluation of water uptake. *J Appl Polym Sci* 93(5):2054–2065
- Banerjee S, Chaurasia G, Pal D, Ghosh AK, Ghosh A, Kaity S (2010a) Investigation on crosslinking density for development of novel interpenetrating polymer network (IPN) based formulation. *J Sci Ind Res* 69:777–784
- Banerjee S, Ray S, Maiti S, Sen KK, Bhattacharyya U, Kaity S, Ghosh A (2010b) Interpenetrating polymer network (IPN): a novel biomaterial. *Int J Appl Pharm* 2(1):28–34
- Bhardwaj V, Harit G, Kumar S (2012) Interpenetrating polymer network (IPN): novel approach in drug delivery. *Int J Drug Dev Res* 4(3):41–54
- Boppana R, Mohan GK, Nayak U, Mutalik S, Sa B, Kulkarni RV (2015) Novel pH-sensitive IPNs of polyacrylamide-g-gum ghatti and sodium alginate for gastro-protective drug delivery. *Int J Biol Macromol* 75:133–143
- Bulut E (2021) Development and optimization of Fe^{3+} -crosslinked sodium alginate-methylcellulose semi-interpenetrating polymer network beads for controlled release of ibuprofen. *Int J Biol Macromol* 168:823–833
- Cao X, Deng R, Zhang L (2006) Structure and properties of cellulose films coated with polyurethane/benzyl starch semi-IPN coating. *Ind Eng Chem Res* 45(12):4193–4199
- Chang Y, Chen S, Yu Q, Zhang Z, Bernards M, Jiang S (2007) Development of biocompatible interpenetrating polymer networks containing a sulfobetaine-based polymer and a segmented polyurethane for protein resistance. *Biomacromolecules* 8(1):122–127
- Chen J, Liu M, Liu H, Ma L, Gao C, Zhu S, Zhang S (2010) Synthesis and properties of thermo- and pH-sensitive poly(diallyldimethylammonium chloride)/poly (N, N-diethylacrylamide) semi-IPN hydrogel. *Chem Eng J* 159(1–3):247–256
- Choudhary S, White JC, Stoppel WL, Roberts SC, Bhatia SR (2011) Gelation behavior of polysaccharide-based interpenetrating polymer network (IPN) hydrogels. *Rheol Acta* 50(1):39–52
- Chowdary KPR, Rao YS (2004) Mucoadhesive microspheres for controlled drug delivery. *Biol Pharm Bull* 27(11):1717–1724
- Cui L, Jia J, Guo Y, Liu Y, Zhu P (2014) Preparation and characterization of IPN hydrogels composed of chitosan and gelatin cross-linked by genipin. *Carbohydr Polym* 99:31–38
- Das B, Dutta S, Nayak AK, Nanda U (2014) Zinc alginate-carboxymethyl cashew gum microbeads for prolonged drug release: development and optimization. *Int J Biol Macromol* 70:506–515
- Das D, Pham HTT, Lee S, Noh I (2019) Fabrication of alginate-based stimuli-responsive, non-cytotoxic, terpolymeric semi-IPN hydrogel as a carrier for controlled release of bovine albumin serum and 5-amino salicylic acid. *Mater Sci Eng C* 98:42–53
- Dragan ES (2014) Design and applications of interpenetrating polymer network hydrogels. A review. *Chem Eng J* 243:572–590
- Dromel PC, Singh D, Andres E, Likes M, Kurisawa M, Alexander-Katz A, Spector M, Young M (2021) A bioinspired gelatin-hyaluronic acid-based hybrid interpenetrating network for the enhancement of retinal ganglion cells replacement therapy. *NPJ Regen Med* 6(1):1–12
- Eswaramma S, Rao KK (2017) Synthesis of dual responsive carbohydrate polymer based IPN microbeads for controlled release of anti-HIV drug. *Carbohydr Polym* 156:125–134
- Eswaramma S, Reddy NS, Rao KK (2017) Carbohydrate polymer based pH-sensitive IPN microgels: synthesis, characterization and drug release characteristics. *Mater Chem Phys* 195:176–186

- Fitzgerald MM, Bootsma K, Berberich JA, Sparks JL (2015) Tunable stress relaxation behavior of an alginate-polyacrylamide hydrogel: comparison with muscle tissue. *Biomacromolecules* 16(5):1497–1505
- Gallagher S, Florea L, Fraser KJ, Diamond D (2014) Swelling and shrinking properties of thermo-responsive polymeric ionic liquid hydrogels with embedded linear pNIPAAm. *Int J Mol Sci* 15(4):5337–5349
- Ganguly S, Maity T, Mondal S, Das P, Das NC (2017) Starch functionalized biodegradable semi-IPN as a pH-tunable controlled release platform for memantine. *Int J Biol Macromol* 95:185–198
- Gao S, Zhang L (2001) Semi-interpenetrating polymer networks from castor oil-based polyurethane and nitrokonjac glucomannan. *J Appl Polym Sci* 81(9):2076–2083
- Goczkowski M, Gobin M, Hindié M, Agniel R, Larreta-Garde V (2019) Properties of interpenetrating polymer networks associating fibrin and silk fibroin networks obtained by a double enzymatic method. *Mater Sci Eng C* 104:109931
- Guo W, Douma L, Hu MH, Eglin D, Alini M, Šećerović A, Grad S, Peng X, Zou X, D'Este M (2022) Hyaluronic acid-based interpenetrating network hydrogel as a cell carrier for nucleus pulposus repair. *Carbohydr Polym* 277:118828
- Hago E-E, Li X (2013) Interpenetrating polymer network hydrogels based on gelatin and PVA by biocompatible approaches: synthesis and characterization. *Adv Mater Sci Eng* 2013:1–8
- Hasnain MS, Ray P, Nayak AK (2020) Alginate-based interpenetrating polymer networks for sustained drug release. In: Nayak AK, Hasnain MS (eds) *Alginates in drug delivery*. Elsevier, London, pp 101–128
- Hoare TR, Kohane DS (2008) Hydrogels in drug delivery: progress and challenges. *Polymer* 49(8):1993–2007
- Huang Y, Zhao X, Zhang Z, Liang Y, Yin Z, Chen B, Bai L, Han Y, Guo B (2020) Degradable gelatin-based IPN cryogel hemostat for rapidly stopping deep noncompressible hemorrhage and simultaneously improving wound healing. *Chem Mater* 32(15):6595–6610
- Jalil A, Khan S, Naeem F, Haider MS, Sarwar S, Riaz A, Ranjha NM (2017) The structural, morphological and thermal properties of grafted pH-sensitive interpenetrating highly porous polymeric composites of sodium alginate/acrylic acid copolymers for controlled delivery of diclofenac potassium. *Des Monomers Polym* 20(1):308–324
- Khalid I, Ahmad M, Minhas MU, Barkat K (2018) Preparation and characterization of alginate-PVA-based semi-IPN: controlled release pH-responsive composites. *Polym Bull* 75(3):1075–1099
- Khurma JR, Nand AV (2008) Temperature and pH sensitive hydrogels composed of chitosan and poly (ethylene glycol). *Polym Bull* 59(6):805–812
- Kim SJ, Yoon SG, Kim SI (2004) Synthesis and characteristics of interpenetrating polymer network hydrogels composed of alginate and poly (diallyldimethylammonium chloride). *J Appl Polym Sci* 91(6):3705–3709
- Kulkarni AR, Soppimath KS, Aminabhavi TM, Rudzinski WE (2001) In-vitro release kinetics of cefadroxil-loaded sodium alginate interpenetrating network beads. *Eur J Pharm Biopharm* 51(2):127–133
- Kulkarni RV, Sreedhar V, Mutalik S, Setty CM, Sa B (2010) Interpenetrating network hydrogel membranes of sodium alginate and poly (vinyl alcohol) for controlled release of prazosin hydrochloride through skin. *Int J Biol Macromol* 47(4):520–527
- Kulkarni RV, Mutalik S, Mangond BS, Nayak UY (2012) Novel interpenetrated polymer network microbeads of natural polysaccharides for modified release of water soluble drug: in-vitro and in-vivo evaluation. *J Pharm Pharmacol* 64(4):530–540
- Kumar Giri T, Thakur D, Alexander A, Badwaik H, Krishna Tripathi D (2012) Alginate based hydrogel as a potential biopolymeric carrier for drug delivery and cell delivery systems: present status and applications. *Curr Drug Deliv* 9(6):539–555

- Kumar P, Singh I (2010) Formulation and characterization of tramadol-loaded IPN microgels of alginate and gelatin: optimization using response surface methodology. *Acta Pharma* 60(3):295
- Lakshmi Narayana Reddy C, Swamy BY, Prasad CV, Subha M, Rao KC (2010) Controlled release of chlorpheniramine maleate through IPN beads of sodium alginate-g-methylmethacrylate. *J Appl Polym Sci* 118(4):2342–2349
- Lee F, Kurisawa M (2013) Formation and stability of interpenetrating polymer network hydrogels consisting of fibrin and hyaluronic acid for tissue engineering. *Acta Biomater* 9(2):5143–5152
- Li X, Xu S, Wang J, Chen X, Feng S (2009) Structure and characterization of amphoteric semi-IPN hydrogel based on cationic starch. *Carbohydr Polym* 75(4):688–693
- Liu Y, Cui Y, Yin G, Ma H (2009) Synthesis, characterization, and drug release behaviour of novel soy protein/poly (acrylic acid) IPN hy-drogels. *Iran Polym J* 18:339–348
- Lohani A, Singh G, Bhattacharya SS, Verma A (2014) Interpenetrating polymer networks as innovative drug delivery systems. *J Drug Deliv* 2014:583612
- Lou J, Stowers R, Nam S, Xia Y, Chaudhuri O (2018) Stress relaxing hyaluronic acid-collagen hydrogels promote cell spreading, fiber remodeling, and focal adhesion formation in 3D cell culture. *Biomaterials* 154:213–222
- Madolia H (2013) Preparation and evaluation of stomach specific IPN hydrogels for oral drug delivery: a review. *J Drug Deliv Ther* 3(2):131–140
- Mallikarjuna Reddy K, Ramesh Babu V, Krishna Rao K, Subha M, Chowdoji Rao K, Sairam M, Aminabhavi T (2008) Temperature sensitive semi-IPN microspheres from sodium alginate and N-isopropylacrylamide for controlled release of 5-fluorouracil. *J Appl Polym Sci* 107(5):2820–2829
- Matricardi P, Pontoriero M, Coviello T, Casadei MA, Alhaique F (2008) In situ cross-linkable novel alginate-dextran methacrylate IPN hydrogels for biomedical applications: mechanical characterization and drug delivery properties. *Biomacromolecules* 9(7):2014–2020
- Matricardi P, Di Meo C, Coviello T, Hennink WE, Alhaique F (2013) Interpenetrating polymer networks polysaccharide hydrogels for drug delivery and tissue engineering. *Adv Drug Deliv Rev* 65(9):1172–1187
- Md Nasir NI, Abdulmalek E, Zainuddin N (2020) Preparation and optimization of water-soluble cationic sago starch with a high degree of substitution using response surface methodology. *Polymers* 12(11):2614
- Mishra B, Upadhyay M, Bakde B (2020) pH sensed interpenetrating polymeric network: application in drug delivery. In: Jana S (ed) *Interpenetrating polymer network: biomedical applications*. Springer, Singapore, pp 119–141
- Mohan YM, Geckeler KE (2007) Polyampholytic hydrogels: poly (N-isopropylacrylamide)-based stimuli-responsive networks with poly (ethyleneimine). *React Funct Polym* 67(2):144–155
- Mukhopadhyay P, Sarkar K, Soam S, Kundu PP (2013) Formulation of pH-responsive carboxymethyl chitosan and alginate beads for the oral delivery of insulin. *J Appl Polym Sci* 129(2):835–845
- Nayak AK, Hasnain MS (2020) *Alginates in drug delivery*. Academic, London
- Nayak AK, Pal D (2017) Tamarind seed polysaccharide: an emerging excipient for pharmaceutical use. *Indian J Pharm Educ Res* 51:S136–S146
- Park S-R, Kang J-H, Ahn DA, Suh MC (2018) A cross-linkable hole transport material having improved mobility through a semi-interpenetrating polymer network approach for solution-processed green PHOLEDs. *J Mater Chem C* 6(29):7750–7758
- Park S, Edwards S, Hou S, Boudreau R, Yee R, Jeong KJ (2019) A multi-interpenetrating network (IPN) hydrogel with gelatin and silk fibroin. *Biomater Sci* 7(4):1276–1280
- Patri M, Reddy C, Narasimhan C, Samui A (2007) Sequential interpenetrating polymer network based on styrene butadiene rubber and polyalkyl methacrylates. *J Appl Polym Sci* 103(2):1120–1126
- Prajapati VD, Gandhi AK, Patel KK, Patel BN, Chaudhari AM, Jani GK (2015) Development and optimization of modified release IPN macromolecules of oxcarbazepine using natural polymers. *Int J Biol Macromol* 73:160–169

- Qi X, Wei W, Li J, Liu Y, Hu X, Zhang J, Bi L, Dong W (2015) Fabrication and characterization of a novel anticancer drug delivery system: salectan/poly (methacrylic acid) semi-interpenetrating polymer network hydrogel. *ACS Biomater Sci Eng* 1(12):1287–1299
- Rabiee A, Ershad-Langroudi A, Jamshidi H (2014) Polyacrylamide-based polyampholytes and their applications. *Rev Chem Eng* 30(5):501–519
- Raina N, Rani R, Khan A, Nagpal K, Gupta M (2020) Interpenetrating polymer network as a pioneer drug delivery system: a review. *Polym Bull* 77(9):5027–5050
- Ramaraj B, Radhakrishnan G (1994) Hydrogel capsules for sustained drug release. *J Appl Polym Sci* 51(6):979–988
- Rastegari A, Mottaghitab F, Farokhi M (2020) Fabrication technology of chitosan-based IPN: drug delivery application. In: Jana S (ed) *Interpenetrating polymer network: biomedical applications*. Springer, Singapore, pp 55–78
- Reddy P, Rao KM, Rao K, Shchipunov Y, Ha C-S (2014) Synthesis of alginate based silver nanocomposite hydrogels for biomedical applications. *Macromol Res* 22(8):832–842
- Reddy OS, Subha M, Jithendra T, Madhavi C, Rao KC (2021) Fabrication and characterization of smart karaya gum/sodium alginate semi-IPN microbeads for controlled release of D-penicillamine drug. *Polym Polym Compos* 29(3):163–175
- Samanta HS, Ray SK (2014) Synthesis, characterization, swelling and drug release behavior of semi-interpenetrating network hydrogels of sodium alginate and polyacrylamide. *Carbohydr Polym* 99:666–678
- Shaikh MAJ, Gilhotra R, Pathak S, Mathur M, Iqbal HM, Joshi N, Gupta G (2021) Current update on psyllium and alginate incorporate for interpenetrating polymer network (IPN) and their biomedical applications. *Int J Biol Macromol* 191:432–444
- Silan C, Akcali A, Otkun MT, Ozbey N, Butun S, Ozay O, Sahiner N (2012) Novel hydrogel particles and their IPN films as drug delivery systems with antibacterial properties. *Colloids Surf B: Biointerfaces* 89:248–253
- Wang W, Wang Q, Wang A (2011) pH-responsive carboxymethylcellulose-g-poly (sodium acrylate)/polyvinylpyrrolidone semi-IPN hydrogels with enhanced responsive and swelling properties. *Macromol Res* 19(1):57–65
- Wang Z, Shi H, Wang F, Wang A, He Q, Cuan S (2021) Synthesis of cassava starch-g-acrylic acid/dimethylaminopropyl methacrylamide: a new hydrogel for brine solution. *Carbohydr Polym* 266:118109
- Xiao W, He J, Nichol JW, Wang L, Hutson CB, Wang B, Du Y, Fan H, Khademhosseini A (2011) Synthesis and characterization of photocrosslinkable gelatin and silk fibroin interpenetrating polymer network hydrogels. *Acta Biomater* 7(6):2384–2393
- Yang J, Chen J, Pan D, Wan Y, Wang Z (2013) pH-sensitive interpenetrating network hydrogels based on chitosan derivatives and alginate for oral drug delivery. *Carbohydr Polym* 92(1):719–725
- Yang C, Xue R, Zhang Q, Yang S, Liu P, Chen L, Wang K, Zhang X, Wei Y (2017) Nanoclay cross-linked semi-IPN silk sericin/poly (NIPAm/LMSH) nanocomposite hydrogel: an outstanding antibacterial wound dressing. *Mater Sci Eng C* 81:303–313
- Yazdi MK, Ganjali MR, Rezapour M, Zarrintaj P, Habibzadeh S, Saeb MR (2021) Ionically gelled polysaccharide-based interpenetrating polymer network systems for drug delivery. In: Nayak AK, Pal D, Hasnain MS (eds) *Ionically gelled biopolysaccharide based systems in drug delivery*. Springer, Singapore, pp 121–133
- Yin L, Fei L, Tang C, Yin C (2007) Synthesis, characterization, mechanical properties and biocompatibility of interpenetrating polymer network–super-porous hydrogel containing sodium alginate. *Polym Int* 56(12):1563–1571
- Zhang Y, Liu J, Huang L, Wang Z, Wang L (2015a) Design and performance of a sericin-alginate interpenetrating network hydrogel for cell and drug delivery. *Sci Rep* 5(1):1–13

- Zhang Q, Wang X, Mu Q, Liu P, Jia S, Chen L, Zhang X, Wang K, Wei Y (2015b) Genipin-cross-linked silk sericin/poly (N-isopropylacrylamide) IPN hydrogels: color reaction between silk sericin and genipin, pore shape and thermo-responsibility. *Mater Chem Phys* 166:133–143
- Zhao J, Zhao X, Guo B, Ma PX (2014) Multifunctional interpenetrating polymer network hydrogels based on methacrylated alginate for the delivery of small molecule drugs and sustained release of protein. *Biomacromolecules* 15(9):3246–3252
- Zoratto N, Matricardi P (2018) Semi-IPNs and IPN-based hydrogels. In: Pal K, Banerjee I (eds) *Polym gels*. Elsevier, London, pp 91–124
- Zou Z, Zhang B, Nie X, Cheng Y, Hu Z, Liao M, Li S (2020) A sodium alginate-based sustained-release IPN hydrogel and its applications. *RSC Adv* 10(65):39722–39730

Alginate Based Micelle in Biomedical Applications



P. R. Sarika and Nirmala Rachel James

Abstract Polymer micelles have widespread applications in the biomedical field including drug and gene delivery and diagnostic imaging. Their specific properties such as biocompatibility, high stability, high accumulation efficiency due to enhanced permeability and retention effect, and the capability to solubilize hydrophobic drugs make them suitable for a variety of applications. Polymer micelles can be engineered by surface modifications to make them appropriate for specific applications. For example, incorporating targeting agents on the surface of the micelle enhances site-specific gene or drug delivery. Multifunctional micelles can be made by designing their polymer structure either by attaching stimuli-sensitive functional groups, targeting agents, or incorporating imaging agents into the micellar structure. Both biopolymers and synthetic polymer-based micelles have been developed and utilized for drug delivery. Biopolymer-based delivery systems provide better biocompatibility, and biodegradability than synthetic ones. In this chapter, biopolymer-alginate-based micelles, their formation, properties, and various applications have been explained in detail. Alginate is a hydrophilic polymer obtained from brown algae and has been extensively used for making nanocarriers for various biomedical applications including tissue engineering, drug delivery, wound dressings, targeted cancer therapy, and diagnostic imaging. We have summarized the micelle formation mechanism, properties of the micelles, structural modifications of alginate required for the micelle formation, and the method of the delivery mechanism. In addition, the recent progress of multifunctional alginate micelle for cancer therapy and imaging applications is also outlined.

Keywords Alginate · Micelle · Self-assembly · Stimuli-responsive

P. R. Sarika

Department of Chemical Engineering, American University of Sharjah, Sharjah, United Arab Emirates

e-mail: sreghunadh@aus.edu

N. R. James (✉)

Department of Chemistry, Indian Institute of Space Science and Technology, Thiruvananthapuram, Kerala, India

e-mail: nirmala@iist.ac.in

1 Introduction

Polymeric micelles are formed by the self-assembly of amphiphilic block polymers containing hydrophilic and hydrophobic moieties in their structure, in an aqueous environment. Micelles with definite size and shape can be prepared by choosing selective polymerization methods such as ring-opening polymerization, controlled radical polymerization, etc. These specific synthetic routes help to achieve amphiphilic polymers with a fixed block ratio, molecular weight, and structure. The self-assembly is governed by various interactions such as van der Waals forces, hydrophobic interaction, hydrogen bonding, guest-host interactions, electrostatic interactions, etc. (Mai and Eisenberg 2012).

Hydrophobic interactions are one of the common driving forces for the self-assembly of amphiphilic copolymers. Various copolymers such as block, graft, hyperbranched, and star copolymers, are widely used for preparing micelles for drug delivery applications due to their ability to form micelles with low CMC and high loading efficiency (Ke et al. 2014). Block polymers such as poly(ethylene glycol)-poly(D,L-lactide) block copolymers (Yasugi et al. 1999), poly(ethylene glycol)-poly(amino acid) (Bae and Kataoka 2009), poly(N-isopropyl acrylamide)-DNA block copolymers (Isoda et al. 2011) have been used for the preparation of micelles. The size and hydrophobic drug loading efficiency of the block copolymer micelle are dependent on the hydrophobicity of the moieties on the polymers. Varshosaz et al. investigated the effect of chain length of the hydrophobic moiety on its CMC and drug loading efficiency (Varshosaz et al. 2012). They found that increase in hydrophobic chain length decreases the CMC of the micelle and also increases its drug loading efficiency. Several researchers studied the importance of hydrophobicity of the amphiphiles on the loading efficiency of the anticancer drug paclitaxel (Kemink et al. 2008; Guo et al. 2009; Lee et al. 2009; Tan et al. 2009).

Hydrogen bonding interactions are another type of driving force for the micelle formation and its properties. The introduction of hydrogen bonding functionalities on the hydrophobic block of amphiphilic polymers lowers the CMC, increases the loading efficiency, and stabilizes the micelle. The introduction of ureas on various diblock polymers increases its drug loading capacity (Ho et al. 2010; Tan et al. 2010).

Ionic interactions between oppositely-charged cations and anions are also used to form micelles for drug delivery applications. Micelles formed through ionic interactions can incorporate ionic drugs, peptides, proteins, and nucleic acids. They can also be successfully used for gene delivery applications. In these types of micelles, three types of ionic interactions are possible- interaction between oppositely charged polymers, between cationic and functional groups on polymers, and between the incorporated cargo and the polymers. The common name for micelle formed by the ionic interaction is polyionic/polyelectrolyte complex micelle (PIC). PIC micelles are excellent in the delivery of proteins and peptides as they carry negative or positive charges depending on the pH. These ionic charges aid them in encapsulating inside the PIC micelle through ionic interactions. Kataoka and coworkers introduced

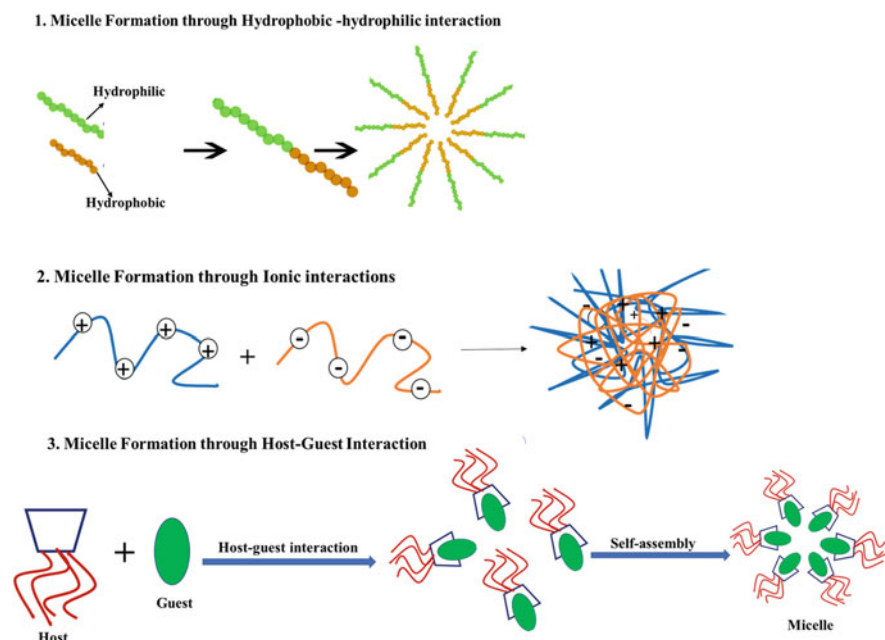


Fig. 1 Formation of micelle through different interactions

the first PIC micelles in 1995 (Segments 1995). They prepared PIC core-shell micelles lysozyme and poly(ethylene glycol)-poly(aspartic acid) block copolymers and used them for the encapsulation of enzymes (Harada and Kataoka 1998). Chen et al. reviewed the formation mechanism, factors affecting the formation, and the various applications of PIC micelle (Chen and Stenzel 2018). Capelôa et al. prepared a PIC micelle through the self-assembly of polypept(o)ide-based triblock polymers polysarcosine, poly(S-ethylsulfonyl-L-cysteine), and a poly(L-lysine) for the delivery of siRNA (Capelôa et al. 2021).

Micelles can also be formed through host-guest interactions. β -cyclodextrin is an excellent candidate for forming host-guest structures as they can accommodate hydrophobic drugs in their hydrophobic inner cavity through supramolecular interactions (Swiech et al. 2012; Nayak and Gopidas 2015). Complexes formed through supramolecular interactions have high colloidal stability, high loading efficiency, and long blood circulation time (Kang et al. 2005; Chen et al. 2007). Kost et al. prepared stereo complexed micelles based on polylactides (PLAs) with β -cyclodextrin (β -CD) for the delivery of doxorubicin through host-guest interactions (Kost et al. 2019). Mechanisms of the formation of micelle through various interactions are shown in Fig. 1.

Self-assembly occurs when the amphiphile reaches or above its critical micellar concentration (CMC). CMC is the minimum concentration required for an amphiphilic polymer in solution to form a micelle. CMC of micelles vary depending on the molecular weight of hydrophobic and hydrophilic polymer blocks and their chemical

characteristics (Biswas et al. 2016). The stability of a micelle in biological environment depends on its CMC. Micelles having low CMC have very high thermodynamic stability and the kinetic stability of the micelle relies on the interactions between the core and the encapsulated drug and the balance between hydrophobic and hydrophilic moieties (Ke et al. 2014). The introduction of more hydrophobic groups in the amphiphilic polymer chain decreases the CMC, which leads to high thermodynamic stability (Atanase 2021). The kinetic stability also depends on the glass transition temperature (T_g) of the hydrophobic core of the micelle.

Polymeric micelles are an excellent candidate for delivering hydrophobic drugs and imaging agents. The hydrophobic core of the micelle accommodates hydrophobic drugs efficiently and the hydrophilic shell of the micelle protects the core from the biological environment. Small particle size and enhanced permeability and retention effect of polymer micelles provide prolonged blood circulation of hydrophobic drugs (Biswas et al. 2016). Polymer micelles can be used for targeted drug delivery by incorporating specific receptors on the shell of the micelles (Deng et al. 2012). Micelles can also be used for stimuli-responsive drug delivery by introducing stimuli-sensitive functional groups in its structure (Wei et al. 2009; Liu et al. 2013). However, polymer micelles are less stable in biological environment as they are easily susceptible to pH, ionic strength, and temperature (Owen et al. 2012; Zhou et al. 2016). Stable micelles are essential for controlling premature drug release and boosting tumor targeting efficiency. Recently, various cross-linking methods are adopted to increase the stability and efficiency of polymer micelles (Ke et al. 2014). Covalent cross-linking increases the stability, but has a poor response to external stimuli (Van Nostrum 2011). Hence, non-covalent interactions such as hydrogen bonding, hydrophobic interaction, π - π stacking, and metal coordination, host-guest interaction are prominent in micelle formation (Mavila et al. 2016). Both synthetic and biopolymers are used in preparation of hydrophilic and hydrophobic blocks in micelle. Polyethylene glycol (PEG) has been extensively used to form hydrophilic shell of micelle (Li et al. 2019). Polymers such as poly(N-vinyl-2-pyrrolidone) (PVP) (Luss et al. 2018), poly(vinyl alcohol) (Sosnik et al. 2021), and poly(vinyl alcohol-co-vinyl oleate) co-polymer (Luppi et al. 2002), and polyethyleneimine (Sarkar et al. 2021) have been used as hydrophilic blocks. Commonly used hydrophobic moieties are propylene oxide (Rikiyama et al. 2018), caprolactone (Sabzi et al. 2020), L-lysine (Akai et al. 2018), aspartic acid (Singh et al. 2019), and D,L-lactic acid (Tam et al. 2019).

Biopolymeric drug delivery systems are preferred over synthetic ones due to their biodegradability, antibacterial activity, low immunogenicity, and biocompatibility (Gopi and Amalraj 2016). Polysaccharides, proteins, and peptides have been extensively used for preparing various biobased nanoparticles for drug delivery applications. Gelatin, silk fibroin, collagen, and albumin are examples of commonly used animal proteins for preparing drug delivery systems. Polysaccharides are macromolecules formed by glycosidic linkages between monosaccharides. Polysaccharides such as chitosan, alginate, cellulose, and pullulan are widely used for forming various delivery systems. Atanase recently reviewed the preparation and characterization of various biopolymers-based micellar drug delivery systems (Atanase

2021). This chapter mainly focuses on alginate-based micellar system and their various biomedical applications.

2 Alginate: Structure and Its Properties

Synthetic and natural polymers have been used in various biomedical applications including drug and gene delivery, and tissue engineering, in implants and stents, for decades. Materials designed and developed from natural resources always have superiority due to their biocompatibility and ability to mimic the extracellular matrices. Sodium alginate adorns a significant position among numerous natural polymers exploited in various biomedical applications. Countless nanomaterials (He et al. 2022), macroparticles (Priyan et al. 2022), drug delivery systems (Lakkakula et al. 2021), hydrogels (Ghauri et al. 2022), and scaffolds (Haghighat et al. 2021) have been developed from alginate for several medical uses due to its nontoxicity, biocompatibility, and cost-effectiveness. About 70% of the biosynthesized alginate has been used in the pharmaceutical and biomedical industries.

Alginate is an anionic polymer extracted mainly from brown algae by aqueous alkali treatments (Clark et al. 1936). It is composed of β -D-mannuronic acid (M) and α -L-guluronic acid (G) units which are arranged as either consecutive G residues (GGGGGG), or consecutive M residues (MMMMMM), or alternating M and G residues (GMGMGM). Depending on the origin/source of the polymer, the ratio of guluronate to mannuronate varies in alginate structure (Lee and Mooney 2012). The chemical structure and properties of alginate are shown in Fig. 2. Larsen et al. provided the first explanation of the structure and various building blocks of alginate (Haug and Larsen 1966; Larsson et al. 1967).

Physicochemical properties of the alginate depend on its molecular weight, functional groups present, and the sequence and ratio of mannuronate and guluronate

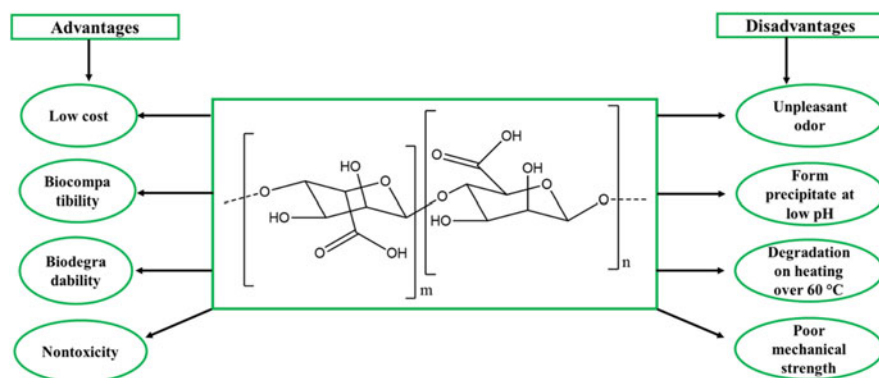


Fig. 2 Structure and advantages and disadvantages in the properties of alginate

groups. Alginate contains free -OH groups and -COOH groups in its structure and its number varies with source and geographical location. Alginate with molecular weight ranging from 32,000 to 400,000 g/mol is available in the market. Since it has poor solubility in water, the viscosity of the alginate solution increases with molecular weight. The selection of alginate with a definite molecular weight is crucial for various applications as the high viscosity could impact the processing.

2.1 Applications of Alginate in the Food Industry and Biological Field

Alginate has been used as a thickening agent, gel-forming, and stabilizing agent in food formulations. It has been used to make films and coatings used for protecting food from microorganisms and increasing its shelf life. Alginate alone or in conjunction with other biopolymers has been used to preserve natural substances, such as natural extracts, fruit and vegetable purees, essential oils, and vitamins. Alginate-based coatings help to reduce food waste and food poisons, and spoilage. Several papers and reviews have been published on the application of alginate in the food industry (Brownlee et al. 2005; Parreidt et al. 2018; Gheorghita Puscaselu et al. 2020). Alginate has been extensively used in the biomedical field due to its biocompatibility, non-toxicity, low cost, and biodegradability. In the biomedical field, it has been extensively used in tissue engineering for the reconstruction of bone, skin, and cartilage (Venkatesan et al. 2015; Hu and Lo 2021; Rekha and Trinath 2021). In biomedicine and the pharmaceutical industry, it has been used for the delivery of anticancer drugs, protein wound dressings, and cell reconstruction. It is also used in tablet manufacturing as a coating material to provide protection and stabilization.

Alginate has been modified, cross-linked, and converted into various sizes, and shapes suitable for diverse biomedical applications. Physical or chemical cross-linking of alginate polymer chain results in hydrogels and the properties of the gels depend on the molecular weight, composition, type of crosslinking, and cross-linking density (Lee and Mooney 2012). Alginate could turn into a gel in presence of divalent cations and these gels could be used for wound healing (Raman et al. 2019), tissue engineering (Distler et al. 2020), and drug delivery (Nayak and Hasnain 2020) applications. Numerous cations including H^+ , Ca^{2+} , Ba^{2+} , Cu^{2+} , Sr^{2+} , Zn^{2+} , Fe^{2+} , Mn^{2+} , Al^{3+} , and Fe^{3+} have been used for gel formation with alginate. Hu et al. recently reviewed the ion-gelation methods and applications of alginate gels (Hu et al. 2021). The G-blocks in alginate predominantly participate in the gel formation and the M-blocks form very strong junctions. MG- blocks also participate in ionic crosslinking and form weak junctions as well. Even though other cations have been used for ionic cross-linking with alginate, Ca^{2+} is the mostly used (Pawar and Edgar 2012). Polyelectrolyte complexes prepared with chitosan and alginate, are another type of nanoparticles prepared by the ionic cross-linking method (Kilicarslan

et al. 2018). Positively charged amino groups of chitosan were cross-linked with negatively charged carboxylate groups of alginate. Other than chitosan, poly-L-lysine, Eudragit E100, and cationized gelatin nanoparticles are also being used for polyionic complex preparation (Sarika and James 2016; Tang et al. 2022).

Among various kinds of alginate-based systems used in biomedical applications, the most prominent form is nanoparticles/nanomaterials. The nanoparticles are mainly used as carriers for delivering less bioavailable drugs, especially hydrophobic cancer drugs. Several methods such as emulsification (Sarika et al. 2015), solvent extractions (Paques et al. 2014), ionic cross-linking, electrospinning (Taemeh et al. 2020; Doderio et al. 2021), self-assembly (Ayub et al. 2019) techniques have been adopted for preparing nanoparticles. Among all the methods used for preparing alginate nanoparticles, the ionic gelation method is the most widely used due to its simplicity easiness, and low cost. This method also does not require specialized equipment and uses less toxic reagents. Alginate-based nanoparticles have been used for drug delivery, insulin delivery, antibiotics delivery, and gene therapy. Niculescu et al. recently reviewed various preparation methods and applications of alginate-chitosan nanoparticles (Niculescu 2022). Sarika et al. prepared cationized gelatin-alginate nanoparticles through electrostatic interaction between anionic alginate and cationic modified gelatin. The nanoparticle has been used for the delivery of curcumin (Sarika and James 2016). Paques et al. have summarized the available methods for the formation of alginate nano-aggregates, nanospheres, and nanocapsules in a recent review (Paques et al. 2014). Alginate has been used as a coating material for magnetic nanoparticles and other nanocarriers. A film of alginate is formed around the nanoparticle by layer-by-layer bottom-up coating technique, to produce a core-shell system. The layers are formed by the electrostatic interaction between oppositely charged polymers that occurs on flat substrates. In some layer-by-layer techniques, the substrates are removed to obtain hollow capsules. Bioactive compounds can be loaded into his hollow space or can be incorporated into the layers.

Nanofibers are another type of alginate nanosystems used in biomedical applications. Fibers can be prepared by solution blowing, force spinning, template synthesis, phase separation, and electrospinning. The nanofibers formed through the electrospinning method have a high surface-to-volume ratio and a porous non-intertwined 3D network. The electrospun mats can mimic the extracellular matrix in the body, thereby acting as a suitable substrate for tissue engineering applications as they allow easy nutrient and gas exchange. Alginate-based nanofibers prepared by electrospinning method have found applications in drug delivery. Ashraf et al. prepared collagen (Col)/sodium alginate (SA)/ polyethylene oxide (PEO)/Rhodotorula mucilaginosa sp. GUMS16 produced exopolysaccharides (EPS) nanofibers by biaxial electrospinning technique. The nanofiber with a diameter of 910 ± 89 nm shows high cell viability and confirms its application in tissue engineering (Sara et al. 2022).

Alginate-based nanocomposites have been extensively used in pharmaceutical and biomedical applications due to their biocompatibility, and swelling behavior. They are modified by incorporating nanoparticles and forming hydrogels to

ameliorate their properties and widen the application range. Metal nanoparticles such as gold, zinc oxide, copper, copper oxide, and silver have antimicrobial activity (Elena et al. 2020). Nanoparticles tend to agglomerate in aqueous solution, especially in physiological fluids, which may reduce their activity. Stabilization of nanoparticles with polymers and surface ligands helps to mitigate the aggregation tendency. The formation of hydrogel with polymers stabilizes the surface of the nanoparticle. Many nanoparticle-alginate hydrogels have been prepared to increase the stability and thereby the biological activity of nanoparticles. Porter et al. protect the antimicrobial activity of silver nanoparticles by incorporating them into alginate hydrogel. The antibacterial property of the silver nanoparticle was enhanced and the silver nanoparticle-alginate gels showed significant bacterial death on the hydrogel biofilms (Porter et al. 2021). Cao et al. recently developed a nanocomposite alginate hydrogel drug delivery system, by the interpenetration of poly(N-isopropyl acrylamide) (PNIPAM) and alginate polymer networks. Graphene oxide and Fe_3O_4 nanoparticles have been incorporated into the polymer networks to accomplish NIR light and magnetic, and pH-responsive drug release (Cao et al. 2021).

Nanogels are nanometer-sized counterparts of hydrogels that have all the advantages of hydrogels and the additional benefits of the nanosize. Alginate-based nanogels are equally important and have widespread applications as hydrogels. Alginate nanogel drug delivery systems have been used for the delivery of many anticancer and anti-inflammatory drugs. Salvati et al. reviewed the conditions to generate alginate nanogels (Salvati et al. 2021). Several reviews and journal articles have been published on the preparation methods and applications of alginate nanogels (Movahedi et al. 2020; Li et al. 2021; Su et al. 2022). Various alginate-based delivery systems are shown in Fig. 3.

3 Alginate Based Micelle

Micelles are formed by the self-assembly of hydrophobic and hydrophilic moieties in the structure of an amphiphilic polymer in an aqueous solution. Alginate is hydrophilic and requires structural modifications to form a micellar structure. Various types of modifications are performed on alginate to make it suitable for micelle formation. It has been modified with hydrophobic polymers, drug molecules, and functional groups for self-assembling to a micellar structure.

3.1 *Alginate Micelle Formation by Grafting Hydrophobic Materials*

Hydrophobic alkyl chains have been grafted to the hydrophilic alginate to make it an amphiphile. These alkyl chains on the alginate propel self-aggregation into a micelle

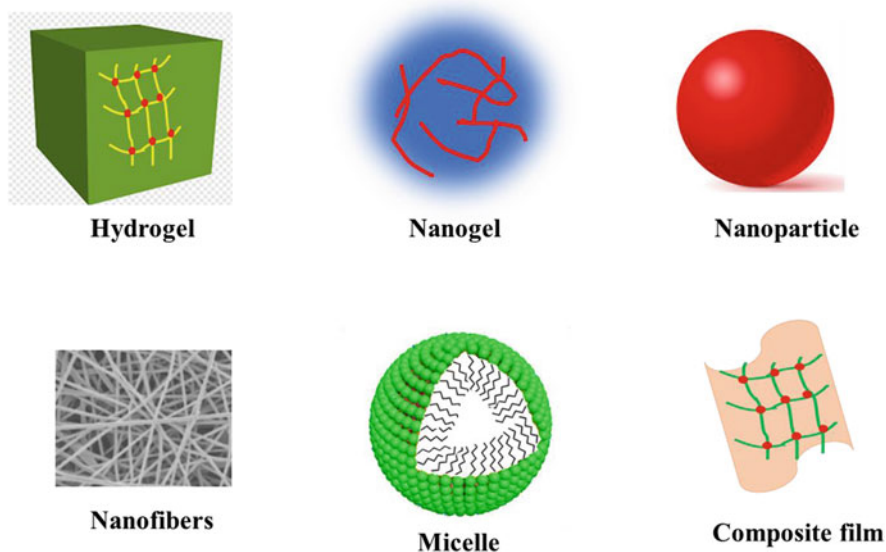


Fig. 3 Various delivery systems based on Alginate

with polar heads adaptable to an aqueous phase and a hydrophobic tail compatible with an oil phase. Hydrophobic drugs can be easily incorporated into the inner core of this micellar structure. Alginate contains hydroxyl and carboxyl moieties in its structure and hydrophobic groups can be easily attached to the polymer through these functional groups via esterification, amidation, and acetylation reactions. Grafting hydrophobic moieties onto the chains of alginate may improve its properties such as hydrophobicity, molecular flexibility, and biological characteristics. The resulting amphiphilic alginate polymer can self-assemble to micelle in aqueous media and they can be used for delivering hydrophobic drugs and molecules. The rigid structure of alginate resulting from intramolecular hydrogen bonding between the carboxyl and hydroxyl groups doesn't favor hydrophobic drug loading. The hydrophobic modification of alginate and the attachment of responsive functional groups stretch the chains and help to accommodate hydrophobic drugs and other molecules.

Dodecyl glycidyl ether is a hydrophobic alkyl chain that has been used extensively for the formation of alginate micelle. Dodecyl glycidyl ether conjugated alginate self assembles to micelle in an aqueous solution at a concentration of 0.4 mg/mL (Yu et al. 2014) and is used for the delivery of drugs clofazimine and Amphotericin B (Meng et al. 2015). Wu et al. optimized the synthesis conditions of dodecyl glycidyl ether-alginate micelle by orthogonal method (Wu et al. 2017). Colloidal properties of this micelle such as surface tension, ζ -potential, and viscosity were also analyzed (Wu et al. 2018). Methyl methacrylate is another hydrophobic group used for creating polymer micelle. Bio-inspired molecules have attracted great attention in biomedical applications due to their less toxicity compared to synthetic

ones. Manna et al. prepared an amino acid-inspired hydrophobic molecule for conjugating with sodium alginate to form an amphiphile and the corresponding micelle. Methyl methacrylate attached to glycine was grafted on sodium alginate through reversible addition–fragmentation chain transfer (RAFT) polymerization to obtain an amphiphilic copolymer. The amphiphile self assembles to micelle in aqueous solutions with CMC of 0.08 mg/mL and size ~200 nm. The micelle showed high loading ability and controlled release behavior toward the hydrophobic drug, indomethacin (Manna et al. 2022). The hydrophilicity of alginate is changed by SN2 reaction using 1-bromohexane as the hydrophobic modifier and the synthesized hexyl alginate ester derivative self-aggregated to micelle with an average hydrodynamic diameter of 416 nm. The loading efficiency of the micelle was demonstrated by performing the sustained release of a pesticide, λ -Cyhalothrin. The studies proved the ability of the micelle as a promising carrier for eco-friendly pesticide formulations (Chen et al. 2022). Hydrophobic octyl amine was grafted on the alginate backbone via the oxidation-reductive amination reaction. The amphiphile finds application in the delivery of the hydrophobic drug ibuprofen (Chen et al. 2021).

To widen the biomedical applications and to improve the properties of alginate, its structure has been modified by various chemical reactions including phosphorylation, esterification, amidation, and oxidation. All these chemical modification require catalysts or coupling agents to boost the reactivity of the functional groups. Ugi condensation reaction improves the properties of alginate without using any additional coupling agent or catalysts. Qian Li et al. modified alginate using hydrophobic oleoyl chloride in water and studied its physicochemical properties in aqueous solutions (Li et al. 2011).

3.2 Alginate Based Prodrug Micelle

Drug delivery by micelle can be achieved by two methods: encapsulating hydrophobic drugs into the hydrophobic core of the micelle by physical interaction or by chemically conjugating the drug on the hydrophilic polymer to form an amphiphile and subsequent release from the resulting micelle in the biological environment. Many hydrophobic drugs have been attached to alginate through easily labile groups for the facile delivery of the drugs. The resulting prodrug self-assembles to micelle or nanoparticles and protects the drug from harsh biological conditions and prevents its premature release. Prodrugs also increase the solubility, bioavailability, and drug loading efficiency of hydrophobic drugs. They also provide long blood circulation time and supports enhanced drug release to the target tissues. The enhanced permeability and retention (EPR) effect of the prodrugs helps to accumulate in tumor sites effectively. Targeting molecules or ligands attached to the prodrug can direct the system toward the active site and provides better therapeutic efficiency. Prodrugs have other advantages such as prolonged blood circulation time, and high drug loading efficiency. Prodrugs are of two classes: carrier-linked prodrugs and precursor prodrugs. Details of prodrug design strategies, mechanisms, advances, and future

perspectives have been reviewed extensively (Introduction 2000; Xu and Mcleod 2001; Abet et al. 2017; Zhang et al. 2021).

Curcumin is a hydrophobic drug with anti-cancer, antioxidant, antineoplastic, antimicrobial, and anti-inflammatory properties (Ali et al. 2006; Ak and Gülçin 2008). Various drug delivery systems have been developed for the delivery of curcumin to cancer cells. Curcumin was delivered using alginate by encapsulating into alginate micelle and by conjugating to the alginate. Manju et al. conjugated curcumin to alginate by esterification reaction between the hydroxyl groups on curcumin and the carboxylic acid groups on the alginate (Manju and Sreenivasan 2011). Wang et al. analyzed the anti-inflammatory activities of alginate curcumin conjugate in ulcerative colitis treatment. The commensal anaerobic flora present in the colon produces esterase which can split the ester bonds present in the conjugate and triggers curcumin release. The released curcumin inhibits the TLR4 expression in the colonic epithelial cell and reduces the inflammation (Wang et al. 2021). Lachowicz et al. analyzed the blood biocompatibility of the alginate-curcumin conjugate (Lachowicz et al. 2019). Karabasz et al. analyzed the in vivo toxicity and antitumor activity of alginate-curcumin conjugate in a mouse tumor model. Their studies showed that the conjugate did not have a significant impact on tumor cells and caused only a moderate reduction in tumor size. Thus, further studies are required for assessing the medical applications of this conjugate (Karabasz et al. 2019). Polymeric micelles based on chitosan, alginate, maltodextrin, pluronic F127, pluronic P123, and tween 80, which were formed by thin-film hydration method could reduce the elevated blood glucose level and lipid profile in diabetic induced mice (Akbar et al. 2018). John et al. prepared Alginate/Pluronic® tri-block copolymer P123 micelles with a size less than 100 nm for the delivery of curcumin. Curcumin was encapsulated inside the hydrophobic core of the micelle. Curcumin was released from the micelle by diffusion mechanism and it showed in vitro cytotoxicity in L 929 cancer cells (John and George 2014).

Gao et al. designed a pH-sensitive alginate prodrug by conjugating hydrophobic doxorubicin through covalent linkage. In aqueous solutions, this prodrug could self-assemble into a nanoparticle micelle with a size of 146 nm. Another hydrophobic drug curcumin was encapsulated inside the micelle through hydrophobic interaction. Both drugs showed enhanced release at acidic pH 5.4. The developed prodrug exhibited co-delivery of multiple drugs into cancer cells with a much-improved safety profile (Gao et al. 2017). Yan et al. developed a pH/reduction responsive dual responsive alginate prodrug for the delivery of 6-thioguanine (6-TG), an anti-metabolite drug for myelocytic leukemia therapy and other immunotherapy. The drug was conjugated to the polymer through a Schiff base linkage formed between the amino groups in 6-TG and the aldehyde groups present in the alginate. The Schiff-base bonds break at acidic pH and provide pH-sensitive drug delivery. In addition to the Schiff base linkages, the thiol groups on the drug can form self-cross-linked disulfide bonds. In an aqueous solution, the prodrug self-assembled to a cross-linked nanostructure. The disulfide linkages are sensitive to the glutathione present in the cancer cells. This dual responsive prodrug system could be a new delivery system for leukemia and immunotherapy (Gao et al. 2017).

In addition to the anticancer drugs, other hydrophobic agents such as vitamins and antioxidants are also conjugated into the hydrophilic polymers for their efficient delivery and activity. Ye et al. conjugated antioxidant α -tocopherol to the alginic acid chains and the resulting prodrug could self-assemble to a nanostructure in an aqueous solution (Ye et al. 2017). Photoactivated pesticide phloxine B was conjugated to alginate (SA) via esterase/GSH sensitive phenolic ester bond. The resulting conjugate self-assembled to micelle in an aqueous solution and showed enhanced release of the pesticide at esterase-6 or GSH stimulus (Yin et al. 2020).

4 Applications of Alginate Micelle in Drug Delivery

In cancer therapy, targeted drug delivery enhances the therapeutic efficacy as the nanosized delivery systems can accumulate at the tumor site with minimum side effects. The drugs were delivered to the tumor sites by three mechanisms: stimuli-responsive delivery and passive and active targeting. Active targeting is achieved by conjugating receptor responsive moieties on the polymer chain and passive targeting is accomplished by nanosized carriers through their enhanced permeability and retention effect (EPR). Drug release can also be achieved by external triggering signals including pH, heat, magnetic fields, ultrasound, and light (Do et al. 2019).

4.1 *Stimuli-Responsive Alginate Micellar Drug Delivery System*

Stimuli-responsive drug delivery has been popular since the 1970s and continues its success in this century. Drug delivery using any polymeric carrier is generally uncontrolled as it may release the drug before reaching the tumor site. The uncontrolled and fast delivery causes toxic side effects and minimizes its concentration in the targeted tumor area. In other words, fast decreases the effectiveness of the drug and also favors the uncontrolled growth of tumors. The design of the carriers with site-specific or responsive functional groups may overcome these drawbacks. Stimuli-responsive delivery systems take advantage of external stimuli including pH, temperature, light, enzyme, magnetic field, and ultrasound. The delivery systems can release their cargo in response to the above-mentioned stimuli in a controlled manner. Responsive delivery systems offer a controlled and a targeted delivery. The main criteria while designing a carrier is that it must be stable and should retain the cargo till the delivery. Besides, the drug should be released in a controlled manner at a suitable time at a definite release site. Many of the alginate micelles are designed to deliver the drugs in response to a wide range of stimuli. The design and controlled release of some of them are detailed in the coming sections.

4.1.1 Temperature Responsive Alginate micelle

Temperature responsive micelles are formed by attaching temperature-sensitive groups to the polymer structure. One of the highly exploited temperature-responsive polymers is poly(N-isopropyl acrylamide) (PNIPAM), which has a lower critical solution temperature (LCST) in an aqueous solution at 32 °C. This polymer is water-soluble below its LCST and becomes insoluble above 32 °C due to the prominent hydrophobic interactions. PNIPAM is grafted to the alginate structure through a free radical polymerization reaction and the graft copolymer self-assembles to a micelle in presence of divalent metal ions (Ba^{2+} , Zn^{2+} , Co^{2+}). The micelles are formed by the electrostatic interactions between divalent cationic metal ions and anionic SA-g-PNIPAM and could be used for delivery of 5-fluorouracil. The size of the micelle varies with the type of divalent ion and the micelle with a large size shows high drug loading efficiency. The release rate of 5-fluorouracil can be controlled by varying the temperature, ionic strength and pH (Yu et al. 2016).

Alginate-poly(N-isopropyl acrylamide) micelles have been used for the delivery of several micelles. Ahn et al. used the micelle for delivery of doxorubicin. Alginate-g-PNIPAM self-assembled to micelle in distilled water due to the increased hydrophobicity of the temperature-responsive group. Doxorubicin was incorporated inside the core of the micelle and it showed controlled release at 37 °C (Ahn et al. 2014).

4.1.2 pH-Sensitive Alginate Micelle

Responsive/controlled drug delivery systems were designed to overcome the drawbacks of traditional carriers. The controlled systems offer cargo delivery at predetermined intervals and predetermined doses in response to various stimuli. The pH range of body fluids is different in various parts of the body. A delivery system, which can sense this pH difference could be used as a pH-responsive drug carrier. pH-responsive polymers can respond to changes in pH by exhibiting changes in their solubility, surface activity, chain conformation, and configuration. pH-responsive polymers are widely used in gene delivery, drug delivery, chromatography, sensor, and membrane synthesis due to their peculiar responsive properties.

Polymers containing acidic or basic functional groups are responsive to pH and ionic strength of the surrounding medium. Hence, polymers holding these functional groups can swell or shrink in response to the pH. For example, acidic polymers will shrink at low pH due to the deprotonation of the acid groups and the swelling will happen at high pH (Veeranna et al. 2011). The pH-responsiveness of polymers allows them to tune their self-assembly behavior, polyelectrolyte nature, and hydrophilicity phase separation. Several reviews have been published on various pH-sensitive drug delivery systems (He et al. 2013; Liu et al. 2014; Wang et al. 2018; Saadat et al. 2021).

Many polymeric micelles have been developed from polymers containing ionizable groups and are able to protonate or deprotonate in response to the pH change in the environment. Several pH-sensitive drug delivery systems for cancer therapy have been developed by making use of the low pH in the cancer tissues. Due to the high glycolysis rate in cancer cells, the pH in cancer tissues is in the range of 5.7 to 7.8, which is lower than the other normal tissues in the body (Stubbs et al. 2000). Endosomes and lysosomes show even a lower pH at the subcellular level. Many pH-responsive delivery systems were designed for controlled delivery of cancer drugs by making use of a low pH gradient of the tumor tissues (Shi et al. 2020).

Polyelectrolyte complex micelles (PEC) are another class of micelles formed by the interaction between oppositely charged electrolytes. pH-sensitive alginate micelles have been developed by exploiting the ability of the weakly ionizable groups in alginate to exhibit an expansion-contraction transition, when a difference in pH occurs. As a result of its anionic nature, alginate can interact with polycations through electrostatic interaction to form polyelectrolyte complexes (Sæther et al. 2008). Li et al. prepared polyelectrolyte complex micelle from chitosan-g-poly (N-isopropyl acrylamide) (PNIPAM) and sodium alginate-g-poly (Nisopropylacrylamide-co-N-vinyl-pyrrolidone) for pH-sensitive delivery of 5-fluorouracil. The drug is loaded inside the core of the micelle through hydrogen bonding between O, N, and F atoms in 5-fluorouracil and the PEC micelle. Since the micelle contains both temperature-sensitive and pH-sensitive groups, the drug release was increased at higher or lower pH and the temperature above LCST of PNIPAM (Sæther et al. 2008).

Li et al. described the synthesis of another alginate-based polyelectrolyte complex micelle and its application for the delivery of a photosensitizer. Alginate was hydrophobically modified with cholesterol and it was complexed with deoxycholic acid group conjugated chitosan derivative through electrostatic interaction. The micelle was used for the protected delivery of a photosensitizer, photosan. The UV-Vis and fluorescence spectroscopy studies indicated the protection efficiency of the micelle. The results indicated the potential of their future application in the protection of photosan, during the blood circulation process in vivo (Li et al. 2014).

4.1.3 Multi-responsive Alginate Micelle for Drug Delivery

Multi-responsive delivery systems can offer better performance and they wider the application areas of these materials. They respond to multiple stimuli and provide better activity than single responsive systems. In addition, multi -responsive systems allow us to precisely tune the release kinetics of the cargo to fit its therapeutic window (Zhuang et al. 2015). Multi-responsive systems can integrate many strategies in a single system which helps to overcome a series of pathological barriers more effectively than single stimuli-responsive delivery vehicle. A recent review by Jia et al. summarizes the advantages of muti-responsive delivery systems for cancer therapy (Jia et al. 2021).

Dodecyl glycidyl ether modified alginate micelles have been used for pH and enzyme responsive release of hydrophobic drug doxorubicin. Dodecyl glycidyl ether-alginate self-assembled to micelle in aqueous solutions and the doxorubicin is encapsulated into the micelle by dialysis method. At acidic pH, the carboxyl groups in the dodecyl glycidyl ether backbone chain get protonated and the hydrophobic interaction between the drug and the hydrophobic groups is reduced, which in turn promotes the doxorubicin release. α -L-fucosidase (AFU) is an enzyme overexpressed in various cancerous tissues, including hepatocellular carcinoma (Waidely et al. 2017). The glycosidic groups in the alginate were cleaved in the presence of AFU, under acidic pH leading to the disassembly of the micelles and the subsequent doxorubicin release (Gao et al. 2020). Tang et al. prepared electrolyte and pH-responsive alginate micelle for the controlled release of acetamiprid. The hydrophobic groups were introduced to alginate via the Ugi reaction without the aid of a catalyst. The amphiphile self-assembled to micelle in aqueous solution and release acetamiprid when there are changes in the Na⁺ concentration and pH of the solution (Tang et al. 2018).

4.1.4 Receptor-Mediated Delivery by Alginate Micelle

Cancer cells overexpress certain receptors, targeting those receptors provide enhanced and selective accumulation of drugs on the cancer cells. For example, hepatocarcinoma cells overexpress asialoglycoprotein receptors, targeting these receptors by groups or molecules which can recognize them provides an accumulation of the drug. Galactosyl or lactosyl moieties can selectively identify the asialoglycoprotein receptors. Sarika et al. conjugated galactose moieties on alginate for site-specific delivery of curcumin from the alginate-curcumin conjugate. The delivery systems with targeting molecules show increased drug release (Sarika et al. 2016).

5 Alginate Micelle for Imaging Applications

Recently, non-invasive imaging technologies such as CT (computerized tomography), gamma-scintigraphy, magnetic resonance (MR), ultrasonography, and nuclear imaging have been used for imaging the vascular wall to diagnose atherosclerosis. Contrasting agents are usually used to get clear and enhanced images of specific tissue areas and to absorb specific signals from areas of interest than surrounding areas. Contrasting agents used in each diagnostic technique are different in terms of their chemical nature, and sensitivity (Torchilin 1999). Nanoparticles have been used for imaging applications due to their long residence time, cell tracking efficiency, biostability, and tunable biodistribution capability (Nishiyama and Kataoka 2006). They can provide simultaneous targeting and imaging compared to conventional imaging dyes. The most frequently used diagnostic moieties for scintigraphy, MRI,

and X-ray computed tomography are ^{111}In or $^{99\text{m}}\text{Tc}$, Gd, and organic iodine respectively. Low molecular weight small molecules containing the above-mentioned nanoparticles are used in the majority of imaging applications. While certain applications including blood pool imaging, tumor, and lymph nodes imaging require stable high molecular weight carriers to provide long-term imaging and protection from the reticuloendothelial system.

Polymeric micelles can protect the imaging agents in their inner core and their potential as carriers for imaging agents has been already established in clinical diagnostics (Trubetskoy 1999). Nanoparticle encapsulated polymer micelles are used as MRI and CT contrast agents. They could provide simultaneous cancer imaging and photothermal damage to the cancer cells (Yang et al. 2013). In some cases, fluorescent polymer amphiphiles are prepared by attaching imaging agents/fluorescent molecules to the polymer chain, and the micelles formed from these amphiphiles can provide imaging and also can act as a drug carrier. Pressly et al. used DOTA-conjugated poly(methyl methacrylate-co-methacryloxysuccinimide-graft poly(ethylene glycol)) (PMMA-co-PMASI-g-PEG) micelle to encapsulate ^{64}Cu for positron emission tomography (PET) imaging.

Alginate micelles have been used for encapsulating or conjugating nanoparticles or fluorescent molecules for imaging applications. Ahn et al. prepared Alginate-g-Poly(N-isopropyl acrylamide) micelle for temperature-sensitive delivery of doxorubicin to the cancer cells. Tagging the micelle with a fluorescent dye may help to see the distribution of the micelle in the biological system. The micelle was tagged with a near-infrared fluorescent (NIRF) dye for analyzing the distribution. Dye-conjugated alginate-g-PNIPAAm micelles help to visualize the tumor margin (Ahn et al. 2014). Lin et al. prepared amphiphilic alginate-based fluorescent polymer nanoparticles through the Ugi one-pot condensation. The conjugate showed excellent biocompatibility, surfactivity, and multicolor bioimaging properties. Their imaging efficiency is evaluated by visualizing the plant stomata with these nanoparticles (Lin et al. 2021a). They have also used the fluorescent micelles to study the stability of Pickering emulsions. The amphiphilic polymer can regulate the thickness of interface film by calcium ions and the fluorescence properties of the micelle help to visualize the stability of the emulsion (Lin et al. 2021b).

Tawfik et al. developed amphiphilic alginate-PEG conjugate and used for capping CdTe quantum dots. The capped quantum dot Opto sensor displayed a high selectivity and sensitivity for ibuprofen. This non-toxic and environmentally friendly optical sensor provides a green and simple analysis of low levels of IBP (Tawfik et al. 2018). Jiang et al. prepared sodium alginate-based fluorescent amphiphile for imaging applications. The conjugate is prepared via an ultrafast one-pot multicomponent reaction with microwave irradiation assistance. In this reaction, the oxidized sodium alginate is conjugated with a fluorescent aggregation-induced emission (AIE) dye. These AIE-active micelles have great potential in biomedical imaging applications (Jiang et al. 2018).

6 Conclusions and Future Perspectives

Biopolymer-based nanoformulations are superior to other delivery systems due to their biocompatibility, non-toxicity, low cost, bioavailability, and hydrophilicity. The latest developments in alginate-based micelles and their various biomedical applications are summarized in this chapter. Many techniques have been developed for the formation of micelle and encapsulation of drugs and imaging agents in the core of the micelle. This chapter also details the moieties or functional groups used for structural modifications of alginate and the techniques for modifications. Over the years, many advancements have been made in the alginate micellar systems for achieving stability, higher drug loading efficiency, and for enhanced and controlled drug release. Though alginate micelles have many applications, it has been enormously used for hydrophobic drug delivery. Structural modifications or improvements are still required to exploit its potential in gene therapy and theragnostics.

References

- Abet V et al (2017) Prodrug approach: an overview of recent cases. *Eur J Med Chem* 127:810–827. <https://doi.org/10.1016/j.ejmech.2016.10.061>
- Ahn D-G, Lee J, Park S-Y, Kwark Y-J, K. Y. L. (2014) Doxorubicin-loaded alginate-g-poly (N-isopropylacrylamide) micelles for cancer imaging and therapy. *ACS Appl Mater Interfaces* 6(24):22069–22077
- Ak T, Gülçin I (2008) Antioxidant and radical scavenging properties of curcumin. *Chem Biol Interact* 174(1):27–37. <https://doi.org/10.1016/j.cbi.2008.05.003>
- Akai H et al (2018) PEG-poly(L-lysine)-based polymeric micelle MRI contrast agent: Feasibility study of a Gd-micelle contrast agent for MR lymphography. *J Magn Reson Imaging* 47(1): 238–245. <https://doi.org/10.1002/jmri.25740>
- Akbar MU et al (2018) In-vivo anti-diabetic and wound healing potential of chitosan/alginate/maltodextrin/pluronic-based mixed polymeric micelles: Curcumin therapeutic potential. *Int J Biol Macromol* 120:2418–2430. <https://doi.org/10.1016/j.ijbiomac.2018.09.010>
- Ali BH et al (2006) Some biological properties of curcumin: a review. *Nat Prod Commun* 1(6): 509–521. <https://doi.org/10.1177/1934578x0600100613>
- Atanase LI (2021) Micellar drug delivery systems based on natural biopolymers. *Polymers* 13(3): 1–33. <https://doi.org/10.3390/polym13030477>
- Ayub AD et al (2019) Biocompatible disulphide cross-linked sodium alginate derivative nanoparticles for oral colon-targeted drug delivery. *Artif Cells Nanomed Biotechnol* 47(1): 353–369. <https://doi.org/10.1080/21691401.2018.1557672>
- Bae Y, Kataoka K (2009) Intelligent polymeric micelles from functional poly(ethylene glycol)-poly (amino acid) block copolymers. *Adv Drug Deliv Rev* 61(10):768–784. <https://doi.org/10.1016/j.addr.2009.04.016>
- Biswas S et al (2016) Recent advances in polymeric micelles for anti-cancer drug delivery. *Eur J Pharm Sci* 83:184–202. <https://doi.org/10.1016/j.ejps.2015.12.031>
- Brownlee IA et al (2005) Alginate as a source of dietary fiber. *Crit Rev Food Sci Nutr* 45:497–510. <https://doi.org/10.1080/10408390500285673>
- Cao Y, Cheng Y, Zhao. G (2021) Near-infrared light-, magneto-, and pH-responsive GO–Fe₃O₄/poly(N-isopropylacrylamide)/alginate nanocomposite hydrogel microcapsules for controlled drug release. *Langmuir* 37(18):5522–5530

- Capelôa L et al (2021) ABC-triblock copolymers for siRNA delivery cross-linkable polyion complex micelles from polypept(o)ide-based ABC-triblock copolymers for siRNA delivery. *Macromol Rapid Commun* 43:e2100698. <https://doi.org/10.1002/marc.202100698>
- Chen F, Stenzel MH (2018) Polyion complex micelles for protein delivery * polyion complex micelles for protein delivery. *Aust J Chem* 71(10):768–780. <https://doi.org/10.1071/CH18219>
- Chen L et al (2007) Enantiomeric PLA—PEG block copolymers and their stereocomplex micelles used as rifampin delivery. *J Nanopart Res* 9:777–785. <https://doi.org/10.1007/s11051-006-9103-8>
- Chen X, Zhu Q, Liu Z, Zhang T, Gong C, Li J, Yan H, Lin Q (2022) Self-aggregate performance of hexyl alginate ester derivative synthesized via SN2 reaction for controlled release of λ -cyhalothrin. *Polym Bull*:1–20. <https://doi.org/10.1007/s00289-022-04103-7>
- Chen X et al (2021) The molecular structure and self-assembly behavior of reductive amination of oxidized alginate derivative for hydrophobic drug delivery. *Molecules* 26(19):5821
- Clark DE, Green HC, Kelco C (1936). Algnc acid and process of makng same. <https://patentimages.storage.googleapis.com/ce/04/2e/23bb938502c916/US2036922.pdf>
- Deng C et al (2012) Biodegradable polymeric micelles for targeted and controlled anticancer drug delivery: promises, progress and prospects. *Nano Today* 7(5):467–480. <https://doi.org/10.1016/j.nantod.2012.08.005>
- Distler T et al (2020) Ionically and enzymatically dual cross-linked oxidized alginate gelatin hydrogels with tunable stiffness and degradation behavior for tissue engineering. *ACS Biomater Sci Eng* 6(7):3899–3914. <https://doi.org/10.1021/acsbiomaterials.0c00677>
- Do HD et al (2019) Advances on non-invasive physically triggered nucleic acid delivery from nanocarriers. *Adv Drug Deliv Rev* 138:3–17. <https://doi.org/10.1016/j.addr.2018.10.006>
- Doderio A et al (2021) An up-to-date review on alginate nanoparticles and nanofibers for biomedical and pharmaceutical applications. *Adv Mater Interfaces* 8(22):2100809. <https://doi.org/10.1002/admi.202100809>
- Elena S et al (2020) Metal-based nanoparticles as antimicrobial agents: an overview. *Nanomaterials* 10(2):292
- Gao C et al (2017) pH-responsive prodrug nanoparticles based on a sodium alginate derivative for selective co-release of doxorubicin and curcumin into tumor cells. *Nanoscale* 9(34):12533–12542. <https://doi.org/10.1039/c7nr03611f>
- Gao X et al (2020) A smart drug delivery system responsive to pH/enzyme stimuli based on hydrophobic modified sodium alginate. *Eur Polym J* 133:109779. <https://doi.org/10.1016/j.eurpolymj.2020.109779>
- Ghauri ZH et al (2022) Novel pH-responsive chitosan/sodium alginate/PEG based hydrogels for release of sodium ceftriaxone. *Mater Chem Phys* 277:125456. <https://doi.org/10.1016/j.matchemphys.2021.125456>
- Gheorghita Puscaselu R, Lobiuc A, Dimian M, Covasa M (2020) Alginate: from food industry to biomedical applications and management of metabolic disorders. *Polymers (Basel)* 12(10):2417
- Gopi S, Amalraj A (2016) Effective drug delivery system of biopolymers based on nanomaterials and hydrogels—a review. *Drug Des* 5(2):1000129. <https://doi.org/10.4172/2169-0138.1000129>
- Guo XD et al (2009) Computational studies on self-assembled paclitaxel structures: Templates for hierarchical block copolymer assemblies and sustained drug release. *Biomaterials* 30(33):6556–6563. <https://doi.org/10.1016/j.biomaterials.2009.08.022>
- Haghighat M, Iranbakhsh A, Baharara J, Ebadi M, Sotoodehnejadnematalahi F (2021) Effect of β -carotene on the differentiation potential of ciliary epithelium-derived MSCs isolated from mouse eyes on alginate-based scaffolds. *Exp Eye Res* 202:108346
- Harada A, Kataoka K (1998) Novel polyion complex micelles entrapping enzyme molecules in the core: preparation of narrowly-distributed micelles from lysozyme and poly(ethylene glycol)-poly(aspartic acid) block copolymer in aqueous medium. *Macromolecules* 31(1):288–294

- Haug A, Larsen B (1966) A study of the constitution of alginic acid by partial hydrolysis. In: Proceedings of the Fifth International Seaweed Symposium, Halifax, August 25–28, 1965, pp 271–277. <https://doi.org/10.1016/B978-0-08-011841-3.50043-4>
- He S et al (2022) Sonochemical fabrication of reduction-responsive alginate-based nanocapsules with folate targeting for drug delivery. *Colloids Surf A: Physicochem Eng Asp* 639:128349. <https://doi.org/10.1016/j.colsurfa.2022.128349>
- He X, Li J, Jiang C (2013) pH-sensitive drug-delivery systems for tumor targeting. *Ther Deliv* 4(12):1499–1510. <https://doi.org/10.4155/tde.13.120>
- Ho S et al (2010) Hydrogen bonding-enhanced micelle assemblies for drug delivery. *Biomaterials* 31(31):8063–8071. <https://doi.org/10.1016/j.biomaterials.2010.07.018>
- Hu C et al (2021) Ions-induced gelation of alginate: mechanisms and applications. *Int J Biol Macromol* 177:578–588. <https://doi.org/10.1016/j.ijbiomac.2021.02.086>
- Hu T, Lo ACY (2021) Collagen—alginate composite hydrogel: application in tissue engineering and biomedical sciences. *Polymers (Basel)* 13(11):1852
- Isoda K et al (2011) Reactive & functional polymers RAFT-generated poly (N-isopropylacrylamide)–DNA block copolymers for temperature-responsive formation of polymer micelles. *React Funct Polym* 71(3):367–371. <https://doi.org/10.1016/j.reactfunctpolym.2010.11.020>
- Jia R, Teng L, Gao L, Su T, Fu L, Qiu Z, Bi Y (2021) Advances in multiple stimuli-responsive drug-delivery systems for cancer therapy. *Int J Nanomedicine* 16:1525–1551
- Jiang R et al (2018) Ultrafast construction and biological imaging applications of AIE-active sodium alginate-based fluorescent polymeric nanoparticles through a one-pot microwave-assisted Döbner reaction. *Dyes Pigments* 153(February):99–105. <https://doi.org/10.1016/j.dyepig.2018.02.008>
- John F, George J (2014) Curcumin encapsulated alginate/pluronic block copolymer micelles as a promising therapeutic agent. *Pharm Biosci J* 2(3):6–12. <https://doi.org/10.20510/ukjpb/2/i3/91160>
- Kang N, Perron M-E, Prud'homme RE, Zhang Y, Gaucher G, Leroux J-C (2005) Stereocomplex block copolymer micelles: core—shell nanostructures with enhanced stability. *Nano Lett* 5(2): 315–319
- Karabasz A et al (2019) Analysis of toxicity and anticancer activity of micelles of sodium alginate-curcumin. *Int J Nanomedicine* 14:7249–7262. <https://doi.org/10.2147/IJN.S213942>
- Ke X et al (2014) Role of non-covalent and covalent interactions in cargo loading capacity and stability of polymeric micelles. *J Control Release* 193:9–26. <https://doi.org/10.1016/j.jconrel.2014.06.061>
- Kemmink J et al (2008) The effect of core composition in biodegradable oligomeric micelles as taxane formulations. *Eur J Pharm Biopharm* 68:596–606. <https://doi.org/10.1016/j.ejpb.2007.08.014>
- Kilicarslan M et al (2018) Preparation and evaluation of clindamycin phosphate loaded chitosan/alginate polyelectrolyte complex film as mucoadhesive drug delivery system for periodontal therapy. *Eur J Pharm Sci* 123(March):441–451. <https://doi.org/10.1016/j.ejps.2018.08.007>
- Kost B, Brzeziński M, Cieślak M, Królewska-Golińska K, Makowski T, Socka M, T. B. (2019) Stereocomplexed micelles based on polylactides with β -cyclodextrin core as anti-cancer drug carriers. *Eur Polym J* 120:109271
- Lachowicz D et al (2019) Blood-compatible, stable micelles of sodium alginate—curcumin bioconjugate for anti-cancer applications. *Eur Polym J* 113(January):208–219. <https://doi.org/10.1016/j.eurpolymj.2019.01.058>
- Lakkakula JR et al (2021) A comprehensive review on alginate-based delivery systems for the delivery of chemotherapeutic agent: doxorubicin. *Carbohydr Polym* 259:117696. <https://doi.org/10.1016/j.carbpol.2021.117696>
- Larsson K et al (1967) Studies on the reduction of uronic acid groups in alginic acid. *Acta Chem Scand* 21:691–704. <https://doi.org/10.3891/acta.chem.scand.38b-0015>

- Lee ALZ et al (2009) The co-delivery of paclitaxel and herceptin using cationic micellar nanoparticles. *Biomaterials* 30(5):919–927. <https://doi.org/10.1016/j.biomaterials.2008.10.062>
- Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. *Progr Polym Sci (Oxford)* 37(1):106–126. <https://doi.org/10.1016/j.progpolymsci.2011.06.003>
- Li C, Obireddy SR, Lai W (2021) Preparation and use of nanogels as carriers of drugs. *Drug Deliv* 28(1):1594–1602. <https://doi.org/10.1080/10717544.2021.1955042>
- Li Q et al (2011) Preparation and characterization of nanoparticles based on hydrophobic alginate derivative as carriers for sustained release of vitamin D3. *J Agric Food Chem* 59(5):1962–1967
- Li HJ et al (2014) Protection of photoactivity of photosensitizers by amphiphilic polysaccharide micelles. *Chin J Polym Sci* 32(10):1413–1418. <https://doi.org/10.1007/s10118-014-1517-7>
- Li H et al (2019) Histology and antitumor activity study of PTX-loaded micelle, a fluorescent drug delivery system prepared by PEG-TPP. *Chin Chem Lett* 30(5):1083–1088. <https://doi.org/10.1016/j.cclet.2019.01.003>
- Lin F, Liu H et al (2021a) Amphiphilic alginate-based fluorescent polymer nanoparticles: fabrication and multifunctional applications. *Int J Biol Macromol* 183:2152–2161. <https://doi.org/10.1016/j.ijbiomac.2021.05.211>
- Lin F, Zhao X et al (2021b) Interfacial regulation and visualization of Pickering emulsion stabilized by Ca^{2+} –triggered amphiphilic alginate-based fluorescent aggregates. *Food Hydrocoll* 119: 106843. <https://doi.org/10.1016/j.foodhyd.2021.106843>
- Liu Y et al (2013) pH-sensitive polymeric micelles triggered drug release for extracellular and intracellular drug targeting delivery. *Asian J Pharm Sci* 8(3):159–167. <https://doi.org/10.1016/j.ajps.2013.07.021>
- Liu J et al (2014) pH-sensitive nano-systems for drug delivery in cancer therapy. *Biotechnol Adv* 32(4):693–710. <https://doi.org/10.1016/j.biotechadv.2013.11.009>
- Luppi B et al (2002) Poly(vinylalcohol-co-vinylolate) for the preparation of micelles enhancing retinyl palmitate transcutaneous permeation. *Drug Deliv* 9(3):147–152. <https://doi.org/10.1080/10426500290095647>
- Luss AL et al (2018) Nanosized carriers based on amphiphilic poly-N-vinyl-2-pyrrolidone for intranuclear drug delivery. *Nanomedicine* 13(7):703–715. <https://doi.org/10.2217/nnm-2017-0311>
- Mai Y, Eisenberg A (2012) Self-assembly of block copolymers. *Chem Soc Rev* 41(18):5969–5985. <https://doi.org/10.1039/c2cs35115c>
- Manju S, Sreenivasan K (2011) Conjugation of curcumin onto hyaluronic acid enhances its aqueous solubility and stability. *J Colloid Interface Sci* 359(1):318–325. <https://doi.org/10.1016/j.jcis.2011.03.071>
- Manna K et al (2022) Amino acid inspired alginate-based pH sensitive polymeric micelles via reversible addition—fragmentation chain transfer polymerization. *ACS Appl Polym Mater* 4(6): 4432. <https://doi.org/10.1021/acsapm.2c00393>
- Mavila S et al (2016) Intramolecular cross-linking methodologies for the synthesis of polymer nanoparticles. *Chem Rev* 116(3):878–961. <https://doi.org/10.1021/acs.chemrev.5b00290>
- Meng Y et al (2015) Amphiphilic alginate as a drug release vehicle for water-insoluble drugs. *Colloid J* 77(6):754–760. <https://doi.org/10.1134/S1061933X15060149>
- Movahedi P et al (2020) Toxicology in vitro folate conjugated hyaluronic acid coated alginate nanogels encapsulated oxaliplatin enhance antitumor and apoptosis efficacy on colorectal cancer. *Toxicol In Vitro* 65:104756. <https://doi.org/10.1016/j.tiv.2019.104756>
- Nayak AK, Hasnain MS (2020) Ionotropically gelled alginate particles in sustained drug release. In: Nayak AK, Hasnain MS (eds) *Alginates in drug delivery*. Academic Press, London, pp 203–230. <https://doi.org/10.1016/b978-0-12-817640-5.00009-1>
- Nayak N, Gopidas KR (2015) Unusual self-assembly of a hydrophilic β -cyclodextrin inclusion complex into vesicles capable of drug encapsulation and release. *J Mater Chem B* 3(17): 3425–3428
- Niculescu A (2022) Applications of chitosan-alginate-based nanoparticles—an up-to-date review. *Nanomaterials* 12(2):186

- Nishiyama N, Kataoka K (2006) Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery. *Pharmacol Ther* 112(3):630–648. <https://doi.org/10.1016/j.pharmthera.2006.05.006>
- Ntroduction I (2000) Targeted prodrug design to optimize drug delivery. *AAPS PharmSci* 2(1): 48–58
- Owen SC, Chan DPY, Shoichet MS (2012) Polymeric micelle stability. *Nano Today* 7(1):53–65. <https://doi.org/10.1016/j.nantod.2012.01.002>
- Paques JP et al (2014) Preparation methods of alginate nanoparticles. *Adv Colloid Interface Sci* 209:163–171. <https://doi.org/10.1016/j.cis.2014.03.009>
- Parreidt TS, Müller K, Schmid M (2018) Alginate-based edible films and coatings for food packaging applications. *Foods* 7(10):170. <https://doi.org/10.3390/foods7100170>
- Pawar SN, Edgar KJ (2012) Alginate derivatization: a review of chemistry, properties and applications. *Biomaterials* 33(11):3279–3305. <https://doi.org/10.1016/j.biomaterials.2012.01.007>
- Porter GC et al (2021) AgNP/alginate nanocomposite hydrogel for antimicrobial and antibiofilm applications. *Carbohydr Polym* 251:117017. <https://doi.org/10.1016/j.carbpol.2020.117017>
- Priyan I et al (2022) International Journal of Biological Macromolecules Preparation of microspheres by alginate purified from *Sargassum horneri* and study of pH-responsive behavior and drug release. *Int J Biol Macromol* 202:681–690. <https://doi.org/10.1016/j.ijbiomac.2022.01.171>
- Raman SP et al (2019) Alginate aerogels carrying calcium, zinc and silver cations for wound care: fabrication and metal detection. *J Supercrit Fluids* 153:104545. <https://doi.org/10.1016/j.supflu.2019.104545>
- Rekha D, Trinath S (2021) Alginate and its application to tissue engineering. *SN Appl Sci* 3(1): 1–19. <https://doi.org/10.1007/s42452-020-04096-w>
- Rikiyama K et al (2018) Micellization of poly(ethylene oxide)-poly(propylene oxide) alternating multiblock copolymers in water. *Polymer* 156(June):102–110. <https://doi.org/10.1016/j.polymer.2018.09.047>
- Saadat M et al (2021) Drug delivery of pH-sensitive nanoparticles into the liver cancer cells. *J Drug Deliv Sci Technol* 63:102557. <https://doi.org/10.1016/j.jddst.2021.102557>
- Sabzi A et al (2020) Targeted co-delivery of curcumin and doxorubicin by citric acid functionalized poly(ϵ -caprolactone) based micelle in MDA-MB-231 cell. *Colloids Surf B: Biointerfaces* 194: 111225. <https://doi.org/10.1016/j.colsurfb.2020.111225>
- Sæther HV et al (2008) Polyelectrolyte complex formation using alginate and chitosan. *Carbohydr Polym* 74(4):813–821. <https://doi.org/10.1016/j.carbpol.2008.04.048>
- Salvati B, Santagapita P, Perullini M (2021) Exploring the conditions to generate alginate nanogels. *J Sol-Gel Sci Technol* 7:1–9. <https://doi.org/10.1007/s10971-021-05631-w>
- Sara S et al (2022) Fabrication and characterization of biaxially electrospun collagen/alginate nanofibers, improved with *Rhodotorula mucilaginosa* sp. GUMS16 produced exopolysaccharides for wound healing applications. *Int J Biol Macromol* 196:194–203. <https://doi.org/10.1016/j.ijbiomac.2021.11.132>
- Sarika PR, James NR (2016) Polyelectrolyte complex nanoparticles from cationised gelatin and sodium alginate for curcumin delivery. *Carbohydr Polym* 148:354–361. <https://doi.org/10.1016/j.carbpol.2016.04.073>
- Sarika PR et al (2015) Nanogels based on alginic aldehyde and gelatin by inverse miniemulsion technique: synthesis and characterization. *Carbohydr Polym* 119:118–125. <https://doi.org/10.1016/j.carbpol.2014.11.037>
- Sarika PR et al (2016) Galactosylated alginate-curcumin micelles for enhanced delivery of curcumin to hepatocytes. *Int J Biol Macromol* 86:1–9. <https://doi.org/10.1016/j.ijbiomac.2016.01.037>
- Sarkar P, Ghosh S, Sarkar K (2021) Folic acid based carbon dot functionalized stearic acid-g-polyethyleneimine amphiphilic nanomicelle: Targeted drug delivery and imaging for triple negative breast cancer. *Colloids Surf B: Biointerfaces* 197:111382. <https://doi.org/10.1016/j.colsurfb.2020.111382>
- Segments P (1995) Formation of polyion complex micelles in an aqueous milieu from a pair of oppositely-charged block copolymers with poly(ethylene glycol) segments. *Macromolecules* 28(15):5294–5299

- Shi Z, Li Q, Mei L (2020) pH-Sensitive nanoscale materials as robust drug delivery systems for cancer therapy. *Chin Chem Lett* 31(6):1345–1356. <https://doi.org/10.1016/j.ccllet.2020.03.001>
- Singh A et al (2019) Delivery of docetaxel to brain employing piperine-tagged PLGA-aspartic acid polymeric micelles: improved cytotoxic and pharmacokinetic profiles. *AAPS PharmSciTech* 20(6):1–9. <https://doi.org/10.1208/s12249-019-1426-8>
- Sosnik A, Shabo RB, Halamish HM (2021) Cannabidiol-loaded mixed polymeric micelles of chitosan/poly(Vinyl alcohol) and poly(methyl methacrylate) for trans-corneal delivery. *Pharmaceutics* 13(12):2142. <https://doi.org/10.3390/pharmaceutics13122142>
- Stubbs M et al (2000) Causes and consequences of tumour acidity and implications for treatment. *Mol Med Today* 6(1):15–19. [https://doi.org/10.1016/S1357-4310\(99\)01615-9](https://doi.org/10.1016/S1357-4310(99)01615-9)
- Su M et al (2022) Microfluidic synthesis of manganese-alginate nanogels with self-supplying H₂O₂ capability for synergistic chemo/chemodynamic therapy and boosting anticancer immunity. *Chem Eng J* 435(P2):134926. <https://doi.org/10.1016/j.cej.2022.134926>
- Swiech O, Mieczkowska A, Chmurski K, Bilewicz R (2012) Intermolecular interactions between doxorubicin and β -cyclodextrin. *J Phys Chem B* 116(6):1765–1771
- Taemeh MA et al (2020) Fabrication challenges and trends in biomedical applications of alginate electrospun nanofibers. *Carbohydr Polym* 228:115419. <https://doi.org/10.1016/j.carbpol.2019.115419>
- Tam YT et al (2019) Poly(ethylene glycol)-block-poly(D,L-lactic acid) micelles containing oligo (lactic acid) 8-paclitaxel prodrug: in vivo conversion and antitumor efficacy. *J Control Release* 298(February):186–193. <https://doi.org/10.1016/j.jconrel.2019.02.017>
- Tan JPK et al (2009) Hierarchical supermolecular structures for sustained drug release. *Small* 5(13): 1504–1507. <https://doi.org/10.1002/smll.200801756>
- Tan JPK et al (2010) Delivery of anticancer drugs using polymeric micelles stabilized by hydrogen-bonding urea groups. *Macromol Rapid Commun* 31(13):1187–1192. <https://doi.org/10.1002/marc.201000105>
- Tang L et al (2022) Microencapsulation of functional ovalbumin and bovine serum albumin with polylysine-alginate complex for sustained protein vehicle's development. *Food Chem* 368: 130902. <https://doi.org/10.1016/j.foodchem.2021.130902>
- Tang Y, Chen K, Li J, Feng Y, Yu G, Wang L, Zhao X, Yang P, Q. Z. (2018) Electrolyte and pH-sensitive amphiphilic alginate: synthesis, self-assembly and controlled release of acetamiprid. *RSC Adv Royal Soc Chem* 8(56):32193–32199. <https://doi.org/10.1039/c8ra05503c>
- Tawfik SM et al (2018) Enhanced fluorescence of CdTe quantum dots capped with a novel nonionic alginate for selective optosensing of ibuprofen. *Sens Actuators B Chem* 256:243–250. <https://doi.org/10.1016/j.snb.2017.10.092>
- Torchilin VP (1999) Polymeric micelles in diagnostic imaging. *Colloids Surf B: Biointerfaces* 16(1–4):305–319. [https://doi.org/10.1016/S0927-7765\(99\)00081-8](https://doi.org/10.1016/S0927-7765(99)00081-8)
- Trubetsky VS (1999) Polymeric micelles as carriers of diagnostic agents. *Adv Drug Deliv Rev* 37(1–3):81–88. [https://doi.org/10.1016/S0169-409X\(98\)00100-8](https://doi.org/10.1016/S0169-409X(98)00100-8)
- Van Nostrum CF (2011) Covalently cross-linked amphiphilic block copolymer micelles. *Soft Matter* 7(7):3246–3259. <https://doi.org/10.1039/c0sm00999g>
- Varshosaz J, Hasanzadeh F, Eslamdoost M (2012) Optimization of self-assembling properties of fatty acids grafted to methoxy poly(ethylene glycol) as nanocarriers for etoposide. *Acta Pharma* 62:31–44. <https://doi.org/10.2478/v10007-012-0006-1>
- Veeranna B, Srujana N, Gupta NV (2011) pH sensitive drug delivery systems: a review. *Am J Drug Discov Dev* 1(1):24–48. <https://doi.org/10.3923/ajdd.2011.24.48>
- Venkatesan J et al (2015) Alginate composites for bone tissue engineering: a review. *Int J Biol Macromol* 72:269–281. <https://doi.org/10.1016/j.ijbiomac.2014.07.008>
- Waidely E, Al-Youbi AO, Bashammakh AS, El-Shahawi MS, Leblanc RM (2017) Alpha-L-fucosidase immunoassay for early detection of hepatocellular carcinoma. *Anal Chem* 89(17): 9459–9466

- Wang Y et al (2021) Commensal flora triggered target anti-inflammation of alginate-curcumin micelle for ulcerative colitis treatment. *Colloids Surf B: Biointerfaces* 203:111756. <https://doi.org/10.1016/j.colsurfb.2021.111756>
- Wang Z et al (2018) Mechanisms of drug release in pH-sensitive micelles for tumour targeted drug delivery system: a review. *Int J Pharm* 535(1–2):253–260. <https://doi.org/10.1016/j.ijpharm.2017.11.003>
- Wei H et al (2009) Thermo-sensitive polymeric micelles based on poly(N-isopropylacrylamide) as drug carriers. *Progr Polym Sci (Oxford)* 34(9):893–910. <https://doi.org/10.1016/j.progpolymsci.2009.05.002>
- Wu J et al (2017) Synthesis and micelle properties of the hydrophobic modified alginate. *Int J Polym Mater Polym Biomater* 66(14):742–747. <https://doi.org/10.1080/00914037.2016.1263956>
- Wu Z et al (2018) Colloid properties of hydrophobic modified alginate: surface tension, ζ -potential, viscosity and emulsification. *Carbohydr Polym* 181:56–62. <https://doi.org/10.1016/j.carbpol.2017.10.052>
- Xu G, Mcleod HL (2001) Strategies for enzyme/prodrug cancer therapy. *Clin Cancer Res* 7(11):3314–3324
- Yang H et al (2013) Micelles assembled with carbocyanine dyes for theranostic near-infrared fluorescent cancer imaging and photothermal therapy. *Biomaterials* 34(36):9124–9133. <https://doi.org/10.1016/j.biomaterials.2013.08.022>
- Yasugi K et al (1999) Preparation and characterization of polymer micelles from poly(ethylene glycol)-poly(D,L-lactide) block copolymers as potential drug carrier. *J Control Release* 62:89–100
- Ye F, Astete CE, Sabliov CM (2017) Entrapment and delivery of α -tocopherol by a self-assembled, alginate-conjugated prodrug nanostructure. *Food Hydrocoll* 72:62–72. <https://doi.org/10.1016/j.foodhyd.2017.05.032>
- Yin Y et al (2020) A sodium alginate-based nano-pesticide delivery system for enhanced in vitro photostability and insecticidal efficacy of phloxine B. *Carbohydr Polym* 247(January):116677. <https://doi.org/10.1016/j.carbpol.2020.116677>
- Yu N et al (2016) Thermo-sensitive complex micelles from sodium alginate-graft-poly(N-isopropylacrylamide) for drug release. *Int J Biol Macromol* 86:296–301. <https://doi.org/10.1016/j.ijbiomac.2016.01.066>
- Yu Y et al (2014) Preparation and characterization of biosurfactant based on hydrophobically modified alginate. *Colloid J* 76(5):622–627. <https://doi.org/10.1134/S1061933X14050160>
- Zhang Y et al (2021) Nanoparticulation of prodrug into medicines for cancer therapy. *Adv Sci* 8:2101454. <https://doi.org/10.1002/advs.202101454>
- Zhou W et al (2016) Factors affecting the stability of drug-loaded polymeric micelles and strategies for improvement. *J Nanoparticle Res* 18(9):1–18. <https://doi.org/10.1007/s11051-016-3583-y>
- Zhuang J, Gordon M, Ventura J, Li L, Thayumanavan S (2015) Multi-stimuli responsive macromolecules and their assemblies. *Gerontology* 61(6):515–525. <https://doi.org/10.1039/c3cs60094g>. Multi-Stimuli

Alginate Based Polyelectrolyte Complexes for Drug Delivery and Biomedical Applications



Parneet Kaur Deol, Amritpal Kaur, Jasleen Kaur Kooner,
Amoljit Singh Gill, Mandeep Singh, and Indu Pal Kaur

Abstract Alginate is one of the most abundant natural biopolymers on earth. It has found immense application in controlled drug delivery owing to its promising profile with respect to safety and biocompatibility, which is missing with several other polymers of interest. It has an inherent mucoadhesive property and can hold substantial amount of water in its matrix to form hydrogels. Alginate is anionic in nature due to presence of carboxylic groups in its polymeric backbone. It can readily crosslink with cations (cationic polymers or ions) to form polyelectrolyte complexes. Latter find applications in the areas ranging from controlled drug delivery to wound dressing scaffold or tissue implants.

The present chapter gives an extensive overview of alginate-based polyelectrolyte complexes including factors affecting their generation. The last part of the chapter focuses on elaborating their biomedical applications including drug delivery.

Keywords Scaffold · Tissue regeneration · Wound dressing · Mucoadhesive · Polycation · Polyanion

1 Alginate

Alginate is the most prevalent biopolymer on the globe and in marine environment. Algae like *Macrocystis pyrifera*, *Laminaria hyperborea*, *Ascophyllum nodosum*, and numerous bacterial strains (*Azotobacter*, *Pseudomonas*) are natural sources of this anionic polysaccharide (Sachan et al. 2009). It is found in nature as divalent alginic acid salts. Alginic acid itself is insoluble in water, but its monovalent salts and esters

P. K. Deol · A. Kaur · J. K. Kooner
G.H.G. Khalsa College of Pharmacy Gurusar Sadhar, Ludhiana, Punjab, India

A. S. Gill
I. K. Gujral Punjab Technical University, Kapurthala, Punjab, India

M. Singh · I. P. Kaur (✉)
University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, Punjab, India
e-mail: indupalkaur@pu.ac.in

are water soluble. Commercial alginate is derived solely from algal sources, however, recently microbial fermentation is reported as a more reliable alternative source of alginate with better defined physicochemical features (Szekalska et al. 2016). Sodium alginate is one of the most thoroughly studied alginate in the pharmaceutical and biomedical fields, and its monograph is included in all official pharmacopoeias.

Chemically, alginate is made up of irregular blocks of -D-mannuronic acid (M) and 1–4 connected -L-guluronic acid (G) residues. The length of the G and M blocks, molecular mass and M/G ratio are all essential parameters that influence the physical properties and gelling capabilities of alginate. All three of the above-mentioned factors tend to alter as the alginate source changes, resulting in a variety of alginate types (Qin 2008). More than 200 distinct types of alginates have recently been observed and isolated from nature. It has been observed that increasing the number of G blocks increases alginate's ability to produce gels. Alginate obtained from *Laminaria hyperborean* is reported to show high G content of 60% (Sahoo and Biswal 2021). On the other hand, increase in MG and M blocks enhances gel flexibility (Lee and Mooney 2012). A high number of M blocks are also reported to enhance immunogenicity of alginate gels and implants (Abasalizadeh et al. 2020).

The molecular weight of commercially sold sodium alginate ranges from 32,000 to 400,000 g/mol. The physical qualities of the resulting gels can be improved by varying the molecular weight of alginate. High molecular weight alginate produces viscous solutions which generally are difficult to manage and are undesirable for processing (Labowska et al. 2021). Proteins or cells mixed with a high viscosity alginate solution are at danger of being damaged by the high shear forces created during mixing and injection into the body (Lee and Mooney 2012). For practical purposes, a blend of high and low molecular weight alginate polymers issued to alter the viscosity of the solution. The pH of the media too influences the viscosity of the alginate solution (Caetano et al. 2016). At low pH, the carboxylate groups in the alginate backbone become protonated and form hydrogen bonds which increase the viscosity of alginate solutions. With increase in pH, protonation reduces, thus reducing the viscosity (Reddy 2021).

The USFDA classifies alginate as generally regarded as safe (GRAS) chemicals. Oral administration of alginate does not elicit any immunological response, and it is reported to be nontoxic and biodegradable (Espevik et al. 1993). Recently, alginate has been widely explored for tissue engineering applications (Sahoo and Biswal 2021). However, because of its natural origin, its composition and purity play a very crucial role in selecting a suitable alginate for such applications. High M content in alginates was found to be immunogenic and 10 times more powerful in triggering cytokine production (Lee and Mooney 2012). Contaminants left in the alginate after processing and purification may elicit immunogenic reaction at the injection or implantation site. Being derived from natural sources, it may also contain contaminants such as heavy metals, endotoxins, proteins, and polyphenolic chemicals (Lopez-Mendez et al. 2021; Deol et al. 2020).

Alginate is inherently nondegradable in mammals because they lack the enzyme, alginase, that can cleave the polymer chains of alginates (Flores et al. 2014). Although cross-linked alginate gels dissolve in dissolution media through exchange

of divalent ions (responsible for reduced alginate solubility) with monovalent ions like sodium ions, the average molecular weights of several commercially available alginates exceed the renal clearance threshold, and thus are unlikely to be entirely eliminated from the body (Reddy 2021). Partial oxidation of alginate chains, using oxidizing agents like sodium periodate, is an appealing method for improving alginate's biodegradability in physiological settings (Sahoo and Biswal 2021). Partial oxidation of alginate reduces its molecular weight without affecting its gelling property (Wang et al. 2022). Oxidized alginate can easily get excreted from the body thus improving its clearance. Further, oxidation of alginate contributes towards increase in its reactivity by increasing the number of reactive groups in its framework. It was observed that oxidized alginate can readily crosslink with various cationic polymers or ions to form complexes (Lee and Mooney 2012).

2 Polyelectrolyte Complexes (PECs)

Polyelectrolytes are macromolecules with a number of charged groups or low molar mass counter ions (LMMC) covalently bonded to them when they are dissolved in a polar solvent like water (Chakraborty et al. 2022). In its uncharged state, a polyelectrolyte behaves like other macromolecules, but dissociation of even a small fraction of its ionic groups causes dramatic changes in its physicochemical properties like chain structure, diffusion coefficients, solution viscosity, polarizability, and miscibility (Dakhara and Anajwala 2011). LMMCs are strongly bonded to the polymer ion group in the solid state and are mobile and solvated in polar liquids. Overall, the polymeric chain does not have any net charge. In bulk solution only a small proportion of the LMMC can migrate away from the polymer due to excessive charge accumulation on polyelectrolyte which holds LMMCs near to the polymer via electrostatic attraction. When another polymer or counter ion of higher molecular mass is mixed with the polyelectrolyte, polyelectrolyte complexes (PECs) with limited aqueous solubility are formed spontaneously (Chremos and Douglas 2018). The active components are molecularly encased in the PECs. This encapsulation not only improves the stability of entrapped active but also alters latter's dissolution profile, hence releasing the active in a controlled manner (Nikolova et al. 2021).

The formation of PECs involves the following three steps (Fig. 1):

1. Formation of primary complex;
2. Creation of secondary complex by the production of new intra-complex linkages;
3. Inter-complex aggregation.

On mixing oppositely charged polyelectrolyte solutions electrostatic interactions are established and leads to development of primary complexes. The secondary phase takes about an hour and involves creation of complex structures like microparticles

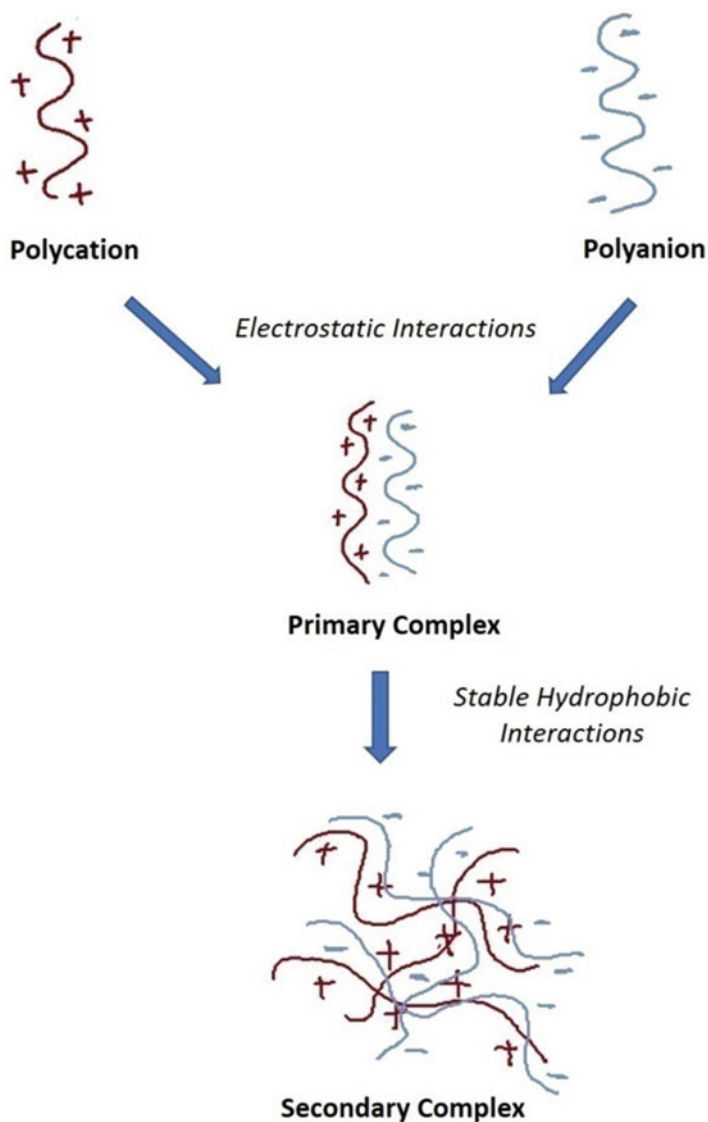


Fig. 1 Various phases of PEC generation

and nanoparticles (Nikolova et al. 2021). In this phase, highly energy efficient complexation takes place and stable intermolecular interactions take place. The third stage involves aggregation of secondary complexes. Hydrophobic bonds are formed during this stage (Dakhara and Anajwala 2011).

3 Alginate Based PECs

Various natural, semisynthetic, and synthetic biocompatible polymers have been widely exploited in the development of polymer-based drug delivery carriers for sustained drug release. Because of its nontoxicity, biodegradability, plentiful availability, ecofriendliness, and low cost, sodium alginate is one of the most researched biocompatible polymers. Alginate is an anionic polysaccharide. It contains carboxylic groups, which are responsible for the polymer's negative charge (Lankalapalli and Kolapalli 2009). It can easily form PECs with cationic ions and based on the source of cation (polymer or ion), alginate-based PECs are classified into two categories:

- Alginate-cationic polymer PECs
- Alginate-metal ion PECs

3.1 *Alginate-Cationic Polymer PECs*

Alginate is an anionic polymer owing to the presence of carboxylic groups in its structure. These carboxylic groups have remarkable sensitivity to external pH stimuli. They remain protonated below the pK_a of alginate, i.e., pH 3.4. Above its pK_a it starts to ionize and contribute towards negative charge of alginate polymer. Alginates can readily crosslink with cationic polymers such as chitosan, gelatin, starch, and poly-L-lysine to form PECs. The following sections will individually discuss such PECs (Aguero et al. 2017).

3.1.1 Alginate-Chitosan PECs

Chitosan is a cationic unbranched polysaccharide made up of D-glucosamine and N-acetyl-D-glucosamine copolymers joined by α -(1–4)-glycosidic bond. Chitosan can spontaneously interact with anionic polymers (like alginate) to create a PEC due to its positively charged amino groups and high charge density under acidic environments (Mateescu et al. 2015). The resulting PECs are normally insoluble in water, although they are intrinsically hydrophilic and have a high proclivity for hydration and can produce hydrogels (Muzzarelli and Muzzarelli 2005). Chitosan solubilizes at low pH while alginate dissolves at neutral pH (Mateescu et al. 2015). Since orally administered delivery systems encounter varied pH ranging from low (pH 1–2) in stomach to neutral or slightly alkaline (pH 6.5–8) in intestine, the chitosan-alginate PECs addresses the issue as alginate reduces the dissolution of chitosan at low pH and the chitosan reduces the dissolution of alginate at neutral pH (Martau et al. 2019) resulting in a sustained and targeted release. Further, biocompatibility and mucoadhesivity of chitosan also makes these PECs a promising candidate for oral delivery of actives (George and Abraham 2006).

Coacervation is the most commonly employed method of preparation of alginate-chitosan PECs. It involves mixing an acidic aqueous solution of chitosan (polycation) with an aqueous solution of alginate. Due to the induction of electrostatic contacts between these two oppositely charged polysaccharides, PECs are produced. Various research groups have modified the basic process of alginate-chitosan PECs generation to optimize drug encapsulation, solubility, release and stability. Table 1 gives the basic details of such reports. Some studies mention synthesis of calcium alginate pre-gel prior to the addition of chitosan. This not only improves the gel strength but also ensures generation of robust polymeric network (Mateescu et al. 2015). Alginate-chitosan films are also reported by some research groups (Kulig et al. 2016). Nature of chitosan used in development of PECs governs the surface morphology of developed films. Best films were developed using low molecular weight chitosan in a ratio 1:3 with alginate (Yan et al. 2001). Kilicarslan et al. produced clindamycin phosphate encapsulated alginate-chitosan PEC films for localized periodontal therapy. The created PEC films had increased swelling, adhesiveness, and drug content and were able to prolong drug release significantly (Kilicarslan et al. 2018). Similar findings were reported by others (Kulig et al. 2016). Alginate-chitosan composite nanoparticles encapsulating various types of medicines have been described by a number of researchers (Niculescu and Grumezescu 2022). The pH of the gastrointestinal tract segment is important in governing release pattern of encapsulated drug from alginate-chitosan PECs. The percentage swelling of alginate-chitosan is small at the acidic pH of the stomach; however, as the pH increases throughout the intestinal tract, the percentage swelling increases proportionally, releasing the encapsulated drug in a controlled manner (Mateescu et al. 2015; Hu et al. 2016). The ratio of alginate to chitosan is varied to develop colon targeted systems (Mladenovska et al. 2007; Tavakol et al. 2009; Segale et al. 2016). Carboxymethyl chitosan-alginate PEC hydrogels with outstanding hygroscopicity modifying capability are also reported. A 450 times increase in hydrogel swelling is reported when the weight ratio of carboxymethyl chitosan was increased in alginate hydrogel. The developed hydrogels demonstrated excellent cytocompatibility and found potential usage in development of wound dressings (Lv et al. 2018).

3.1.2 Alginate-Gelatin PECs

Gelatin is a protein made from denaturized collagen. Collagen denaturation happens when hydrogen interactions break down, causing the triple helices to split from one another and form a random structure (Zhang et al. 2006). Gelatin is nontoxic, biodegradable, biocompatible, and nonimmunogenic. It can be treated at a variety of pH levels, is easily soluble in biological fluids, and is a good film-forming substance that produces robust, flexible film. It is also capable of forming thermally reversible gels (Devi et al. 2016).

Chemically, gelatin is amphoteric as it has both carboxylic (anionic) and amino acid groups (cationic). The negatively charged carboxylic acid groups of manuronic

Table 1 List of various alginate-chitosan PECs

Delivery system	Encapsulated drug/active	Rationale of study	Reference
Chitosan coated calcium alginate beads	Chlorpheniramine maleate	Prolonged/controlled drug release	Alfatama et al. (2021)
	Acrylamidase		Bedade et al. (2019)
	Verapamil		Mateescu et al. (2015)
	BSA		Hu et al. (2016)
	Amoxicillin		Arora and Budhiraja (2012)
	Albendazole		Wang et al. (2011)
	5- fluorouracil		Azhar and Olad (2014)
	Sulfasalazine	Colonic delivery of drug	Tavakol et al. (2009)
	Insulin	Improve drug stability	Onal and Zihnioğlu (2002), Jaafar and Hamid (2019), Chen et al. (2019)
	BSA		Hu et al. (2016)
Microspheres/ Microparticles/ Microcapsules	Paracetamol	Tastemasking	Almurisi et al. (2020)
	Insulin	Improving drug stability	Zhang et al. (2011)
	Diltiazem chlorhydrate		Sultana et al. (2009)
	BSA		Coppi et al. (2001)
	Diltiazem chlorhydrate	Improving mucoadhesive property	Sultana et al. (2009)
	Quercetin	Prolonged/controlled drug release	Frent et al. (2022)
	Diclofenac sodium		Omer et al. (2021)
	Nitrofurantoin		Hari et al. (1996)
	Celecoxib	Colon targeted system	Segale et al. (2016)
	5-aminosalicylic acid		Mladenovska et al. (2007)
Tablets	Haemoglobin	Improved drug loading and encapsulation	Silva et al. (2006)
	Theophylline	Prolonged/controlled drug release	Li et al. (2013), Jin et al. (2020)
Nanoparticles	Timolol maleate		Tonnesen and Karlsen (2002)
	Gatifloxacin	Improved drug loading and encapsulation	Ravisankar et al. (2015)
	Curcumin diethyl diglutarate	Prolonged/controlled drug release	Sorasitthyanukarn et al. (2019)
	Curcumin diglutaric acid		Sorasitthyanukarn et al. (2018)
	Quercetin		Mukhopadhyay et al. (2018)
	Crocine		Rahaiee et al. (2017)
	Nifedipine		Li et al. (2008)

(continued)

Table 1 (continued)

Delivery system	Encapsulated drug/active	Rationale of study	Reference
Films	Lovastatin		Thai et al. (2020)
	Clindamycin phosphate	Localised drug delivery	Kilicarslan et al. (2018)
	Ketotifen fumarate	Controlled release	Lefnaoui et al. (2018)
Gels	Ornidazole		Pei et al. (2008)
	<i>Haliotis iris</i>	High porosity	Massana Roquero et al. (2021)
	Diclofenac sodium	Sustained release	Nikolova et al. (2021)
	Urease	Enzyme immobilization	Hermanto et al. (2019)
	Protein	Improved absorptive capacity	Somo et al. (2017)

BSA bovine serum albumin

and guluronic acid units in alginate interact electrostatically and bind with the positively charged amino groups of gelatin to form a PEC coacervate (Mateescu et al. 2015). Researchers have used alginate-gelatin PECs for a variety of applications ranging from a coating material (implants) to carrier systems (hydrogels, microparticles) for targeted and controlled drug release. Xiao et al. (2009) coated titanium implant with gentamicin loaded gelatin-alginate PEC. The coating was found to prolong the release of water-soluble drug (gentamicin) for 10 days in pH dependent manner (Li et al. 2008; Singh et al. 2022). Developed PEC not only helped in delivering the drug to the site of infection but also improved implant performance by reducing bacterial colonization and formation of biofilms on the implant surface. Gelatin-alginate PECs were also reported to prolong the release of another water soluble drug, indomethacin for 14 h by encapsulating the same in alginate-gelatin coacervates (Joseph and Venkataram 1995). Sodium alginate and gelatine have also been used to successfully encapsulate various oils like olive and sunflower oil. Devi N et al. reported olive oil loaded alginate-gelatin microparticles with glutaraldehyde as a crosslinker. The system resulted in controlled release of encapsulated oil. The effectiveness of encapsulation was found to improve as the concentration of oil, polymers, and glutaraldehyde were raised (Devi et al. 2012). Various studies involving alginate-gelatin PECs are listed in Table 2.

3.1.3 Alginate-Starch PECs

Starch is an excellent polymer because it is biodegradable, non-toxic, and biocompatible. Furthermore, it is inexpensive and is widely available. Linear amylose and branched amylopectin form the backbone of starch. The amylopectin region is responsible for its crystalline appearance while amylose contributes to its amorphous character (Thomas et al. 2021). Cationized starches are major industrial derivatives of starches that have been given a positive ionic charge by adding ammonium,

Table 2 List of various alginate-gelatin PECs

Delivery system	Encapsulated drug/active	Rationale of study	Reference
Coated implants	Gentamicin	Prolonged/controlled drug release	Li et al. (2008)
Hydrogel	Transglutaminase	Prolonged/controlled drug release	Sun et al. (2021)
	Silver	Localized drug delivery	Diniz et al. (2020)
Microparticles/coacervates	Sunflower oil	Prolonged/controlled drug release	Devi et al. (2016)
	Olive oil		
	Indomethacin		

Table 3 List of various alginate-starch PECs

Delivery system	Drug encapsulated	Rationale of study	Reference
Hydrogel patches	Rhodamine B	Localized drug delivery	Bom et al. (2020)
Nanoparticles	Theophylline	Prolonged/controlled drug release	Thomas et al. (2021)
Beads	Aceclofenac	Prolonged/controlled drug release	Malakar et al. (2013)
	Trifluralin		Onyido et al. (2012)
	L-phenylalanine		Kim et al. (2005)

amino, imino, sulfonium, or phosphonil groups (Malakar et al. 2013). It is through these groups that anionic alginates form PECs with starch. Starch is reported to improve the efficacy of alginate hydrogels by regulating drug release from its matrix and modifying various physical properties of alginate (Onyido et al. 2012). Starch has been shown to operate as a structural component in the PEC as it controls the shrinking capacity of alginate (Zhang et al. 2019). Researchers have developed various delivery systems (patches, nanoparticles and beads) by combining these two polymers (Table 3) to control/prolong the release of loaded active.

3.1.4 Alginate-Polylysine PECs

Polylysine is a water soluble, biocompatible polymer of amino acid lysine. It is a natural broad-spectrum antimicrobial, effective against both gram-positive and gram-negative bacteria (Zheng et al. 2021). Amino groups in polylysine are involved in self-gelling with carboxylic groups of alginate via Schiff base reaction (Ge et al. 2022). Polylysine-alginate nanoparticles are reported for sustained release of loaded antigen (Bovine serum albumin). The developed particles did not show any cytotoxicity and were readily taken up by target cells versus free antigen (Yuan et al. 2018). Polylysine has been widely employed to produce alginate-polylysine

microcapsules via polycation membrane with alginate (Leick et al. 2011). Developed microcapsules reported improvement in drug encapsulation, its in vitro release profile and stability (Orive et al. 2006). Cai et al. (2015) developed alginate-polylysine films for improving the shelf life and preservation quality of Japanese sea bass (Fish). Significant improvement in evaluated parameters was recorded (Cai et al. 2015).

Ren et al. (2016) compared binding capacity of chitosan and polylysine with alginate. It was observed that polylysine rapidly binds to alginate vis-à-vis chitosan due to smaller molar hydrodynamic volume of former. Polylysine is reported to have higher binding capacity and higher binding stoichiometry to sodium alginate versus chitosan. Further polylysine forms readily reversible complexes with alginate with nine times higher binding enthalpy than that for chitosan (Ren et al. 2016).

3.1.5 Other Alginate-Polymer PECs

Alginate-Eudragit PECs: Eudragit is a cationic copolymer made up of 2-dimethylaminoethyl methacrylate, methyl methacrylate, and n-butyl methacrylate with a mean relative molecular mass of 150,000. Dimethylaminoethyl methacrylate groups contribute 20–25% of all the methacrylates and is present roughly in a ratio of 2:1:1 to butyl methacrylate and methyl methacrylate groups (Jeganathan and Prakya 2015). Eudragit readily forms PECs with sodium alginate. Mucoadhesive properties of alginate are combined with pH sensitive dissolution of eudragit E to develop site-specific delivery of actives. Researchers have used alginate-eudragit PEC for coating purposes to minimize drug losses in upper gastro intestinal tract (Chawla et al. 2012; Sonavane and Devarajan 2007). Eudragit-alginate nanoparticles developed via coacervation indicated a shelf life of about 1 month (Sepúlveda-Rivas et al. 2019).

3.2 Alginate-Metal Ion PECs

Alginate readily undergoes ion-induced gelation. Its salt with monovalent cation (Na^+) is having good water solubility. Once it comes in contact with divalent (Ca^{2+} , Ba^{2+} , Cu^{2+} , Sr^{2+} , Fe^{2+} , Zn^{2+} , Mg^{2+}) or trivalent (Al^{3+} , Fe^{3+}) cations, the monovalent ions get replaced by later to form hydrogel via formation of an egg box structure as proposed by Grant et al. (1973).

Alginate hydrogels produced by different cations vary in various properties viz. gel strength, viscoelasticity and biocompatibility (Hu et al. 2021). Bivalent alkaline earth cations like Mg^{2+} , Ca^{2+} and Sr^{2+} form ionic bonds with alginate while all other multivalent ions form complex uronates by strong coordinate-based interactions (Peric-Hassler and Hunenberger 2010). The former produce reversible gels while the gels formed via strong coordinate based interactions are irreversible having high binding forces (Brus et al. 2017). Idota et al. arranged all cations based on their binding constant 'K' which indicate their affinity towards alginate. It was found that

Sr^{2+} had the highest affinity and K^{+} the lowest affinity for alginate (Idota et al. 2016). Further, it was found that the 'K' value of divalent ions is more than monovalent ions thus, they readily replace the later in a solution to form hydrogels.

The M/G ratio and its configurations differ significantly depending on the molecular weight of the alginate. Polyvalent cations have more affinity for G blocks as compared to M blocks. Thus, alginates having higher M/G ratio form porous, leaky gels while lower M/G ratio ascertains formation of stronger gels (Ramos et al. 2018).

Calcium alginate is one of the most explored PECs and the calcium alginate scaffolds are reported to efficiently encapsulate even viable cells like fibroblasts. It was found that these scaffolds steadily disintegrate, without losing volume, for over 21 days. Further, cells were found to be viable after being liberated from the scaffold even after 21 days (Hunt et al. 2012). Calcium alginate nanoparticles loaded with bovine serum albumin were developed to sustain the release of loaded protein for extended period (Nesamony et al. 2012). Similarly, calcium alginate films were developed to exhibit good water vapor barrier characteristics. Effect of alginate composition and contact time with crosslinker calcium ion was determined. It was found that choosing an appropriate immersion period in calcium solution was important in the production of ideal films. Further, G block rich alginate forms stronger films as compared to M block rich ones (Olivas and Barbosa-Canovas 2008). Various calcium alginate PECs based delivery systems and their details are given in Table 4.

Other divalent cations are also reported to fabricate alginate PECs. Folic acid loaded copper alginate PECs were developed to protect folic acid from acidic environment of stomach. Copper alginate is a gastro-resistant substance which releases the encapsulated actives at $\text{pH} > 5$, therefore it mainly releases the loaded active in intestine (Camacho et al. 2019). Another group reported development of ferric alginate PECs and evaluated effect of alginate composition on PEC properties (Machida-Sano et al. 2012). In vitro cell adhesion and proliferation studies suggested that initial cell adhesion was not influenced by M or G block content in ferric alginate PECs. Further both PECs had equivalent protein adsorption capabilities. However, as the culture time preceded, high-G content in ferric alginate PECs promoted superior cell proliferation. It was observed that the mode of cross-linkage between ferric ions and alginate varies depending on alginate composition. PECs formed using high M alginate loses ionic crosslinkage with time and thus records lower degree of cell growth (Machida-Sano et al. 2012). Different batches of beads, altering the ratios of calcium and magnesium ions, were prepared to determine the effect of adding Mg^{2+} cations to calcium alginate chains to crosslink them and generate mixed heteroionic calcium/magnesium alginate beads. Although developed beads were found to absorb water quickly at $\text{pH} 7.2$, the system with the largest amount of Mg^{2+} , had the highest swelling rate at both low ($\text{pH} 1.2$) and high pH ($\text{pH} 7.2$). It was concluded that as there was no significant alginate- Mg^{2+} interactions, the Na^{+} ions present in the phosphate buffer exchange was more quickly enhanced the rate of swelling (Sanchez-Ballester et al. 2019).

Table 4 List of various calcium alginate PECs

Beads	Trimetazidine	Prolonged/controlled drug release	Mandal et al. (2010)
	Sulphamethoxazole		Badwan et al. (1985)
	Famotidine		Satishbabu et al. (2010)
	5-fluorouracil	Colonic delivery of drug	Agarwal et al. (2015)
	Tinidazole		Chekwube et al. (2022)
	Ceftriaxone	Improved drug stability	Patel et al. (2016)
Microspheres/Microparticles/Microcapsules	Astaxanthin	Improving drug stability	Lin et al. (2016)
	Probiotics		Mathews (2017)
	Prednisolone	Improving mucoadhesive property	Wittaya-areekul et al. (2006)
	Amoxicillin		Arora and Budhiraja (2012)
	Metformin hydrochloride	Prolonged/controlled drug release	Szekalska et al. (2018)
	Catechin		Kim et al. (2016)
	Cyclosporine A	Colon targeted system	Oshi et al. (2021)
	Mangostins		Mulia et al. (2020)
	Insulin	Improved drug loading and encapsulation	Goswami et al. (2014)
	Lidocaine		Park et al. (2004)
Tablets	Acetazolamide	Prolonged/control led drug release	Barzegar-Jalali et al. (2013)
	Metronidazole		Sriamornsak et al. (2007)
Nanoparticles	Theophylline	Improved drug loading and encapsulation	Thomas et al. (2019)
	Clindamycin	Prolonged/controlled drug release	Gowri et al. (2021)
Film	Ciprofloxacin	Localised drug delivery	Dong et al. (2006)
	Aloe vera		Pereira et al. (2013)
Gels	MSCs	Improved absorptive capacity	Schmitt et al. (2015)
	Protamine		Wang et al. (2019)

MSC mesenchymal stem cells

4 Factors Affecting Generation of PECs

Alginate gelation is carried out in a safe environment with nontoxic reactants. It can produce gels either by replacing soluble monovalent ions from the G chain with divalent ions (like Ca^{+2}) via “egg-box” formation or by lowering the pH below the pKa of alginate monomers using lactones like d-gluconolactone (Fu et al. 2011). The properties of the produced gels are influenced by various factors, broadly classified into two categories: (i) *nature and composition of alginate and counterion (polymer/metal ion)*, and (ii) *environmental factors*. Alginate gels with a large number of poly Gblock units are known to be more brittle, stiff, and mechanically stable. They are highly porous and fail to re-swell after drying (Morch et al. 2008). On the other hand, M block rich alginate gels are softer and elastic, with less shrinkability and porosity (Abasalizadeh et al. 2020). Molecular weight of polymer and its counter ion too influence the properties of developed PECs. Hu et al. reported effect of molecular weight of chitosan on particle size of developed PECs. It was observed that with increase in molecular weight of chitosan from 12,000 to 46,000 g/mole, particle size of developed PECs almost doubled from 135 to 279 nm, respectively (Hu et al. 2012). Further, Muller et al. reported that with increase in polyelectrolyte concentration, significant increase in primary PEC particles per volume was recorded. It was observed that increased polyelectrolyte concentration reduced the electrostatic repulsion between like-charged primary PEC particles (Muller et al. 2011).

Gelation rate governs the mechanical properties of the developed gel. Slower gelation ensures mechanically uniform gel structure. The type of counter ion and its form (salt) plays a crucial role in determining gelation rate. Chloride salt of calcium is responsible for the rapid and uncontrolled gelation of alginate (Crossingham et al. 2014). Other calcium salts viz. Calcium sulphate and calcium carbonate, due to its limited solubility, prolongs gelation time (Khromova 2006). The final size of PECs is influenced by the ionic strength of the polyelectrolytes. An increase in ionic strength causes a decrease in average diameter, which could be related to improved chain flexibility (Pardeshi et al. 2016). Dautzenberg et al. (1982) reported that PECs formed using both polyanion and polycation of identical charge density were simple and compact. As the difference in charge density was increased the particle adopted a loose fluctuating structure (Dautzenberg et al. 1982).

The pH of the medium can also affect the formation and stability of PECs (Lankalapalli and Kolapalli 2009). Interaction between the two oppositely charged polymers must take place in a pH-appropriate environment with a high degree of polymer ionization. Fukuda and Kikuchi (1977) reported the effect of change in pH on the coagulation tendency of developed PECs. They observed that at low pH, mutual electrostatic repulsion predominates and thus reduces the tendency of PECs to coagulate. On the other hand, at higher pH, because of electrostatic attractions, PEC coagulates to form compact hydrogels (Kikuchi and Fukuda 1977).

5 Biomedical Applications of Alginate Based PECs

5.1 As a Wound Dressing Material

Alginate based PECs in the form of sponges, hydrogels, films, and electrospun mats, are promising wound healing substrates with numerous benefits. They have hemostatic capacity and can absorb exudates and assist in wound healing (Aderibigbe and Buyana 2018). Further, non-toxicity, biocompatibility, non-immunogenicity and easy availability advocate its use in development of dressings. Various marketed alginate PEC based wound dressings are listed in Table 5. They are reported to elevate levels of IL-6 and TNG- α (alpha) and induce monocytes to produce collagen and thus assist in healing process (Sun and Tan 2013).

Table 5 List of alginate PEC based marketed products

S. No.	Formulation	Brand name (company)	Composition	Reference
1.	Wound Dressings	Algicell™ Ag (Integra LifeSciences, Princeton, NJ))	Dressing containing 1.4% ionic silver	fda.report (n.d.) Integra LifeSciences (2022)
		Biostep™ Ag (Smith and Nephew, Inc., Largo, FL)	Calcium alginate-collagen and ethylenediaminetetraacetic acid (EDTA) dressing containing silver ion	Smith-nephew.com (2022)
		Flaminal® Hydro and Forte (Flen Health)	Alginate; macrogol; enzyme system (glucose oxidase, lactoperoxidase, glucose, guaiacol); potassium sorbate; potassium iodide	www.flenhealth.com (n.d.)
		Tromboguard® (TRICOMED SA, Poland)	Alginate, Chitosan, Polyurethane	Kucharska et al. (2011)
		ActivHeal AquaFiber® (MedLogic Global Ltd)	Calcium alginate and carboxymethylcellulose	Timmons (2008)
		Sorbsan® (CreedMed UK)	Calcium alginate	Creedmed (2022)
		Capgel™	Collagen and alginate	Bosak et al. (2019)
2.	Solution for treating acid reflux	Gaviscon Double Action Liquid (Reckitt Benckiser Healthcare Limited, United Kingdom)	Sodium alginate, sodium bicarbonate and calcium carbonate	Gaviscon (n.d.)

5.1.1 Hydrogels

Alginate hydrogels are nontoxic and absorb a lot of water to build a hydrogel network on the wound surface keeping the surroundings moist. The wound tissues do not adhere to alginate hydrogels and their removal does not result in additional wound damage (Zhang and Zhao 2020). Alginate PEC hydrogels can provide sustained release of the loaded actives. The later will be released through diffusion or polymer erosion (Aderibigbe and Buyana 2018). Encapsulated drugs will remain protected from environmental stresses and unwanted degradation thus improving local drug availability (Oveissi et al. 2020; Lv et al. 2018). Oveissi et al. reported ambystoma mexicanum epidermal lipoxigenase enzyme (AmbLOXe) loaded hydrogel for wound healing application (Oveissi et al. 2020). AmbLOXe-loaded pectin nanoparticles (AmbLOXe Pec-NPs) were produced and embedded in an alginate hydrogel. Being a protein, AmbLOXe is prone to degradation. Loading in a delivery system was found to provide protection against degradation. The pH sensitive carboxymethyl chitosan/alginate PECs hydrogels are also reported. The swelling ratios of hydrogel were altered by adjusting the weight ratios of the polymer (s). In vitro release at different pH revealed that highest release of encapsulated drug (Bovine serum albumin) was recorded at pH 7.4 (Lv et al. 2018).

5.1.2 Foams

The alginate-based foam dressing is also reported by various researchers. Foams can absorb exudates to form a gel, which keeps the wound moist. Moisture at the wound site speeds up the healing process. It promotes cell migration, promotes re-epithelialization, reduces dehydration, boosts fibroblast and keratinocyte growth, breaks down dead tissue and boosts collagen formation and supports angiogenesis (Oh et al. 2020). Curcumin loaded alginate foam developed for infected wounds displayed longer hydration and significant in vitro antimicrobial activity (81% inactivation of viable *Escherichia coli*) (Hegge et al. 2011). Alginate foams for antibacterial photodynamic treatment of infected wounds are also reported (Valeron Bergh et al. 2017).

5.1.3 Films

Alginate PECs can be fabricated as films. The latter show excellent permeability to water vapor and gases (carbon dioxide, and oxygen), and protect the wound from bacterial infections (Varaprasad et al. 2020). Alginate-chitosan films with controlled release of silver sulfadiazine have been developed as a wound dressing (Meng et al. 2010). Achitosan/alginate/silicone gel PEC film containing silicone to improve the tensile strength of the developed films and encapsulating thymol and β -carotene as an aesthetic and anti-inflammatory is also reported (Pires et al. 2018).

5.1.4 Nanofibers

Nanofibers offer large specific surface area and high porosity and can better mimic extracellular matrix and help to promote epithelial cell proliferation. Electrospinning technique can be used to make alginate nanofibers. Zheng et al. reported alginate-Zinc PECs based composite fibers with excellent mechanical characteristics and high breaking strength with antimicrobial activity (Zhang et al. 2021). Mokhena et al. reported silver nanoparticle loaded chitosan-alginate composite nanofibers. Silver nanoparticles are antibacterial in nature. Loading them onto porous nanofibers enables them to exhibit their activity for longer duration of action (Teboho and Luyt 2017). Alginate nanofibers with zinc oxide nanoparticles were also shown to have high antibacterial characteristics and wound healing applications. The developed fibers were coupled using a polycaprolactone electrospun scaffold in order to produce a multilayered framework capable of promoting cell viability and providing protection against external environment (Doderio et al. 2021).

5.2 As a Drug Delivery System

Alginate is an excellent mucoadhesive, that can stick to the mucosa of the intestine for a long time after getting hydrated (Swain et al. 2012). Extended stay because of this can be exploited to target loaded drug to a particular mucus tissue viz. gastrointestinal, buccal, nasal, and ocular (Kamath et al. 1994). Kesavan and coworkers worked on improving the mucoadhesive properties of alginate by formulating its PECs with sodium carboxymethyl cellulose (Kesavan et al. 2010). Gatifloxacin was loaded in the developed PECs and showed better mucoadhesion and controlled release for ocular delivery. Increased concentration of sodium carboxymethyl cellulose was found to improve mucoadhesive property of developed system and controlled the initial burst release of loaded active. Significantly better performance in terms of in vitro antimicrobial activity was also reported (Mishra and Gilhotra 2008; Kesavan et al. 2010). Improved ocular residence of gatifloxacin via alginate nanoparticles is also reported (Motwani et al. 2008). Glicazide loaded calcium alginate-isaphghula mucoadhesive beads with superior mucoadhesive properties and improved (>70%) drug loading are also reported (Nayak et al. 2010). Alginate is a hydrocolloid which plays an important role in the design of a controlled-release product. It gets readily hydrated resulting in a high-viscosity hydrocolloidal layer. This creates a diffusion barrier, preventing encapsulated molecules from migrating (Kumar et al. 2014). Researchers have tried both polymer barrier systems and polymer matrix systems with alginate (Hariyadi and Islam 2020). Exposure of alginate based PECs to the dissolution medium, modulates drug release both via diffusion through matrix swelling and dissolution/erosion at the matrix periphery (Sriamornsak et al. 2007). The exact mechanism of drug release is controlled by various factors including pH of the media and osmotic pressure in the system. Both

these factors control the swelling rate of alginate-PEC matrix, which in turn governs the release mechanism. At low pH, alginates show minimal swelling and the drug release is prominently controlled by matrix erosion. As the pH increases, both erosion and diffusion contribute to drug release. Researchers have reported different alginate based PECs with the intent to control the drug release (Witzler et al. 2021; Unagolla and Jayasuriya 2018; Boi et al. 2020; Wang and Zhang Newby 2018). Site specific delivery of drugs using alginate PECs as carrier has also been reported. Chitosan coating of alginate PECs were done with the intent to deliver the content to colon. The percentage swelling of alginate-chitosan is small at the acidic pH of the stomach; however, as one climbs through the intestinal tract, the pH increases and so does the percentage swelling, releasing the encapsulated drug in a controlled manner (Mateescu et al. 2015; Hu et al. 2015). Similarly, eudragit-alginate PECs are also reported to provide pH-controlled release of actives (Yusif et al. 2014).

Further, the alginate PEC encapsulation is found to improve the stability of loaded drugs by providing protection from degradation due to storage and environmental stresses (Ahmad Raus et al. 2021). Drugs, especially those having proteinaceous origin are prone to degradation in acidic environment of stomach. Through successful encapsulation in alginate-based PECs their shelf life and bioavailability can be improved (Sankalia et al. 2007).

Alginate based PECs have also been used to taste mask bitter drugs and thus enhance patient compliance. Almurisi et al. (2020) reported chitosan coated alginate beads for taste masking paracetamol. Results suggested that coating of alginate microparticles with chitosan significantly reduced the release of paracetamol below the threshold concentration (2 mg/mL) eliciting bitter taste (Almurisi et al. 2020). Table 5 enlists few marketed alginate PEC based products.

5.3 *In Tissue Engineering*

Tissue engineering is a significant method for restoring, maintaining, or improving damaged tissues or organs by producing live and functional tissue finding attention of the scientific community. In general, it entails the propagation of cells loaded onto or enclosed in material that promotes cell growth and differentiation (Han et al. 2020). These cell material complexes are implanted into the body to propagate tissue formation. Alginate has been thoroughly investigated as a material for tissue engineering applications. Researchers have used it to develop various scaffolds for engineering bone, cartilage, liver or ocular tissues (Chen and Liu 2016). Alginate alone has a poor mechanical property and does not offer suitable conditions for cell adhesion and proliferation (Lee and Mooney 2012). This limits its usage as sole polymer for tissue engineering applications. However, its property to form PECs with cationic polymers enables researchers to play with its properties and widen the horizon of its scope and application (Kutlusoy et al. 2017). Patil et al. (2017) reported development of 3D porous hybrid scaffold utilizing chitosan-alginate PECs. The developed PECs were coated with hydroxyapatite to improve

compressive and mechanical strength of the scaffold. The developed scaffold was found to support the proliferation of MG63 osteosarcoma cells (Patil et al. 2017; Gill et al. 2019).

Researchers have used alginate based PECs to deliver drugs to different grafted tissues. Bioactive recombinant human BMP-2 (a growth factor) loaded strontium alginate PECs have been reported earlier. Results revealed robust bone formation and a biomechanically solid fusion after 6 weeks (Abbah et al. 2012). Superiority of alginate based PECs in tissue engineering applications are reported by various other groups (Dvir-Ginzberg et al. 2003; Ma et al. 2019).

6 Concluding Remarks

Alginate is well researched natural biopolymer in the fields of biomaterials science and drug delivery. This anionic polysaccharide is versatile due to its biocompatibility and structural functionality. It can readily form polyelectrolyte complexes with counter ions (cationic polysaccharides or other cations). Various parameters like nature and composition of alginate and counter ion, charge density, gelation rate and the pH at which complex formation takes place influence the nature and strength of generated PECs. Researchers have used these PECs for a variety of biomedical applications including fabrication of wound dressings and tissue scaffolds, and controlled drug delivery applications. Alginate based PECs are reported to have superior mucoadhesive properties which contribute towards improved drug residence at delivery site. Improvement in stability of encapsulated drug and taste masking of bitter drugs are also reported for the alginate based PECs.

Acknowledgement Dr. Indu Pal Kaur and Dr. Parneet Kaur Deol acknowledge the financial assistance provided by DST SERB under the CRG Scheme (CRG/2019/002768).

References

- Abasalizadeh F, Moghaddam SV, Alizadeh E, Akbari E, Kashani E, Fazljou SMB, Torbati M, Akbarzadeh A (2020) Alginate-based hydrogels as drug delivery vehicles in cancer treatment and their applications in wound dressing and 3D bioprinting. *J Biol Eng* 14:8–8
- Abbah SA, Liu J, Lam R, Cho J, Wong H-K (2012) In vivo bioactivity of rhBMP-2 delivered with novel polyelectrolyte complexation shells assembled on an alginate microbead core template. *J Control Release* 162:364–372
- Aderibigbe BA, Buyana B (2018) Alginate in wound dressings. *Pharmaceutics* 10:42
- Agarwal T, Narayana SN, Pal K, Pramanik K, Giri S, Banerjee I (2015) Calcium alginate-carboxymethyl cellulose beads for colon-targeted drug delivery. *Int J Biol Macromol* 75:409–417. <https://doi.org/10.1016/j.ijbiomac.2014.12.052>
- Agüero L, Zaldivar-Silva D, Pena L, Dias ML (2017) Alginate microparticles as oral colon drug delivery device: a review. *Carbohydr Polym* 168:32–43

- Ahmad Raus R, Wan Nawawi WMF, Nasaruddin RR (2021) Alginate and alginate composites for biomedical applications. *Asian J Pharm Sci* 16:280–306
- Alfatama M, Lim LY, Wong TW (2021) Chitosan oleate-tripolyphosphate complex-coated calcium alginate bead: physicochemical aspects of concurrent core-coat formation. *Carbohydr Polym* 273:118487
- Almurisi SH, Doolaanea AA, Akkawi ME, Chatterjee B, Sarker MZI (2020) Taste masking of paracetamol encapsulated in chitosan-coated alginate beads. *J Drug Deliv Sci Technol* 56: 101520
- Arora S, Budhiraja RD (2012) Chitosan-alginate microcapsules of amoxicillin for gastric stability and mucoadhesion. *J Adv Pharm Technol Res* 3:68–74
- Azhar F, Olad A (2014) A study on sustained release formulations for oral delivery of 5-fluorouracil based on alginate-chitosan/montmorillonite nanocomposite systems. *Appl Clay Sci* 101:288–296
- Badwan AA, Abumaloo A, Sallam E, Abukalaf A, Jawan O (1985) A sustained release drug delivery system using calcium alginate beads. *Drug Dev Ind Pharm* 11:239–256
- Barzegar-Jalali M, Hanaee J, Omid Y, Ghanbarzadeh S, Ziaee S, Bairami-Atashgah R, Adibkia K (2013) Preparation and evaluation of sustained release calcium alginate beads and matrix tablets of acetazolamide. *Drug Res (Stuttg)* 63(2):60–64. <https://doi.org/10.1055/s-0032-1331755>
- Bedade DK, Sutar YB, Singhal RS (2019) Chitosan coated calcium alginate beads for covalent immobilization of acrylamidase: process parameters and removal of acrylamide from coffee. *Food Chem* 275:95–104
- Boi S, Rouatbi N, Dellacasa E, Di Lisa D, Bianchini P, Monticelli O, Pastorino L (2020) Alginate microbeads with internal microvoids for the sustained release of drugs. *Int J Biol Macromol* 156: 454–461
- Bom S, Santos C, Barros R, Martins AM, Paradiso P, Cláudio R, Pinto PC, Ribeiro HM, Marto J (2020) Effects of starch incorporation on the physicochemical properties and release kinetics of alginate-based 3D hydrogel patches for topical delivery. *Pharmaceutics* 12:719
- Bosak A, Kwan MWC, Willenberg A, Perle KMDL, Weinstein D, Hines RB, Schultz GS, Ross EA, Willenberg BJ (2019) Capillary alginate gel (Capgela™) for the treatment of full-thickness dermal wounds in a hypoxic mouse model. *Int J Polym Mater Polym Biomater* 68:1108–1117
- Brus J, Urbanova M, Czernek J, Pavelkova M, Kubova K, Vyslouzil J, Abbrent S, Konefal R, Horsky J, Vetchy D, Vyslouzil J, Kulich P (2017) Structure and dynamics of alginate gels cross-linked by polyvalent ions probed via solid state NMR spectroscopy. *Biomacromolecules* 18: 2478–2488
- Caetano LA, Almeida AJ, Goncalves LMD (2016) Effect of experimental parameters on alginate/chitosan microparticles for BCG encapsulation. *Mar Drugs* 14:90
- Cai L, Cao A, Bai F, Li J (2015) Effect of ϵ -polylysine in combination with alginate coating treatment on physicochemical and microbial characteristics of Japanese sea bass (*Lateolabrax japonicus*) during refrigerated storage. *LWT Food Sci Technol* 62:1053–1059
- Camacho DH, Uy SJY, Cabrera MJF, Lobregas MOS, Fajardo T (2019) Encapsulation of folic acid in copper-alginate hydrogels and its slow in vitro release in physiological pH condition. *Food Res Int* 119:15–22. <https://doi.org/10.1016/j.foodres.2019.01.053>
- Chakraborty G, Bhattarai A, De R (2022) Polyelectrolyte-dye interactions: an overview. *Polymers* 14:598
- Chawla A, Sharma P, Pawar P (2012) Eudragit S-100 coated sodium alginate microspheres of naproxen sodium: formulation, optimization and in vitro evaluation. *Acta Pharma* 62(4): 529–545. <https://doi.org/10.2478/v10007-012-0034-x>
- Chekhubwe E, Onyia O, Ezegbe A, Kalu A, Ugochi E, Ike O (2022) Formulation and evaluation of tinidazole-loaded alginate micro-beads for colon delivery. *Afr J Pharm Res Dev* 13:76–86
- Chen F-M, Liu X (2016) Advancing biomaterials of human origin for tissue engineering. *Prog Polym Sci* 53:86–168
- Chen T, Li S, Zhu W, Liang Z, Zeng Q (2019) Self-assembly pH-sensitive chitosan/alginate coated polyelectrolyte complexes for oral delivery of insulin. *J Microencapsul* 36:96–107

- Chremos A, Douglas JF (2018) Polyelectrolyte association and solvation. *J Chem Phys* 149: 163305–163305
- Coppi G, Iannuccelli V, Leo E, Bernabei M, Camerini R (2001) Chitosan-alginate microparticles as a protein carrier. *Drug Dev Ind Pharm* 27:393–400
- Creedmed (2022) <https://sorbsan.co.uk/>. Accessed 21 Oct 2022
- Crossingham YJ, Kerr PG, Kennedy RA (2014) Comparison of selected physico-chemical properties of calcium alginate films prepared by two different methods. *Int J Pharm* 473(1-2):259–269. <https://doi.org/10.1016/j.ijpharm.2014.06.043>
- Dakhara SL, Anajwala CC (2011) Polyelectrolyte complex: a pharmaceutical review. *Syst Rev Pharm* 1:121
- Dautzenberg H, Linow KJ, Philipp B (1982) Zur Bildung wasserloslicher Polysalze (Symplexe) aus anionischen und kationischen Copolymeren des Acrylamids. 2. Mitt.: einfluß der Ladungsdichte und des Umsatzgrades auf die Struktur der Symplexe. *Acta Polymerica* 33:619–625
- Deol PK, Gill AS, Prajapati S, Kaur IP (2020) Additive manufacturing and nanotherapeutics: present status and future perspectives in wound healing. In: Rai M (ed) *Nanotechnology in skin, soft tissue, and bone infections*. Springer International Publishing, Cham, pp 205–220
- Devi N, Hazarika D, Deka C, Kakati D (2012) Study of complex coacervation of gelatin A and sodium alginate for microencapsulation of olive oil. *J Macromol Sci* 49:936–945
- Devi N, Deka C, Maji T, Kakati D (2016) Gelatin and gelatin-polyelectrolyte complexes: drug delivery. In: Mishra M (ed) *Encyclopedia of biomedical polymers and polymeric biomaterials*. CRC Press, Boca Raton, FL
- Diniz FR, Maia RCAP, Rannier Andrade L, Andrade LN, Vinicius Chaud M, Da Silva CF, Correa CB, De Albuquerque Junior RLC, Pereira Da Costa L, Shin SR, Hassan S, Sanchez-Lopez E, Souto EB, Severino P (2020) Silver nanoparticles-composing alginate/gelatin hydrogel improves wound healing in vivo. *Nanomaterials (Basel)* 10(2):390
- Dodero A, Alberti S, Gaggero G, Ferretti M, Botter R, Vicini S, Castellano M (2021) An up-to-date review on alginate nanoparticles and nanofibers for biomedical and pharmaceutical applications. *Adv Mater Interfaces* 8:2100809
- Dong Z, Wang Q, Du Y (2006) Alginate/gelatin blend films and their properties for drug controlled release. *J Membr Sci* 280:37–44
- Dvir-Ginzberg M, Gamlieli-Bonshtein I, Agbaria R, Cohen S (2003) Liver tissue engineering within alginate scaffolds: effects of cell-seeding density on hepatocyte viability, morphology, and function. *Tissue Eng* 9:757–766
- Espevik T, Otterlei M, Skjak-Braek G, Ryan L, Wright SD, Sundan A (1993) The involvement of CD14 in stimulation of cytokine production by uronic acid polymers. *Eur J Immunol* 23(1): 255–261. <https://doi.org/10.1002/eji.1830230140>
- Flores C, Diaz-Barrera A, Martinez F, Galindo E, Pena C (2014) Role of oxygen in the polymerization and de-polymerization of alginate produced by *azotobacter vinelandii*. *J Chem Technol Biotechnol* 90:356–365
- Frent O, Duteanu N, Teusdea A, Ciocan S, Vicas L, Tunde J, Muresan M, Pallag A, Paula I, Marian E (2022) Preparation and characterization of chitosan-alginate microspheres loaded with quercetin. *Polymers* 14:490
- Fu S, Thacker A, Sperger DM, Boni RL, Buckner IS, Velankar S, Munson EJ, Block LH (2011) Relevance of rheological properties of sodium alginate in solution to calcium alginate gel properties. *AAPS PharmSciTech* 12(2):453–460. <https://doi.org/10.1208/s12249-011-9587-0>
- Fukuda H, Kikuchi Y (1977) Polyelectrolyte complexes of sodium dextran sulfate with chitosan. *Die Makromolekulare Chemie: Macromol Chem Phys* 178(10):2895–2899
- Ge L, Li Z, Han M, Wang Y, Li X, Mu C, Li D (2022) Antibacterial dialdehyde sodium alginate-polylysine microspheres for fruit preservation. *Food Chem* 387:132885
- George M, Abraham T (2006) Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan—a review. *J Control Release* 114:1–14

- Gill AS, Deol PK, Kaur IP (2019) An update on the use of alginate in additive biofabrication techniques. *Curr Pharm Des* 25(11):1249–1264. <https://doi.org/10.2174/1381612825666190423155835>
- Goswami S, Bajpai J, Bajpai AK (2014) Calcium alginate nanocarriers as possible vehicles for oral delivery of insulin. *J Exp Nanosci* 9:337–356
- Gowri M, Latha N, Suganya K, Murugan M, Rajan M (2021) Calcium alginate nanoparticle crosslinked phosphorylated polyallylamine to the controlled release of clindamycin for osteomyelitis treatment. *Drug Dev Ind Pharm* 47(2):280–291. <https://doi.org/10.1080/03639045.2021.1879835>
- Grant GT, Morris ER, Rees DA, Smith PJ, Thom D (1973) Biological interactions between polysaccharides and divalent cations: the egg-box model. *FEBS Lett* 32:195–198
- Han F, Wang J, Ding L, Hu Y, Li W, Yuan Z, Guo Q, Zhu C, Yu L, Wang H, Zhao Z, Jia L, Li J, Yu Y, Zhang W, Chu G, Chen S, Li B (2020) Tissue engineering and regenerative medicine: achievements, future, and sustainability in Asia. *Front Bioeng Biotechnol* 8:83
- Hari PR, Chandy T, Sharma CP (1996) Chitosan/calcium alginate microcapsules for intestinal delivery of nitrofurantoin. *J Microencapsul* 13(3):319–329. <https://doi.org/10.3109/02652049609026019>
- Hariyadi DM, Islam N (2020) Current status of alginate in drug delivery. *Adv Pharmacol Pharm Sci* 2020:8886095
- Hegge AB, Andersen T, Melvik JE, Bruzell E, Kristensen S, Tonnesen HH (2011) Formulation and bacterial phototoxicity of curcumin loaded alginate foams for wound treatment applications: studies on curcumin and curcuminoides XLII. *J Pharm Sci* 100:174–185
- Hermanto D, Mudasir M, Siswanta D, Kuswandi B, Ismillayli N (2019) Polyelectrolyte complex (PEC) of the alginate-chitosan membrane for immobilizing urease. *J Math Fund Sci* 51:309–319
- Hu Y, Yang T, Hu X (2012) Novel polysaccharides-based nanoparticle carriers prepared by polyelectrolyte complexation for protein drug delivery. *Polym Bull* 68:1183–1199
- Hu Y, Zheng M, Dong X, Zhao D, Cheng H, Xiao X (2015) Preparation and characterization of alginate-hyaluronic acid-chitosan based composite gel beads. *J Wuhan Univ Technol Mater Sci Ed* 30:1297–1303
- Hu Y, Peng J, Ke L, Zhao D, Zhao H, Xiao X (2016) Alginate/carboxymethyl chitosan composite gel beads for oral drug delivery. *J Polym Res* 23:129
- Hu C, Lu W, Mata A, Nishinari K, Fang Y (2021) Ions-induced gelation of alginate: mechanisms and applications. *Int J Biol Macromol* 177:578–588
- Hunt N, Shelton R, Henderson D, Grover L (2012) Calcium-alginate hydrogel-encapsulated fibroblasts provide sustained release of vascular endothelial growth factor. *Tissue Eng Part A* 19:905–914
- Idota Y, Kogure Y, Kato T, Yano K, Arakawa H, Miyajima C, Kasahara F, Ogihara T (2016) Relationship between physical parameters of various metal ions and binding affinity for alginate. *Biol Pharm Bull* 39:1893–1896
- Integra LifeSciences (2022) <https://www.woundsource.com/product/algicell-agantimicrobial-alginate-dressing>. Accessed 21 Oct 2022
- Jaafar HMM, Hamid AK (2019) Chitosan-coated alginate nanoparticles enhanced absorption profile of insulin via oral administration. *Curr Drug Deliv* 16:672–686
- Jeganathan B, Prakya V (2015) Interpolyelectrolyte complexes of eudragit® EPO with hypromellose acetate succinate and eudragit® EPO with hypromellose phthalate as potential carriers for oral controlled drug delivery. *AAPS PharmSciTech* 16:878–888
- Jin L, Qi H, Gu X, Zhang X, Zhang Y, Mao S (2020) Effect of sodium alginate type on drug release from chitosan-sodium alginate-based in situ film-forming tablets. *AAPS PharmSciTech* 21(2):55. <https://doi.org/10.1208/s12249-019-1549-y>
- Joseph I, Venkataram S (1995) Indomethacin sustained release from alginate-gelatin or pectin-gelatin coacervates. *Int J Pharm* 126:161–168
- Kamath KR, Park K, Sugbrick J, Boylen JC (1994) Encyclopedia of pharmaceutical technology. Marcel Dekker, New York

- Kesavan K, Nath G, Pandit JK (2010) Sodium alginate based mucoadhesive system for gatifloxacin and its in vitro antibacterial activity. *Sci Pharm* 78:941–957
- Khromova Y (2006) The effect of chlorides on alginate gelation in the presence of calcium sulfate. *Colloid J* 68:115–119
- Kikuchi Y, Fukuda H (1977) Polyelectrolyte complex consisting sodium dextran sulfate and 2-(diethylamino)ethyl-dextran hydrochloride. *Nippon Kagaku Kaishi* 1977:1051–1054
- Kilicarslan M, Ilhan M, Inal O, Orhan K (2018) Preparation and evaluation of clindamycin phosphate loaded chitosan/alginate polyelectrolyte complex film as mucoadhesive drug delivery system for periodontal therapy. *Eur J Pharm Sci* 123:441–451. <https://doi.org/10.1016/j.ejps.2018.08.007>
- Kim YJ, Park HG, Yang YL, Yoon Y, Kim S, Oh E (2005) Multifunctional drug delivery system using starch-alginate beads for controlled release. *Biol Pharm Bull* 28(2):394–397. <https://doi.org/10.1248/bpb.28.394>
- Kim ES, Lee J-S, Lee HG (2016) Calcium-alginate microparticles for sustained release of catechin prepared via an emulsion gelation technique. *Food Sci Biotechnol* 25:1337–1343
- Kucharska M, Niekaszewicz A, Ciechańska D, Gąsiorowski T, Struszczyk M, Pluta A, Rogaczewska A, Witczak E, Płoszaj I, Gulbas-Diaz A, Fortuniak K, Tarkowska S (2011) Tromboguard - first aid wound dressing. *Progr Chem Appl Chitin Deriv* 16:121–130
- Kulig D, Zimoch-Korzycka A, Jarmoluk A, Marycz K (2016) Study on alginate-chitosan complex formed with different polymers ratio. *Polymers* 8:167
- Kumar K, Dhawan N, Sharma H, Vaidya S, Vaidya B (2014) Bioadhesive polymers: novel tool for drug delivery. *Artif Cells Nanomed Biotechnol* 42(4):274–283. <https://doi.org/10.3109/21691401.2013.815194>
- Kutlusoy T, Oktay B, Kayaman-Apohan N, Suleymanoglu M, Erdem S (2017) Chitosan-co-hyaluronic acid porous cryogels and their application in tissue engineering. *Int J Biol Macromol* 103:366–378
- Labowska MB, Cierluk K, Jankowska AM, Kulbacka J, Detyna J, Michalak I (2021) A review on the adaption of alginate-gelatin hydrogels for 3D cultures and bioprinting. *Materials (Basel, Switzerland)* 14:858
- Lankalapalli S, Kolapalli VRM (2009) Polyelectrolyte complexes: a review of their applicability in drug delivery technology. *Indian J Pharm Sci* 71:481–487
- Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. *Prog Polym Sci* 37:106–126
- Lefnaoui S, Moulai-Mostefa N, Yahoum MM, Gasmi SN (2018) Design of antihistaminic transdermal films based on alginate-chitosan polyelectrolyte complexes: characterization and permeation studies. *Drug Dev Ind Pharm* 44:432–443
- Leick S, Kemper A, Rehage H (2011) Alginate/poly-L-lysine capsules: mechanical properties and drug release characteristics. *Soft Matter* 7:6684–6694
- Li P, Dai Y-N, Zhang J-P, Wang A-Q, Wei Q (2008) Chitosan-alginate nanoparticles as a novel drug delivery system for nifedipine. *Int J Biomed Sci* 4:221–228
- Li L, Wang L, Shao Y, Ni R, Zhang T, Mao S (2013) Drug release characteristics from chitosan-alginate matrix tablets based on the theory of self-assembled film. *Int J Pharm* 450(1–2):197–207. <https://doi.org/10.1016/j.ijpharm.2013.04.052>
- Lin S-F, Chen Y-C, Chen R-N, Chen L-C, Ho H-O, Tsung Y-H, Sheu M-T, Liu D-Z (2016) Improving the stability of astaxanthin by microencapsulation in calcium alginate beads. *PLoS One* 11:e0153685
- Lopez-Mendez TB, Santos-Vizcaino E, Pedraz JL, Orive G, Hernandez RM (2021) Cell microencapsulation technologies for sustained drug delivery: latest advances in efficacy and biosafety. *J Control Release* 335:619–636
- Lv X, Zhang W, Liu Y, Zhao Y, Zhang J, Hou M (2018) Hygroscopicity modulation of hydrogels based on carboxymethyl chitosan/alginate polyelectrolyte complexes and its application as pH-sensitive delivery system. *Carbohydr Polym* 198:86–93

- Ma F, Pang X, Tang B (2019) Alginate/chondroitin sulfate based hybrid hydrogel with different molecular weight and its capacity to regulate chondrocytes activity. *Carbohydr Polym* 206:229–237
- Machida-Sano I, Ogawa S, Ueda H, Kimura Y, Satoh N, Namiki H (2012) Effects of composition of iron-cross-linked alginate hydrogels for cultivation of human dermal fibroblasts. *Int J Biomater* 2012:820513
- Malakar J, Nayak A, Das A (2013) Modified starch (cationized)-alginate beads containing aceclofenac: formulation optimization using central composite design. *Starch - Starke* 65: 603–612
- Mandal S, Kumar S, Krishnamoorthy B, Basu S (2010) Development and evaluation of calcium alginate beads prepared by sequential and simultaneous methods. *Braz J Pharm Sci* 46:785–793
- Martau GA, Mihai M, Vodnar DC (2019) The use of chitosan, alginate, and pectin in the biomedical and food sector-biocompatibility, bioadhesiveness, and biodegradability. *Polymers* 11:1837
- Massana Roquero D, Bollella P, Katz E, Melman A (2021) Controlling porosity of calcium alginate hydrogels by interpenetrating polyvinyl alcohol-diboronate polymer network. *ACS Appl Polym Mater* 3:1499
- Mateescu MA, Ispas-Szabo P, Assaad E (2015) 4 - Chitosan-based polyelectrolyte complexes as pharmaceutical excipients. In: Mateescu MA, Ispas-Szabo P, Assaad E (eds) *Controlled drug delivery*. Woodhead Publishing, London, pp 127–161
- Mathews S (2017) Microencapsulation of probiotics by calcium alginate and gelatin and evaluation of its survival in simulated human gastro-intestinal condition. *Int J Curr Microbiol App Sci* 6: 2080–2087
- Meng X, Tian F, Yang J, He C-N, Xing N, Li F (2010) Chitosan and alginate polyelectrolyte complex membranes and their properties for wound dressing application. *J Mater Sci Mater Med* 21:1751–1759
- Mishra D, Gilhotra R (2008) Design and characterization of bioadhesive in-situ gelling ocular inserts of gatifloxacin sesquihydrate. *Daru* 16:1–8
- Mladenovska K, Slaveska R, Janevik E, Ristoski T, Pavlova M, Kavrakovski Z, Glavas Dodov M, Goracinova K (2007) Colon-specific delivery of 5-aminosalicylic acid from chitosan-Ca-alginate microparticles. *Int J Pharm* 342:124–136
- Morch YA, Holtan S, Donati I, Strand B, Skjak-Braek G (2008) Mechanical properties of C-5 epimerized alginates. *Biomacromolecules* 9:2360–2368
- Motwani S, Chopra S, Talegaonkar S, Kohli K, Ahmad F, Khar R (2008) Chitosan-sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: formulation, optimisation and in-vitro characterisation. *Eur J Pharm Biopharm* 68:513–525
- Mukhopadhyay P, Maity S, Mandal S, Chakraborti AS, Prajapati AK, Kundu PP (2018) Preparation, characterization and in vivo evaluation of pH sensitive, safe quercetin-succinylated chitosan-alginate core-shell-corona nanoparticle for diabetes treatment. *Carbohydr Polym* 182:42–51
- Mulia K, Singarimbun AC, Krisanti EA (2020) Optimization of chitosan-alginate microparticles for delivery of mangostins to the colon area using box-behnken experimental design. *Int J Mol Sci* 21:873
- Muller M, Keller B, Frohlich J, Poeschla S, Torger B (2011) Polyelectrolyte complex nanoparticles of (ethyleneimine) and poly (acrylic acid): preparation and applications. *Polymers* 3:762–778
- Muzzarelli R, Muzzarelli C (2005) Chitosan chemistry: relevance to the biomedical sciences. In: Heinze T (ed) *Polysaccharides I. Advances in polymer science*, vol 186. Springer, Berlin, pp 151–209
- Nayak A, Hasnain M, Beg S, Alam M (2010) Mucoadhesive beads of gliclazide: design, development and evaluation. *Sci Asia* 36:319–325
- Nesamony J, Singh P, Nada S, Shah Z, Kolling B (2012) Calcium alginate nanoparticles synthesized through a novel interfacial cross-linking method as a potential protein drug delivery system. *J Pharm Sci* 101:2177–2184

- Niculescu A-G, Grumezescu AM (2022) Applications of chitosan-alginate-based nanoparticles-an up-to-date review. *Nanomaterials* (Basel, Switzerland) 12:186
- Nikolova D, Simeonov M, Tzachev C, Apostolov A, Christov L, Vassileva E (2021) Polyelectrolyte complexes of chitosan and sodium alginate as a drug delivery system for diclofenac sodium. *Polym Int* 71:668–678
- Oh G-W, Nam SY, Heo S-J, Kang D-H, Jung W-K (2020) Characterization of ionic cross-linked composite foams with different blend ratios of alginate/pectin on the synergistic effects for wound dressing application. *Int J Biol Macromol* 156:1565–1573
- Olivas GI, Barbosa-Canovas GV (2008) Alginate-calcium films: water vapor permeability and mechanical properties as affected by plasticizer and relative humidity. *LWT Food Sci Technol* 41:359–366
- Omer AM, Ahmed MS, El-Subruiti GM, Khalifa RE, Eltaweil AS (2021) pH-sensitive alginate/carboxymethyl chitosan/aminated chitosan microcapsules for efficient encapsulation and delivery of diclofenac sodium. *Pharmaceutics* 13:338
- Onal SI, Zihnioglu F (2002) Encapsulation of insulin in chitosan-coated alginate beads: oral therapeutic peptide delivery. *Artif Cells Blood Substit Immobil Biotechnol* 30:229–237
- Onyido I, Sha'ato R, Nnamonu L (2012) Environmentally friendly formulations of trifluralin based on alginate modified starch. *J Environ Prot* 3(9):1085–1093
- Orive G, Tam SK, Pedraz JL, Halle J-P (2006) Biocompatibility of alginate-poly-L-lysine microcapsules for cell therapy. *Biomaterials* 27:3691–3700
- Oshi MA, Lee J, Kim J, Hasan N, Im E, Jung Y, Yoo JW (2021) pH-responsive alginate-based microparticles for colon-targeted delivery of pure cyclosporine A crystals to treat ulcerative colitis. *Pharmaceutics* 13(9):1412. <https://doi.org/10.3390/pharmaceutics13091412>
- Oveissi F, Tavakoli N, Minaian M, Mofid MR, Taheri A (2020) Alginate hydrogel enriched with ambystoma mexicanum epidermal lipoxygenase-loaded pectin nanoparticles for enhanced wound healing. *J Biomater Appl* 34:1171–1187
- Pardeshi C, Kulkarni A, Vanjari Y, Sancheti K, Patel H, Belgamwar V, Surana S (2016) Polyelectrolyte complexes: mechanisms, critical experimental aspects, and applications. *Artif Cells Nanomed Biotechnol Int J* 44:1615–1625
- Park SB, Kang HW, Haam S, Park HY, Kim WS (2004) Ca-alginate microspheres encapsulated in chitosan beads. *J Microencapsul* 21(5):485–497. <https://doi.org/10.1080/02652040410001729269>
- Patel N, Lalwani D, Gollmer S, Injeti E, Sari Y, Nesamony J (2016) Development and evaluation of a calcium alginate based oral ceftriaxone sodium formulation. *Progr Biomater* 5:117–133
- Patil T, Saha S, Biswas A (2017) Preparation and characterization of HAp coated chitosan-alginate PEC porous scaffold for bone tissue engineering. *Macromol Symp* 376:1600205
- Pei HN, Chen XG, Li Y, Zhou HY (2008) Characterization and ornidazole release in vitro of a novel composite film prepared with chitosan/poly(vinyl alcohol)/alginate. *J Biomed Mater Res A* 85(2):566–572. <https://doi.org/10.1002/jbm.a.31223>
- Pereira RB, Carvalho A, Vaz DC, Gil MH, Mendes A, Băirtolo, P. (2013) Development of novel alginate based hydrogel films for wound healing applications. *Int J Biol Macromol* 52:221–230
- Peric-Hassler L, Hunenberger P (2010) Interaction of alginate single-chain polyguluronate segments with mono- and divalent metal cations: a comparative molecular dynamics study. *Mol Simul* 36:778–795
- Pires AL, Motta L, Dias A, de Sousa H, Moraes AN, Braga M (2018) Towards wound dressings with improved properties: effects of poly(dimethylsiloxane) on chitosan-alginate films loaded with thymol and beta-carotene. *Mater Sci Eng C* 93:595–605
- Qin Y (2008) Alginate fibres: an overview of the production processes and applications in wound management. *Polym Int* 57:171–180
- Rahaiee S, Hashemi M, Shojaosadati S, Moini S, Razavi S (2017) Nanoparticles based on crocin loaded chitosan-alginate biopolymers: antioxidant activities, bioavailability and anticancer properties. *Int J Biol Macromol* 99:401–408

- Ramos PE, Antoinio AV, Josei AT, Lorenzo MP, Marta MA, Miguel AC, Pedro S (2018) Effect of alginate molecular weight and M/G ratio in beads properties foreseeing the protection of probiotics. *Food Hydrocoll* 77:8–16
- Ravisankar S, Dey N, Francis AP, Pandian K, Devasena T (2015) Preparation and characterization of gatifloxacin encapsulated chitosan nanoparticles for ocular drug delivery. *Int J Innovative Res Sci Eng Technol* 4(1):28–33
- Reddy S (2021) Alginates—a seaweed product: its properties and applications. In: Deniz I, Imamoglu E, Keskin-Gundogdu T (eds) *Properties and applications of alginates*. IntechOpen, London, pp 19–38
- Ren Y, Xie H, Liu X, Bao J, Yu W, Ma X (2016) Comparative investigation of the binding characteristics of poly-L-lysine and chitosan on alginate hydrogel. *Int J Biol Macromol* 84:135–141. <https://doi.org/10.1016/j.ijbiomac.2015.12.008>
- Sachan N, Pushkar S, Jha A, Bhattacharya A (2009) Sodium alginate: the wonder polymer for controlled drug delivery. *J Pharm Res* 2:1191–1199
- Sahoo DR, Biswal T (2021) Alginate and its application to tissue engineering. *SN Appl Sci* 3:30
- Sanchez-Ballester N, Soulaïrol I, Bataille B, Sharkawi T (2019) Flexible heteroionic calcium-magnesium alginate beads for controlled drug release. *Carbohydr Polym* 207:224–229
- Sankalia MG, Mashru RC, Sankalia JM, Sutariya VB (2007) Reversed chitosan–alginate polyelectrolyte complex for stability improvement of alpha-amylase: optimization and physicochemical characterization. *Eur J Pharm Biopharm* 65:215–232
- Satishbabu BK, Sandeep VR, Ravi RB, Shrutnag R (2010) Formulation and evaluation of floating drug delivery system of famotidine. *Indian J Pharm Sci* 72:738–744
- Schmitt A, Rodel P, Anamur C, Seeliger C, Imhoff AB, Herbst E, Vogt S, van Griensven M, Winter G, Engert J (2015) Calcium alginate gels as stem cell matrix-making paracrine stem cell activity available for enhanced healing after surgery. *PLoS One* 10:e0118937
- Segale L, Giovannelli L, Mannina P, Pattarino F (2016) Calcium alginate and calcium alginate-chitosan beads containing celecoxib solubilized in a self-emulsifying phase. *Scientifica* 2016: 5062706
- Sepúlveda-Rivas S, Fritz HF, Valenzuela C, Santiviago CA, Morales JO (2019) Development of novel EE/alginate polyelectrolyte complex nanoparticles for lysozyme delivery: physicochemical properties and in vitro safety. *Pharmaceutics* 11:103
- Silva CM, Ribeiro ANJ, Figueiredo M, Ferreira D, Veiga F (2006) Microencapsulation of hemoglobin in chitosan-coated alginate microspheres prepared by emulsification/internal gelation. *AAPS J* 7:E903–E913
- Singh M, Gill AS, Deol PK, Agrawal A, Sidhu SS (2022) Drug eluting titanium implants for localised drug delivery. *J Mater Res*. <https://doi.org/10.1557/s43578-022-00609-y>
- Somo SI, Khanna O, Brey EM (2017) Alginate microbeads for cell and protein delivery. *Methods Mol Biol* 1479:217–224. https://doi.org/10.1007/978-1-4939-6364-5_17
- Sonavane G, Devarajan P (2007) Preparation of alginate nanoparticles using eudragit E100 as a new complexing agent: development, in-vitro, and in-vivo evaluation. *J Biomed Nanotechnol* 3:160–169
- Sorasiththyanukarn FN, Muangnoi C, Ratnatilaka Na Bhuket P, Rojsitthisak P, Rojsitthisak P (2018) Chitosan/alginate nanoparticles as a promising approach for oral delivery of curcumin diglutamic acid for cancer treatment. *Mater Sci Eng C* 93:178–190
- Sorasiththyanukarn FN, Ratnatilaka Na Bhuket P, Muangnoi C, Rojsitthisak P, Rojsitthisak P (2019) Chitosan/alginate nanoparticles as a promising carrier of novel curcumin diethyl diglutamate. *Int J Biol Macromol* 131:1125–1136
- Sriamornsak P, Thirawong N, Korkerd K (2007) Swelling, erosion and release behavior of alginate-based matrix tablets. *Eur J Pharm Biopharm* 66:435–450
- Sultana Y, Mall S, Maurya P, Kumar D, Das M (2009) Preparation and in vitro characterization of diltiazem hydrochloride loaded alginate microspheres. *Pharm Dev Technol* 14:321–331
- Sun J, Tan H (2013) Alginate-based biomaterials for regenerative medicine applications. *Materials (Basel, Switzerland)* 6:1285–1309

- Sun CK, Ke CJ, Lin YW, Lin FH, Tsai TH, Sun JS (2021) Transglutaminase cross-linked gelatin-alginate-antibacterial hydrogel as the drug delivery-coatings for implant-related infections. *Polymers (Basel)* 13(3):414. <https://doi.org/10.3390/polym13030414>
- Swain DS, Behera A, Beg S, Patra C, Dinda S, Sruti J, Rao M (2012) Modified alginate beads for mucoadhesive drug delivery system: an updated review of patents. *Recent Pat Drug Deliv Formul* 6:259–277
- Szekalska M, Pucilowska A, Szymanska E, Ciosek P, Winnicka K (2016) Alginate: current use and future perspectives in pharmaceutical and biomedical applications. *Int J Polym Sci* 2016: 7697031
- Szekalska M, Sosnowska K, Czajkowska-Ko nik A, Winnicka K (2018) Calcium chloride modified alginate microparticles formulated by the spray drying process: a strategy to prolong the release of freely soluble drugs. *Materials (Basel)* 11(9):1522. <https://doi.org/10.3390/ma11091522>
- Tavakol M, Vasheghani-Farahani E, Dolatabadi-Farahani T, Hashemi-Najafabadi S (2009) Sulfasalazine release from alginate-N, O-carboxymethyl chitosan gel beads coated by chitosan. *Carbohydr Polym* 77:326–330
- Teboho M, Luyt A (2017) Electrospun alginate nanofibres impregnated with silver nanoparticles: preparation, morphology and antibacterial properties. *Carbohydr Polym* 165:304–312
- Thai H, Thuy Nguyen C, Thi Thach L, Thi Tran M, Duc Mai H, Thi Thu Nguyen T, Duc Le G, Van Can M, Dai Tran L, Long Bach G, Ramadass K, Sathish CI, Van Le Q (2020) Characterization of chitosan/alginate/lovastatin nanoparticles and investigation of their toxic effects in vitro and in vivo. *Sci Rep* 10:909
- Thomas D, Nair VV, Latha MS, Thomas KK (2019) Theoretical and experimental studies on theophylline release from hydrophilic alginate nanoparticles. *Future J Pharm Sci* 5:2
- Thomas D, Mathew N, Nath MS (2021) Starch modified alginate nanoparticles for drug delivery application. *Int J Biol Macromol* 173:277–284
- Timmons JP (2008) ActivHeal AquaFiber®: a new soft, conformable highly-absorbent dressing for use with chronic wounds. *Wounds UK* 4(3):88–91
- Tonnesen HH, Karlsen J (2002) Alginate in drug delivery systems. *Drug Dev Ind Pharm* 28(6): 621–630. <https://doi.org/10.1081/ddc-120003853>
- Unagolla JM, Jayasuriya AC (2018) Drug transport mechanisms and in vitro release kinetics of vancomycin encapsulated chitosan-alginate polyelectrolyte microparticles as a controlled drug delivery system. *Eur J Pharm Sci* 114:199–209
- Valeron Bergh VJ, Johannessen E, Andersen T, Tonnesen HH (2017) Evaluation of porphyrin loaded dry alginate foams containing poloxamer 407 and β -cyclodextrin-derivatives intended for wound treatment. *Pharm Dev Technol* 23:761–770
- Varaprasad K, Jayaramudu T, Kanikireddy V, Toro Aedo C, Sadiku R (2020) Alginate-based composite materials for wound dressing application: a mini review. *Carbohydr Polym* 236: 116025
- Wang Q, Zhang Newby B-M (2018) Layer-by-layer polyelectrolyte coating of alginate microgels for sustained release of sodium benzoate and zosteric acid. *J Drug Deliv Sci Technol* 46:46–54
- Wang F-Q, Li P, Zhang J-P, Wang A-Q, Wei Q (2011) pH-sensitive magnetic alginate-chitosan beads for albendazole delivery. *Pharm Dev Technol* 16:228–236
- Wang T, Zheng Y, Shi Y, Zhao L (2019) pH-responsive calcium alginate hydrogel laden with protamine nanoparticles and hyaluronan oligosaccharide promotes diabetic wound healing by enhancing angiogenesis and antibacterial activity. *Drug Deliv Transl Res* 9(1):227–239. <https://doi.org/10.1007/s13346-018-00609-8>
- Wang H, Chen X, Wen Y, Li D, Sun X, Liu Z, Yan H, Lin Q (2022) A study on the correlation between the oxidation degree of oxidized sodium alginate on its degradability and gelation. *Polymers* 14(9):1679
- Wittaya-Areekul S, Krueenate J, Prahsarn C (2006) Preparation and in vitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone. *Int J Pharm* 312(1-2):113–118. <https://doi.org/10.1016/j.ijpharm.2006.01.003>

- Witzler M, Vermeeren S, Kolevatorov RO, Haddad R, Gericke M, Heinze T, Schulze M (2021) Evaluating release kinetics from alginate beads coated with polyelectrolyte layers for sustained drug delivery. *ACS Appl Bio Mater* 4:6719–6731
- Xiao J, Zhu Y, Liu Y, Zeng Y, Xu F (2009) A composite coating of calcium alginate and gelatin particles on Ti6Al4V implant for the delivery of water soluble drug. *J Biomed Mater Res B Appl Biomater* 89B(2):543–550. <https://doi.org/10.1002/jbm.b.31246>
- Yan XL, Khor E, Lim L (2001) Chitosan-alginate films prepared with chitosans of different molecular weights. *J Biomed Mater Res* 58:358–365
- Yuan J, Guo L, Wang S, Liu D, Qin X, Zheng L, Tian C, Han X, Chen R, Yin R (2018) Preparation of self-assembled nanoparticles of ϵ -polylysine-sodium alginate: a sustained-release carrier for antigen delivery. *Colloids Surf B Biointerfaces* 171:406–412. <https://doi.org/10.1016/j.colsurfb.2018.07.058>
- Yusif R, Abu Hashim I, El-Dahan M (2014) Some variables affecting the characteristics of Eudragit E-sodium alginate polyelectrolyte complex as a tablet matrix for diltiazem hydrochloride. *Acta Pharm (Zagreb, Croatia)* 64:89–104
- Zhang M, Zhao X (2020) Alginate hydrogel dressings for advanced wound management. *Int J Biol Macromol* 162:1414–1428
- Zhang ZK, Li GY, Shi B (2006) Physicochemical properties of collagen, gelatin and collagen hydrolysate derived from bovine limed split wastes. *J Soc Leather Technol Chem* 90:23–28
- Zhang Y, Wei W, Lv P, Wang L, Ma G (2011) Preparation and evaluation of alginate-chitosan microspheres for oral delivery of insulin. *Eur J Pharm Biopharm* 77(1):11–19. <https://doi.org/10.1016/j.ejpb.2010.09.016>
- Zhang S, Li Z, Lin L, Zhang L, Wei C (2019) Starch components, starch properties and appearance quality of opaque kernels from rice mutants. *Molecules (Basel, Switzerland)* 24:4580
- Zhang H, Cheng J, Ao Q (2021) Preparation of alginate-based biomaterials and their applications in biomedicine. *Mar Drugs* 19:264
- Zheng M, Pan M, Zhang W, Lin H, Wu S, Lu C, Tang S, Liu D, Cai J (2021) Poly(α -l-lysine)-based nanomaterials for versatile biomedical applications: current advances and perspectives. *Bioact Mater* 6:1878–1909

Alginate-Based Inhalable Particles for Controlled Pulmonary Drug Delivery



Hao-Ying Li

Abstract The drug delivery to the lungs for the treatment of diseases has a very long history, and it has become the priority for respiratory care and attracts much rapidly increasing attention for dealing with local and systemic diseases. The pulmonary delivery can offer a number of prominent advantages, including rapid on-site treatment for local diseases, low dose required, avoidance of systemic side effects, suitability for delivery of biologics, and exceptional patient compliance. In order to elongate the time of drug release for continuous treatment of diseases in the lungs, polymers are explored to prepare delivery systems and are engineered as particles of inhalable size. Alginate is a natural polysaccharide with remarkable biocompatibility and has been extensively utilized as an excipient for drug delivery. Recently, Alginate draws research interests as a fresh additive to prepare inhalable particles for pulmonary drug delivery. The major methods to prepare alginate-based inhalable particles consist of the traditional spray-drying, the ionotropic gelation, and the combination of different technologies. The in-vitro aerosolization performance is investigated and alginate-based particles show satisfactory aerodynamic particle size distribution, suggesting an ideal drug delivery to the lungs. For the determination of drug release profiles, a variety of methods have been developed. In comparison with pure drug substance, the particles modified by alginate show a sustained drug release profile, which can elongate the time of drug release from minutes to several days, implying a great potential for drug delivery to the lungs for the continuous treatment of respiratory diseases.

Keywords Pulmonary delivery · In-vitro aerosolization · Sustained release · Alginate

H.-Y. Li (✉)

Institute of Pharmaceutical Science, King's College London, London, UK

e-mail: hao-ying.li@kcl.ac.uk

1 The Brief History of Drug Delivery to the Lungs

The drug delivery to the lungs can be dated back to thousands of years ago and have been recorded in different countries including Egypt, China, India and Greece, for the treatment of lung diseases such as breathlessness and asthma. The original approaches are to burn or boil herbs to generate smokes/vapors for inhalation, including the herbs of Ma-Huang (later confirmed with ephedrine contained), stramonium, hemp, opium, and tobaccos. The word of ‘inhaler’ was first introduced by English physician John Mudge in his 1778 book of ‘A Radical and Expeditious Cure for a Recent Catarrhus Cough’ for elucidating his own invention of Mudge Inhaler. This device utilizes hot water to generate mists of volatile drugs that are subsequently delivered to the lungs through inhalation (Mudge 1778), and it was subsequently well used for the respiratory delivery of herbs, medicines and chemicals such as ether for anesthesia. During nineteenth century, a French physician Sales-Girons created a ‘powered’ inhaler where an air pump was utilized to force the solutions through a jet nozzle against a plate to produce mists for inhalation, which was presented in Medical Academy, Paris in 1858 (Sales-Girons 1858) and is generally considered as the first pressurized inhaler. Sooner later, Newton patented the first dry powder inhaler (Newton 1864) and claimed two important characteristics of inhalable dry powders: the powders must be fined and should be maintained at dry state. The official landmark of inhalation therapy appeared in 1867, at which British Pharmacopeia listed five medications for inhalation delivery (General Medical Council 1867).

The modern inhaled drugs started from 1950’s with the ground-breaking invention of pressurized metered dose inhaler (pMDI) by Riker (3 M) for the pulmonary delivery of isoproterenol sulfate (Medihaler[®]-iso) and adrenalin (Medihaler[®]-epi), approved by FDA and launched to market in 1956 (Crompton 2006). The pMDIs utilized the chlorofluorocarbon (CFC) as propellant which is liquefied under pressure but will be vaporized when released to generate aerosols for inhalation. However, The CFCs have green-house effect and severely deplete the ozone in the atmosphere; these deteriorated side effects become a big concern and stimulate the generation of dry powder inhalers (DPIs). The first modern DPI is Spinhaler[®] was developed by Fisons and commercialized approximately 1970’s (Stein and Thiel 2017). DPIs have become the most active field over years as it is more creative in the design of inhaler device, formulations and their integration, in comparison with other inhalation delivery systems. The Montreal Protocol signed in 1987 histrionically changed the inhalation pharmaceutical product industry (Federal Register 1994), by encouraging the findings and acceptance of new environmentally friendly propellants of hydrofluoroalkanes (HFAs) to replace hazardous CFCs, by stimulating the development of new HFA pMDI products, and by tremendously motivating the creation of new dry powder inhalers. The drug delivery to the lungs has been considered as an effective routine for the delivery of chemical drugs and biologics for the treatment of local (e.g., asthma, COPD) and systemic (e.g., diabetes and migraine) diseases (Nokhodchi and Martin 2015).

1.1 Lung Structure and Function

Generally, human respiratory tract can be classified as three dissimilar units of the upper respiratory tract, the conducting airways and the alveolar regions. The upper respiratory tract refers to the airway starts from the mouth/nose to the upper trachea. From the trachea to the alveolar sacs, the human airway tree has 23 generations of bifurcates in average. The conducting airways consist of 0–14 generations, extend from the trachea (generation 0), bronchi, bronchioles to the terminal bronchioles (generation 14), followed which is the 15th generation of transitional bronchioles with gas exchange surface that lead to the pulmonary regions (Weibel 2009). The alveolar regions start from 16 to 23 generations, consisting of the respiratory bronchioles, alveolar ducts and alveolar sacs that are blind-ending, bubble-like and surrounded by blood capillaries. The generations of 15–23 is defined as acinus that is the major site for gas exchange, where the oxygen penetrates into the blood systems and carbon dioxide is expelled within a very short time. The reasons to achieve such rapid gas exchange are based on structural fundamentals of alveolar regions. The human lung of a healthy adult has about 300–500 million of sacs, and the total area can be as large as 140 m², and the blood flow is over 5 L/min in the alveolar region (Tsuji et al. 2005). Additionally, the thickness of monolayer of epithelium cells that separate the sac air and the capillary blood is generally less than 200 nm (Patton and Byron 2007), which greatly favors the rapid transportation of substances between sac air and the blood circulation.

1.2 Particle Deposition in the Lungs

Primarily, there are three major mechanisms for particle deposition in the respiratory tract, classified as inertial impaction, gravitational sedimentation, and Brownian diffusion. Inertial impaction refers to that the large particles (>5 µm) following the inhalation airflow have sufficient inertial momentum that makes them deviate from the streamlines at the sudden change of airway (e.g., upper airway and bifurcates in lower respiratory tract) and consequently be impacted on airway walls. Gravitational sedimentation states that the particles' fall and subsequent settlement on airway wall are caused by gravity, which typically happens in small airways and alveolar cavities where the distance the particle transverses is minor. Particle deposition by Brownian diffusion originates from particle random motions caused by their collisions with air molecules, which characteristically occurs in alveolar regions in which the velocities of airflow are very gentle. For inertia impaction and gravitational sedimentation, the increase of particle size will enhance their effects for particle deposition; while Brownian diffusion will dominate particle deposition for those particles with the sizes less than 0.5 µm (Darquenne 2020).

1.3 Pulmonary Drug Delivery Systems

The pulmonary drug delivery systems are generally classified as nebulized aerosols, pMDIs, and DPIs. The nebulized aerosols refers to that the inhalation drug solutions or suspensions are aerosolized by nebulizers in which external energies (i.e., ultra-sonic, pressurized gas flow) are employed to generate mists that are subsequently delivered to the lungs following patient's inhalation airflow. The apparent advantages associated with nebulizers might be that there are no requirements on patients for the drug aerosolization, which makes it particularly suitable for pediatrics, elderly and patients with limited lung functions (Martin and Finlay 2015). In pMDIs, the drug is dissolved or dispersed in liquefied propellant in a canister, and the inner pressure will drive drug solution or suspension metered by a valve come out to form an aerosol for inhalation. A large range of medications have been formulated as pMDI products for lung delivery, including beta 2-antagonists, muscarinic antagonists, and corticosteroids, with an annual sales over 400 m units (Newman 2005; Newhouse 2009). The pMDI major drawbacks associated with patients claim to the required coordination between the patient inhalation and device actuation for effective pulmonary drug delivery, which might become very difficult for pediatrics, elderly and patients with limited lung functions (Thompson et al. 1994); additionally, the newly developed propellants of HFAs are still greenhouse gas, contributing to global warming (Leach 2005). Fortunately, these problems can be satisfactorily solved by DPIs. For DPIs, the dry powders are pre-metered in blisters or capsules or are device metered using a powder dispenser. The blisters or capsules are packaged or inserted (when in use) into a designed inhaler device; The drug particles are aerosolized subsequently delivered to the lungs by patients inhalation airflow, which avoid the coordination between patient inhalation and device actuation (Prime et al. 1997; Islam and Gladki 2008). In addition, the drugs in dry powder state are more stable, and the drug amount in each dose can be very large. Based on these advantages, DPIs have been gaining very rapid development for the pulmonary delivery of chemical drugs and particularly biologics such as genetic materials, proteins and peptides for the treatment of local and systemic diseases (Atkins 2005; Islam and Cleary 2012).

1.4 Scientific Motivations to Explore Polymers for Pulmonary Delivery

The fates of drug particles after deposited in the lungs consist of being dissolved in the lung fluid then absorbed quickly, being cleared from the lungs by mucociliary escalator mechanism and eventually expelled by coughing or swallowing, or being 'consumed' by alveolar macrophages for elimination. Additionally, although the lungs are much unblemished in comparison with gastrointestinal tract, the biologics delivered to the lung still take the risks to be digested, as nearly all liver metabolizing

enzymes are also presented in the lungs (Lippmann et al. 1980; Gehr et al. 2006; Patton et al. 2010). In current situation, only lactose is officially approved as carrier in DPIs and it has very limited function for solving these problems. Therefore, these challenges largely stimulate academic researchers to explore polymers as excipients for the construction of novel nano- and micro- particles for inhalation, with the aim to enhance the dispersibility of particles and therefore increase the lung delivery efficiency, and to elongate the time of drug release, avoid mucociliary clearance hence prolong the retention time, and protect biotherapeutics after the drug delivered to the lungs. The progress in any of these features will proclaim the values of polymers in inhalable formulations for respiratory drug delivery (Pham et al. 2021; Rytting et al. 2008).

1.5 Alginates

Alginates are a collection of natural, non-toxic and biodegradable polysaccharides that is plentiful in nature and exists in marine brown algae (phaeophyceae) for their structure maintenance and in bacteria strains as capsular polysaccharide. The name of alginate is usually considered as an overall term referring to the whole group of alginic acids, alginate salts and their derivatives. In chemical structure, alginates consist of α -L-guluronic (G) and β -D-mannuronic (M) residues with 1 $\rightarrow$ 4 linked to form polyanionic copolymers with linear chain; The sequences of G and M are variable and depended on the organisms and tissues that they are obtained from; And they can be organized either as successive blocks or in an arbitrary dissemination (Fig. 1) (Szekalska et al. 2016). The GG-blocks offer the competence of gel-forming, MM and MG blocks provide the flexibility of polymer chain in the order of MM < MG. The fraction, dimension and distribution of GG, MM and MG blocks will define the physiochemical properties of alginate molecules, and bestow alginates with strength and flexibility. The molecular weight of alginate may vary according to difference resources, and the commercial available alginate generally has the molecular weight of approximately 32–400 K of Daltons, which grants alginate have a broad range of viscosity that can be very high and possess exceptional gelling abilities (Lee and Mooney 2012).

In terms of water solubility of alginate, there are a number of influential factors including the types of metal cations, pH values, and the total ionic strength of solution (Brink et al. 2009). The monovalent (e.g., K^+ , Na^+ , NH_4^+) alginate salts can be naturally solubilized in cold and hot water, while the divalent (e.g., Ca^{2+} , Zn^{2+} and Ba^{2+}) and trivalent (e.g., Fe^{3+} , Al^{3+}) alginate salts are only swelling but insoluble in water. Based on this unique characteristic, the soluble monovalent alginate such as sodium alginate can be purposively selected to interact with divalent or trivalent metal cations to generate insoluble gelled alginate through a process of ionic gelation, based on which the alginate-metal particles can be prepared for drug delivery (Gwon et al. 2015; Hu et al. 2021).

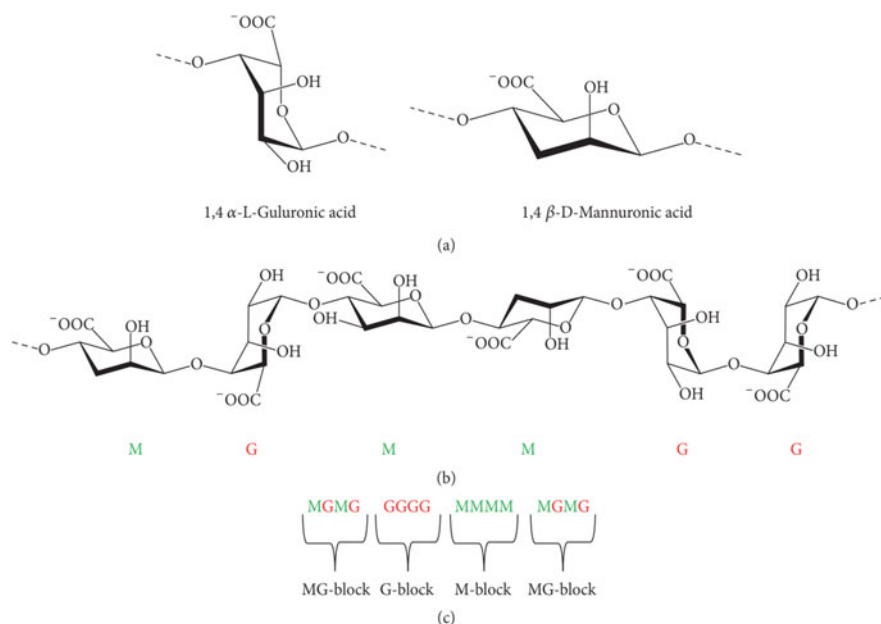


Fig. 1 The chemical structures of alginate: (a) monomers, (b) chain conformation, and (c) block distribution (Szekalska et al. 2016). Reprinted with permission from Hindawi

2 Current Development of Alginate-Based Particles for Inhalation

As early as 2005, Ahmad et al. reported the use of alginate to prepare nanoparticles for the pulmonary delivery of combined drugs (i.e., isoniazid, rifampicin, and pyrozinamide) with the aim for the treatment of tuberculosis. This alginate-based nanoparticle was evaluated in in-vitro aerosolization performance and in-vitro drug release (Ahmad et al. 2005). Since then, the use of alginate in inhalation are gradually attractive researchers' attention, with a few work has been published (Sivadas et al. 2008; El-Sherbinya and Smyth 2010; Alipour et al. 2010; Schoubben et al. 2010). During the following decade (2011–2020), a lot more interesting research studies have been carried out and published in a range of international journals, 15 major investigations on alginate for inhalation drug delivery have been organized as shown in Table 1.

The drugs that are utilized in the alginate formulations consist of a wide range of active pharmaceutical ingredients (APIs). A number of antibiotics/anti-inflammatory medications were utilized in the formulations including paclitaxel (Alipour et al. 2010), xocycyclinhyclate (Mishra and Mishra 2012), isoniazid (Tiwari et al. 2012), roflumilast (Mahmoud et al. 2018), ciprofloxacin (Hariyadi et al. 2019), tobramycin (Hill et al. 2019) and the combination of rifampicin with isoniazid (Garg et al. 2016), and rifampicin with ascorbic acid (Scolari et al. 2020), for the treatment of lung

Table 1 The analysis of research studies related to the alginate-based inhalable nano- and micro- particles

Year	Drug	Target disease	Preparation methods	Particle size	Research studies	Reference
2005	Isoniazid	Tuberculosis	IG	200–300 nm	In-vitro aerosolization	Ahmad, et al.
	Rifampicin Pyrazinamide				In-vivo release	
2008	BSA	–	SD	2–5 μm	In-vitro aerosolization In-vitro release	Sivadas, et al.
2010	BSA	–	SD/IG	2–5 μm	In-vitro aerosolization In-vitro release	El-Sherbiny and Smyth
2010	Paclitaxel	Lung cancer	EIG	2–10 μm	In-vitro aerosolization In-vitro release	Alipour, et al.
2010	Bacitracin BSA	Lung infection	SD/IG	6–9 μm	In-vitro aerosolization In-vitro release	Schoubben, et al.
2011	BSA	–	IG/SD	4–5 μm	In-vitro aerosolization In-vitro release	Sivadas and Cryan
2012	BSA	–	SD-IG	2–5 μm	In-vitro aerosolization In-vitro release	Möbus, et al.
2012	Doxycycline hyclate	Lung infection	SD	1–5 μm^*	In-vitro aerosolization In-vitro release	Mishra and Mishra
2012	Isoniazid	Tuberculosis	SD	2–6 μm	In-vitro release In-vivo pharmacokinetics	Tiwari, et al.
2012	BSA	–	SD/IG	4–16 μm	In-vitro release	Moebus, et al.
2014	Insulin	Diabetes	SD	5 μm^*	In-vitro aerosolization In-vitro release	Rohani, et al.
2017	Sodium chromoglycate	Asthma	SD	2–10 μm	In-vitro aerosolization In-vitro release	Gallo, et al.

(continued)

Table 1 (continued)

Year	Drug	Target disease	Preparation methods	Particle size	Research studies	Reference
2016	Rifampicin	Tuberculosis	IG/SD	1–2 μm	In-vivo aerosolization	Garg, et al.
	Isoniazid				In-vitro release	
2017	DNase	Lung infection	IG/FD	3–5 μm^*	In-vitro release	Islan, et al.
	Levofloxacin				In-vivo pharmaco-kinetics	
2018	Roflumilast	Lung infection	NSD	0.6–4 μm	In-vitro aerosolization	Mahmoud, et al.
					In-vitro release	
2019	Ciprofloxacin HCl	Lung infection	Spray/IG/FD	1–2 μm	In-vitro release	Hariyadi, et al.
2019	Sildenafil citrate	Pulmonary arterial hypertension	SD	2–10 μm	In-vitro aerosolization	Shahin, et al.
					In-vitro release	
					In-vivo lung/systemic kinetics	
2019	–	–	EIG/CO ₂	1–100 μm , high porous	In-vitro aerosolization	Athamneh, et al.
2019	Tobramycin	Lung infection	IG	400–500 nm	In-vitro release	Hill, et al.
2020	Rifampicin	Lung infection	IG	300–400 nm	In vivo study: intratracheal instillation, Wistar rats	Scolari, et al.
	Ascorbic acid					

IG ionotropic gelation, SD spray drying, FD freeze drying, NSD nano spray drying, EIG emulsifying ionotropic gelation

inflammation or infections and related lung diseases (e.g., tuberculosis, COPD, etc.). Apart from that, other medications are also employed to prepare the inhalable alginate particles, including sodium cromoglycate (Alipour et al. 2016) and sildenafil citrate (Shahin et al. 2019), with the aim for the cure of asthma and pulmonary arterial hypertension respectively. Biomacromolecules are also investigated in alginate formulations, consisting of insulin (Rohani et al. 2014) aimed for the therapy of diabetes, or simply the BSA as a model protein (Sivadas and Cryan 2011; Möbus et al. 2012; Moebus et al. 2012). In term of the particle size, three studies developed the nanoparticles with the size range less than 200–300 nm (Ahmad et al. 2005), 300–400 nm (Scolari et al. 2020), and 400–500 nm (Hill et al. 2019). All other research studies prepared the microparticles with the size less than 10 μm , except the data reported by Athamneh et al. who fabricated novel highly porous particles with excellent flowability and low mass median aerodynamic diameter despite that their physical dimension can be as large as 100 μm in diameter (Athamneh et al. 2019).

Among all these listed publications, Shahin et al. systematically investigate the alginate effects on the pulmonary delivery and release characteristics of sildenafil citrate by in-vitro aerosolization, in-vitro release, and in-vivo lung/systemic kinetics (Shahin et al. 2019). Four studies directly investigated the impact of alginate on the in-vitro release profiles of chemical drugs and proteins (Schoubben et al. 2010; Moebus et al. 2012; Hariyadi et al. 2019; Hill et al. 2019). Scolari et al. focused on the in-vivo study, delivering alginate-based rifampicin and ascorbic acid formulations by intratracheal instillation, and investigated the particle distribution in lungs (Scolari et al. 2020). All other studies investigated the impact of alginate on the respiratory delivery and dissolution of a range of APIs through both in-vitro aerosolization and in-vitro release studies.

3 Preparation Methods of Alginate-Modified Inhalable Particles

As mentioned previously, the deposition of particles are closely related to their aerodynamic diameters. The aerosolized particles with the size over than 10 μm are tended to be deposited in the mouth and throat through inertial impaction, while the particles have the size less than 5 μm can be inhaled into the lower respiratory tract and deposited in the conducting airways. For those particles that have the size smaller than 2 μm , they may escape from the inertial impaction and gravitational sedimentation in the upper respiratory tract and conducting airway, and they are more likely to follow the inhalation airflow and go down deeper into the alveolar region that is the major site for drug absorption for generation of local and systemic therapeutic effects.

So, for effective drug delivery to the lungs, it is highly desirable to prepare drug particles with the size less than 5 μm . In terms of alginates as excipients, the methods that can be utilized to prepare particles with the size in the inhalable range consist of

spray drying, ionotropic gelation, and their combination or combined with other physicochemical technologies (i.e., emulsions).

3.1 *Spray Drying*

Spray drying is a one-step process to turn the fluids (i.e., solutions, suspensions, emulsions and pastes) into dry particulate forms such as powders, agglomerates or granules, through spraying the fluids into a hot drying medium (e.g., air, nitrogen, etc.). The spray drying process consists of four continuous steps: atomization of fluids into millions of droplets, contact of droplets with heated air, drying of droplets to create dry particles, implementation of gas-solid separation devices to recover dry powders. For laboratory-scale mini spray-dryer, the atomization of feed is achieved by co-current nozzle by which the fluid is broken up into droplets by pressurized air. The dimension of nozzle opening plays an important role in determining the size of droplets and further of the final dry particles; and the smaller size of nozzle opening will provoke droplets with reduced size and subsequently finer dry powders (Seville et al. 2007).

The moisture content of the final spray-dried particles is basically influenced by the components in the formulations and the operation parameters during the spray-drying. The addition of hydrophilic components in the formulation tend to augment the moisture content of final spray-dried products; and the polysaccharides would facilitate the increase of moisture content in comparison with mono-, di- and oligosaccharides, as evidenced by many previous studies (Li et al. 2003, 2005a, b, 2010; Li and Birchall 2006; Li and Seville 2010; Jiang et al. 2017). Further, the operation parameters also have impact on the moisture content of spray-dried powders, including the inlet/outlet temperature, the aspiration, and the feed flowrate; the increase inlet/out temperature and the aspiration and the decrease of feed flowrate will reduce the moisture contents of the final spray-dried powders. For the collection of spray-dried powders, the high performance cyclone has smaller size in comparison with normal cyclone and therefore will generate stronger centrifuge force for particles at the same linear speed leading to those particles at smaller sizes can be isolated from the airflow and get recovered (Maa et al. 1998; Maury et al. 2005).

Spray-drying can offer a number of advantages to prepare particles for inhalation. It is a one-step process and therefore can produce dry particulates from wet states within a very short time. In addition, the size, morphology and other key physicochemical properties can be modified and improved by adjusting the spray-drying fluids including optimizing components and employing suspensions and emulsions, to create spray-dried particles with particular characteristics including high surface roughness and therefore low inter-particulate contact (Li et al. 2005b; Li and Birchall 2006), improved flowability and dispersibility (Li et al. 2003, 2005a; Yang et al. 2015a), low density and high porosity (Weers and Tarara 2014) and nanoparticles (Li and Xu 2017). Apart from that, the specific drug spray-dried powders show amazing flowability, dispersibility in the airflow and ideal lung deposition without

the aid of carrier (Yang et al. 2015b). More excitingly, spray drying has been proved to be a reliable approach to prepare biomacromolecules for inhalation, including DNA (Li et al. 2003, 2005a, b; Li and Birchall 2006), RNA (Keil and Merkel 2019), proteins and peptides (Li and Seville 2010; Li et al. 2010; Kane et al. 2013), where the biotherapeutics can effectively maintain their chemical structure and biological activities.

On the preparation of alginate-based particles for inhalation, Mishra and Mishra utilized the spray drying technology to prepare microparticles for pulmonary delivery of doxycycline hyclate, a medication having potency for the treatment of a variety of bacterial infections. The spray drying solutions are water-ethanol:cosolvent system with dissolved drug, leucine (dispersibility enhancer), lactose and muco-adhesive excipients including alginate (20% w/w in final product). The parameters for operation are inlet/outlet temperatures: 140 °C/76 °C, aspiration airflow 28 m³/h, the air pressure for atomization 20 bar. The spray-dried alginate-modified particles, observed by SEM, show the size of 1–5 µm with smooth spherical shape, and have the moisture content of 5.03% w/w and tapped density of 0.502 g/cm³ (Mishra and Mishra 2012). Gallo et al. explored polysaccharides including alginate sodium as excipients to prepare spray-dried powders of sodium cromoglycate for pulmonary delivery. The spray-drying solution (100 mL) contains drug and a polysaccharide (1 vs. 0.16 g), and is spray-dried (0.5 mm nozzle) under the conditions of inlet temperature 110 °C, aspiration 35 m³/h, atomization air pressure 6 bar and flowrate 600 L/h. The spray-dried alginate-cromoglycate particles have the size in the range of 2–9 µm and moisture content of 7.01% w/w (Gallo et al. 2017). Shahin et al. utilized the mini spray-dryer (0.7 mm nozzle) to prepare inhalable powders of sildenafil citrate for the treatment of pulmonary arterial hypertension, and sodium carboxymethyl cellulose, sodium alginate, and sodium hyaluronate polymers were added as excipient with adjusting ratios for optimization. The spray-dried powders have the size of 2–11 µm, while the formulation with only alginate, alginate/carboxymethyl cellulose (0.5: 0.5 w/w), and alginate/carboxymethyl cellulose/hyaluronate (0.375: 0.375: 0.25 w/w/w) showed the small particle size of 2.07, 2.05 and 2.20 µm respectively, suggesting the addition of alginate effectively reduced the particle size of spray-dried powders (Shahin et al. 2019). For the respiratory delivery of proteins and peptides, a range of biocompatible polymers including alginate were screened to prepare spray-dried powders of model protein BSA (polymer: BSA = 100: 1 w/w). The mini spray dryer (0.5 mm nozzle) was utilized and operated at the inlet temperature of 45–140 °C and the feed flow rate of 4–5 mL/min. The spray-dried alginate-based BSA particles have the perfect spherical shape with smooth surface, with the size of 3.23 µm, a tapped density of 0.22 g/cm³, and moisture content of 6.73% w/w (Sivadas et al. 2008).

Nano spray-drying also has been employed for the preparation of alginate-modified dry powders for inhalation. In comparison with mini spray dryer, the nano spray dryer utilizes the high frequency vibration of piezoelectric effect to drive the liquids through a micronized orifice to generate the droplets for drying, and the spray-dried powders are collected by electrostatic force generated by a high-voltage electric field. The nano spray-drying was operated to prepare alginate-based

nano-/micro- particles of roflumislast. The o/w emulsions were prepared for spray-drying, where the oil phase isopropyl myristate with calcium beta-glycerophosphate and the water phase containing alginate, drug and other excipients are emulsified by surfactant of Tween 80 under vortex. The operation conditions for nano spray drying are: the hole size for atomization: 7 μm , the feeding rate: 25 mL/h, aspiration: 110 L/min, and the inlet/outlet temperatures: 110 $^{\circ}\text{C}$ /51 $^{\circ}\text{C}$. It was reported that the nano spray-dried yields were 34–68% w/w, and the nano spray-dried particles had the size in the range of 0.6–3 μm .

3.2 Ionotropic Gelation

The scientific rationale of ionotropic gelation for particle preparation is based on the insoluble nature when the alginate polyanion chain interacted with divalent (e.g., Ca^{2+} , Zn^{2+}) or trivalent (e.g., Fe^{3+} , Al^{3+}) metal cations. Calcium ions are usually utilized to interact with alginate linear anionic chain for the generation of particles as they can be obtained easily with lost cost. The chemical interaction of calcium ions with the alginate linear chain has been well understood and identified as the well-known ‘egg-box’ zig-zag structural features (Fig. 2) (Fang et al. 2007). Scientifically, the gel formation is based on the particular and robust interactions between long stretches of G units and divalent cation of Ca^{2+} . The $\alpha\text{-L-(1,4)}$ -linked guluronic

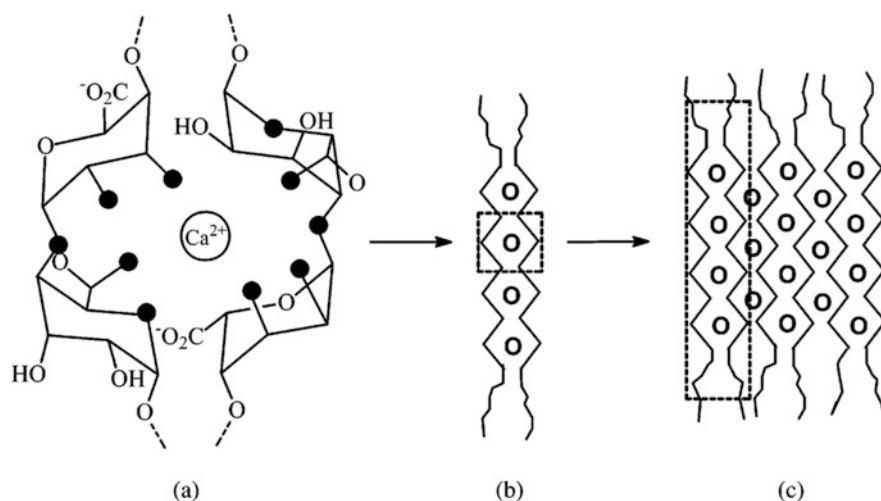


Fig. 2 Schematic illustration of the hierarchical structure of alginate-calcium ‘egg-box’ and junction zones. (a) Coordination of calcium ion in a cavity produced by two pairs of guluronate sequences along different alginate chains; (b) ‘Egg-box’ dimer; (c) lined-up ‘Egg-box’ multimer. The solid black dots symbolize the oxygen atoms potentially stabilizing calcium ions, while the open circle represent the calcium ions (Fang et al. 2007). Reprinted with permission from ACS Publications

acid (GG-block) adopts a characteristic zigzag shape in polymer chain, which works like a pocket to facilitate the accommodation of Ca^{2+} . The chemical bonding of Ca^{2+} with two pairs of GG originated from different alginate chain will thereafter fix these two polymer chain together and subsequently construct steady junction sectors. As such principle, the gel strength will be undoubtedly enhanced as the growth of total content of G units and of the average dimension of G block in the gelling polymer chain (Cao et al. 2020; Draget et al. 1997; Hu et al. 2021).

Ahmad et al. added calcium chloride into sodium alginate solution containing the drugs of isoniazid, rifampicin and pyrazinamide (drug: alginate = 7.5: 1 w/w) and with minor chitosan as additional binder to prepare alginate-based nanoparticles loaded with combined drugs. These alginate-based nanoparticles have the mean size of 235.5 nm with polydispersity index of 0.439, and drug encapsulation efficiency of 70–90% w/w (Ahmad et al. 2005). Hill et al. employed the very similar process to prepare tobramycin-loaded alginate nanoparticles and reported the particle size 400–500 nm with polydispersity index of 0.26–0.31; it is also reported that the use of additional crosslinker of chitosan can dramatically increase the drug loading efficiency (Hill et al. 2019). Following the comparable approach, rifampicin was loaded into alginate-chitosan nanoparticles with the particle size of approximately 300 nm, polydispersity of 0.22–0.23, and drug loading efficiency of 50% w/w (Scolari et al. 2020).

3.3 The Combined Technologies

A number of research combined spray drying with ionotropic gelation to prepare inhalable alginate-modified particles. For the combination of technologies, there are two ways reported - to implement ionotropic gelation first then followed by spray-drying, and vice versa. The first approach can generate products of nanoagglomerates in microparticles, the vice versa way can modify the surface morphology of spray-dried microparticles.

In terms of nano-agglomerate-in-microparticle, Sivadas and Cryan prepared the alginate solution containing elastin and BSA-FITC (elastin: alginate = 1: 6 w/w, BSA-FITC: polymer = 1:100 w/w), in which CaCl_2 solution was dropped to generate the drug-loaded nanoparticles. This suspension was subsequently spray-dried under the parameters of inlet/out temperature: 170 °C/56 °C, feed flow rate 22% and aspiration 75%. The SEM images clearly showed nanosized particles agglomerated together to generate the spray-dried microparticles that had the size of 4–5 μm and tapped density of 0.09 g/cm^3 (Sivadas and Cryan 2011). Garg et al. reported a comparative method by using calcium alginate nanoparticles to trap rifampicin and isoniazid, then the suspension was spray-dried with mannitol and leucine as addition excipients to generate microparticles. The drug-loaded alginate nanoparticles had the size <600 nm, while the spray-dried agglomerates had the size 1–2 μm with polydispersity index of 0.10–0.22, moisture contents of approximately 4–7% w/w, spray-dried yields of 26–50% w/w, and drug entrapment efficiency over

50% w/w (Garg et al. 2016). In addition, other combined technologies are also developed. Islan et al. prepare the alginate calcium nanoparticles loaded with DNase and levofloxacin, then freeze-dried to produce microparticles with the size of 3–5 μm visualized by SEM and encapsulation efficiency of drug in the range of 21–68% w/w (Islan et al. 2017). Hariyadi et al. utilized a device to generate sprays of ciprofloxacin-alginate solution into CaCl_2 solution for gelation, and then the suspensions are freeze-dried to generate dry powders. The microparticles had the size 1–2 μm , moisture content 2–3% w/w, drug loading 75–80% (Hariyadi et al. 2019).

In the other way of preparing spray-dried powders then followed by ionotropic gelation, El-Sherbinya and Smyth synthesized PEG-g-NPHCs copolymers by self-assembly forming into drug-loaded nanoparticles, subsequently encapsulated into respirable sodium alginate spray-dried microparticles that soaked into CaCl_2 solutions for ionotropic gelation and freeze-dried to get the final dry powders. The microparticles showed the size of 2–3 μm , moisture contents of 5–11% w/w, bulk density of 0.2–0.7 g/cm^3 , and the encapsulation efficiency of 50–90% w/w (El-Sherbinya and Smyth 2010). Schoubben et al. spray dried the sodium alginate solution containing bacitracin and bovine serum albumin to prepare dry powders that were subsequently put into calcium chloride solution for external gelation. The microparticles treated by external gelation showed more spherical shape with improved smooth surface; and the particle size distribution became narrower although the mean size was comparable to untreated microparticles (Schoubben et al. 2010).

Möbus, et al. reported a novel method, where the spray drying of aqueous alginate solutions containing BSA, $\text{Zn}(\text{NH}_3)_4\text{SO}_4$ and supplementary excipients. Upon ammonia evaporation, the alginate was cross-linked by Zn^{2+} cations, indicating that the alginate ionotropic gelation and the generation of spray-dried microparticles may take place simultaneously. The alginate- ZnSO_4 particles showed irregular shapes with much wrinkled surface and had the size of 2.8–13 μm , based on the formulations (Möbus et al. 2012). More interestingly, Athamneh et al. developed a novel combined technology to prepare high porous particles. The principle is to make water in oil emulsion where the water droplets contained alginate that will be in controlled ionotropic gelation by the sustained release of Ca^{2+} from the reaction of CaCO_3 with acetic acid. Then break the emulsion and replace the water by organic solvent for freeze-drying. The generated alginate particles show the porosity as high as 99% and the density as low as 0.035 g/cm^3 . Despite their large size distribution of 1–100 μm , these porous particles are expected to have amazing flowability for drug delivery to the lung (Athamneh et al. 2019).

4 In-Vitro Aerosolization Performance

The in-vitro aerosolization performance is a critical characteristic of inhaled drug products, as in mandatory the drug particles are required to be effectively aerosolized and the aerosols essentially need to demonstrate desirable aerodynamic particle size

distribution, in order to achieve the targeted regions in the lungs. In addition, the in-vitro measurement of aerosol performance provides an anticipation and eventually proposes the drug deposition in human respiratory tract, through the establishment of in-vitro and in-vivo correlation (IVIVC, Mitchell et al. 2007; Chow et al. 2021), which will subsequently make it a possibility to presume a clinical response to the pharmaceutically active agents. Currently, cascade impactors are well-recognized devices to assess in-vitro aerosolization performance of inhaled drugs, as these facilities are at laboratory scale and able to produce meaningful data in clinical context, and have been authorized by FDA to control the quality of inhaled products during their development and manufacturing (Tougas et al. 2013; USP 2012).

4.1 Introduction of Cascade Impactors

The working principle of cascade impactor is utilizing inertial forces to separate particles from airstream and thereafter to accomplish powder classification according to their aerodynamic diameters. For the assessment of aerosols, the cascade impactor is operated at a constant flowrate. As the aerosol passes through jets, the flow speed will be increased; the particles with higher inertia are subsequently deviated from the streamlines and impacted on the collection plate (Fig. 3) (Marple and Willeke 1976; Djoumi et al. 2018). The multiple stages of impactor have sequentially decreasing sizes of jets thereafter initiating increasing speed of airflow for the separation of powders with reducing sizes stage by stage. Each stage of the impactor has a specific cut-off diameter which is dependent on the device design and the airflow rate for sampling aerosols (Mercer et al. 1970; Newton et al. 1977; Tougas et al. 2013).

Generally, four approved cascade impactors are employed for determination of aerosolization performance, and they are Twin-Stage Impinger (TSI), Multi-Stage Liquid Impinger (MSLI), Anderson Cascade Impactor (ACI) and Next Generation Impactor (NGI). The cut-off diameters for their individual stage at different airflow

Fig. 3 Principle of inertial impaction (Djoumi et al. 2018). Reprinted with open access permission from MDPI

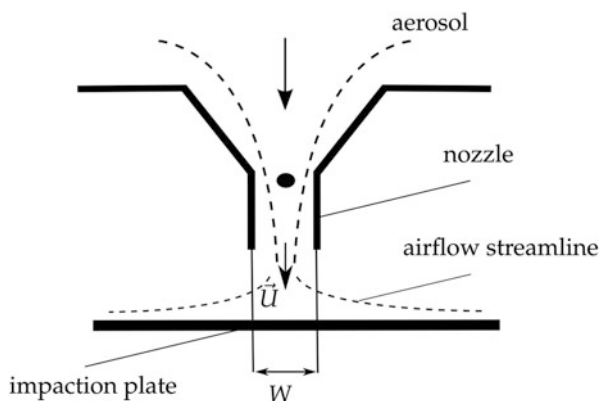


Table 2 Cut-off diameters of cascade impactors (Data from company digital manual)

Apparatus	TSI	MSLI				ACI (non-viable)			NGI			
Flow rate (L/min)	60	30	60	80	100	28.3	60	90	15	30	60	100
		Cut-off diameter (μm)										
Stage												
–2								8.0				
–1							8.6	6.5				
0	6.4					9.0	6.5	5.2				
1		16.9	13.0	11.8	10.4	5.8	4.4	3.5	14.10	11.72	8.06	6.12
2		9.3	6.8	5.6	4.9	4.7	3.2	2.6	8.61	6.40	4.46	3.42
3		4.5	3.1	2.7	2.4	3.3	1.9	1.7	5.39	3.99	2.82	2.18
4		2.5	1.7	1.4	1.2	2.1	1.2	1.0	3.30	2.30	1.66	1.31
5						1.1	0.55	0.22	2.08	1.36	0.94	0.72
6						0.7	0.26		1.36	0.83	0.55	0.40
7						0.4			0.98	0.54	0.34	0.24
F/MOC									0.70	0.36	0.14	0.07

ACI Anderson cascade impactor, *F* filter, *MSLI* multi-stage liquid impinger, *MOC* micro-orifice collector, *NGI* next generation impactor, *TSI* twin-stage impinger

are summarized in Table 2. NGI is a newly developed device, offering low inter-stage particle loss and convenient handling and particle collection for industrial use. For the operation, the plates are coated by oils or polymers to avoid their bouncing back. From the deposition data, a number of parameters can be calculated to present the aerosolization performance of inhaled products, including recovered dose (RD), recovered fraction (RF), emitted dose (ED), emitted fraction (EF), respirable dose (RD), respirable fraction (RF), fine particle dose (FPD), fine particle fraction (FPF), mass median aerodynamic diameter (MMAD), and geometric standard deviation (GSD). These parameters, together with the original in-vitro deposition pattern, are working together to symbolize the Aerodynamic Particle Size Distribution (APSD) of an inhalation aerosol. For the drug delivery to the lungs, a delivery system is required to aerosolize the drug for inhalation; the common delivery systems are nebulizers, pMDIs and DPIs that have been summarized previously in this chapter.

4.2 In-Vitro Aerosolization Performance of Inhalable Alginate Particles

For the pulmonary delivery of alginate particles, two delivery systems have been reported including the DPIs and nebulizers that are summarized as below.

4.2.1 DPI Delivery System

Möbus et al. employed the MSLI to investigate the APSD of zinc–alginate microparticles and reported the ED of 86–97% w/w and FPF of 1.9–36.2% w/w, varying due to additional excipients (Möbus et al. 2012). Sivadas et al. blended the spray-dried BSA-loaded alginate microparticle with mannitol (as a carrier) to form dry powder formulations, and aerosolized into ACI where each plate is coated by Tween 80 to prevent particle bounding back. The alginate-based microparticles showed the MMAD of 4.1 μm , GSD of 1.88 μm , and FPF of 20.7% w/w that is much higher than those of chitosan-, PLGA-, or Gelatin- based spray-dried microparticles (Sivadas et al. 2008). The nano-agglomerate in spray-dried microparticles was assessed by ACI to investigate their APSD with MMAD of 3.65 μm and GSD of 1.84 μm reported (Sivadas and Cryan 2011). Mishra and Mishra reported the spray-dried microparticles of a range of polymers loaded with doxycycline hyclate were examined by ACI to investigate their aerosolization performance. The alginate-based spray-dried microparticles showed the RF of 93.6% w/w, EF of 86.8% w/w, FPF of 27.3%, and the MMAD and GSD of 5.02 and 1.68 μm respectively, which were much inferior to those microparticles modified by sodium carboxymethyl cellulose and starch (FPF > 50% w/w) (Mishra and Mishra 2012). Garg et al. prepared the nanoaggregate-in-microparticle spray-dried alginate-based powders, and employ ACI to investigate their aerosolization performance. At optimized formulation, the MMADs were 1.58 and 1.23 μm for rifampicin - and isoniazid - loaded spray-dried powders respectively (Garg et al. 2016). By nano spray-drying emulsions, Mahmoud et al. prepared roflumilast-loaded nano- and micro- particles that were tested by ACI to determine APSD. For the test, silicone oil coatings were applied on the plate surface to reduce particle bouncing back. The drug powders were loaded into capsules, inserted into Aerolizer[®] DPI, and fired into ACI at the flow rate of 60 L/min with a flow-duration of 4 s. The optimized formulation showed the FPF of 42.7% w/w, MMAD of 2.48 μm and GSD of 1.91 μm (Mahmoud et al. 2018). The non-viable ACI was also employed to evaluate the aerosolization performance of spray-dried polymeric microparticles loaded with sildenafil citrate. The powders were put into hydroxypropyl methylcellulose hard capsules and actuated by using low air resistance mono-dose dry powder inhaler. The alginate only spray-dried powders generated a FPF of 24.29% w/w, MMAD of 4.66 μm , GSD of 1.70 μm . While the alginate- sodium carboxymethyl cellulose (0.5:0.5 w/w) demonstrated an increased FPF of 30.04% w/w, a comparable MMAD of 4.68 μm and GSD of 1.60 μm (Shahin et al. 2019).

El-Sherbiny and Smyth utilized the NGI to assess the APSD of spray-dried alginate microparticles, and reported the MMAD of 1.0–1.7 and 1.5–2.6 μm for plain and BSA-loaded powders (El-Sherbiny and Smyth 2010). Alipour et al. aerosolized paclitaxel-loaded microparticles into NGI and calculated the EF, FPF, MMAD and GSD as 92% w/w, 13.9% w/w, 5.9 and 1.84 μm respectively (Alipour et al. 2010). Rohani et al. made use of Spinhaler[®] to aerosolize insulin-loaded spray-dried microparticles into NGI (plated coated by hydroxypropyl methylcellulose gel)

under the flowrate of 60 L/min for the APSD analysis. The data were revealed as RF of 81–87% w/w, FPF of 52–81% w/w, MMAD of 2.5–4.6 μm and GSD of 1.77–3.68 μm , and the micropartilces formulated by mannitol, sodium alginate, insulin and sodium citrate showed the superior aerosolization performance (Rohani et al. 2014). The spray-dried polymeric microparticles loaded with Sodium Cromoglycate were aerolized into NGI by using Breezhaler[®] dry powder device at 60 L/min for 4 s. The alginate-based spray-dried microparticles showed the EF of 87% w/w, FPF of 31% w/w, MMAD of 4.16 μm and GSD 1.66 μm , suggesting an inferior aerosolization performance to those microparticles modified by sodium carboxymethylcellulose (Gallo et al. 2017).

4.2.2 Nebulizer Delivery System

Ahmad et al. nebulized the alginate nanoparticles load with isoniazid, rifampicin and pyrazinamide into ACI for the assessment of aerosolization performance, and reported the RF (particle size: 0.4–2.1 μm), MMAD and GSD were 80.5% w/w, 1.1 μm and 1.71 μm respectively (Ahmad et al. 2005).

5 The Dissolution Process

5.1 Theory of Dissolution

By definition, dissolution is a process of a mass transfer of particles from the surface of a solid into a dissolution medium, which involves the solute molecule isolated from solid phase and accommodated by vacancy among solvent molecules to generate a homogenous solution (Kamlet et al. 1986; Gao and Cao 2008). Based on diffusion layer model, the rate of dissolution can be mathematically determined according to Noyes–Whitney equation, which is state as:

$$\frac{dC}{dt} = \frac{DS_w}{Vh}(C_s - C) \quad (1)$$

Noyes–Whitney equation links the rate of drug dissolution (dC/dt) with the diffusion coefficient of drug substance (D), the exposed surface area of drug solid state (S_w), the volume of solution (V), the layer thickness for drug diffusion (h), and the difference of drug concentrations of solubility (C_s) and of dissolved drug substance in bulk solution (C) at the time of t . This theory discloses that the dissolution rate is controlled by drug diffusion. .

5.2 *Motivations on In-Vitro Dissolution Testing of Inhaled Drugs*

For solid oral dosage forms, the in-vitro dissolution testing has been well established as an effective means to control the consistency of product quality in drug development and manufacturing, and as an approach to create the model of in the IVIVC, in combination with the pharmacokinetic data (Fotaki et al. 2009; Fotaki and Vertzoni 2010). The IVIVC empowers dissolution data become an essential means to assess the changes of formulations and manufacturing process after product approval, and to evaluate generics during the development and manufacturing process. However, for orally inhaled products, neither regulatory requirements nor pharmacopeia techniques are existed in current official documents for in-vitro dissolution test. Furthermore, the Inhalation Advisory Panel of USP judged no convincing evidence on dissolution “kinetically and/or clinically crucial for currently approved” orally inhaled products (Gray et al. 2008). Despite of that, the academic and industrial interests are increasing considerably to develop techniques for in-vitro dissolution testing of inhaled products.

The driving forces to develop techniques for in-vitro dissolution of inhaled drugs may come from the following aspects. (1) The need for comprehensive evaluation of inhaled products. By regulatory guidance, the inhaled products are required to be assessed on the dose content uniformity and APSD; and the in-vivo studies are implemented as needed to understand the pharmacokinetics and/or pharmacodynamics of inhaled products. As the complex of realistic oropharyngeal geometry and the difference of breathing pattern, the APSD does not accurately reflect the particle deposition in human respiratory tract. This gap need to be filled up by exploring technologies for testing in-vitro drug dissolution of inhaled products, in order to build up comprehensive quality criteria (Riley et al. 2012; Radivojev et al. 2019). (2) The need of developing a standard dissolution method for quality control. In-vitro dissolution test can work as a powerful tool to generate product quality information, identify formulation factors to influence the drug bioavailability, and to categorize the variables affecting post-approval changes. Besides, in-vitro dissolution test can powerfully control the quality and its consistency for generic and innovator inhaled products over the whole life cycle. There is a need to develop a standardized dissolution test method to meet these needs (Son and McConville 2009). (3) The need for establishing IVIVC. The in-vitro dissolution data will assist the construction of a realistic and full landscape of IVIVC, and thereafter make it promising to use in-vitro data to predict the in-vivo particle performance and therapeutic effects, which will tremendously benefit many aspects across the drug development, manufacturing and quality control (Gerde et al. 2021; Riley et al. 2012).

5.3 *Factors to Influence Drug Dissolution in the Lungs*

After the deposition of drug particles in the lungs, their dissolution into the lining fluid of respiratory tract is expected for the generation of desired therapeutic effects. According to Noyes-Whitney dissolution model, the drug dissolution is driven by the solubility of drug substance, the solid surface area wetted by dissolution fluid and the concentration of dissolved drug substance in the bulk solution. These factors are further controlled by the physicochemical states of inhaled drugs and the on-site environment of respiratory tract that fundamentally refers to the dissolution medium of lung fluids and their conditions (i.e., thickness, compositions, temperature, and hydrodynamics). The physicochemical states of inhaled drug substance are very complex and consist of a range of parameters including chemical structure that fundamentally influences the lipophilicity, hydrophilicity and solubility, and the engineering-related characteristics of particle size distribution, shape, morphology, surface roughness, porosity and the polymorphs (crystalline/amorphous states and their ratios) (Chow et al. 2007; Shetty et al. 2020). All these factors are working together to determine the drug dissolution in the lungs.

For the inhaled drug particles, the lung lining fluid is the dissolution medium, and its characteristics of composition, viscosity, temperature and the speed of movement will generate an influence on the drug dissolution and solubility. The increase of viscosity of a dissolution medium will lessen the diffusion coefficient, and as a result reduce the rate of drug dissolution according the Noyes – Whitney equation. The increase of temperature can empower solvent molecules with more kinetic energy, which will more effectively break apart solute molecules, facilitate the isolation of solute molecules from the solid phase and their entry into the solvent for dissolution. Additionally, the higher temperature can increase the movement speed of solute molecules in the solvent, and thereafter escalate the diffusion coefficient, which will consequently benefit the rate of drug dissolution, as determined by the equation. Moreover, the agitation, stirring and fluid movement would generate disturbance to the dissolution system, which subsequently increases the solute-solvent molecule contact chances, and facilitates the quick distribution of solvent molecules around solute. Furthermore, the increase of disturbance level has proved to alleviate the thickness of stagnant layer and reduce the diffusion gradient and hence enhance the drug dissolution (Ahuja and Scypinski 2001).

On particle size, it is well recognized that the reduced particle size will enhance the dissolution rate and solubility of drugs (Kumar et al. 2022). The reason for that is the drug particles with much reduced size will massively enlarge the specific surface area for exposure to dissolution medium, which therefore boost the dissolution rate according to Noyes-Whitney equation. The reduction of particle size is a classical approach to improve the drug dissolution and solubility, as it is safe and has no changes of drug chemical structure. Apart from that, the increase of particle porosity has also well recognized as another way to improve drug dissolution and solubility, because the porous particles have massively increased specific surface area to augment their exposure to dissolution medium. Furthermore, the density of porous

particles can be very low, which offers these particles, even at immense physical dimensions, with considerably reduced aerodynamic diameter and thereafter strongly enhances the particle aerosolization with improved lung deposition in the peripheral regions (Edwards et al. 1997; Weers and Tarara 2014). In terms of the polymorphs, the drug at amorphous state can offer greater solubility than the crystalline form, as the amorphous drug is thermodynamically unstable; and therefore the drugs with poorly solubility can be purposively fabricated into amorphous state for improvement of solubility, bioavailability and therapeutic effects (Censi and Martino 2015).

On the lipophilicity and hydrophilicity, the drug chemical structure determines the lipophilicity and hydrophilicity that will generate significant impact on drug solubility, regulate the level of drug affinity to tissue, and finally determine the extent of therapeutic effects on site. The stronger lipophilicity will increase drug partition in the plasma membranes and thereafter reduce the drug release and diffusion to the targets. In comparison with salmeterol, formoterol has relative water solubility which empowers it diffuse speedily to the beta-2 adrenergic receptors and generates rapid (<3 min) bronchodilation like short-acting beta-2 antagonist (SABA) (Benhamou et al. 2001). While, the more lipophilic salmeterol need to take a significantly longer time (~20 min) to engender onset action. Apart from that, formoterol and salmeterol are classified as long-acting beta-2 antagonists, as both of them have sufficient lipophilicity that allows them to remain in respiratory tissues and to take a much longer duration of action than the highly water soluble short-acting beta-2 antagonists (SABAs) such as salbutamol sulphate. As an aqueous solution, the lung fluid has 96% of water, where the lipophilic drug will undoubtedly have poor solubility (Celik et al. 1999). Conversely, the lipids and proteins contained in the lung fluid are facilitators to promote the wetting and solubility of poorly soluble drugs, and as a result, to assist their dissolution in the lung fluid. Apart from that, the lung surfactants can furthermore boost the solubility of lipophilic drug, as evidenced by examining the dissolution of glucocorticoids (Wiedmann et al. 2000; Liao and Wiedmann 2003).

5.4 Technical Challenges Related to Dissolution Testing of Inhaled Drugs

5.4.1 Challenges Related to Human Lung Fluid

The complexity of human respiratory tract makes it become challenging to replicate such environment in-vitro. The complicated lung structure has been presented above in this chapter. The complexity of lung fluid will be particularly discussed in this section as it is an actual medium for drug dissolution. The challenges associated with lung fluid development for in-vitro drug dissolution consist of the following important aspects.

1. The lung fluid in human respiratory tract has a small volume of about 10–30 mL. However, the lung fluid at small volume actually covers a very large lung area and subsequently turns into a layer with the thickness as thin as 5–10 μm in conducting airways and less than 100 nm in the alveoli (Patton and Byron 2007; Olsson et al. 2011), therefore only a trivial volume of lung fluid is present as dissolution medium at a specific site in the lungs. Additionally, the thin layer is highly possible to be stagnant. Undoubtedly, both actual conditions of lung fluid detrimentally inhibit the on-site drug dissolution. In in-vitro test, it is extremely challenging to simulate the thin layer and with various thickness of lung fluid; And it is hard or even not possible to fully dissolve a drug with very low solubility (e.g., budesonide, salmeterol, et al.) by using a minor volume of dissolution medium.
2. The components of lung lining fluid are complicated and gradually changing from bronchus to alveolus (Pouwels et al. 2021). It is known that there are abundant endogenous surfactants in alveolar lining layer such as phosphatidylcholine (DPPC), which can facilitate the wetting of poorly soluble drug particles. However, the components of lung fluid are too complicated, and the exact composition of the lung aqueous fluids and surfactants has not been established accurately (Marques et al. 2011). Furthermore, escalator clearance mechanism turns the insoluble drug particles into a risk of being cleared off, which thereafter becomes a barrier for particle diffusion and drug dissolution. All these aspects are very difficult to be imitated outside the human body, and it is therefore highly challenging to generate in-vitro data that can reliably reflect the actual drug dissolution in the lungs.

5.4.2 Challenges Related to Experimental Operations

There are two apparent challenges associated with the experimental operations for in-vitro dissolution testing of inhaled drug. One is related to the method for particle collection from drug aerosols, and the other is associated with the apparatus for in-vitro dissolution testing of inhaled particles (Riley et al. 2012).

1. In order to reliably reveal the drug dissolution in the lungs, the particles collected for in-vitro dissolution testing should be in the comparable size range as those deposited in the lower respiratory tract, which is the particles with the aerodynamic diameter less than 5 μm . Additionally, in comparison with the whole respirable fraction, the use of specific fraction of aerosols with particular aerodynamic size range is likely to generate dissimilar data that is more accurately to reflect the drug dissolution profile in a defined respiratory tract. One example is that the dry powder inhaler formulations generally consist of micronized API particles blended with large lactose particles as carriers, the API particles are required to be detached from lactose carrier for lung delivery, and collected at specific aerodynamic size range for dissolution test.
2. Currently, there are no apparatus that has been approved by FDA for the determination of in-vitro dissolution testing of inhaled products. Even more,

there are no apparatus that are well agreed by the researchers in this field and can satisfactorily simulate the complexity of lung geometry and physiological environment for drug dissolution. So, researchers have carried out the dissolution studies of inhaled drugs by using a variety of tools including Franz Cell, Transwell, Flow-Through Cell (USP IV Apparatus), Paddle Dissolution Apparatus (USP II Apparatus), Basket Dissolution Apparatus (USP I Apparatus). The common problems associated with these methods are uncontrollable particle state in medium, loss of particles on sampling, lack of differentiation in dissolution data among different formulations, and possibly immense dissolution alteration by using different facilities for testing (Mercuri and Fotaki 2020).

5.5 The Current Situation for Drug Dissolution of Inhaled Alginate-Based Powders

5.5.1 The Dissolution Media

For the investigation of particle dissolution in lungs, a number of dissolution media have been simulated over the years, aimed to prepare solutions or fluids with the same components and at the same concentrations as those contained in actual human lung fluids. According to the literature, different simulated lung fluids have been developed and utilized for the drug dissolution of inhaled drugs in-vitro, including the phosphate-buffered saline (PBS), Gamble's solution, modified Gamble's solution and artificial lysosomal fluid (ALF). As a standard physiological solution, PBS has the comparable ion strength and pH (7.4) value to human serum and is simple in composites; it is therefore widely utilized in biological, pharmaceutical and biomedical research. PBS is particularly preferable for the dissolution test of alginate-based drug formulation, as the phosphate anions can facilitate the release of calcium cations that is widely utilized for gelling alginate for drug encapsulation. Gamble's solution (pH 7.4) is comprised of cations (i.e., sodium, potassium, calcium, and magnesium) and anions (i.e., bicarbonate, carbonate, sulfate, organic acids, chloride, and monohydrogen phosphate) (Gamble 1942). The Gamble's solution was further modified with one way by the addition of dipalmitoylphosphatidylcholine (DPPC) (pH 7.4), proteins (i.e. albumin, mucin), amino acids (i.e. glycine, L-cysteine, glutathione) and others. DPPC is major phospholipid of lung surfactant benefits declining air-water interface tension of the terminal airways and improves lungs defensive function. As the major lung surfactant, the widely existing DPPCs may generate interactions with drug molecules, which will impact the fate of inhaled drugs *in vivo*. *While the addition of amino acids and proteins can adjust the viscosity of simulated lung fluid; Moreover, they may have an important effect to facilitate the drug dissolution, as the recent report proved that the addition of BSA in the simulated lung fluid can approximately double the solubility of poorly soluble drug of budesonide* (Radivojev et al. 2021). ALF is also a complex artificial biological medium, with the purpose to simulate the phagocytosis-associated

Table 3 Compositions of simulated human lung fluids (g/L)

Type	PBS	Original Gamble solution	Modified Gamble solution	ALF
NaCl	8	6.019	0.2	3.21
NaHCO ₃		2.604	2.7	
KCl	0.2	0.298	0.298	
Na ₂ HPO ₄	1.44	0.142	0.15	0.071
Na ₂ SO ₄		0.071	0.072	0.039
CaCl ₂ ·2H ₂ O		0.4	0.256	0.128
MgCl ₂ ·6H ₂ O		0.203		0.05
C ₆ H ₅ O ₇ Na ₃ ·2H ₂ O		0.097		0.077
C ₂ H ₃ O ₂ Na·3H ₂ O		0.9526		
KH ₂ PO ₄	0.24			
Sodium lactate				0.085
Disodium tartrate				0.09
NH ₄ Cl			6.02	
Sodium pyruvate				0.172
Glycine			0.376	0.059
Citric acid				20.8
NaOH				6.0
L-Cysteine			0.122	
DPPC			0.1	
Ascorbic acid			0.018	
Uric acid			0.016	
Glutathione			0.03	
Albumin			0.26	
Mucin			0.5	
pH	7.4	7.4	7.4	4.5

PBS phosphate-buffered saline, *ALF* artificial lysosomal fluid

interstitial conditions in the lungs, and the pH value is adjusted as 4.5 to mimic the phagolysosomal acidity of alveolar and interstitial macrophages (Marques et al. 2011; Pelfrene et al. 2017) (Table 3).

5.5.2 Apparatus for Drug Dissolution of Inhaled Products

For the alginate-based inhalable particles, four major methods/apparatuses have been reported for the drug dissolution testing, and they are dialysis membranes, Franz diffusion cell, basket dissolution apparatus (USP I Apparatus), paddle dissolution apparatus (USP II Apparatus). These methods/apparatuses are discussed in detail for the evaluation the drug dissolution from alginate-based inhaled drugs.

5.5.2.1 Dialysis Membranes

Dialysis membranes are widely utilized for the investigation of drug releases from alginate-based inhaled drugs. The general procedure is to disperse the inhalable particles into a small volume of the dissolution medium to form a suspension that is subsequently transferred into a pre-treated dialysis bag selected; the dialysis bag is selected with a specific cut-off molecular weight to allow that the released drugs from particles can go through the membrane while other materials are 'cut-off' for avoidance the potential interference of drug detection. The dialysis bag was then soaked into a big volume of dissolution medium contained in beaker or dissolution apparatus flask for drug release.

Mishra and Mishra reported the dialysis membrane (Molecular Weight Cut-Off, MWCO: 6–8 kDa) was filled with 5 mL of PBS with DX- alginate inhalable microparticles suspended inside, tied to the paddle of USP type II dissolution apparatus, stirred (50 rpm) in 500 mL of PBS at 37 °C. The pure drug was nearly entirely dissolved in approximately 4 h. The addition of alginate in the formulation can effectively extend the time to 10 h with 50% w/w of drug released and to 48 h with 80% w/w. More amazingly, no initial burst of drug release can be observed for alginate-based formulation (Mishra and Mishra 2012). The fast release of pure drug (i.e., terbutaline sulfate) was further confirmed to dialysis bag dissolution testing; and the use of alginate can effectively slow down the dissolution rate and thereafter extend the time for drug release (Moebus et al. 2012). Garg et al. utilized dialysis membrane (MWCO: 1 kDa) with 10 mL suspension inside and soaked into 100 mL of PBS in a beaker, shaking at 37 °C for investigation of INH and RIF release. The release behavior of RIF and INH from the particulate formulation followed a biphasic pattern of an initial burst and a following sustained release. Results of in vitro release study demonstrated that 80–90% of plain drugs were released after 2 h whereas an initial burst release of RIF and INH about 30–40% from optimized formulations up to 4 h followed by more gradual and sustained release pattern about 90% for the following 60 h (Garg et al. 2016).

Mahmoud et al. placed 1 mL drug microparticle suspension into dialysis membrane that was further immersed into 50 mL of ethanolic PBS (30% v/v; pH 7.4), shaking at 37 °C for test of roflumilast release. More controlled drug release patterns were observed with the L1 and L2 formulations, where <45% of the drug was released within the first hour. This confirms the successful cross links between the alginate molecules and the calcium ions present in the cross linking agent (β - GP), which in turn trapped the drug inside the particles resulting in more controlled release pattern for the drug (Mahmoud et al. 2018).

Hill et al. investigated the drug release by incubating the particles (3 mg) in dialysis membranes (1 mL) with a 10 kDa MWCO, soaked into 5 mL of PBS, agitated at 37 °C. A typical biphasic release was observed with 18.9% of the entrapped tobramycin released within the first 24 h, although the overall release was limited to 25.4% of the total amount of loaded tobramycin over the course of the experiment. The optimal formulation of the tobramycin-loaded alginate/chitosan particles was tested against live cultures of *P. aeruginosa* (Fig. 2). The unloaded

particles showed no activity against *P. aeruginosa*, tobramycin alone had a Minimum inhibitory concentration (MIC) of 1.5 µg/mL, and the tobramycin-loaded particles showed activity in a dose dependent manner as expected, with a somewhat elevated MIC of 6.25 µg/mL, which was likely to be due to the rate of diffusion of tobramycin from the particles. While this would still be compatible with the delivery of a clinically relevant dosage (of 300 mg twice daily) to be delivered as the biopolymers.

5.5.2.2 Franz Cell Diffusion

A Franz cell apparatus consists of upper donor and lower receptor compartments, separated by a semipermeable membrane (Fig. 4a). For the test of drug release, the inhalable drug particles are directly placed the membrane, and the receptor is filled full of dissolution medium. The drug particles then become wetted due to absorbing dissolution medium but not submersed; the drug start to release and diffuse across the membrane into the receptor dissolution medium, and the sample is collected from the side arm at scheduled time intervals for detection.

Sivadas et al. utilized a Franz diffusion cell to monitor in-vitro protein release with pH 7.4 PBS as dissolution medium that was maintained at 37 °C. Microparticles were deposited on the membrane at the air–liquid interface established by the filter. The dissolution data revealed the polymers including alginate can achieve the target of sustain the release of BSA. Further, the Franz Cell method together with PBS as dissolution solution was employed to investigate the influence of elastase on the release profiles of model protein of BSA from alginate-based formulations (Sivadas and Cryan 2011). Möbus et al. adapted Franz Cell to mimic situation of drug release on mucosal surface, and utilized PBS or modified simulated lung fluid as dissolution medium. The BSA-only microparticle had a very rapid drug release, while the addition of alginate-zinc complex would tremendously slow down the release of BSA, demonstrating a super capability to encapsulate BSA by forming complexation with zinc and the hydrogel network protein diffusion. It was also confirmed that the alginate-based microparticles can also achieve the sustained release of small chemical molecules such as terbutaline sulfate. Rohani et al. also employed Franz diffusion cell for the analysis of insulin release from spray-dried microparticles; the PBS was utilized as the dissolution medium. The alginate-based spray-dried microparticles showed a sustained release of insulin more than 6 h, much longer than that (~30 min) of the mannitol-modified spray-dried that showed an instant release of insulin (Rohani et al. 2014).

5.5.2.3 USP Apparatus Type I

USP apparatus I, also known as rotating basket apparatus, is made up of a glass vessel, inside which a cylindrical basket is contained and attached to a rotating stirrer by an axle (Fig. 4b). For the testing of drug dissolution, the drug at a certain dosage

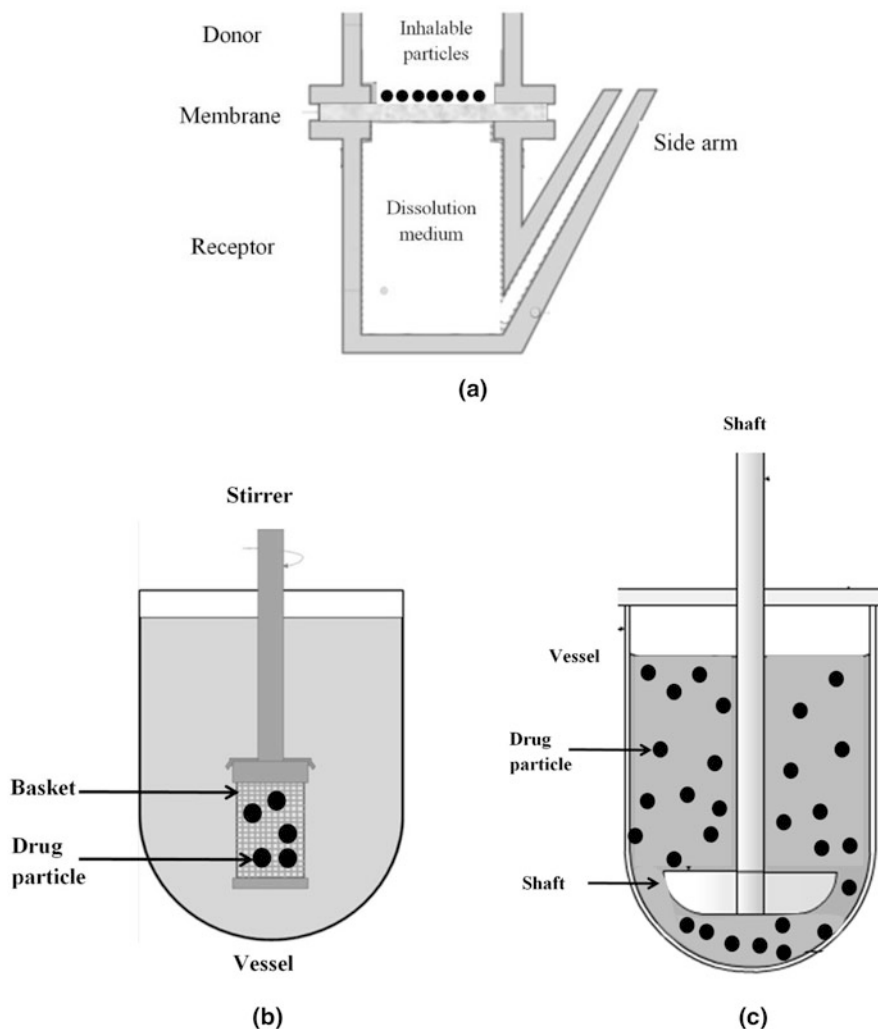


Fig. 4 The schematic diagrams of USP Apparatus used for the investigation of dissolution of inhaled drugs. (a) Franz Diffusion Cell; (b) Basket Dissolution Apparatus; (c) Paddle Dissolution Apparatus

form is placed into the basket that will be immersed into the dissolution medium, and the basket is driven to be rotated at a speed of 50–100 rpm to facilitate the drug release. Typically, the temperature is conserved at 37 °C. This apparatus is not usually accepted for testing the drug release from inhaled products, as the large volume of dissolution medium is needed, incomparable to the actual small volume of lung fluid; and as the drug particles are hard to be managed in the solution due to the possible particle aggregates formed to block the basket mesh and the potential of particle escaping from the basket and floating up on the surface of dissolution medium.

Very few studies have been reported to employ this apparatus for drug release testing of inhaled products. On alginate inhalable particles, Shahin et al. recently reported the use of USP type I dissolution apparatus with PBS as dissolution medium at reduced volume to investigate the drug release from spray-dried microparticles for inhalation. For the test, the microparticles dispersed in PBS, poured into dialysis bag that is secured and placed into the basket for dissolution test at 37 °C. The data revealed that the use of alginate can not only elongate the time of sildenafil release to as long as 24 h, but also the increasing content of the alginate in the formulations can unceasingly escalate the cumulative release of sildenafil.

5.5.2.4 USP Apparatus Type II

USP Apparatus II, also named as Paddle Dissolution Apparatus, has the same assembly as basket type, just replacing the basket by a paddle that has a freely-rotating metallic shaft (Fig. 4c). By using this apparatus, the inhalable drug particles are directly dispersed into the dissolution medium for drug releasing, which causes a number of drawbacks including the adherence of drug particles to the interior vessel surface, the particle floating to the solution surface further making the particle be poorly wetted, and the heterogeneous dispersion of particles in the solution. Furthermore, as a large amount of dissolution medium is needed as required in the pharmacopeia, the drug release from inhalable particles, usually at micronized size, can be too quick to get meaningful results for comparison. As Apparatus I, this apparatus is rarely utilized in a direct way for the analysis of drug release for inhalation drug particles. On the investigation of drug release of alginate-based inhalation powders, there is one report about the use of USP Apparatus II for the testing of drug release. The spray-dried powders (80 mg) were dispersed into 500 mL of PBS at 37 °C and 50 rpm. Unsurprisingly, the spray-dried particles, even with the presence of alginate, have a complete drug release within 15 min. Although the use of CMC can extend the release time, the total time of release was still not over than 60 min (Gallo et al. 2017). This is agreed to our work previously reported (Xu et al. 2013, 2014); where spray-dried powders made of di-, oligo- and poly- saccharides respectively demonstrated a drug release time no more than an hour, confirming the rapid drug release profile by using paddle apparatus.

5.5.2.5 Other Methods

Other reports revealed that the inhalable powders were just simply dispersed in dissolution medium for drug release. The centrifuge or filtration was utilized to separate the particles and get the samples with released drug inside for the quantification. By employing this method, in-vitro release profiles of ciprofloxacin HCl, LV and DNase were revealed, and the incorporation of alginate into the formulation can elongate the time of drug release to several days (Islan et al. 2017; Hariyadi et al. 2019).

6 Conclusion

Alginate is recently explored as a new excipient in pharmaceutical formulations for controlled pulmonary drug delivery. For effective drug delivery to the lungs, the major methods for preparing inhalable alginate-based particles consist of spray-drying, ionotropic gelation and the combined technologies. The spray drying technology can produce alginate-based microparticles, at optimized formulations, generally with the mean size of 2–5 microns, suggesting a potential to be delivered to the lower respiratory tract. The ionotropic gelation method usually uses calcium ions to interact with alginate polyanionic chains to form ‘egg-box’ structure and zig-zag conformation, the insoluble nature of calcium alginate will induce the formation of nanoparticles (200–500 nm as reported) with drug encapsulated inside. A number of research studies combined the technologies of spray-drying and ionotropic gelation together to produce inhalable alginate-based powders. There are two ways for technique combination; one is to use spray-drying first, followed by ionotropic gelation, and the other is vice versa. The implementation of ionotropic gelation on spray-dried microparticles can improve shape more spherical, the particle surface with extra smooth, and the particle size distribution much narrower. The other way of spray-drying nanosuspension containing alginate-based nanoparticles generated by ionotropic gelation can produce complex structure nanoagglomerates, where the alginate-based nanoparticles are aggregated together to generate spray-dried microparticles. Another combined technology has also been developed by uniting emulsifying ionotropic gelation with CO₂ drying technology to produce highly porous microparticles, as a platform to prepare inhalable particles for drug delivery to the lungs. For the in-vitro aerosolization performance, two delivery systems of dry powder inhaler and nebulizer have been developed. The alginate-based particles were aerosolized by inhalers or nebulizers, and impacted on different stages of cascade impactors (e.g. MSLI, ACI or NGI). The dry powder inhalers were widely utilized for the pulmonary delivery of alginate-based microparticles, all alginate-based microparticles show satisfying aerosolization performance generally with the FPF of 20–50% w/w, and MMAD of 1–5 μm . The alginate nanoparticles, produced by ionotropic gelation in solution, can be delivered to the lung by nebulizer, with very high delivery efficiency (FPF > 80% w/w) and an ideal MMAD (~ 1 μm), suggesting a majority of alginate-based drug nanoparticles deposited in the peripheral regions of the lungs. Furthermore, a variety of methods have been developed for the investigation of in-vitro drug dissolution of inhaled drug particles. The use of alginate in the formulation can effectively elongate the time of drug release from minutes for drug substance to several days, strongly suggesting a great potential to generate sustained release system of inhaled drugs for continuous treatment of respiratory diseases.

References

- Ahmad Z, Sharma S, Khuller GK (2005) Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis. *Int J Antimicrob Agents* 26:298–303
- Ahuja S, Scypinski S (eds) (2001) *Handbook of modern pharmaceutical analysis*. Academic, London
- Alipour S, Montaseri H, Tafaghodi M (2010) Preparation and characterization of biodegradable paclitaxel loaded alginate microparticles for pulmonary delivery. *Colloids Surf B Biointerfaces* 81:521–529
- Alipour S, Montaseri H, Khalili A et al (2016) Non-invasive endotracheal delivery of paclitaxel-loaded alginate microparticles. *J Chemother* 28:411–416
- Athamneh T, Amin A, Benke E (2019) Alginate and hybrid alginate-hyaluronic acid aerogel microspheres as potential carrier for pulmonary drug delivery. *J Supercrit Fluids* 150:49–55
- Atkins PJ (2005) Dry powder inhalers: an overview. *Respir Care* 50:1304–1312
- Benhamou D, Cuvelier A, Muir JF et al (2001) Rapid onset of bronchodilation in COPD: a placebo-controlled study comparing formoterol (ForadilAerolizer) with salbutamol (Ventodisk). *Respir Med* 95:817–821
- Brink P, Zwijnenburg A, Smith G (2009) Effect of free calcium concentration and ionic strength on alginate fouling in cross-flow membrane filtration. *J Membr Sci* 345:207–216
- Cao L, Lu W, Mata A, a. (2020) Egg-box model-based gelation of alginate and pectin: a review. *Carbohydr Polym* 242:116389
- Celik G, Kayacan O, Beder S et al (1999) Formoterol and salmeterol in partially reversible chronic obstructive pulmonary disease: a crossover, placebo-controlled comparison of onset and duration of action. *Respiration* 66:434–439
- Censi R, Martino PD (2015) Polymorph impact on the bioavailability and stability of poorly soluble drugs. *Molecules* 20:18759–18776
- Chow AHL, Tong HHY, Chattopadhyay P et al (2007) Particle engineering for pulmonary drug delivery. *Phar Res* 24:411–437
- Chow MYT, Tai W, Chang RYK et al (2021) In vitro-in vivo correlation of cascade impactor data for orally inhaled pharmaceutical aerosols. *Adv Drug Deliv Rev* 177:113952
- Crompton G (2006) A brief history of inhaled asthma therapy over the last fifty years. *Prim Care Respir J* 15:326–331
- Darquenne C (2020) Deposition mechanisms. *J Aerosol Med Pulm Drug Deliv* 33:181–185
- Djoumi L, Vanotti M, Blondeau-Patissier V (2018) Real time cascade impactor based on surface acoustic wave delay lines for pm10 and pm2.5 mass concentration measurement. *Sensors (Basel)* 18:255
- Draget KI, Skjak-Braek G, Smidsrod O (1997) Alginate based new materials. *Int J Biol Macromol* 21:47–55
- Edwards DA, Hanes J, Caponetti G et al (1997) Large porous particles for pulmonary drug delivery. *Science* 276:1868–1871
- El-Sherbinya IM, Smyth HDC (2010) Biodegradable nano-micro carrier systems for sustained pulmonary drug delivery: (I) Self-assembled nanoparticles encapsulated in respirable/swellable semi-IPN microspheres. *Int J Pharm* 395:132–141
- Fang Y, Al-Assaf S, Phillips GO et al (2007) Multiple steps and critical behaviors of the binding of calcium to alginate. *J Phys Chem B* 111:2456–2462
- Federal Register (1994) The Montreal Protocol on substances that deplete the ozone layer, Final Act (Nairobi: UNEP 1987). FR 59:56276–56298
- Fotaki N, Aivaliotis A, Butler J et al (2009) A comparative study of different release apparatus in generating in vitro-in vivo correlations for extended release formulations. *Eur J Pharm Biopharm* 73:115–120
- Fotaki N, Vertzoni M (2010) Biorelevant dissolution methods and their applications in in vitro-in vivo correlations for oral formulations. *Open Drug Deliv J* 4:2–13

- Gallo L, Bucala V, Ramirez-Rigo MV (2017) Formulation and characterization of polysaccharide microparticles for pulmonary delivery of sodium cromoglycate. *AAPS PharmSciTech* 18:1634–1645
- Gamble JL (1942) Chemical anatomy, physiology and pathology of extracellular fluid: a lecture syllabus. Harvard University Press, Cambridge
- Gao S, Cao C (2008) A new approach on estimation of solubility and *n*-octanol/water partition coefficient for organohalogen compounds. *Int J Mol Sci* 9:962–977
- Garg T, Goyal AK, Rath G et al (2016) Spray-dried particles as pulmonary delivery system of anti-tubercular drugs: design, optimization, in vitro and in vivo evaluation. *Pharm Dev Technol* 21:951–960
- Gehr P, Blank F, Rothen-Rutishauser BM (2006) Fate of inhaled particles after interaction with the lung surface. *Paediatr Respir Rev* 7:S73–S75
- General Medical Council (1867) British pharmacopoeia. Spottiswoode, London
- Gerde P, Malml M, Selg E (2021) In vitro to ex vivo in vivo correlation (IVIVC) of dissolution kinetics from inhaled particulate solutes using air blood barrier models Relation between in vitro design, lung physiology and kinetic output of models. *J Aerosol Sci* 151:105698
- Gray VA, Hickey AJ, Balmer P et al (2008) The inhalation ad hoc advisory panel for the USP performance tests of inhalation dosage forms. *Pharm Forum* 34:1068–1074
- Gwon SH, Yoon J, Seok HK et al (2015) Gelation dynamics of ionically crosslinked alginate gel with various cations. *Macromol Res* 23:1112–1116
- Hariyadi D, Hendradi E, Kurniawan TD (2019) Alginate microspheres encapsulating ciprofloxacin HCl: characteristics, release and antibacterial activity. *Int J Pharm Res Health Sci* 7:3020–3027
- Hill M, Twigg M, Sheridan EA et al (2019) Alginate/chitosan particle-based drug delivery systems for pulmonary applications. *Pharmaceutics* 11:379
- Hu C, Lu W, Mata A et al (2021) Ions-induced gelation of alginate: mechanisms and applications. *Int J Biol Macromol* 177:578–588
- Islam N, Cleary MJ (2012) Developing an efficient and reliable dry powder inhaler for pulmonary drug delivery—a review for multidisciplinary researchers. *Med Eng Phys* 34:409–427
- Islam N, Gladki E (2008) Dry powder inhalers (DPIs)—a review of device reliability and innovation. *Int J Pharm* 360:1–11
- Islan GA, Ruiz ME, Morales JF et al (2017) Hybrid inhalable microparticles for dual controlled release of levofloxacin and DNase: physicochemical characterization and in vivo targeted delivery to the lungs. *J Mater Chem B* 5:3132–3144
- Jiang WZ, Cai Y, Li HY (2017) Chitosan-based spray-dried mucoadhesive microspheres for sustained oromucosal drug delivery. *Powder Technol* 312:124–132
- Kamlet MJ, Doherty RM, Abboud JLM et al (1986) Solubility—a new look. *Chemtech* 16:566–576
- Kane C, O’Neil K, Conk M (2013) Inhalation delivery of protein therapeutics. *Inflamm Allergy Drug Targets* 12:81–87
- Keil TWM, Merkel OM (2019) Dry powder inhalation of siRNA. *Ther Deliv* 10:265–267
- Kumar R, Thakur AK, Chaudhari P et al (2022) Particle size reduction techniques of pharmaceutical compounds for the enhancement of their dissolution rate and bioavailability. *J Pharm Innov* 17:333–352
- Leach CL (2005) The CFC to HFA transition and its impact on pulmonary drug development. *Respir Care* 50:1201–1208
- Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. *Prog Polym Sci* 37:106–126
- Li HY, Birchall JC (2006) Chitosan-modified dry powder formulations for pulmonary drug delivery. *Pharm Res* 23:941–950
- Li HY, Neill H, Innocent R et al (2003) Enhanced dispersibility and deposition of spray-dried powders for pulmonary gene therapy. *J Drug Target* 11:425–432
- Li HY, Seville PC (2010) Novel pMDI formulations for pulmonary delivery of proteins. *Int J Pharm* 385:73–78

- Li HY, Seville PC, Williamson IJ et al (2005a) The use of amino acids to enhance the aerosolisation of spray-dried powders for pulmonary gene therapy. *J Gene Med* 7:343–353
- Li HY, Seville PC, Williamson IJ et al (2005b) The use of absorption enhancers to enhance the dispersibility of spray-dried powders for pulmonary gene therapy. *J Gene Med* 7:1035–1043
- Li HY, Song X, Seville PC (2010) The use of sodium carboxymethylcellulose in the preparation of spray-dried proteins for pulmonary drug delivery. *Eur J Pharm Sci* 40:56–61
- Li HY, Xu EY (2017) Innovative pMDI formulations of spray-dried nanoparticles for efficient pulmonary drug delivery. *Int J Pharm* 530:12–20
- Liao X, Wiedmann TS (2003) Solubilization of cationic drugs in lung surfactant. *Pharm Res* 20:1858–1863
- Lippmann M, Yeates DB, Albert RE (1980) Deposition, retention, and clearance of inhaled particles. *Br J Ind Med* 37:337–362
- Maa YF, Nguyen PA, Sit K et al (1998) Spray-drying performance of a bench-top spray dryer for protein aerosol powder preparation. *Biotechnol Bioeng* 60:301–309
- Mahmoud AA, Elkasabgy NA, Abdelkhalek AA (2018) Design and characterization of emulsified spray dried alginate microparticles as a carrier for the dually acting drug roflumilast. *Eur J Pharm Sci* 122:64–76
- Marple VA, Willeke K (1976) Impactor design. *Atmos Environ* 10:891–896
- Marques MRC, Loebenberg R, Almukainzi M (2011) Simulated biological fluids with possible application in dissolution testing. *Dissolution Technol* 18(3):15–28. <https://doi.org/10.14227/DT180311P15>
- Martin AR, Finlay WH (2015) Nebulizers for drug delivery to the lungs. *Expert Opin Drug Deliv* 12:889–900
- Maury M, Murphy K, Kumar S et al (2005) Effects of process variables on the powder yield of spray-dried trehalose on a laboratory spray-dryer. *Eur J Pharm Biopharm* 59:565–573
- Mercer TT, Tillery MI, Newton GJ (1970) A multi-stage, low flow rate cascade impactor. *Aerosol Sci* 1:9–15
- Mercuri A, Fotaki N (2020) In-vitro dissolution for inhalation products. In: Fotaki N, Klein S (eds) *In vitro drug release testing of special dosage forms*. Wiley, West Sussex, pp 119–153
- Mishra M, Mishra B (2012) Mucoadhesive microparticles as potential carriers in inhalation delivery of doxycycline hyclate: a comparative study. *Acta Pharm Sin B* 2:518–526
- Mitchell J, Newman S, Chan HK (2007) In vitro and in vivo aspects of cascade impactor tests and inhaler performance: a review. *AAPS PharmSciTech* 8:237–248
- Möbus K, Siepmann J, Bodmeier R (2012) Zinc–alginate microparticles for controlled pulmonary delivery of proteins prepared by spray-drying. *Eur J Pharm Biopharm* 81:121–130
- Moebus K, Siepmann J, Bodmeier R (2012) Novel preparation techniques for alginate–poloxamer microparticles controlling protein release on mucosal surfaces. *Eur J Pharm Sci* 45:358–366
- Mudge J (1778) A radical and expeditious cure for a recent catarrhus cough. Allen, London
- Newhouse M (2009) Advantages of pressurized canister metered dose inhaler. *J Aerosol Med* 4:139–150
- Newman SP (2005) Principles of metered-dose inhaler design. *Respir Care* 50:1177–1190
- Newton A (1864). *Inhaling apparatus*. UK patent 1161
- Newton GJ, Raabe OG, Mokler BV (1977) Cascade impactor design and performance. *J Aerosol Sci* 8:339–347
- Nokhodchi A, Martin GP (eds) (2015) *Pulmonary drug delivery: advances and challenges*. Wiley, West Sussex
- Olsson B, Bondesson E, Borgström L et al (2011) Pulmonary drug metabolism, clearance, and absorption. In: Smyth H, Hickey A (eds) *Controlled pulmonary drug delivery*. Springer, New York, pp 21–50
- Patton JS, Brain JD, Davies LA et al (2010) The particle has landed—characterizing the fate of inhaled pharmaceuticals. *J Aerosol Med Pulm Drug Deliv* 23:S71–S87
- Patton JS, Byron PR (2007) Inhaling medicines: delivering drugs to the body through the lungs. *Nat Rev Drug Discov* 6:67–74

- Pelfrene A, Cave MR, Wragg J et al (2017) In vitro investigations of human bioaccessibility from reference materials using simulated lung fluids. *Int J Environ Res Public Health* 14:112
- Pham DT, Chokamonsirikun A, Phattaravorakarn V et al (2021) Polymeric micelles for pulmonary drug delivery: comprehensive review. *J Mater Sci* 56:2016–2036
- Pouwels SD, Burgess JK, Verschuuren E et al (2021) The cellular composition of the lung lining fluid gradually changes from bronchus to alveolus. *Respir Res* 22:285
- Prime D, Atkins PJ, Salter A et al (1997) Review of dry powder inhalers. *Adv Drug Deliv Rev* 26: 51–58
- Radivojev S, Luschin-Ebengreuth G, Pinto JT et al (2021) Impact of simulated lung fluid components on the solubility of inhaled drugs and predicted *in vivo* performance. *Int J Pharm* 606: 120893
- Radivojev S, Zellnitz S, Paudel A et al (2019) Searching for physiologically relevant in vitro dissolution techniques for orally inhaled drugs. *Int J Pharm* 556:45–56
- Riley T, Christopher D, Arp J et al (2012) Challenges with developing in vitro dissolution tests for orally inhaled products (OIPS). *AAPS PharmSciTech* 13:978–989
- Rohani SSR, Abnous K, Tafaghodi M (2014) Preparation and characterization of spray-dried powders intended for pulmonary delivery of Insulin with regard to the selection of excipients. *Int J Pharm* 465:464–478
- Ryting E, Nguyen J, Wang XY et al (2008) Biodegradable polymeric nanocarriers for pulmonary drug delivery. *Expert Opin Drug Deliv* 5:629–639
- Sales-Girons J (1858) *Thérapeutique respiratoire. Traité théorique et pratique des salles de respiration nouvelles (à l'eau minérale pulvérisée) dans les établissements thermaux, pour le traitement des maladies de poitrine*. Victor Masson, Paris
- Schoubben A, Blasi P, Giovagnoli S et al (2010) Development of a scalable procedure for fine calcium alginate particle preparation. *Chem Eng J* 160:363–369
- Scolari IR, Paez PL, Musri MM et al (2020) Rifampicin loaded in alginate/chitosan nanoparticles as a promising pulmonary carrier against *Staphylococcus aureus*. *Drug Deliv Transl Res* 10:1403–1417
- Seville PC, Li HY, Learoyd TP (2007) Spray-dried powders for pulmonary drug delivery. *Crit Rev Ther Drug Carrier Syst* 24:307–360
- Shahin HI, Vinjamuri BP, Mahmoud AA (2019) Design and evaluation of novel inhalable sildenafil citrate spray-dried microparticles for pulmonary arterial hypertension. *J Control Release* 302: 126–139
- Shetty N, Cippola D, Park H et al (2020) Physical stability of dry powder inhaler formulations. *Expert Opin Drug Deliv* 17:77–96
- Sivadas N, Cryan SA (2011) Inhalable, bioresponsive microparticles for targeted drug delivery in the lungs. *J Pharm Pharmacol* 63:369–375
- Sivadas N, O'Rourke D, Tobin A et al (2008) A comparative study of a range of polymeric microspheres as potential carriers for the inhalation of proteins. *Int J Pharm* 358:159–167
- Son YJ, McConville JT (2009) Development of a standardized dissolution test method for inhaled pharmaceutical formulations. *Int J Pharm* 382:15–22
- Stein SW, Thiel CG (2017) The history of therapeutic aerosols: a chronological review. *J Aerosol Med Pulm Drug Deliv* 30:20–41
- Szekalska M, Bowska AP, Nska ES et al (2016) Alginate: current use and future perspectives in pharmaceutical and biomedical applications. *Inter J Polym Sci* 2016:7697031. <https://doi.org/10.1155/2016/7697031>
- Thompson CJ, Irvine MT, Grathwohl CK et al (1994) Misuse of metered-dose inhalers in hospitalized patients. *Chest* 105:715–717
- Tiwari S, Chaturvedi AP, Tripathi YB et al (2012) Microspheres based on mannoseylated lysine-co-sodium alginate for macrophage-specific delivery of isoniazid. *Carbohydr Polym* 87:1575–1582
- Tougas TP, Mitchell JP, Lyapustine SA (eds) (2013) *Good cascade impactor practices*, AIM and EDA for orally inhaled products. Springer, New York

- Tsujino I, Kawakami Y, Kaneko A (2005) Comparative simulation of gas transport in airway models of rat, dog, and human. *Inhal Toxicol* 17:475–485
- USP (2012) <601> Aerosols, nasal sprays, metered-dose inhalers, and dry powder inhalers. USP 35:232–252
- Weers J, Tarara T (2014) The PulmoSphere™ platform for pulmonary drug delivery. *Ther Deliv* 5: 277–295
- Weibel ER (2009) What makes a good lung? *Swiss Med Wkly* 139:375–386
- Wiedmann TS, Bhatia R, Wattenberg LW (2000) Drug solubilisation in lung surfactant. *J Control Release* 65:43–47
- Xu EY, Guo J, Xu Y et al (2014) Influence of excipients on spray-dried powders for inhalation. *Powder Technol* 256:217–223
- Xu EY, Guo J, Yang M et al (2013) Spray-dried inhalation powders for sustained drug release. *J Control Release* 172:e28–e29
- Yang XF, Xu Y, Qu DS et al (2015a) The influence of amino acids on aztreonam spray-dried powders for inhalation. *Asian J Pharm Sci* 10:541–548
- Yang XF, Xu Y, Qu DS et al (2015b) Excipient-free, spray-dried powders for pulmonary aztreonam delivery. *J Drug Deliv Sci Technol* 28:7–10

Biomedical Applications of Alginate in the Delivery System for Natural Products



Jia Wang and Haixia Chen

Abstract Due to the numerous pharmacological activities, natural products have been employed extensively in the treatment of many diseases. However, the clinical applications of some active substances are limited due to their water-insoluble, low bioavailability and poor stability characteristics. Natural biopolymer alginate has beneficial traits such as biodegradability, biocompatibility and non-toxicity. Given these attractive characteristics, alginate has been extensively studied for the development of various delivery systems for natural bioactive ingredients, such as hydrogels, beads, floating drug delivery system, microspheres, nanoparticles, nanofibers, micelles, liposomes. Due to their high viscosity and pH sensitivity, alginates can be used in oral drug delivery, sustained drug release, targeting drug delivery and wound dressings and so on. This chapter will concentrate on the development of alginate-based natural product delivery systems, describe the biomedical applications of these systems, and offer helpful recommendations for future alginate-based natural product delivery system design.

Keywords Natural products · Alginate · Delivery systems · Biomedical applications

1 Introduction

As biologically active pharmacophores, natural compounds made by plants, fungus, bacteria, protozoans, insects, and animals have been identified (Strohl 2000). Natural products have been utilized as herbal remedies for treating infection, inflammation, cancer, and other disorders since ancient times because of their amazing pharmacological effects and wide range of biological activities (Watkins et al. 2015).

In the current pharmaceutical market, approximately 30% of the major pharmaceutical products are based on natural products or their derivatives. A seminal point

J. Wang · H. Chen (✉)

Tianjin Key Laboratory for Modern Drug Delivery and High-Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, China

e-mail: chenhx@tju.edu.cn

in the industrialization of natural products was with the discovery of penicillin in 1928 and its irreplaceable role during World War II (Strohl 2000). Since then, the pharmaceutical business has grown to highly value natural products for their unique qualities, such as their extraordinary chemical variety, chemical and biological capabilities with macromolecular precision, and lower toxicity (Bonifacio et al. 2014). Natural products' primary benefit is the development of unique molecular structures and functions (Beutler 2019). Elucidating the chemical structure of natural products has become a hotspot for researchers. Based on it, an increasing number of new medicines with higher efficacy and fewer side effects could be developed (Bonifacio et al. 2014). In addition, considering the advantages of natural drugs, such as abundant resources, long-term practice, low price, high efficiency and low toxicity, the biological activities of natural products have been widely studied to explore the potential drug candidates for the treatment of diseases, including cancer, diabetes, inflammatory, and microbial diseases (Crittenden et al. 2006; Dahan et al. 2010; Masuelli et al. 2014; Li et al. 2021b).

Despite there are various merits of natural products and so many drugs used today come from them, the research and development on natural product-based drug has declined in the pharmaceutical industry over the past two decades (Beutler 2019). The trend of declination could be due to that most of the pharmaceutical companies have favored using high throughput screening method to identify and prioritize chemical leads from synthetic compound libraries for drug discovery instead of spending a lot of time for extraction, isolation and purification to get monomer compounds (Bonifacio et al. 2014). In addition, a more significant problem with using natural items as medicine is their limited bioavailability, which accounts for the failure of many natural treatments in clinical trials (Watkins et al. 2015). It has been reported that the main active constituents in natural resource, such as flavonoids, polyphenols, tannins and terpenoids have high water-solubility, while owing to their high molecular sizes and inability to cross lipid membranes, these ingredients could not be absorbed into the body and exhibit low bioavailability (Bonifacio et al. 2014; Thilakarathna and Rupasinghe 2013). Additionally, some necessary ingredients are infrequently employed because they are unattractive or incompatible with other ingredients in the formulation (Ajazuddin and Saraf 2010). In order to increase the effectiveness of bioactive natural compounds and shield them from degradation before they reach the target location, there has been an increased effort over the past few decades to construct vehicles for these molecules (Khan et al. 2018).

Natural polymers have been widely applied for pharmaceutical and biomedical purpose due to the unique biodegradability, the high degree of compatibility and biological activity (Gheorghita Puscaselu et al. 2020). Alginates, collagen, and gelatin, which are hydrogel-based natural polymers, are frequently utilized to encapsulate medications for controlled release and targeted administration. Because the hydrogel matrices contain hydrophilic groups like -OH, -COOH, -CONH, -SO₃H, and -CONH₂, these polymers might absorb a lot of water without losing their structural integrity (Andrianov and Payne 1998; Choukaife et al. 2020). Alginates are among them, and research has shown that they have chelating, biocompatible, immunogenic, and mucoadhesive qualities. They are also non-toxic, abundant in

sources, and simple to obtain (Rinaudo 2008; Sun and Tan 2013). Based on these favorable properties, alginates have become the most important biomaterials for drug delivery (Zhang et al. 2021).

Alginates are collections of linear anionic polysaccharides that are obtained from various bacterial strains and brown sea algae (Zhang et al. 2020a). After filtration, the extract can be collected by adding either of the sodium or calcium chlorides for alginate precipitation (Kothale et al. 2020). Alginates are copolymers that are homopolymeric MM or GG blocks and heteropolymeric MG or GM blocks that include different amounts of -D-mannuronic acid (M) and -L-guluronic acid (G) residues linked by 1,4-glycosidic bonds (Zhang et al. 2021; Liew et al. 2006). The occurrence, proportions and distributions of blocks vary in alginates from different sources and influence the properties of the alginate (Jana et al. 2016). Alginate can be easily altered chemically and biochemically to produce a wide variety of alginate derivatives since it possesses a variety of derivable groups on its molecular chains (Pawar and Edgar 2012). The alginates modification mainly includes acetylation, phosphorylation, sulfation, hydrophobic modification etc. Furthermore, some alginates can be modified as attachment of cell signaling molecules, and the covalent crosslinking of alginates can yield matrices with improved mechanical properties (Fig. 1).

Since the alginates can be made in neutral or charged forms, a wide range of chemicals can be used with them (Jain and Bar-Shalom 2014). Alginate and its derivatives have the capacity to conjugate hydrophobic or hydrophilic moieties to increase the effectiveness of their drug encapsulation due to the hydroxyl and carboxyl groups of each G and M residue (Khan et al. 2018). The alginate matrix is highly hydrated as a result of intermolecular interaction, and it can form a highly viscous gelatinous layer that can serve as a drug diffusion barrier to accomplish controlled-release of pharmaceuticals (Liew et al. 2006). Multivalent cations such as Ca^{2+} , Zn^{2+} , Pb^{2+} , Cd^{2+} , Al^{3+} , etc. can initiate cross-linking of alginate under gentle conditions, forming stable reversible gels. Considering the alginate gels can be heat treated without melting, it can be used for the entrapment of sensitive compounds (Jana et al. 2013). Additionally, the hydrogel alginate matrix exhibits pH-dependent swelling behaviors due to its pH responsiveness (shrinks at low pH and swells at higher pH values). Alginates are hence widely applicable for the targeted and regulated distribution of medications in the gastrointestinal (GI) tract (Bakhshi et al. 2017; Reis et al. 2006). Based on the new process technology, combined with the clinical uses of the drugs, alginates can be investigated in a variety of ways, including as a coating material and in controlled-release drug delivery systems such as hydrogels, beads, floating drug delivery systems, microspheres, nanoparticles, nanofibers, micelles, and liposomes (Gheorghita Puscaselu et al. 2020; Jain and Bar-Shalom 2014). Uyen et al. generated a type of curcumin loaded alginate microspheres with controlled size using an emulsification/gelation method (Uyen et al. 2020). Mukhopadhyay et al. synthesized a novel pH responsive chitosan/alginate microparticle for the oral delivery of quercetin to diabetic animals (Mukhopadhyay et al. 2016). In Yang and Chiang's study, a kind of self-emulsified alginate beads (SEABs) were successfully prepared to control the release of

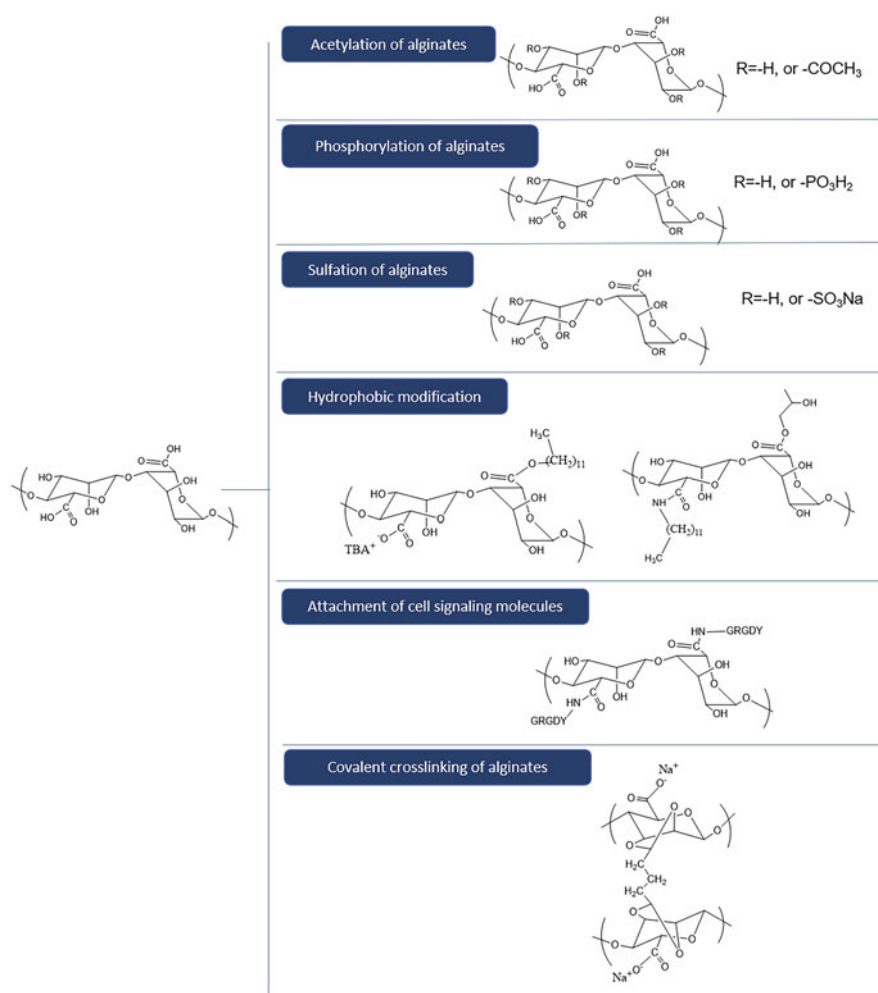


Fig. 1 Representative alginate derivatization structures

γ -oryzanol/algae oil in GI tract (Yang and Chiang 2019). Gamboa et al. used the spray freeze-drying method to create chitosan and alginate nano-composite carriers for targeted colonic administration of prednisolone and inulin (Gamboa et al. 2015). Alginates have been utilized widely in recent years to encapsulate natural materials, such as polysaccharides like pullulan (Xiao and Lim 2018), polyphenols like resveratrol (Saralkar and Dash 2017), alkaloid like caffeine (Morrish et al. 2020), etc. Besides, these alginates encapsulated natural products have been extensively tested and proved to be helpful for the treatment of various diseases, such as tumors, skin inflammations, diabetes, and others (Conti et al. 2013; Abu Bakar et al. 2018; Li et al. 2021b).

In this chapter, we mainly focused on the formation of alginate-based delivery systems containing natural products. The biomedical and pharmaceutical applications of alginates incorporated with bioactive natural ingredients were discussed. Alginate-based delivery strategies for natural bioactive compounds are expected to become more popular in the future. The purpose of this chapter is to offer helpful guidelines for future alginate-based natural product delivery system design and production.

2 Alginate-Based Delivery System of Natural Products

Alginate meets the safety requirements of biocompatibility, biodegradation and toxicity, and has been widely used to incorporate natural products with good encapsulation efficiency and loading capacity, and to deliver the substances to the target site, meanwhile protect the substances from the environment (strong acidity, oxygen, UV light, etc.) and improve their bioavailability (Reig-Vano et al. 2021; Wang et al. 2018; Guedes Silva et al. 2021; Kothale et al. 2020). Alginate hydrogel delivery systems offer good physical and mechanical qualities in particle size, swelling, viscosity, and alginate-based core-shell particles could be utilized to regulate the release of loaded pharmaceuticals, among other benefits of the alginate-based delivery system (Li et al. 2021a). Given the significant advantages of alginate in drug delivery, recent studies aim to investigate various alginate platforms (Fig. 2), such as hydrogels, beads, floating drug delivery system, microspheres, nanoparticles, nanofibers, micelles, liposome, especially those of micro- or nanosized for the delivery of natural products in the treatment of disease (He et al. 2020).

The quick in vitro release of the entrapped substance is a key drawback of alginate as a delivery strategy for low molecular weight natural compounds since the alginate matrices generated are porous. The synthetic gel network has a low density and does not offer the requisite barrier effect even at the highest concentration of alginate aqueous solutions, making it difficult to precisely control the

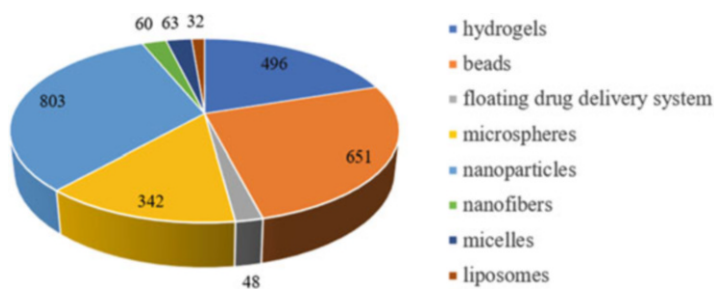


Fig. 2 Articles about alginate-based delivery systems containing natural products published in the last decade

payload's release rate (Crittenden et al. 2006). Therefore, alginate has been modified to improve the targeted therapeutic effect of drugs or combined with other polymers, such as chitosan, gum, pectin or synthetic polymers, like Poly vinyl alcohol (PVA), carboxymethyl cellulose sodium salt (CMC) or Poly-L-Lysine to provide an additional protection measure for drugs and to strengthen the suitable performance qualities of the alginate hydrogels (Matricardi et al. 2008; Belscak-Cvitanovic et al. 2015; Fathi et al. 2014). Table 1 summarized various alginate-based delivery systems containing different bioactive natural products and their applications, respectively.

2.1 Hydrogels

When a polymer absorbs water, it changes state from sol to gel, forming hydrogels (Rafiq et al. 2018). Alginates have excellent gel-forming capacity, especially under the secure and gentle condition, alginate hydrogels could be easily formed. The varied G:M ratios, types of ionic cross-linkers, and concentrations of the alginate solution could affect the mechanical strength and porosity of the produced alginate hydrogels (Cohen et al. 1997). Alginate hydrogels' special qualities have made it possible to employ them as a matrix for the trapping of cells, enzymes, and proteins for applications in bioprocessing, drug delivery, and chemical sensing (Srivastava and Mcshane 2005; Jain and Bar-Shalom 2014). Alginate hydrogels are widely utilized nowadays as delivery systems for regulating the release of a variety of natural compounds, such as caffeine, hesperidin, dandelion polyphenols, etc. (Hadley and Silva 2019; Busic et al. 2018; Zhong et al. 2013).

Due to its quick metabolism in the gastrointestinal tract, caffeine, a well-known natural product with a variety of activities in stimulation of the central nervous system, peripheral vasoconstriction, and relaxation of the smooth muscle, has been extensively researched on its effective delivery systems (McLellan et al. 2005). Belscak-Cvitanovic et al. prepared alginate hydrogel based systems consisting of different natural polysaccharides, pectin, carrageenan, chitosan and psyllium for assessment of the best formulation of immobilization of caffeine to realize the controlled release of caffeine (Belscak-Cvitanovic et al. 2015). The results showed that the psyllium or chitosan combined hydrogel beads with higher sodium alginate concentration would be the optimal carrier systems for encapsulation of caffeine. Nikoo et al. validated the alginate hydrogels coated with chitosan could be employed for the encapsulation of low molecular components and discovered that electrostatic interaction between alginate and chitosan provided an excellent barrier against caffeine leakage (Nikoo et al. 2018). Tsirigotis-Maniecka et al. prepared hesperidin, one of the most advantageous bioflavonoids with various biological activities loaded ALG/CMC hydrogel systems considering the poor water solubility, low oral bio-availability and instability in human gastrointestinal tract of hesperidin (Tsirigotis-Maniecka et al. 2017). The hesperidin encapsulated in double-compartment ALG/CMC hydrogel systems exhibited higher stability in simulated gastric juice

Table 1 Examples of alginate-based delivery systems containing natural products

Delivery system	Alginate and other materials	Substances	Application	References
Hydrogels	Alginate, Sodium carboxymethyl cellulose, CaCO ₃ , Liquid paraffin, Tween 80	Hesperidin	Design a sustained-release delivery system	Tsirigotis-Maniecka et al. (2017)
Hydrogels	Alginate, Cocoa powder, Carob, CaCl ₂	Dandelion polyphenols	Evaluate a potential of new natural fillers for structuring alginate network aimed for encapsulation of dandelion polyphenols	Busic et al. (2018)
Hydrogels	Alginate, Whey protein isolate, CaCl ₂	Linseed oil nanoemulsion (LON)	Fabricate calcium alginate hydrogels filled with linseed oil nanoemulsion	Rahiminezhad et al. (2020)
Hydrogels	Sodium alginate, Poly (D, L-lactic), 18β-glycyrrhetic acid, poly(vinyl alcohol), BSA, CaCl ₂	18 β-glycyrrhetic	Create a novel drug delivery platform that can load and release the compounds concurrently	Zhong et al. (2013)
		Acid and bovine serum albumin		
Beads	Alginate, CaCl ₂ , Eudragit® S-100 (SE)	Curcumin	Exhibit how SE-Cur laden alginate beads might be used to transport drugs that are poorly soluble to the colon	Sookkasem et al. (2015)
Beads	Alginate, CaCl ₂ , Inulin, Arabic gum, chitosan	Tea polyphenols	Study the influence of composition of carriers on encapsulation efficiency and stability of polyphenols	Li et al. (2021c)
Beads	Sodium alginate, Tween 20, CaCl ₂	Astaxanthin	Encapsulation of astaxanthin in calcium alginate beads is a promising way to enhance the stability of Astaxanthin	Lin et al. (2016)
Beads	Alginate, CaCl ₂	γ-oryzanol	Design an effective controlled delivery system for γ-oryzanol	Kai-Min and Chiang (2019)
Floating drug delivery system	Sodium alginate, CaCl ₂ , CaCO ₃ , Acetic acid	Haskap berry extract	Create floating microspheres that can transfer anthocyanins through	Celli et al. (2017)

(continued)

Table 1 (continued)

Delivery system	Alginate and other materials	Substances	Application	References
			gastroretentive systems	
Floating drug delivery system	Alginate, Low density materials (polypropylene foam powder, oils, various solubilizers)	Curcumin	Study the robust alginate floating systems as delivery systems for curcumin that are targeted for the stomach	Treesinchai et al. (2019)
Microspheres	Alginate, Liquid paraffin oil, CaCl ₂	Curcumin	Development of the controlled size curcumin loaded alginate microspheres for in therapeutic delivery via parenteral injection route	Uyen et al. (2020)
Microspheres	Alginate/gelatin, Span 80, Tween 80, Cross-linking agents (EDC/NHS)	Berberine hydrochloride	Design bioadhesive microspheres to increase the oral bioavailability of berberine hydrochloride	Zhang and Liu (2016)
Nanoparticles	Fe ₃ O ₄ Magnetic Nanoparticles, Sodium alginate, Chitosan	Curcumin	Targeted Delivery of Curcumin into Human Breast Cancer Cells	Song et al. (2018)
Nanoparticles	Alginate, Cashew gum, CaCl ₂	Lippia sidoides essential oil	Synthesis of a bio-polymer mixture for essential oil encapsulation	de Oliveira et al. (2014)
Nanoparticles	Sodium alginate, Polysorbate 80, CaCl ₂	Curcumin	Design curcumin loaded alginate nanoparticles to improve the solubility and bioavailability of curcumin	Govindaraju et al. (2019)
Nanoliposome	Soyalecithin and cholesterol, Sodium alginate, CaCl ₂ , Eudragit S100s	Capsaicin	Create liposome-encapsulated alginate beads that are covered in a layer of Eudragit S-100 to deliver capsaicin to the colon	Giri et al. (2017)
Micelles	Oxidized sodium alginate,	Curcumin	Targeted administration of curcumin to hepatocytes via	Sarika et al. (2016)

(continued)

Table 1 (continued)

Delivery system	Alginate and other materials	Substances	Application	References
	modified lactobionic acid		asialoglycoprotein receptor on hepatocytes	
Film	Sodium alginate, CaCl ₂	Ageratum conyzoides extract (ACE)	Design a SA film loaded with ACE to enhance its properties and potential application as wound dressing materials	Abu Bakar et al. (2018)
Film	Pectin, Sodium alginate, Curcumin, CaCl ₂	Curcumin	Study the effect of different drug delivery media on the release rate of curcumin	Pan et al. (2019)
Patch	Sodium alginate, PVA, TiO ₂	Curcumin	Create patches that are antibacterial against both gram-positive and gram-negative bacteria	Selvi et al. (2018)

and pH-triggered release behavior in simulated intestinal juice. The release time of hesperidin in the double-compartment ALG/CMC hydrogel systems could reach to 22 h. In order to study the potential of new plant-derived functional fillers on natural product's efficient delivery. Whey protein isolate (WPI), cocoa powder (CP), and carob (C) were employed by Busic et al. to fortify an alginate hydrogel matrix used to encapsulate an aqueous extract of dandelion leaf (Busic et al. 2018). The prepared alginate hydrogel beads encasing dandelion polyphenols demonstrated extremely effective entrapment of polyphenols (>88%), delayed release of polyphenols in simulated gastrointestinal conditions, and maintained its antioxidant capacity (Busic et al. 2018). Alginate is also thought to be a suitable approach for functional lipids that are rich in polyunsaturated fatty acids. Rahiminezhad et al. fabricated calcium alginate hydrogels filled with linseed oil nanoemulsion (LON) with whey protein isolate. Results showed that less lipid oxidation products were produced in LON and the degradation of alpha-linolenic acid could be delayed after the LON' encapsulation on the alginate beads (Rahiminezhad et al. 2020). Tan et al. created a novel biohybrid microgel with polyelectrolyte complexes (PECs) based on polysaccharides for anthocyanins. The PECs composed of chondroitin sulfate and chitosan were prepared to encapsulate anthocyanins, a natural pigment with high antioxidant activity, by sequential copigmentation and encapsulation technique. The anthocyanins-loaded PECs were further incorporated into alginate microgel particles through emulsification/internal gelation methods. Results showed that adding PECs effectively shielded the microgel from freeze-drying stress and improved its ability for reconstitution after rehydrating. For the regulated delivery of anthocyanins and

other hydrophilic bioactive chemicals, the PECs-inclusive microgels may be a desirable vehicle (Tan et al. 2018).

2.2 Beads

By adding an aqueous solution of sodium alginate drop-by-drop to an aqueous solution of calcium chloride, one can produce alginate beads with predictable form and size (Kim and Lee 1992). The content, sequence structure, and molecular size of the alginate polymers all had a significant impact on the physical characteristics of the alginate beads (Martinsen et al. 1989). The inclusion of acetic acid and hydrochloric acid has a considerable impact on the swelling performance and pH responsiveness of alginate beads (Kothale et al. 2020). Various technologies and additional materials were studied to enable formulated sodium alginate beads to contain appropriate swelling performance, pH responsiveness, and to become an effective drug delivery carrier (Tiwari et al. 2019).

The self-emulsion approach was used to integrate the γ -oryzanol into oil-entrapped alginate beads, improving both its solubility and bioavailability. The self-emulsified alginate beads effectively arrest the γ -oryzanol's release in simulated stomach fluid and showed higher release content in the small intestine condition (Kai-Min and Chiang 2019). The alginate beads with controllable size were prepared by electrospray technique through adjusting the working voltage. The gallic acid, a mononuclear water-soluble phenolic compound was further encapsulated in the alginate beads. Results indicated that the formulated alginate beads could protect gallic acid from autoxidation. Gallic acid release might be affected by alginate bead size and loading amount, and the release in simulated intestinal juice is higher than that in gastric condition (Li et al. 2016). The quinic acid, a food constituent having anti-caries and anti-gingivitis properties, was incorporated with two mucoadhesive polymers, alginate and chitosan by coacervation to form an adhesive microbeads delivery system. Due to its cationic behavior in an acidic environment, such as the oral cavity, chitosan was chosen as the coating material for alginate microbeads. In order to prevent caries and gingivitis, the synthesized microbeads adhesive delivery method demonstrated extended quinic acid release and might be employed as an active ingredient in chewing gum or mouthwash (Conti et al. 2013).

2.3 Floating Drug Delivery System

In order to deliver oral medications to the stomach, particularly for those with a small solubility and absorption window in the stomach or upper small intestine, floating drug delivery systems (FDDS) have been developed. FDDS has a lower bulk density than gastric juice and floats for a long time in the stomach. FDDS could thus be

employed for local therapy of disorders such as peptic ulcer, gastric cancer, and *H. pylori* infection, and so on (Kriangkrai et al. 2014; Sungthongjeen et al. 2008).

Based on a gas-generating system, floating alginate beads with tetrahydrocurcumin (THC) self-emulsifying drug delivery system (SEDDS) were created to increase the solubility of THC and extend its stomach residence period. Results showed these beads presented an increase in the dissolution rate of THC with a longer floating lag time (Sriraksa et al. 2012). Ionotropic gelation was used to create curcumin floating beads with low-density materials such as polypropylene foam powder and oil. And the addition of low-density oils and solubilizers could help to increase the floating properties and curcumin release of beads. The alginate-based curcumin floating system could be used for prolonging the presence of curcumin in the stomach, reducing its degradation and improving its therapeutic effect of gastric diseases (Treesinchai et al. 2019). Celli et al. developed floating alginate microspheres as gastroretentive systems for the oral delivery of anthocyanins (ACNs) existed in haskap berries extracts. Employing calcium carbonate as the gas-generating chemical, the floating microspheres were created utilizing the ionotropic gelation process. The concentration of acetic acid used in the gelation media significantly affects the floatation capacity of the synthesized microspheres. The floating microspheres that were created in the presence of 10% acetic acid could be employed as gastroretentive delivery systems for ACNs to reach their absorption window (Celli et al. 2017).

2.4 Microspheres

Drugs, vaccines, antibiotics, and hormones can be released using microparticles in a regulated manner. A microsphere's large surface area makes it easier to assess the properties of diffusion and mass transfer (Kothale et al. 2020). Microspheres are free-flowing particles with sizes ranging from 1 to 1000 μm that are often made of protein and synthetic polymer. Microparticles have characteristic of large surface area, could resulting in an easier diffusion and transfer (Kothale et al. 2020). Also the microsphere could encapsulate the small molecules drugs and control its release to lessen toxicity and increase the drug's effectiveness.

Berberine hydrochloride (BBH) is an active isoquinoline alkaloid with the treatment of duodenal and benign gastric ulcers caused by bacteria. Due to its poor water-solubility, the BBH was loaded on the chitosan-coated alginate/gelatin microspheres by water-in-oil emulsification technique for its sustained delivery (Zhang and Liu 2016). Lin et al. created chuanxiong-loaded alginate microspheres in order to create a precise dosage approach for natural product oral administration in zebrafish, which is known as a superior animal model for pharmacology studies. The results indicated the release rate of chuanxiong was controlled by the drug loading in simulated intestinal fluid and proved the potential application of alginate as potential platform in natural products' delivery (Lin et al. 2016). Curcumin-loaded alginate microspheres (Cur-AMs) were created using a water-in-oil emulsion process followed by

emulsion droplet gelation. Cur-AMs was sensitive to *S. aureus* ATCC 12600 and resistant to *E. coli* ATCC 25922, and it demonstrated sustained release properties (Uyen et al. 2020). Catechin-loaded alginate microparticles were prepared for efficient sustained release of catechin, a major polyphenolic compound of green tea, through an emulsion gelation method using alginate, oil, and oleic acid ester as an emulsifier. The release of catechin encapsulated in alginate microparticles was sustained under acidic circumstances and increased as the pH of the release medium increased (Kim et al. 2016). In order to create effective and biosafe polymeric carriers for researching the medicinal uses of the bioflavonoid quercetin in antidiabetic research, Mukhopadhyay et al. incorporated quercetin to two polymers, sodium alginate and modified chitosan derivative (succinyl chitosan) by ionic cross-linking. The quercetin-loaded microparticles exhibited pH sensitive properties, resulting in the protective effect of encapsulated quercetin in harsh environment. Toxicity study was performed through oral administration of quercetin-loaded microparticles in rats, and confirmed its safety. An in vivo pharmacological study also demonstrated that oral treatment of quercetin-loaded microparticles had a greater anti-diabetic impact in diabetic rat models than free quercetin (Mukhopadhyay et al. 2016).

2.5 Nanoparticles

Nanoparticles are particulate dispersions or solid particles with sizes ranging from 10 to 1000 nm that can entrap pharmaceuticals or attach them to the nanoparticle's matrix (Severino et al. 2019; Khatami et al. 2018). With the characteristics of small in size and shape, the nanoparticles could easily link with the tissues, cells and delivery the drugs to the target area (Jain et al. 2018).

Govindaraju et al. prepared curcumin-loaded alginate-polysorbate 80 nanoparticles through ionotropic gelation technique and investigated its bioavailability in fit human volunteers. According to the results, curcumin's oral bioavailability improved by five times after being encapsulated, and its maximum plasma levels also dramatically increased (Govindaraju et al. 2019). Curcumin diethyl diglutarate (CDG), a new ester prodrug of curcumin, was synthesized by Sorasitthiyanukarn et al. and encapsulated by chitosan/alginate nanoparticles (CANPs) through o/w emulsification and ionotropic gelation. The enhanced CDG-CANPs increased the digestibility, biocompatibility, and cellular absorption of CDG and had stability for up to 3 months at 4 °C (Sorasitthiyanukarn et al. 2019). In order to encapsulate curcumin more efficiently, hydrophobic alginate derivative, oleate alginate ester (OAE) was developed by the modification of alginate with methyl. Simple sonication was used to create OAE nanoparticles that were loaded with curcumin (Cur-OAE Nps). Results indicated that Cur-OAE Nps exhibit higher aqueous solubility and stability of curcumin and showed slow and continuous cytotoxic effect on MCF-7 cells. The sustained cell absorption of curcumin from Cur-OAE Nps was time- and concentration-dependent (Raja et al. 2015). By using

an ionotropic gelation process, curcumin-loaded alginate-gum Arabic nanoparticles (Cur/ALG-GANPs) were produced. The cytotoxicity assay results showed that Cur/ALG-GANPs had a greater anticancer effect against human liver cancer cells (HepG2) than against colon cancer (HT29), lung cancer (A549), and breast cancer (MCF7) cells (Hassani et al. 2020). Essential oils have been shown to exhibit a variety of biological actions, including antibacterial, antifungal, antioxidant, and anticancer characteristics (Natrajan et al. 2015). However, due to the unstable, volatile and water-insoluble characteristic of essential oils, their applications are limited. Various studies have been focused on the development of drug carrier platform for the encapsulation of essential oils. Through the use of spray-drying technique, alginate/cashew gum nanoparticles were created for the encapsulation of lippiasidoides essential oil, a base oil with antibacterial and antimicrobial properties (de Oliveira et al. 2014). Chitosan-alginate nanocapsules loaded with essential oils (lemongrass and turmeric oil) were synthesized by Natrajan et al. Results showed that the oil-loaded nanocapsules had higher antiproliferative properties in A549 cells than unloaded free oil, and the nanocapsules were hemocompatible, suggesting their potential use for biomedical applications (Natrajan et al. 2015).

2.6 Nanofibers

Nanofibers, one of the common alginate-based nanomaterials, have been attracted for its biomedical and pharmaceutical applications (Mokhena et al. 2020). The process of electrospinning is well recognized for producing nanoscale fibers. The surface area to volume ratio of electrospun nanofibers is higher, allowing for the inclusion of various components.

Scaffolds made of PVA and sodium alginate act remarkably well for the aim of healing wounds (Selvi et al. 2018). Rafiq et al. developed three different essential oils (cinnamon, clove and lavender) loaded sodium alginate/PVA antibacterial nanofibers by electrospinning. The nanofibers with 1.5% cinnamon oil had the greatest zone of inhibition, measuring 2.7 cm. When compared to plain cotton gauze, nanofibrous coated cotton gauze absorbed more fluid and could be used to improve wound dressings in the future (Rafiq et al. 2018). Also, lavender oil, a potent antibacterial and anti-inflammatory natural substance, were incorporated in alginate nanofibers and showed a sustained release for more than 2 days (Hajiali et al. 2016).

2.7 Micelles

Amphiphilic macromolecules that self-assemble into polymer micelles could serve as solubilizers for the delivery of insoluble medicines. Amphiphilic compounds typically form structured nanoscale core-shell structures in aqueous medium when

the concentration surpasses the critical micelle concentration (Lu and Park 2012). The core of the micelle acts as a reservoir for the drug, and the outer shell protects the stability of the colloid from in vivo protein adsorption (Deshmukh et al. 2017). Due to the high hydrophilicity of alginate, it is necessary to modify its structure to make it hydrophobic to form amphiphilic copolymer for micelle preparation (He et al. 2020).

Through the thin-film hydration approach, curcumin-loaded mixed polymeric micelles based on chitosan, alginate, maltodextrin, pluronic F127, pluronic P123, and tween 80 were created (Akbar et al. 2018). The antidiabetic effect of the micelles incorporated with curcumin were further studied in Bisphenol A induced diabetics rats. The results indicated that the synthesized curcumin-based formulations have exhibited advantageous hypoglycemic activity and wound healing capacity as compared to free curcumin (Akbar et al. 2018).

2.8 Liposomes

Liposomes are biocompatible and biodegradable carriers with features similar to biological cell membranes that have been widely exploited for anticancer medication delivery (Bardania et al. 2017). Liposomes are capable of incorporating both hydrophilic and lipophilic medicines, resulting in the unique structure where hydrophobic substances loaded in the bilayer of liposomes and hydrophilic compounds entrapped in the inner aqueous phase (Giri et al. 2017). However, Liposomes are often obtained as suspensions, which are prone to oxidation and hydrolysis during storage (Ghaleshahi and Rajabzadeh 2020). Due to the existence of digestive enzymes and bile salts, the lipid bilayer structure of liposomes could be degraded in the gastrointestinal environment (Karn et al. 2011). The alginate has been reported as coating material for liposomes to protect them during transit. And meanwhile the liposome may shield hydrophilic and lipophilic bioactive substances from environmental degradation (Li et al. 2021a; Balanc et al. 2016). Liposomes were firstly developed based soyalecithin and cholesterol through thin film hydration technique. Capsaicin, an alkaloid of chilli peppers with efficacy on relieving pain, was prepared and loaded in the liposomes, then further encapsulated in alginate hydrogel beads by ionotropic gelation methods, and last coated with anionic polymer Eudragit S-100. Results of in vitro release study showed that Eudragit coated alginate beads can delivery capsaicin through the gastrointestinal tract at a very low rate in the stomach and intestine and prolonged release in colonic region (Giri et al. 2017). Resveratrol, epigallocatechin gallate (EGCG), and pantothenic acid were also added to the liposome, which was subsequently enveloped in alginate particles. Results showed that the alginate matrix-loaded liposome with the aforementioned bioactive components could greatly increase the stability of free medicines (Balanc et al. 2016; Istenic et al. 2016; Ota et al. 2018).

3 Biomedical and Pharmaceutical Applications of Alginates in the Natural Products

Alginate was classified as a GRAS (Generally Recognized as Safe) material by the United States Food and Drug Administration (USFDA) since it has not yet been reported to cause substantial immunological reactions when administered orally (Li et al. 2021a; Agüero et al. 2017). This characterization makes it easier to transfer the research outcomes of alginate based delivery system from bench to bedside (Reig-Vano et al. 2021).

3.1 Oral Drug Delivery

Oral administration systems based on alginate are anticipated to increase the bioavailability of natural compounds, particularly those with low water solubility. Due to the advantageous characteristics of alginate, including bioadhesiveness, mucoadhesiveness and biocompatibility, alginate-based drugs systems can be easily introduced to organisms by oral route of administration (Reig-Vano et al. 2021). The high mucoadhesiveness of alginate also help to prolong its residence time in the gastrointestinal tract. Moreover, due to its ability to affect the swelling level of the alginate gel and the rate at which bioactive substances are released into the gastrointestinal system, alginate's pH responsiveness plays an unrivaled role in the design of alginate-based oral drug delivery (Morrish et al. 2020). Alginate shows shrink in acidic conditions and expands in neutral and weakly alkaline conditions. When the alginate matrix swells, active compounds could diffuse and surface dissolves. Drugs loaded alginate could protect the encapsulated compounds in the stomach, at low pH values, and exhibit sustained release of compounds in intestine (Dhamecha et al. 2019).

A modified double emulsion process was employed to create the calcium alginate microspheres, which had a high encapsulation effectiveness when used to encapsulate Astaxanthin (AST). The synthesized microspheres could protect AST in simulated gastric fluid and significantly release in alkaline solution. Cellular studies indicated that alginate-loaded AST could inhibit HepG2 cells and meanwhile having little harm to normal cells. Its possible mechanism is to repair glucose metabolism abnormality by blocking aerobic glycolysis and increasing the tricarboxylic acid cycle, leading in suppression of HepG2 cell proliferation (Zhang et al. 2020b). The caffeine-loaded whey peptides nanoparticles were added into the alginate and exhibited gastrointestinal protective effect of free caffeine. Because of the alginate's ion-mediated cross-linking, only 15% of the loaded caffeine was released in the simulated gastric condition (Bagheri et al. 2014).

3.2 Targeted Drug Delivery

3.2.1 Cancer-Targeted Drug Delivery

Targeted drug delivery systems for cancer therapy have been developed to enhance therapeutic efficacy with minimized side effects. The targeting characteristics of the system enable the drugs could accumulate at the cancer area and reduce the damage to the healthy cells (He et al. 2020; Hema et al. 2018). One method of delivering hydrophobic medications to their target sites is to embed them in hydrophilic particles such as calcium alginate (Saralkar and Dash 2017). The alginate-based natural product delivery systems for cancer therapy are created based on the primary targeted drug delivery methodologies, such as passive targeting, active targeting, and stimuli-responsive release (Saralkar and Dash 2017).

The enhanced permeation and retention (EPR) effect serves as the foundation for passive targeting strategy (Torchilin 2000). The EPR effect can be achieved through synthesizing nanoparticles with size less than 200 nm. Curcumin and resveratrol are two phytochemicals that present significant anticancer activity. According to numerous research, the combination of curcumin and resveratrol is more effective against hematological malignancies, in vivo lung cancer tests, and in vitro-in vivo head and neck carcinoma investigations (Saralkar and Dash 2017; Kelkel et al. 2010; Liu et al. 2015; Masuelli et al. 2014). Through the use of emulsification and crosslinking technologies, Saralkar et al. created calcium alginate nanoparticles that were loaded with curcumin and resveratrol. The formulation was an ideal candidate to display the EPR effect for passive tumor targeting because the alginate-based nanoparticles were only about 50 nm in size. Cell studies indicated that drug-loaded nanoparticles exhibit higher cytotoxic effects on DU145 cells. It was also discovered that alginate nanoparticles were safe for intravenous delivery (Saralkar and Dash 2017). Active targeting strategy is mainly based on the combination of ligand receptors, antigen antibodies and other forms of molecular recognition to achieve precise distribution to particular cells, tissues, or organs (He et al. 2020; Chattopadhyay et al. 2012; Slavoff and Saghatelian 2012). Sarika et al. prepared galactosylated alginate-curcumin micelle with size in nano meter range by self-assembling for enhanced delivery of curcumin to hepatocytes. The highly hydrophobic curcumin is attached to hydrophilic alginate chain, and the formed amphiphilic structure, resulting in micelles in aqueous medium. The results of cell experiment indicated that the galactose moiety improve the target delivery of curcumin into HepG2 cells via ASGPR mediated endocytosis and promote curcumin cellular uptake, accumulation and cytotoxic effects on HepG2 cells (Sarika et al. 2016). In reaction to changes in the cancer cells' microenvironment, such as those caused by changes in temperature, pH, or a particular ion, stimuli-responsive release strategies typically entail a phase transition (Zhang et al. 2016). For instance, magnetic nanoparticles (MNPs) have been touted as a promising substance for targeted drug delivery, with benefits such as the ability to visualize the targeting process, quick targeting, and magnetic force-induced aggregation of drug carriers at the desired places (Song et al. 2018). MNPs

can be created by precipitating iron ions in a bio-based matrix, resulting in a small size and a large surface area. Water-soluble biopolymers, e.g., alginate and chitosan have been widely used for surface modification of MNPs through polyelectrolyte layer-by-layer deposition (Amani et al. 2019). A novel magneto-responsive nanoplatform was developed by layering MnFe_2O_4 nanoparticles with chitosan and sodium alginate for magnetic controlled release of curcumin, a hydrophobic chemical utilized in cancer treatment. The encapsulated curcumin can be given remotely, and its release can be regulated to specific sites using an alternating magnetic field to optimize efficiency and limit toxicity (Jardim et al. 2018).

3.2.2 Colon-Targeted Drug Delivery

Inflammatory bowel diseases (IBD), which include Crohn's disease, ulcerative colitis, and colon cancer, are chronic autoimmune disorders. Natural products have been extensively researched as prospective treatment agents for IBD due to their high efficacy, low toxicity, and broad spectrum of biological activity (Li et al. 2022; Besednova et al. 2020; Wei et al. 2020). Furthermore, colon-target drug delivery systems have been investigated in order to administer drugs in the colonic region at the appropriate concentrations and at the appropriate time. The optimum oral colon-target drug delivery systems should be able to postpone drug release in the upper GI tract, stomach, and small intestine, then release as much as possible in the lower GI tract (Sookkasem et al. 2015). Due to the advantage of pH sensitivity and remarkable adhesive ability, alginate platforms have been appropriate options for targeting of natural products in the colon area (Dias et al. 2017; Zhang et al. 2017). Because of the presence of carboxylic groups in the alginate structure, alginate has a high pH sensitivity. The carboxylic acid groups are in an insoluble structure in the condition of $\text{pH} < 3.4$, and changed to the ionized form when $\text{pH} > 4.4$, leading to an increase of negative charges. Furthermore the polymer chain expanded and hydrophilic matrix swelled following the increase of electrostatic repulsion, and being highest around $\text{pH} 7.4$ (Dias et al. 2017). Therefore, alginate can block material release in the stomach while achieving slow, swelling-controlled release in simulated small intestinal fluid ($\text{pH} 6.8$). In addition, with the help of bacterial enzymes, the alginate structure was destroyed and leading to local delivery of drugs to the lower intestine (Dahan et al. 2010; Santhanes et al. 2018). Alginate has been extensively used in the design of oral colon-specific medication delivery systems.

To establish the colon-target plug-controlled capsule delivery system, Zhang et al. developed curcumin-loaded alginate beads comprising a self-microemulsifying drug delivery system (SMEDDS). The capsule exhibited a pulsatile drug-release profile with a specified lag period followed by a sustained-release phase (Zhang et al. 2017). Sookkasem et al. developed self-emulsifying curcumin (SE-Cur)-loaded calcium alginate beads through ionotropic gelation and coated the beads with Eudragit® S-100 for colon targeting. The alginate beads successfully prevented curcumin release in simulated stomach and intestinal fluids while instantly released the medication in simulated colonic fluid for more than 60% within 12 h. The

SE-Cur produced by the beads also shown cytotoxicity against human colon cancer cell lines (HT-29) as well as antioxidant properties (Sookkasem et al. 2015). Li et al. structured alginate-based delivery systems consisting of inulin, Arabic gum, or chitosan to select the best formulation for encapsulation of tea polyphenols (TP). The results showed that alginate beads coated with chitosan had the highest encapsulation efficiencies of TP and the most retarded release of TP in both water and simulated gastrointestinal conditions, while alginate beads coated with inulin had the best protection capacity of TP from degradation during storage (Li et al. 2021c).

3.3 *Would Healing*

Alginate-based wound dressing is well known in the literature and commercially in wound treatment (Abu Bakar et al. 2018). Alginate based wound dressing exhibits significant advantage over the conventional wound dressing. Alginate dressing provides a moist wound environment, which aids in wound healing (Kothale et al. 2020). Furthermore, the natural products, with antimicrobial, anti-inflammatory and antioxidant activities loaded on the alginate dressing enable to improve the wound healings.

Abu Bakar et al. prepared *Ageratum conyzoides* extract (ACE) and incorporated it in sodium alginate films. The inclusion of ACE decreased the values of water vapor transmission rates while increasing the swelling degree and stress of the alginate film. Results indicated that the ACE loaded alginate film has potential to be an ideal wound dressing material (Abu Bakar et al. 2018). Pan et al. developed curcumin-pectin film (PT-Curf) by casting method due to the crosslinking mechanism of pectin-calcium. The sodium alginate was then added to the film, resulting in a blending modification system to increase the performance of PT-Curf. The results confirmed that pectin and sodium alginate were suitable carriers for the hydrophobic drug curcumin, that curcumin was uniformly disseminated in the composite films in diverse crystal forms, and that the pectin-sodium alginate curcumin film performs well in fake body fluids (Pan et al. 2019). Selvi et al. added titanium dioxide (TiO_2) into alginate/PVA scaffold, which is useful to enhance its wound healing activity by photo catalytic property. The curcumin was further incorporated into the polymeric patches due to its anti-inflammatory activity. The patches demonstrated antibacterial efficacy against Gram positive and Gram negative microorganisms. Results proved that curcumin loaded TiO_2 /SA/PVA patches have potential application in wound healing (Selvi et al. 2018). Silva et al. studied the impact of several metal crosslinkers on the polyelectrolyte effect and viscosity properties of polyphenol-loaded alginates systems. Stability assays results of different polyphenols (gallic acid, epicatechin and EGCG) in the presence of alginate-gelling cations indicated that Ca^{2+} , Ba^{2+} and Zn^{2+} could be selected as safer ion crosslinkers. Besides, epicatechin-loaded alginates patch was prepared and its activity was tested on an ex-vivo skin model. Results verified that the patch enables to deliver polyphenol with certain concentrations to skin surface with further fully release. The improved

alginate-based technology may provide a multifunctional strategy for regulated polyphenol administration and wound healing (Silva et al. 2020).

4 Conclusion and Perspectives

Due to the various biological activities and extraordinary pharmacological effects, natural products have been used for many years in medicine to treat a variety of disorders such as infection, inflammation, cancer and so on. Many best-selling medicines today are also natural compounds or their derivatives. While, poor water solubility, stability, and bioavailability are still the main problems restricting the clinical application and commercialization of natural products. Alginate is one of the attractive carrier materials for the development of natural product delivery system because of its excellent functional properties such as ion crosslinking, pH sensitivity, biocompatibility and biodegradation. At present, various natural bioactive substance delivery systems based on alginates have been widely established, such as hydrogel, microbead, floating drug delivery system, microsphere, nano particle, nano fiber, micelle, liposome and so on. These alginates-based systems have been shown to dramatically improve natural product's bioavailability and pharmacological activity, as well as permit targeted medication delivery and controlled release. In addition, in order to increase the stability and targeting of the drug delivery system, some other polymers, such as chitosan, carrageenan, gum, pectin, PVA, CMC have been also added to the alginate-based systems.

Sodium alginate is a biomaterial that is used as a medical implant and a device to promote healing during tissue regeneration in the human body, and as a carrier for cells. It is also suitable for application in biosensor designs, medication carriers, and medical imaging materials. Alginates-based delivery systems containing natural products have showed multiple biomedical and pharmaceutical applications. Alginate is often used in the preparation of wound dressing due to it can provide a humid environment for wound healing, and also used in oral drug delivery, targeted drug delivery. Alginate has been extensively designed as a medium for medication delivery in the treatment of all instances of diseases because of the diverse bioactivities and medicinal benefits of natural goods. However, the application of alginates in natural product delivery still have some problems need to be solved. Although most of the research on alginate-based delivery methods is still on the laboratory scale, it has been clearly established that adding alginate helps to increase the efficacy of natural product distribution. Besides, drug targeting also remains a challenge, and the potential toxicity of alginate nanoparticles requires further study, especially for the targeted drugs used to treat humans' chronic diseases. In addition, the single bioactive ingredient with sodium alginate as carrier has been studied extensively, while a single ingredient can no longer meet the needs of complex disease treatment, so the development of a multifunctional drug delivery system of sodium alginate that can simultaneously carry a variety of drugs with different properties is another problem need to be solved.

References

- Abu Bakar AJ, Ghazali CMR, Amin KAM (2018) Sodium alginate/ageratum conyzoides extract film for wound dressing materials. In: Euroinvent International Conference on Innovative Research (ICIR), Iasi, Romania, 2018 May 17–18. IOP Conf Ser Mater Sci Eng 374:012087. <https://doi.org/10.1088/1757-899x/374/1/012087>
- Aguero L, Zaldivar-Silva D, Pena L et al (2017) Alginate microparticles as oral colon drug delivery device: a review. Carbohydr Polym 168:32–43. <https://doi.org/10.1016/j.carbpol.2017.03.033>
- Ajazuddin, Saraf S (2010) Applications of novel drug delivery system for herbal formulations. Fitoterapia 81(7):680–689. <https://doi.org/10.1016/j.fitote.2010.05.001>
- Akbar MU, Zia KM, Akash MSH et al (2018) In-vivo anti-diabetic and wound healing potential of chitosan/alginate/maltodextrin/pluronic-based mixed polymeric micelles: curcumin therapeutic potential. Int J Biol Macromol 120:2418–2430. <https://doi.org/10.1016/j.ijbiomac.2018.09.010>
- Amani S, Mohamadnia Z, Mandavi A (2019) pH-responsive hybrid magnetic polyelectrolyte complex based on alginate/BSA as efficient nanocarrier for curcumin encapsulation and delivery. Int J Biol Macromol 141:1258–1270. <https://doi.org/10.1016/j.ijbiomac.2019.09.048>
- Andrianov AK, Payne LG (1998) Polymeric carriers for oral uptake of microparticulates. Adv Drug Deliv Rev 34(2–3):155–170. [https://doi.org/10.1016/s0169-409x\(98\)00038-6](https://doi.org/10.1016/s0169-409x(98)00038-6)
- Bagheri L, Madadlou A, Yarmand M et al (2014) Spray-dried alginate microparticles carrying caffeine-loaded and potentially bioactive nanoparticles. Food Res Int 62:1113–1119. <https://doi.org/10.1016/j.foodres.2014.05.040>
- Bakhshi M, Ebrahimi F, Nazarian S et al (2017) Nano-encapsulation of chicken immunoglobulin (IgY) in sodium alginate nanoparticles: in vitro characterization. Biologicals 49:69–75. <https://doi.org/10.1016/j.biologicals.2017.06.002>
- Balanc B, Trifkovic K, Dordevic V et al (2016) Novel resveratrol delivery systems based on alginate-sucrose and alginate-chitosan microbeads containing liposomes. Food Hydrocolloids 61:832–842. <https://doi.org/10.1016/j.foodhyd.2016.07.005>
- Bardania H, Tarvirdipour S, Dorkoosh F (2017) Liposome-targeted delivery for highly potent drugs. Artif Cell Nanomed Biotechnol 45(8):1478–1489. <https://doi.org/10.1080/21691401.2017.1290647>
- Belscak-Cvitanovic A, Komes D, Karlovic S et al (2015) Improving the controlled delivery formulations of caffeine in alginate hydrogel beads combined with pectin, carrageenan, chitosan and psyllium. Food Chem 167:378–386. <https://doi.org/10.1016/j.foodchem.2014.07.011>
- Besednova NN, Zaporozhets TS, Kuznetsova TA et al (2020) Extracts and marine algae polysaccharides in therapy and prevention of inflammatory diseases of the intestine. Mar Drugs 18(6):289
- Beutler JA (2019) Natural products as a foundation for drug discovery. Curr Protoc Pharmacol 86(1):e67. <https://doi.org/10.1002/cpph.67>
- Bonifacio BV, da Silva PB, Ramos MAD et al (2014) Nanotechnology-based drug delivery systems and herbal medicines: a review. Int J Nanomedicine 9:1–15. <https://doi.org/10.2147/ijn.S52634>
- Basic A, Belscak-Cvitanovic A, Cebin AV et al (2018) Structuring new alginate network aimed for delivery of dandelion (*Taraxacum officinale* L.) polyphenols using ionic gelation and new filler materials. Food Res Int 111:244–255. <https://doi.org/10.1016/j.foodres.2018.05.034>
- Celli GB, Ghanem A, Brooks MS (2017) Development and evaluation of floating alginate microspheres for oral delivery of anthocyanins—a preliminary investigation. Food Sci Nutr 5(3): 713–721. <https://doi.org/10.1002/fsn3.451>
- Chattopadhyay N, Fonge H, Cai Z et al (2012) Role of antibody-mediated tumor targeting and route of administration in nanoparticle tumor accumulation in vivo. Mol Pharm 9(8):2168–2179. <https://doi.org/10.1021/mp300016p>
- Choukaife H, Doolaanea AA, Alfatama M (2020) Alginate nanoformulation: influence of process and selected variables. Pharmaceuticals 13(11):335. <https://doi.org/10.3390/ph13110335>
- Cohen S, Lobel E, Trevoda A et al (1997) A novel in situ-forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. J Control Release 44(2–3):201–208

- Conti B, Colzani B, Papetti A et al (2013) Adhesive microbeads for the targeting delivery of anticaries agents of vegetable origin. *Food Chem* 138(2–3):898–904. <https://doi.org/10.1016/j.foodchem.2012.11.097>
- Crittenden R, Weerakkody R, Sanguansri L et al (2006) Synbiotic microcapsules that enhance microbial viability during nonrefrigerated storage and gastrointestinal transit. *Appl Environ Microbiol* 72(3):2280–2282. <https://doi.org/10.1128/AEM.72.3.2280-2282.2006>
- Dahan A, Amidon GL, Zimmermann EM (2010) Drug targeting strategies for the treatment of inflammatory bowel disease: a mechanistic update. *Expert Rev Clin Immunol* 6(4):543–550. <https://doi.org/10.1586/eci.10.30>
- De Oliveira EF, Paula HCB, de Paula RCM (2014) Alginate/cashew gum nanoparticles for essential oil encapsulation. *Colloids Surface B Biointerfaces* 113:146–151. <https://doi.org/10.1016/j.colsurfb.2013.08.038>
- Deshmukh AS, Chauhan PN, Noolvi MN et al (2017) Polymeric micelles: basic research to clinical practice. *Int J Pharm* 532(1):249–268. <https://doi.org/10.1016/j.ijpharm.2017.09.005>
- Dhamecha D, Movsas R, Sano U et al (2019) Applications of alginate microspheres in therapeutics delivery and cell culture: past, present and future. *Int J Pharm* 569:118627
- Fathi M, Martín Á, McClements DJ (2014) Nanoencapsulation of food ingredients using carbohydrate based delivery systems. *Trends Food Sci Tech* 39(1):18–39. <https://doi.org/10.1016/j.tifs.2014.06.007>
- Gamboa A, Araujo V, Caro N et al (2015) Spray freeze-drying as an alternative to the ionic gelation method to produce chitosan and alginate nano-particles targeted to the colon. *J Pharm Sci* 104(12):4373–4385. <https://doi.org/10.1002/jps.24617>
- Ghaleshahi AZ, Rajabzadeh G (2020) The influence of sodium alginate and genipin on physico-chemical properties and stability of WPI coated liposomes. *Food Res Int* 130:108966. <https://doi.org/10.1016/j.foodres.2019.108966>
- Gheorghita Puscaselu R, Lobiuc A, Dimian M et al (2020) Alginate: from food industry to biomedical applications and management of metabolic disorders. *Polymers* 12(10):2417. <https://doi.org/10.3390/polym12102417>
- Giri TK, Bhowmick S, Maity S (2017) Entrapment of capsaicin loaded nanoliposome in pH responsive hydrogel beads for colonic delivery. *J Drug Deliv Sci Technol* 39:417–422. <https://doi.org/10.1016/j.jddst.2017.05.002>
- Govindaraju R, Karki R, Chandrashekarappa J et al (2019) Enhanced water dispersibility of curcumin encapsulated in alginate-polysorbate 80 nano particles and bioavailability in healthy human volunteers. *Pharm Nanotechnol* 7(1):39–56. <https://doi.org/10.2174/2211738507666190122121242>
- Guedes Silva KC, Feltre G, Dupas Hubinger M et al (2021) Protection and targeted delivery of β -carotene by starch-alginate-gelatin emulsion-filled hydrogels. *J Food Eng* 290:110205
- Hadley DJ, Silva EA (2019) Thaw-induced gelation of alginate hydrogels for versatile delivery of therapeutics. *Ann Biomed Eng* 47(8):1701–1710
- Hajiali H, Summa M, Russo D et al (2016) Alginate-lavender nanofibers with antibacterial and anti-inflammatory activity to effectively promote burn healing. *J Mater Chem B* 4(9):1686–1695
- Hassani A, Mahmood S, Enezei HH et al (2020) Formulation, characterization and biological activity screening of sodium alginate-gum Arabic nanoparticles loaded with curcumin. *Molecules* 25(9):2244. <https://doi.org/10.3390/molecules25092244>
- He LL, Shang ZH, Liu HM et al (2020) Alginate-based platforms for cancer-targeted drug delivery. *Biomed Res Int* 2020:1487259. <https://doi.org/10.1155/2020/1487259>
- Hema S, Thambiraj S, Shankaran DR et al (2018) Nanoformulations for targeted drug delivery to prostate cancer: an overview. *J Nanosci Nanotechnol* 18(8):5171–5191
- Istenic K, Korosec RC, Ulrich NP (2016) Encapsulation of (–)-epigallocatechin gallate into liposomes and into alginate or chitosan microparticles reinforced with liposomes. *J Sci Food Agr* 96(13):4623–4632. <https://doi.org/10.1002/jsfa.7691>
- Jain A, Kumari R, Tiwari A et al (2018) Nanocarrier based advances in drug delivery to tumor: an overview. *Curr Drug Targets* 19(13):1498–1518

- Jain D, Bar-Shalom D (2014) Alginate drug delivery systems: application in context of pharmaceutical and biomedical research. *Drug Dev Ind Pharm* 40(12):1576–1584
- Jana S, Das A, Nayak AK et al (2013) Aceclofenac-loaded unsaturated esterified alginate/gellan gum microspheres: in vitro and in vivo assessment. *Int J Biol Macromol* 57:129–137
- Jana S, Sen KK, Gandhi A (2016) Alginate based nanocarriers for drug delivery applications. *Curr Pharm Design* 22:3399–3410
- Jardim KV, Francisco Palomec-Garfias A, Garcia Andrade BY et al (2018) Novel magneto-responsive nanoplatforms based on MnFe_2O_4 nanoparticles layer-by-layer functionalized with chitosan and sodium alginate for magnetic controlled release of curcumin. *Mat Sci Eng C Mater Biol Appl* 92:184–195. <https://doi.org/10.1016/j.msec.2018.06.039>
- Kai-Min Y, Chiang P-Y (2019) Preparation and evaluation of release formulation of γ -oryzanol/algae oil self-emulsified with alginate beads. *Mar Drugs* 17(3):156
- Karn PR, Vanić Z, Pepić I et al (2011) Mucoadhesive liposomal delivery systems: the choice of coating material. *Drug Dev Ind Pharm* 37(4):482–488. <https://doi.org/10.3109/03639045.2010.523425>
- Kelkel M, Jacob C, Dicato M et al (2010) Potential of the dietary antioxidants resveratrol and curcumin in prevention and treatment of hematologic malignancies. *Molecules* 15(10):7035–7074. <https://doi.org/10.3390/molecules15107035>
- Khan W, Abtew E, Modani S et al (2018) Polysaccharide Based Nanoparticles. *Isr J Chem* 58(12):1315–1329. <https://doi.org/10.1002/ijch.201800051>
- Khatami M, Alijani HQ, Sharifi I (2018) Biosynthesis of bimetallic and core-shell nanoparticles: their biomedical applications-a review. *IET Nanobiotechnol* 12(7):879–887. <https://doi.org/10.1049/iet-nbt.2017.0308>
- Kim CK, Lee EJ (1992) The controlled release of blue dextran from alginate beads. *Int J Pharm* 79(1):11–19
- Kim ES, Lee J-S, Lee HG (2016) Calcium-alginate microparticles for sustained release of catechin prepared via an emulsion gelation technique. *Food Sci Biotechnol* 25(5):1337–1343. <https://doi.org/10.1007/s10068-016-0210-8>
- Kothale D, Verma U, Dewangan N et al (2020) Alginate as promising natural polymer for pharmaceutical, food, and biomedical applications. *Curr Drug Deliv* 17(9):755–775. <https://doi.org/10.2174/1567201817666200810110226>
- Kriangkrai W, Puttipipatkachorn S, Srimornsak P et al (2014) Impact of anti-tacking agents on properties of gas-entrapped membrane and effervescent floating tablets. *AAPS PharmSciTech* 15(6):1357–1369
- Li D, Wei Z, Xue C (2021a) Alginate-based delivery systems for food bioactive ingredients: an overview of recent advances and future trends. *Compr Rev Food Sci Food Saf* 20(6):5345–5369. <https://doi.org/10.1111/1541-4337.12840>
- Li J, Kim SY, Chen X et al (2016) Calcium-alginate beads loaded with gallic acid: preparation and characterization. *Lwt-Food Sci Technol* 68:667–673. <https://doi.org/10.1016/j.lwt.2016.01.012>
- Li J, Wu H, Jiang K et al (2021b) Alginate calcium microbeads containing chitosan nanoparticles for controlled insulin release. *Appl Biochem Biotech* 193(2):463–478. <https://doi.org/10.1007/s12010-020-03420-9>
- Li Q, Duan M, Hou D et al (2021c) Fabrication and characterization of Ca(II) -alginate-based beads combined with different polysaccharides as vehicles for delivery, release and storage of tea polyphenols. *Food Hydrocoll* 112:106274. <https://doi.org/10.1016/j.foodhyd.2020.106274>
- Li Q, Li LY, Li QQ et al (2022) Influence of natural polysaccharides on intestinal microbiota in inflammatory bowel diseases: an overview. *Foods* 11(8):1084. <https://doi.org/10.3390/foods11081084>
- Liew CV, Chan LW, Ching AL et al (2006) Evaluation of sodium alginate as drug release modifier in matrix tablets. *Int J Pharm* 309(1–2):25–37. <https://doi.org/10.1016/j.ijpharm.2005.10.040>
- Lin L-J, Chiang C-J, Chao Y-P et al (2016) Development of alginate microspheres containing chuanxiong for oral administration to adult zebrafish. *Biomed Res Int* 2016:4013071. <https://doi.org/10.1155/2016/4013071>

- Liu Y, Wu YM, Yu Y et al (2015) Curcumin and resveratrol in combination modulate drug-metabolizing enzymes as well as antioxidant indices during lung carcinogenesis in mice. *Hum Exp Toxicol* 34(6):620–627. <https://doi.org/10.1177/0960327114551396>
- Lu Y, Park K (2012) Polymeric micelles and alternative nanonized delivery vehicles for poorly soluble drugs. *Int J Pharm* 453(1):198–214
- Martinsen A, Skjak-Braek G, Smidsrod O (1989) Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate gel beads. *Biotechnol Bioeng* 33(1):79–89. <https://doi.org/10.1002/bit.260330111>
- Masuelli L, Di Stefano E, Fantini M et al (2014) Resveratrol potentiates the in vitro and in vivo anti-tumoral effects of curcumin in head and neck carcinomas. *Oncotarget* 5(21):10745–10762. <https://doi.org/10.18632/oncotarget.2534>
- Matricardi P, Meo CD, Coviello T et al (2008) Recent advances and perspectives on coated alginate microspheres for modified drug delivery. *Expert Opin Drug Del* 5(4):417–425. <https://doi.org/10.1517/17425247.5.4.417>
- McLellan TM, Kamimori GH, Voss DM et al (2005) Caffeine maintains vigilance and improves run times during night operations for special forces. *Aviat Space Environ Med* 76(7I):647–654
- Mokhena TC, Mochane MJ, Mtibe A et al (2020) Electrospun alginate nanofibers toward various applications: a review. *Materials (Basel)* 13(4):934. <https://doi.org/10.3390/ma13040934>
- Morrish C, Teimouri S, Istivan T et al (2020) Molecular characterisation of hot moulded alginate gels as a delivery vehicle for the release of entrapped caffeine. *Food Hydrocoll* 109(2):106142
- Mukhopadhyay P, Maity S, Chakraborty S et al (2016) Oral delivery of quercetin to diabetic animals using novel pH responsive carboxypropionylated chitosan/alginate microparticles. *RSC Adv* 6(77):73210–73221. <https://doi.org/10.1039/c6ra12491g>
- Natrajan D, Srinivasan S, Sundar K et al (2015) Formulation of essential oil-loaded chitosan-alginate nanocapsules. *J Food Drug Anal* 23(3):560–568. <https://doi.org/10.1016/j.jfda.2015.01.001>
- Nikoo AM, Kakhodaee R, Ghorani B et al (2018) Electrospray-assisted encapsulation of caffeine in alginate microhydrogels. *Int J Biol Macromol* 116:208–216. <https://doi.org/10.1016/j.ijbiomac.2018.04.167>
- Ota A, Istenic K, Skrt M et al (2018) Encapsulation of pantothenic acid into liposomes and into alginate or alginate-pectin microparticles loaded with liposomes. *J Food Eng* 229:21–31. <https://doi.org/10.1016/j.jfoodeng.2017.06.036>
- Pan J, Cao D, Ma X et al (2019) Preparation, characterization and in vitro release properties of pectin-based curcumin film. *Korean J Chem Eng* 36(5):822–827. <https://doi.org/10.1007/s11814-019-0238-6>
- Pawar SN, Edgar KJ (2012) Alginate derivatization: a review of chemistry, properties and applications. *Biomaterials* 33(11):3279–3305. <https://doi.org/10.1016/j.biomaterials.2012.01.007>
- Rafiq M, Hussain T, Abid S et al (2018) Development of sodium alginate/PVA antibacterial nanofibers by the incorporation of essential oils. *Mater Res Express* 5(3):035007. <https://doi.org/10.1088/2053-1591/aab0b4>
- Rahiminezhad Z, Gahruei HH, Esteghlal S et al (2020) Oxidative stability of linseed oil nano-emulsions filled in calcium alginate hydrogels. *Lwt-Food Sci Technol* 127:109392. <https://doi.org/10.1016/j.lwt.2020.109392>
- Raja MA, Liu C, Huang Z (2015) Nanoparticles based on oleate alginate ester as curcumin delivery system. *Curr Drug Deliv* 12(5):613–627. <https://doi.org/10.2174/1567201812666150511095029>
- Reig-Vano B, Tylkowski B, Montane X et al (2021) Alginate-based hydrogels for cancer therapy and research. *Int J Biol Macromol* 170:424–436. <https://doi.org/10.1016/j.ijbiomac.2020.12.161>
- Reis CP, Neufeld RJ, Vilela S et al (2006) Review and current status of emulsion/dispersion technology using an internal gelation process for the design of alginate particles. *J Microencapsul* 23(3):245–257. <https://doi.org/10.1080/02652040500286086>

- Rinaudo M (2008) Main properties and current applications of some polysaccharides as biomaterials. *Polym Int* 57(3):397–430. <https://doi.org/10.1002/pi.2378>
- Santhanes D, Teng LY, Sheng FS et al (2018) Exploiting the versatility of oral capsule formulations based on high M-alginate for targeted delivery of poorly water soluble drugs to the upper and lower GI tract. *J Drug Deliv Sci Technol* 46:384–391. <https://doi.org/10.1016/j.jddst.2018.05.024>
- Saralkar P, Dash AK (2017) Alginate nanoparticles containing curcumin and resveratrol: preparation, characterization, and in vitro evaluation against DU145 prostate cancer cell line. *AAPS PharmSciTech* 18(7):2814–2823. <https://doi.org/10.1208/s12249-017-0772-7>
- Sarika PR, James NR, Kumar APR et al (2016) Galactosylated alginate-curcumin micelles for enhanced delivery of curcumin to hepatocytes. *Int J Biol Macromol* 86:1–9. <https://doi.org/10.1016/j.ijbiomac.2016.01.037>
- Selvi RT, Prasanna APS, Niranjana R et al (2018) Metal oxide curcumin incorporated polymer patches for wound healing. *Appl Surf Sci* 449:603–609. <https://doi.org/10.1016/j.apsusc.2018.01.143>
- Severino P, da Silva CF, Andrade LN et al (2019) Alginate nanoparticles for drug delivery and targeting. *Curr Pharm Design* 25(11):1312–1334. <https://doi.org/10.2174/1381612825666190425163424>
- Silva J, Vanat P, Marques-da-Silva D et al (2020) Metal alginates for polyphenol delivery systems: studies on crosslinking ions and easy-to-use patches for release of protective flavonoids in skin. *Bioact Mater* 5(3):447–457. <https://doi.org/10.1016/j.bioactmat.2020.03.012>
- Slavoff SA, Saghatelian A (2012) Discovering ligand-receptor interactions. *Nat Biotechnol* 30(10):959–961. <https://doi.org/10.1038/nbt.2373>
- Song W, Su X, Gregory DA et al (2018) Magnetic alginate/chitosan nanoparticles for targeted delivery of curcumin into human breast cancer cells. *Nano* 8(11):907. <https://doi.org/10.3390/nano8110907>
- Sookkasem A, Chatpun S, Yuenyongsawad S et al (2015) Alginate beads for colon specific delivery of self-emulsifying curcumin. *J Drug Deliv Sci Technol* 29:159–166. <https://doi.org/10.1016/j.jddst.2015.07.005>
- Sorasithyanukarn FN, Bhuket PRN, Muangnoi C et al (2019) Chitosan/alginate nanoparticles as a promising carrier of novel curcumin diethyl diglutarate. *Int J Biol Macromol* 131:1125–1136. <https://doi.org/10.1016/j.ijbiomac.2019.03.120>
- Sriraksa S, Sermkaew N, Setthacheewakul S et al (2012) Floating alginate beads as carriers for self-emulsifying system containing tetrahydrocurcumin. *Biomater Appl* 506:517–520
- Srivastava R, Mcshane MJ (2005) Application of self-assembled ultra-thin film coatings to stabilize macromolecule encapsulation in alginate microspheres. *J Microencapsul* 22(4):397–411
- Strohl WR (2000) The role of natural products in a modern drug discovery program. *Drug Discov Today* 5(2):39–41. [https://doi.org/10.1016/s1359-6446\(99\)01443-9](https://doi.org/10.1016/s1359-6446(99)01443-9)
- Sun J, Tan H (2013) Alginate-based biomaterials for regenerative medicine applications. *Materials* 6(4):1285–1309. <https://doi.org/10.3390/ma6041285>
- Sunghthongjeen S, Sriamornsak P, Puttipipatkachorn S (2008) Design and evaluation of floating multi-layer coated tablets based on gas formation. *Eur J Pharm Biopharm* 69(1):255–263
- Tan C, Celli GB, Lee M et al (2018) Polyelectrolyte complex inclusive biohybrid microgels for tailoring delivery of copigmented anthocyanins. *Biomacromolecules* 19(5):1517–1527. <https://doi.org/10.1021/acs.biomac.8b00352>
- Thilakarathna SH, Rupasinghe HPV (2013) Flavonoid bioavailability and attempts for bioavailability enhancement. *Nutrients* 5(9):3367–3387. <https://doi.org/10.3390/nu5093367>
- Tiwari A, Jain A, Verma A et al (2019) Alginate-based composites in drug delivery application. In: Hasnain MS, Nayak AK (eds) *Alginates: versatile polymers in biomedical applications and therapeutics*. Apple Academic Press, Oakville, pp 457–482
- Torchilin VP (2000) Drug targeting. *Eur J Pharm Sci Suppl* 11(2):S81–S91. [https://doi.org/10.1016/S0928-0987\(00\)00166-4](https://doi.org/10.1016/S0928-0987(00)00166-4)

- Treesinchai S, Puttipipatkachorn S, Pitaksute T et al (2019) Development of curcumin floating beads with low density materials and solubilizers. *J Drug Deliv Sci Technol* 51:542–551
- Tsirigotis-Maniecka M, Gancarz R, Wilk KA (2017) Polysaccharide hydrogel particles for enhanced delivery of hesperidin: fabrication, characterization and in vitro evaluation. *Colloids Surface A* 532:48–56. <https://doi.org/10.1016/j.colsurfa.2017.07.001>
- Uyen NTT, Hamid ZAA, Thi LA et al (2020) Synthesis and characterization of curcumin loaded alginate microspheres for drug delivery. *J Drug Deliv Sci Technol* 58:1–12. <https://doi.org/10.1016/j.jddst.2020.101796>
- Wang B, Akanbi TO, Agyei D et al (2018) Coacervation technique as an encapsulation and delivery tool for hydrophobic biofunctional compounds. In: Grumezescu AM, Holban AM (eds) *Role of materials science in food bioengineering*. Academic, London, pp 235–261. <https://doi.org/10.1016/B978-0-12-811448-3.00007-3>
- Watkins R, Wu L, Zhang C et al (2015) Natural product-based nanomedicine: recent advances and issues. *Int J Nanomedicine* 10:6055–6074. <https://doi.org/10.2147/ijn.S92162>
- Wei N, Xca B, Rxab C et al (2020) Polysaccharides from natural resources exhibit great potential in the treatment of ulcerative colitis: a review. *Carbohydr Polym* 254:117189
- Xiao Q, Lim LT (2018) Pullulan-alginate fibers produced using free surface electrospinning. *Int J Biol Macromol* 112:809–817. <https://doi.org/10.1016/j.ijbiomac.2018.02.005>
- Yang KM, Chiang P-Y (2019) Preparation and evaluation of release formulation of γ -oryzanol/algae oil self-emulsified with alginate beads. *Mar Drugs* 17(156):1–10
- Zhang CG, Wang WX, Zhao XM et al (2020a) Preparation of alginate oligosaccharides and their biological activities in plants: a review. *Carbohydr Res* 494:108056. <https://doi.org/10.1016/j.carres.2020.108056>
- Zhang HT, Cheng JQ, Ao Q (2021) Preparation of alginate-based biomaterials and their applications in biomedicine. *Mar Drugs* 19(5):264. <https://doi.org/10.3390/md19050264>
- Zhang X, Li W, Dou X et al (2020b) Astaxanthin encapsulated in biodegradable calcium alginate microspheres for the treatment of hepatocellular carcinoma in vitro. *Appl Biochem Biotech* 191(2):511–527. <https://doi.org/10.1007/s12010-019-03174-z>
- Zhang X, Yang X, Ji J et al (2016) Tumor targeting strategies for chitosan-based nanoparticles. *Colloids Surface B Biointerfaces* 148:460–473
- Zhang Y, Bai Y, Chen H et al (2017) Preparation of a colon-specific sustained-release capsule with curcumin-loaded SMEDDS alginate beads. *RSC Adv* 7(36):22280–22285. <https://doi.org/10.1039/c6ra27693h>
- Zhang Y, Liu H (2016) Development of bioadhesive microspheres for oral bioavailability enhancement of berberine hydrochloride. *Int J Polym Sci* 2016:1–7. <https://doi.org/10.1155/2016/4235832>
- Zhong D, Liu Z, Xie S et al (2013) Study on poly(D,L-lactic) microspheres embedded in calcium alginate hydrogel beads as dual drug delivery systems. *J Appl Polym Sci* 129(2):767–772. <https://doi.org/10.1002/app.38797>

Alginate in Cancer Therapy



Vikas, Abhishesh Kumar Mehata, Chandrasekhar Singh,
Ankit Kumar Malik, Aseem Setia, and Madaswamy S. Muthu

Abstract According to WHO World Health Organization, Cancer <https://www.who.int/news-room/fact-sheets/detail/cancer> (2020), cancer is the major cause of death worldwide, with more than 10 million fatalities. The most prevalent malignancies are breast, lung, colorectal, and prostate. There are many drugs that have been used to treat various forms of cancer. However, practically all medications are cytotoxic, meaning they can harm healthy cells with cancerous cells, resulting in significant non-specific toxicity. For cancer treatment, it is necessary to deliver the anti-neoplastic drug in optimal concentration in tumor cells. Alginate, chitosan, gelatin, and hyaluronic acid are biodegradable polymers frequently used to deliver anticancer treatments to improve efficacy while minimizing side effects. According to the current research, alginate is a biocompatible, non-toxic, and relatively cost-effective natural polysaccharide with physicochemical properties that make it acceptable for cancer medicine delivery vehicles. Alginate-based drug delivery systems can be used with passive and active targeted techniques to administer anticancer drugs. In addition, the function of alginate in 3-D scaffolds for the cell-culture techniques, including cytotoxicity and internalization studies were discussed. Theranostic applications of alginate-based nanomedicine were also investigated in order to examine cancer diagnostics and therapy. This chapter compiles with the roles of alginate and its drug-delivery systems and their significance in cancer research and treatment.

Keywords Alginate · Anti-cancer drugs · Theranostic nanomedicine · 3D scaffold

Vikas · A. K. Mehata · C. Singh · A. K. Malik · A. Setia · M. S. Muthu (✉)
Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology
(BHU), Varanasi, Uttar Pradesh, India
e-mail: msmuthu.phe@itbhu.ac.in

1 Introduction

Cancer is a group of disorders characterized by the uncontrolled division of aberrant tumor cells (Matthews et al. 2022). When we consider the magnitude and complexity of cancer's diversity, it is easy to become overwhelmed (Hanahan 2022). The primary target of any cancer treatment is to slow down tumor development, control metastases, and prevent recurrence following eradication, all while extending the patient's survival. Surgery, chemotherapy, and radiotherapy are standard cancer treatment options (Sala et al. 2022; Simpson and Scholefield 2008). Each treatment method has its own set of limitations, and none of them is sufficient to produce satisfactory therapeutic results (Demaria et al. 2017; Vakalopoulos et al. 2015; Wirsdörfer et al. 2018). Because tumor lesions are strongly connected with normal body tissues, their full eradication is nearly impossible in most circumstances. Chemotherapy has a limited role in cancer treatment due to drug resistance and the adverse side effects of chemotherapeutic medications accumulating in normal tissue. Hypoxic tumors are showing resistance to radiotherapy and it is linked to radiation-induced adverse effects (Brown and Wilson 2004). Nanotechnology is gaining popularity due to its effectiveness in the diagnosis and treatment of many types of cancer tumors (Duo et al. 2022; Jadid et al. 2021; Tang et al. 2021). Increasing the solubility of drugs in the aqueous phase and can improve the pharmacodynamic and pharmacokinetic aspects of medications in nanocarriers for cancer therapy. Nanocarriers improve medication stability by lowering drug concentration in normal (non-targeted) tissues while slowing drug breakdown in circulation and enhancing drug accumulation in malignant tissues. Nanotechnology-based drug delivery can be produced using a polymeric, lipidic and metallic material such as alginate, chitosan, polyethylene glycol, silver nitrate, etc. Polymeric nanocarriers were chosen based on their distinct physicochemical properties. Both synthetic and naturally derived polymeric materials can be used to make nanocarriers. Natural and manufactured nanoparticles each have their own set of benefits and drawbacks. Natural polymeric molecules have vital properties such as biodegradability, biocompatibility, hydrophilicity, high stability, and non-toxicity.

Furthermore, after in-vivo biodegradation, no hazardous by-products are formed. Recently, alginate has gained a lot of attention as a carrier in polymeric and nanocarriers (Jana et al. 2016; Vikas et al. 2021). This book chapter reviews the literature on alginate and its various synthesized forms in the context of drug delivery systems and their functions in cancer research, diagnostics, and therapy.

2 Molecular Structure and Properties of Sodium-Alginate

Alginates, a naturally occurring anionic polymer, can be obtained from two different algae (majorly from *Laminaria hyperborean*, *Ascophyllum nodosum*, etc. and a minimal degree from *Laminaria digitate*, *Laminaria japonica*, etc.) and bacteria

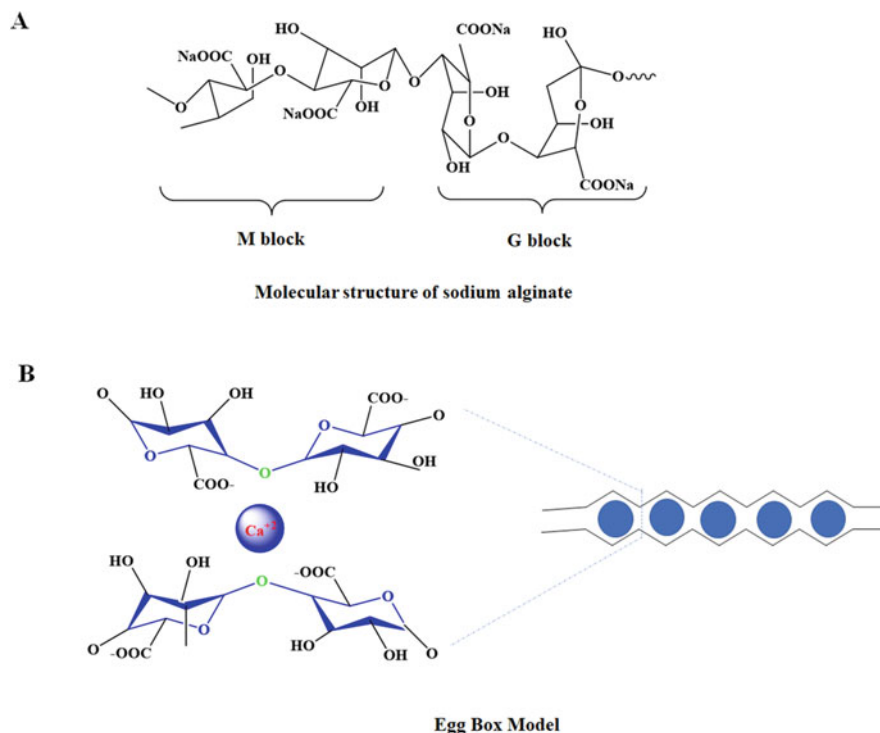


Fig. 1. (A) Molecular structure of the sodium alginate (B) Egg box structure of the calcium alginate complex

(mucoid strains of *Pseudomonas aeruginosa* or *Azotobacter vinelii*). Bacterial alginates differ from algal alginates in that they contain O-acetyl groups that are not found in the architecture of algal alginates, and they have larger molecular masses than algal polymers. These differences in molecular mass can be due to the origin and source of alginate, as well as the sequencing and co-polymer makeup (Mehata et al. 2022). Alginate has been widely studied and employed in a variety of biological applications (Rizg et al. 2022; Tan and Takeuchi 2007). Alginate is an unbranched polysaccharide made up of repeated units of -D-mannuronic acid (M) and -L-guluronic acid (G) linked by a 1–4 linkage (Fig. 1). It contains MMM and GGG chain homosequences interleaved with MGM heterosequences. Alginate makes gel when sodium ions in guluronic acids are replaced by divalent cations like Ca^{2+} (which work as cross-linking agents) or the Na^+ in the guluronic acids are lowered. Easy handling, versatility, and chemical stability are crucial qualities for fabrication of drug carriers. As a result, alginate has been utilized to create a variety of drug delivery systems, including hydrogels, microparticles, porous scaffolds etc. (Wulf et al. 2022). Because of their biocompatibility, water solubility, and bioadhesive properties, alginate nanocarriers have much potential as drug delivery systems (Li et al. 2022). Alginate based drug delivery systems improves the cellular-

internalization and accumulation of the anti-neoplastic drug in tumor cells (Gao et al. 2017b).

2.1 Crosslinking

Physical/chemical crosslinking of polymer chains can be used to create an alginate matrix. The well-known 3-D “Egg box” structure is formed due to the interaction of guluronate carbonyl groups with multivalent cations (e.g., Ca^{2+}) (Wang et al. 2019) (Fig. 1b). Alginates, along with other polymers including polyacrylamide, polyacrylic acid, gelatin, chitosan, and PVA, are used to make hydrogels and composite materials (Gami et al. 2022; Hackenhaar et al. 2022; Raji et al. 2022; Sadeghianmaryan et al. 2022). The ability of various cations to crosslink with alginates is strongest for trivalent cations, while for divalent cations, the order of crosslinking ability is $\text{Pb}^{2+} > \text{Cu}^{2+} > \text{Cd}^{2+} > \text{Ba}^{2+} > \text{Sr}^{2+} > \text{Ca}^{2+}$. Although the Ca^{2+} ion has relatively weaker interaction strength, it is the most widely utilized crosslinking agent. Nowadays, the use of ionic crosslinking is still popular because it provides a suitable way for trapping active compounds while keeping their biological activity for practical implementation. Calcium chloride is an effective ionic crosslinker for bioencapsulation. Although CaCO_3 and CaSO_4 are more commonly utilized in tissue engineering scaffolds due to their slower gelation and hence more homogenous matrix (Younes et al. 2017; Correia et al. 2013). Few literatures have been reported for alginate based microparticles produced using ionic and covalent crosslinkers. For example, Fenn et al., prepared methacrylate-alginate sub-microspheres for doxorubicin hydrochloride administration and reported their improved mechanical properties. The hydrophobic portion of methacrylate was integrated into alginate chains by using dodecyl trimethylammonium bromide (DTAB), and microspheres were then synthesized using water in oil emulsification (Fenn et al. 2016). The crosslinking process was split into two stages. In the first step, methacrylated-alginate microspheres loaded with doxorubicin were prepared, followed by a second ionic crosslinking stage using CaCl_2 . In a study, researchers investigated the impact of dual crosslinker agents on doxorubicin in-vitro release properties and intracellular delivery efficiency, finding that adding calcium to the microsphere reduced drug loss and improved the drug loading capacity, and inferred that green light as a crosslinker was sufficient for microsphere fabrication as an anticancer therapeutic delivery.

2.2 pH-Sensitiveness

Alginate has high pH sensitivity, which is an important factor in delivering the drug for anticancer medications. Since the pH affects a wide range of parameters, including fabrication, impurity separation, hydrogel synthesis, swell-ability, drug

release, and bio-degradation rate. It has been noted that the carboxy functional group of the alginate has displayed an important role in the imparting sensitivity in the alginate polymers towards external pH stimuli. The carboxylic acid groups are non-ionized (single bond -COOH), and when pH falls below its pKa ($\text{pH} < 3.4$), resulting in an insoluble structure that helps to prevent the degradation of the drug delivery in the microenvironment of cancer.

2.3 Mucoadhesiveness

Alginate is a polymer with good mucoadhesive properties due to its low surface tension. The presence of free -COOH and -OH groups scattered in the backbone of alginate can be attributed to its strong mucoadhesiveness. However, the mucoadhesion properties of the alginate can be upgraded by chemical alteration or by using another polymer in combination, such as chitosan.

Mucin-1, a transmembrane glycoprotein found to be overexpressed in most cancer types, therefore, a drug-delivery system of alginate with enhanced mucoadhesion properties can produce synergistic effects in cancer therapy (Viswanadh and Muthu 2018; Yi et al. 2005).

2.4 Biocompatibility

Various additional contaminants are obtained during the extraction of alginate from natural sources, which can produce a foreign body reaction. Biocompatibility is another important factor to consider because commercial-grade alginate has been known to elicit an immunological response (Xu et al. 2016). The extent of biocompatibility is determined by the composition and purity of the alginate. According to Tam et al., the mannuronate/guluronate content or inherent viscosity are the key parameters influencing the biocompatibility of purified alginate gels (Tam et al. 2011). Dusseault et al., reported the biological compatibility of alginate by evaluating the levels of impurities and contaminants present in the polymer (Dusseault et al. 2006). The United States Food and Drug Administration (US FDA) considers alginate to be generally recognized as safe (GRAS) material because it has not been shown to cause a significant immunological response (George and Abraham 2006).

3 Targeting Strategies of Alginate-Based Nanomedicines for Cancer Therapy

Both active and passive drug-delivery targeting strategies can be applied with alginate-based nanomedicines. The endothelial cells that line the tumor blood vessels have a huge gap junction (up to 200 nm), whereas normal tissue has a much smaller gap junction (2–4 nm). The colloidal nanomedicine in this range (≤ 200 nm) accumulates in tumor tissue only, a process called the EPR (enhanced permeability and retention) effect (Bazak et al. 2014). Numerous examples of alginate nanomedicine with a passive targeting strategy have been reported, including exemestane-loaded alginate nanoparticles (Jayapal and Dhanaraj 2017), curcumin-iron oxide nanoparticles coated with alginate and hydroxyapatite (Nobahari et al. 2022), alginate-stabilized doxorubicin-loaded nanodroplets (Baghbani et al. 2016). Furthermore, the overexpression of specific types of receptors in cancer is a significant trait for active targeting and involves interactions between relevant receptors and ligand-decorated nanomedicines, followed by enhanced endocytosis and drug release (Attia et al. 2019). A large variety of targeting ligands for active cancer-targeted nanomedicine have been discovered. Nanomedicines with such targeting ligands can target cancer selectively and improve the therapeutic efficiency of anticancer drugs. Various targeting ligands, such as folate, transferrin, aptamers, and monoclonal antibodies, can facilitate the active targeting of nanomedicines (Din et al. 2017; Jahan et al. 2017; Yu et al. 2017). Pre-conjugation or post-conjugation strategies can be used to conjugate nanomedicines with targeting ligands (Yu et al. 2010). There are various examples of alginate-based nanomedicine being used as an active targeting approach in cancer therapy, such as folate-mediated self-assembled phytosterol-alginate nanoparticles (Wang et al. 2015), cetuximab conjugated calcium alginate beads (Abdellatif et al. 2020) and folate conjugated and hyaluronic acid-coated alginate nanogels.

4 Drug Delivery Systems of Alginate in Cancer Treatment

Over the years, alginate has been used as a polymer in broad categories of therapeutic delivery systems. Alginate possesses all of the necessary features for a polymer to serve as a carrier system, including biocompatibility, easy biotransformation, drug encapsulation, targeting efficiency and controlled drug release. Alginate is a pH-sensitive polymeric material that functions as a thickener and gelling agent, which is required for medications to be released in a regulated and sustained manner. Because its glycosidic bond is unaffected by lysozyme, alginate serves as a good alternative to other polymeric materials such as chitosan. In addition to hydrogels, nanogels, microparticles, nanoparticles, and microspheres, alginate has been utilized to deliver anticancer drugs in a number of forms (Lakkakula et al. 2021). Many researchers have already discussed using sodium alginate as an

anticancer drug carrier for paclitaxel, doxorubicin, tamoxifen, curcumin, etc. (Shaikh et al. 2022). In a recent study, Abbasalizadeh et al., developed a hydrogel based on alginate and chitosan loaded with curcumin and chrysin for evaluating its cytotoxicity against lung and breast cancer cell lines. The developed drug delivery system has reduced viability and induced apoptosis in both A549 and T47D cell lines (Abbasalizadeh et al. 2022).

4.1 Beads of Hydrogel

Hydrogel beads are 3-D, cross-linked networks of hydrophilic polymers shaped into spheres with diameters ranging from 0.5 to 1.0 mm. Various cross-linking processes, including chemical and irradiation, are used to synthesize beads. Beads are a type of multiparticulate drug delivery system that can be used to achieve controlled drug delivery, improve bioavailability and target therapeutic delivery to specific areas. Beads can also provide benefits such as limiting therapeutic range fluctuation, reducing adverse effects, reducing dose frequency, and boosting patient compliance. Alginate can form hydrogel beads by combining with other polymers, such as chitosan, a natural polysaccharide, to create hydrogels for drug administration (Khodarahmi et al. 2022). The beads of chitosan-sodium alginate hydrogel were produced by crosslinking of repeating units of chitosan and sodium alginate. The beads were developed by using various proportions of chitosan and sodium alginate. The amount of chitosan in hydrogel beads was affected by the presence of water; the higher the water content, higher the chitosan concentration showed less swelling. Madeo et al., developed an alginate hydrogel by incorporating curcumin and graphene oxide for the localised therapy of squamous cell carcinoma. In this study, curcumin was encapsulated inside graphene oxide nanosheets followed by mixing with Ca^{2+} based cross-linked alginate hydrogel, resulting in hybrid hydrogels. The in-vitro cytotoxicity study of prepared hydrogel exhibited excellent cytotoxicity and enhanced cellular uptake in 4T1 and B16 cells. Therefore, it can be used as a localized therapy for squamous cell carcinoma-affected areas (Madeo et al. 2022).

4.2 Nanohydrogels

Nanohydrogels (or nanogels) are 3-D hydrogel materials made from crosslinked swellable polymer networks on a nanoscale. Nanogels may hold a large amount of water without actually dissolving in the aqueous medium. Nanogels can be produced from natural or synthetic polymers, or a combination of the two. Nanogels, like hydrogels, have high biocompatibility and the potential to improve the delivery of anticancer (Manivong et al. 2022). Hossenifar et al., describe a simple and direct method for making pressure-sensitive nanogels that appear to be a suitable carrier for

5-fluorouracil for colon cancer. Alginate is crosslinked with modified β -cyclodextrin as a crosslinker to create alginate-cyclodextrin hydrogels. Then, using an emulsification process, nanoparticles are created. By combining 5-fluorouracil in an aqueous solution with the nanoparticles, it easily loads into the alginate-cyclodextrin nanogel. The prepared nanogels exhibited enhanced cytotoxicity and cellular uptake in HT-29 cells. Therefore, the developed nanogels can be employed as an excellent candidate to overcome the inefficiency of 5-fluorouracil in the treatment of cancer (Hosseinifar et al. 2018). Pei et al., recently developed the theranostic by crosslinking modified oxidized alginate with folate-terminated poly (ethylene glycol) and rhodamine B-terminated poly (ethylene glycol) (RhB-PEG-NH₂). Owing to the exterior folic acid moieties, disulphide crosslinking framework, and Schiff base linkage for doxorubicin, the folate receptor-mediated targeted and pH/reduction simultaneous responsiveness subcellular induced releasing of doxorubicin was realized. Because of the desirable targeting of intracellular triggered release, the cytotoxicity and cellular uptake study clearly demonstrated that the majority of doxorubicin was released and deposited in the cell nucleus and cytotoxic to tumor cells effectively (Pei et al. 2018).

4.3 Stimuli-Responsive Hydrogels

Stimulus-sensitive hydrogels have the remarkable capacity to change volume abruptly from collapsed to inflated states in response to external stimuli, making them suitable as sensors and effectors. Many stimulus-sensitive hydrogels have been investigated, including those that respond to external stimuli such as temperature, pressure, light and pH. The diffusion coefficient, porosity, and tortuosity are the variables that control drug release from hydrogels. Sun et al., designed a crosslinking technique to generate a novel nanogel comprised of keratin and alginate that can respond to a dual stimulus. Keratin provided the crosslinked framework and bio-responsive features, while alginate improved morphology, stability, and encapsulation efficiency. The doxorubicin-loaded sodium alginate and keratin crosslinked nanogel showed improved cellular uptake in 4T1 and B16 cells, and in-vitro cytotoxicity analysis demonstrated its inhibitory effects on tumor cells equivalent to doxorubicin. In addition, in-vivo biological study demonstrated that the doxorubicin nanogels were safer than the drug itself (Sun et al. 2017). Abdelrahim and co-workers used alginate-conjugated polydopamine hydrogel. Polydopamine's catechol moiety attaches to the boronic acid group of bortezomib (BTZ) anticancer drug therapy, releasing the therapeutic agent in a pH-dependent manner. In tumor tissue, BTZ detaches from the catechol ring of polydopamine, and therefore the alginate hydrogels specifically release the drug in the cancer microenvironment (Rezk et al. 2019).

4.4 *pH-Responsive Hydrogel*

Through an acid-catalyzed acetalization reaction, the hydroxyl group of alginates can be crosslinked with bifunctional glutaraldehyde, resulting in a hydrogel having an acetal-linked matrix. Because the carboxylate groups in alginate were not involved in the reaction, the resulting hydrogel had pH-responsive swelling/contraction properties. In contrast, glutaraldehyde undergoes parallel oxidative breakdown, yielding non-reactive glutaric acid, whereas the aldol-polymerization, yielding polyglutaraldehyde, that competes with monomeric glutaraldehyde for the acetalization reaction. Because the rates of reactions enhance with temperature and pH, at low glutaraldehyde or acid catalyst concentrations, the competitive reactions can often overwhelm the acetalization reaction (cross-linking reaction), and causing the actual cross-linking density to deviate from the expected one (Chan et al. 2009). Because of the environment-sensitive volumetric transitions in the hydrogel membrane, stimuli-responsive porous hydrogels with pore sizes ranging from micrometers to nanometers are of particular interest for accurate mass transport regulation that may be modified by altering the pore size. Phase separation of polyvinyl alcohol (PVA) and sodium alginate mixture caused by evaporation of aqueous solutions during spin-cast deposition of polymer films can be used to construct nanoporous thin-film membranes. PVA has been shown to form intramolecular hydrogen bonds and intermolecular hydrogen bonds with carboxyl functionalized polymers, such as polyacrylic acid (Gopishetty et al. 2012). Peng et al., designed the alginate-based super paramagnetic iron oxide nanoparticles with magnetic targeting for pH-responsive release of the anticancer drug doxorubicin in tumor-cell (hep G2) microenvironments. The magnetic nanoparticles of the alginate nanoparticles exhibited higher cytotoxicity toward the cancer cells (hep G2 cells) as compared to control cells because of the acidic microenvironment of the cancer cells and due to the application of an extra magnetic field (Peng et al. 2016).

4.5 *Thermoresponsive Hydrogel*

The thermoresponsive hydrogel, which responds to temperature variation, is one of the most prevalent and well-studied carriers for therapeutic delivery (Zhuang et al. 2022). The thermoresponsive hydrogel can be synthesized into micro or nanoparticles (organic or inorganic origin) by combining different polymers. The resulting system represents a novel approach to local and long-term drug delivery at a specific body site. By introducing a local rise in temperature to the system, it is feasible to achieve on-demand drug release (Lacroce and Rossi 2022). Poly (N-isopropylacrylamide) (PNIPAM) has a lower critical solution temperature (LCST) of 32 °C, poly(N,N-diethylacrylamide) (PDEAAM) has an LCST between 25 °C to 32 °C, poly(N-vinylcaprolactam) (PVCL) has an LCST between 25°C to 35 °C (Bütün et al. 2001). The phase transition that occurs in reaction to temperature

variations is quick and reversible. The hydrophobic isopropyl group and the hydrophilic amide group are both present in PNIPAM. When the temperature is below LCST, PNIPAM is hydrophilic, with the chains extended and hydrated in water, and when the temperature is above LCST, the chains dehydrate and collapse. With the addition of salts, surfactants, or co-polymerization with various hydrophilic or hydrophobic co-monomers, the LCST of PNIPAM might be adjusted. The co-polymerization with a hydrophobic monomer lowers the LCST, while co-polymerization with a hydrophilic monomer raises it (Pelton 2010).

Sodium alginate is a better choice for forming thermoresponsive hydrogels using PNIPAM due to its biocompatibility, biodegradability, safety, chelatable, and chemically modifiable nature. Brown seaweed produces alginate, a water-soluble carbohydrate. Two G-block aligned areas could generate a diamond-shaped hole with the perfect architecture for supporting binding divalent cations like Ca^{2+} to produce mechanically crosslinked hydrogels. Hydrogels made of alginate and PNIPAM have previously been developed using a semi-interpenetration network of Ca^{2+} as a physical crosslinker and glutaraldehyde as a chemical crosslinking agent, or grafting PNIPAM to alginate.

Furthermore, temperature change affects the self-assembled micelles of alginate-g-PNIPAM loaded with doxorubicine to accumulate passively at the tumor site due to the increased permeation and retention effect (Stilhano et al. 2016). Recently, Zhuang et al., developed drug-loaded graphene-incorporated calcium alginate (GCA) microspheres that were loaded into the thermosensitive injectable gel. As per the findings, the drug-loaded GCA/PPP hydrogels are injectable. Their in-vitro biological studies revealed that the drug-loaded GCA/PPP hydrogels were biocompatible with improved anti-tumor activity when exposed to NIR light, indicating that they can be used to treat metastatic tumors (Zhuang et al. 2022).

4.6 Magnetic Hydrogels

Owing to the cost-effective and biocompatibility of the alginate-based hydrogel, it has been preferred over numerous gels in the drug delivery approach. Alginate hydrogels can be doped with magnetic particles to create “smart” materials. Magnetically sensitive species incorporated into hydrogel structures may provide additional properties such as stimuli-responsive activity, better thermosensitive properties, or adjustable rheological properties without compromising biocompatibility. As a result, magnetic hydrogels are becoming increasingly helpful in therapeutic delivery, notably as scaffolds for soft tissue engineering, where the aforementioned benefits are critical. The type, size, shape, and concentration of the inserted magnetic particles are then primarily responsible for the rheological properties of magnetic hydrogels (also known as ferrogels) in the presence of a magnetic field. For example, with a moderate magnetic field, small particles (e.g., nanoparticles) have a modest attraction to each other, whereas larger particles (e.g., microparticles) can interact significantly (even in a weak magnetic field). In

the case of strong magnetic fields, it can cause dramatic changes in the viscoelasticity of magnetic hydrogels made up of microparticles. Magnetic hydrogels are a fascinating study subject, but they are still in their early stages. Several papers on ferrogels do not completely reflect their significant potential in terms of current and emerging biomedical concerns (Barczak et al. 2020). Jahanban-Esfahlan et al., developed a hydrogel-based drug delivery system loaded with magnetic nanoparticles as a smart therapeutic system for effective tumor therapy. Because of the applied external magnetic field and the pH-dependent release of the drug at the cancer site, the prepared alginate-gelatine magnetic nanoparticles of doxorubicin showed increased cytotoxicity in Hela cells (Jahanban-Esfahlan et al. 2020).

4.7 *Injectable Hydrogel*

Injectable hydrogels are 3-D hydrophilic polymeric matrixes having a high affinity for body fluids that can be injected directly into the body with a syringe or through a catheter (Bidarra et al. 2014). In the biomedical field, injectable hydrogels have been proposed as a platform for tissue engineering and medicinal delivery (Liu et al. 2017; Norouzi et al. 2016). Ferreira et al., looked at anti-vascular endothelial growth factor (anti-VEGF) therapy for solid tumors and found it to be a promising strategy. However, the difficulty of delivering anti-VEGF agents at high drug concentrations and maintaining regional therapeutic concentrations at the tumor site limits their benefits. The researchers constructed a delivery method for regulated and anti-angiogenic treatment under tumor microenvironmental conditions using a bevacizumab-loaded alginate hydrogel to eliminate these challenges. The resulting 3-D hydrogel assembly offers drug stability and a system that can be administered as a flowable solution, allowing for the establishment of a depot following local administration (Ferreira et al. 2017).

4.8 *Microparticles*

Various alginate-based drug delivery systems have been developed, including polymeric microparticles, nanoparticles, micelles, and beads. Antitumor drugs can be put into alginate-based microparticles (Batyrbekov et al. 2009). Microparticles are spherical particles with a diameter of 1–1000 μm that are encased in a semi-permeable polymeric membrane and contain a bioactive ingredient. Various procedures, such as atomization, emulsification, and droplet production, can be used to fabricate alginate microparticles. An alginate solution is dropped into a divalent cation solution (ionotropic gelation), and this approach is frequently supplemented with other techniques published in the literature. Bai et al., synthesized alginate microparticles loaded with disulfiram and superparamagnetic iron oxide, which showed a lot of potential for treating ovarian cancer with two treatments at the

same time, namely chemotherapy and hyperthermia (Bai et al. 2021). In another investigation, gallic acid-containing microspheres were developed using varying ratios of sodium alginate, which had an entrapment efficiency ranging from 11.26% to 72.64%, whereas their cytotoxicity study was conducted against the Caco-2 cells (Celep et al. 2022).

4.9 Alginate Nanoparticles

To date, numerous methods for making alginate-based nanoparticles are available (Niculescu and Grumezescu 2022). The ion-gelation approach has been widely used to make alginate-based nanocarrier systems. Covalent crosslinking techniques can be used (Xu et al. 2021). However, the crosslinkers used in these techniques, such as glutaraldehyde and epichlorohydrin, are toxic and may cause side effects. Despite this, other methods provide a more accessible and straightforward way to prepare nanoparticulate drug delivery systems from alginate. For instance, depositing a layer of alginate on the nanodroplets of material must be emulsified to develop a drug delivery system. Ionic/covalent crosslinking stabilizes the resulting nanoparticles, which are subsequently thoroughly dried. The electrospraying approach, which involves applying an electric field to an alginate-based liquid to trigger the production of nanoparticles that are harvested in an appropriate crosslinking media, is an additional alternative approach. Saralkar et al., developed curcumin and resveratrol-loaded calcium alginate nanoparticles by using crosslinker. The drug-loaded nanoparticles of alginate exhibited an enhanced cytotoxic effect on the DU145 cells, although the prepared alginate nanoparticles did not exhibit hemolysis and were considered safe for intravenous administration (Saralkar and Dash 2017).

4.10 Alginate-Drug Conjugates

Since the structure of the alginate comprises both hydroxyl and carboxylic groups, it can be used to conjugate anticancer drugs through chemical reactions or electrostatic interactions. Wang et al. (2014) conjugated the lipophilic drug cisplatin with the alginate by combining their equimolar fractions in the deuterium oxide. The resultant conjugate of cisplatin and alginate showed the enhanced water solubility of the drug (Wang et al. 2014). Similarly, Cheng et al., synthesized the alginate-doxorubicin complex by using a Schiff base reaction, which was used to prepare self-assembled nanocarriers when rehydrated with the aqueous medium. Their in-vitro cytotoxicity investigations of the nanoparticles revealed remarkable cytotoxicity against MCF-7 cell lines, whereas the human breast epithelial cell line MCF-10A demonstrated an enhanced safety profile. These findings indicate that a pH-sensitive prodrug-nanoparticle system might be a simple and effective platform for selective co-delivery of several anticancer drugs to tumor cells (Gao et al. 2017a). Furthermore, Pawar et al.,

used carbodiimide crosslinkers to prepare an alginate-doxorubicin conjugate, which was then loaded into polycaprolactone microparticles using a solvent evaporation technique. The prepared microparticles showed enhanced cytotoxic activity of doxorubicin and higher cellular uptake against MDA-MB-231 cells (Pawar et al. 2018).

4.11 Alginate-Based Hybrid Nanogels

So far, nanogels have so far been made by either polymerizing monomers or using microemulsion or inverse microemulsion techniques (Soni et al. 2016). However, the nanogels prepared by these techniques are complicated and require many purification stages to remove the unreacted monomers and surfactants. Additionally, the techniques as mentioned earlier have disadvantages when encapsulating hydrophobic guest molecules (Keskin et al. 2021). As a result, a simple and effective strategy to address these concerns must be devised. Preparing nanogels by crosslinking hydrophilic polymer chains with an oppositely charged polymer via ionic or covalent interactions could be a viable option. On the other hand, nanogels synthesized by ionic cross-linking oppositely charged polymers are fragile and prone to dissolution when pH is too acidic or too basic. As the irreversible chemical linkages are established with covalent cross-linking, nanogels with a permanent network can be developed. Such nanogels allow water or other bioactive groups to pass through without dissolving, thus resolving the stability concerns. The choice of polymeric system is one of the most important aspects of nanogel synthesis. Polymers obtained from natural sources are used to create nanogels that are biocompatible and biodegradable. Matai and Gopinath developed alginate-poly(amidoamine) dendrimer hybrid nanogels for the delivery of epirubicin hydrochloride. To achieve the conjugation reaction, the carboxylic group of the alginate was activated by 1-ethyl-3-(3-dimethylamino propyl), a carbodiimide crosslinker, and then poly(amidoamine)dendrimer was added. Finally, the remaining carboxylate groups were cross-linked using calcium chloride. The prepared nanogels exhibited a higher apoptotic capability by cytotoxicity and cell cycle analysis, while cellular uptake studies revealed increased internalization in MCF-7 cells. As a consequence of the current study, the hybrid nanogels of epirubicin improved its anticancer activity (Matai and Gopinath 2016).

4.12 Alginate-Based Polyelectrolyte Complex

Polyelectrolytes are macromolecular compounds with various ionizable functional groups with varying molecular weights and chemical compositions. The partial or complete dissociation of polyelectrolytes in aqueous solutions results in the development of charges on the macromolecules. To make polyelectrolyte complexes,

oppositely charged polyelectrolytes are combined in solution simultaneously. The polyelectrolyte complex of the alginate and the chitosan are extensively studied for the drug delivery approaches in cancer therapy. The preparation of the polyelectrolyte complex of the alginate and the chitosan can be done in various methods. Alginate and chitosan are oppositely charged polymers; following gradual mixing of the solution of the two polymers in the presence of Ca^{2+} lead to the formation of a polyelectrolyte complex by electrostatic interaction (Martău et al. 2019). Chitosan is a positively charged deacetylated derivative of chitin derived from crustacean shells, whereas alginate is a negatively charged, linear co-polymer (Cheung et al. 2015). The main drawback of chitosan in the drug delivery system is that it swells and begins to break down in an acidic environment (Szymańska and Winnicka 2015). Since the microenvironment of cancer is inherently acidic, it is possible for the chitosan nanomedicine to break down before endocytosis (Jhaveri et al. 2021). In contrast to chitosan, alginate shrinks in an acidic medium and swells in an alkaline medium (Thai et al. 2020). Since both the polymers are oppositely charged in nature, they can be used in polyelectrolyte complex reactions to form hybrid-polymeric drug delivery systems (Alnaief et al. 2020; Ishihara et al. 2019). The polyelectrolyte complex of the chitosan-alginate overcomes the shortcomings of chitosan, including poor mechanical strength and stability at lower pH, while maintaining its biocompatible, biodegradable, and non-toxic properties (Potaś et al. 2020). During the formation of polyelectrolyte complexes, ionic gelation occurs between the amino group ($-\text{NH}_2$) of the chitosan and carboxylic group ($-\text{COOH}$) of the alginate (Hamman 2010). Today, various publications on the use of chitosan and alginate polyelectrolyte complexes loaded with doxorubicin, paclitaxel, and 5-fluorouracil in anticancer drug delivery systems are available (Sun et al. 2019; Vasiliu et al. 2019; Yoncheva et al. 2019).

5 Theranostic Application of Alginate-Based Nanomedicine in Cancer

Theranostics refers to nanoscale or molecular-level diagnostic and therapeutic agents. Theranostics systems integrate diagnostic and therapeutic agents into their structure (Muthu et al. 2017; Sonali et al. 2018). Theranostics are gaining popularity since they are tailored treatments that can be employed for diagnostic imaging with no or minimal alterations to aid in personalized medicine. Thus, theranostics and image-guided therapy (IGT) are closely related, and theranostics are a subclass of IGT in which both diagnosis and treatment functions are given to a unified platform. Molecular pretargeting is a critical theranostics approach (Jha et al. 2020; Mehata et al. 2021). In pretargeted IGT, a target-specific naturally derived bioligand identifies the target, which is then accompanied by a nanoscale or molecular therapeutics fraction that forms therapeutic complexes by *in situ* coupling processes (Mehata et al. 2020; Moghissi and Dixon 2017). Pretargeted drug delivery systems can be

employed as theranostics for diagnosis and therapy if labeled with advanced imaging probes. Optical and nuclear imaging methods have primarily been employed in pretargeted theranostics proof-of-concept investigations (Narendra et al. 2020). Pretargeting is a relatively new idea in theranostics, and it usually necessitates a validated amplification of surface receptors on the targeting cells/tissue. Furthermore, biological origin or semisynthetic bioligands must be present in the receptors to serve as pretargeting constituents. As a result, pretargeting theranostics are currently restricted to a few cancer types that overexpress cell-surface markers on the cell membrane (Mehata et al. 2019).

In a study, Pei et al., developed a theranostic nanomedicine surface-functionalized with folic acid and covalently linked with doxorubicin and rhodamine B, cross-linked modified oxidized alginate with cystamine. Developed theranostic nanomedicine has the capability of releasing loaded doxorubicin in response to changes in pH and reduction potential of the cell, as in the case of cancer cells. Folic acid functionalization acts as a targeting ligand for targeted delivery of the theranostic nanomedicine to cancer cells. Additionally, cellular uptake and cytotoxicity studies demonstrated that the developed nanoparticles were effectively uptaken by cancer cells and accumulated in the nuclei of the cancer cells. Moreover, doxorubicin was released inside the cells and produced anticancer activity. Furthermore, theranostic nanomedicine was able to track cancer cells in real-time due to the presence of the rhodamine B dye (Pei et al. 2018).

In another study, Peng et al., developed cancer-targeted theranostic nanomedicine for early diagnosis of cancer with improved therapeutic efficacy. The developed alginate-based drug delivery system was integrated with superparamagnetic iron oxide nanoparticles and doxorubicin. The fabricated theranostic system was capable of providing contrast agents for magnetic resonance imaging and stimulus-responsive delivery of doxorubicin to the cancer cells. Additionally, developed nanosystems were biocompatible and non-toxic to the normal cells (Peng et al. 2019).

Similarly, Lengert et al., developed a silver alginate-based microparticulate drug delivery system with calcium carbonate as a core. In this study, they have immobilized the sodium alginate in the highly porous calcium carbonate pores via cross-linking with the aid of silver ions. The silver ions play dual roles in this study; primarily, they act as cross-linking agents and on the other hand, they form silver nanoparticles. Additionally, silver ions help in enhancing the sensitivity of the ultrasound leading to the production of homogeneous dispersed silver nanoparticles. Transmission electron microscopy revealed that, silver nanoparticles were present on the shell of the alginate microparticles, in the twin structure. The particle size has a significant influence on the controlled delivery of cargo from alginate microparticles by ultrasound. It was established that such particles might be used as a framework for label-free molecular detection via surface-enhanced Raman diffraction. The cytotoxicity and cellular uptake investigations done in this study demonstrated that microcontainers have a low degree of toxicity and a high rate of cellular uptake. The above-mentioned features are the building elements of a theranostic

system, in which detecting and distant release can be accomplished with the same carrier (Lengert et al. 2017).

Recently, Pan et al., developed $\text{Ca}^{2+}/\text{Mg}^{2+}$ stimuli-responsive Indocyanine Green (ICG) – alginate in situ hydrogels for localized cancer photothermal therapy. The developed formulation was biocompatible with strong ICG fixation ability. The ICG – alginate formulation has good photothermal therapeutic efficacy with minimization of ICG side effect that occurs due to the diffusion of the ICG to the surrounding tissues. The free ICG and ICG-alginate were intratumorally injected into mice and observed for any leakage; the ICG-alginate formulation does not show any type of leakage, indicating that the prepared formulation successfully formed gel inside tumors (Fig. 2IIA). In-vivo intratumoral metabolism of the ICG and ICG-alginate demonstrated that ICG-alginate has slower metabolism compared to the free ICG (Fig. 2IIB). The in-vivo fluorescent study was performed on the mice after administration of the ICG and ICG-alginate formulations to investigate their fixation capability (Fig. 2IIC). In-vivo imaging demonstrated that free ICG was quickly distributed to the body, whereas ICG-alginate was slowly penetrated to the neighboring tissue, indicating that ICG-alginate has strong fixation capability. Thus, ICG-alginate's strong fixation and accumulation capabilities indicated that ICG-alginate has strong potential for the improvement of photothermal therapy in cancer (Pan et al. 2019).

In a separate study, Jia et al., reported the synthesis of doxorubicin-loaded theranostic nanomedicine which was synthesized by using carbon dots as a cross-linker for PEGylated oxidized alginate. It has been observed that theranostic nanomedicine does not show the release of doxorubicin at physiological pH (simulated body fluid), indicating that premature drug release at physiological pH can be prevented. Further, an in-vitro release study in the simulated tumor microenvironment media demonstrated the higher or burst release of doxorubicin. Moreover, the MTT study suggested that theranostic nanomedicine could inhibit cancer cell growth effectively due to its nuclear-targeted delivery of doxorubicin. Furthermore, a cellular fluorescence study demonstrated the potential of the theranostic nanomedicine for the imaging and therapy of cancer (Jia et al. 2016).

Combining the diagnostic agents and therapeutic molecules in the single drug delivery system has displayed an emerging and promising strategy for advancing cancer therapy. Moreover, designing a theranostic system for targeted imaging and therapy of cancer with improved clinical efficacy remains an unmet clinical need. Addisu et al., developed pH-responsive theranostic nanomedicine based on mixed lanthanide materials. The delivery system was formed by a simple metal-ligand interaction for concurrent tumor cell imaging and therapy. They have developed theranostic nanomedicine based on the alginate-polydopamine complexed with terbium/europium or dysprosium/erbium oxide nanoparticles. These nanoparticles were loaded with doxorubicin and functionalized with folic acid for tumor targeting and anticancer delivery. The developed nanoparticles were biocompatible and were confirmed by incubating these nanoparticles with zebrafish. Further, targeted nanoparticles have demonstrated higher cellular penetration and accumulation inside tumor spheroids (Fig. 3) compared to free doxorubicin (Addisu et al. 2019).

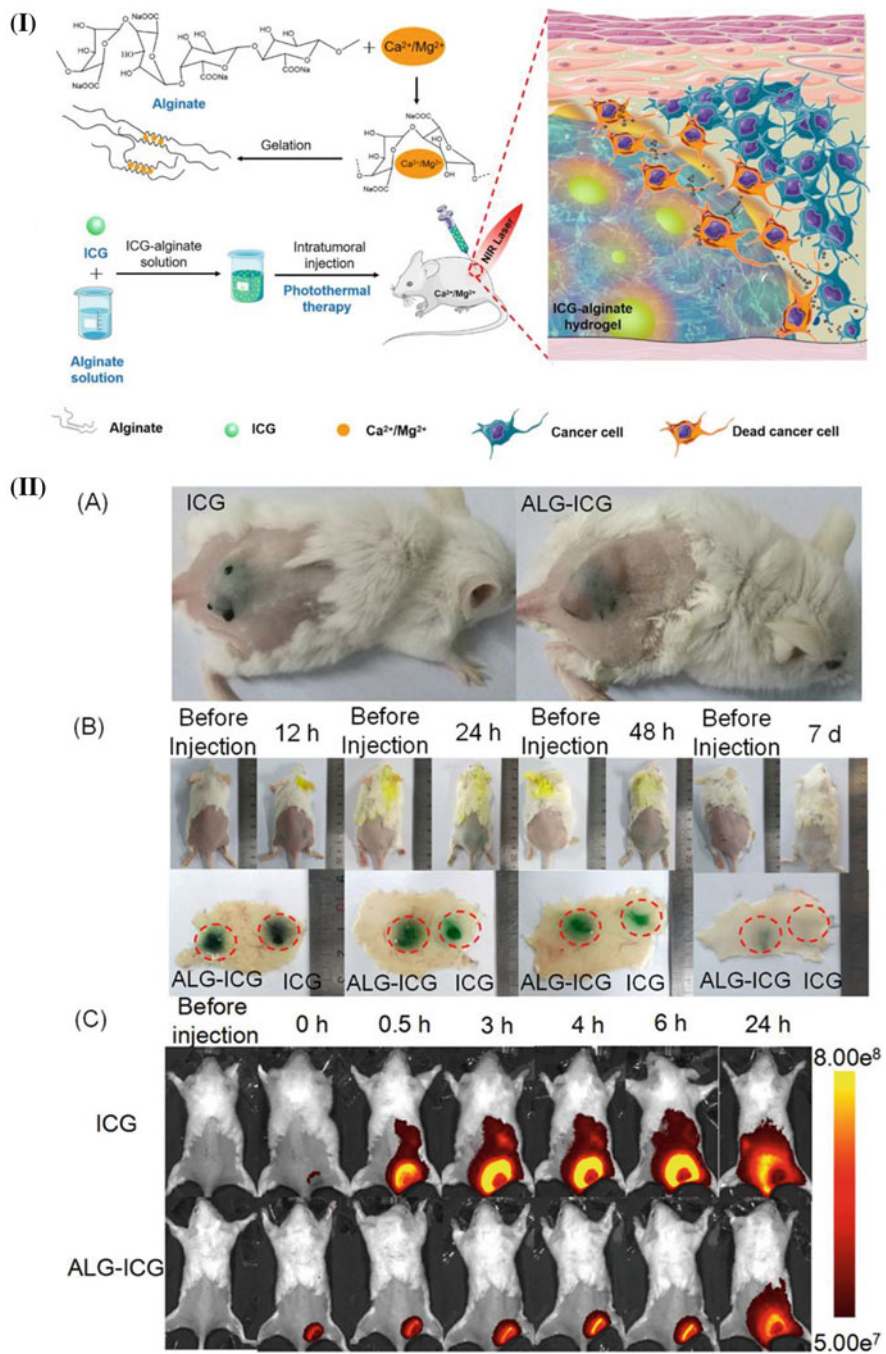


Fig. 2 (I) Graphical representation of the preparation of the ICG-alginate based hydrogel for in-vivo imaging and photothermal therapy of cancer. (II) (A) Intertumoral leakage of ICG and ICG-alginate (B) in-vivo metabolism of the ICG and ICG-alginate (C) in-vivo tumor imaging and

Overall, it has been observed that most of the alginate-based theranostic nanomedicines were prepared by using doxorubicin as an anticancer drug. Doxorubicin has fluorescence properties that can be helpful for the detection and imaging of cancer cells and anticancer activity. Additionally, we have presented various application of alginate-based drug delivery systems for cancer therapy in Table 1.

6 Limitations and Challenges of the Alginate-Based Drug Delivery System

Sodium alginate is abundantly accessible, environmentally safe, and inexpensive to manufacture. Various additional benefits of the alginate including biocompatibility, biodegradability, and non-toxic, have led to the production of a variety of alginate-based nanomedicine that is utilized in the food and biomedical industries. The most common salt of alginate is sodium alginate. Alginate, on the other hand, has a variety of drawbacks related to its qualities, including poor stability, low mechanical and barrier qualities, incompatibility with heavy metals, and high-temperature instability, some of which are irreversible. These qualities can be enhanced by integrating alginate with other natural polymers, particularly protein-based biodegradable polymers or synthetic polymers, and by changing or applying the procedures used during manufacture (Gheorghita Puscaselu et al. 2020). There is a considerable quantity of alginate substance in the environment due to the presence of algae in the marine environment. About 30,000 tonnes of commercial alginate are produced each year, with fewer than 10% of them being biosynthesized. As a result, renewable alginate-based polymers have a lot of design limitations and challenges (Gheorghita Puscaselu et al. 2020) (Fig. 4).

7 Factor Influencing Physicochemical Properties of Alginate Nanomedicine

Alginate is a popular alternative amongst pharmaceutical additives for producing improved therapeutic delivery systems for oral administration because of its high biocompatibility and is certified as a food ingredient by the US Food and Drug Administration. The researcher community's enthusiasm for alginate coincided with the nanomedicine-driven medicinal breakthrough. The physicochemical features of the alginate notably viscosity, thermostability, sol-gel transition, pH-responsiveness, and drug release, might help researchers to better understand its possible uses.

Fig. 2 (continued) photothermal therapy produced by using ICG and ICG-alginate formulation. Reproduced with permission from ref. (Pan et al. 2019), Fig. 5, (American Chemical Society®)

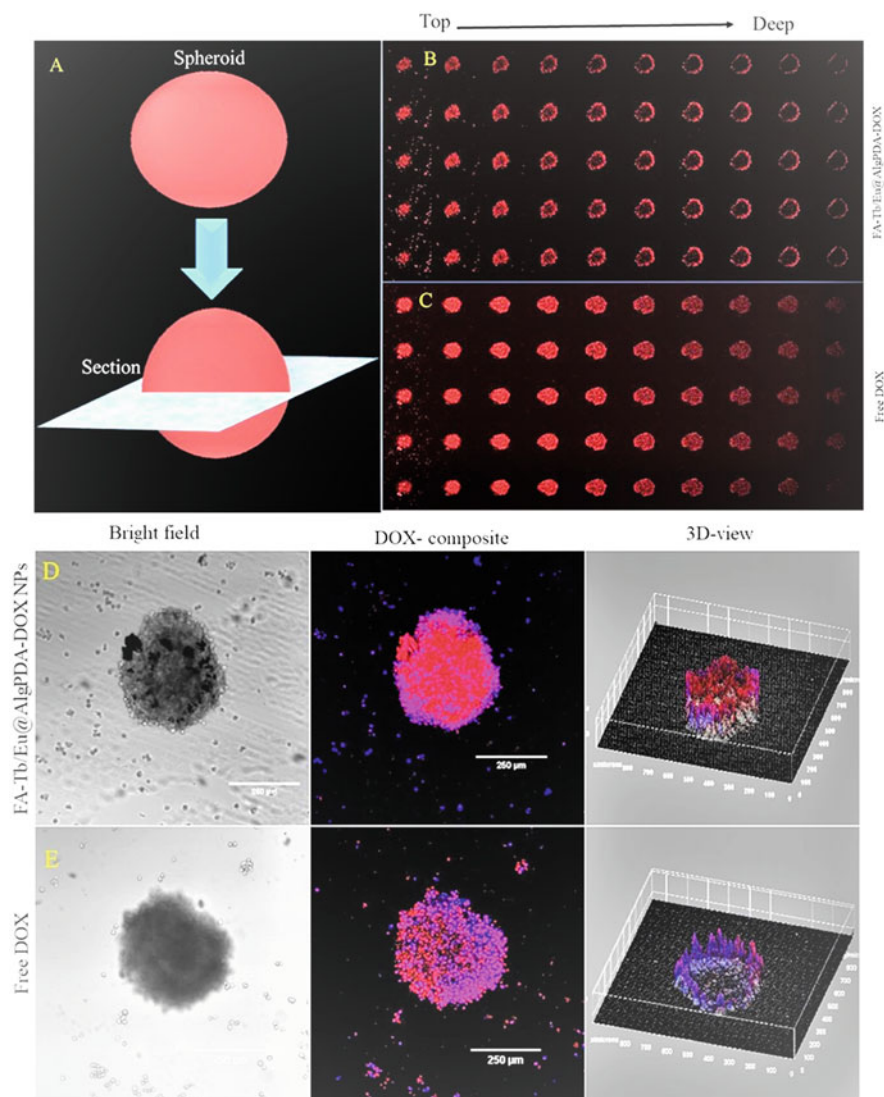


Fig. 3 (A) Graphical representation of the spheroids has been presented. Confocal microscopy images of HeLa cell spheroids incubated with (B) FA-Tb/Eu@AlgPDA-DOX NPs or (C) free DOX. Distribution of (D) FA-Tb/Eu@AlgPDA-DOX NPs or (e) free DOX in the spheroids. Reproduced with permission from ref. (Addisu et al. 2019), fig. 12, (American Chemical Society®)

Alginate nanomaterials characteristics are influenced by various parameters, including alginate, surfactant, cross-linker concentrations, stirring time and speed, and pH value (Choukaife et al. 2020).

Table 1 Applications of the various alginate-based drug delivery systems in cancer treatment

Formulation	Therapeutic agent	Type of study	Outcome	Ref.
Alginate-hydrogel	Bevacizumab	Chick embryo chorioallantoic membrane assay	pH-independent improved anti-angiogenic activity	Ferreira et al. (2017)
Chitosan/alginate bio-composite	Copper-nanoparticles	In-vitro cytotoxicity against breast cancer cell-line (UACC-3133), Antioxidant study	Enhanced cytotoxicity, antioxidant activities against DPPH	Xu et al. (2022)
Alginate-chitosan Hydrogel	Curcumin, chrysin	MTT assay against lung (A549) and breast (T47D) cancer cell lines	Improved cytotoxicity as compare to Curcumin, chrysin or both	Abbasalizadeh et al. (2022)
Alginate-based macroporous hydrogel matrix	Radiation dose of 25 Gy	3D distribution of F98 cells within the matrices, Cell survival after irradiation	The F98 cells were distributed throughout the matrices	Solano et al. (2021)
			All F98 cells entrapped in the matrix were eliminated with radiation	
Alginate-nanogel	Cisplatin	In-vivo anticancer efficacy on ovarian cancer mouse model with KRAS mutations	Enhanced anticancer efficacy of cisplatin	Yamaguchi et al. (2021)
Alginate/k-carrageenan oral microcapsules	Extracted novel Mushroom polysaccharides	Cytotoxicity study against colon cancer (Caco-2) cells	Exhibited efficient cytotoxicity (74.09%)	El-Deeb et al. (2022)
Alginate nanogel	Cisplatin, gold nanoparticles	Anticancer efficacy in CT26 colorectal tumor model	Enhanced anticancer efficacy of cisplatin	Mirrahimi et al. (2019)
Alginate-cyclodextrin nanogel	5-fluorouracil	Cellular uptake and apoptosis assay in colon cancer cells (HT-29)	Enhanced cellular uptake, higher cell death	Hosseini et al. (2018)
Fucoidan/alginate hydrogels	Photodynamic therapy (PDT) using chlorin e6 (Ce6)	In-vitro cytotoxicity and migration study in colon cancer	promising targeting approach-PDT for treating colon cancer	Shanmugapriya et al. (2020)
Liposomes loaded alginate beads	Oxaliplatin	Biodistribution study in NUDE/SCID mice	Higher accumulation of the oxaliplatin in colon tissue	Bansal et al. (2016)

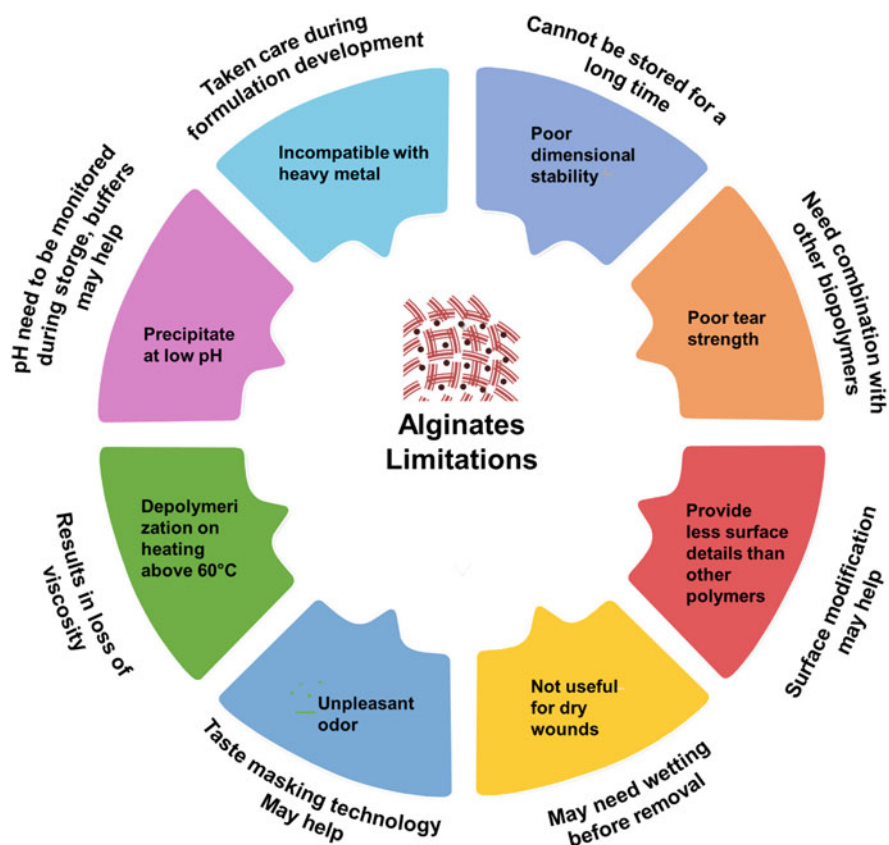


Fig. 4 Limitations and challenges of alginates as the polymer used for the development of drug delivery carriers

8 Alginate-Based 3D Cell Culture Techniques

The advent of 3D cell culture technology will lead to advancements in the research of numerous physiologically relevant techniques such as organogenesis, tissue morphogenesis, hypoxia, drug discovery, cell-based assays, and therefore minimized animal utilization. The potential of 3D cell culture techniques to imitate tissue architecture using single cells or co-cultures is an advanced technique over 2D monolayer cultures. 3D culture techniques have a significant influence on the prediction of pharmacological efficacy comparable to the real in-vivo response. For cell immobilization, there are numerous ways and modifications of 3D cell culture. There are numerous approaches and variants of 3D cell culture for cell immobilization. For the development of 3D tumor models, of several matrices have been investigated. Collagen, hyaluronic acid, alginate, peptide, and hydrogel-based scaffolds are most often employed in biomedical applications. Alginate-based

scaffolds have gained much interest for 3D cell culture for anticancer research. Alginate scaffold has several advantages, including being animal-free, being stable at room temperature, and having a flexible porosity. Collagen, fibrin, and hyaluronic acid are examples of animal-derived scaffolds that have bioactivity. Alginate, on the other hand, is bioinert and lacks inherent bioactivity. The bioactive capabilities of the alginate can be enhanced by adding peptides or employing composite hydrogels like alginate–collagen. Because continuous culture for a long time can lead to the development of hypoxia-induced necrotic regions, which can lead to inconsistencies in the results obtained, the formation of multicellular spheroids that mimic the in-vivo tumor microenvironment without compromising the respiratory features. Alginate scaffolds allow the formation of multicellular tumor spheroids due to their porous nature. The unique properties of the alginate scaffold prompted major research into the development of 3D tumor models, and a large number of papers demonstrate the economic potential of products based on the alginate matrix. AlgiMatrix[®] is a 3D cell culture product of the alginate. Godugu et al., demonstrate that this culture system can be used as an in-vitro tumor model for anticancer medication testing. They employed multiple anticancer drugs to treat human non-small cell lung cancer cell lines. The IC₅₀ values of anti-neoplastic agents were substantially higher when comparing 2D culture models to AlgiMatrix[®] systems, indicating that 3D culture is a better model for in vitro anticancer drug cytotoxicity testing (Godugu et al. 2013; Viswanadh et al. 2020).

9 Future Perspectives

A variety of polymers (natural and synthetic) are widely used in the fabrication of drug delivery systems for a wide range of biological applications. Due to the unique origin and properties of alginate, such as biodegradability, biocompatibility, and non-toxicity, it is one of the most promising polymeric materials. Alginate-based drug delivery systems offer a wide range of applications in cancer therapy, including the administration of chemotherapeutic agents. Anticancer drugs can be delivered to specific cancer sites using alginate-based nanomedicines using active and passive targeting approaches. Chemical modification of polymers has also been used to develop desirable drug delivery systems. Besides, other polymers like chitosan can be integrated with alginate to enhance anticancer drug delivery strategies. Substantial research is being conducted to develop targeted drug delivery systems that may be used in various biological applications. Alginate matrices have been used to encapsulate cells and tissues that would be transplanted. Alginate-based 3D gels and scaffolds have been recognized as a central matrix for simulating a more natural tumor microenvironment. Furthermore, 3D systems provide a more accurate representation of physiologic design; specific cellular interactions, and improving functional features. However, human trials of alginate-based delivery systems will still require considerable in-vivo investigations. A number of anticancer agents, including paclitaxel, cisplatin, and methotrexate, have been reported to be delivered using

alginate-based drug delivery systems. In the future, modified alginate based anticancer drug delivery systems could revolutionize cancer diagnosis and treatment.

10 Conclusion

Most of the anticancer drugs used clinically have lots of drawbacks, including significant dose-related adverse effects and a poor therapeutic index. The primary objective of the targeted drug delivery system is to release the anti-neoplastic agents into the cancer microenvironment in the required concentrations. The nanomedicine of alginate loaded with anticancer drugs enhances therapeutic efficiency while decreasing side effects. The molecular chain of the alginate consists of a large number of free hydroxyl and carboxyl groups. Therefore, the drug delivery system of alginate may be cross-linked with targeted ligands such as folate and monoclonal antibodies using a pre-conjugation and post-conjugation technique to improve anticancer efficacy. Anticancer agents can be delivered more efficiently and have fewer adverse effects because of advancements in alginate-based drug delivery. The application of alginate in combination with other polymers like chitosan has the potential to speed up cancer therapy. Because of their porous nature, alginate scaffolds facilitate the development of multicellular tumor spheroids. Furthermore, the alginate scaffold has unique properties that aid in forming 3D tumor models, allowing researchers to analyze the in-vitro cytotoxicity of anticancer drugs and cancer nanomedicine with greater precision than traditional 2D culture approaches. As a result, alginate-based drug delivery systems have a promising future in cancer research, treatment and diagnosis.

Acknowledgements The authors are grateful to the Ministry of Education (Govt of India) for granting a teaching assistantship. The authors have no other relationships or financial conflicts with any organization or company.

Conflict of Interest The authors acknowledge that they have no known conflicting personal or financial interests that might have profoundly affected the findings of this study.

References

- Abbasalizadeh F, Alizadeh E, Bagher Fazljou SM, Torbati M, Akbarzadeh A (2022) Anticancer effect of alginate-chitosan hydrogel loaded with curcumin and chrysin on lung and breast cancer cell lines. *Curr Drug Deliv* 19(5):600–613
- Abdellatif AAH, Ibrahim MA, Amin MA, Maswadeh H, Alwehaibi MN, Al-Harbi SN et al (2020) Cetuximab conjugated with octreotide and entrapped calcium alginate-beads for targeting somatostatin receptors. *Sci Rep* 10(1):4736
- Addisu KD, Hsu WH, Hailemeskel BZ, Andrgie AT, Chou HY, Yuh CH et al (2019) Mixed lanthanide oxide nanoparticles coated with alginate-polydopamine as multifunctional nanovehicles for dual modality: targeted imaging and chemotherapy. *ACS Biomater Sci Eng* 5(10):5453–5469

- Alnaief M, Obaidat RM, Alsmadi MM (2020) Preparation of hybrid alginate-chitosan aerogel as potential carriers for pulmonary drug delivery. *Polymers (Basel)* 12(10):2223
- Attia MF, Anton N, Wallyn J, Omran Z, Vandamme TF (2019) An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. *J Pharm Pharmacol* 71(8):1185–1198
- Baghbani F, Moztarzadeh F, Mohandesi JA, Yazdian F, Mokhtari-Dizaji M (2016) Novel alginate-stabilized doxorubicin-loaded nanodroplets for ultrasounic theranosis of breast cancer. *Int J Biol Macromol* 93(Pt A):512–519
- Bai M-Y, Yu M-H, Wang T-T, Chen S-H, Wang Y-C (2021) Plate-like alginate microparticles with disulfiram–SPIO–coencapsulation: an in vivo study for combined therapy on ovarian cancer. *Pharmaceutics* 13(9):1348
- Bansal D, Gulbake A, Tiwari J, Jain SK (2016) Development of liposomes entrapped in alginate beads for the treatment of colorectal cancer. *Int J Biol Macromol* 82:687–695
- Barczak M, Borowski P, Gila-Vilchez C, Alaminos M, González-Caballero F, López-López MT (2020) Revealing importance of particles' surface functionalization on the properties of magnetic alginate hydrogels. *Carbohydr Polym* 247:116747
- Batyrbekov YO, Rakhimbaeva D, Musabekov K, Zhubanov B (2009) Alginate based microparticle drug delivery systems for the treatment of eye cancer. *MRS Online Proc Library* 1209:304
- Bazak R, Hourri M, Achy SE, Hussein W, Refaat T (2014) Passive targeting of nanoparticles to cancer: a comprehensive review of the literature. *Mol Clin Oncol* 2(6):904–908
- Bidarra SJ, Barrias CC, Granja PL (2014) Injectable alginate hydrogels for cell delivery in tissue engineering. *Acta Biomater* 10(4):1646–1662
- Brown JM, Wilson WR (2004) Exploiting tumour hypoxia in cancer treatment. *Nat Rev Cancer* 4(6):437–447
- Bütün V, Armes S, Billingham N (2001) Synthesis and aqueous solution properties of near-monodisperse tertiary amine methacrylate homopolymers and diblock copolymers. *Polymers* 42(14):5993–6008
- Celep AGS, Demirkaya A, Solak EK (2022) Antioxidant and anticancer activities of gallic acid loaded sodium alginate microspheres on colon cancer. *Curr Appl Phys* 40:30–42
- Chan AW, Whitney RA, Neufeld RJ (2009) Semisynthesis of a controlled stimuli-responsive alginate hydrogel. *Biomacromolecules* 10(3):609–616
- Cheung RC, Ng TB, Wong JH, Chan WY (2015) Chitosan: an update on potential biomedical and pharmaceutical applications. *Mar Drugs* 13(8):5156–5186
- Choukaife H, Doolaanea AA, Alfatama M (2020) Alginate nanoformulation: influence of process and selected variables. *Pharmaceutics (Basel)* 13(11):335
- Correia CR, Sher P, Reis RL, Mano JF (2013) Liquified chitosan–alginate multilayer capsules incorporating poly (L-lactic acid) microparticles as cell carriers. *Soft Matter* 9(7):2125–2130
- Demaria M, O'Leary MN, Chang J, Shao L, Liu S, Alimirah F et al (2017) Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. *Cancer Discov* 7(2):165–176
- Din FU, Aman W, Ullah I, Qureshi OS, Mustapha O, Shafique S et al (2017) Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. *Int J Nanomedicine* 12:7291–7309
- Duo Y, Liu Q, Zhu D, Zhang B, Luo G, Wang F-B et al (2022) Proof of concept for dual anticancer effects by a novel nanomaterial-mediated cancer cell killing and nano-radiosensitization. *Chem Eng J* 429:132328
- Dusseau J, Tam SK, Ménard M, Polizu S, Jourdan G, Yahia LH et al (2006) Evaluation of alginate purification methods: effect on polyphenol, endotoxin, and protein contamination. *J Biomed Mater Res A* 76(2):243–251
- El-Deeb NM, Ibrahim OM, Mohamed MA, Farag MM, Farrag AA, El-Aassar M (2022) Alginate/k-carrageenan oral microcapsules loaded with *Agaricus bisporus* polysaccharides MH751906 for natural killer cells mediated colon cancer immunotherapy. *Int J Biol Macromol* 205:385–395

- Fenn SL, Miao T, Scherrer RM, Floreani RA (2016) Dual-cross-linked methacrylated alginate sub-microspheres for intracellular chemotherapeutic delivery. *ACS Appl Mater Interfaces* 8(28):17775–17783
- Ferreira NN, Ferreira LM, Miranda-Gonçalves V, Reis RM, Seraphim TV, Borges JC et al (2017) Alginate hydrogel improves anti-angiogenic bevacizumab activity in cancer therapy. *Eur J Pharm Biopharm* 119:271–282
- Gami F, Algethami N, Ragab H, Tarabiah A (2022) Structural, optical and electrical studies of chitosan/polyacrylamide blend filled with synthesized selenium nanoparticles. *J Mol Struct* 1257:132631
- Gao C, Tang F, Gong G, Zhang J, Hoi MPM, Lee SMY et al (2017a) pH-Responsive prodrug nanoparticles based on a sodium alginate derivative for selective co-release of doxorubicin and curcumin into tumor cells. *Nanoscale* 9(34):12533–12542
- Gao C, Tang F, Zhang J, Lee SM, Wang R (2017b) Glutathione-responsive nanoparticles based on a sodium alginate derivative for selective release of doxorubicin in tumor cells. *J Mater Chem B* 5(12):2337–2346
- George M, Abraham TE (2006) Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan—a review. *J Control Release* 114(1):1–14
- Gheorghita Puscaselu R, Lobiuc A, Dimian M, Covasa M (2020) Alginate: from food industry to biomedical applications and management of metabolic disorders. *Polymers (Basel)* 12(10):2417
- Godugu C, Patel AR, Desai U, Andey T, Sams A, Singh M (2013) AlgiMatrix™ based 3D cell culture system as an in-vitro tumor model for anticancer studies. *PLoS One* 8(1):e53708
- Gopishetty V, Tokarev I, Minko S (2012) Biocompatible stimuli-responsive hydrogel porous membranes via phase separation of a polyvinyl alcohol and Na-alginate intermolecular complex. *J Mater Chem B* 22(37):19482–19487
- Hackenhaar CR, Rosa CF, Flores EEE, Santagapita PR, Klein MP, Hertz PF (2022) Development of a biocomposite based on alginate/gelatin crosslinked with genipin for β -galactosidase immobilization: performance and characteristics. *Carbohydr Polym* 291:119483
- Hamman JH (2010) Chitosan based polyelectrolyte complexes as potential carrier materials in drug delivery systems. *Mar Drugs* 8(4):1305–1322
- Hanahan D (2022) Hallmarks of Cancer: new Dimensions. *Cancer Discov* 12(1):31–46
- Hosseinfar T, Sheybani S, Abdouss M, Hassani Najafabadi SA, Shafiee AM (2018) Pressure responsive nanogel base on alginate-cyclodextrin with enhanced apoptosis mechanism for colon cancer delivery. *J Biomed Mater Res A* 106(2):349–359
- Ishihara M, Kishimoto S, Nakamura S, Sato Y, Hattori H (2019) Polyelectrolyte complexes of natural polymers and their biomedical applications. *Polymers (Basel)* 11(4):672
- Jadid MFS, Shademan B, Chavoshi R, Seyyedsani N, Aghaei E, Taheri E et al (2021) Enhanced anticancer potency of hydroxytyrosol and curcumin by PLGA-PAA nano-encapsulation on PANC-1 pancreatic cancer cell line. *Environ Toxicol* 36(6):1043–1051
- Jahan ST, Sadat SMA, Walliser M, Haddadi A (2017) targeted therapeutic nanoparticles: an immense promise to fight against cancer. *J Drug Deliv* 2017:9090325
- Jahanban-Esfahlan R, Derakhshankhah H, Haghshenas B, Massoumi B, Abbasian M, Jaymand M (2020) A bio-inspired magnetic natural hydrogel containing gelatin and alginate as a drug delivery system for cancer chemotherapy. *Int J Biol Macromol* 156:438–445
- Jana S, Kumar Sen K, Gandhi A (2016) Alginate based nanocarriers for drug delivery applications. *Curr Pharm Des* 22(22):3399–3410
- Jayapal JJ, Dhanaraj S (2017) Exemestane loaded alginate nanoparticles for cancer treatment: formulation and in vitro evaluation. *Int J Biol Macromol* 105(Pt 1):416–421
- Jha A, Viswanadh MK, Burande AS, Mehata AK, Poddar S, Yadav K et al (2020) DNA biodots based targeted theranostic nanomedicine for the imaging and treatment of non-small cell lung cancer. *Int J Biol Macromol* 150:413–425
- Jhaveri J, Raichura Z, Khan T, Momin M, Omri A (2021) Chitosan nanoparticles-insight into properties, functionalization and applications in drug delivery and theranostics. *Molecules* 26(2):272

- Jia X, Pei M, Zhao X, Tian K, Zhou T, Liu P (2016) PEGylated oxidized alginate-DOX prodrug conjugate nanoparticles cross-linked with fluorescent carbon dots for tumor theranostics. *ACS Biomater Sci Eng* 2(9):1641–1648
- Keskin D, Zu G, Forson AM, Tromp L, Sjollem J, van Rijn P (2021) Nanogels: a novel approach in antimicrobial delivery systems and antimicrobial coatings. *Bioact Mater* 6(10):3634–3657
- Khodarahmi M, Abbasi H, Kouchak M, Mahdavinia M, Handali S, Rahbar N (2022) Nanoencapsulation of aptamer-functionalized 5-Fluorouracil liposomes using alginate/chitosan complex as a novel targeting strategy for colon-specific drug delivery. *J Drug Deliv Sci Technol* 71:103299
- Lacroce E, Rossi F (2022) Polymer-based thermoresponsive hydrogels for controlled drug delivery. *Expert Opin Drug Deliv*. <https://doi.org/10.1080/17425247.2022.2078806>
- Lakkakula JR, Gujarathi P, Pansare P, Tripathi S (2021) A comprehensive review on alginate-based delivery systems for the delivery of chemotherapeutic agent: doxorubicin. *Carbohydr Polym* 259:117696
- Lengert E, Saveleva M, Abalymov A, Atkin V, Wuytens PC, Kamyshinsky R et al (2017) Silver alginate hydrogel micro- and nanocontainers for theranostics: synthesis, encapsulation, remote release, and detection. *ACS Appl Mater Interfaces* 9(26):21949–21958
- Li S, Zhang H, Chen K, Jin M, Vu SH, Jung S et al (2022) Application of chitosan/alginate nanoparticle in oral drug delivery systems: prospects and challenges. *Drug Deliv* 29(1): 1142–1149
- Liu M, Zeng X, Ma C, Yi H, Ali Z, Mou X et al (2017) Injectable hydrogels for cartilage and bone tissue engineering. *Bone Res* 5(1):1–20
- Madeo LF, Sarogni P, Cirillo G, Vittorio O, Voliani V, Curcio M et al (2022) Curcumin and graphene oxide incorporated into alginate hydrogels as versatile devices for the local treatment of squamous cell carcinoma. *Mater Chem Phys* 15(5):1648
- Manivong S, Garcia Ac A, Patten SA, Fernandes JC, Benderdour M, Banquy X et al (2022) Chitosan-based nanogels: synthesis and toxicity profile for drug delivery to articular joints. *Nano* 12(8):1337
- Martão GA, Mihai M, Vodnar DC (2019) The use of chitosan, alginate, and pectin in the biomedical and food sector-biocompatibility, bioadhesiveness, and biodegradability. *Polymers (Basel)* 11(11):1837
- Matai I, Gopinath P (2016) Chemically cross-linked hybrid nanogels of alginate and PAMAM dendrimers as efficient anticancer drug delivery vehicles. *ACS Biomater Sci Eng* 2(2):213–223
- Matthews HK, Bertoli C, de Bruin RA (2022) Cell cycle control in cancer. *Nat Rev Mol Cell Biol* 23(1):74–88
- Mehata AK, Bharti S, Singh P, Viswanadh MK, Kumari L, Agrawal P et al (2019) Trastuzumab decorated TPGS-g-chitosan nanoparticles for targeted breast cancer therapy. *Colloids Surf B Biointerfaces* 173:366–377
- Mehata AK, Dehari D, Gupta A, Rabin DC, Miya A (2021) Multifunctional liquid crystal nanoparticles for cancer therapy. *Curr Nanomater* 6(1):4–16
- Mehata AK, Suseela MNL, Gokul P, Malik AK, Viswanadh MK, Singh C et al (2022) Fast and highly efficient liquid chromatographic methods for qualification and quantification of antibiotic residues from environmental waste. *Microchem J* 179:107573
- Mehata AK, Viswanadh MK, Priya V, Muthu MS (2020) Dendritic cell-targeted theranostic nanomedicine: advanced cancer nanotechnology for diagnosis and therapy. *Nanomedicine (Lond)* 15(10):947–949
- Mirrahimi M, Abed Z, Beik J, Shiri I, Dezfali AS, Mahabadi VP et al (2019) A thermo-responsive alginate nanogel platform co-loaded with gold nanoparticles and cisplatin for combined cancer chemo-photothermal therapy. *Pharmacol Res* 143:178–185
- Moghissi K, Dixon K (2017) Image-guided surgery and therapy for lung cancer: a critical review. *Future Oncol* 13(26):2383–2394
- Muthu MS, Mehata AK, Viswanadh MK (2017) Upconversion nanotheranostics: emerging designs for integration of diagnosis and therapy. *Nanomedicine (Lond)* 12(6):577–580

- Narendra, Mehata AK, Viswanadh MK, Sonkar R, Pawde DM, Priya V et al (2020) Formulation and in vitro evaluation of upconversion nanoparticle-loaded liposomes for brain cancer. *Ther Deliv* 11(9):557–571
- Niculescu A-G, Grumezescu AM (2022) Applications of chitosan-alginate-based nanoparticles—an up-to-date review. *Nano* 12(2):186
- Nobahari M, Shahanipour K, Fatahian S, Monajemi R (2022) Curcumin-loaded iron oxide nanoparticles coated with sodium alginate and hydroxyapatite and their cytotoxic effects against the HT-29 and MCF-7 cancer cell lines. *Jundishapur J Nat Pharm Prod* 17(1):e114572
- Norouzi M, Nazari B, Miller DW (2016) Injectable hydrogel-based drug delivery systems for local cancer therapy. *Drug Discov Today* 21(11):1835–1849
- Pan H, Zhang C, Wang T, Chen J, Sun SK (2019) In situ fabrication of intelligent photothermal indocyanine green-alginate hydrogel for localized tumor ablation. *ACS Appl Mater Interfaces* 11(3):2782–2789
- Pawar V, Borse V, Thakkar R, Srivastava R (2018) Dual-purpose injectable doxorubicin conjugated alginate gel containing polycaprolactone microparticles for anti-cancer and anti-inflammatory therapy. *Curr Drug Deliv* 15(5):716–726
- Pei M, Jia X, Zhao X, Li J, Liu P (2018) Alginate-based cancer-associated, stimuli-driven and turn-on theranostic prodrug nanogel for cancer detection and treatment. *Carbohydr Polym* 183:131–139
- Pelton R (2010) Poly(N-isopropylacrylamide) (PNIPAM) is never hydrophobic. *J Colloid Interface Sci* 348(2):673–674
- Peng N, Ding X, Wang Z, Cheng Y, Gong Z, Xu X et al (2019) Novel dual responsive alginate-based magnetic nanogels for onco-theranostics. *Carbohydr Polym* 204:32–41
- Peng N, Wu B, Wang L, He W, Ai Z, Zhang X et al (2016) High drug loading and pH-responsive targeted nanocarriers from alginate-modified SPIONs for anti-tumor chemotherapy. *Biomater Sci* 12:1802–1813
- Potaś J, Szymańska E, Winnicka K (2020) Challenges in developing of chitosan—based polyelectrolyte complexes as a platform for mucosal and skin drug delivery. *Eur Polym J* 140:110020
- Raji M, El Foujji L, Mekhzoum MEM, El Achaby M, Essabir H, Bouhfid R (2022) pH-indicative films based on chitosan–PVA/sepiolite and anthocyanin from red cabbage: application in milk packaging. *J Bionic Eng* 19(3):837–851
- Rezk AI, Obiweluozor FO, Choukrani G, Park CH, Kim CS (2019) Drug release and kinetic models of anticancer drug (BTZ) from a pH-responsive alginate polydopamine hydrogel: towards cancer chemotherapy. *Int J Biol Macromol* 141:388–400
- Rizg WY, Naveen NR, Kurakula M, Bukhary HA, Safhi AY, Alfayez E et al (2022) QbD supported optimization of the alginate-chitosan nanoparticles of simvastatin in enhancing the anti-proliferative activity against tongue carcinoma. *Gels* 8(2):103
- Sadeghianmaryan A, Naghieh S, Yazdanpanah Z, Sardroud HA, Sharma N, Wilson LD et al (2022) Fabrication of chitosan/alginate/hydroxyapatite hybrid scaffolds using 3D printing and impregnating techniques for potential cartilage regeneration. *Int J Biol Macromol* 204:62–75
- Sala P, Bogliolo S, Barra F, Fazio A, Maramai M, Cassani C et al (2022) Neoadjuvant chemotherapy followed by radical surgery versus concurrent chemo-radiotherapy in the treatment of locally advanced cervical cancer: a multicenter retrospective analysis. *J Investig Surg* 35(2):308–314
- Saralkar P, Dash AK (2017) Alginate nanoparticles containing curcumin and resveratrol: preparation, characterization, and in vitro evaluation against DU145 prostate cancer cell line. *AAPS PharmSciTech* 18(7):2814–2823
- Shaikh MAJ, Alharbi KS, Almalki WH, Imam SS, Albratty M, Meraya AM et al (2022) Sodium alginate based drug delivery in management of breast cancer. *Carbohydr Polym* 292:119689
- Shanmugapriya K, Kim H, Kang HW (2020) Epidermal growth factor receptor conjugated fucoidan/alginate loaded hydrogel for activating EGFR/AKT signaling pathways in colon cancer cells during targeted photodynamic therapy. *Int J Biol Macromol* 158:1163–1174

- Simpson J, Scholefield JH (2008) Treatment of colorectal cancer: surgery, chemotherapy and radiotherapy. *Surgery* 26(8):329–333
- Solano AG, Dupuy J, Theriault H, Liberelle B, Faucheux N, Lauzon M-A et al (2021) An alginate-based macroporous hydrogel matrix to trap cancer cells. *Carbohydr Polym* 266:118115
- Sonali, Viswanadh MK, Singh RP, Agrawal P, Mehata AK, Pawde DM et al (2018) Nanotheranostics: emerging strategies for early diagnosis and therapy of brain cancer. *Nano* 2(1):70–86
- Soni KS, Desale SS, Bronich TK (2016) Nanogels: an overview of properties, biomedical applications and obstacles to clinical translation. *J Control Release* 240:109–126
- Stillhano RS, Madrigal JL, Wong K, Williams PA, Martin PK, Yamaguchi FS et al (2016) Injectable alginate hydrogel for enhanced spatiotemporal control of lentivector delivery in murine skeletal muscle. *J Control Release* 237:42–49
- Sun X, Liu C, Omer AM, Yang LY, Ouyang XK (2019) Dual-layered pH-sensitive alginate/chitosan/kappa-carrageenan microbeads for colon-targeted release of 5-fluorouracil. *Int J Biol Macromol* 132:487–494
- Sun Z, Yi Z, Zhang H, Ma X, Su W, Sun X et al (2017) Bio-responsive alginate-keratin composite nanogels with enhanced drug loading efficiency for cancer therapy. *Carbohydr Polym* 175:159–169
- Szymańska E, Winnicka K (2015) Stability of chitosan-a challenge for pharmaceutical and biomedical applications. *Mar Drugs* 13(4):1819–1846
- Tam SK, Dusseault J, Bilodeau S, Langlois G, Hallé JP, Yahia LH (2011) Factors influencing alginate gel biocompatibility. *J Biomed Mater Res A* 98(1):40–52
- Tan WH, Takeuchi S (2007) Monodisperse alginate hydrogel microbeads for cell encapsulation. *Adv Mater* 19(18):2696–2701
- Tang L, Li J, Zhao Q, Pan T, Zhong H, Wang W (2021) Advanced and innovative nano-systems for anticancer targeted drug delivery. *Pharmaceutics* 13(8):1151
- Thai H, Thuy Nguyen C, Thi Thach L, Thi Tran M, Duc Mai H, Thi Thu Nguyen T et al (2020) Characterization of chitosan/alginate/lovastatin nanoparticles and investigation of their toxic effects in vitro and in vivo. *Sci Rep* 10(1):909
- Vakalopoulos I, Dimou P, Anagnostou I, Zeginiadou T (2015) Impact of cancer and cancer treatment on male fertility. *Hormones* 14(4):579–589
- Vasiliu S, Racovita S, Popa M, Ochiz L, Peptu CA (2019) Chitosan-based polyelectrolyte complex hydrogels for biomedical applications. In: Mondal MIH (ed) *Cellulose-based superabsorbent hydrogels*. Springer International Publishing, Cham, pp 1695–1725
- Vikas, Viswanadh MK, Mehata AK, Sharma V, Priya V, Varshney N et al (2021) Bioadhesive chitosan nanoparticles: dual targeting and pharmacokinetic aspects for advanced lung cancer treatment. *Carbohydr Polym* 274:118617
- Viswanadh MK, Muthu MS (2018) Targeted bioadhesive nanomedicine: an effective approach for synergistic drug delivery to cancers. *Nanomedicine (Lond)* 13(12):1401–1403
- Viswanadh MK, Vikas JA, Reddy Adena SK, Mehata AK, Priya V et al (2020) Formulation and in vivo efficacy study of cetuximab decorated targeted bioadhesive nanomedicine for non-small-cell lung cancer therapy. *Nanomedicine (Lond)* 15(24):2345–2367
- Wang J, Wang M, Zheng M, Guo Q, Wang Y, Wang H et al (2015) Folate mediated self-assembled phytosterol-alginate nanoparticles for targeted intracellular anticancer drug delivery. *Colloids Surf B Biointerfaces* 129:63–70
- Wang S-y, Li J, Zhou Y, D-q L, G-m D (2019) Chemical cross-linking approach for prolonging diclofenac sodium release from pectin-based delivery system. *Int J Biol Macromol* 137:512–520
- Wang Y, Zhou J, Qiu L, Wang X, Chen L, Liu T et al (2014) Cisplatin-alginate conjugate liposomes for targeted delivery to EGFR-positive ovarian cancer cells. *Biomaterials* 35(14):4297–4309
- World Health Organization (2020) Cancer. <https://www.who.int/news-room/fact-sheets/detail/cancer>
- Wirsdörfer F, De Leve S, Jendrosseck V (2018) Combining radiotherapy and immunotherapy in lung cancer: can we expect limitations due to altered normal tissue toxicity? *Int J Mol Sci* 20(1):24

- Wulf A, Mendgaziev RI, Fakhrullin R, Vinokurov V, Volodkin D, Vikulina AS (2022) Porous alginate scaffolds designed by calcium carbonate leaching technique. *Adv Funct Mater* 32(14): 2109824
- Xu D, Li E, Karmakar B, Awwad NS, Ibrahim HA, Osman H-EH et al (2022) Green preparation of copper nanoparticle-loaded chitosan/alginate bio-composite: investigation of its cytotoxicity, antioxidant and anti-human breast cancer properties. *Arab J Chem* 15(3):103638
- Xu D, Qiao T, Wang Y, Wang QS, Cui YL (2021) Alginate nanogels-based thermosensitive hydrogel to improve antidepressant-like effects of albiflorin via intranasal delivery. *Drug Deliv* 28(1):2137–2149
- Xu X, Bi D, Wan M (2016) Characterization and immunological evaluation of low-molecular-weight alginate derivatives. *Curr Top Med Chem* 16(8):874–887
- Yamaguchi K, Hiraike O, Iwaki H, Matsumiya K, Nakamura N, Sone K et al (2021) Intraperitoneal administration of a cisplatin-loaded nanogel through a hybrid system containing an alginic acid-based nanogel and an in situ cross-linkable hydrogel for peritoneal dissemination of ovarian cancer. *Mol Pharm* 18(11):4090–4098
- Yi H, Wu LQ, Bentley WE, Ghodssi R, Rubloff GW, Culver JN et al (2005) Biofabrication with chitosan. *Biomacromolecules* 6(6):2881–2894
- Yoncheva K, Merino M, Shenol A, Daskalov NT, Petkov PS, Vayssilov GN et al (2019) Optimization and in-vitro/in-vivo evaluation of doxorubicin-loaded chitosan-alginate nanoparticles using a melanoma mouse model. *Int J Pharm* 556:1–8
- Younes M, Aggett P, Aguilar F, Crebelli R et al (2017) Re-evaluation of alginic acid and its sodium, potassium, ammonium and calcium salts (E400–E404) as food additives. *EFSA J* 15(11): e05049
- Yu B, Tai HC, Xue W, Lee LJ, Lee RJ (2010) Receptor-targeted nanocarriers for therapeutic delivery to cancer. *Mol Membr Biol* 27(7):286–298
- Yu HP, Aljuffali IA, Fang JY (2017) Injectable drug-loaded nanocarriers for lung cancer treatments. *Curr Pharm Des* 23(3):481–494
- Zhuang Y, Kan Z, Jiang Y, He M, Zhang Y, Sun X et al (2022) Constructing an on-demand drug release system composed of thermosensitive PPP hydrogel and drug-laden alginate/graphene microspheres to treat tumorous defect. *J Mater Sci Mater Electron* 57(7):4754–4770

Alginate Carriers in Wound Healing Applications



Lisette Agüero and Marcos L. Dias

Abstract Daily life activities exposure our body to injury, fire, insect bites, and diverse domestic or traffic accidents. On answer to damage caused, the wound healing process is automatically activated to restore natural biological functions, but in many cases professional health care treatment is required. Alginate wound dressing is so far the most advantageous and flexibility material to care infected wound, being the most commercial available dressing. In this chapter, we expose and discuss the unique properties and functionality of alginate polysaccharide as a carrier in wound healing management. The recent innovative solutions concerning to enhancing simultaneously mechanical and antimicrobial properties are also summarized. At the same time, alternatives to decrease antimicrobial resistant emphasizing in versatile combinations of fibers, polymers and bioactive agents as potential biomimetic strategy is highlighted. Finally, some physicochemical and biological assays are presented to make evaluation process more rational, selective, efficient and cost-effective.

Keywords Alginate · Wound healing · Fiber · Antibiotic resistance · Drug release

1 Introduction

Wound is a disruption of the integrity of anatomical human tissue, including skin, mucous membrane and organ tissues. It is caused by diverse sources such as insect bites, surgical incisions, infections, burns, among others. There are many classifications systems for wound based on their location, etiology, nature of injury, depth,

L. Agüero (✉)

Departamento de Biomateriales Poliméricos, Centro de Biomateriales, Universidad de La Habana, La Habana, Cuba
e-mail: lisette@biomat.uh.cu

M. L. Dias

Universidade Federal do Rio de Janeiro, Instituto de Macromoléculas Professora Eloisa Mano, Rio de Janeiro, RJ, Brazil

and their appearance (Kuznetsova et al. 2020). For example, wounds are generally classified as wounds without tissue loss (e.g., in surgery) and wounds with tissue loss such as burn wounds. Another terminology is based on the layers involved: superficial wounds that cover only the epidermis, partial thickness wounds that include only the epidermis and dermis, while full-thickness wounds involve only the subcutaneous fat or deep tissue. According to US Centers for Disease Control and Prevention there are four classes: Class 1 corresponds to wounds considered to be clean (uninfected, no inflammation is presented, and are primarily close); Class 2 is considered to be clean-contaminated (lack unusual contamination, e.g., urinary tract); Class 3 is attributed to wound to be contaminated (fresh, open wound or leakage from the gastrointestinal tract); and Class 4 that is considered to be dirty-infected (from improperly cared for traumatic wounds) (Herman and Bordoni 2021). Although it is very difficult to find a common wound classification in the literature, all of them are directed to identify the proper treatment and improve personalized patient care.

In the same sense, wound healing is a complex and dynamic biological process related with repair and tissue regeneration of damaged wound. It involves a series of events as hemostasis, inflammation, proliferations and remodeling through interactions of immune cells (neutrophils, monocytes, lymphocytes, macrophages), non-immune cells (fibroblast, endothelial, keratinocytes), soluble mediators (cytokines, growth factor), and extracellular matrix components (Norouzi et al. 2015; Stojadinovic et al. 2008). The effective reparative process of damaged wound should be achieved in the shortest time to prevent infections and minimize pain, discomfort and scar. In that direction, the evolution of wound healing materials began in the use grease, waxes and plasters as bandages, continuing by gauze-cotton/gauze-composite products, and finally with the expansion to natural and synthetic polymers. Among the numerous materials used during this trajectory, the intrinsic and beneficial properties of polysaccharides (e.g., alginate, chitosan, cellulose and collagen) offer numerous advantages making them preferred to design and produce biomaterials for wound healing (Pan et al. 2021; Sahana and Rekha 2018).

2 Alginate Properties in Wound Healing

Alginate is a term that includes alginic acid and its salts, being sodium alginate the most used in biomedical applications. This biopolymer from marine brown algae is composed of alternating blocks of α -L-guluronic acid (G) and β -D-mannuronic acid (M) residues in varying proportions and arrangements. The versatile properties of alginate such as biocompatibility, natural hemostat, and high absorption capacity remark its potentiality as biomaterial in different areas (Alvarez-Lorenzo et al. 2013; Spadari et al. 2017).

The material used in wound management demands specific requirements for effective therapy. As shown in Fig. 1, alginate possess attractive and versatile properties that statement its preference in wound healing treatments, being the

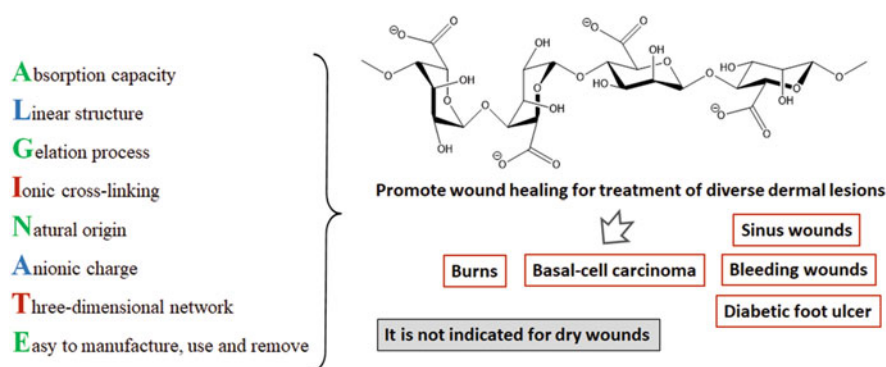


Fig. 1 Schematic representation of alginate chemical structure and its distinctive properties to promote effective wound healing in different dermal lesions

most commercially-available biomaterial in this field (Agüero et al. 2017; Barbu et al. 2021):

- **High absorption capacity:** As hydrogel due to the presence of abundance carboxyl (-COOH) and hydroxyl (-OH) groups in its chemical structure (Fig. 1), it can swell with the absorption of large quantity of water or biological fluids, stopping bleeding and absorbing exudates. It is an essential property for wound healing applications, which allow to maintain adequate physiological environment avoiding dehydration of wound dressing, defending the tissue to the invasion by microorganism and diminishing the pain throughout exchange. Moreover, it contributes to keep cooling the wound with a marked reduction of pain and therefore high treatment acceptability.
- **Gelation process:** In presence of multivalent cations, mainly Ca^{2+} ions, alginate forms instantaneous gel structure with diverse geometries in aqueous medium, at room temperature and gentle stirring. These ecofriendly conditions provide easy manufacture of alginate wound-care products with good cost-effective, whereas the variety of dressing format offers a wide range of use in specific anatomical areas.
- **Ionic crosslinking:** The ionic bridges between multivalent cations and guluronic blocks (G) appears forming well-known “egg box” architecture, which is responsible to retain wet integrity during functional action. Besides that, porosity material formed provides adequate water vapor and oxygen exchange across the wound surface.
- **Natural origin:** It is abundant renewable resources from sea, which reduces production and commercialization cost, and with the added benefit for tourism sector by the concept to clean the excess of Sargassum (Sargasso Sea) that arrive in the Caribbean beaches.
- **Anionic charge:** Polyelectrolyte complexes are formed by interaction of anionic charge of alginate and cationic macromolecules such as chitosan, cationic

peptides and ammonium quaternary copolymers enhancing or incorporating new properties (e.g., mechanical or biological).

- **Three-dimensional network (3D):** This conformation creates adequate reservoir to encapsulate active compounds and allows cells growth with beneficial effect on tissue regeneration and wound healing.
- **Easy to apply and remove:** The wound healing process is very painful because it involves damage to sensory nerves. Soft consistency of alginate hydrogel leads easy handling permitting its application in a precisely controlled manner, and it can be easily removed by saline irrigation without damage of the newly formed tissue.

Other relevant properties include biocompatibility, solubility in cold water, degradability, and it can be manufactured under sterile conditions. Alginate-based wound dressing is not indicated for dry wounds due to impractical action, since in this situation dressing will require constantly rehydration.

3 Alginate Action in Wound Dressing

The wound-care products are the most extensively researched and commercially exploited to date (Stevens and Chaloner 2005), being alginate hydrogel widely used in multipurpose wound dressing. Traditional wound dressings such as cotton wool and gauzes differ in their grade of absorbency, with a primary function to protect wound against opportunistic pathogens (Yeung and Kennedy 2019). Their benefits are easy to handle, great ability to absorb fluids and reasonable price, but it has a lot of inconvenience for wound management associated to rapid dehydration of wound surface, peeling, damage of newly formed epithelium during removal process and they are not effective against the bacterial contamination (Memci et al. 2019; Abasalizadeh et al. 2020).

In contrast, alginate-based wound dressing maintains the moisture of the wound, allow to oxygen exchange, absorb wound exudates, accelerate granulation and re-epithelialization, reduce pain, odor and healing time, and prevent bacterial infections (Zhang et al. 2021). When Ca-alginate dressing is directly applied to wound, it is transformed into soft gel by exchange of significant proportions of the calcium ions with sodium ions present in exuding or infected wound. The progressive absorption of exudate causes swelling and expansion of network trapping the bacteria within as partial arrangement, resulting in a reduction of infection and the state of inflammation in the wound. The calcium released during process acts as a hemostatic agent.

The ionic crosslinking is frequently used to obtain Ca-alginate dressing by dropping the water-sodium alginate solution into a bath containing calcium ions through fine nozzles (Goh et al. 2012). Although strontium and barium form stronger gel, calcium chloride as a source of Ca^{2+} ion is more regularly used due to its biocompatibility and biological role in the human organism. On the other hand,

different compositions and arrangements are obtained for alginate from several species of brown algae, which determinate gel formation, its use according to dermal lesion and the removal process. Based on this, modulating composition and concentration of alginate as well as content of crosslinking agent, alginate dressings are manufactured in different presentations such as hydrogel, films, hydrocolloids, foams nanofibers, membranes and sponges (Kuznetsova et al. 2020). For instance, dressings fabricated using alginate rich in G-blocks [e.g., *Laminariahyperborea* algae, 70% of G content (Paul and Sharma 2015)] react readily with sodium ions and forms stiffer, brittle and mechanically more stable gels. Taking into consideration the greater interaction of G-blocks with crosslinker, a lower content of calcium chloride is required during technological process. Contrarily, dressings obtained from alginate with a higher number of M-blocks [e.g., *Macrocystispyrifer* algae, 61% of M content (Paul and Sharma 2015)] have higher elasticity and more soft consistency. In this case, higher Ca^{2+} content is employed in manufacture process. Another important parameter in terms of dressing fabrication is molecular weight by approach that its increase can improve the physical properties of gel (Paques et al. 2014). Nevertheless, excessive high molecular weight in alginate solution leads to extreme viscosity, which could be cause obstruction of equipment, make more difficult to dissolve alginate and to obtain continuous fiber by electrospinning method. The fusion of a high and low molecular weight alginate polymer is an alternative to alleviate this limitation (Cattelan et al. 2020).

The relative proportions and distribution of M/G units in alginate has also influence in its use according to characteristics of wound. Wound could be shallow or deep (cavities), large, narrow or small, with a wide variety of secreting lesion, presence of bleeding, and acute or chronic. Thus, depending on the condition of the wound the healthcare professional personnel will select adequate dressing. For example, flat sheets and pads can be used for superficial or large wounds, whereas rope, packing and ribbon can be used for cavities and narrow wounds. Alginates with high relation M/G provide elastic gels that allow adjustment different forms and dressing texture (Łabowska et al. 2019).

In the case of relationship between composition and removal process, pain is a critical point to be considered. Pain is one of the most debilitating symptoms of patient suffering from chronic and post-surgical wounds, especially during wound debridement and dressing exchange (e.g., burn wounds). It causes discomfort, stress, anxiety and poor quality of life to the patient. Dressing with higher content of M-blocks is more absorbent material with soft texture as consequence is more easy to removal by saline irrigation (e.g., SorbsanTM). If the wound is highly exudative, this type of dressing requires more frequent changing. The use of high G content in dressing fabrication leads a firmer structure, which can it removal intact from infected wound (e.g., Kaltostat[®]). Nevertheless, the nursing personnel recommend to irrigate with water or sterile saline solution to reduce skin irritation.

4 Alginate as Bioactive Agent Carrier

A facile method to fabricate 3D structure of Ca-alginate hydrogels in favorable conditions leads alginate as a promising carrier technology with desirable controlled and sustained drug delivery. Growth factors, nucleic acids and several hydrophilic and hydrophobic drugs have been released successfully from alginates matrices with a high stability during the processing and storage stages.

Since infection is the most common complication in a wound, bioactive agent with antimicrobial properties constitutes a vital component in therapeutic formulations. Numerous pathogenic bacteria are frequent in wound infections including *Staphylococcus aureus* (*S. aureus*), *Streptococcus pyogenes* (*S. pyogenes*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Proteus mirabilis* (Kaiser et al. 2021). Microbial contamination of the wound can occur in a fraction of seconds, and its ability to persist for long period of time within the wound impacts negatively on repair processes. Although, some studies report the antimicrobial activity of alginate polysaccharide, it has insufficient bioactive action during wound healing process, especially in chronic wound (Raus et al. 2021). Several materials as silver, copper, antibiotics, honey and growth factors are incorporated into alginate dressing aiming to restore the damaged site.

4.1 Antimicrobials

4.1.1 Silver

Silver ions have a long history as an effective antimicrobial agent against a wide range of bacteria, fungi and certain viruses, even at low concentration (Liang et al. 2022; Chen and Schluesener 2008). Silver nitrate, silver sulfadiazine and colloidal silver in monoatomic ionic state (Ag^+) have been frequently used to treat wounds. There are three different modes of this action: (1) silver cations can form pores and puncture the bacterial cell wall by reacting with the peptidoglycan component; (2) silver ions can enter into the bacterial cell, both inhibiting cellular respiration and disrupting metabolic pathway resulting in generation of reactive oxygen species, and (3) once in the bacterial cell, silver ions disrupt DNA and its replication cycle. (Sim et al. 2018). The silver incorporated into alginate wound dressings has demonstrated a highlighting bioavailability of ionic silver in adequate periods of time. In fact, silver alginate wound dressing have greatly improved in efficacy compared to standard dressing. In an interesting work, Hooper et al. examined and compared the antimicrobial properties of commercial RESTORE silver alginate dressing with an equivalent silver-free alginate dressing. Nine typical wound microorganism including *S. aureus*, *S. pyogenes*, *Candida albicans* (*C. albicans*) and *Escherichia coli* (*E. coli*) were tested by combination of in vitro culture ($\log_{10}$ reduction and COZI assays) and confocal laser scanning microscopy. Compared with the silver-free

dressings, log₁₀ reduction assay showed an excellent antimicrobial activity of RESTORE silver alginate dressing. Moreover, it had activity against all Gram-positive, Gram-negative and fungi tested in a range of 5 to 12 days. Confocal laser scanning microscopy as a complementary tool confirmed visualization the bacteria sequestered inside of dressing and demonstrated the broad-spectrum antimicrobial effect of silver ions. (Hooper et al. 2012). Extending this work, Percival's group evaluated antimicrobial efficacy of a silver alginate dressing in 115 wound isolates routinely from intensive care patients at West Virginia University Hospital. The authors found that silver alginate dressing was effective inhibiting the growth of Gram-positive and Gram-negative bacteria, but it was more effective on Gram-positive bacteria. Additionally, silver alginate dressing confirmed antimicrobial efficacy within the biofilm phenotypic state, which is more resistant to antimicrobial action (Percival et al. 2011).

The initial use of silver as silver nitrate solution or silver sulfadiazine cream in the treatment of burn wounds was not completely satisfactory, since it required repeated application and failed providing a sustained antimicrobial activity. In that direction, Opananon et al. evaluated clinical efficacy of AskinaCalgitrol Ag[®] as alginate silver dressing in patients with partial-thickness burn wounds, using as a control patients treated with 1% silver sulfadiazine (topical administration). The results showed that patient treated with AskinaCalgitrol Ag[®] had lower number of wound dressing change, decreased of level of pain and nursing time, as well as exhibited shorter healing time (7 days) in comparison with control group (14 days) (Opananon et al. 2010). For this reason, the incorporation silver compounds in micro and nanoparticles afforded as a promising drug delivery strategy. Submicron architecture as beneficial innovation provides prolonged bioactive agent release, offers protection of drug sensitive to light (e.g., prevent oxidation of silver), as well as the possibility of different silver combinations such as colloidal silver, which is a mixture of silver ions and silver nanoparticles suspended in an aqueous medium. In a representative work, Sharma et al. incorporated silver nanoparticles in sodium alginate-chitosan film as potential antibacterial platform. The blended film was found to be highly effective against Gram-positive and Gram-negative bacteria tested, with more activity against Gram-positive bacteria. The combination of alginate with chitosan contributed to enhance mechanical properties by polyelectrolyte complex formation (Sharma et al. 2012). For the same purpose, Singh and Singh reported the synthesis of polyvinyl pyrrolidone/alginate hydrogel containing nanosilver by gamma radiation, as a clean method for wound dressing fabrication. The antimicrobial efficacy of hydrogel with different concentrations of nanosilver was tested by log reduction assay. The results showed strong antimicrobial effect and complete inhibition of microbial growth at 70 ppm nanosilver concentration against *P. aeruginosa*, *S. aureus* (bacteria) and *C. albicans* (fungi) (Singh and Singh 2012). Actisorb[®] and Acticoat[®] are examples of alginate commercial wound dressing containing silver nanoparticles (Sim et al. 2018; Atiyeh et al. 2007).

The introduction commercial antibiotics in the clinical practice replaced the use of silver in this field, but the increasing prevalence of bacterial resistance to antimicrobial agents returned the silver's use as an essential therapeutic option

(Möhler et al. 2017). Silver has demonstrated antimicrobial activity including antibiotic-resistant bacteria (Lansdown 2002; Percival and McCarty 2014).

4.1.2 Zinc

Zinc is an important micronutrient with extent multifaceted crucial functions for human health related to bone metabolism, the central nervous system, wound healing, among others. It constitutes an important component in cell physiology, generates immuno-modulatory and antimicrobial effects, as well as the activation of matrix metalloproteinase, which facilitates auto debridement and keratinocyte migration during wound repair (Qin 2008). The zinc sulfate (ZnSO_4) and zinc oxide (ZnO) have been widely used in wound healing by topical formulation due to its antioxidant and antibacterial properties. Other form of application includes a solution of zinc chloride (ZnCl_2), which is indicated in total parenteral nutrition to maintain zinc levels. Deficiency of zinc in human body can manifest clinically as many disorders including renal disease, mental and growth retardation, dermatitis and impaired wound healing. Indeed, zinc deficiency may decrease rate of fibroplasia, epithelialization, and collagen synthesis; compromises wound strength and immune response, as consequence increase the patient's susceptibility to skin breakdown (Kogan et al. 2017). Although, the common cause of zinc deficiency is malnutrition, the exudate from wound produces loss of zinc. Thereby, zinc supplementation is used to enhance healing in zinc-deficient patients, and it is included in daily diet as a standard regimen for severe burn. On the other hand, dressing impregnated with zinc can be a potential option for patients that experiments allergic reactions of silver sulfadiazine in burns treatments or are sensitive to silver.

Zinc oxide is recognized as safe by US Food and Drug Administration and various studies have been revealed that combination biopolymers with zinc oxide nanoparticles leads effective antimicrobial capacity (Lin et al. 2018). In a representative work, Mohandas et al. prepared a composite bandage formed between alginate and zinc oxide nanoparticles by freeze-dry technique. The porous ZnO -composite bandage showed controlled degradation profile and faster blood clotting ability in comparison with commercial Kaltostat[®] dressing and bandages without ZnO , which was used as control. The dressing prepared exhibited excellent antimicrobial activity against *S. aureus*, methicillin resistant *S. aureus*, *C. albicans* and *E. coli* (Mohandas et al. 2015). In another work, Raguvaran et al. synthesized zinc oxide nanoparticles-loaded sodium alginate-gum acacia hydrogels as potential wound healing biomaterial. Antimicrobial properties and biocompatibility of designed hydrogel were tested on *P. aeruginosa*, *Bacillus cereus* and peripheral blood mononuclear/fibroblast cells, respectively. The authors found that hydrogel matrix containing zinc oxide nanoparticles preserved the antibacterial and healing effects, while a high concentration of zinc oxide were toxic to cells. The incorporation zinc oxide nanoparticles within sodium alginate-gum acacia hydrogels significantly reduced this toxicity (Raguvaran et al. 2017). In a recent work, Cleetus et al. prepared 3D printed alginate composite containing zinc oxide nanoparticles for antibacterial purpose. Bacterial

resistance tested on *Staphylococcus epidermidis* indicated that the addition of zinc oxide nanoparticles into alginate gel decreased bacterial growth compared to free-alginate gel. The authors concluded that the incorporation zinc oxide nanoparticles had dual action due to enhanced of mechanical properties composite, which is beneficial for retention of structural stability (Cleetus et al. 2020). Curasorb[®] Zinc is an example of alginate commercial dressing impregnated with zinc solution, while Dermagran[®] Hydrophilic is supply in zinc ointment formulation (Lansdown et al. 2007).

4.1.3 Antibiotics

Since the discovery penicillin as a first documented antibiotic, the antimicrobial agents save many patient's lives each year. Nevertheless, it is well known the emergence of bacterial resistance to antimicrobial drugs has become a serious problem in health care. Based on their high capacity to inhibit growth or kill a broad range of microorganisms and with the intention to solve this problem, the initial action was focused on finding new sources of antibiotics. In that line, the progress in organic synthesis field has been provided an extent antibiotic family such as Cephalosporins, Tetracyclines and Quinolones. In all cases, the core structure of each family remains intact preserving its biological activity, while the external functional groups have been modified to improve its properties, emerging until fourth-generation molecules (Fischbach and Walsh 2009). But, slow development of new antibiotics to replace those that become ineffective (Carmona-Ribeiro and de Melo Carrasco 2013) and its indiscriminate use lead to date an increase bacterial resistance (Zilberman and Elsner 2008).

Antibiotics therapy is frequently used in wound care, taking into consideration that wound infections to be usually polymicrobial and require a broad-spectrum of antibiotics by topical and systematic administration. In particular, biofilm formation as a local manifestation of wound infection is strongly adherent to the surrounding tissue, impeding adequate concentration of antimicrobial agent at the infected site, as consequence prolonging the wound healing period. Thus, the compact nature of biofilm structure leads extremely resistant to immune system and antibiotics, where bacteria within biofilm are up to 1000 times more resistant to conventional antimicrobial agents. Additionally, many antibiotics cannot be used in chronic wounds treatment due to their toxicity on human tissues.

A critical aspect of diagnosis to wound is determine when the infection is localized or systematic. There are many factors that influences in the risk of infection, and inadequate management results in fibrosis, chronic wound and loss of tissue functioning or amputation. In early stage of infection (minor wound infection) it may be enough to keep wound clean, dry and apply an antibiotic ointment or cream as local therapy. Localized antimicrobial delivery are preferred because of maintain higher antibiotic concentration in infected site for a long period, reducing the duration of treatment and avoid systematic toxicity, which results in health and economic benefits for patient. Antibiotic available for topical therapy

includes aminoglycosides (neomycin and gentamicin), sulfonamides (sulfacetamide sodium), polypeptides antibiotics (bacitracin and tyrothricin), and metronidazole. Nevertheless, the persistence of inflammation, swelling, erythema and pain are indicative the higher level of infection, being necessary combined antimicrobial therapy.

The common antibiotic pathogens resistant in wound treatments cover methicillin/oxacillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and extended-spectrum β -lactamases (ESBLs). In order to reduce abuse of antibiotics and minimize the risk of antibiotics resistance, different strategies should remark our attention: (1) maximizing topical delivery using silver and/or zinc; (2) the encapsulation of antibiotic molecules in hydrogel platforms with various morphologies; and (3) the combination antibiotic with silver and/or zinc, and natural materials within wound dressing. For example, Severino et al. encapsulated semi-synthetic polymixin B sulfate antibiotic into solid lipid nanoparticles using anionic sodium alginate. The polyelectrolyte complex formed by ionotropic gelation increased the lipophilicity of polymixin B sulfate and showed to be less toxic than free-antibiotic after tested on HaCat and NIH/3T3 cell lines. Moreover, solid lipid nanoparticles containing semi-synthetic antibiotic exhibited to enhance minimal inhibitory concentration on *P. aeruginosa* test strains (Severino et al. 2015). On the other hand, natural materials such as honey, plant extracts and essential oils have been also added into alginate matrices to kill and inhibit the bacterial growth. Gómez Chabala et al. reported alginate-chitosan membranes impregnated with silver nanoparticles and *Aloe vera* by immersion technique. The antibacterial capacity against *S. aureus* and *P. aeruginosa* was evaluated by Kirby Bauer disk diffusion method using five blanks: unloaded hydrophilic matrix, *Aloe vera*, silver nanoparticles and two antibiotics (gentamicin and tetracycline). The results showed an increase of antimicrobial activity with presence of *Aloe vera* in all test. The combination of silver nanoparticles and *Aloe vera* exhibited an inhibitory capacity greater than gentamicin and closer to tetracycline (Gómez Chabala et al. 2017). In addition, natural products aim to reduce antibiotic resistant by its use as topical treatment through gel, cream or ointment formulations. Especially case corresponds to burn wounds, where the complete healing process could be extremely large period and the skin remains sensible to light and moderate temperatures during months or years. *Aloe vera* has been demonstrated wound healing and anti-inflammatory properties, allowing to complete treatment with excellent results, and avoids excess use the antibiotic agents.

4.2 Growth Factors

Growth factors are natural polypeptides secreted by cells, and responsible to promote various cellular process. There is no doubt respect to the crucial role of growth factors in the adequate wound management, being essential in the recruitment of inflammatory cells to the wound, cellular influx for local debridement, proliferation

of fibroblast, formation of granulation tissue, collagen and extracellular matrix, among others (Caetano et al. 2014; Norouzi et al. 2015). Nevertheless, systematic administration of growth factors is considered unpractical due to their extremely low bioviability and stability in parenteral route, requiring high doses administration during clinical treatment. Their application is also associated with the elevated pressure blood and thrombolytic events. Thereby, taking advantages of microencapsulation techniques alginate delivery systems have been developed to encapsulate growths factors (Bahadoran et al. 2020):

- Vascular endothelial growth factor (VEGF) is a significant promoter of endothelial proliferation and angiogenesis. It has low permeation and a high concentration of VEGF in human body causes vascular leakage, being necessary its controlled and sustained release. In order to reach controlled release of VEGF, Song et al. produced alginate/silk fibroin fibers for wound healing employing a novel wheel spinning method. Alginate/silk fibroin fibers released VEGF in controlled manner in comparison with VEGF loaded-alginate fibers and VEGF loaded-alginate/silk microspheres (Song et al. 2021). In another study, Chou et al. combined alginate and Pluronic to develop interpenetrating polymeric network (IPN) template as drug delivery platform. The authors found that IPN provided soft and elastic thermosensitive structure able to retain their form even after absorbing a large amount of wound exudate. Calcium alginate network as external layer promoted the stability of VEGF and controlled its release during topical application in wound. The growth factor delivered from IPN template stimulated vascular endothelial cell proliferation and promoted granulation tissue formation for wound recovering (Chou et al. 2020).
- Basic fibroblast growth factor (bFGF) is a representative member of the heparin-binding growth factors and plays an important role in stimulation of cell growth and tissue repair. It is fragile in physiological conditions, requiring protection for avoid premature inactivation. In an original work, Tanihara et al. fabricated a novel artificial extracellular matrix by covalent crosslinking of alginate and heparin in presence of ethylenediamine. The results demonstrated that hydrogel released bFGF for 1 month in physiological conditions and simulated the regeneration of skin (Tanihara et al. 2001). Additionally, systematic administration of bFGF causes unexpected adverse effects, being necessary its slowly release. Based on this criterion, Downs et al. entrapped proteins growth factors (acidic FGF and bFGF, epidermal growth factor) into alginate beads to provide slow release delivery system. In all cases the biological activity of the proteins was retained and more bFGF was entrapped within beads due to its higher positive charge able to interact with anionic alginate. In an in vivo murine model, all angiogenic factors in alginate beads caused quantifiable neovascularization when were injected subcutaneously (Downs et al. 1992).
- Epidermal growth factor (EGF) is involved in keratinocyte migration, fibroblast proliferation and differentiation, as well as granulation tissue formation. It is susceptible to proteolytic degradation losing its bioactivity, and adequate EGF release rate minimize scar formation. Considering previous studies carried out in

their scientific group, Jeong et al. prepared gelatin-alginate coacervates containing EGF to accelerate diabetic foot ulcer healing. The efficacy of growth factor was evaluated with respect to the viability and migration of keratinocytes on HaCat cell, as well as using the streptozotocin-induced diabetic mouse model. The results showed that EGF-polymer coacervates enhanced in vitro migration of keratinocytes, accelerated wound healing in streptozotocin-induced diabetic mouse, and reduced level of proinflammatory cytokines IL-1, IL-6 and THF- α (Jeong et al. 2020). In another work, Hu et al. prepared a 3D porous *N*-carboxylmethyl chitosan/alginate hydrogel for effective protein delivery. Dual crosslinking by polysaccharides electrostatic interaction and divalent calcium chelation allowed achieve similar mechanical properties to skin tissue. Good swelling, slow release of EGF, an increase of cell proliferation, and wound closure in vivo experiments were appreciated from different assays (Hu et al. 2018).

- Platelet-derived growth factor (PDGF) induces angiogenic effect, mitogen for connective cells, and vessel maturation. In diabetic foot ulcers treatments, it needs to be released internally in the wound at specific periods for its effectiveness. In a representative work, Nardini et al. fabricated a freeze-dried sponge by combination alginate, sericin and platelet lysate as bioactive wound dressing. The highest level of the growth factor release occurred within 48 h, and delivery process was modulated by presence of sericin due to its interaction with protein. The platelet lysate loaded-biomembrane formed was biocompatible (cytotoxicity test), capable to protect cells against oxidative stress and induced proliferation. Moreover, in a mouse skin lesion model, the freeze-dried sponge containing platelet lysate induced and accelerated formation of granulation tissue and new collagen deposition, providing a more rapid skin regeneration (Nardini et al. 2020).

Examples of commercial products are Fiblast® (bFGF, spray solution), Regranex® (PDGF, topical gel), Heberprot-P® and Easyef® (EGF, lyophilized powder and spray solution/ointment, respectively).

As mentioned before, wound healing is a complex and high coordinate process where intervene multiple biological factors. Indeed, an insufficient healing rate (chronic wound), an excessive healing (scar tissue formation), an inadequate angiogenic response, as well as the alteration in balance of extracellular matrix components are some pathological deficiencies of wound healing process. In this respect, the combined therapy is more effective reducing risk of side effects, and providing inherent advantages in wound healing management. For example, in an original and advanced work, Lin et al. combined polysaccharides, techniques and bioactive agents to prepare biocompatible hydrogels as drug codelivery system. The hydrophilic double membrane formed by chemically modified cellulose nanocrystals as the inner layer, and sodium alginate as external layer showed controlled and sustained drug release. Antibiotic ceftazidime hydrate was incorporated in the alginate external layer for rapid release, while EGF was added in the internal membrane for prolonged release. Scanning electron microscopy characterization confirmed that polysaccharides combination enhanced structural stability through electrostatic interaction

between cationic cellulose nanocrystals and anionic alginate (Lin et al. 2016). In another interesting and extent work, advanced bioactive alginate-based dressing was development by Ahmed et al. For achieve effective treatment of common infection in diabetic foot ulcers, ciprofloxacin as antibacterial and fluconazole as antifungal agents were combined within films and lyophilized wafers. Evaluation of designed systems by turbid metric, and Kirby Bauer disk diffusion method demonstrated completely eradication of *E. coli*, *S. aureus* and *P. aeruginosa*, and reduction of *C. albicans* within 24 h. Moreover, drug combination supported its use against mixed infections (*C. albicans* + *E. coli*, *C. albicans* + *S. aureus*, *C. albicans* + *P. aeruginosa*), and exhibited bactericidal activity (ZOI assay) (Ahmed et al. 2021).

5 Fabrication of Alginate Carrier Dressing

5.1 Production

The design, development and characterization are the route to obtain a biomaterial with specific properties according to its future application. In the case of alginate dressing construction, each type differs in composition, size, morphology, and absorbency capacity. Although ionic interaction mechanism allows diversity geometries in alginate hydrogels, fibers (micro and nanofibers) are more used in dressing manufacture because of their high surface area, relatively easy of handling products fabrication and their ability to retain mechanical integrity in wet state (Knill et al. 2004). Moreover, its unique “gel blocking” property remarks fiber’s preference. After incorporation enough water inside structure of alginate dressing, the space between the fibers are closed (gel blocking), prohibiting liquid from lateral spreading and preventing maceration of the areas surrounding the wound surface. This cohesive gel action facilitates wound healing process. The majority commercial available dressings are based on calcium alginate fibers, whereas calcium and sodium blend is used to accelerate gel formation in some formulations (Table 1). Additionally, the commercial alginate bioactive dressings by incorporation silver or zinc are also reported.

Alginate fibers are typically prepared by wet spinning, electrospinning and microfluidic techniques, providing potential new platform for the innovative drug delivery system, wound healing, and surgical reconstruction.

5.1.1 Wet Spinning Method

Traditional wet spinning method is based on extruding water soluble sodium alginate solution into an aqueous calcium chloride coagulation bath to produce insoluble calcium alginate hydrogel. According to a wide range of experimental parameters including composition, shape molding and syringe, microparticles or

Table 1 Some commercially available alginate wound dressing

Alginate dressing	Composition	Formulation	Applications	Reference
AlgiDERM® (Bard)	Calcium alginate	Fibers	Highly exudative wounds, partial or full thickness wounds	Dabiri et al. (2016)
	High G			
Algicell®/ Algicell® Ag (Integra LifeSciences Corp.)	Calcium alginate	Hydrogel	Moderate to heavy exudate such as diabetic foot ulcers, leg ulcer, arterial ulcers, pressure ulcers, donor sites and traumatic and surgical wounds	Aderibigbe and Buyana (2018)
	Blending G and M	Hydrogel + Ag		
Algosteril® (Johnson & Johnson)	Calcium alginate	Fibers	Moderate and high exudate, surgical and traumatic wounds, chronic wounds, burns, infected wounds	Williams (1999)
	High G			
Curasorb™/ Curasorb™ Zinc (Kendall Company)	Calcium alginate	Fibers	Moderately to heavily draining wounds, blending wounds, deep wounds cavities, diabetic, arterial and venous ulcers, pressure ulcers, infected wounds, red or yellow wounds. Abrasions, lacerations and skin tears	Stojadinovic et al. (2008)
	High G (68%)	Fibers + Zn		
Kalginat® (DeRoyal)	Calcium alginate	Fibers	Arterial, pressure, venous insufficiency and diabetic ulcers; superficial wounds and burns; lacerations, cuts and abrasions; donor sites, and post-surgical incisions	Dabiri et al. (2016)
Kaltostat® (ConvaTec)	Calcium/Sodium alginate	Fibers	Moderately to highly exudative chronic and acute wounds, and wounds with minor bleeding	Barbu et al. (2021)
	High G (80%)			
Melgisorb®/ Melgisorb® Ag (Mölnlycke)	Calcium/Sodium alginate	Fibers	Moderately to heavily exudative partial- to full-thickness wounds.	Williams (1998)
	High M (60%)	Fibers + Ag		

(continued)

Table 1 (continued)

Alginate dressing	Composition	Formulation	Applications	Reference
Health Care UK, LLC)			Pressure, venous, arterial and diabetic ulcers, donor sites, postoperative wounds; dermal lesions and traumatic wounds	
Seasorb [®] / Seasorb Ag (Coloplast Sween Corp.)	Calcium alginate (85%)/ Carboxymethylcellulose (15%)	Fibers	Moderate to heavy exudative leg ulcers and granulating wounds	Szekalska et al. (2016)
	High G			
Sorbsan [™] / Sorbsan Ag	Calcium alginate	Fibers	Leg ulcers, pressure areas, donor sites and other granulation wounds	Kuznetsova et al. (2020)
	High M (63%)	Fibers + Ag		

alginate microfibers are instantaneously formed in favorable conditions. A highly swelling structure as hydrofibers (e.g., Carboflex[®], Exufiber[®]) is obtained using wet spinning method. The contact of hydrofiber with exudate form a continuous gel, where its progressive swell leads fully hydrate fibers, being indistinguishable from each other. As a result, this type of dressing is useful for heavy exudate wound and uncontrollable bleeding. The bioactive agent is usually added in alginate solution before hydrogel formation, but the immersion technique can be used in case of natural products.

5.1.2 Microfluidic Methods

In the case of microfluidic techniques, microfibers are formed by extruding the sodium alginate solution vertically into cylinder container, previously loaded with calcium chloride solution. The precise control of dimensional and morphological characteristics of resultant microfiber has been achieved due to the formation of stable laminar flow upon injection into microchannel system. Extending droplet extrusion/precipitation concept and with the propose to obtain lower size particles, as well as homogeneous and scalable production, alginate beads and microparticles have been widely prepared by spinning disk atomization, vibrating nozzle devices, coaxial air flow techniques, as well as others. Similar to wet spinning, bioactive agent is incorporated in alginate sodium solution before dropping action.

5.1.3 Electrospinning Method

Electrospinning is a modern, attractive and efficient fiber production technique, which is based on the effect of high voltage on polymer solutions (e.g., coaxial and emulsion electrospinning). It creates 3D scaffolds containing random oriented or aligned ultrafine fiber structure (nanofibers) with a diameter generally lower than 10 μm . This conformation allows to create wound dressings with highly interconnected nanoporous structure that can mimic the native skin environment, facilitates water and oxygen exchange, as well as removal of metabolic waste. It also gives the possibility to blending fibers reinforcing the strength of scaffold, and reducing cost of dressing. Thus, in the fabrication of SeaSorb[®] dressing, fibers of alginate and carboxymethyl cellulose are intertwined. Sodium alginate cannot be electrospun by itself in aqueous solution, but its blending with a suitable polymer such as polyethylene oxide (PEO) or polyvinyl alcohol allows the fibers formation employing this method. Indeed, Saquing et al. studied the required polymers concentrations to obtain alginate fibers by electrospinning using scanning electron microscopy (SEM) as powerful tool. The authors found that alginate must be blended with a high molecular weight of electrospinnable polymers (more than 600 kDa for PEO). The addition of PEO reduced electrical conductivity and surface tension of alginate solution, which facilitated its fiber formation. Moreover, SEM micrographs showed adequate molecular entanglement as carrier polymer in presence of PEO and Triton X-100 as surfactant (Saquing et al. 2013). In electrospinning techniques bioactive agents can be attached inside to nanofibers through intermolecular interactions or by introduction solution containing drug loaded nanoparticles into template of alginate fibers. As a result, high encapsulation of bioactive agents into nanofibrous scaffold has been obtained, especially in the formation of core-shell structure in which the core containing encapsulated bioactive agent is covered by polymer matrix by coaxial electrospinning.

5.2 Characterizations

Characterization as a final phase to validate functionality of biomaterial designed covers physicochemical, mechanical and biological evaluations (Fig. 2).

5.2.1 Swelling Process: Absorption Capacity

Swelling is a key alginate property in wound management and it is referred to the high capability of this polysaccharide to absorb liquids due to the presence of abundance carboxyl (-COOH) and hydroxyl (-OH) groups in its chemical structure. The mechanism occurs through interactions between hydrophilic groups and the water molecules, exposing multiple interconnected cavities as reservoir of bioactive

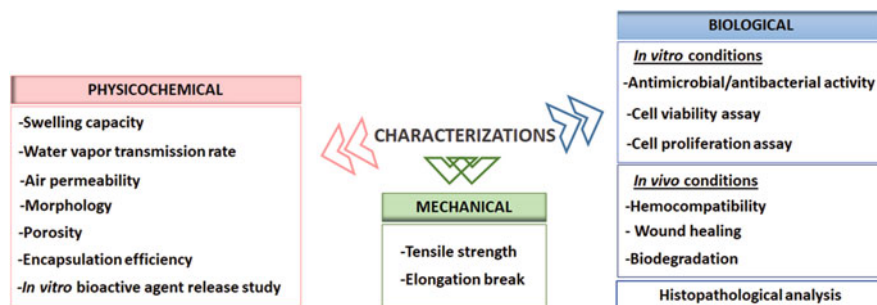


Fig. 2 Schematic representation of different physicochemical, mechanical and biological characterizations

agent and physiological fluids. Thereby, in contact with exudate alginate hydrogel experiments a progressive swelling, and simultaneous expansion-diffusion process appears allowing to penetrate fluids into its 3D porous matrix. This swelling behavior is affected for several parameters such as chemical composition (concentration and type of alginate), content of crosslinking agent, and environmental pH. In this sense, the high alginate concentration in dressing implies more amounts of hydrophilic groups with subsequent more capacity to absorb water or exudate, resulting in a higher swelling behavior.

As expected, dressing with higher M blocks is more absorbent and permeable than those rich in G blocks attributed to the polymeric chains flexibility (Goh et al. 2012). A great increase swelling ability has been also appreciated in alginate dressing manufactured with calcium alginate/carboxymethylcellulose fibers due to the highest hydrophilic group content by contribution of both polysaccharides, and the fibers rearrange. Regarding this fact, Qin. reported a comparative study of seven commercially available alginate wound dressing in terms of absorbency capacity. Alginate wound dressing containing alginate/carboxymethylcellulose fibers (Urgosob™) showed a higher absorption in water and normal saline solution (0.9% w/w) compared with the other samples (Qin 2006). In contrast, crosslinking density is inversely proportional to the degree of swelling, since it is responsible to retain 3D conformation in a specific environment. The lower crosslinking density provides more flexibility polymer chains and higher swelling ratio is reached, whereas an increase in the amount of crosslinker leads more compact structure reducing mobility of polymeric chains and swelling properties of hydrogel. It is important to notice that combination of fibers provides entangled conformation (hydrophobic crosslinking) resulting in dressing with stronger mechanical strength, while very lower weight of the polymers and crosslinker concentration cause the collapse of swollen alginate fibers due to insufficient entanglement.

Swelling capacity of alginate dressing is measured by immersion dried sample in water, sodium saline solution or a solution to simulate human blood and exudate (Miraftab et al. 2002), and is determined gravimetrically through sample weight

changes. For that, at specific time intervals sample is removed from the medium and weighted. The swelling ratio (W_s) is calculated using following equation:

$$W_s = \frac{W_t - W_0}{W_0}$$

where W_t is the weight of dressing piece in swollen state at the time t , and W_0 represents the initial weight of dried dressing piece. The swelling ratio is usually given in percentage and similar terms as degree swelling or swelling index appear in the literature.

The incorporation of bioactive nanoparticles in fibers also affects swelling abilities of alginate dressing. The nanoparticles act as reinforce material occupying the free space within porous structure, causing reduction in the absorption of water and fluids. For instance, Mohandas et al. reported a significant decreased in the swelling ratios of alginate bandages with increment of zinc oxide nanoparticle concentration (Mohandas et al. 2015). Similar behavior was evidenced by Zhang et al. during preparation fiber dressing by blending nano-silica or hydroxyapatite with alginate using microfluidic spinning method (Zhang et al. 2017). In summary, swelling ratio measurements allows to quantify the extent of crosslinking in order to reach adequate balance between fluid absorption capacity and the integrity of alginate dressing network. Moreover, it is useful to predict the release rate of many different types of bioactive agents for practical use in wound healing.

5.2.2 Water Vapor Transmission Rate (WVTR)

It is well known that alginate polysaccharide produces highly porous structure, which is also important property for adequate water vapor and oxygen exchange across the wound surface. The ability of a dressing to regulate water loss can be evaluated by determination of WVTR, also known as moisture vapor transmission rate through different Standard test. In the in vitro experiments, sample is incubated into the solution (water method) or exposed only to moisture vapor employing the cylinder inverted (desiccant method) at specified conditions of temperature and relative humidity. Wound dressing with optimal WVTR value is necessary to guarantee wound healing. An extremely high WVTR may lead dehydration of wound, while very low WVTR may cause the accumulation of wound exudates, skin maceration and infection (Xu et al. 2016). The variability of test conditions between Standard methods difficulty comparison of reported results in the literature. For instance, Mokhena and Luyt carried out the experiments by ASTM 398–03, where sample was fixed into adhesive card and placed between two chambers (Mokhena and Luyt 2017). To realize WVTR assay, Güner et al. followed protocols of ASTM E96 using desiccant method with saturated sodium chloride solution (Güner et al. 2018), whereas Hasatsri et al. 2018 employed water method based on BS EN 13726–2, British standard 7209 with slightly modification (Hasatsri et al.

2018). The WVTR ($\text{g/m}^2\text{h}$ or $\text{g/m}^2/\text{day}$) is calculated according to the following equation:

$$WVTR = \frac{M_1 - M_0}{A \times t}$$

where M_1 is the weight of the sample (assembly) after incubation time, M_0 represents the weight of the sample (assembly) at the beginning of the assay, A is the test area of the sample in m^2 , and t correspond to test time of the experiment (24 h).

For comparative propose WVTR value of normal skin and injured skin removes have been used as a control, but high variability in data reported was also found. Thereby, analyzing all research information reviewed and some commercial products, we suggest the range 2000–2500 $\text{g/m}^2/\text{day}$ as acceptable value for adequate level of moisture without risking wound dehydration. The determination of WVTR values for electrospun alginate nanofibers and electrospun alginate nanofibers coated with chitosan was carried out by ASTM 398–03. Although, the results showed that chitosan coating process slightly decreased WVTR (1586–1373 $\text{g/m}^2/\text{day}$) and reduced the porosity of the fiber mats, all samples showed values within required range (Mokhena and Luyt 2017). Oxidized alginate gelatin hydrogels showed WVTR $2086 \pm 124 \text{ g/m}^2/\text{day}$ using ASTM standards E 96–00 (Balakrishnan et al. 2005). Algisite[®] as calcium alginate dressing (Smith & Nephew) was found 1267 $\text{g/m}^2\text{h}$ following protocol of British Standard 7209. In addition, comparative analysis with polyurethane foams commercial dressings remarked alginate dressing as highly permeable material for water vapor (Yang and Hu 2017).

5.2.3 Drug Release

Sustained and controlled release of bioactive agent in infected site facilitates regeneration functional of skin, improves efficiency of treatment, and minimize nursing time and healthcare costs. Thereby bioactive products as dressing containing therapeutic substance are considered crucial for the maintenance clinical benefits in wound treatment.

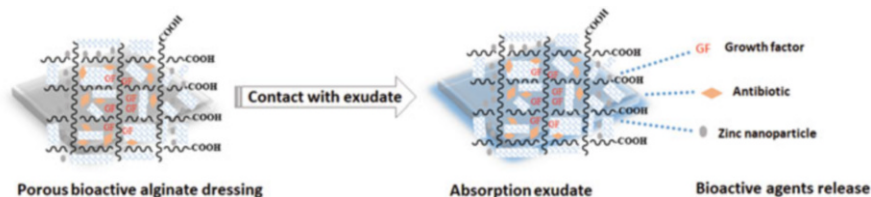
Bioactive agent release process from alginate dressing is governed by diffusion mechanism and generally presents initial burst effect. In other applications burst effect is undesirable, but could be advantageous in wound treatments, especially in chronic wound due to high level of infection. Diffusion mechanisms occur in two stages: at first the bioactive agent localized in external surface is rapidly release to medium (burst effect) by the contact between exudate and alginate dressing. In the second stage after 3D network swells, the porous open up absorbing exudate and releasing bioactive agent into surrounding tissue. Depending on flexibility of fibers or mobility polysaccharide chains, the maximum exposure of potential hydrophilic groups, amount of fluid medium, and crosslinking density, the rate of diffusion will be low or fast leading sustained or immediately release of therapeutic agent. As similar to swelling process, bioactive agents released from alginate dressing tends to

decrease with increase of high G fraction in sodium alginate, lower porosity and higher crosslinking density. All these factors have been exploited covering a wide range of release prolonged actions such as combination fibers and nanoparticles, core-shell fibers structure, and the co-delivery of different bioactive agents in simultaneous or sequential manner (Table 2). Moreover, many different types of

Table 2 Representative examples of different bioactive agents within alginate for wound healing applications

Composition	Bioactive agent	Highlight	Reference
Alginate/PEO	Ciprofloxacin (antibiotic)	Stable and cylindrical alginate nanofibers as antibiotic delivery system	Kyzioł et al. (2017)
Pluronic F-127 (Fibers)			
Alginate/PVA (3D bioprinting scaffold)	Bovine serum albumin (protein)	Porous structure with high absorption capacity and encapsulation efficiency, fast release for protein and sustained release of growth factor over 7 days	Luo et al. (2017)
	Bone morphogenetic protein 2		
	(Growth factor)		
Tegagel 3M [®] (Calcium alginate fibers)	–	The fibers are hydroentangled leading a lowest absorption capacity in comparison with other commercial dressing.	Qin (2004)
Methacrylated heparin alginate/PEO (Nanofibers)	Fibroblast growth factor-2 (FGF-2) (Growth factor)	To confer additional bioactivity, heparin was covalently coupled to alginate, and FGF-2 was released in sustained manner during 1 week	Jeong et al. (2012)
Alginate (Hybrid hydrogel beads and nanofibers)	Fluorenylmethylcarbonyldiphenylalanine (Peptide)	Sustained and controlled docetaxel release, enhanced stability in water and phosphate buffered solution	Xie et al. (2016)
	Docetaxel (Antineoplastic)		
Acrylamide/Alginate/Chitosan	Honey (natural antimicrobial)	Combination of acrylamide, alginate, chitosan and honey showed adequate properties in terms of drug release and healing activity	Kurahde et al. (2013)
Acrylamide/Alginate/Gelatin	Povidone iodine (antiseptic)		
Acrylamide/Chitosan/Gelatin (Hydrogel sponges)			
Alginate (Fibers)	Brananferulate (polysaccharide Sterigel [®] with biological activity)	Combination between polysaccharides and alginate fibers enhanced tenacity, breaking extension and wound healing properties	Miraftab et al. (2002)
Alginate (Films)	Asiaticoside (from <i>Centellaasiatica</i> plant)	For combined therapy using traditional medicine as active wound dressing	Sikareepaisan et al. (2011)

bioactive agents have been successful released from alginate scaffold (Zhang et al. 2020; Thakur et al. 2008).



In general, the *in vitro* bioactive agent release study is carried out immersed bioactive agent loaded-sample previously weighed (films, membranes, micro/nanoparticles, micro/nanofibers and dressing pieces) in a specific volume medium at room temperature or 37 °C. The water and simulated wound fluid (SWF) are frequent release mediums used during evaluation. The SWF containing 0.02 M calcium chloride, 0.4 M sodium chloride, 0.08 M tris-methylamine and 2% (w/v) bovine serum albumin in deionized water is adjusted to pH 7.5 to mimic chronic wound conditions, with pH value reported in the range of 7.2 to 8.9. At regular intervals, aliquots of bioactive agent are extracted from medium and the absorbency is measured in corresponding equipment.

- Silver: Quantification of the silver released in water is carried out by atomic absorption spectrometry. The sample containing silver is previously digested with concentrated nitric acid (65%) in excess or heating in a mixture of concentrated sulfuric and nitric acid (Stojkowska et al. 2018)
- Zinc oxide: The total zinc content released from zinc oxide nanoparticles is estimated using UV spectrophotometer.
- Antibiotics: The amount of antibiotic released to medium can be determined by UV spectrophotometer or High performance liquid chromatography (HPLC) (Jannesari et al. 2011; López-Iglesias et al. 2020).
- Growth factor: The content of growth factor released into the medium is generally determined by enzyme-linked immunoabsorbent assay (ELISA) (Sahoo et al. 2010).
- Natural extracts: The diversity of these materials allow to use diverse analytical techniques for quantification extracts of natural bioactive molecules (e.g., honey by UV (Schuhladen et al. 2021), Asiaticoside by HPLC (Sikareepaisan et al. 2011)).

Different slight modifications have been realized in protocols experiments according to characteristics of sample and bioactive agents. For instance, different containers such as Transwell membranes/dishes (Corning, USA) or beakers.

Other physicochemical and mechanical characterizations include: (1) composition, microstructure, and interactions between functional groups or compounds by Fourier transform infrared (FTIR) and X-Ray Diffraction (XRD); (2) surface morphology, fiber diameter, nanoparticle size and pore size by SEM; (3) thermal behavior and residual moisture content by thermogravimetric analysis (TGA) and

differential scanning calorimetry (DSC), (4) tensile strength, Young's moduli and elongation break by mechanical assays, and (5) air permeability and wettability.

5.2.4 Biological Evaluation

The alginate dressings in contact with human body should be provide safety in use, which is validated through a diverse set of biological experiments (ISO, ASTM). Based on this, alginate hydrogels are considered biocompatible by many experimental and clinical studies (Thomas 2000). A detail overview of biocompatibility of alginate polysaccharide, the ionic and covalent crosslinkers used during fabrication process, and alginate scaffold has been reported/referred in a recent review by Agüero. Thereby, herein we are focuses on biological evaluation of bioactive alginate dressing for wound healing applications.

5.2.4.1 Antibacterial Activity

After incorporation bioactive agent in alginate scaffold is necessary to corroborate the biological activity efficacy, even considering the favorable ecofriendly conditions of manufacture process. Antibacterial activity test is a well-established procedure can be realizing under in vitro and in vivo conditions according to protocols described in US Pharmacopeia (USP). Nevertheless, antibacterial activity of dressing containing bioactive agent is mostly evaluated using in vitro test due to simplicity, low cost, and the ability to analyze numerous microorganism and antimicrobial agents rapidly with reproducible results (Wiegand et al. 2015; Balouiri et al. 2016). Methods such as disk diffusion, well diffusion and agar dilution are frequently employed, where samples are tested in contact with major microorganism that causes skin infections. At the early stage, the most common microorganism implicated are *S. aureus* and *E. coli*, while fungi (*C. albicans*) and mixed infections (bacteria + fungi) appear in the advanced stage. For instance, the recurrent mixed infection by *Pseudomonas* and *C. albicans* tends to invade deeper layers of skin causing significant tissue damage, becoming in a challenge in the treatment of infected wound. At this point, determination of minimum inhibitory concentration (MIC) as quantitative analysis has been established like standard experimental routine by broth or well diffusion method. The antibacterial activity of bioactive alginate-based microparticles (Shi et al. 2019), films or membranes (Ghadiri et al. 2014), nanofibers/nanohydrogels (Tarun and Gobi 2012; Kyzioł et al. 2017) and dressing (Wiegand et al. 2015) against diverse microorganisms have been measured by determination of clear zone of inhibition (ZOI) around the sample (halo) after 24 h of incubation at 37 °C. The diameter of ZOI is associated to the rate the agent's antimicrobial efficacy, thus the larger ZOI correspond to the more effective agent. Other testing for antibacterial activity includes Japanese Industrial Standard 1902: 2002, cytofluorometric and bioluminescent methods, but the use of two last one's techniques is limited because of require specific and modern equipment, as well as further evaluation for reproducibility and standardization.

5.2.4.2 In Vitro Cytotoxicity

Cytotoxicity, cell viability, and proliferation under in vitro conditions are conducted to evaluate biocompatibility of potential alginate biomaterials. The experiments are carried out in robust established cell lines recommended by International Standard Organization (ISO) such as human umbilical vein endothelial, human keratinocytes HaCaT, murine fibroblasts NIH/3T3, and normal human dermal fibroblast cells, among others. After specific time of incubation (24, 48, 72 h or days), the parameters are assessed by Alamar Blue, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) or 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) methods. By comparison with the control group the ability of cells to reduce MTT/MTS is analyzed, thus an increase in the metabolic activity of cells adhered should be observed as indicative not toxic effects of sample for the model cell line used. The values above 70% cell viability are considered non-cytotoxic relative to cell tested. For example, chitosan/alginate multilayer films displayed uniform distribution of viable cells with viability of $96 \pm 4.2\%$, being considered cytocompatibility for human adipose stem cells (Hatami et al. 2017). Resazurin dye (7-hydroxi-3H-phenozacin-3-one 10 oxide) is another colorimetric assay has been reported to evaluate cytotoxic of alginate-based nanohydrogels (Chopra et al. 2015; Raguvaran et al. 2017). In this case, the reduction of blue (resazurin) to pink color (resorufin) correspond directly to bacterial growth and the reduction of cytotoxicity. On the other hand, cell proliferation study is performed by DNA quantification considering as good result when a high value in DNA content is observed with increasing culture time, which indicates the increase in number of cells (proliferation). Additionally, for further evaluation cellular morphology, distribution, adhesion and membrane mechanical integrity are examined by fluorescence and confocal microscopic techniques.

5.2.4.3 In Vivo Wound Healing

It is well known that the in vivo experiments should be only performed after an exhaustive in vitro experiments, and in accordance with Ethical Guide for Animal Experimentation, as well as under approved by the corresponding Institutional Animal Ethical Committee in each country. In this line, we are found a lower number of scientific reports concerning in vivo assays in comparison with the number of in vitro studies.

In order to evaluate practical functionality of alginate hydrogels as wound dressing, in vivo wound healing experiment is performed as representative assay. The rate of wound contraction, inflammation, infection and healing time are some parameters have been considerate as measure of the wound healing (Kurahde et al. 2013). For instance, the efficacy of chitosan-alginate membrane in wound healing process during 21 days was performed in adult male Wistar rats. The animal samples were collected for histological studies that included inflammatory infiltrate, blood

vessel and fibroblast (Caetano et al. 2014). After confirmed cytocompatibility of bioglass/oxidized sodium alginate hydrogel in human umbilical vein endothelial and human dermal fibroblast, the effect of alginate composite on wound closure was evaluated in a mouse model. An adequate adhesiveness, lack of leakages and infections, as well as the intact epidermis layer formation in lacerated site was appreciated by comparison with traditional suture method. These results suggest bioglass/oxidized sodium alginate hydrogel as potential candidate for wound healing applications (Gao et al. 2019). A similar concept was followed by Rahman et al. that also assessed wound healing efficiency of chitosan-alginate biocomposite in full-thickness wounds using a mice model. After comparison with normal surgical gauge bandage as a control, granulation was appreciated more extents in the wound treated with the biocomposite. Moreover, a complete wound closure was observed (99%) with the respect to control (30%) after 10 days of wound healing process. The increase of epithelization and collagen regeneration were examined by histological analysis (Rahman et al. 2020).

5.2.4.4 Hemocompatibility Assay

Considering the different deep of wounds which require incorporation pieces of dressing and bleeding as one of common symptoms of wounds infected, hemocompatibility assays are essential in the evaluation of biomaterial. In a representative and extent work, alginate-pectin hydrogels for diabetic wound healing was evaluated by hematology assay. After realize to body weight of animals and tissue harvesting, the whole blood was collected and different physiological parameters was determined such as hemoglobin concentration (Hb), red blood cell count (RBC), packed cell volume (PVC), mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), the mean corpuscular hemoglobin concentration (MCHC), reticulocyte count (RDW), total white blood cells (WBCs), and differential leukocytes count (neutrophils, lymphocytes, monocytes). The experiments were carried out on diabetic and non-diabetic male Wistar rats at 7, 14 and 21 days. The results indicated the absence of any adverse reaction after topical administration of alginate-pectin hydrogel dressing (Rezvani et al. 2021).

Another in vivo experiments include the evaluation of biodegradation and hemostasis.

6 Conclusions

The inherent properties of alginate polysaccharide remark its preferential place in wound management, being the most commercially available wound product. The high absorption capacity of biological fluids, good biocompatibility and porous three-dimensional architecture provide adequate environment for treat diverse dermal lesions. In this chapter, the role of each property, extrusion techniques as widest

fibers dressing fabrication, and recent strategies in the development of advanced alginate dressing was discussed. The action of combining as an essential aspect in future development of bioactive alginate dressing was also highlighted, focusing on blending alginate with other polymers, in the combination of fibers with diverse entanglements, and the incorporation of various bioactive molecules into a same 3D network. At the same time, representative *in vitro* and *in vivo* experiments are exposed proving biocompatibility and functionality if a wide range of alginate hydrogels as potential wound dressing. Alginate-based wound products with good stability, biocompatibility, exudate-retaining ability and antimicrobial properties remain as advantageous opportunity for the wound treatment.

Conflict of Interest The authors declare not conflict of interest.

References

- Abasalizadeh F, Moghaddam SV, Alizadeh E et al (2020) Alginate-based hydrogels as drug delivery vehicles in cancer treatment and their applications in wound dressing and 3D bioprinting. *J Biol Eng* 14:1–22
- Aderibigbe BA, Buyana B (2018) Alginate in wound dressing. *Pharmaceutics* 10:42
- Agüero L, Zaldivar-Silva D, Peña L et al (2017) Alginate microparticles as oral colon drug delivery devices: a review. *Carbohydr Polym* 168:32–43
- Ahmed A, Getti G, Boateng J (2021) Medicated multi-targeted alginate-based dressings for potential treatment of mixed bacterial-fungal infections in diabetic foot ulcer. *Int J Pharm* 606:120903
- Alvarez-Lorenzo C, Blanco-Fernandez B, Puga AM et al (2013) Crosslinked ionic polysaccharides for stimuli-sensitive drug delivery. *Adv Drug Deliv Rev* 65:1148–1171
- Atiyeh BS, Costagliola M, Hayek SN et al (2007) Effect of silver on burn wound infection control and handling: review of the literature. *Burns* 33:139–148
- Bahadoran M, Shamloo A, Nokoorani YD (2020) Development of a polyvinyl alcohol/sodium alginate hydrogel-based scaffold incorporating bFGF-encapsulated microspheres for accelerated wound healing. *Sci Rep* 10:1–18
- Balakrishnan B, Mohanty M, Umashankar PR et al (2005) Evaluation of an *in situ* hydrogel wound dressing based on oxidized alginate and gelatin. *Biomaterials* 26:6335–6342
- Balouiri M, Sadiki M, Ibsouda SK (2016) Methods for *in vitro* evaluating antimicrobial activity: a review. *J Pharm Anal* 6:71–79
- Barbu A, Neamtu B, Zăhan M et al (2021) Current trends in advanced alginate-based wound dressings for chronic wounds. *J Pers Med* 11:890
- Caetano GF, Frade MAC, Andrade TAM et al (2014) Chitosan-alginate membranes accelerate wound healing. *J Biomed Mater Res B* 103:1013–1022
- Carmona-Ribeiro AM, de Melo Carrasco LD (2013) Cationic antimicrobial polymers and their assemblies. *Int J Mol Sci* 14:9906–9946
- Cattelan G, Guerrero Gerbolés A, Foresti R et al (2020) Alginate formulations: current developments in the race for hydrogel-based cardiac regeneration. *Front Biong Biotechnol* 8:414
- Chen X, Schluesener HJ (2008) Nanosilver: a nanoproduct in medical application. *Toxicol Lett* 176:1–12
- Chopra M, Bernela M, Kaur P et al (2015) Alginate/gum acacia bipolymeric nanohydrogels-promising carrier for Zinc oxide nanoparticles. *Int J Biol Macromol* 72:827–833
- Chou HY, Weng CC, Lai JY et al (2020) Design of an interpenetrating polymeric network hydrogel made of calcium-alginate from a thermos-sensitive pluronic template as a thermal-ionic reversible wound dressing. *Polymers* 12:2138

- Cleetus CM, Primo FA, Fregoso G et al (2020) Alginate hydrogels with embedded ZnO nanoparticles for wound healing therapy. *Int J Nanomedicine* 15:5097–5111
- Dabiri G, Damstetter E, Phillips T (2016) Choosing a wound dressing based on common wound characteristics. *Adv Wound Care* 5:32–41
- Downs EC, Robertson NE, Riss TL et al (1992) Calcium alginate beads as a slow-release system for delivering angiogenic molecules *in vivo* and *in vitro*. *J Cell Physiol* 152:422–429
- Fischbach MA, Walsh CT (2009) Antibiotics for emerging pathogens. *Science* 325:1089–1093
- Gao L, Zhou Y, Peng J et al (2019) A novel dual-adhesive and bioactive hydrogel activated by bioglass for wound healing. *NPG Asia Mater* 11:1–11
- Ghadiri M et al (2014) Antibiotic eluting clay mineral (Laponite®) for wound healing application: an *in vitro* study. *J Mater Sci Mater Med* 25:2513–2526
- Goh CH, Heng PWS, Chan LW (2012) Alginates as a useful natural polymer for microencapsulation and therapeutic applications. *Carbohydr Polym* 88:1–12
- Gómez Chabala LF, Cuartas CEE, López MEL (2017) Release behavior and antibacterial activity of chitosan/alginate blends with *aloe vera* and silver nanoparticles. *Mar Drugs* 15:328
- Güner OZ, Cam C, Arabacioglu B et al (2018) Theophylline-loaded pectin-based hydrogels. I. Effect of medium pH and preparation conditions on drug release. *J Appl Polym Sci* 135:46731
- Hasatsri S, Pitiratanaworarat A, Swangwit S et al (2018) Comparison of the morphological and physical properties of different absorbent wound dressings. *Dermatol Res Pract* 2018:9367034. <https://doi.org/10.1155/2018/9367034>
- Hatami J, Silva SG, Oliveira MB et al (2017) Multilayered films produced by layer-by-layer assembly of chitosan and alginate as potential platform for the formation of human adipose-derived stem cell aggregates. *Polymers* 9:440
- Herman TF, Bordoni B (2021) Wound classifications. StatPearls, Treasure Island, FL
- Hooper SJ, Percival SL, Hill KE et al (2012) The visualisation and speed of kill of wound isolates on a silver alginate dressing. *Int Wound J* 9:633–642
- Hu Y, Zhang Z, Li Y et al (2018) Dual-crosslinked amorphous polysaccharide hydrogels based on chitosan/alginate for wound healing applications. *Macromol Rapid Commun* 39:1800069
- Jannesari M, Varshosaz J, Morshed M et al (2011) Composite poly(vinyl alcohol)/poly(vinyl acetate) electrospun nanofibrous mats as a novel wound dressing matrix for controlled release drugs. *Int J Nanomedicine* 6:993–1003
- Jeong SI, Jeon O, Krebs MD et al (2012) Biodegradable photo-crosslinked alginate nanofibre scaffolds with tuneable physical properties, cell adhesivity and growth factor release. *Eur Cell Mater* 24:331–343
- Jeong S, Kim B, Park M et al (2020) Improved diabetic wound healing by EGF encapsulation in gelatin-alginate coacervates. *Pharmaceutics* 12:334
- Kaiser P, Wächter J, Windbergs M (2021) Therapy of infected wounds: overcoming clinical challenges by advanced drug delivery systems. *Drug Deliv Transl Res* 11:1545–1567
- Knill CJ, Kennedy JF, Mistry J et al (2004) Alginate fibres modified with unhydrolysed and hydrolysed chitosans for wound dressings. *Carbohydr Polym* 55:65–76
- Kogan S, Sood A, Granick M (2017) Zinc and wound healing: a review of zinc physiology and clinical applications. *Wounds* 29:102–106
- Kurhade ST, Momin M, Khanerkar P et al (2013) Novel biocompatible honey hydrogel wound healing sponge for chronic ulcers. *Int J Drug Deliv* 5:353–361
- Kuznetsova TA, Andryukov BG, Besednova NN et al (2020) Marine algae polysaccharides as basis for wound dressings, drug delivery, and tissue engineering: a review. *J Mar Sci Eng* 8:481
- Kyzioł A, Michna J, Moreno I et al (2017) Preparation and characterization of electrospun alginate nanofibers loaded with ciprofloxacin hydrochloride. *Eur Polym J* 96:350–360
- Łabowska MB, Michalak I, Detyna J (2019) Methods of extraction, physicochemical properties of alginates and their applications in biomedical field—a review. *Open Chem* 17:738–762
- Lansdown AB (2002) Silver I: its antibacterial properties and mechanism of action. *J Wound Care* 11:125–130

- Lansdown AB, Path FRC, Mirastschijski U et al (2007) Zinc in wound healing: theoretical, experimental and clinical aspects. *Wound Rep Reg* 15:2–6
- Liang Y, Liang Y, Zhang H et al (2022) Antibacterial biomaterials for skin wound dressing. *Asian J Pharm Sci* 17:353–384. <https://doi.org/10.1016/j.ajps.2022.01.001>
- Lin N, Gèze A, Wouessidjewe D et al (2016) Biocompatible double-membrane hydrogels form cationic cellulose nanocrystals and anionic alginate as complexing drugs codelivery. *ACS Appl Mater Interfaces* 8:6880–6889
- Lin PH, Sermersheim M, Li H et al (2018) Zinc in wound healing modulation. *Nutrients* 10:16
- López-Iglesias C, Quílez C, Barros J et al (2020) Lidocaine-loaded solid lipid microparticles (slmps) produced from gas-saturated solutions for wound applications. *Pharmaceutics* 12:870
- Luo Y, Luo G, Gelinsky M et al (2017) 3D bioprinting scaffold using alginate/polyvinyl alcohol bioinks. *Mater Lett* 189:295–298
- Memic A, Abudula T, Mohammed HS et al (2019) Latest progress in electrospun nanofibers for wound healing applications. *ACS Appl Bio Mater* 2:952–969
- MirafTAB M, Qiao Q, Kennedy JF et al (2002) Advanced wound care materials: developing an alginate fibre containing branan ferulate. *J Wound Care* 11:353–356
- Mohandas A, Sudheesh Kumar PT, Raja B et al (2015) Exploration of alginate hydrogel/nano zinc oxide composite bandages for infected wounds. *Int J Nanomedicine* 10:53–66
- Möhler JS, Kolmar T, Synnatschke K et al (2017) Enhancement of antibiotic-activity through complexation with metal ions- combined ITC, NMR, enzymatic and biological studies. *J Inorg Biochem* 167:134–141
- Mokhena TC, Luyt AS (2017) Electrospun alginate nanofibres impregnated with silver nanoparticles: preparation, morphology and antibacterial properties. *Carbohydr Polym* 165: 304–312
- Nardini M, Perteghella S, Mastracci L et al (2020) Growth factor delivery system for skin regeneration: an advanced wound dressing. *Pharmaceutics* 12:120
- Norouzi M, Boroujeni SM, Omidvarkordshouli N et al (2015) Advances in skin regeneration: application of electrospun scaffolds. *Adv Healthc Mater* 4:1114–1133
- Opasanon S, Muangman P, Namviriyachote N (2010) Clinical effectiveness of alginate silver dressing in outpatient management of partial-thickness burns. *Int Wound J* 7:467–471
- Pan Z, Ye H, Wu D (2021) Recent advances on polymeric hydrogels as wound dressings. *APL Bioeng* 5:011504
- Paques JP, van der Linden E, van Rijn CJ (2014) Preparation methods of alginate nanoparticles. *Adv Colloid Interface Sci* 209:163–171
- Paul W, Sharma CP (2015) Alginate: wound dressing. In: Mishra M (ed) *Encyclopedia of biomedical and polymeric biomaterials*. Taylor & Francis, New York, pp 134–146
- Percival SL, McCarty SM (2014) Silver and alginates: role in wound healing and biofilm control. *Adv Wound Care* 4:407–414
- Percival SL, Slone W, Linton S et al (2011) The antimicrobial efficacy of a silver alginate dressing against a broad spectrum of clinically relevant wound isolates. *Int Wound J* 8:237–243
- Qin Y (2004) Absorption characteristics of alginate wound dressing. *J Appl Polym Sci* 91:953–957
- Qin Y (2006) The characterization of alginate wound dressings with different fibers and textiles structures. *J Appl Polym Sci* 100:2516–2520
- Qin Y (2008) Alginate fibres: an overview of the production processes and application in wound management. *Polym Int* 57:171–180
- Raguvaran R, Manuja BK, Chopra MM et al (2017) Sodium alginate and gum acacia hydrogels of ZnO nanoparticles show wound healing effect on fibroblast cells. *Int J Biol Macromol* 96:185–191
- Rahman MA, Islam MS, Haque P et al (2020) Calcium ion mediated rapid wound healing by nano-ZnO doped calcium phosphate chitosan-alginate biocomposites. *Materialia* 13:100839
- Raus RA, Nawawi WMFW, Nasaruddin RR (2021) Alginate and alginate composites for biomedical applications. *Asian J Pharm Sci* 16:280–306

- Rezvanian M, Ng SF, Alavi T (2021) In-vivo evaluation of alginate-pectin hydrogel film loaded with Simvastatin for diabetic wound healing in Streptozotocin-induced diabetic rats. *Int J Biol Macromol* 171:308–319
- Sahana TG, Rekha PD (2018) Biopolymers: applications in wound healing and skin tissue engineering. *Mol Biol Rep* 45:2857–2867
- Sahoo S, Ang LT, Goh JCH et al (2010) Growth factor delivery through electrospun nanofibers in scaffold for tissue engineering applications. *J Biomed Mater Res A* 93:1539–1550
- Saouq CD, Tang C, Monian B et al (2013) Alginate-polyethylene oxide blend nanofibers and the role of the carrier polymer in electrospinning. *Ind Eng Chem Res* 52:8692–8704
- Schuhladen K, Raghu SN, Liverani L et al (2021) Production of a novel poly(e-caprolactone)-methylcellulose electrospun wound dressing by incorporating bioactive glass and Manuka honey. *J Biomed Mater Res* 2:180–192
- Severino P, Chaud MV, Shimojo A et al (2015) Sodium alginate-cross-linked polymyxin B sulphate-loaded solid lipid nanoparticles: antibiotic resistance test and HaCaT and NIH/3T3 cell viability studies. *Colloids Surf B Biointerfaces* 129:191–197
- Sharma S, Sanpui P, Chattopadhyay A et al (2012) Fabrication of antibacterial silver nanoparticle-sodium alginate-chitosan composite films. *RSC Adv* 2:5837–5843
- Shi M, Zhang H, Song T et al (2019) Sustainable dual release of antibiotic and growth factor from pH-responsive uniform alginate composite microparticles to enhance wound healing. *ACS Appl Mater Interfaces* 11:22730–22744
- Sikareepaisan P, Ruktanonchai U, Supaphol P (2011) Preparation and characterization of asiaticoside-loaded alginate films and their potential for use as effectual wound dressing. *Carbohydr Polym* 83:1457–1469
- Sim W, Barnard RT, Blaskovich MAT et al (2018) Antimicrobial silver in medicinal and consumer applications: a patent review of the past decades (2007–2017). *Antibiotics (Basel)* 7:93
- Singh R, Singh D (2012) Radiation synthesis of PVP/alginate hydrogel containing nanosilver as wound dressing. *J Mater Sci Mater Med* 23:2649–2658
- Song J, Chen Z, Liu Z et al (2021) Controllable release of vascular endothelial growth factor (VEGF) by wheel spinning alginate/silk fibroin fiber for wound healing. *Mater Des* 212:110231
- Spadari CDC, Lopes LB, Ishida K (2017) Potential use of alginate-based carriers as antifungal delivery system. *Front Microbiol* 8:97
- Stevens J, Chaloner D (2005) Urgosrob™ dressing: management of acute and chronic wounds. *Br J Nur* 14:S22–S28
- Stojadinovic A, Carlson JW, Schultz GS (2008) Topical advances in wound care. *Gynecol Oncol* 111:S70–S80
- Stojkowska J, Djurdjevic Z, Jancic I et al (2018) Comparative *in vivo* of novel formulations based on alginate and silver nanoparticles for wound treatments. *J Biomater Appl* 32:1197–1211
- Szekalska M, Pucilowska A, Szymańska E et al (2016) Alginate: current use and future perspectives in pharmaceutical and biomedical applications. *Int J Polym Sci* 2016:7697031. <https://doi.org/10.1155/2016/7697031>
- Tanihara M, Suzuki Y, Yamamoto E et al (2001) Sustained release of basic fibroblast growth factor and angiogenesis in a novel covalently crosslinked gel of heparin and alginate. *J Biomed Mater Res* 56:216–221
- Tarun K, Gobi N (2012) Calcium alginate/PVA blended nanofibre matrix for wound dressing. *Indian J Fibre Text Res* 37:127–132
- Thakur RA, Florek CA, Kohn J et al (2008) Electrospun nanofibrous polymeric scaffold with targeted drug releases profiles for potential application as wound dressing. *Int J Pharm* 364:87–93
- Thomas S (2000) Alginate dressing in surgery and wound management—part 3. *J Wound Care* 9:163–166
- Wiegand C, Abel M, Ruth P et al (2015) In vitro assessment of the antimicrobial activity of wound dressings: influence of the test method selected and impact of the pH. *J Sci Mater Med* 26:1–13

- Williams C (1998) Melgisorb: a highly absorbent calcium/sodium alginate dressing. *Br J Nurs* 7: 975–976
- Williams C (1999) Algosteril calcium alginate dressing for moderate/highly exudate. *Br J Nurs* 8: 313–317
- Xie Y, Zhao J, Huang R et al (2016) Calcium-ion-triggered co-assembly of peptide and polysaccharide into a hybrid hydrogel for drug delivery. *Nanoscale Res Lett* 11:1–9
- Xu R, Xia H, He W et al (2016) Controlled water vapor transmission rate promotes wound-healing via wound re-epithelialization and contraction enhancement. *Sci Rep* 6:1–12
- Yang Y, Hu H (2017) Spacer fabric-based exuding wound dressing—part II: comparison with commercial wound dressing. *Text Res J* 87:1481–1493
- Yeung RA, Kennedy RA (2019) A comparison of selected physico-chemical properties of calcium alginate fibers produced using two different types of sodium alginate. *J Mech Behav Biomed Mater* 90:155–164
- Zhang X, Huan C, Zhao Y et al (2017) Preparation and characterization of nanoparticle reinforced alginate fibers with high porosity for potential wound dressing application. *RSC Adv* 7:39349–39358
- Zhang Y, Holl E, Chun M et al (2020) Just a flesh wound? A detail review of modern dressings. *Health Sci J* 14:730
- Zhang H, Cheng J, Ao Q (2021) Preparation of alginate-based biomaterials and their applications in biomedicine. *Mar Drugs* 19:264
- Zilberman M, Elsner JJ (2008) Antibiotic-eluting medical devices for various applications. *J Control Release* 130:202–215

Alginate as Support Material in Enzyme Immobilization



Zahra Ashkan, Sahar Zahirinejad, Roohullah Hemmati, and Ali Dinari

Abstract Despite the extensive application of enzymes in various industries, their instability and non-reusability cause to their limitation. The immobilization of enzymes can be used as a remedy for these limitations. However, supports and immobilization methods affect the activity and stability of enzymes. Here, the effects of alginate support on immobilized enzymes are investigated. Alginate is an unbranched heterogeneous copolymer comprised of 1,4'-linked β -D-Mannuronic acid (M) and α -L-Guluronic acid (G) residues. Enzyme immobilization on alginate support can improve their stability and increase their reusability significantly. Enzymes entrapment through the alginate and its derivatives is an effective method for immobilization. Availability, biocompatibility, resistance against microbial contamination, non-toxicity, and low price, make it an ideal candidate. Green alginate synthesized nanoparticles, and their combination with other materials (organic/inorganic) have introduced a variety of new applications in drug delivery. Moreover, directed immobilization methods, directed mutagenesis-based methods, and the methods based on recombinant fusion protein technology have paved the way for new strategies for the attachment of enzymes on some supports based on alginate for various pharmaceutical and industrial applications.

Keywords Alginate · Support material · Enzyme immobilization · Drug delivery

Z. Ashkan · S. Zahirinejad

Department of Biology, Faculty of Basic Sciences, Shahrekord University, Shahrekord, Iran

R. Hemmati (✉)

Department of Biology, Faculty of Basic Sciences, Shahrekord University, Shahrekord, Iran

Biotechnology Research Institute, Shahrekord University, Shahrekord, Iran

e-mail: Roohullah.hemmati@sku.ac.ir

A. Dinari

Research Center for Nanorobotics in Brain, Gwangju Institute of Science and Technology (GIST), Gwangju, Republic of Korea

1 Alginate

Alginate is a biological compound, which has found many applications in biomedical sciences and engineering. It is widely used due to its outstanding properties such as high biocompatibility and easy gelling. Alginate hydrogels, for instance, can be prepared using a variety of cross-linking techniques, and because of the structural resemblance between them and extracellular matrices found in live tissues, they have very high applications in wound healing, and delivery of cell transplantation, small proteins, and chemical drugs (Sahoo and Biswal 2021; Varaprasad et al. 2020). Moreover, alginate is widely used in various technologies including various biomolecules immobilization methods and cell transplantation in tissue engineering. The basic objective of tissue engineering is to give patients who are experiencing the organ or tissue failure an artificial tissue or a suitable substitute. For this purpose, hydrogels are exploited to deliver specific cells to a target area aiming at tissue regeneration (Lee and Mooney 2012).

2 Structure and Properties of Alginate

Alginate is a linear, unbranched polysaccharide made up of 1,4'-linked-D-Mannuronic acid (M) and -L-Guluronic acid (G) residues. However, until Fischer and Dorfel identified L-Guluronic acid residues, D-Mannuronic acid was considered to be the only major component of alginates (Joseph et al. 2019). Alginate is now known as the complete family of linear copolymers containing G and M residues (Pawar and Edgar 2012). According to the percentage of precipitations, along with calcium and manganese salts and the variable ratio of glucuronate to manuronate depending on the type of natural component present, alginates are copolymers (Haug 1959). Blocks may consist of consecutive residues G (GGG GGG) and M (MMM MMM) or alternating residues G and M (GMG MGM). The blocks come from many sources, and they vary in length as well as M and G contents. Different types of alginates can be found and more than 200 different alginates are being produced (Qin 2008; Tønnesen and Karlsen 2002). The chemical structure of alginate has been shown in Fig. 1. Alginate is the main part of algae like kelp and is also an exopolysaccharide produced by some bacteria like *Pseudomonas*. Nevertheless, commercial alginates currently come only from algae. Alginate's sequences and molecular weight depend on the source and species of copolymers (Gombotz and Wee 1998). G blocks contain 60% of *L. hyperborean* and in other commercially available alginates contain 14–31% of seaweeds (Qin 2008). It is believed that only alginate's G-blocks play a key role in the formation of intermolecular cross-links in the presence of divalent cations (e.g., Ca^{+2}) to form hydrogels. Composition (e.g., M/G ratio), length of G-blocks, molecular weight, and sequence are critical factors influencing the physical characteristics of alginate and its resultant hydrogels. Alginate gels' mechanical characteristics typically are improved as G-block length

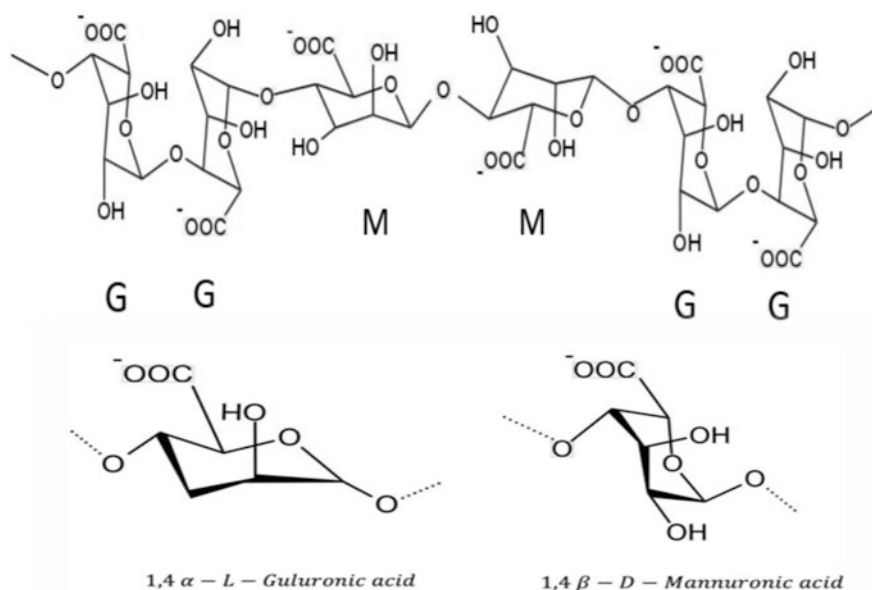


Fig. 1 Chemical structure of alginate

and molecular weight are increased. Different organisms such as bacteria and seaweeds are used as alginate sources and resultant alginate polymers have a wide range of chemical structures (Larsen and Haug 1971). Some alginates include a lot of G blocks, and the resulting gels are relatively rigid. Alginate with a high G content is typically favored for applications requiring a stiffer structure, whereas alginate with a high M content is typically suitable for applications requiring flexible structures. It can be concluded that the versatile property of alginate results from the higher affinity of G residues for divalent ions (Draget et al. 1997). The most popular alginates from brown algae *Laminaria Digitata*, *Laminaria Japonica*, *Laminaria Hyperborea*, *Ascophyllum nodosum*, and *Macrocystis Pyrifera* have been used for cellular encapsulation (Pawar and Edgar 2012). A problem in the application of alginate is the varying degrees of biocompatibility. Various factors such as the type of alginates, coating method, and the purity of alginates are considered as the main reasons for the variable biocompatibility of capsules and their acceptance by the host (Haug 1959; Kong et al. 2002). The factors affect capsule porosity as a measure of cell survival, as well as stiffness which in turn may affect cell differentiation. Although alginate's biocompatibility has been thoroughly assessed both in vitro and in vivo, its composition is still up for discussion (Otterlei et al. 1991). Numerous of these discrepancies are likely caused by the varying purities of the alginate examined in various investigations. For this, in some studies, alginates with a high M content have been more immunogenic than those with a higher G content and have been approximately 10 times more potent in inducing cytokine production (Otterlei et al. 1991). Conversely, in other studies, few immune responses around

alginate implants have been reported (Velings and Mestdagh 1995; Zimmermann et al. 1992).

2.1 *Physical Characteristics*

Alginate chemical derivatizations and design strategies depend on three critical parameters as follows:

- (1) Solubility (2) reactivity (3) determination of specifications.

2.1.1 Solubility

For derivatization, alginates can be dissolved in mixed, inorganic, or organic-organic solutions. The type of reagents required for modification depends on the solvent system selected. Aside from these, the solubility of alginate in the solvent system can affect the derivative replacement pattern (Draget 2009). Three factors determine how soluble alginates are in the water include: the presence of various ions in the solvent, the environment's ionic strength, and the solvent's pH. The pH must be above a specified level in order to dissolve the alginate and the carboxylic acid groups be deprotonated. Alginate's composition, solubility, viscosity, and chain extension are all impacted by variations in the ionic strength of the medium. Alginate gel formation occurs in the presence of dual cations such as Ba^{2+} , Ca^{2+} , Sn^{2+} and thus it is necessary to have an organic solvent free of cross-linking ions to accelerate the dissolution of alginate. Tetrabutylammonium salt (TBA) must be formed in order to enhance the solubility of alginates in organic mediums. Recently, it was observed that TBA-alginate completely dissolved in polar aprotic solvents that contained tetrabutylammonium fluoride (Pawar and Edgar 2011). The solubility of alginates strongly depends on the status of the carboxylic acid groups in the backbone. In any solvent system, including water, alginic acid is not entirely soluble when the carboxylic acid groups are protonated.

Although sodium alginate is soluble in water, it is not entirely soluble in any of investigated organic mediums. TBA-alginate is completely soluble in water, ethylene glycol, and aprotic polar solvents. However, it cannot be dissolved in any other solvent system (Pawar and Edgar 2011; Pawar and Edgar 2012).

2.1.2 Reactivity

Alginates can be altered in two secondary OH positions: C-2 and C-3 for one and C-6 for the other. One of the two types of functional groups can be easily modified by utilizing the difference in reactivity between the two types. It is difficult to modify the C-2 or C-3 hydroxyl groups selectively because of the small changes in their reactivity. In addition, the reaction may be controlled by modifying the selection of

M or G residues (Onsøyen 1997). Alginate chelation with divalent cations to produce hydrogels is a significant challenge in ion cross-linking reactivity. Gel formation is performed by the interaction between G-blocks, which form strong bonds in the presence of divalent cations. In addition to blocks, MG blocks also participate in the formation of weak connections. Therefore, alginate with a high G content produces stiffer gels. In the order shown, alginates lose their affinity for divalent ions: $\text{Pb} > \text{Cu} > \text{Cd} > \text{Ba} > \text{Sn} > \text{Ca} > \text{Co, Ni, Zn} > \text{Mn}$ (Mørch et al. 2006). However, calcium is the most common cation used to induce alginate gel formation in the lab. The cross-linkings of calcium alginate are done in two ways. The first method is a diffusion-based method in which cross-linking ions are dispersed from an external reservoir into an alginate solution. The second approach is a controlled one in which the cross-linking ions are released into the alginate solution after being released from an ion source that exist is in the solution.

2.1.3 Determination of Specifications

In order to completely understand the substitution patterns produced for alginate derivatives, it is sometimes necessary to have a large number of samples of alginate with a comparable M/G ratio. To fully comprehend the replacement patterns, M-, G-, or MG-enriched alginate derivatives might also be needed. Table 1 lists M/G ratio and some physical parameters such as molecular weight and molar mass of the various types of alginates. The lack of commercial availability of alginates with regulated sequences may impair the complete structural properties of the derivatives. Use of advanced analytical techniques is crucial because the alginate polysaccharide backbone is so complicated. Numerous alginate derivatives have been applied in a variety of biomedical applications up to this point. For instance, amphiphilic alginate derivatives with a hydrophobic fraction are synthesized by conjugation of alginate with long chain alkyls (Murata et al. 2004). These compounds have the potential to be used in numerous drug delivery applications since they can self-assemble into particles and gels in aqueous conditions. Amphiphilic derivatives of sodium alginate are prepared by conjugating long alkyl chains to the spine of alginate by forming an ester bond. Aqueous solutions of these alginate derivatives in a semi-dilute diet can be helpful for cartilage repair and regeneration (Haug and Larsen 1966; Pawar and Edgar 2012).

2.2 Chemical Properties

Under acidic conditions, polysaccharides undergo hydrolytic decomposition. The mechanism of glycosidic bond hydrolysis is explained by Timell (Timell 1964). It consists of three steps: (1) Protonation of glycosidic oxygen to form conjugate acid. (2) Conjugated acid hydrolysis and the formation of a non-reducing terminal group

Table 1 M/G ratio and physical parameters of different alginates

Species	M/G ratio	Molar masses ($\times 10^{-5}$ g/mol)	Molecular weight (kDa)	Ref
<i>S. vulgare</i> (Brazil)	1.27	1.94	—	Torres et al. (2007)
<i>S. fluitans</i> (Cuba)	0.52	—	—	Davis et al. (2004)
<i>S. oligocystum</i> (Australia)	0.62	—	—	Davis et al. (2004)
<i>S. muticum</i> (England)	0.31	—	—	Davis et al. (2003)
<i>S. dentifolium</i> (Egypt)	0.52	6.06	—	Larsen et al. (2003)
<i>S. latifolium</i> (Egypt)	0.82	4.16	141–221	Larsen et al. (2003); Fawzy et al. (2017)
<i>S. fluitans</i> (Florida)	1.18	—	—	Torres et al. (2007)
<i>S. turbinarioides</i>	—	—	553	Fenoradosoa et al. (2010)
<i>S. angustifolium</i>	—	—	557	Borazjani et al. (2017)
<i>L. hyperborea</i> stipe	—	—	400	Draget et al. (1994)
<i>L. hyperborea</i> leaf	—	—	710	Draget et al. (1994)
Phaeophyceae class	—	—	300	Costa et al. (2018)
<i>Sargassum pallidum</i>	1.26	—	—	Minghou et al. (1984)
<i>Laminaria japonica</i>	2.26	—	—	Minghou et al. (1984)
<i>Turbinaria ornata</i>	0.89	—	—	Minghou et al. (1984)

as well as a carbonium-oxonium ion. (3) The rapid addition of water to the carbonium-ammonium ion and the formation of a reducing group (Haug et al. 1963).

Sodium alginate in a dry powder shape can be put away for a few months in a cool, dry to put away from light without debasement. By keeping it in a freezer, its half-life can be increased up to several years. Alginic acid decomposes faster than sodium salts. This is thought to be due to the increased degradation rate of the molecule and intramolecular catalysis by C-5 carboxyl groups. Enzymatic degradation of alginate by lyase occurs by a β -removal mechanism, which leads to the formation of unsaturated compounds. They follow a similar path of degradation when they are exposed to highly alkaline environments. The extent of degradation increases significantly above pH 10 and below pH 5. At pH above 10, the degradation rate is more due to the removal mechanism, however, below five more degradations is related to acid-catalyzed hydrolysis. The β -removal mechanism involves the removal of protons at the C-5 position, which is enhanced by the removal of the electron from its carbonyl group at the C-6 position (Haug et al. 1963). The effect of

electron removal is regulated when the carboxyl group C-6 is ionized, and proton removal from C-5 is more difficult than it is when the carboxyl group is protonated (Haug et al. 1967). However, the deprotonation rate is still high enough and causes relatively rapid degradation at higher pH. Some brown algae-derived alginates have different quantities of phenolic chemicals in them. The rate of algal alginates degradation is much higher in species that contain higher amounts of phenolic. It is important to note that a number of reducing compounds, including hydrazine sulfate, ascorbic acid, hydroquinone, cysteine, sodium sulfite, and sodium hydrogen sulfite, speed up the breakdown of alginates. The mechanism of degradation included peroxide formation, which produces free radicals, and subsequently leads to the fragmentation of the alginate chains (Larsen and Haug 1958; Smidsrod et al. 1963).

3 Mechanism of Alginate Biosynthesis

Lin and Hassid studied alginate biosynthesis firstly and identified the enzymatic activity required for mannuronate synthesis in a cell-free system of *Fucus gurdnerii* (Ertesvåg and Valla 1998). The word alginate is based on the word “alga”. Alginate can be found extensively in brown algae and can be isolated from the cell wall of brown algae such as *Ascophyllum nodosum*, *Laminaria Hyperborea* and *Macrocystis pyrifera*. In addition to brown algae, alginate can also be found in some red algae and isolated from the polysaccharide capsule of several bacteria such as *Pseudomonas* and *Azotobacter* (Draget et al. 1994; Laurienzo 2010; Sosnik 2014; Steinbüchel and Rhee 2005). For many years, it has been important for the scientific community to understand the biosynthetic pathway of alginates in order to develop drugs to treat *P. aeruginosa* infections that are exacerbated by the overproduction of alginates, and also to produce bacterial alginates. Unlike the algal alginate biosynthesis pathway, bacteria alginate biosynthesis is well known. Some strains of *P. aeruginosa* can secrete large amounts of alginate that are used to produce thick, highly structured biofilms. At the same time, *Azotobacter* produces stiffer alginates (usually containing a more proportion of G residues), which is proximally associated with the cell and allows the formation of cysts resistant to drought (Hay et al. 2013). In general, *A. vinelandii* and *P. aeruginosa* have analogous gene cluster that is conserved among alginate-producing bacteria; however, they are different in terms of regulatory mechanisms and epimerization. Most involved genes in alginate production are arranged in the bacterial genome except for *algC*, genes such as *algA*, *algD*, *alg8*, *alg44*, *algI*, *algF*, *algX*, *algJ* (*algV*), *algG*, *algE* (*algJ*), *algK*, and *algL*, which are located in the alginate operon (Gene name of the pigeon presented in parentheses). Transcription of these genes is under the strict control of one *algD* upstream promoter and two internal promoters. Therefore, differential transcription of upstream genes to internal promoters has been proposed as a mechanism that may control the stoichiometry of protein subunits in a complex of several proteins, resulting in different combinations of alginates under different

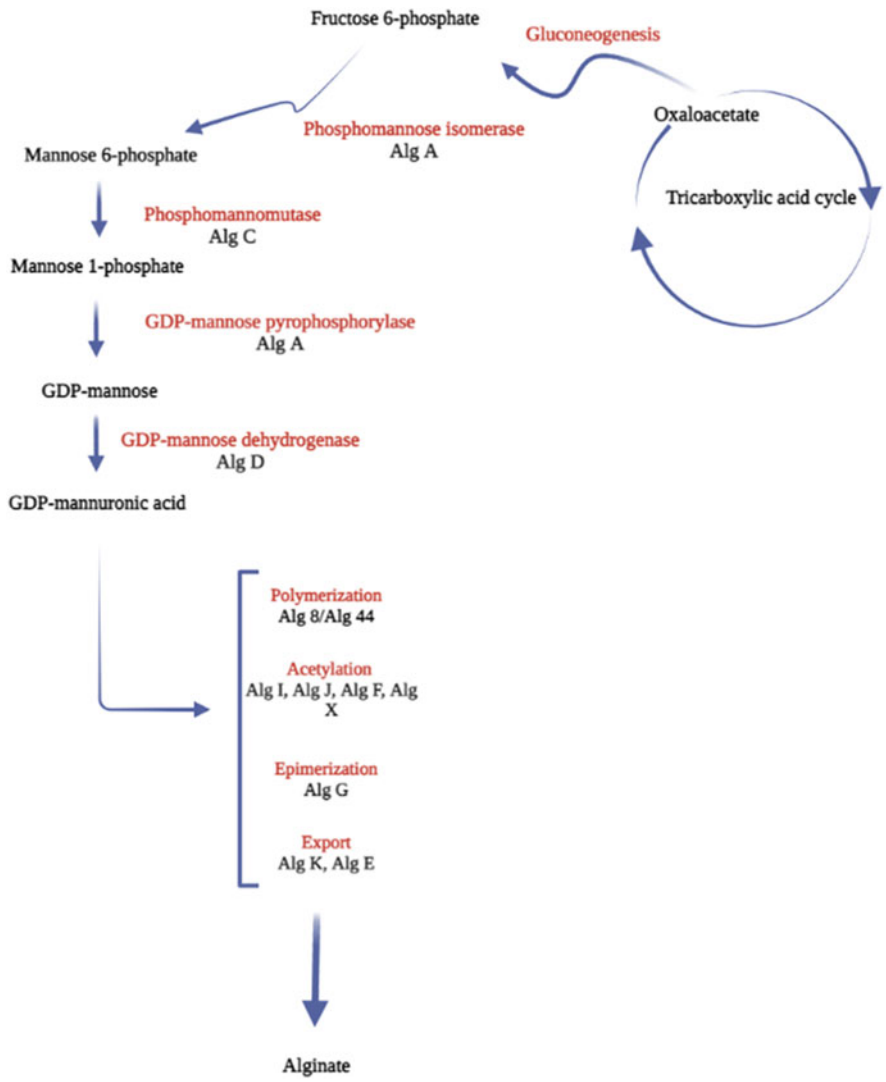


Fig. 2 Biosynthesis pathway of alginate

environmental conditions. Alginate biosynthesis begins with the preparation of the active precursor guanosine diphosphate (GDP) -manuronic acid and other steps are completely shown and explained in Fig. 2 (Hay et al. 2013; May and Chakrabarty 1994; Rehm and Valla 1997).

4 Mechanism of Alginate Decomposition

Alginate has wide use in pharmaceutical and food industries due to its special and unique physical and chemical properties. However, the low solubility of Alginate in water and the high viscosity of the solution limit its applications. Organic synthesis, biosynthesis, or the hydrolysis of glycosidic bonds can acquire alginate oligosaccharides, oligomers containing 2 to 25 monomers. Due to its shorter chain length, AOS has better solubility compared with alginates, with higher molecular weight than monomers. Additionally, due to unique bioactivity, AOS has been shown to have immunomodulatory, antioxidant, antimicrobial, prebiotic, antitumor, antihypertensive, antidiabetic, anticoagulant, and other activities (Liu et al. 2019). Many methods can be used to decompose alginates, including physical, chemical, and enzymatic methods. Physical methods such as ultrasound, ultraviolet, and gamma-ray are often used for the decomposition of Alginate (Cheng et al. 2020). Gamma radiation is one of the best methods of degradation in terms of energy efficiency. Radiation polymerization of alginate does not require any special control of temperature, pH, or additives. By adjusting the dose and dose rate and using gamma radiation AOS with different molecular weights can be produced (Liu et al. 2019). Based on a study conducted by Lee et al. in 2003 and the effect of γ radiation on alginate decomposition was investigated. In the latter study, alginate was irradiated with gamma-60Co in the dose range of 10 to 500 kGy, and subsequent changes in color, viscosity, molecular weight, etc., were measured. At a dose of 100 kGy, gamma radiation reduced the molecular weight of crude Alginate from 25,000 to 300,000. Aside from this, no significant discoloration observed in irradiated samples up to this dose, but by doubling the radiation dose severe browning occurred (Lee et al. 2003). In another study by Nagasawa et al. alginates were tested in a solid or aqueous solution with gamma rays of Co60 in the dose range of 20–500 kGy, and degradation was reported in both solid and solution forms. Degradation was significantly greater in the solute state compared with the solid-state. For example, the molecular weight of alginate in 1% (w/v) solution was reduced from 6×10^{-5} to 8×10^{-3} for 0 kGy and 20 kGy radiation dose respectively, however, while equivalent degradation of Solid requires 500 kGy radiation dose. The later degradation was also followed by a change in color to dark brown for highly degraded alginate (Nagasawa et al. 2000). Hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) are the most common chemicals used to modify alginates by chemical methods. Alginate lyases play a key role in the decomposition of Alginate through an enzymatic pathway (Cheng et al. 2020). Based on the analysis of their hydrophobic components, the polysaccharide lyase family, which includes alginate lyase, is divided into 42 subfamilies. Alginate lyase degrades the glycosidic bond of alginate through the β -elimination reaction and then degrades the alginate to form unsaturated uronic acid oligosaccharides with non-regenerative ends and unsaturated uronic acid monomers (Shankar et al. 2016; Wong et al. 2000). Lyase can be isolated from different sources such as seaweed, marine mollusks, terrestrial and marine bacteria, and fungi (Gacesa 1992; Zhu and Yin 2015). Alginate lyases have different

substrate preferences; these properties of alginate lyases are related to how different they are in terms of their amino acid sequence and the distribution of the monosaccharide group in the substrate. Therefore, these enzymes can be classified into poly M-specific lyase, Poly G-specific lyase, and Poly MG-specific lyase, depending on their substrate preferences (Zhu and Yin 2015). Most of the identified alginate lyases have been reported to have poly M-specific lyase activity, although these enzymes can affect other blocks as well, they usually don't show a lot of activity. Bacterial alginates usually have more effect on G blocks than other alginate lyases; however, enzymes derived from other organisms have a greater effect on M blocks. In the meantime, some of the alginate lyases have bifunctional activity and affect both M-block and G-block alginate connections (Lamppa and Griswold 2013). Alginate lyases can be found in both alginate-producing and non-alginate-producing organisms. In non-alginate-producing organisms, this enzyme acts as a source of carbon. Therefore, microorganisms that use alginate as a carbon source support their cellular metabolism with the help of the extracellular lyase alginate enzyme (Kim et al. 2011). Alginate lyase, decomposes alginates through the removal of beta-glycosidic bonds, creating a double bond between the C4 and C5 carbons of sugar rings. At the time of removal, the 4-O-glycoside bond will be cleaved, and oligosaccharides containing 4-deoxy-L-erythro-hex-4-enopyranuronic acid will be produced as non-regenerative agents (Wong et al. 2000; Zhang et al. 2004).

5 Purification of Alginate

Alginate as a natural polymer is utilized in drug delivery, cell delivery and tissue engineering. In any case, commercial alginate contains an expansive number of pollutions that might cause side effects in humans. Therefore, alginate purification is very important for the use of alginate in biomedical fields. In a previous study, chloroform, butanol, and activated charcoal were used to purify alginate, which is a simple yet effective method of purification. In spite of the fact that commercial alginates are profoundly biocompatible, they might contain shifting amounts of contaminants such as polyphenols, endotoxins, and proteins. As a result, these alginates exhibit cytotoxicity when exposed to a range of susceptible cells, including hybridoma cells. Many research groups have developed their purifying technique and achieved rather decent outcomes (Lee and Palsson 1990). The findings demonstrate that, albeit to various degrees, all techniques of purification lower the quantity of known contaminants, such as polyphenols, endotoxins, and proteins. An increase in the viscosity of alginate solutions was observed. Despite the overall efficiency in reducing contaminant levels, all purified alginates contained relatively high amounts of residual proteins (Selimoglu and Elibol 2010). For example, the same procedure used for raw materials was used to purify commercial sodium alginate; Using macrophage cell lines from mice (Raw264) and bone marrow stromal cells (BMSC), respectively, the toxicity and the biocompatibility of sodium alginate derived from algae were examined. Raw264 and bone marrow stromal cell growth

are both inhibited by the presence of contaminants, as well as the increased production of nitric oxide by macrophages. All these effects were reversed by alginate purification. As a result, alginate treatment improves biocompatibility, bone induction as well as decrease in its toxicity (Dusseault et al. 2006; Selimoglu et al. 2012).

6 Alginate Modification

Alginate chains have many free hydroxyl and carboxyl groups that change surface charge distribution in proportion to pH and thereby alginate is a good candidate for chemical functionalization. Some properties like biological properties, physico-chemical, hydrophobicity, and solubility can be modified by functionalizing the hydroxyl and carboxyl groups (Yang et al. 2011). Various studies demonstrated that alginate derivatives such as oxidized, acetylated, phosphorylated, aminated, and sulfated alginates have many potential applications (Gomez et al. 2006). These include tissue engineering, *P. aeruginosa* biofilm architecture (Pawar 2017), bone tissue engineering (Chamberlain et al. 1946), reducing the level of neutral sterols and lipids action in the digestive system (Coleman et al. 2011), and acting as blood-compatible anticoagulants (Alban et al. 2002) respectively.

6.1 Oxidation

Recent research shows that oxidation is a popular method for alginate modification. The oxidized alginates show more reactivity and decompose when they are used as support material in controlled drug delivery (Boontheekul et al. 2005; Kong et al. 2004). Sodium periodate oxidizes -OH groups of alginates in C-2 and C-3 positions of uronic sodium alginate units accompanied by cleavage of the carbon-carbon bond, resulting in the formation of two aldehyde groups at the ends of each oxidized monomer unit. As a result, greater rotational freedom along the backbone can be observed. During oxidation, the light exclusion is essential to limit the side reaction (Yang et al. 2011). In a study by Gomez et al. oxidized alginates were synthesized at 25 °C for a full day in an aqueous sodium alginate solution in the presence of sodium periodate in the dark. Based on the results, alginate chain oxidation reduced the polymer stiffness by breaking the C2-C3 bond and subsequent chain breakage (Gomez et al. 2006).

6.2 Acetylation

Alginate acetylation is done both naturally and artificially. The biosynthesis of alginates has been well studied in bacterial organisms and the results indicate that

a critical stage in this action is the partial acetylation of the polymer backbone. This process has paramount importance because on one hand acetylation is a control mechanism to inhibit the epimerization of M to G residues and on another hand acetylated alginate plays a key biological role in bacteria (Pawar 2017). In early 1946, a report was published by Chamberlain et al. This study discussed the importance of hydrogen bonding in alginates. The used acetylating agent was glacial acetic acid, and swelling of alginate fibers with water is an indispensable prerequisite before acetylation because, in the absence of water, the hydrogen bond between the alginate fibers is so strong that existing hydroxyl groups don't react. Loss of molar mass due to alginate degradation that occurs during the process is related to the acidic conditions created (Chamberlain et al. 1946).

6.3 Phosphorylation

Phosphorylated alginates are interested due to their ability to promote the production of hydroxyapatite in bone tissue engineering. In a study conducted by Coleman et al. the phosphorylation of alginate was evaluated using the urea/phosphate method and analyzed by FTIR and NMR spectroscopy. N1R D1 and D2 techniques were used to assess the replacement patterns in phosphorylated alginates accurately, and it was found that substitution occurs on both M and G residues. For M residues, mainly phosphorylation occurred at the M3 position; however, M2 and the terminal polysaccharide groups are phosphorylated in a small amount. Still, the exact location of the phosphorylation on the G residue was not precisely determined. Since the reaction is performed in an acidic medium, the molecular weight is reduced to a quarter of the initial value after the completion of the reaction. Reduction in the molecular weight can affect the ability of the polymer to form cross-linked calcium hydrogels and so phosphorylated alginate fraction needs to be mixed with the original and unmodified Alginate fraction. When the two fractions were mixed, it was found that hydrogels containing phosphorylated alginate sodium salt product (PAIg) were more resistant to Ca^{+2} ions (Coleman et al. 2011).

6.4 Sulfation

When polysaccharides are sulfated, they show high blood compatibility and can act as blood-compatible anticoagulants. Heparin is a natural glycosaminoglycan widely used to treat blood clotting (Alban et al. 2002; Linhardt 2003). In a 2014 study, alginates with similar molecular weights and homogeneous sequences (Poly-G, poly-MG, and Poly-M) were sulfated and structurally determined using NMR and elemental analysis. This study showed that alginates are sulfated in a similar way to heparin. It binds to hepatocyte growth factors on the surface of myeloma cells and causes displacement. Polysulfate alginate (poly-MG) showed the highest relative

degree of hepatocyte growth factor (HGF) inhibition, interaction, and maintenance of the high activity at low sulfate degree (DS) values (Arlov et al. 2014).

6.5 Amidation

In comparison to other polysaccharides, alginate has more activity thanks to carboxyl groups. The high content of carboxyl groups throughout the length of the alginate chain leads to production derivatives with a high degree of substitution. Modification of alginate through an Amidation process composed of two-step. First, methyl esterification, and second, aminolysis (amino-de-alkoxylation). Analytical methods confirmed the alginate with high or moderate substituted N-alkyl amides, hydrazide, and hydroxamic acid (Taubner et al. 2017). Labre et al. reported that alginate oligosaccharides with an average molecular weight of 5 kDa show better stability against alginate lyases when they are amidated by 4 (4,6-dimethoxy-1,3,5-triazin-2-yl) 4 methyl morpholinium chloride in an aqueous medium. In this study, alanine, leucine, serine, mannose, and rhamnose were used for Amidation (Labre et al. 2018).

7 Alginate Nanoparticles

Nanoparticles, nanospheres, and nanocapsules are nanoscale frameworks (10–100 nm) and can maintain and carry proteins, drugs, and other compounds by dissolving, trapping, or joining them to the matrix. Some methods which are used to synthesize nanoparticles as follows:

1. Nano-aggregations as nano-sized colloidal systems in which the drugs are physically scattered and can have diverse morphologies.
2. Nano capsules as vesicular frameworks in which the drugs are restricted to an oily or fluid core encompassed by a polymeric film.
3. Nanospheres as particles with jelly centers in which the drug or target part is physically scattered and encircled.
4. Nano capsules by internal structures which are the combination of nano capsules and Nanospheres. They first prepare a Nanosphere and then an additional shell forms on the common surface of the Nanosphere (Severino et al. 2019).

In addition, according to the formation of alginate nanoparticles, they are divided into two groups: (1) Complexation, where the formation of a complex occurs in an aqueous solution and the formation of alginate nanoparticles occurs on the common surface of an oil drop and finally, alginate nano capsules are formed. Cross-linker is calcium from calcium chloride or a polyelectrolyte with the opposite charge (e. g Poly-L-lysine). (2) Using oil emulsification alginate nanospheres can be produced (Lambert et al. 2001; Shi 2018).

Emulsification of alginate is the dispersion of emulsion droplets in an oil phase to solidify in order to create nanospheres (Paques et al. 2014). Compared with the nozzle-based methods, this technique is simple and low-cost (Holban and Grumezescu 2016; Uyen et al. 2020). Generally, gelation takes place in two steps include alginate-in-oil (w/o) emulsion and the gelation of the emulsified beads of alginate through covalent bonds or ionic interactions. Altogether, the gelation of alginate occurs in two internal and external gelation ways. In this case, the release of cations from the inner core of emulsified alginate droplets is necessary for internal gelation (Pestovsky and Martínez-Antonio 2019). Furthermore, the water-insoluble CaCO_3 is used for alginate emulsification (Choukaife et al. 2020). Finally, micro and nanospheres with a soft core and a rigid outside matrix are produced through external gelation (Leong et al. 2016).

7.1 Alginate-Based Hydrogel Nanoparticles

As mentioned earlier, non-toxic alginic acid is highly soluble in water, has a tendency to form a gel with high porosity in suitable conditions, and has high biocompatibility. Cross-linking and formation of polymer networks in hydrogel structured drug carriers, including micro-and nanoparticles culminated with the addition of cross-ions to alginate (Tønnesen and Karlsen 2002). The most popular salt employed by researchers is calcium chloride, however, any cation might start the reaction sequence. Preparation methods can control the gelling process, this leads to the desired size ranges, which depend on the concentration and viscosity of the alginate, the concentration of the cross-ion, and the rate at which the cross-ions are added to the alginate solution (Shi 2018).

Rajaonarivony et al. reported in 1993 a new drug carrier based on sodium alginate (Pandey and Khuller 2006; Rajaonarivony et al. 1993). They created alginate nanoparticles in sodium alginate solution that were generated by adding calcium chloride, followed by polylysine, and ranged in size from 250 to 850 nm. The concentrations of cross-ions and polymer solutions in this investigation were lower than those typically used for gel formation. In addition, they showed that high loading capacity could be achieved, with more than 50 mg of drug per 100 mg of alginate using doxorubicin as a model drug. Alginate-based nanoparticle studies have been more popular since the late 1990s. The use of therapeutic agents such as insulin, anti-tuberculosis drugs as well as antifungal drugs has shown promising results toward gel-based carriers. The size range of alginate nanoparticles depends on the order of the addition of ions to the alginate. A few studies have demonstrated the benefits of including a polyelectrolyte complex step in this strategy (Pandey and Khuller 2006). Sarmiento et al. Showed via addition of chitosan polyelectrolyte complex to the alginate nanoparticles, increases the efficiency of insulin loading (Sarmiento et al. 2007). They prepared particles within the nanometer size range under ideal conditions with a burden capacity of 14.3%. In another study, Reis et al. loaded dextran polysaccharide as a complexing factor and insulin on

alginate-dextran nanospheres through nano emulsion scattering and consequent gel arrangement. The produced particles had a one-sided size distribution and ranged in size from 267 nm to 2.76 μm , with an insulin capsulation yield of 82.5% (Reis et al. 2007; Shi 2018).

7.2 *Alginate Magnetic Nanoparticles*

Magnetic nanoparticles are widely used because of their unique properties, which are especially promising in medicine. Alginate, among all the polymers, has a number of remarkable characteristics that make it suitable as a support for the entrapment and the delivery of a variety of molecules and biomolecules (Ciofani et al. 2008). In this regard, Ciofani et al. Reported magnetic alginate nanoparticles prepared through the emulsion, lattice for effective drug release, and precise targeting (Chomoucka et al. 2010; Ciofani et al. 2009).

7.3 *Application of Alginate Nanoparticles in Drug Delivery*

Compared with conventional dosage forms, controlled-release drug delivery systems provide numerous advantages, including reduced toxicity, increased efficacy, and increased patient compliance and comfort. These properties increase the efficiency of treatments (Ravi Kumar and Kumar 2001). The biodegradability of alginate is the most important advantage of using it as a matrix for controlled release. The low molecular weight molecules, antigens, antibiotics, polysaccharides (heparin), peptide hormones (like insulin or growth hormone), and enzymes can all be delivered by alginate under controllable conditions (Ravi Kumar and Kumar 2001; Shilpa et al. 2003).

8 Enzyme Immobilization on Alginate Supports

Enzymes act as biological catalysts and specifically reduce the activation energy of reactions and thereby increase the rate of biochemical reactions. Enzymes have been used in many industries, such as chemical, pharmaceutical, cosmetic, textile, food and etc., due to their natural applications and extensive potential. But the applications of enzymes are fraught with problems. Generally, the instability of enzymes can be considered as their main weakness, which in the case of temperature and pH fluctuations, their half-life is reduced. In addition, the purification process of enzymes is very costly. Therefore, the immobilization of enzymes on the different supports with varying shapes and sizes is what we can call the most efficient and successful way to solve these problems. As we know, enzyme immobilization causes

an increase in the stability of the enzyme versus environmental changes, prevents or minimizes product contamination, and as a result increases the reusability of enzymes (Ashkan et al. 2021; Zahirinejad et al. 2021). Alginate is a copolymer containing manuronic acid and guluronic acid. This polymer has a negative charge and forms a gel in the presence of many divalent cations under controlled conditions. In general, due to their low toxicity, Ca^{+2} ions are mostly used for such purposes. Alginates are commercially available in the form of water-soluble sodium alginate and have been used in the food and pharmaceutical industries for more than 65 years as thickeners, emulsifiers, and film-forming agents. One of the common ways for enzyme immobilization is by trapping enzymes in Alginate. The main advantage of using Alginate is that this gel allows the passage of macromolecules due to its porosity and high diffusion rate. In addition to this advantage, this gel is a non-toxic, inexpensive, available, versatile, heat-resistant substance, compatible with living tissues, economical, and improves the stability of the enzyme and its functional properties. Alginate itself can be stored well in low pH environments and allows the catalytic process to be performed in an acidic environment by the immobilized enzyme (Anwar et al. 2009; Doğaç et al. 2015; Yang et al. 2014). Immobilization of enzymes on alginate supports improves the use of enzymes for industrial applications. For example, the undesirable turbidity of the final product is one of the most important problems during the industrial production of fruit juice (Siebert 2006). The cause of instant turbidity in freshly produced fruit juice backs is the suspension of polysaccharide particles such as cellulose, hemicellulose, pectin, starch, and lignin (Sorrivas et al. 2006). Among these, pectin plays an important role and causes turbidity and unwanted solid suspensions in juices. In the commercial production of juices, pectinases are often used to break down and remove pectin. Therefore, commercial pectinase has an essential role in juice technology and has a favorable and significant effect on improving the yield, clarification, and filterability of juices. Like other free enzymes, free pectinases are not sufficiently stable under operating conditions. The impossibility of multiple reuses in an industrial process, enzyme contamination in the final food product, and the additional step of purification of the enzyme from the products are other problems for the application of free pectinase in juice clarification. Therefore, the immobilization strategy is used to solve the problems of expensive enzymes. Immobilization of pectinase on calcium alginate microspheres was inspected to solve pectinase problems, and it was found that the immobilized enzyme has better stability compared to the free enzyme and also maintained 63% of its initial activity after three successive reactions. Reduction of apple juice turbidity and improvement of heat and freezing capacity of apple juice after the use of immobilized pectinase was also reported (Deng et al. 2019).

Lactase is an essential enzyme to treat lactose intolerance problems. The natural form of this enzyme is soluble and unstable for reusing. Zawawi et al. have compared the productivity and stability of lactase immobilized on calcium alginate with the free enzyme. Thermal stability was measured at different temperatures. After 21 days, the immobilized enzyme showed a better stability where it retained up to 62% of enzyme activity and showed a significant improvement in reusability (Zawawi et al. 2020). In order to show that starch hydrolysis at a liquefaction

temperature of 95 °C may be recycled, Tee et al. coupled a thermostable amylase to calcium alginate via a covalent connection. After seven consecutive hydrolysis cycles, the covalently bound enzyme produced 53 g of starch degradation per mg of bound protein (conditions: 10 min at 95 °C in the stirred batch reactor). At pH values of 5.5 and 6.0, respectively, the maximum activity of the free enzyme and the immobilized one were observed. The K_m value of the immobilized amylase was 2.5 times greater than free one. Because of its high immobilization capacity on calcium alginate, immobilized amylase is a good choice for the industrial starch hydrolysis process (Tee and Kaletunç 2009). In biotechnology, laccases are hampered by some issues related to their recovery and inactivation. In research was done by Olajuyigbe et al. purified laccase immobilized on calcium and copper alginate beads. The optimum pH related to free laccase and immobilized one was recorded at 5.0 and 6.0 respectively. Free laccase optimum activity was at 40 °C; however, those of Ca and Cu alginate immobilized laccases were appeared at 60 °C and 50 °C. The immobilized enzyme demonstrated greater operational stability after 21 days at 4 °C in comparison to the free enzyme. Additionally, the immobilized laccase was reused successfully for three cycles with residual activity exceeding 70%. In the later study, improved thermal stability and kinetic parameters, along with the increased reusability of immobilized laccase from *C. Fabiani* with potential application in bioremediation were revealed (Olajuyigbe et al. 2019). The enzyme endo- β -1,4-xylanase immobilized on calcium alginate can also be used in the paper and pulp industry. Endo- β -1,4-xylanase immobilization demonstrated greater stability at high temperatures than free enzyme. According to the data, the rate of activity remained at 18% and 9%, respectively, at 70 °C and 80 °C. In addition, the enzyme was immobilized for up to five reaction cycles and showed sufficient recycling efficiency. This indicates the enzyme can be an acceptable candidate in the industry of paper and pulp (Bibi et al. 2015). Soybean (*Glycine max*) is used widely as a source of different proteins for industrial uses. Soybean is rich in protein content and its byproducts are valuable in the feed and food industries. It has been reported that due to its easy digestibility, soybean is a valuable product to lactating dairy cows and pigs. Among various proteins contained in soybean urease is abundantly expressed in its seeds. In a study by Kumar et al. they immobilized soybean seed urease on alginate and found that the support could enhance the enzyme stability, with a half-life of 80 days. These hydrogels containing immobilized urease have uses in the biomedical, biotechnological, agricultural, and environmental fields. For example, they can be used in artificial kidney machines (Kumar et al. 2009). In a study which has done by Malhotra, Ishita, and Seemi Farhat Basir, the enzyme of invertase was immobilized in Ca^{+2} -alginate and Ca^{+2} -alginate-kappa-carrageenan support using the entrapment method. The optimum pH values for immobilized and free invertase were found to be 5.5 and 4.5, respectively. Free and immobilized forms of enzyme share the same optimum hydrolysis temperature. Invertase immobilized on Ca^{+2} -alginate-kappa-carrageenan support revealed the highest reusability, thermal stability, storage stability, and pH, after 3 months at 4 °C (Malhotra and Basir 2020).

8.1 *Common Methods of Enzyme Immobilization on Alginate*

8.1.1 **Entrapment**

Physical entrapment of the enzyme in alginate supports has been proposed as a relatively simple, inexpensive, rapid, and safe technique compared with other immobilization methods. The support also has high porosity for the support and product diffusion, good mechanical strength, and most importantly provides the required functional groups for immobilization (Kumar et al. 2012; Sunita 2014; Talekar and Chavare 2012). Enzymes entrapment by alginate is an effective method for enzyme immobilization due to its biocompatibility, non-toxicity, cheapness, availability, and resistance to microbial contamination therefore it is more widely used than other methods. Aside from these properties, calcium alginate as a support has paramount importance for enzyme entrapment because the trapped enzymes on calcium alginate support retain most of their activity. Therefore, this support has been used for the immobilization of different types of enzymes such as protease (Anwar et al. 2009), cellulose (Viet et al. 2013), polygalacturonase (Buga et al. 2010), Lipase (Bhushan et al. 2008; Hertzberg et al. 1992), Alkaline Protease (Qamar et al. 2020), α -amylase (Dey et al. 2003), and other enzymes which are summarized in Table 2. For example, in a study by Anwar et al. the immobilization of protease enzyme on calcium alginate support was investigated, and it was reported that most of activity of the immobilized enzyme was retained for a longer time compared with that of the free enzyme (Anwar et al. 2009). In another research in 2013, cellulase was immobilized on calcium alginate granules, and the enzyme showed higher stability against changes in pH and temperature compared to the free enzyme. It was also reported that the immobilized enzyme could tolerate more acidic pH and higher temperatures than the free enzyme. Immobilized cellulase can be reused multiple times (Viet et al. 2013). The immobilization of polygalacturonase obtained from *Aspergillus niger* on calcium alginate was also investigated by Buga et al. Taken together, the optimal pH of the immobilized polygalacturonase is 4.5 and can be reused up to three cycles; however, most of its activity retained (Buga et al. 2010). In addition to calcium alginate, immobilization of enzymes on other supports via the trapping method can improve the properties of enzymes. For example, immobilization of Phenol oxidase on copper alginate gel (Palmieri et al. 1994), Proteases on Alginate-chitosan (Rezakhani et al. 2014), Lipase on Alginate/polyvinyl alcohol Beads (Bonine et al. 2014), and Levan-xylanase on sodium alginate beads (Jampala et al. 2017) improved the activity, stability, and thermal stability of the enzymes.

Table 2 The immobilization of different enzymes on alginate and alginate derivatives supports

Enzyme	Support	Immobilization method	Immobilization effects	Ref
Phenol oxidase	CU-AL.G	Entrapment	Improvement of enzyme activity in the higher pH range	Palmieri et al. (1994)
			Increment of the oxidation rate of different supports by the immobilized enzyme	
Proteases	Ca-AL	Entrapment	Increment of retention of enzyme activity	Anwar et al. (2009)
			Increment of enzyme reusability	
Proteases	AL-CHIT	Entrapment	Improvement of stability and activity of the immobilized enzyme	Rezakhani et al. (2014)
Lipase	Ca-AL	Entrapment	Increment of enzyme stability	Bhushan et al. (2008)
			Increment of enzyme reusability without activity reduction	
Lipase	Ca-AL	Entrapment	Significant increment of enzyme stability	Hertzberg et al. (1992)
Lipase	Ca-AL.GB	Entrapment	Increment of enzyme reusability	Won et al. (2005)
Lipase	Ca-AL.B	Entrapment	Improvement of enzyme thermal stability	Bonine et al. (2014)
			Possibility of reusing the immobilized enzyme	
			Retaining 50% of enzyme activity after 5 cycles	
Lipase	PVA-AL	Entrapment	Improvement of enzyme thermal stability	Bonine et al. (2014)
			Increment of enzyme reusability	
Alkaline Protease	Ca-AL	Entrapment	Improvement of pH and thermal stability of the enzyme	Qamar et al. (2020)
			Increment of Vmax of the enzyme	
			Increment of enzyme reusability	
Amylase	Na-AL	Entrapment	Increment of enzyme affinity with the support	Ningsih et al. (2020)
			Increment of enzyme reusability	

(continued)

Table 2 (continued)

Enzyme	Support	Immobilization method	Immobilization effects	Ref
α -amylase	Ca-AL	Entrapment	• Increment of enzyme activity and functional stability	Dey et al. (2003)
			• Retaining 85% of the initial activity of the enzyme after seven reuses	
α -amylase	Ca-AL	Entrapment	• Improvement of pH and optimum temperature of the enzyme	Talekar and Chavare (2012)
			• Reduction of support affinity and maximum enzyme reaction rate	
			• Retaining 35% of enzyme activity after 10 reuses	
α -amylase	Ca-AL.B	Entrapment	• Increment of enzyme reusability	Ertan et al. (2007)
			• Loss of only 35% of enzyme activity after seven reuses	
Cellulase	Ca-AL	Entrapment	• Improvement of pH and optimum temperature of the enzyme	Viet et al. (2013)
			• Possibility of placing the immobilized enzyme in acidity and temperature higher than the free enzyme	
			• Possibility of reusing the enzyme	
Yeast alcohol dehydrogenase	BM/AL.B	Entrapment	• Retaining 86.6% of enzyme activity after 12 reuses	Ai et al. (2013)
Alcohol dehydrogenase	AL-CHIT. B	Entrapment	• Improvement of operational and thermal stability of the enzyme	Li and Li (2010)
Inulinase	AL-CHIT. B	Entrapment	• Retaining 76% of enzyme activity after 6 days	Missau et al. (2014)
Phospholipase A1	PVA-AL	Entrapment	• Improvement of pH and optimum temperature of the enzyme	Zhan et al. (2013)
			• Retaining more than half of the initial activity in the eighth cycle	

(continued)

Table 2 (continued)

Enzyme	Support	Immobilization method	Immobilization effects	Ref
			Retaining more than 70% of the initial activity after 6 weeks	
Naringinase	PVA	Entrapment	Retaining 70% of enzyme activity after eight reuses	Nunes et al. (2010)
Araujiaia	Ca-AL.B	Entrapment	Stability of immobilized enzyme over a wide range of pH and temperature	Quiroga et al. (2011)
			Retaining 95% of initial activity of the immobilized enzyme after 6 weeks	
			Retaining 78% of enzyme activity after 20 reuses	
Laccase	AL-CHIT. MC	Entrapment	Increment of the staining efficiency of immobilized enzyme	Lu et al. (2007)
			Retaining 35.73% of enzyme activity after three reaction cycles	
Laccase	Ca-AL.B and CU-AL.B	Entrapment	Improvement of optimal pH of the enzyme	Olajuyigbe et al. (2019)
			Retaining 70% of enzyme activity after three reaction cycles	
Urease	AL.B	Entrapment	Improvement of enzyme stability	Kumar et al. (2009)
Pectinase	Ca-AL. MS	Entrapment	Improvement of enzyme stability	Deng et al. (2019)
			Increment of optimum temperature	
			Retaining 63% of enzyme activity after three reactions	
			Reduction of apple juice turbidity and improvement of heat and freezing capability	
Polygalacturonase	Ca-AL	Entrapment	Improvement of optimal pH of the enzyme	Buga et al. (2010)
			Increment of enzyme reusability	

(continued)

Table 2 (continued)

Enzyme	Support	Immobilization method	Immobilization effects	Ref
Endo- β -1,4-xylanase	Ca-AL	Entrapment	• Increment of the stability of the enzyme immobilized at higher temperatures	Bibi et al. (2015)
			• Increment of enzyme reusability	
Pectinase	P-HAP/ Ca-AL. COM		• Increment of thermal stability and pH tolerance of the enzyme	Qi et al. (2020)
			• Increment of stability of enzyme storage	
Phytase	PVA-Na-AL		• Increment of catalytic activity of the enzyme	Duru Kamaci and Peksel (2021)
			• Optimal increment of enzyme pH and temperature	
			• Increment of immobilized enzyme activity over a wide range of pH and temperature	
Levan-xylanase	Na-AL.B	Entrapment	• Increment of enzyme activation energy	Jampala et al. (2017)
			• Increment of stability and retention of enzyme activity	
Glucose isomerase	Ca-AL. GB	Entrapment	• Retaining 86% of the initial activity of the enzyme after 6 weeks	Tumturk et al. (2008)
			• Retaining 85% of enzyme activity after 22 repeated uses	
Glucose oxidase	Ca-AL-CHIT. MS		• Increment of retention of enzyme activity	Wang et al. (2011)
Horseradish peroxidase	PVA-A	Cross-linking	• Increment of thermal stability of the enzyme	Bilal et al. (2017)
			• Increment of enzyme reusability	
Glucose oxidase	Ca-AL.G	Cross-linking	• Increment of K_m and decrement of V_{max} after enzyme immobilization	Blandino et al. (2001)
Endo- β -glucanase	Ca-AL.B		• Increment of the half-life of immobilized enzyme	Busto et al. (1998)
			• Retaining 75% of enzyme activity	
Xylanase	GA-a- Ca-AL.B	Cross-linking		

(continued)

Table 2 (continued)

Enzyme	Support	Immobilization method	Immobilization effects	Ref
			- Improvement of pH and optimum temperature of the enzyme - Improvement of stability of the immobilized enzyme in the presence of additives - Increment of reusability of the enzyme after immobilization for up to 5 cycles	Kumar et al. (2017)
Lignin peroxidase	Ca-AL.B	Covalent	- Effective decolorization of the biocatalytic system in five consecutive operations - Retaining color removal efficiency of more than 80% after 5 cycles	Bilal and Iqbal (2019)
Inulinase	GF-AL	Covalent	- Optimal increment of enzyme pH and temperature - Retaining 95% of enzyme activity after 20 reuses	Elnashar et al. (2009)
α -amylase	Ca-AL	Covalent	Increment of reusability of the enzyme after immobilization for up to 10 cycles	Tee and Kaletunç (2009)
Xylanase	GA-AL.B	Covalent	- Increment of K_m, V_{max}, optimal pH, and temperature - Increment of enthalpy and change of free energy	Pal and Khanum (2011)

CU-AL.G copper alginate gel, *Ca-AL* calcium alginate, *Ca-AL.GB* calcium alginate gel beads, *Ca-AL.G* calcium alginate gel, *AL.B* alginate beads, *Ca-AL.B* calcium alginate beads, *PVA* polyvinyl alcohol, *PVA-AL* polyvinyl alcohol-alginate, *Na-AL* Na-alginate, *Na-AL.B* sodium alginate beads, *CU-AL.B* copper alginate beads, *AL-CHIT* alginate-Chitosan, *AL-CHIT.B* alginate-Chitosan beads, *GA-AL.B* glutaraldehyde-alginate beads, *GA-a-C-AL.B* glutaraldehyde-activated calcium alginate beads, *AL-CHIT.MC* alginate-Chitosan microcapsules, *GF-AL* grafted alginate, *Ca-AL.MS* calcium alginate microspheres, *Ca-AL-CHIT.MS* calcium alginate-Chitosan microspheres, *P-HAP/Ca-AL.COM* porous hydroxyapatite/calcium alginate composite, *PVA-Na-AL* polyvinyl alcohol-sodium alginate, *BM/AL.B* boehmite/alginate hybrid beads

8.2 Covalent Bond

Using this method, enzyme leakage from the support is minimized, and it is possible to reuse it more than other available immobilization methods. This method also

increases the half-life of enzymes and their thermal stability. An increase in enzyme stability due to covalent attachment results from the limited movement and collision of the immobilized enzyme molecules. Directed immobilization of enzymes on the surface of the supports also increases the binding of enzymes and the loading capacity of the enzyme. On the other hand, the covalent attachment immobilization method also has its own disadvantages, for instance, decreased enzyme activity due to the disproportionate orientation of the enzyme on carriers in a way that the enzyme active site is attached to the support and the activity is drastically decreased after immobilization (Mohamad et al. 2015; Zhan et al. 2013). In a study conducted by Pal, Ajay, and Farhath Khanum, xylanase enzyme immobilization on Glutaraldehyde-alginate beads was investigated. According to their results, immobilized xylanase showed higher thermal stability which could be attributed to an increase in enthalpy (H°) and free energy change (G°) after covalent attachment, also, the immobilized xylanase was reused successfully for five cycles with residual activity exceeding 85% (Pal and Khanum 2011).

8.2.1 Cross-Linking

In the cross-linking method, enzyme molecules accumulate on themselves by forming covalent bonds after the addition of functional cross-linking agents. This can lead to minimizing leakage and increasing the operational stability of enzymes, but this method causes the enzyme to have a strong bond with the support which reduces the enzyme activity and is costly (Ashkan et al. 2021). In a study by Bilal, Muhammad et al. horseradish peroxidase was immobilized on self-made polyvinyl alcohol (PVA)-alginate grains in the presence of sodium nitrate as a cross-linker. The immobilized enzyme showed considerable improvements in reusability and thermal stability. The results of this study demonstrated that HRP immobilized on alginate-PVA could be used as an economical green catalyst in biotechnology and industries, especially for dye-containing industrial waste effluents or the purification of textile dyes (Bilal et al. 2017).

9 Drug Immobilization on Alginate Support and Drug Release

Alginates are used in various applications due to special properties such as viscosity, biocompatibility, biodegradability, non-cytotoxicity, and facilitated drug release. Some drugs such as peptides, proteins, antibodies, and small molecules can be loaded inside or on the surface depending on the properties of the nano-supports. Usually, during the formation of the nano-supports, drugs are encapsulated in the supports through the non-covalent binding of the drug to the matrix (Rai et al. 2019). In contrast, in covalent attachment, drugs and nano-supports are held together by

covalent bonds (Ranghar et al. 2014). Drug immobilization in solid carriers has been increased as a therapeutic strategy; however, the ideal carrier itself must be biodegradable and non-toxic. Therefore, substances such as gelatin, albumin, and alginate are used as ideal drug carriers due to their low toxicity and biocompatibility (Rai et al. 2019). Nano-support properties and immobilization methods have significant effects on the performance and the function of the immobilized drugs. The supports have a large surface area that facilitates effective drug loading and reduces mass transfer resistance (Aldobaev et al. 2018). Nanohydrogels based on alginate and chitosan are among the most used polymers as drug nanocarriers. The reason is that alginate-based nanohydrogels have different properties such as hydrophilicity, flexibility, adaptability, the ability to absorb large volumes of water, biocompatibility, and appropriate size (Ahsan et al. 2018). Thus, the alginate hydrogels can be used to deliver the drugs in a targeted and controlled way (Hassan et al. 2019). In practice, alginate nanomaterials were used according to a method presented by Ciofani et al. to deliver several drugs with a prolonged release time (approx. 5–6 days, after the first burst) (Ciofani et al. 2008). Alginate nanoparticles can be prepared through covalent cross-linking, emulsification, spray drying, self-assembling, ionic gelation, and polyelectrolyte complexation for drug delivery (Hasnain et al. 2020). Alginate has also been used to make nanogels, in which nanoparticles are cross-linked and then swelled by large amounts of solvent (Vicario-de-la-Torre and Forcada 2017). In a previous study, oxidized alginate was covalently coupled to doxorubicin by the Schiff base reaction to provide a novel drug delivery system. Recently, venlafaxine-loaded alginate nano-support for the intranasal treatment of depression was used to assess the possible use of alginate matrix for brain drug delivery (Elzoghby et al. 2016; Haque et al. 2014). In another study, magnetic nanoparticles with a core-shell structure (doxorubicin-gelatin = core and Fe_3O_4 -alginate = shell) were developed as specialized drug delivery systems for cancer treatment. The results showed that anticancer drugs in alginate magnetic nanoparticles have the potential to be used for targeted drug delivery and cancer therapy (Huang et al. 2020).

10 Future Perspective

Specific attention has been paid to biopolymers and particular alginates in the medical and pharmaceutical industries in recent years. Alginate is a natural compound that has advantages over synthetic polymers such as availability, non-toxicity, biocompatibility, biodegradability, low cost, and the ability to form gels in mild, aqueous conditions. All these benefits make Alginate a useful biomaterial for biomedical applications, especially targeted drug delivery and the delivery of other biologically active compounds (Shilpa et al. 2003). In addition, due to its favorable properties, it has become a suitable support for enzyme immobilization. The combination of Alginate with other low-risk organic/inorganic supports can improve the properties of resultant hybrid nano-supports for enzyme immobilization. For example, immobilization of chlorophyllase I and lipase on magnetized alginate support, or

immobilization of catalase by encapsulation in Fe_3O_4 /alginate magnetic composite support showed that enzymatic activity, thermal stability, and pH resistance are improved after immobilization, and it is possible to reuse these enzymes multiple times (Yang et al. 2014). Organic-inorganic hybrid composites have the properties of organic and minerals simultaneously with unique properties. Organic materials are responsible for the appearance of specific physical and chemical properties (optical, electrical, reactivity); however, minerals have mechanical strength properties, increase thermal stability, and also allow the modification of the refractive index (Oshiro Junior et al. 2016). It can be foreseen that organic-mineral compounds that demonstrate the unique physicochemical properties of their components will be widely used in targeted medicine and drug delivery. Although Alginate plays a pivotal role in the immobilization of enzymes and proteins, it has disadvantages such as leakage of immobilized proteins from the surface of alginate grains, limited the immobilization of lower molecular weight molecules such as peptides, loss of mechanical properties of cross-linked alginates over time, failure of immobilization of some enzymes such as chymotrypsin on the support. Of course, to overcome the limitations of immobilization on Alginate surface, chemically modified alginates, polyelectron polymerization and granules coated with chitosan alginate can be used. In addition, in order to reduce enzyme leakage and increase immobilization efficiency, cross-linking of enzymes using some other polymers is performed (Chaudhari et al. 2015). Alginate can include electrostatic interactions due to its negative surface charge. Tielen et al. investigated the electrostatic interactions between lipase (LPS) and alginate and recorded a significant increase in the stability of the immobilized lipase. Naturally, this model of interaction between LPS and Alginate has advantages such as preventing LPS from being rapidly diluted into the surrounding environment. Interaction between LPS and Alginate can also enhance the activity by directing the catalytic site of the enzyme to lipid supports (Tielen et al. 2013). In addition to the option of an appropriate support, choosing the most appropriate immobilization method is very important. Although the entrapment method has many advantages such as being relatively simple, safe, inexpensive, and rapid, the leakage of enzymes included in the matrix network is often observed and is not ignorable. However, there is another method that leads to a decrease in enzyme activity which is known as the cross-linking method. The later method creates a strong bond between enzymes and nano-supports. Due to the tight bond between enzymes and nano-supports in the covalent attachment method, enzymes remain attached to nano-surfaces. Nevertheless, enzyme immobilization has some disadvantages such as aggregation of enzymes on nano-surfaces or the inactivation of enzymes due to the blocking of the active sites by nano-surfaces. To overcome these challenges, some new methods have been introduced such as oriented immobilization or site-directed immobilization. Enzyme orientation determines a location on which an enzyme active site is attached to nano-surface. In fact, the attachment of enzyme active sites to nano-surfaces may produce steric hindrance which in turn leads to the inactivation of the immobilized enzyme. Both aforementioned immobilization methods (oriented and site-directed) result in the immobilization of enzymes or proteins in a targeted manner (Ashkan et al. 2021; Zahirinejad et al. 2021).

Recently some studies on the use of oriented or site-directed immobilization methods in enzyme immobilization have been reported; however, these two methods are more commonly used for non-enzymes proteins. For example, immobilized Polygalacturonase (PG) on the Calcium Alginate-Coated Polypyrrole/Silver Nanocomposite supports improved the stability of enzyme to temperature and pH relative. The immobilized Polygalacturonase was highly achievable (91% and 68%) after 5 and 10 cycles. The immobilized Polygalacturonase is ideal for industrial and biotechnological applications due to its improved catalytic efficiency (V_{max}/K_m), storage stability, and reusability (Almulaiky and Al-Harbi 2022). The technology of the oriented immobilization and recombinant fusion proteins of enzymes have gained increasing attention in recent years (Kumada 2014). Lead (Pb) is detrimental to human health and can even cause fatalities. It is needed to remediate Pb from the environment. In order to investigate the biosorption of Pb (II) from an aqueous solution Keshav et al. studied the use of a Pb-specific recombinant fusion metalloprotein. PbrD is a lead binding protein, which can be exclusively found in *Cupriavidus metallidurans* CH₃, that binds intracellular Pb (II) ions and decrease their toxic effects. In the aforementioned study, a recombinant form of PbrD (rPbrD) was immobilized by a cross-linked method on Ca⁺²-alginate nanoparticles. The findings of this study showed that the proposed nano-adsorbent is highly suitable for the removal of concentrated Pb ions found in industrial effluent or acid mine drainage (Keshav et al. 2019).

Today, natural polymers such as Alginate are used more as drug carriers compared with synthetic polymers due to the various properties mentioned earlier (Jain and Bar-Shalom 2014; Lu et al. 2006; Shelke et al. 2014). The use of nanotechnology and nano-sized particulate in targeted drug delivery has advantages such as targeted delivery, sustainable drug delivery, increased bioavailability, and the reduction of side effects (Fina et al. 2017). On the other hand, the green method of nanoparticle synthesis is the one-step method, an efficient, cost-effective, and environmentally friendly which is why it is so popular (Ashkan et al. 2021). Nanoparticles and nano surfaces have the advantage of size-dependent intestinal absorption (Thomas et al. 2018). In a study conducted by Deepa et al. using a green method, rifampicin-loaded alginate nanoparticles were synthesized. According to the results of the study, the nanoparticles did not show any toxicity in albino Wistar rats (Thomas et al. 2020). In another study, alginate-cellulose hybrid nanoparticles were produced by the green method to control the oral delivery of rifampicin. The nanoparticles showed pH-dependent swelling and drug-releasing properties in vitro. In this study, only 15% of rifampicin was released after 2 h, indicating that the nanoparticles produced from the drug protect well against gastric conditions (Thomas et al. 2018). These results indicate the high potential of green alginate synthesized nanoparticles in drug delivery. It seems that alginate hybrid with other organic and inorganic materials can open a new window for drug delivery. On the other hand, developments in fusion protein technology and molecular biology techniques will pave the way for the stronger attachment of the enzymes on the alginate-based or alginate derivatives matrices for industrial applications.

References

- Ahsan SM et al (2018) Chitosan as biomaterial in drug delivery and tissue engineering. *Int J Biol Macromol* 110:97–109
- Ai Q et al (2013) Fabrication of boehmite/alginate hybrid beads for efficient enzyme immobilization. *Ind Eng Chem Res* 52(42):14898–14905
- Alban S, Schauerte A, Franz G (2002) Anticoagulant sulfated polysaccharides: part I. Synthesis and structure–activity relationships of new pullulan sulfates. *Carbohydr Polym* 47(3):267–276
- Aldobaev V, Prezent M, Zavarzin I (2018) Shortened single-walled carbon nanotubes modification as design of nano-structural drug delivery system for pharmaceutical substances. *Russ Chem Bull* 67(11):2098–2102
- Almulaiky YQ, Al-Harbi SA (2022) Preparation of a calcium alginate-coated polypyrrole/silver nanocomposite for site-specific immobilization of polygalacturonase with high reusability and enhanced stability. *Catal Lett* 152(1):28–42
- Anwar A et al (2009) Calcium alginate: a support material for immobilization of proteases from newly isolated strain of bacillus subtilis KIBGE-HAS. *World Appl Sci J* 7(10):1281–1286
- Arlov Ø et al (2014) Heparin-like properties of sulfated alginates with defined sequences and sulfation degrees. *Biomacromolecules* 15(7):2744–2750
- Ashkan Z et al (2021) Immobilization of enzymes on nanoinorganic support materials: an update. *Int J Biol Macromol* 168:708–721
- Bhushan I et al (2008) Immobilization of lipase by entrapment in Ca-alginate beads. *J Bioact Compat Polym* 23(6):552–562
- Bibi Z, Qader SAU, Aman A (2015) Calcium alginate matrix increases the stability and recycling capability of immobilized endo- β -1, 4-xylanase from *Geobacillus stearothermophilus* KIBGE-IB29. *Extremophiles* 19(4):819–827
- Bilal M, Iqbal HM (2019) Lignin peroxidase immobilization on Ca-alginate beads and its dye degradation performance in a packed bed reactor system. *Biocatal Agric Biotechnol* 20:101205
- Bilal M et al (2017) Novel characteristics of horseradish peroxidase immobilized onto the polyvinyl alcohol-alginate beads and its methyl orange degradation potential. *Int J Biol Macromol* 105:328–335
- Blandino A, Macias M, Cantero D (2001) Immobilization of glucose oxidase within calcium alginate gel capsules. *Process Biochem* 36(7):601–606
- Bonine BM, Polizelli PP, Bonilla-Rodríguez GO (2014) Immobilization of a plant lipase from *Pachira aquatica* in alginate and alginate/PVA beads. *Enzyme Res* 2014:738739
- Boonthekul T, Kong H-J, Mooney DJ (2005) Controlling alginate gel degradation utilizing partial oxidation and bimodal molecular weight distribution. *Biomaterials* 26(15):2455–2465
- Borazjani NJ et al (2017) Effects of extraction methods on molecular characteristics, antioxidant properties and immunomodulation of alginates from *Sargassum angustifolium*. *Int J Biol Macromol* 101:703–711
- Buga ML, Ibrahim S, Nok AJ (2010) Physico-chemical characteristics of immobilized polygalacturonase from *Aspergillus niger* (SA6). *Afr J Biotechnol* 9(52):8934–8943
- Busto M, Ortega N, Perez-Mateos M (1998) Characterization of microbial endo- β -glucanase immobilized in alginate beads. *Acta Biotechnol* 18(3):189–200
- Chamberlain N, Cunningham G, Speakman J (1946) Alginic acid diacetate. *Nature* 158(4016):553–553
- Chaudhari SA, Kar JR, Singhal RS (2015) Immobilization of proteins in alginate: functional properties and applications. *Curr Org Chem* 19(17):1732–1754
- Cheng D et al (2020) Characteristics and applications of alginate lyases: a review. *Int J Biol Macromol* 164:1304–1320
- Chomoucka J et al (2010) Magnetic nanoparticles and targeted drug delivering. *Pharmacol Res* 62(2):144–149
- Choukaife H, Doolaanea AA, Alfatama M (2020) Alginate nanoformulation: influence of process and selected variables. *Pharmaceuticals* 13(11):335

- Ciofani G et al (2008) Magnetic driven alginate nanoparticles for targeted drug delivery. *Curr Nanosci* 4(2):212–218
- Ciofani G et al (2009) A bi-modal approach against cancer: magnetic alginate nanoparticles for combined chemotherapy and hyperthermia. *Med Hypotheses* 73(1):80–82
- Coleman RJ et al (2011) Phosphorylation of alginate: synthesis, characterization, and evaluation of in vitro mineralization capacity. *Biomacromolecules* 12(4):889–897
- Costa MJ et al (2018) Physicochemical properties of alginate-based films: effect of ionic crosslinking and mannuronic and guluronic acid ratio. *Food Hydrocoll* 81:442–448
- Davis TA et al (2003) Metal selectivity of *Sargassum* spp. and their alginates in relation to their α -L-guluronic acid content and conformation. *Environ Sci Technol* 37(2):261–267
- Davis TA et al (2004) Extraction, isolation and cadmium binding of alginate from *Sargassum* spp. *J Appl Phycol* 16(4):275–284
- Deng Z et al (2019) Immobilization of pectinases into calcium alginate microspheres for fruit juice application. *Food Hydrocoll* 89:691–699
- Dey G, Bhupinder S, Banerjee R (2003) Immobilization of alpha-amylase produced by *Bacillus circulans* GRS 313. *Braz Arch Biol Technol* 46(2):167–176
- Doğaç YI, Çınar M, Teke M (2015) Improving of catalase stability properties by encapsulation in alginate/Fe₃O₄ magnetic composite beads for enzymatic removal of H₂O₂. *Prep Biochem Biotechnol* 45(2):144–157
- Drægt K, Bræk GS, Smidsrød O (1994) Alginic acid gels: the effect of alginate chemical composition and molecular weight. *Carbohydr Polym* 25(1):31–38
- Drægt KI (2009) Alginates. In: Phillips GO, Williams PA (eds) *Handbook of hydrocolloids*. Elsevier, Cambridge, pp 807–828
- Drægt KI, Skjåk-Bræk G, Smidsrød O (1997) Alginate based new materials. *Int J Biol Macromol* 21(1–2):47–55
- Duru Kamaci U, Peksel A (2021) Enhanced catalytic activity of immobilized phytase into polyvinyl alcohol-sodium alginate based electrospun nanofibers. *Catal Lett* 151(3):821–831
- Dusseault J et al (2006) Evaluation of alginate purification methods: effect on polyphenol, endotoxin, and protein contamination. *J Biomed Mater Res A* 76(2):243–251
- Elashar MM, Danial EN, Awad GE (2009) Novel carrier of grafted alginate for covalent immobilization of inulinase. *Ind Eng Chem Res* 48(22):9781–9785
- Elzoghby OA et al (2016) Natural polymeric nanoparticles for brain-targeting: implications on drug and gene delivery. *Curr Pharm Des* 22(22):3305–3323
- Ertan F, Yagar H, Balkan B (2007) Optimization of α -amylase immobilization in calcium alginate beads. *Prep Biochem Biotechnol* 37(3):195–204
- Ertesvåg H, Valla S (1998) Biosynthesis and applications of alginates. *Polym Degrad Stab* 59(1–3): 85–91
- Fawzy MA et al (2017) Optimization of alginate alkaline extraction technology from *Sargassum latifolium* and its potential antioxidant and emulsifying properties. *Carbohydr Polym* 157:1903–1912
- Fenoradosoa TA et al (2010) Extraction and characterization of an alginate from the brown seaweed *Sargassum turbinarioides* Grunow. *J Appl Phycol* 22(2):131–137
- Fina F et al (2017) Selective laser sintering (SLS) 3D printing of medicines. *Int J Pharm* 529(1–2): 285–293
- Gacesa P (1992) Enzymic degradation of alginates. *Int J Biochem* 24(4):545–552
- Gombotz WR, Wee S (1998) Protein release from alginate matrices. *Adv Drug Deliv Rev* 31(3): 267–285
- Gomez CG et al (2006) Synthesis and characterization of a β -CD-alginate conjugate. *Polymer* 47(26):8509–8516
- Haque S et al (2014) Development and evaluation of brain targeted intranasal alginate nanoparticles for treatment of depression. *J Psychiatr Res* 48(1):1–12
- Hasnain MS et al (2020) Alginate nanoparticles in drug delivery. In: Nayak AK, Hasnain MS (eds) *Alginates in drug delivery*. Elsevier, London, pp 129–152

- Hassan ME et al (2019) Impact of immobilization technology in industrial and pharmaceutical applications. *3 Biotech* 9(12):1–16
- Haug A (1959) Fractionation of alginic acid. *Acta Chem Scand* 13:601–603
- Haug A, Larsen B (1966) A study on the constitution of alginic acid by partial acid hydrolysis. In: *Proceedings of the Fifth International Seaweed Symposium*, Halifax, August 25–28, 1965. Elsevier
- Haug A, Larsen B, Smidsrod O (1967) Alkaline degradation of alginate. *Acta Chem Scand* 21(10):2859–2870
- Haug A, Smidsrod O, Larsen B (1963) Degradation of alginates at different pH values. *Acta Chem Scand* 17:1466–1468
- Hay ID et al (2013) Microbial alginate production, modification and its applications. *Microb Biotechnol* 6(6):637–650
- Hertzberg S et al (1992) Alginate as immobilization matrix and stabilizing agent in a two-phase liquid system: application in lipase-catalysed reactions. *Enzym Microb Technol* 14(1):42–47
- Holban AM, Grumezescu AM (2016) Nanoarchitectonics for smart delivery and drug targeting. William Andrew, Oxford
- Huang C-H et al (2020) Doxorubicin–gelatin/Fe₃O₄–alginate dual-layer magnetic nanoparticles as targeted anticancer drug delivery vehicles. *Polymers* 12(8):1747
- Jain D, Bar-Shalom D (2014) Alginate drug delivery systems: application in context of pharmaceutical and biomedical research. *Drug Dev Ind Pharm* 40(12):1576–1584
- Jampala P et al (2017) Immobilization of levan-xylanase nanohybrid on an alginate bead improves xylanase stability at wide pH and temperature. *Int J Biol Macromol* 95:843–849
- Joseph JJ, Sangeetha D, Shivashankar M (2019) In vitro release and cytotoxic studies of novel alginate nanocarrier for the antitumor drug: sunitinib. *Regen Eng Transl Med* 5(2):220–227
- Keshav V, Franklyn P, Kondiah K (2019) Recombinant fusion protein PbrD cross-linked to calcium alginate nanoparticles for Pb remediation. *ACS Omega* 4(16):16816–16825
- Kim HS, Lee C-G, Lee EY (2011) Alginate lyase: structure, property, and application. *Biotechnol Bioprocess Eng* 16(5):843–851
- Kong H-J, Lee KY, Mooney DJ (2002) Decoupling the dependence of rheological/mechanical properties of hydrogels from solids concentration. *Polymer* 43(23):6239–6246
- Kong HJ et al (2004) Controlling rigidity and degradation of alginate hydrogels via molecular weight distribution. *Biomacromolecules* 5(5):1720–1727
- Kumada Y (2014) Site-specific immobilization of recombinant antibody fragments through material-binding peptides for the sensitive detection of antigens in enzyme immunoassays. *Biochim Biophys Acta* 1844(11):1960–1969
- Kumar D, Muthukumar M, Garg N (2012) Kinetics of fungal extracellular [alpha]-amylase from *Fusarium solani* immobilized in calcium alginate beads. *J Environ Biol* 33(6):1021
- Kumar S, Dwevedi A, Kayastha AM (2009) Immobilization of soybean (*Glycine max*) urease on alginate and chitosan beads showing improved stability: analytical applications. *J Mol Catal B Enzym* 58(1–4):138–145
- Kumar S et al (2017) Improved enzyme properties upon glutaraldehyde cross-linking of alginate entrapped xylanase from *Bacillus licheniformis*. *Int J Biol Macromol* 98:24–33
- Labre F et al (2018) DMTMM-mediated amidation of alginate oligosaccharides aimed at modulating their interaction with proteins. *Carbohydr Polym* 184:427–434
- Lambert G, Fattal E, Couvreur P (2001) Nanoparticulate systems for the delivery of antisense oligonucleotides. *Adv Drug Deliv Rev* 47(1):99–112
- Lampsa JW, Griswold KE (2013) Alginate lyase exhibits catalysis-independent biofilm dispersion and antibiotic synergy. *Antimicrob Agents Chemother* 57(1):137–145
- Larsen B, Haug A (1958) Chemical composition of the brown alga *Ascophyllum nodosum* (L.) Le Jol.: presence of reducing compounds in *ascophyllum nodosum*. *Nature* 181(4617):1224–1224
- Larsen B, Haug A (1971) Biosynthesis of alginate: part I. Composition and structure of alginate produced by *Azotobacter vinelandii* (Lipman). *Carbohydr Res* 17(2):287–296

- Larsen B et al (2003) Characterization of the alginates from algae harvested at the Egyptian Red Sea coast. *Carbohydr Res* 338(22):2325–2336
- Laurienzo P (2010) Marine polysaccharides in pharmaceutical applications: an overview. *Mar Drugs* 8(9):2435–2465
- Lee DW et al (2003) Effect of γ -irradiation on degradation of alginate. *J Agric Food Chem* 51(16):4819–4823
- Lee GM, Palsson BO (1990) Simplified method of making alginate-polylysine microcapsules for hybridoma cell culture using RPMI 1640 medium. *Biotechnol Tech* 4(5):341–344
- Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. *Prog Polym Sci* 37(1):106–126
- Leong J-Y et al (2016) Advances in fabricating spherical alginate hydrogels with controlled particle designs by ionotropic gelation as encapsulation systems. *Particuology* 24:44–60
- Li G-Y, Li Y-J (2010) Immobilization of *Saccharomyces cerevisiae* alcohol dehydrogenase on hybrid alginate–chitosan beads. *Int J Biol Macromol* 47(1):21–26
- Linhardt RJ (2003) Claude S. Hudson Award address in carbohydrate chemistry. Heparin: structure and activity. *J Med Chem* 46(13):2551–2564
- Liu J et al (2019) Alginate oligosaccharides: production, biological activities, and potential applications. *Compr Rev Food Sci Food Saf* 18(6):1859–1881
- Lu B et al (2006) Mitoxantrone-loaded BSA nanospheres and chitosan nanospheres for local injection against breast cancer and its lymph node metastases: I: formulation and in vitro characterization. *Int J Pharm* 307(2):168–174
- Lu L, Zhao M, Wang Y (2007) Immobilization of laccase by alginate–chitosan microcapsules and its use in dye decolorization. *World J Microbiol Biotechnol* 23(2):159–166
- Malhotra I, Basir SF (2020) Immobilization of invertase in calcium alginate and calcium alginate-kappa-carrageenan beads and its application in bioethanol production. *Prep Biochem Biotechnol* 50(5):494–503
- May TB, Chakrabarty A (1994) *Pseudomonas aeruginosa*: genes and enzymes of alginate synthesis. *Trends Microbiol* 2(5):151–157
- Minghou J et al (1984) Studies on the M: G ratios in alginate. In: Bird CJ, Ragan MA (eds) Eleventh international seaweed symposium. Springer, Dordrecht, pp 554–556
- Missau J et al (2014) Immobilization of commercial inulinase on alginate–chitosan beads. *Sustain Chem Process* 2(1):1–6
- Mohamad NR et al (2015) An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes. *Biotechnol Biotechnol Equip* 29(2):205–220
- Mørch YÅ et al (2006) Effect of Ca^{2+} , Ba^{2+} , and Sr^{2+} on alginate microbeads. *Biomacromolecules* 7(5):1471–1480
- Murata H et al (2004) Physical properties and compatibility with dental stones of current alginate impression materials. *J Oral Rehabil* 31(11):1115–1122
- Nagasawa N et al (2000) Radiation-induced degradation of sodium alginate. *Polym Degrad Stab* 69(3):279–285
- Ningsih D et al (2020) Improved reuse and affinity of enzyme using immobilized amylase on alginate matrix. *J Phys Conf Ser* 1494:012028
- Nunes MA et al (2010) Immobilization of naringinase in PVA–alginate matrix using an innovative technique. *Appl Biochem Biotechnol* 160(7):2129–2147
- Olajuyigbe FM, Adetuyi OY, Fatokun CO (2019) Characterization of free and immobilized laccase from *Cyberlindnera fabianii* and application in degradation of bisphenol A. *Int J Biol Macromol* 125:856–864
- Onsøyen E (1997) Alginates. In: Imeson AP (ed) Thickening and gelling agents for food. Springer, New York, pp 22–44
- Oshiro Junior JA et al (2016) Drug delivery systems obtained from silica based organic-inorganic hybrids. *Polymers* 8(4):91
- Otterlei M et al (1991) Induction of cytokine production from human monocytes stimulated with alginate. *J Immunother* 10(4):286–291

- Pal A, Khanum F (2011) Covalent immobilization of xylanase on glutaraldehyde activated alginate beads using response surface methodology: characterization of immobilized enzyme. *Process Biochem* 46(6):1315–1322
- Palmieri G et al (1994) A new enzyme immobilization procedure using copper alginate gel: application to a fungal phenol oxidase. *Enzym Microb Technol* 16(2):151–158
- Pandey R, Khuller G (2006) Nanotechnology based drug delivery system (s) for the management of tuberculosis. *Indian J Exp Biol* 44(5):357–366
- Paques JP et al (2014) Nanospheres of alginate prepared through w/o emulsification and internal gelation with nanoparticles of CaCO_3 . *Food Hydrocoll* 40:182–188
- Pawar SN (2017) Chemical modification of alginate. In: Venkatesan J, Kim S-K, Anil S (eds) *Seaweed polysaccharides*. Elsevier, Amsterdam, pp 111–155
- Pawar SN, Edgar KJ (2011) Chemical modification of alginates in organic solvent systems. *Biomacromolecules* 12(11):4095–4103
- Pawar SN, Edgar KJ (2012) Alginate derivatization: a review of chemistry, properties and applications. *Biomaterials* 33(11):3279–3305
- Pestovsky YS, Martínez-Antonio A (2019) The synthesis of alginate microparticles and nanoparticles. *Drug Des Intellect Prop Int J* 3:293–327
- Qamar SA, Asgher M, Bilal M (2020) Immobilization of alkaline protease from *Bacillus brevis* using Ca-alginate entrapment strategy for improved catalytic stability, silver recovery, and dehairing potentialities. *Catal Lett* 150(12):3572–3583
- Qi D et al (2020) Immobilization of pectinase onto porous hydroxyapatite/calcium alginate composite beads for improved performance of recycle. *ACS Omega* 5(32):20062–20069
- Qin Y (2008) Alginate fibres: an overview of the production processes and applications in wound management. *Polym Int* 57(2):171–180
- Quiroga E et al (2011) Performance improvement of araujiain, a cystein phytoprotease, by immobilization within calcium alginate beads. *Process Biochem* 46(4):1029–1034
- Rai A, Comune M, Ferreira L (2019) Nanoparticle-based drug delivery systems: promising approaches against bacterial infections. In: Ahmad I, Rumbaugh KP, Ahmad S (eds) *Antibacterial drug discovery to combat MDR*. Springer, Singapore, pp 605–633
- Rajonarivony M et al (1993) Development of a new drug carrier made from alginate. *J Pharm Sci* 82(9):912–917
- Ranghar S et al (2014) Nanoparticle-based drug delivery systems: promising approaches against infections. *Braz Arch Biol Technol* 57(2):209–222
- Ravi Kumar MN, Kumar N (2001) Polymeric controlled drug-delivery systems: perspective issues and opportunities. *Drug Dev Ind Pharm* 27(1):1–30
- Rehm B, Valla S (1997) Bacterial alginates: biosynthesis and applications. *Appl Microbiol Biotechnol* 48(3):281–288
- Reis CP et al (2007) Nanoparticulate delivery system for insulin: design, characterization and in vitro/in vivo bioactivity. *Eur J Pharm Sci* 30(5):392–397
- Rezakhani N et al (2014) Immobilization of protease in biopolymers (mixture of alginate-chitosan). *Arch Adv Biosci* 5(4). <https://doi.org/10.22037/jps.v5i4.7858>
- Sahoo DR, Biswal T (2021) Alginate and its application to tissue engineering. *SN Appl Sci* 3(1): 1–19
- Sarmiento B et al (2007) Insulin-loaded nanoparticles are prepared by alginate ionotropic pre-gelation followed by chitosan polyelectrolyte complexation. *J Nanosci Nanotechnol* 7(8): 2833–2841
- Selimoglu SM, Elibol M (2010) Alginate as an immobilization material for MAb production via encapsulated hybridoma cells. *Crit Rev Biotechnol* 30(2):145–159
- Selimoglu SM et al (2012) Purification of alginate and feasible production of monoclonal antibodies by the alginate-immobilized hybridoma cells. *J Biosci Bioeng* 113(2):233–238
- Severino P et al (2019) Alginate nanoparticles for drug delivery and targeting. *Curr Pharm Des* 25(11):1312–1334

- Shankar T et al (2016) Purification, characterization and immobilization of alginase produced by bacillus sp associated with sargassum wightii. *Universal J Microbiol Res* 4(1):11–22
- Shelke NB et al (2014) Polysaccharide biomaterials for drug delivery and regenerative engineering. *Polym Adv Technol* 25(5):448–460
- Shi Y (2018) Functionalized silica nanostructures: degradation pathways and biomedical application from 2D to 3D. Sorbonne Université, Paris
- Shilpa A, Agrawal S, Ray AR (2003) Controlled delivery of drugs from alginate matrix. *J Macromol Sci Polym Rev* 43(2):187–221
- Siebert KJ (2006) Haze formation in beverages. *LWT Food Sci Technol* 39(9):987–994
- Smidsrod O, Larsen B, Haug A (1963) Influence of reducing substances on rate of degradation of alginates. *Acta Chem Scand* 17(5):1473–1474
- Sorriwas V, Genovese D, Lozano J (2006) Effect of pectinolytic and amylolytic enzymes on apple juice turbidity. *J Food Process Preserv* 30(2):118–133
- Sosnik A (2014) Alginate particles as platform for drug delivery by the oral route: state-of-the-art. *ISRN Pharm* 2014:926157
- Steinbüchel A, Rhee SK (2005) Polysaccharides and polyamides in the food industry: properties, production, and patents. Wiley-VCH, Weinheim
- Sunita S (2014) A comparative study on immobilization of alpha amylase enzyme on different matrices. *Int J Plant Animal Environ Sci* 4(3):192–198
- Talekar S, Chavare S (2012) Optimization of immobilization of α -amylase in alginate gel and its comparative biochemical studies with free α -amylase. *Recent Res Sci Technol* 4(2):1–5
- Taubner T, Marounek M, Synytsya A (2017) Preparation and characterization of amidated derivatives of alginic acid. *Int J Biol Macromol* 103:202–207
- Tee BL, Kaletunç G (2009) Immobilization of a thermostable α -amylase by covalent binding to an alginate matrix increases high temperature usability. *Biotechnol Prog* 25(2):436–445
- Thomas D, KurienThomas K, Latha M (2020) Preparation and evaluation of alginate nanoparticles prepared by green method for drug delivery applications. *Int J Biol Macromol* 154:888–895
- Thomas D, Latha M, Thomas KK (2018) Synthesis and in vitro evaluation of alginate-cellulose nanocrystal hybrid nanoparticles for the controlled oral delivery of rifampicin. *J Drug Deliv Sci Technol* 46:392–399
- Tielen P et al (2013) Interaction between extracellular lipase LipA and the polysaccharide alginate of pseudomonas aeruginosa. *BMC Microbiol* 13(1):1–12
- Timell T (1964) The acid hydrolysis of glycosides: I. General conditions and the effect of the nature of the aglycone. *Can J Chem* 42(6):1456–1472
- Tønnesen HH, Karlsen J (2002) Alginate in drug delivery systems. *Drug Dev Ind Pharm* 28(6):621–630
- Torres MR et al (2007) Extraction and physicochemical characterization of Sargassum vulgare alginate from Brazil. *Carbohydr Res* 342(14):2067–2074
- Tumturk H et al (2008) Immobilization of glucose isomerase in surface-modified alginate gel beads. *J Food Biochem* 32(2):234–246
- Uyen NTT et al (2020) Fabrication of alginate microspheres for drug delivery: a review. *Int J Biol Macromol* 153:1035–1046
- Varaprasad K et al (2020) Alginate-based composite materials for wound dressing application: a mini review. *Carbohydr Polym* 236:116025
- Velings NM, Mestdagh MM (1995) Physico-chemical properties of alginate gel beads. *Polym Gels Netw* 3(3):311–330
- Vicario-de-la-Torre M, Forcada J (2017) The potential of stimuli-responsive nanogels in drug and active molecule delivery for targeted therapy. *Gels* 3(2):16
- Viet TQ, Minh NP, Dao DTA (2013) Immobilization of cellulase enzyme in calcium alginate gel and its immobilized stability. *Am J Res Commun* 1(12):254–267
- Wang X, Zhu K-X, Zhou H-M (2011) Immobilization of glucose oxidase in alginate-chitosan microcapsules. *Int J Mol Sci* 12(5):3042–3054

- Won K et al (2005) Optimization of lipase entrapment in Ca-alginate gel beads. *Process Biochem* 40(6):2149–2154
- Wong TY, Preston LA, Schiller NL (2000) Alginate lyase: review of major sources and enzyme characteristics, structure-function analysis, biological roles, and applications. *Annu Rev Microbiol* 54(1):289–340
- Yang C-H et al (2014) Immobilization of *Brassica oleracea* chlorophyllase 1 (BoCLH1) and *Candida rugosa* lipase (CRL) in magnetic alginate beads: an enzymatic evaluation in the corresponding proteins. *Molecules* 19(8):11800–11815
- Yang J-S, Xie Y-J, He W (2011) Research progress on chemical modification of alginate: a review. *Carbohydr Polym* 84(1):33–39
- Zahirinejad S et al (2021) Nano-organic supports for enzyme immobilization: scopes and perspectives. *Colloids Surf B: Biointerfaces* 204:111774
- Zawawi FSM et al (2020) Enzyme activity and stability of lactase immobilized on two different supports: calcium alginate and magnetic chitosan. *Mal J Fund Appl Sci* 16(4):413–417
- Zhan J, Jiang S, Pan L (2013) Immobilization of phospholipase A1 using a polyvinyl alcohol-alginate matrix and evaluation of the effects of immobilization. *Braz J Chem Eng* 30(4):721–728
- Zhang R et al (2004) Hypoglycemic effect of *Rehmannia glutinosa* oligosaccharide in hyperglycemic and alloxan-induced diabetic rats and its mechanism. *J Ethnopharmacol* 90(1):39–43
- Zhu B, Yin H (2015) Alginate lyase: review of major sources and classification, properties, structure-function analysis and applications. *Bioengineered* 6(3):125–131
- Zimmermann U et al (1992) Production of mitogen-contamination free alginates with variable ratios of mannuronic acid to guluronic acid by free flow electrophoresis. *Electrophoresis* 13(1):269–274

Alginate in Gene and Vaccine Delivery



Hani Nasser Abdelhamid

Abstract Gene and vaccine delivery offer promising technologies for the treatment of several diseases. However, they suffer from low transfection efficacy, enzyme degradation, and immunogenicity of the gene-based therapeutic agents or vaccines. Thus, several materials were reported as carriers for gene therapeutic agents. Alginates-based materials provide solutions for several challenges of other biomaterials. They exhibit high biocompatibility for many biological cells with low or minimal toxicity, show high stability under different environments, and can proceed quickly into various forms such as beads, capsules, fibers, and hydrogels. Bio-beads of calcium alginate is widely used to encapsulate gene-based therapeutic agents. They can be quickly processed as three-dimensional (3D) scaffolds, hydrogels, capsules, spheres, foams, sponges, and fibers. They can be used as carriers for gene and vaccine delivery. They offer several advantages, such as high biodegradability, good encapsulation efficiency, excellent biocompatibility, and good chelating capacity. The alginate-based system was used for gene delivery for tissue generation, bone generation, cartilage repair, and cancer therapy. Alginate-based biomaterials offered the development of gene-activated bio-inks (GABs) for 3D printing. This book chapter summarizes the applications of alginate as carriers for gene and vaccine delivery.

Keywords Alginate · Gene delivery · Vaccine · Nanocarrier · Regenerative medicine

H. N. Abdelhamid (✉)

Advanced Multifunctional Materials Laboratory, Department of Chemistry, Faculty of Science, Assiut University, Assiut, Egypt

Proteomics Laboratory for Clinical Research and Materials Science, Department of Chemistry, Assiut University, Assiut, Egypt

Nanotechnology Research Centre (NTRC), The British University in Egypt, Cairo, Egypt
e-mail: hany.abdelhamid@aun.edu.eg; hani.nasser@bue.edu.eg

1 Introduction

Alginate is a salt of alginic acid (E400, algin), a hydrophilic polysaccharide (Maity and Das 2022; Sutirman et al. 2021). It can be considered the most abundant biopolymer after cellulose (Kanasan et al. 2017; Dey et al. 2019; Teng et al. 2021). It represents the cell walls of the brown micro-algae *Phaeophyceae*. It can be extracted from *Eclonia*, *Laminaria*, *Lessonia*, *Macrocystis*, *Sargassum*, *Ascophyllum*, and *Durvillea* (Helmiyati 2017; Pawar and Edgar 2012). The biosynthesis of alginic acid was reviewed in (Hay et al. 2010; Gacesa 1998; Remminghorst and Rehm 2006; Núñez et al. 2022). It is widely used for food, biomedical, packaging, and textiles (Dodero et al. 2021; Raguvaran et al. 2022). It exhibits the formation of gels in the presence of Ca^{2+} ions. It exhibits high biocompatibility for both animal and plant cells. It can be used as biomaterial to immobilize bacteria in bioreactors. It can also encapsulate plant somatic embryos as artificial seeds (Chang 1992). It can be used for drug delivery (Bi et al. 2019), treatment of virus infection, i.e., antiviral (Serrano-Aroca et al. 2021; Otto and de Villiers 2020; Pietropaolo et al. 1993; Pardee et al. 2004), tissue engineering (e.g., bone, cartilage, blood vessel, and cornea) (Sahoo and Biswal 2021; Reakasame and Boccaccini 2018; Choe et al. 2019; Augst et al. 2006; Dvir-Ginzberg et al. 2003), regenerative medicine (Xu et al. 2021), wound dressing (Diniz et al. 2020), and implantable electronics (Choi et al. 2021). It can be used to deliver drugs under external stimuli such as pH (Kong 2003), light (Javvaji et al. 2011; Oh et al. 2016; Cui et al. 2013; Chueh et al. 2010), electric fields (Liu et al. 2017), and enzymes (Yang et al. 2018; Hu et al. 2018a). It is a promising carrier for acid-labile drugs (Li et al. 2017). Alginate can be solidified as uniformly spherical particles with tunable particle size via cross-linking with divalent ions such as calcium (Ca^{2+}) ions.

Calcium alginate beads denoted as “bio-beads,” can be easily formed via the cross-linking of alginate and a pinch of calcium salt. It can encapsulate plasmid DNA molecules carrying a reporter gene. Bio-beads of calcium alginate micrometer-sized can be used to encapsulate drugs, small molecules, and pharmaceuticals (Liu et al. 2016; Lee and Mooney 2012). It can encapsulate a single cell (Shao et al. 2020) and enzyme (Yang et al. 2018; Hu et al. 2018a). It be prepared as monodispersed (Tan and Takeuchi 2007). The encapsulation can be achieved using an alginate emulsion using a water/oil (W/O) type emulsion or in a water solution during the bead’s formation. In the presence of DNA, the formed particles can encapsulate the hydrophilic molecules forming solid calcium alginate beads. Alginate microbeads can be used for the incorporation of adipose-derived stem cells (ASCs) (Lee et al. 2010).

Alginate is widely used for processing using advanced technology such as three-dimensional (3D) printing (Maity and Das 2022; Mallakpour et al. 2021; Bedell et al. 2020; Heid and Boccaccini 2020; Sultan et al. 2018). It can be used for 3D printing of osteogenic and vasculogenic patterns for engineering 3D bone tissue (Byambaa et al. 2017) and liver tissue engineering (Mazza et al. 2018). Alginate-based

materials may advance 3D printing for biomedical applications (Tamay et al. 2019). 3D printing using alginate-based materials offers high shape fidelity (Kilian et al. 2020).

Gene and vaccine delivery are highly promising for modern medicine (Niidome and Huang 2002; Dowaidar et al. 1861; Abdelhamid et al. 2019; Abdelhamid et al. 2020; Dowaidar et al. 2022). Gene delivery using a therapeutic agent can be achieved via vectors that are divided into (1) viral and (2) non-viral vectors. Viral vectors are commonly used viruses with the inherent ability to transfer genes into the target cells. They suffer from high security limiting their wide applications compared to non-viral vectors. In general, vectors facilitate the escape of gene therapeutic agents from the endosome into the cytosol.

Vaccination represents one of the promising modern medical technologies that can improve public health and increase life expectancy. It saves 3.5–5 million lives per year according to a recent (2022) report from the World Health Organization (WHO, <https://www.who.int>). The vaccination process was first reported in the 12th century in the Middle East and China (Plotkin 2005). It was transferred to Europe in the 17th century (Tahamtan et al. 2017). The first trial of vaccination may be the treatment of the skin of patients with smallpox that causes disease resistance in another patient (Plotkin 2005). In 1798, Edward Jenner reported the first vaccine by injecting humans with cowpox for protection against smallpox (Smith 2011). Simply, vaccines are composed merely of inactivated, killed, or subunit (e.g., toxins, endotoxins, polysaccharides, proteins) of a pathogen. Vaccination can be also performed using live-attenuated or weakened viruses. The immune system recognizes the antigens from these pathogens leading to an immune response, i.e., the production of antibodies such as Immunoglobulin G (IgG) and immunoglobulin M (IgM). These species are expected to protect the body from another infection. Vaccination is promising for the early against numerous bacterial and viral pathogens. It can be considered as one of the effective methods for protection and disease control, especially for epidemic and pandemic.

This book chapter summarized the application of alginate as the carrier for gene and vaccine delivery. It offered a brief introduction to the chemical structure and properties of alginate. It highlighted the methods used for processing alginate-based materials into different forms, such as beads, capsules, spheres, fibers, threads, and mats. The chemistry of alginate offered several advantages for loading or encapsulation of gene therapeutic agents or vaccines with high loading percentages and strong complexation using simple preparation methods. Alginate showed several biological benefits being highly biocompatible and low toxicity. Alginate-based materials exhibited high cell transfection for many gene therapeutic agents. They can be applied for gene delivery, improving tissue engineering, bone regeneration, cancer treatment, and vaccine delivery.

2 Chemical Structure and Properties of Alginate

Alginic acid or sodium alginate was first extracted in 1883 (Stanford 1883). Alginic acid consist of β -(1-4)-D-mannuronic acid- α -L-guluronic acid, i.e., $[\text{D-ManA}(\beta 1 \rightarrow 4)\text{L-GulA}(\alpha 1 \rightarrow 4)]_n$, n represents the degree of polymerization with chemical formula $(\text{C}_6\text{H}_8\text{O}_6)_n$ (Fig. 1) (Dey et al. 2019; Pawar and Edgar 2012; Farokhi et al. 2020). The ratio of mannuronate:guluronate (m:n ratio, Fig. 1) depends on the source of alginic acid. The blocks of mannuronic (M) acid and guluronic (G) acid are ordered consecutively as G, M, and then interchanging G and M residues. The main active functional groups of alginates are the hydroxyl and carboxylic groups. Alginate represents 22–30%, 25–44%, and 17–33% of the dry mass of *Ascophyllum nodosum*, *Laminaria digitata*, and *L. hyperborean*. The sequence of G and M-building blocks in the biopolymers varies based on the original source used for the material's extraction. Based on these variations, there are more than 200 different categories of alginates. It can be extracted from brown algae (e.g., *Laminaria* (hyperborean, digitata, or japonica), *Ascophyllum nodosum*, and *Macrocystis pyrifera*) or bacteria (*Pseudomonas* and *Azotobacter*) using an alkaline solution of an inorganic base such as sodium hydroxide (NaOH) or sodium carbonate (Na_2CO_3). The extract after NaOH treatment is filtered. The materials can be precipitated using inorganic salts such as calcium chloride (CaCl_2) or barium chloride (BaCl_2). The salt of alginate can be converted into alginic acid using dilute acid such as hydrochloric acid (HCl).

Alginic acids are biopolymers with different molecular weights. Commercial salt of alginic acid is sodium alginate with a molecular weight (M.Wt) range of 32,000 to 400,000 g/mol (Lee and Mooney 2012). Wide molecular weight alginate can be derived based on the biological source used for the extraction. At the same time, alginate with high molecular weight and a high degree of polymerization (DP) can be extracted from bacteria species. The blocks of alginates, i.e., mannuronate and gluruonate are arranged in different orders based on the extraction sources. For example, *Azotobacter* offers alginate with higher guluronic-blocks content than mannuronic acid. Thus, alginate extracted from *Azotobacter* species exhibits stiffer

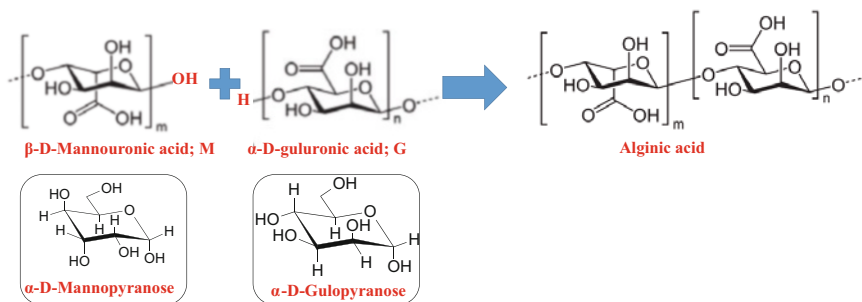


Fig. 1 Chair presentation of alginic acid and their building blocks, M and G. alpha (α) chair presentation of mannopyranose and gulpyranose was also inserted

gel than other bacteria. The properties of alginate-based materials, such as porosity and durability, can be controlled by changing the polymer length of the G-block and using a suitable source of extraction or high molecular weight.

The solubility of alginates in water depends mainly on the pH value of the solution. Alginate solubility is based on the deprotonation of the carboxylic groups. It can be controlled via solvent, pH, ionic strength of the medium, and the presence of gelling ions. Alginate can be dissolved in organic media using a surfactant such as tetrabutylammonium (TBA) salt. The formed complex, i.e., TBA-alginate in polar aprotic solvents or ethylene glycol containing tetrabutylammonium fluoride (TBAF) (Pawar and Edgar 2011).

Alginate can be undergo several reactions such as acetylation (Skjåk-Bræk et al. 1989), phosphorylation (Coleman et al. 2011), and sulfation (Ronghua et al. 2003). The functional groups of alginate can also be cross-linked via covalent cross-linking using reagents such as epichlorohydrin (Moe et al. 1991). The chemical modification improved the properties of alginate, including the material's mechanical properties. They also improved the super-swelling materials.

The viscosity of alginate is high at low pH values, i.e., 3–3.5, because of the protonation of the carboxylic groups that form hydrogen bonding. The mechanical properties of alginate gels improved by increasing the polymer concentration, using high molecular weight (LeRoux et al. 1999), the formation of polyelectrolyte complexes using a cationic such as poly(ethyleneimine) (PEI) (Kong and Mooney 2003) or the formation of cross-linking composite (Eiselt et al. 1999). The mechanical properties can also be improved via the formation of well-ordered gels using low temperature (Drury et al. 2004).

Alginate can be oxidized using oxidants such as sodium periodate (NaIO_4) (Reakasame and Boccaccini 2018). The oxidation breaks the carbon-carbon linkage of the cis-diol group in the urinate. The process causes changes in the chair conformation of alginate, leading to an open-chain form. The chemical structure of the oxidized alginate improves the degradation of the backbone of the alginate. The partial oxidation of alginate shows an insignificant change in the gel-forming ability of alginate in the presence of Ca^{2+} .

Alginate is the salt of alginic acid. The primary salt of alginate is sodium salt (NaAlg); however, other salts of calcium (Ca^{2+}), potassium (K^+), and ammonium (NH_4^+) are also present in the literature. Alginate can also be found as an ester with propylene glycol, i.e., the formation of propylene glycol alginate. Adding inorganic salts of Ca^{2+} and Ba^{2+} to sodium alginate exhibits the gelling capability with high viscosity in an aqueous medium. Divalent ions are mainly bonded to alginate-building blocks causing precipitation. The binding capacity to divalent ions can be arranged in the order of $\text{Mn}^{2+} < \text{Ca}^{2+} < \text{Sr}^{2+} < \text{Ba}^{2+} < \text{Cd}^{2+} < \text{Cu}^{2+} < \text{Pb}^{2+}$ (Siew et al. 2005; Cao et al. 2020a). Ca^{2+} , Ba^{2+} , and Sr^{2+} bind to G and MG-blocks, G and M-blocks, and G-blocks, respectively (Mørch et al. 2006). The gel properties, such as strength and stability, can be improved by using alginates containing high content of G. Divalent ions such as Ca^{2+} ions are linked to the guluronate blocks forming a junction known as the “egg-box” model (Fig. 2) (Boisguérin et al. 2015). The gelation rate can be controlled using insoluble calcium salts such as CaCO_3 (Pawar

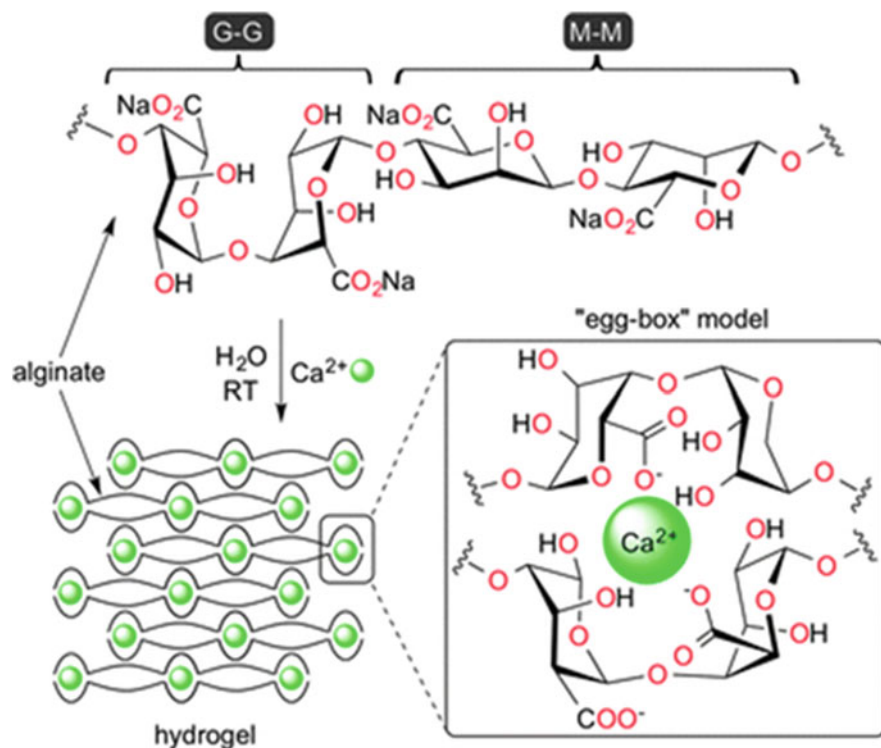


Fig. 2 Egg-box model representation associated with the guluronate sequences of cross-linked alginate with calcium cations. Reproduced with permission under a Creative Commons Attribution 3.0 Supported License from (Boisguérin et al. 2015). Copyright 2015 Royal Society of Chemistry

and Edgar 2012), or temperature (Augst et al. 2006). The high number of G-blocks ensures the good mechanical performance of the gel. The cross-link of sodium alginate with Ca^{2+} ions enhances osteoblasts' differentiation and proliferation (Leslie et al. 2016). It is important to mention also that the gelation of alginate can also be performed via carbon dioxide (CO_2) (Gurikov et al. 2015).

3 Gene Delivery Using Alginate for Tissues Engineering

Tissue (e.g., skin, muscle, cartilage, blood vessels, bladder, and bone) engineering or regenerative medicine aims to form new and viable tissues of damaged tissues. It combines several disciplines such as biomedical, engineering, and biomaterials. The formation of new tissues can be achieved via several methods, including the autologous shape of the body. However, this method takes a long time and does not come with the desired formation rate due to the body's weakness. Thus, tissue

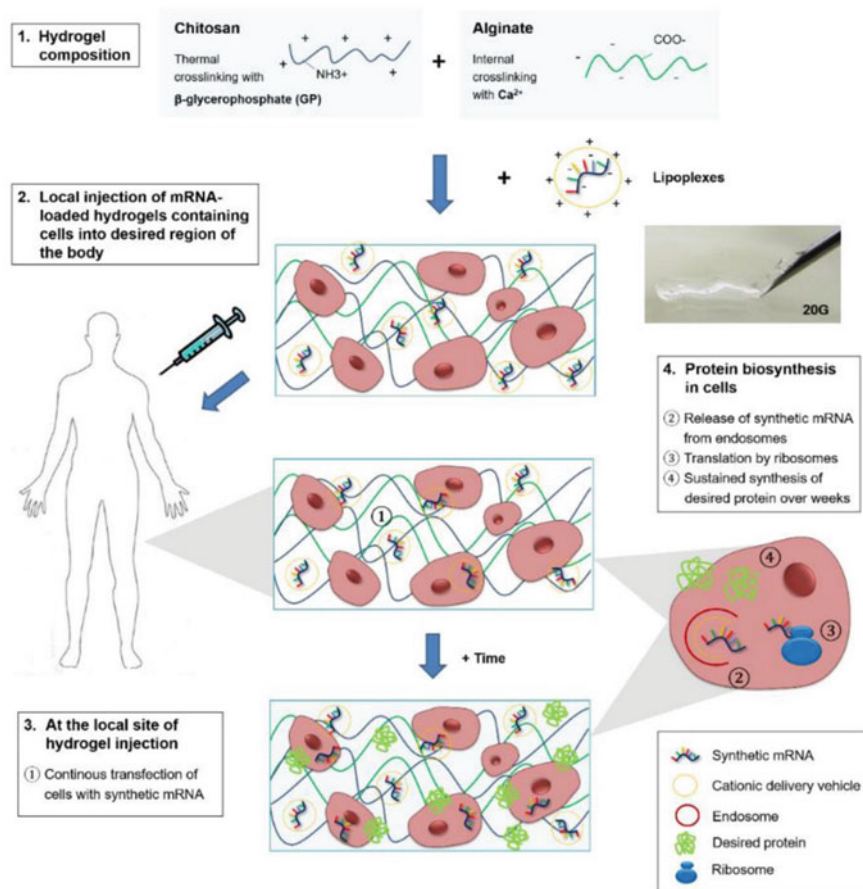


Fig. 3 The chitosan/alginate composite formation and their application for tissue engineering. Figure reprinted with permission from (Steinle et al. 2018), an Open Access article

engineering can be achieved via implemented cells as tissue scaffolds in the damaged area. Organs and tissues can be regenerated via the cell culture into mods or scaffolds of biodegradable biomaterials. Tissue engineering can be improved via the new adventure of biomaterial fields. The delivery of genes can also induce tissue culture, offering regenerated organs. Alginate is one of these biomaterials that can advance tissue engineering.

Alginate, chitosan, and chitosan/alginate were investigated for the gene delivery of humanized Gaussia luciferase (hGLuc) messenger RNA (mRNA, Fig. 3) (Steinle et al. 2018). After 3 weeks, alginate, chitosan, and chitosan/alginate released 79%, 42%, and 70% of the incorporated mRNA. Alginate exhibited the highest gene release and improved cell transfection. Chitosan-alginate hydrogels were injectable and can be used to deliver mRNAs for tissue engineering via invasive local injection. The material was also biodegradable (Fig. 3) (Steinle et al. 2018).

Sodium alginate was reported as gene-activated hydrogels based on plasmid DNA with the gene of vascular endothelial growth factor (VEGF-A) (Bozo et al. 2021). The formed hydrogel acts as a gene-activated biomaterial that can induce reparative myogenesis in the central zone of muscle defect. After 2 weeks of surgery, it can form a more significant number of MyoG⁺ cells and create heavy chains of fetal myosin 7B (MYH7B) muscle fibers. It is a promising biomaterial for the recovery of skeletal muscle after extensive lesions (Bozo et al. 2021).

4 Alginate-Based Gene Delivery for Bone Generation

Trauma and surgical resection cause bone defects. The treatment of bone defects represents a significant challenge in orthopedics. Currently, bone defects can be treated via autologous or allogenic grafts. However, these methods showed disadvantages such as low availability of autologous grafts, the high risk of donor site morbidity, and disease transmission or host rejection. Recent approaches include the delivery of bone morphogenetic proteins (BMPs), for example, bone-forming peptide-1 (BFP-1), which is obtained from the bone morphogenetic protein-7 (BMP-7). The delivery of these reagents promotes bone regeneration via osteoinductive effect and the differentiation of mesenchymal stem cells (MSCs) into osteoblasts. However, the delivery of BMP-2 suffers from the need to deliver enormous amounts of protein. This challenge can be solved using a carrier that offers high delivery efficiency to the area of interest in bone.

Bone generation is a vital topic for regenerative medicine. Bone generation can be achieved via several bone generation strategies, including cell implementation and applying material for growth factor delivery. Bone morphogenetic protein 2 (BMP-2) has been considered an important growth factor. BMP-2 exhibits vigorous osteogenic activity inducing new bone formation. It differentiates multipotent stromal cells (MSCs) toward the osteogenic lineage. However, it lacks along half-life. The use of supraphysiological doses causes severe side effects. Alternatively, gene delivery can provide sustained protein delivery at the physiological concentration. Alginate may advance non-viral gene delivery in cartilage and bone repair (Gonzalez-Fernandez et al. 2018).

Gene delivery could improve the bone generation. Alginate was used to deliver BMP-2 using plasmid DNA for osteogenic differentiation (Wegman et al. 2011). The material can be used to deliver both in vitro and in vivo. As a result of the prolonged presence of BMP-2 using plasmid DNA-based gene therapy. Alginate-based gene delivery system offered high transfection efficiencies of 95% for human multipotent stromal cells (MSCs) and MG-63 cells. Data analysis using alkaline phosphatase (ALP) production revealed high protein levels causing effective inducing of osteogenic differentiation. There was also an increase in the production of collagen I and osteocalcin. The alginate-based gene delivery system offered minimal-invasive delivery of growth factors for bone regeneration (Wegman et al. 2011).

Alginate hydrogels containing calcium phosphate (CaP) and DeoxyriboNucleic Acid (DNA) were reported for bone tissues using MC3T3-E1 preosteoblast cells (Krebs et al. 2010). Alginate-based system in the nano-regime (<200 nm) with a particle size of 100 nm is a promising carriers for tissue engineering. It offered a high DNA incorporation efficiency of 66%. When the nanoparticles were incorporated in alginate hydrogels, a sustained release of cargo DNA was observed. It showed an increased capacity to form bony tissue within a short time of 2.5 weeks. Alginate/DNA/BMPs can be injected subcutaneously into the backs of mice (Krebs et al. 2010).

Alginate-based hydrogel is a promising carrier for delivering BMP-2 plasmid DNA for bone tissue engineering (Wegman et al. 2014). An alginate hydrogel was used for the gene delivery of BMP-2 complementary DNA (cDNA) (Wegman et al. 2014). The formed hydrogel was applied as a non-viral gene-activated matrix using goat multipotent stromal cells (gMSCs). It was also investigated for the implantation in spinal cassettes, ceramic granules, and intramuscularly in goats. After 16 weeks, the hydrogel offered stable transfection of BMP-2. It promoted osteogenic differentiation by forming bon in cell-seeded constructs at an ectopic location.

A bioink using a composite of a tripeptide Arg-Gly-Asp (RGD, Arginine, Glycine, and Aspartate)-Gama (γ)-irradiated alginate and nano-hydroxyapatite (nHA)/bone marrow-derived mesenchymal stem cells (MSCs) was reported for the delivery of plasmid DNA (pDNA) (Cunniffe et al. 2017). The composite was co-printed with a polycaprolactone (PCL) to improve the material's mechanical properties (Cunniffe et al. 2017). The printing can be used for multi-tool biofabrication of various materials. It can be used to create a complex composite implant with tailorable mechanical properties. The printed object can be used to control the gene delivery to induce cell transfection spatially. The material can be used for the delivery of the therapeutic genes BMP2 and TGF- β 3. It induces the in vitro osteogenesis of MSCs and enhance the vascularization and mineralization of tissue for subcutaneous implantation (Cunniffe et al. 2017).

Alginate was reported for the delivery of BMP-2 plasmid DNA (pBMP-2) for multipotent mesenchymal stromal cells (MSCs) (Loozen et al. 2015). The material prolonged the gene expression. It can be used for the formation of bone via in vivo investigation (Loozen et al. 2015).

5 Gene Delivery for Cartilage Repair

Cartilage repair can be achieved by generating ideal biomimetic synthetic chondral and osteochondral replacements. However, this technique suffers several challenges. The used recombinant proteins have a short half-life time and are expensive. The chondrocyte phenotypes are unstable and lack high mechanical properties. They also depend on several key parameters, such as age and weight. The cartilage implementation depends on the tissue's location. Some of these challenges can be overcome via physiological gene therapy. Cartilage repair via physiological gene therapy

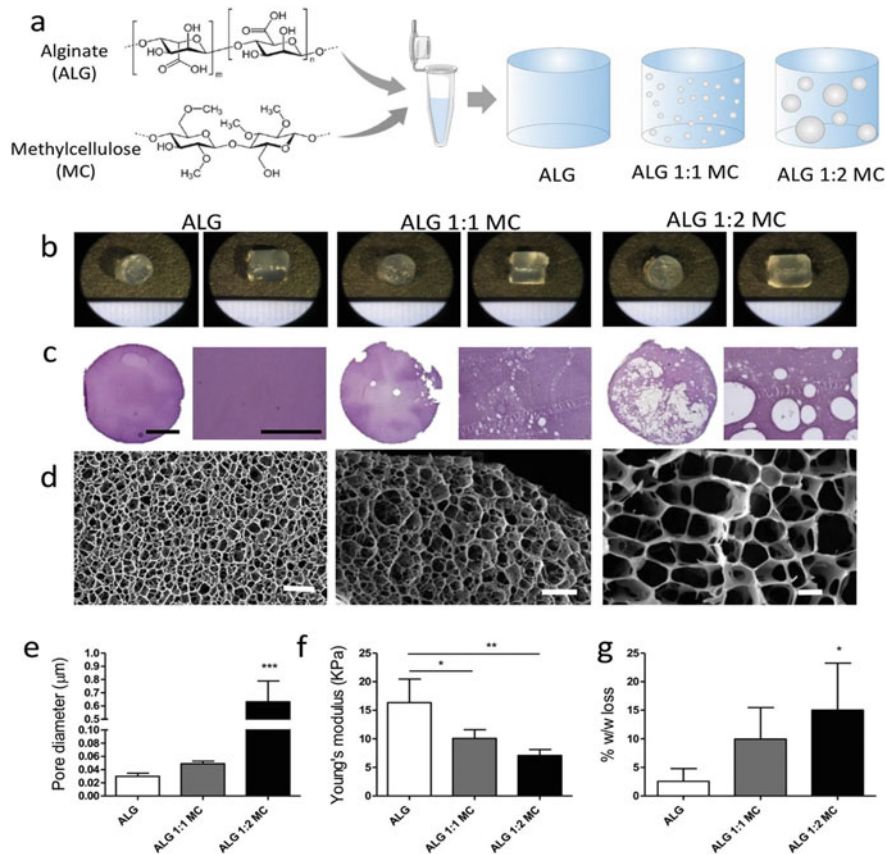


Fig. 4 (a) Synthesis procedure for ALG-MC gels, (b) macroscopic appearance, (c) histological representation through H & E staining, (d) cryo-scanning electron microscopy (SEM) imaging, (e) the pore diameter calculation based using cryo SEM imaging, (f) Young's modulus after 1 day of post-fabrication, and (g) a percentage of wet weight loss after 7 days in aqueous solution. Figure reprinted from (Gonzalez-Fernandez et al. 2019)

allows continuous expression of a gene therapeutic agent that generates an intricate structure of the osteochondral unit in the area of rehabilitation. Gene therapy promotes the generation of new cartilage. However, the direct use of gene therapeutic agents lacks high efficiency. Thus, a carrier is usually required to facilitate the gene delivery. Among several materials, alginate-based materials are promising for gene-delivery of cartilage repair (Yang et al. 2020). The reader can find more details on alginate-based materials for cartilage repair in Ref. (Liu et al. 2022). Alginate-based materials improved the spatiotemporal effects of gene therapeutic agents. They suppress the enzyme degradation and allow control delivery.

Methylcellulose (MC) was used for the pore-forming of alginate (ALG) bio-inks (denoted as ALG-MC, Fig. 4) (Gonzalez-Fernandez et al. 2019). A high concentration of methylcellulose ensures the formation of larger pores that enable a high

release rate and ensure high cell transfection. Alginate-based materials were used to deliver amphipathic peptide-based plasmid DNA (Fig. 4). After *in vivo* implantation for 4 weeks, data analysis revealed the expressions of cartilage-anabolic markers and cellular aggregates. The materials can also be used for zonally positioning stem cells and plasmids encoding for either osteogenic (BMP-2) or chondrogenic (combination of TGF- β 3, BMP2, and SOX9) genes. They exhibited good mechanical properties and enabled reinforced structure. The printed bio-ink offered temporally and spatially controlled delivery of therapeutic genes. The printed objects can be used for host or implanted cells inside constructs with reasonable control and mechanical properties (Gonzalez-Fernandez et al. 2019).

6 Cancer Treatment

Gene delivery can be used for cancer treatment. It can be achieved via the transfection of RNA interference (RNAi) that can be used for the gene silencing mechanism. Direct transfection of gene therapeutic agents suffers from low efficiency and enzymatic degradation. The cancer treatment using alginate hydrogels as a carrier was reviewed in (Reig-Vano et al. 2021; Niculescu and Grumezescu 2022) for further information on the topic.

Chitosan/alginate (Chi/Alg) nanoparticles were reported as a non-viral vector for cell transfection using Smad4 encoding plasmid (Zohri et al. 2021). The materials showed a particle size, loading efficiency, and cumulative release of 111 nm, 43%, and 39%, respectively. The toxicity of Smad4 encoding plasmid-loaded nanoparticles such as chitosan/alginate, lipofectamine 2000, and polyethyleneimine was compared using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, green Fluorescence protein (GFP) assay, flow cytometry, and reverse transcription polymerase chain reaction (RT-PCR). Chi/Alg exhibits high cell biocompatibility compared to Lipofectamine[®] 2000 and polyethyleneimine (PEI). Chi/Alg/Smad4, Lipofectamine, and PEI nanoparticles exhibited transfection efficacy of 29.9%, 35.5% and 30.9%, respectively (Zohri et al. 2021). Chi/Alg exhibit several properties, including high cell biocompatibility, low toxicity, and comparable transfection efficiency to commercial and previously reported non-viral vectors, i.e., Lipofectamine and PEI.

Alginate-based materials advanced the gene-delivery (He et al. 2012). The alginate-graft-polyethyleneimine (PEI, denoted as Alg-g-PEI) showed low cytotoxicity compared to PEI against cells BEL7402, MSC, and RVMSC cells (He et al. 2012). It also exhibited higher cell transfection efficiency for VEGF expression was significantly higher than PEI (M. Wt, 25 K)-mediated VEGF expression (He et al. 2012).

Alginate beads were used for the differentiation of human mesenchymal stem cells (hMSC) using differentiation factor 5 (GDF5) (Bucher et al. 2013). It improved the electroporation mediation of the gene enabling high cell transplantation from hMSCs that can be reimplanted into a degenerated disc for a low back pain patient.

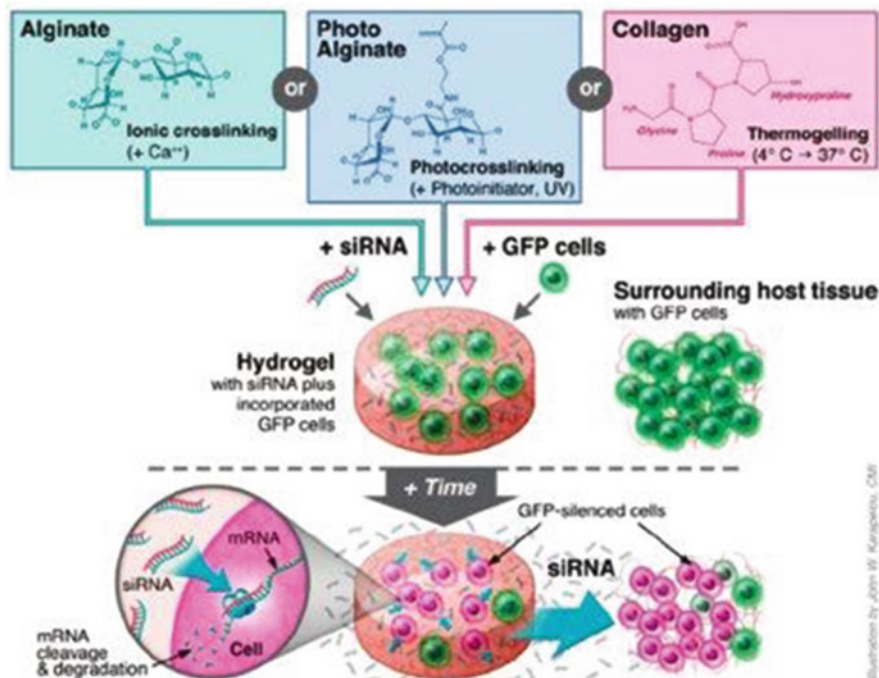


Fig. 5 The synthesis procedure for alginate/photo-alginate/collagen hydrogen and their application for gene delivery of siRNA. Figure reprinted from (Krebs et al. 2009). Copyright belongs to the American Chemical Society (ACS, 2009)

Chemotherapy conjugated gene delivery using alginate-based materials were reported for cancer treatment (Lakkakula et al. 2021). A hybrid system of alginate/calcium carbonate (CaCO_3)/DNA was synthesized via co-precipitation (Zhao et al. 2012a). The materials were prepared via the addition of alginate to CaCO_3 . Alginates modulate the particle size of the formed material. Alginate/ CaCO_3 /DNA was loaded with a chemotherapy drug, doxorubicin hydrochloride (DOX). Alginate/ CaCO_3 /DNA/DOX nanoparticles were tested for the vitrogene transfections of 293-T cells and HeLa cells using pGL3-Luc as a reporter plasmid (Zhao et al. 2012a). Alginate improved the gene transfection efficiency. Alginate/ CaCO_3 /DNA/DOX nanoparticles was also reported for the gene delivery of p53 expression plasmid (Zhao et al. 2012b). It offered a high cell inhibition rate of 80%. It could effectively mediate gene transfection and deliver the drug to the cells (Zhao et al. 2012b).

The alginate-based hydrogel was reported, including alginate, collagen, and siRNA (Fig. 5) (Krebs et al. 2009). Alginate with different cross-linking can be used, such as calcium cross-linked alginate and photo cross-linked alginate (Krebs et al. 2009). The formed hydrogel exhibits high biocompatibility with good transfection efficiency. It can be degradable. It can be used for cancer treatment and regenerative medicine (Fig. 5) (Krebs et al. 2009).

The prepared scaffold is porous scaffold that demonstrated gene delivery in prostate cancer cells for *in vitro* and *in vivo* evaluation (Wang et al. 2015). 3D chitosan/alginate scaffold was reported for the gene delivery of cationic pDNA. Chitosan/alginate scaffold was able to transfect the targeted cell *in vivo*. It is a good *in vitro* platform compared to 2D cultures for gene delivery to prostate cancer cells (Wang et al. 2015).

A ternary composite was reported containing superparamagnetic iron oxide (SPIO) nanoparticles, circular plasmid DNA (pDNA), and PEI (Leung et al. 2015). It was used for the employed for NP/gene transfection into glioblastoma U87MG and U138MG cells. The high transfection performance was recorded using PEI, DNA, and SPIO of 0.2 ng, 0.5 μ g, and 1.0 μ g, respectively (Leung et al. 2015). Alginate improved the transfection efficiency with good performance compared to the commercial non-viral vector Lipofectamine (Leung et al. 2015). Magnetic nanoparticles could improve the gene transfection (Dowaidar et al. 1861; Dowaidar et al. 2017, 2018).

There are several affecting the performance of alginate-based carriers for gene delivery (Douglas and Tabrizian 2005). Small particle size and low molecular weight polymers exhibit the highest transfection efficiency (Douglas and Tabrizian 2005). Alginate composite with small particle size offered DNA efficiency of 60% association (Douglas and Tabrizian 2005). A chitosan–alginate composite core-shell was used to encapsulate enhanced green fluorescent protein (EGFP)-encoded plasmids (You et al. 2006). The average size was measured using dynamic light scattering (DLS). Data analysis revealed a proportional increase in the average particle size with the rise of the proportionally, with the N/P ratios (amino nitrogen of chitosan: phosphate groups of plasmid DNA) in the range of 5 to 20. A composite consisting of chitosan–alginate nanoparticles (average size of 64 nm) exhibited a high gene expression level compared to a polyethyleneimine–DNA composite (You et al. 2006). The gene transfection using alginate-based nanomaterials also depends on other parameters such as incubation temperature, the concentration of the gene-therapeutic agents, and the separation procedure of the synthesized nanocarriers (Aynié et al. 1999).

7 Advantages and Disadvantages of Alginate for Gene Delivery

The chemistry of alginate is essential. Monovalent cations such as sodium ions dissolve the alginate gels. The gel can be also dissolved in the physiological condition of the mammals. However, it cannot be fully eliminated from the human body due to the absence of alginase enzyme. Alginic acid degrades more rapidly than the sodium salt form. Partially oxidized alginate is highly degraded compared to alginate (Bouhadir et al. 2001; Boontheekul et al. 2005). Alginic acid exhibits a

higher degradation rate than sodium alginate because of intramolecular catalysis via the C-5 carboxyl groups (Smidsrød et al. 1966).

Alginate lacks adhesion properties to cells because of the negative charges repulsion between alginic and cell membranes (Smetanajr 1993). Thus, it can be conjugated with laminin (Prang et al. 2006), chitosan (You et al. 2006), or fibronection (Mosahebi et al. 2003) to improve cell adhesion. Alginate improved the gene transfection of other non-viral vectors such as chitosan (Douglas et al. 2006). Alginate/chitosan composite exhibited fourfold higher gene transfection for 293 T cells compared to chitosan nanoparticles alone. It also showed higher transfection efficiency compared to conventional vectors such as Lipofectamine™. The improvement in the gene transfection of alginate-chitosan composite is due to the competition in the binding affinity that decreases the interaction between chitosan and DNA (Douglas et al. 2006). The complexation between alginate and chitosan maintained the integrity of DNA moieties. It reduced the enzymatic degradation of DNA compared to chitosan alone.

Composite containing alginate can be fabricated via several methods, including water-in-oil reverse micro-emulsion template (You et al. 2006) and co-electrospinning (Hu et al. 2018b). It can be performed in different morphology, including core-shell, nano-capsules, nanobeads, nanofibers, and nanospheres (You et al. 2006; Paques et al. 2014). Further information on the synthesis procedure of alginate was reviewed in (Paques et al. 2014). The comprehensive methods for processing alginate ensure the applicability of alginate for many biomedical applications.

The selection of the source used for alginate extraction is the crucial parameter for biomedical applications. The material's biocompatibility depends on the ratio of G: M ratio. For example, alginates with higher content of mannuronate (M)-blocks exhibit high immunogenic properties and offer 10 times the high performance for the cytokine compared to alginates with higher content of guluronic (G)-blocks. Alginate containing high G-block units exhibit higher encapsulation performance for therapeutic agents (O'Brien 2011).

Alginate can be conjugated with other biopolymers such as alginate/chitosan/Fucoidan (Hao et al. 2020; Venkatesan et al. 2014), carboxymethyl chitosan/organic rectorite/alginate (Jiang et al. 2015), alginate/dextran/spermine (Azadi et al. 2016), and polyethyleneimine/heparin/alginate (Mahlicli and Altinkaya 2013). Alginate biocomposite can proceed into different forms such as sponges (Hao et al. 2020), hydrogels (Eiselt et al. 2000), and aerogels (Guastaferrero et al. 2021). Alginate can proceed into three-dimensional (3D) scaffolds (Choe et al. 2019; Furuya et al. 2017). The selection of suitable forms enables high transfection performance and ensures biodegradation properties. Alginate-based carriers were used to deliver recombinant bone morphogenetic proteins (Krebs et al. 2010).

Alginate exhibit high DNA incorporation efficiency (up to 66%) (Krebs et al. 2010). It destroys the electrostatic interaction between DNA and polycations non-viral vectors, e.g., chitosan (Danielsen et al. 2005).

Alginate-based carriers exhibit high gene transfection. Alginate biobeads were tested for protoplast cells isolated from cultured tobacco cells (BY-2) (Sone et al.

2002). It was used to encapsulate a plasmid-modified green fluorescent protein (GFP) gene *CaMV35S-sGFP*. It showed tenfold higher GFP expression after 24 h of incubation. It showed higher transfection compared to a naked plasmid solution (Sone et al. 2002).

Alginate-based biobeads exhibit high storage stability for several weeks (Sone et al. 2002). They can be used for commercial marketing with minimal effort for storage and distribution. Alginate preserves the shape and maintain the mechanical stiffness of the formed objects. These properties may be promising for proceeding the alginate-based materials into capsules and other forms for simple uptake via oral or other administration processes.

Alginate-based biomaterials were reported for the release of nucleic acids, such as small interfering RNA (siRNA), plasmid DNA (pDNA), or antisense oligonucleotide (ON) (Aynié et al. 1999). The analysis of the alginate-based system using radioactive analysis revealed the distribution of the complex in the lung, liver, and spleen (Aynié et al. 1999). These observations indicate the high potential of the alginate-based complex for in vivo treatment. A core-shell of alginate/chitosan was reported for the oral delivery of heparin and enoxaparin (Bagre et al. 2013). Alginate-based nanofibers were reported for the in-situ transfection application (Hu et al. 2018b).

8 Vaccine Delivery Using Alginate-Based Materials

There are several reasons for the disease distribution. The lack of clean water causes an acute enteric infection due to *Vibrio cholerae* defined as Cholera (Ahmad Zamri et al. 2022). Disease such as Grass carp reovirus (GCRV) causes economic problem (Xu et al. 2022). Vaccination offers an excellent solution to face the infection and may save several million lives every year. There is massive progress in vaccination thanks to the renaissance of the purification of microbial species, genetic engineering, and the accumulation of knowledge of immune protection. Figure 6 shows the difference between conventional, i.e., antigenic vaccines (e.g., inactivated, subunits, or live-attenuated pathogens) and DNA vaccines (Franck et al. 2021). In traditional vaccines, the antigen is injected into the immune system via antigen-presenting cells (APCs). On the other side, DNA vaccines is based on the administration of plasmid DNA (pDNA). The transfected pDNA endocytosed and released in the cytosol. Finally, the nucleus uptakes pDNA, leading to transcription and translation into an antigenic protein. Both vaccine pathways, i.e., conventional or DNA vaccines, lead to downstream signaling cascades that evoke an immunological response. The mechanism of action can be found in (Franck et al. 2021). For high cell transfection, there are several carriers that can be used to deliver vaccine into cells. Among these carriers, alginate-based materials are promising (Jazayeri et al. 2021).

Alginate-based materials can be used for vaccine delivery. There are several nanocomposite such as alginate/poly-L-lysine (Freudenberger Catanzaro et al. 2022), alginate/chitosan (Huang et al. 2021; Rocha et al. 2021), and alginate/

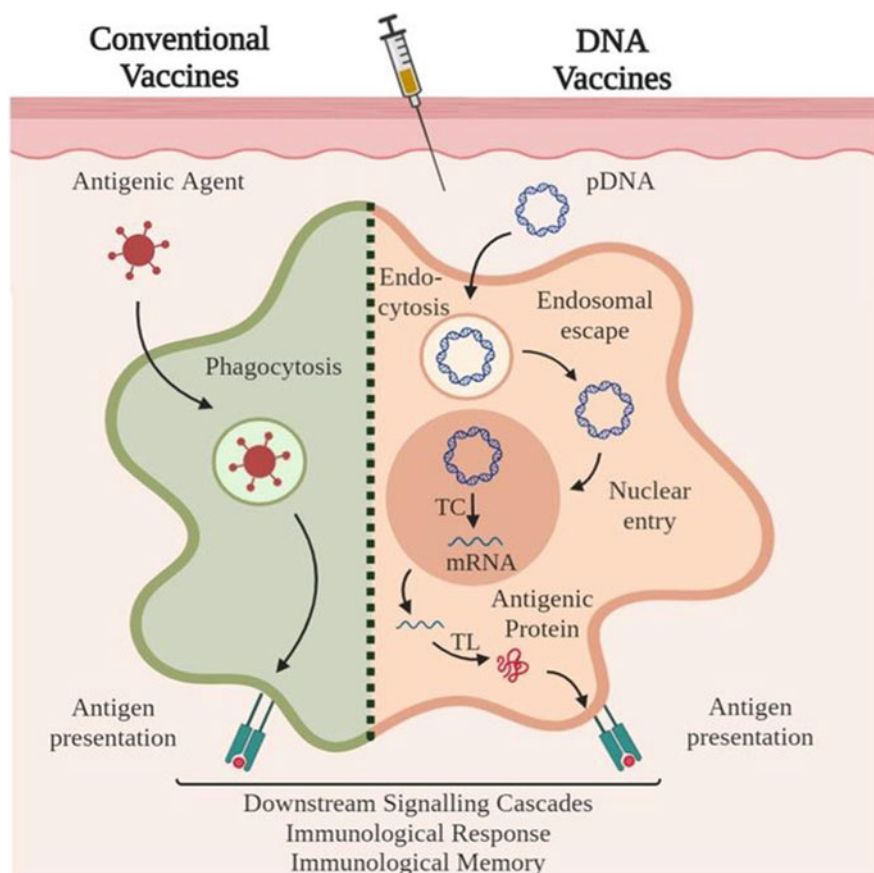


Fig. 6 Conventional and DNA vaccines. Figure reprinted from (Franck et al. 2021), an open access article distributed under the terms of the Creative Commons CC BY license

VP56-3 (Xu et al. 2022). They can be used for the vaccination of legumain DNA vaccines (Liu et al. 2013), flagellin B (FlaB) (Xu et al. 2022), fowl typhoid vaccine (Onuigbo et al. 2018), formalin-killed *L. garvieae* TW-446.B3 (Romalde et al. 2004), glycoprotein (G) gene (Ballesteros et al. 2015), *Pasteurella multocida* and Ovalbumin (OVA) antigens (Bowersock et al. 1999).

Alginate/chitosan nanocomposite was investigated for the vaccine delivery of PPE17 protein and CpG (Najafi et al. 2021). PPE17 is a surface localized protein that can exhibit robust immunoreactivity in a patient with active tuberculosis. While, CpG is an effective adjuvant that induces Th1 responses via the binding Toll-like receptor 9, stimulating Th1 and Th17 immune responses in mice. The vaccine system based on alginate can be administered via intranasal (IN) and subcutaneous (SC) routes (Najafi et al. 2021). Alginate was reported for *Bacillus Calmette–Guérin* (BCG), the only approved vaccine for tuberculosis (Kesarwani et al. 2021).

A capsule of chitosan/alginate capsules (3.5 mm in diameter) of the live probiotic pYG-KHV-ORF81/LR CIQ249 (5.2×10^{10} colony-forming unit (CFU) per gram (CFU/g) was reported for the oral delivery of a live probiotic (*Lactobacillus rhamnosus*) vaccine, pYG-KHV-ORF81/LR CIQ249 (Huang et al. 2021). The used vaccine expresses Koi Herpesvirus (KHV) ORF81 protein mass oral vaccination of Carps against KHV Infection (Huang et al. 2021). The vaccination efficiency was evaluated via IgM levels, lymphocyte proliferation, and expression of immune-related genes. Chitosan/alginate offered a protection rate of 85% for koi carp against KHV (Huang et al. 2021). It exhibited high tolerance against the simulated digestive environments, i.e., simulated gastric condition, i.e., pH 1.5. It also showed high stability against an intestinal fluid environment (i.e., 0.5% of bile salt), and a hypertonic environment (i.e., 9% NaCl) (Huang et al. 2021). Chitosan/alginate-coated calcium phosphate nanoparticles protect the antigens against the acidic condition of the gastrointestinal (GI) environment leading to a high immune response in the small intestine (Cao et al. 2020b).

A microcapsule of alginate/chitosan was used to encapsulate *Listeriamonocytogenes* inactivated by gamma irradiation (KLM- γ) as an adjuvant for the vaccination against *Listeria monocytogenes* infection (Fig. 7) (Rocha et al. 2021). Alginate/chitosan-encapsulated KLM- γ increased the proportion of memory CD4⁺ and CD8⁺ T cells. It induced the production of IL-10 and IFN- γ . Thus, it can increase the lethal infection with lower *L.monocytogenes*-induced hepatic inflammation and necrosis, indicating the high potential to serve as a vaccine against listeriosis compared to the cell without vaccination. The vaccination of the liver of mice with a sublethal dose of *L. monocytogenes* (1×10^5 CFU) was investigated after 4 days (Fig. 7). Data analysis reveals high vaccination performance of alginate-based materials.

Alginate was proposed for the delivery of antigen conjugated exotoxin A (ETA, lipopolysaccharides (Gopal et al. 2013)) to enhance the production of antibodies in the body against bacterial infections such as *Pseudomonas aeruginosa* (Haji Ali Babaei et al. 2022). The vaccine system produced antibodies such as total IgG, IgG1, IgG2a, IgG2b, and IgG3 improving the body's action against the infection by *P. aeruginosa* (Haji Ali Babaei et al. 2022).

Alginate-based was used for DNA vaccine, pVAX-ctxB-tcpA, to treat Cholera (Ahmad Zamri et al. 2022). The pVAX-ctxB-tcpA/alginate was tested using mammalian COS-7 cells. The biological activity was evaluated using quantitative real-time polymerase chain reaction (qRT-PCR) via measuring the cytokine expression level (TNF- α , IFN- γ , IL-6, and IL-10). Data analysis revealed that pVAX-ctxB-tcpA/alginate induced immune response against Cholera. The vaccination proposal suggested the vaccination via the upregulation of gene expression, protein, and cytokine genes, e.g., IFN- γ and IL-10 (Ahmad Zamri et al. 2022). An inorganic composite of selenium (Se)/alginate/chitosan was investigated for the controlled delivery of *Vibrio Cholerae* lipopolysaccharide (LPS; Fig. 8) (Bagheri-Josheghani and Bakhshi 2022). Data analysis revealed a significant increase of the anti-inflammatory cytokines (TGF- β , IL-10) and reduced IL-6 and IL-10 (Fig. 8) (Bagheri-Josheghani and Bakhshi 2022).

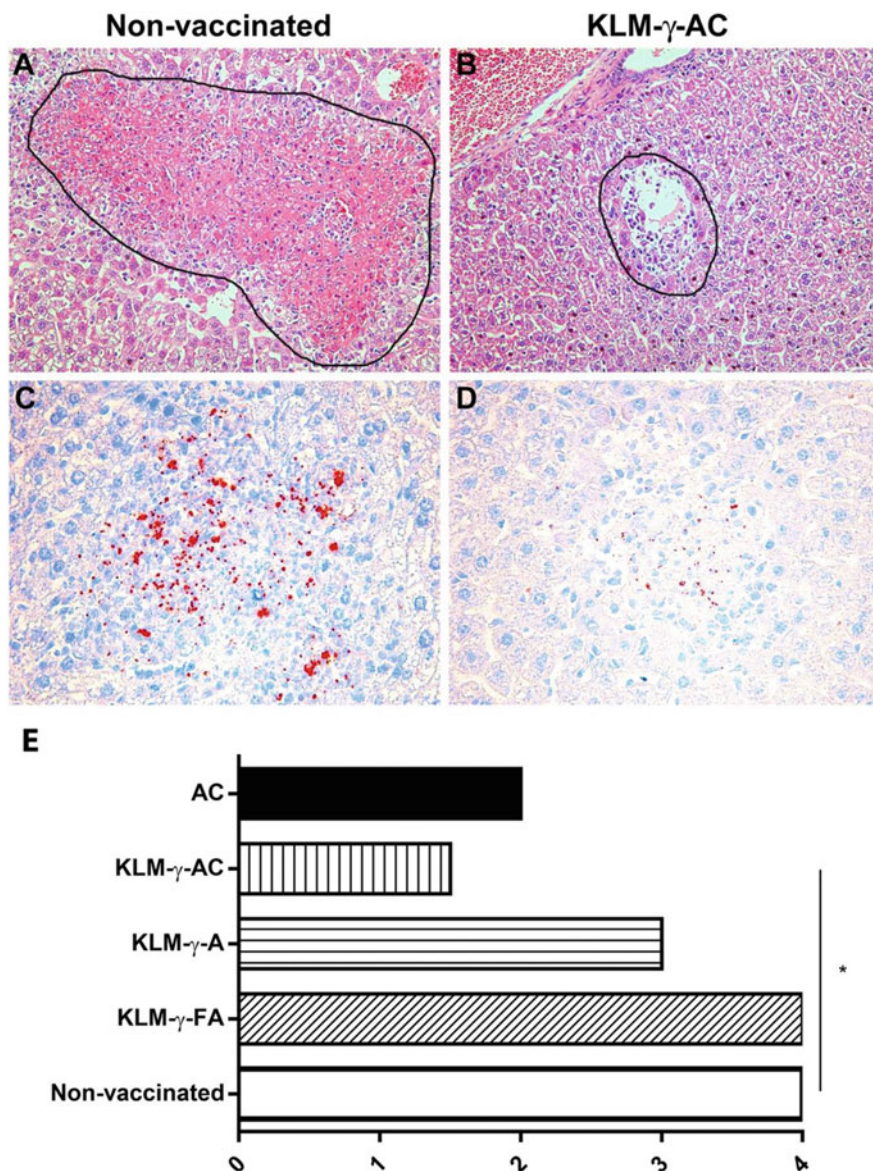


Fig. 7 Liver of mice (a, c) without and (b, d) with vaccination using (a, b) Microscope image, (c, d) Immunolabeling of *L. monocytogenes* (red) in the liver, and (e) Histopathologic score of mice's liver without (non-vaccinated control group, only sterile PBS), and with vaccination using KLM- γ without any adjuvant; KLM- γ -FA (Freund's Adjuvant); and using carriers of Aluminum Hydroxide (KLM- γ -AH); alginate microcapsules (KLM- γ -A); and alginate/chitosan microcapsules (KLM- γ -AC); and AC (empty alginate/chitosan microcapsules). Figure reprinted with permission from (Rocha et al. 2021). Copyright belongs to Elsevier, 2021

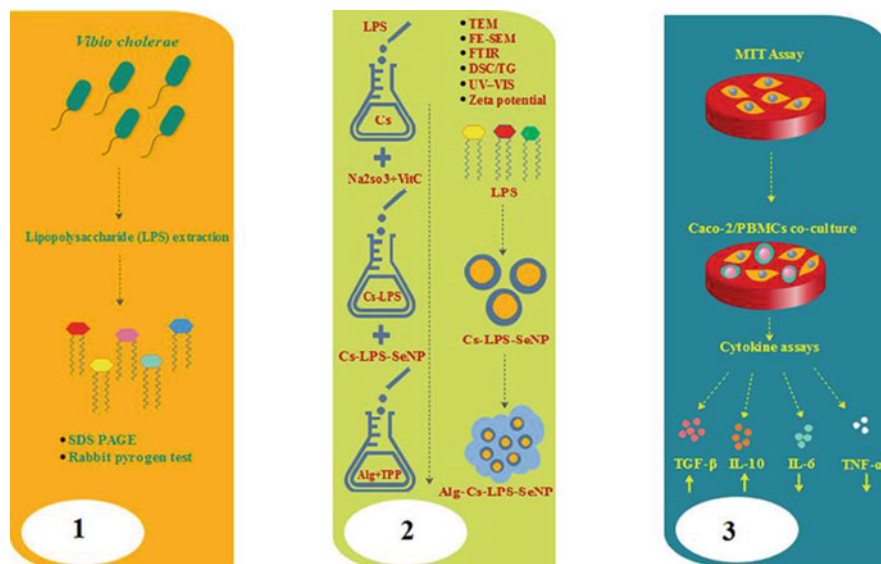


Fig. 8 Overview of vaccine delivery using Se NPs/alginate/chitosan for delivering *Vibrio Cholerae* LPS, including (1) LPS extraction, (2) synthesis of composite and (3) biological evaluation via measuring cytokines TNF- α , IL-6, IL-10, and TGF- β . Figure reprinted with permission from (Bagheri-Josheghani and Bakhshi 2022). Copyright belongs to Elsevier (2020)

Alginate was reported for vaccine delivery of several diseases such as *Francisella tularensis* (Freudenberger Catanzaro et al. 2022), *Ctenopharyngodon Idella* (GCRV) (Xu et al. 2022), *listeriosis* (Rocha et al. 2021), Fish *Lactococcosis* (Romalde et al. 2004), infectious hematopoietic necrosis virus (IHNV) (Ballesteros et al. 2015), and acute toxoplasmosis (Gaafar et al. 2022). Sodium alginate (SA)/antigen (VP56-3) was reported as a carrier for the vaccination against GCRV using as an adjuvant of flagellin B (FlaB) (Xu et al. 2022). Alginate is the most familiar carrier that can encapsulate and effectively deliver a vaccine to the intestine without degradation (Bøggwald and Dalmo 2021). It can be also used for oral administration of pDNA (Nogales et al. 2012). The oral vaccination of fish using chitosan/alginate microparticle showed high immune response with high survival rate via the expressions of IL-6 and IgM (Halimi et al. 2019).

The processing of alginate is easy and can be performed via several methods. 3D printing of alginate was reported for cell transfection using plasmid DNA (Khvorostina et al. 2022). The printed objects can be easily investigated for in vitro and in vivo treatments. They can mediate the in vitro transfection of HEK-293 cells. It can effectively transfect the in vivo model with plasmid DNA-encoding the enhanced green fluorescent protein (pEGFP) (Khvorostina et al. 2022). Thereby, it allowing the implementation of the developed GAS in future tissue engineering applications.

9 Conclusions

Alginate-based materials advanced gene and vaccine delivery. They circumvent several challenges, such as low transfection and enzymatic degradation of naked gene therapeutic agents. They decrease enzymatic degradation, improve cell adhesion, and enhance the clearance of gene-based therapeutic agents in the body. They can proceed quickly into different forms enabling wide applicability. However, alginate is incapable of controlling the rate of degradation and viscoelasticity. Further research should focus on the marketing and commercialization of alginate-based materials for gene and vaccine delivery.

References

- Abdelhamid HN, Dowaidar M, Hällbrink M, Langel Ü (2019) Cell penetrating peptides-hierarchical porous zeolitic imidazolate frameworks nanoparticles: an efficient gene delivery platform. *SSRN Electron J*. <https://doi.org/10.2139/ssrn.3435895>
- Abdelhamid HN, Dowaidar M, Langel Ü (2020) Carbonized chitosan encapsulated hierarchical porous zeolitic imidazolate frameworks nanoparticles for gene delivery. *Microporous Mesoporous Mater* 302:110200. <https://doi.org/10.1016/j.micromeso.2020.110200>
- Ahmad Zamri N, Rusli MEF, Mohamad Yusof L, Rosli R (2022) Immunization with a bicistronic DNA vaccine modulates systemic IFN- γ and IL-10 expression against *Vibrio cholerae* infection. *J Med Microbiol* 71. <https://doi.org/10.1099/jmm.0.001536>
- Augst AD, Kong HJ, Mooney DJ (2006) Alginate hydrogels as biomaterials. *Macromol Biosci* 6: 623–633. <https://doi.org/10.1002/mabi.200600069>
- Aynié I, Vauthier C, Chacun H, Fattal E, Couvreur P (1999) Spongelike alginate nanoparticles as a new potential system for the delivery of antisense oligonucleotides. *Antisense Nucleic Acid Drug Dev* 9:301–312. <https://doi.org/10.1089/oli.1.1999.9.301>
- Azadi SA, Vasheghani-Farahani E, Hashemi-Najafabadi S, Godini A (2016) Co-encapsulation of pancreatic islets and pentoxifylline in alginate-based microcapsules with enhanced immunosuppressive effects. *Prog Biomater* 5:101–109. <https://doi.org/10.1007/s40204-016-0049-3>
- Bagheri-Josheghani S, Bakhshi B (2022) Formulation of selenium nanoparticles encapsulated by alginate-chitosan for controlled delivery of *vibrio cholerae* LPS: a novel delivery system candidate for nanovaccine. *Int J Biol Macromol* 208:494–508. <https://doi.org/10.1016/j.ijbiomac.2022.03.087>
- Bagre AP, Jain K, Jain NK (2013) Alginate coated chitosan core shell nanoparticles for oral delivery of enoxaparin: in vitro and in vivo assessment. *Int J Pharm* 456:31–40. <https://doi.org/10.1016/j.ijpharm.2013.08.037>
- Ballesteros NA, Alonso M, Saint-Jean SR, Perez-Prieto SI (2015) An oral DNA vaccine against infectious haematopoietic necrosis virus (IHNV) encapsulated in alginate microspheres induces dose-dependent immune responses and significant protection in rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol* 45:877–888. <https://doi.org/10.1016/j.fsi.2015.05.045>
- Bedell ML, Navara AM, Du Y, Zhang S, Mikos AG (2020) Polymeric systems for bioprinting. *Chem Rev* 120:10744–10792. <https://doi.org/10.1021/acs.chemrev.9b00834>
- Bi Y, Lin Z, Deng S (2019) Fabrication and characterization of hydroxyapatite/sodium alginate/chitosan composite microspheres for drug delivery and bone tissue engineering. *Mater Sci Eng C* 100:576–583. <https://doi.org/10.1016/j.msec.2019.03.040>

- Bøgwald J, Dalmo RA (2021) Protection of teleost fish against infectious diseases through oral administration of vaccines: update 2021. *Int J Mol Sci* 22:10932. <https://doi.org/10.3390/ijms222010932>
- Boisguérin P, Deshayes S, Gait MJ, O'Donovan L, Godfrey C, Betts CA et al (2015) delivery of therapeutic oligonucleotides with cell penetrating peptides. *Adv Drug Deliv Rev* 87:52–67. <https://doi.org/10.1016/j.addr.2015.02.008>
- Boonthekul T, Kong H-J, Mooney DJ (2005) Controlling alginate gel degradation utilizing partial oxidation and bimodal molecular weight distribution. *Biomaterials* 26:2455–2465. <https://doi.org/10.1016/j.biomaterials.2004.06.044>
- Bouhadir KH, Lee KY, Alsberg E, Damm KL, Anderson KW, Mooney DJ (2001) Degradation of partially oxidized alginate and its potential application for tissue engineering. *Biotechnol Prog* 17:945–950. <https://doi.org/10.1021/bp010070p>
- Bowersock T, Hogenesch H, Suckow M, Guimond P, Martin S, Borie D et al (1999) Oral vaccination of animals with antigens encapsulated in alginate microspheres. *Vaccine* 17:1804–1811. [https://doi.org/10.1016/S0264-410X\(98\)00437-X](https://doi.org/10.1016/S0264-410X(98)00437-X)
- Bozo IY, Mavlikeev MO, Presnyakov EV, Yasinovsky MI, Trofimov VO, Indeykin FA et al (2021) Gene-activated hydrogels based on sodium alginate for reparative myogenesis of skeletal muscle. *Inorg Mater Appl Res* 12:1026–1032. <https://doi.org/10.1134/S2075113321040092>
- Bucher C, Gazdhar A, Benneker LM, Geiser T, Gantenbein-Ritter B (2013) Nonviral gene delivery of growth and differentiation factor 5 to human mesenchymal stem cells injected into a 3D bovine intervertebral disc organ culture system. *Stem Cells Int* 2013:1–9. <https://doi.org/10.1155/2013/326828>
- Byambaa B, Annabi N, Yue K, Trujillo-de Santiago G, Alvarez MM, Jia W et al (2017) Bioprinted osteogenic and vasculogenic patterns for engineering 3D bone tissue. *Adv Healthc Mater* 6:1700015. <https://doi.org/10.1002/adhm.201700015>
- Cao L, Lu W, Mata A, Nishinari K, Fang Y (2020a) Egg-box model-based gelation of alginate and pectin: a review. *Carbohydr Polym* 242:116389. <https://doi.org/10.1016/j.carbpol.2020.116389>
- Cao P, Han FY, Grøndahl L, Xu ZP, Li L (2020b) Enhanced oral vaccine efficacy of polysaccharide-coated calcium phosphate nanoparticles. *ACS Omega* 5:18185–18197. <https://doi.org/10.1021/acsomega.0c01792>
- Chang TMS (1992) Artificial cells in immobilization biotechnology. *Biomater Artif Cells Immobil Biotechnol* 20:1121–1143. <https://doi.org/10.3109/10731199209117340>
- Choe G, Oh S, Seok JM, Park SA, Lee JY (2019) Graphene oxide/alginate composites as novel bioinks for three-dimensional mesenchymal stem cell printing and bone regeneration applications. *Nanoscale* 11:23275–23285. <https://doi.org/10.1039/C9NR07643C>
- Choi Y, Park K, Choi H, Son D, Shin M (2021) Self-healing, stretchable, biocompatible, and conductive alginate hydrogels through dynamic covalent bonds for implantable electronics. *Polymers (Basel)* 13:1133. <https://doi.org/10.3390/polym13071133>
- Chueh B, Zheng Y, Torisawa Y, Hsiao AY, Ge C, Hsiong S et al (2010) Patterning alginate hydrogels using light-directed release of caged calcium in a microfluidic device. *Biomed Microdevices* 12:145–151. <https://doi.org/10.1007/s10544-009-9369-6>
- Coleman RJ, Lawrie G, Lambert LK, Whittaker M, Jack KS, Grøndahl L (2011) Phosphorylation of alginate: synthesis, characterization, and evaluation of in vitro mineralization capacity. *Biomacromolecules* 12:889–897. <https://doi.org/10.1021/bm1011773>
- Cui J, Wang M, Zheng Y, Rodríguez Muñoz GM, del Campo A (2013) Light-triggered cross-linking of alginates with caged Ca^{2+} . *Biomacromolecules* 14:1251–1256. <https://doi.org/10.1021/bm400022h>
- Cunniffe GM, Gonzalez-Fernandez T, Daly A, Sathy BN, Jeon O, Alsberg E et al (2017) Three-dimensional bioprinting of polycaprolactone reinforced gene activated bioinks for bone tissue engineering. *Tissue Eng Part A* 23:891–900. <https://doi.org/10.1089/ten.tea.2016.0498>
- Danielsen S, Maurstad G, Stokke BT (2005) DNA-polycation complexation and polyplex stability in the presence of competing polyanions. *Biopolymers* 77:86–97. <https://doi.org/10.1002/bip.20170>

- Dey P, Ramanujam R, Venkatesan G, Nagarathnam R (2019) Sodium alginate potentiates antioxidant defense and PR proteins against early blight disease caused by *Alternaria solani* in *Solanum lycopersicum* Linn. *PLoS One* 14:e0223216. <https://doi.org/10.1371/journal.pone.0223216>
- Diniz FR, Maia RCAP, Rannier Andrade L, Andrade LN, Vinicius Chaud M, da Silva CF et al (2020) Silver nanoparticles-composing alginate/gelatin hydrogel improves wound healing in vivo. *Nano* 10:390. <https://doi.org/10.3390/nano10020390>
- Dodero A, Alberti S, Gaggero G, Ferretti M, Botter R, Vicini S et al (2021) An up-to-date review on alginate nanoparticles and nanofibers for biomedical and pharmaceutical applications. *Adv Mater Interfaces* 8:2100809. <https://doi.org/10.1002/admi.202100809>
- Douglas KL, Tabrizian M (2005) Effect of experimental parameters on the formation of alginate-chitosan nanoparticles and evaluation of their potential application as DNA carrier. *J Biomater Sci Polym Ed* 16:43–56. <https://doi.org/10.1163/1568562052843339>
- Douglas KL, Piccirillo CA, Tabrizian M (2006) Effects of alginate inclusion on the vector properties of chitosan-based nanoparticles. *J Control Release* 115:354–361. <https://doi.org/10.1016/j.jconrel.2006.08.021>
- Dowaidar M, Abdelhamid HN, Hällbrink M, Zou X, Langel Ü (1861) Graphene oxide nanosheets in complex with cell penetrating peptides for oligonucleotides delivery. *Biochim Biophys Acta Gen Subj* 2017:2334–2341. <https://doi.org/10.1016/j.bbagen.2017.07.002>
- Dowaidar M, Abdelhamid HN, Hällbrink M, Kurrikoff K, Freimann K, Zou X et al (2017) Magnetic nanoparticle assisted self-assembly of cell penetrating peptides-oligonucleotides complexes for gene delivery. *Sci Rep* 7:9159. <https://doi.org/10.1038/s41598-017-09803-z>
- Dowaidar M, Nasser Abdelhamid H, Hällbrink M, Langel Ü, Zou X (2018) Chitosan enhances gene delivery of oligonucleotide complexes with magnetic nanoparticles-cell-penetrating peptide. *J Biomater Appl* 33:392–401. <https://doi.org/10.1177/0885328218796623>
- Dowaidar M, Abdelhamid HN, Langel Ü (2022) Improvement of transfection with peptides using organic and inorganic materials. *Methods Mol Biol* 83:555–567. https://doi.org/10.1007/978-1-0716-1752-6_35
- Drury JL, Dennis RG, Mooney DJ (2004) The tensile properties of alginate hydrogels. *Biomaterials* 25:3187–3199. <https://doi.org/10.1016/j.biomaterials.2003.10.002>
- Dvir-Ginzberg M, Gamlieli-Bonshtein I, Agbaria R, Cohen S (2003) Liver tissue engineering within alginate scaffolds: effects of cell-seeding density on hepatocyte viability, morphology, and function. *Tissue Eng* 9:757–766. <https://doi.org/10.1089/107632703768247430>
- Eiselt P, Lee KY, Mooney DJ (1999) Rigidity of two-component hydrogels prepared from alginate and poly(ethylene glycol)-diamines. *Macromolecules* 32:5561–5566. <https://doi.org/10.1021/ma990514m>
- Eiselt P, Yeh J, Latvala RK, Shea LD, Mooney DJ (2000) Porous carriers for biomedical applications based on alginate hydrogels. *Biomaterials* 21:1921–1927. [https://doi.org/10.1016/S0142-9612\(00\)00033-8](https://doi.org/10.1016/S0142-9612(00)00033-8)
- Farokhi M, Jonidi Shariatzadeh F, Solouk A, Mirzadeh H (2020) Alginate based scaffolds for cartilage tissue engineering: a review. *Int J Polym Mater Polym Biomater* 69:230–247. <https://doi.org/10.1080/00914037.2018.1562924>
- Franck CO, Fanslau L, Bistrovic Popov A, Tyagi P, Fruk L (2021) Biopolymer-based carriers for DNA vaccine design. *Angew Chemie Int Ed Engl* 60:13225–13243. <https://doi.org/10.1002/anie.202010282>
- Freudenberger Catanzaro KC, Lahmers KK, Allen IC, Inzana TJ (2022) Alginate microencapsulation of an attenuated O-antigen mutant of *Francisella tularensis* LVS as a model for a vaccine delivery vehicle. *PLoS One* 17:e0259807. <https://doi.org/10.1371/journal.pone.0259807>
- Furuya DC, da Costa SA, de Oliveira RC, Ferraz HG, Pessoa Junior A, da Costa SM (2017) Fibers obtained from alginate, chitosan and hybrid used in the development of scaffolds. *Mater Res* 20: 377–386. <https://doi.org/10.1590/1980-5373-mr-2016-0509>
- Gaafar MR, El-Mansoury ST, Eissa MM, Shalaby TI, Younis LK, Rashed HA (2022) Effect of alginate nanoparticles on the immunogenicity of excretory-secretory antigens against acute

- toxoplasmosis in murine model. *Acta Trop* 225:106215. <https://doi.org/10.1016/j.actatropica.2021.106215>
- Gacesa P (1998) Bacterial alginate biosynthesis—recent progress and future prospects. *Microbiology* 144:1133–1143. <https://doi.org/10.1099/00221287-144-5-1133>
- Gonzalez-Fernandez T, Kelly DJ, O'Brien FJ (2018) Controlled non-viral gene delivery in cartilage and bone repair: current strategies and future directions. *Adv Ther* 1:1800038. <https://doi.org/10.1002/adtp.201800038>
- Gonzalez-Fernandez T, Rathan S, Hobbs C, Pitacco P, Freeman FE, Cunniffe GM et al (2019) Pore-forming bioinks to enable spatio-temporally defined gene delivery in bioprinted tissues. *J Control Release* 301:13–27. <https://doi.org/10.1016/j.jconrel.2019.03.006>
- Gopal J, Abdelhamid HN, Hua P-Y, Wu H-F (2013) Chitosan nanomagnets for effective extraction and sensitive mass spectrometric detection of pathogenic bacterial endotoxin from human urine. *J Mater Chem B* 1:2463. <https://doi.org/10.1039/c3tb20079e>
- Guastrafiero M, Reverchon E, Baldino L (2021) Agarose, alginate and chitosan nanostructured aerogels for pharmaceutical applications: a short review. *Front Bioeng Biotechnol* 9:688477. <https://doi.org/10.3389/fbioe.2021.688477>
- Gurikov P, Raman SP, Weinrich D, Fricke M, Smirnova I (2015) A novel approach to alginate aerogels: carbon dioxide induced gelation. *RSC Adv* 5:7812–7818. <https://doi.org/10.1039/C4RA14653K>
- Haji Ali Babaei R, Shapoori R, Beig M (2022) Evaluation of alginate-exotoxin a conjugated vaccine immunogenicity against pseudomonas aeruginosa infection in mouse model. *Iran J Med Microbiol* 16:1–16. <https://doi.org/10.30699/ijmm.16.1.1>
- Halimi M, Alishahi M, Abbaspour MR, Ghorbanpoor M, Tabandeh MR (2019) Valuable method for production of oral vaccine by using alginate and chitosan against lactococcus garvieae/streptococcus iniae in rainbow trout (oncorhynchus mykiss). *Fish Shellfish Immunol* 90:431–439. <https://doi.org/10.1016/j.fsi.2019.05.020>
- Hao Y, Zhao W, Zhang L, Zeng X, Sun Z, Zhang D et al (2020) Bio-multifunctional alginate/chitosan/fucoidan sponges with enhanced angiogenesis and hair follicle regeneration for promoting full-thickness wound healing. *Mater Des* 193:108863. <https://doi.org/10.1016/j.matdes.2020.108863>
- Hay ID, Ur Rehman Z, Ghafoor A, Rehm BHA (2010) Bacterial biosynthesis of alginates. *J Chem Technol Biotechnol* 85:752–759. <https://doi.org/10.1002/jctb.2372>
- He W, Guo Z, Wen Y, Wang Q, Xie B, Zhu S et al (2012) Alginate-graft-PEI as a gene delivery vector with high efficiency and low cytotoxicity. *J Biomater Sci Polym Ed* 23:315–331. <https://doi.org/10.1163/092050610X550359>
- Heid S, Boccaccini AR (2020) Advancing bioinks for 3D bioprinting using reactive fillers: a review. *Acta Biomater* 113:1–22. <https://doi.org/10.1016/j.actbio.2020.06.040>
- Helmiyati AM (2017) Characterization and properties of sodium alginate from brown algae used as an ecofriendly superabsorbent. *IOP Conf Ser Mater Sci Eng* 188:012019. <https://doi.org/10.1088/1757-899X/188/1/012019>
- Hu X, Yang J, Chen C, Khan H, Guo Y, Yang L (2018a) Capillary electrophoresis-integrated immobilized enzyme microreactor utilizing single-step in-situ penicillinase-mediated alginate hydrogelation: application for enzyme assays of penicillinase. *Talanta* 189:377–382. <https://doi.org/10.1016/j.talanta.2018.07.019>
- Hu W-W, Wu Y-C, Hu Z-C (2018b) The development of an alginate/polycaprolactone composite scaffold for in situ transfection application. *Carbohydr Polym* 183:29–36. <https://doi.org/10.1016/j.carbpol.2017.11.030>
- Huang X, Ma Y, Wang Y, Niu C, Liu Z, Yao X et al (2021) Oral probiotic vaccine expressing Koi Herpesvirus (KHV) ORF81 protein delivered by chitosan-alginate capsules is a promising strategy for mass oral vaccination of carps against KHV infection. *J Virol* 95:e00415–e00421. <https://doi.org/10.1128/JVI.00415-21>
- Javvaji V, Baradwaj AG, Payne GF, Raghavan SR (2011) Light-activated ionic gelation of common biopolymers. *Langmuir* 27:12591–12596. <https://doi.org/10.1021/la201860s>

- Jazayeri SD, Lim HX, Shameli K, Yeap SK, Poh CL (2021) Nano and microparticles as potential oral vaccine carriers and adjuvants against infectious diseases. *Front Pharmacol* 12:682286. <https://doi.org/10.3389/fphar.2021.682286>
- Jiang L, Lu Y, Liu X, Tu H, Zhang J, Shi X et al (2015) Layer-by-layer immobilization of quaternized carboxymethyl chitosan/organic rectorite and alginate onto nanofibrous mats and their antibacterial application. *Carbohydr Polym* 121:428–435. <https://doi.org/10.1016/j.carbpol.2014.12.069>
- Kanasan N, Adzila S, Azimah Mustaffa N, Gurubaran P (2017) The effect of sodium alginate on the properties of hydroxyapatite. *Procedia Eng* 184:442–448. <https://doi.org/10.1016/j.proeng.2017.04.115>
- Kesarwani A, Sahu P, Jain K, Sinha P, Mohan KV, Nagpal PS et al (2021) The safety and efficacy of BCG encapsulated alginate particle (BEAP) against M.tb H37Rv infection in macaca mulatta: a pilot study. *Sci Rep* 11:3049. <https://doi.org/10.1038/s41598-021-82614-5>
- Khvorostina MA, Mironov AV, Nedorubova IA, Bukharova TB, Vasilyev AV, Goldshtein DV et al (2022) 3D printed gene-activated sodium alginate hydrogel scaffolds. *Gels* 8:421. <https://doi.org/10.3390/gels8070421>
- Kilian D, Ahlfeld T, Akkineni AR, Bernhardt A, Gelinsky M, Lode A (2020) 3D bioprinting of osteochondral tissue substitutes—in vitro-chondrogenesis in multi-layered mineralized constructs. *Sci Rep* 10:8277. <https://doi.org/10.1038/s41598-020-65050-9>
- Kong H (2003) Designing alginate hydrogels to maintain viability of immobilized cells. *Biomaterials* 24:4023–4029. [https://doi.org/10.1016/S0142-9612\(03\)00295-3](https://doi.org/10.1016/S0142-9612(03)00295-3)
- Kong HJ, Mooney DJ (2003) The effects of poly(ethyleneimine) (PEI) molecular weight on reinforcement of alginate hydrogels. *Cell Transplant* 12:779–785. <https://doi.org/10.3727/000000003108747253>
- Krebs MD, Jeon O, Alsberg E (2009) Localized and sustained delivery of silencing RNA from macroscopic biopolymer hydrogels. *J Am Chem Soc* 131:9204–9206. <https://doi.org/10.1021/ja9037615>
- Krebs MD, Salter E, Chen E, Sutter KA, Alsberg E (2010) Calcium phosphate-DNA nanoparticle gene delivery from alginate hydrogels induces in vivo osteogenesis. *J Biomed Mater Res A* 92(3):1131–1138. <https://doi.org/10.1002/jbm.a.32441>
- Lakkakula JR, Gujarathi P, Pansare P, Tripathi S (2021) A comprehensive review on alginate-based delivery systems for the delivery of chemotherapeutic agent: doxorubicin. *Carbohydr Polym* 259:117696. <https://doi.org/10.1016/j.carbpol.2021.117696>
- Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. *Prog Polym Sci* 37:106–126. <https://doi.org/10.1016/j.progpolymsci.2011.06.003>
- Lee CSD, Moyer HR, Gittens RAI, Williams JK, Boskey AL, Boyan BD et al (2010) Regulating in vivo calcification of alginate microbeads. *Biomaterials* 31:4926–4934. <https://doi.org/10.1016/j.biomaterials.2010.03.001>
- LeRoux MA, Guilak F, Setton LA (1999) Compressive and shear properties of alginate gel: effects of sodium ions and alginate concentration. *J Biomed Mater Res* 47:46–53. [https://doi.org/10.1002/\(SICI\)1097-4636\(199910\)47:1<46::AID-JBM6>3.0.CO;2-N](https://doi.org/10.1002/(SICI)1097-4636(199910)47:1<46::AID-JBM6>3.0.CO;2-N)
- Leslie SK, Nicolini AM, Sundaresan G, Zweit J, Boyan BD, Schwartz Z (2016) Development of a cell delivery system using alginate microbeads for tissue regeneration. *J Mater Chem B* 4:3515–3525. <https://doi.org/10.1039/C6TB00035E>
- Leung KC-F, Sham K WY, Chak C-P, Lai JMY, Lee S-F, Wáng Y-XJ et al (2015) Evaluation of biocompatible alginate- and deferroxamine-coated ternary composites for magnetic resonance imaging and gene delivery into glioblastoma cells. *Quant Imaging Med Surg* 5:382–391. <https://doi.org/10.3978/j.issn.2223-4292.2015.03.12>
- Li D, Zhuang J, He H, Jiang S, Banerjee A, Lu Y et al (2017) Influence of particle geometry on gastrointestinal transit and absorption following oral administration. *ACS Appl Mater Interfaces* 9:42492–42502. <https://doi.org/10.1021/acsami.7b11821>

- Liu Z, Lv D, Liu S, Gong J, Wang D, Xiong M et al (2013) Alginic acid-coated chitosan nanoparticles loaded with legumain DNA vaccine: effect against breast cancer in mice. *PLoS One* 8:e60190. <https://doi.org/10.1371/journal.pone.0060190>
- Liu G, Zhou H, Wu H, Chen R, Guo S (2016) Preparation of alginate hydrogels through solution extrusion and the release behavior of different drugs. *J Biomater Sci Polym Ed* 27:1808–1823. <https://doi.org/10.1080/09205063.2016.1237452>
- Liu X, Liu H, Qu X, Lei M, Zhang C, Hong H et al (2017) Electrical signals triggered controllable formation of calcium-alginate film for wound treatment. *J Mater Sci Mater Med* 28:146. <https://doi.org/10.1007/s10856-017-5956-x>
- Liu W, Madry H, Cucchiari M (2022) Application of alginate hydrogels for next-generation articular cartilage regeneration. *Int J Mol Sci* 23:1147. <https://doi.org/10.3390/ijms23031147>
- Loozen LD, van der Helm YJM, Öner FC, Dhert WJA, Kruyt MC, Alblas J (2015) Bone morphogenetic protein-2 nonviral gene therapy in a goat iliac crest model for bone formation. *Tissue Eng Part A* 21:1672–1679. <https://doi.org/10.1089/ten.tea.2014.0593>
- Mahlieli FY, Altinkaya SA (2013) Surface modification of polysulfone based hemodialysis membranes with layer by layer self assembly of polyethyleneimine/alginate-heparin: a simple polyelectrolyte blend approach for heparin immobilization. *J Mater Sci Mater Med* 24:533–546. <https://doi.org/10.1007/s10856-012-4804-2>
- Maity C, Das N (2022) Alginate-based smart materials and their application: recent advances and perspectives. *Top Curr Chem* 380:3. <https://doi.org/10.1007/s41061-021-00360-8>
- Mallakpour S, Azadi E, Hussain CM (2021) State-of-the-art of 3D printing technology of alginate-based hydrogels—an emerging technique for industrial applications. *Adv Colloid Interf Sci* 293: 102436. <https://doi.org/10.1016/j.cis.2021.102436>
- Mazza G, Al-Akkad W, Rombouts K, Pinzani M (2018) Liver tissue engineering: from implantable tissue to whole organ engineering. *Hepatol Commun* 2:131–141. <https://doi.org/10.1002/hep4.1136>
- Moe ST, Skjåk-Bræk G, Smidsrød O (1991) Covalently cross-linked sodium alginate beads. *Food Hydrocoll* 5:119–123. [https://doi.org/10.1016/S0268-005X\(09\)80296-8](https://doi.org/10.1016/S0268-005X(09)80296-8)
- Mørch YA, Donati I, Strand BL, Skjåk-Bræk G (2006) Effect of Ca^{2+} , Ba^{2+} , and Sr^{2+} on alginate microbeads. *Biomacromolecules* 7:1471–1480. <https://doi.org/10.1021/bm060010d>
- Mosahebi A, Wiberg M, Terenghi G (2003) Addition of fibronectin to alginate matrix improves peripheral nerve regeneration in tissue-engineered conduits. *Tissue Eng* 9:209–218. <https://doi.org/10.1089/107632703764664684>
- Najafi A, Ghazvini K, Sankian M, Gholami L, Amini Y, Zare S et al (2021) T helper type 1 biased immune responses by PPE17 loaded core-shell alginate-chitosan nanoparticles after subcutaneous and intranasal administration. *Life Sci* 282:119806. <https://doi.org/10.1016/j.lfs.2021.119806>
- Niculescu A-G, Grumezescu AM (2022) Applications of chitosan-alginate-based nanoparticles—an up-to-date review. *Nano* 12:186. <https://doi.org/10.3390/nano12020186>
- Niidome T, Huang L (2002) Gene therapy progress and prospects: non-viral vectors. *Gene Ther* 9: 1647–1652. <https://doi.org/10.1038/sj.gt.3301923>
- Nogral N, Abdullah S, Shamsudin MN, Billa N, Rosli R (2012) Formation and characterization of pDNA-loaded alginate microspheres for oral administration in mice. *J Biosci Bioeng* 113:133–140. <https://doi.org/10.1016/j.jbiosc.2011.10.003>
- Núñez C, López-Pliego L, Ahumada-Manuel CL, Castañeda M (2022) Genetic regulation of alginate production in *azotobacter vinelandii* a bacterium of biotechnological interest: a mini-review. *Front Microbiol* 13:845473. <https://doi.org/10.3389/fmicb.2022.845473>
- O'Brien FJ (2011) Biomaterials & scaffolds for tissue engineering. *Mater Today* 14:88–95. [https://doi.org/10.1016/S1369-7021\(11\)70058-X](https://doi.org/10.1016/S1369-7021(11)70058-X)
- Oh H, Lu AX, Javvaji V, DeVoe DL, Raghavan SR (2016) Light-directed self-assembly of robust alginate gels at precise locations in microfluidic channels. *ACS Appl Mater Interfaces* 8:17529–17538. <https://doi.org/10.1021/acsami.6b03826>

- Onuigbo E, Iseghohimhen J, Chah K, Gyang M, Attama A (2018) Chitosan/alginate microparticles for the oral delivery of fowl typhoid vaccine: Innate and acquired immunity. *Vaccine* 36:4973–4978. <https://doi.org/10.1016/j.vaccine.2018.05.087>
- Otto DP, de Villiers MM (2020) Layer-by-layer nanocoating of antiviral polysaccharides on surfaces to prevent coronavirus infections. *Molecules* 25:3415. <https://doi.org/10.3390/molecules25153415>
- Paques JP, van der Linden E, van Rijn CJM, Sagis LMC (2014) Preparation methods of alginate nanoparticles. *Adv Colloid Interf Sci* 209:163–171. <https://doi.org/10.1016/j.cis.2014.03.009>
- Pardee KI, Ellis P, Bouthillier M, Towers GH, French CJ (2004) Plant virus inhibitors from marine algae. *Can J Bot* 82:304–309. <https://doi.org/10.1139/b04-002>
- Pawar SN, Edgar KJ (2011) Chemical modification of alginates in organic solvent systems. *Biomacromolecules* 12:4095–4103. <https://doi.org/10.1021/bm201152a>
- Pawar SN, Edgar KJ (2012) Alginate derivatization: a review of chemistry, properties and applications. *Biomaterials* 33:3279–3305. <https://doi.org/10.1016/j.biomaterials.2012.01.007>
- Pietropaolo V, Seganti L, Marchetti M, Sinibaldi L, Orsi N, Nicoletti R (1993) Effect of natural and semisynthetic polymers on rabies virus infection in CER cells. *Res Virol* 144:151–158. [https://doi.org/10.1016/S0923-2516\(06\)80023-3](https://doi.org/10.1016/S0923-2516(06)80023-3)
- Plotkin SA (2005) Vaccines: past, present and future. *Nat Med* 11:S5–S11. <https://doi.org/10.1038/nm1209>
- Prang P, Muller R, Eljaouhari A, Heckmann K, Kunz W, Weber T et al (2006) The promotion of oriented axonal regrowth in the injured spinal cord by alginate-based anisotropic capillary hydrogels. *Biomaterials* 27(19):3560–3569. <https://doi.org/10.1016/j.biomaterials.2006.01.053>
- Raguvaran R, Mondal DB, Sharma DK, Jithin MV, Yadav N (2022) Biomedical applications of alginate nanoparticles. In: Venkatesan J, Kim S-K, Anil S, Rekha PD (eds) *Polysaccharide nanoparticles*. Elsevier, Amsterdam, pp 311–328. <https://doi.org/10.1016/B978-0-12-822351-2.00002-4>
- Reakasame S, Boccaccini AR (2018) Oxidized alginate-based hydrogels for tissue engineering applications: a review. *Biomacromolecules* 19:3–21. <https://doi.org/10.1021/acs.biomac.7b01331>
- Reig-Vano B, Tylkowski B, Montané X, Giamberini M (2021) Alginate-based hydrogels for cancer therapy and research. *Int J Biol Macromol* 170:424–436. <https://doi.org/10.1016/j.ijbiomac.2020.12.161>
- Remminghorst U, Rehm BHA (2006) Bacterial alginates: from biosynthesis to applications. *Biotechnol Lett* 28:1701–1712. <https://doi.org/10.1007/s10529-006-9156-x>
- Rocha CEV, Silva MF, Guedes ACB, Carvalho TP, Eckstein C, Ribeiro NQ et al (2021) Alginate-chitosan microcapsules improve vaccine potential of gamma-irradiated listeria monocytogenes against listeriosis in murine model. *Int J Biol Macromol* 176:567–577. <https://doi.org/10.1016/j.ijbiomac.2021.02.056>
- Romalde JL, Luzardo-Alvárez A, Ravelo C, Toranzo AE, Blanco-Méndez J (2004) Oral immunization using alginate microparticles as a useful strategy for booster vaccination against fish lactococcosis. *Aquaculture* 236:119–129. <https://doi.org/10.1016/j.aquaculture.2004.02.028>
- Ronghua H, Yumin D, Jianhong Y (2003) Preparation and in vitro anticoagulant activities of alginate sulfate and its quaterized derivatives. *Carbohydr Polym* 52:19–24. [https://doi.org/10.1016/S0144-8617\(02\)00258-8](https://doi.org/10.1016/S0144-8617(02)00258-8)
- Sahoo DR, Biswal T (2021) Alginate and its application to tissue engineering. *SN Appl Sci* 3:30. <https://doi.org/10.1007/s42452-020-04096-w>
- Serrano-Aroca A, Ferrandis-Montesinos M, Wang R (2021) Antiviral properties of alginate-based biomaterials: promising antiviral agents against SARS-CoV-2. *ACS Appl Bio Mater* 4:5897–5907. <https://doi.org/10.1021/acsabm.1c00523>
- Shao F, Yu L, Zhang Y, An C, Zhang H, Zhang Y et al (2020) Microfluidic encapsulation of single cells by alginate microgels using a trigger-gellified strategy. *Front Bioeng Biotechnol* 8:583065. <https://doi.org/10.3389/fbioe.2020.583065>

- Siew CK, Williams PA, Young NWG (2005) New insights into the mechanism of gelation of alginate and pectin: charge annihilation and reversal mechanism. *Biomacromolecules* 6:963–969. <https://doi.org/10.1021/bm0493411>
- Skjåk-Bræk G, Zanetti F, Paoletti S (1989) Effect of acetylation on some solution and gelling properties of alginates. *Carbohydr Res* 185:131–138. [https://doi.org/10.1016/0008-6215\(89\)84028-5](https://doi.org/10.1016/0008-6215(89)84028-5)
- Smetanajr K (1993) Cell biology of hydrogels. *Biomaterials* 14:1046–1050. [https://doi.org/10.1016/0142-9612\(93\)90203-E](https://doi.org/10.1016/0142-9612(93)90203-E)
- Smidsrød O, Haug A, Larsen B, von Hofsten B, Williams DH, Bunnenberg E et al (1966) The influence of pH on the rate of hydrolysis of acidic polysaccharides. *Acta Chem Scand* 20:1026–1034. <https://doi.org/10.3891/acta.chem.scand.20-1026>
- Smith KA (2011) Edward Jenner and the small pox vaccine. *Front Immunol* 2:21. <https://doi.org/10.3389/fimmu.2011.00021>
- Sone T, Nagamori E, Ikeuchi T, Mizukami A, Takakura Y, Kajiyama S et al (2002) A novel gene delivery system in plants with calcium alginate micro-beads. *J Biosci Bioeng* 94:87–91. [https://doi.org/10.1016/S1389-1723\(02\)80123-2](https://doi.org/10.1016/S1389-1723(02)80123-2)
- Stanford ECC (1883) On algin, a new substance obtained from some of the commoner species of marine algae. *Chem News* 47:254
- Steinle H, Ionescu T-M, Schenk S, Golombek S, Kunnakattu S-J, Özbek MT et al (2018) Incorporation of synthetic mRNA in injectable chitosan-alginate hybrid hydrogels for local and sustained expression of exogenous proteins in cells. *Int J Mol Sci* 19:1313. <https://doi.org/10.3390/ijms19051313>
- Sultan S, Abdelhamid HN, Zou X, Mathew AP (2018) Cello MOF: nanocellulose enabled 3D printing of metal-organic frameworks. *Adv Funct Mater* 29:1805372. <https://doi.org/10.1002/adfm.201805372>
- Sutirman ZA, Sanagi MM, Wan Aini WI (2021) Alginate-based adsorbents for removal of metal ions and radionuclides from aqueous solutions: a review. *Int J Biol Macromol* 174:216–228. <https://doi.org/10.1016/j.ijbiomac.2021.01.150>
- Tahamtan A, Charostad J, Hoseini Shokouh SJ, Barati M (2017) An overview of history, evolution, and manufacturing of various generations of vaccines. *J Arch Mil Med* 5(3):e12315. <https://doi.org/10.5812/jamm.12315>
- Tamay DG, Dursun Usal T, Alagoz AS, Yucel D, Hasirci N, Hasirci V (2019) 3D and 4D printing of polymers for tissue engineering applications. *Front Bioeng Biotechnol* 7:164. <https://doi.org/10.3389/fbioe.2019.00164>
- Tan W-H, Takeuchi S (2007) Monodisperse alginate hydrogel microbeads for cell encapsulation. *Adv Mater* 19:2696–2701. <https://doi.org/10.1002/adma.200700433>
- Teng K, An Q, Chen Y, Zhang Y, Zhao Y (2021) Recent development of alginate-based materials and their versatile functions in biomedicine, flexible electronics, and environmental uses. *ACS Biomater Sci Eng* 7:1302–1337. <https://doi.org/10.1021/acsbiomaterials.1c00116>
- Venkatesan J, Bhatnagar I, Kim S-K (2014) Chitosan-alginate biocomposite containing fucoidan for bone tissue engineering. *Mar Drugs* 12:300–316. <https://doi.org/10.3390/md12010300>
- Wang K, Kievit FM, Florczyk SJ, Stephen ZR, Zhang M (2015) 3D porous chitosan–alginate scaffolds as an in vitro model for evaluating nanoparticle-mediated tumor targeting and gene delivery to prostate cancer. *Biomacromolecules* 16:3362–3372. <https://doi.org/10.1021/acs.biomac.5b01032>
- Wegman F, Bijenhof A, Schuijff L, Öner F, Dhert W, Alblas J (2011) Osteogenic differentiation as a result of BMP-2 plasmid DNA based gene therapy in vitro and in vivo. *Eur Cells Mater* 21:230–242. <https://doi.org/10.22203/eCM.v021a18>
- Wegman F, Geuze RE, van der Helm YJ, Cumhur Öner F, Dhert WJA, Alblas J (2014) Gene delivery of bone morphogenetic protein-2 plasmid DNA promotes bone formation in a large animal model. *J Tissue Eng Regen Med* 8:763–770. <https://doi.org/10.1002/term.1571>

- Xu M, Qin M, Cheng Y, Niu X, Kong J, Zhang X et al (2021) Alginate microgels as delivery vehicles for cell-based therapies in tissue engineering and regenerative medicine. *Carbohydr Polym* 266:118128. <https://doi.org/10.1016/j.carbpol.2021.118128>
- Xu C, Qiao M, Huo X, Liao Z, Su J (2022) An oral microencapsulated vaccine loaded by sodium alginate effectively enhances protection against GCRV infection in grass carp (*Ctenopharyngodon idella*). *Front Immunol* 13:848958. <https://doi.org/10.3389/fimmu.2022.848958>
- Yang J, Hu X, Xu J, Liu X, Yang L (2018) Single-step in situ acetylcholinesterase-mediated alginate hydrogelation for enzyme encapsulation in CE. *Anal Chem* 90:4071–4078. <https://doi.org/10.1021/acs.analchem.7b05353>
- Yang R, Chen F, Guo J, Zhou D, Luan S (2020) Recent advances in polymeric biomaterials-based gene delivery for cartilage repair. *Bioact Mater* 5:990–1003. <https://doi.org/10.1016/j.bioactmat.2020.06.004>
- You J-O, Liu Y-C, Peng C-A (2006) Efficient gene transfection using chitosan? alginate core-shell nanoparticles. *Int J Nanomedicine* 1:173–180. <https://doi.org/10.2147/nano.2006.1.2.173>
- Zhao D, Zhuo R-X, Cheng S-X (2012a) Alginate modified nanostructured calcium carbonate with enhanced delivery efficiency for gene and drug delivery. *Mol Bio Syst* 8:753–759. <https://doi.org/10.1039/C1MB05337J>
- Zhao D, Liu C-J, Zhuo R-X, Cheng S-X (2012b) Alginate/CaCO₃ hybrid nanoparticles for efficient codelivery of antitumor gene and drug. *Mol Pharm* 9:2887–2893. <https://doi.org/10.1021/mp3002123>
- Zohri M, Arefian E, Akbari Javar H, Gazori T, Aghaee-Bakhtiari SH, Taheri M et al (2021) Potential of chitosan/alginate nanoparticles as a non-viral vector for gene delivery: formulation and optimization using D-optimal design. *Mater Sci Eng C* 128:112262. <https://doi.org/10.1016/j.msec.2021.112262>

Alginate Based Scaffolds in Tissue Engineering and Regenerative Medicine



Debleena Ghosh, Taposi Trishna Neog, Rishik Patra, Kritideepa Nath, and Kishor Sarkar

Abstract Regenerative medicine and tissue engineering appears as a new hope to biomedical world applying the principles of both science and medicine for regrowth of the injured tissue and organs of human body. Alginate is a negatively charged naturally occurring polysaccharide which has profound biomedical utilizations including drug delivery, tissue engineering, healing of different wounded tissues etc. Alginate possesses some attractive behaviors of non-toxicity, biocompatibility and gel forming ability. But owing to few difficulties, Alginate is modified easily by blending it with different other substances (both natural and synthetic materials) via different physiochemical crosslinking techniques. Oxidation of Alginate can be carried out to change its characteristics. As a result of such modifications, the alginate-based constructs are developed with improved cellular activity and mechanical strength which reflect their applications as a good scaffolding substance to mimic the corresponding ECM of the native tissue and restore its original functions. This book chapter gives almost detailed information of the structure properties of Alginate based materials and its applications in tissue engineering and regenerative medicine.

Keywords Regenerative medicine · Tissue engineering · Alginate · Naturally occurring polysaccharide · Non-toxicity · Biocompatibility · Scaffolds

1 Introduction

Regenerative medicine is an interdisciplinary field applying the concepts of science to medicine, biology to engineering to re-establish the functions of damaged part of human body that may lose their activities due to serious injury, or diseases. This promising field of biomedical engineering gives new hope to the therapeutic world to

D. Ghosh · T. T. Neog · R. Patra · K. Nath · K. Sarkar (✉)

Gene Therapy and Tissue Engineering Lab, Department of Polymer Science and Technology, University of Calcutta, Kolkata, West Bengal, India

e-mail: kspoly@caluniv.ac.in

face the challenges of organ transplantation, most accepted way for regeneration of new tissue. Shortage of organ or tissue immune rejection are the major shortcoming of the latter therapy which makes the large percentage of deserving people waited for getting a better treatment. This regenerative medicine ensures the scientists easy development and implantation of tissue, even organ to accelerate the physiological healing process (Dzobo et al. 2018; Sun and Tan 2013). It's a process of repairing or replacing cell, tissue or organs or stimulating the repair mechanism of remedy of the wounded tissue. It consists with a bunch of biomedical approaches and clinical therapies, these are cell therapies (the incorporation of cells mainly stem cells), immunomodulation therapy (reproduction of bioactive molecules) and tissue engineering (transplantation of tissue made in laboratory). But the latter category is affiliated with the purpose of regenerative medicine involving replace, repair and regrowth of damaged tissue (i.e., bone, skin, cartilage, blood vessels etc.). Tissue engineering is relatively quiet novel area which utilizes cell supporting substances, living cell systems as well as proper physiochemical and biological factors fabricating tissue-like material. The success of TE majorly depends on source of cells, growth factors and biocompatible scaffold. This scaffold is a 3D material designed for beneficial cellular interaction which finally supports new tissue formation. A suitable scaffold must contain few important qualities to be useful in TE. Non-toxic behavior, biodegradability, cytocompatibility, porous network with mechanical support are the crucial desirable characteristics of scaffold helping to mimic the extracellular matrix (ECM) of native tissue (Berthiaume et al. 2011). Biomaterials, designing natural porous scaffolds, serve this purpose of replacing tissue traditionally. One of the popular biomaterials is Alginate which has its own huge utilization in wide range of area starting from food industry to medicinal application. Alginate, an anionic polysaccharide, is the most abundant member of biopolymer family found in worldwide and marine domain after cellulose. This naturally occurring material though is named after algae, it is extracted mainly from cell wall of brown algae, brown seaweed and bacteria including *Azotobacter* and *Pseudomonas*. This co-polymer Alginate contains thousands of α -L-guluronate and β -D-manuronate connecting via 1 $\rightarrow$ 4 linkage in alternative manner. And the strength of the gel formed with it depends on the proportion of this sugar contents within it. Some Remarkable features of Alginate such as high hygroscopicity, cytocompatibility and non-toxicity with faster gelation and stabilization helps to make it more flexible, compressible, and mechanically strong which reflects its extensive application in diverse biomedical and pharmaceutical arena. 3D scaffolding substituents are developed from Alginate based polymers, e.g., hydrogels, foams, sponges, fibers, microspheres, and microcapsules. And the constructs can be able to takes part in different field of tissue engineering including tissue repairing, treatment of bone injury and scars, new bone generation. The networks of Alginate scaffold are appropriate for better movements of moieties allowing to deliver drug molecules, protein segments in disciplined manner. And for this similar reason Alginate gels are wonderful candidate for cell delivery, releases of nutrients, thus cultivating variable cell types like stem cell, myoblast fibroblast etc. Anti- antigenic and anti-inflammatory characteristics of Alginate along with pain relieving ability make it suitable to apply in

wound healing, wound repairing and its cell culture maintaining moist microenvironment with minimal infection at the site. Sometimes oral administration of Alginate is also performed in minimal invasive manner. Another interesting property of Alginate is its capacity of sol/gel transition independently to temperature with chelation the bivalent metal ions (like Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} etc.), so ease of preparation of hydrogels are occurred. Despite such outstanding properties, Alginate has some serious drawbacks which are responsible for rejection of scaffold designing with it alone. The main limitations include low cell adhesion and poor in vivo degradability. The modifications of Alginate can be done easily by blending it with different other substances (biomaterials: Collagen, Chitosan, Gelatin, Silk fibroin etc. or synthetic materials: GO, PLGA, etc. or cellular materials: ECM, Stem cell etc.) via different physiochemical crosslinking technique. Sometimes Alginate is oxidized by chemical means to form Alginate di aldehyde (ADA). And these derivatives of Alginate are enriched with improved cellular activity and mechanical strength. These bio composites based on Alginate are easily synthesized by free-drying method which incorporates porous and spongy nature enhancing its tensile support. In this book chapter, comprehensive outline is provided about the properties of alginate and its application in different biomedical fields (Sun and Tan 2013; Sahoo and Biswal 2021; Lin et al. 2021; Lee and Mooney 2012).

2 Details of Alginate

2.1 Structure and Properties

Alginate is a naturally occurring anionic and edible hydrophilic polysaccharide which is derived primarily from brown seaweed and bacteria. Alginates are linear biopolymers consisting blocks of 1,4-linked β -D-mannuronic acid (M) and 1,4 α -L-guluronic acid (G) guluronic acid (G) monomers as shown in Fig. 1 (Sun and Tan 2013). These blocks are composed of three different forms of polymer segments like consecutive G residues, consecutive M residues and alternating MG residues.

The reason of substantially different geometries of the G-block regions, M-block regions and alternating regions is the particular geometries of the monomers and their modes of linkage in the polymer. An example of block structure of alginate:

Alginates do not have regularly repeating units, so the monomer composition is not sufficient to determine the sequential structure of alginates. To obtain the information of the structure of alginate, ^1H and ^{13}C NMR spectroscopy is used and another method circular dichroism (CD) to determine the dyadic composition.

Physical properties as well as mechanical properties of alginate depend on its composition and concentration. Because of the unique sol/gel transition property of alginates can be easily formed into semisolid or solid structure hence these are used as viscosity increasing agents, thickeners, suspension and emulsion stabilizers in food and pharmaceutical industries (Szekalska et al. 2016). The gelling property of alginate not only depends on molecular weight but also related with the structure and

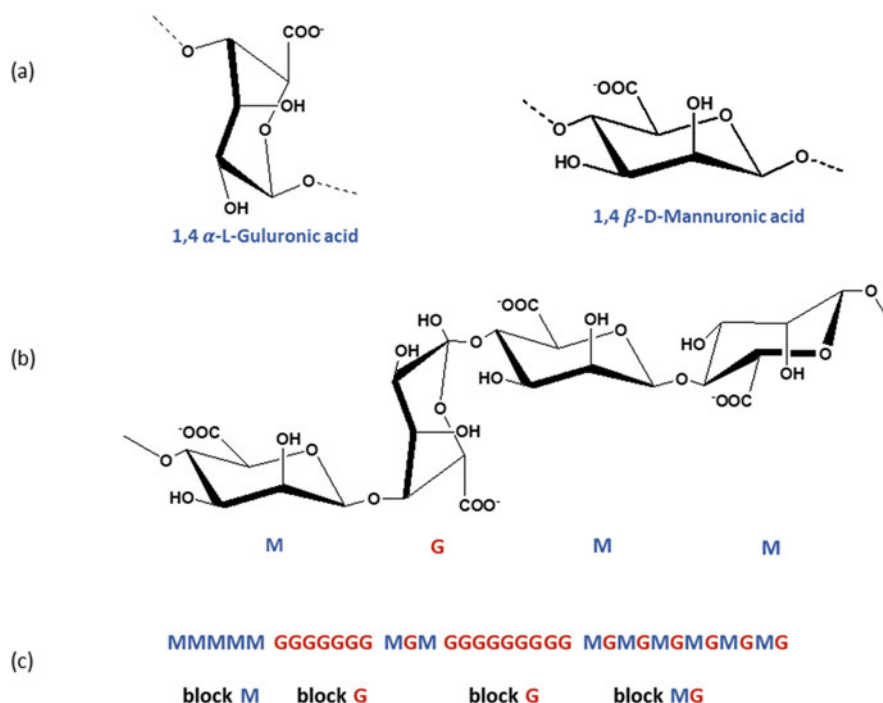


Fig. 1 Structure of alginate: (a) monomers (b) chain conformation (c) block distribution

proportion of M-, G- and MG- blocks. As the repeating units of G- block increases it results mechanically more stable as well as brittle with low shrinkage during gel formation and while high M- block contents results in more soft and elastic gels. Regularity of MG- blocks gives rise to alginate shrinkage and more flexibility. Aqueous solubility of alginate is influenced by the free divalent or trivalent metal cations, solvent pH and total ionic strength of solution. Potassium alginate, sodium alginate and ammonium alginates are soluble in both hot and cold aqueous medium but alginic acid salt with divalent and trivalent metal cations (Ca^{2+} , Zn^{2+} , Ba^{2+} , Fe^{3+} , Al^{3+}) are generally insoluble in aqueous medium. At low pH, alginate shows very less solubility and found stable in the higher pH up to 10 and beyond pH value ($10 <$) causes depolymerization. At acidic pH, alginates with alternative homogeneous structure arrangement (M-G blocks) get precipitated compared to the alginates having more homogeneous structural arrangements (G-G and/or M-M). Ion binding is another unique property compared to other polyanions which is the basis for alginates gelling properties. The affinity of metals increases in the order $\text{Mg} < < \text{Ca} < \text{Sr} < \text{Ba}$. The ion binding nature is connected to guluronic acid residues (G) or molecular weight and the length of G blocks and M/G ratio in the alginate molecular structure (Hasnain et al. 2020). When two chains of G blocks are aligned, cavity like coordination sites are generated to accommodate the divalent and trivalent metal cations. The alginates undergo conformational change after the

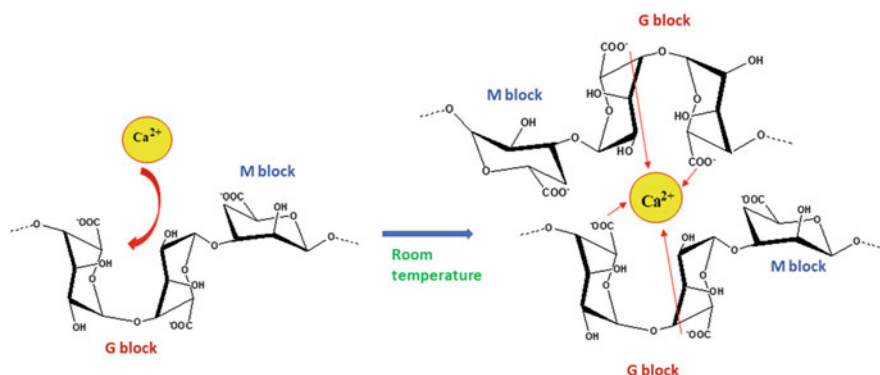


Fig. 2 “Egg-box” model of alginate gelation by ionic interaction of -COO groups and Ca^{2+}

addition of metal cations by the dimerization of the alginate chains and higher aggregation property of dimmers which can be described by so-called Egg-box model as shown in Fig. 2 (Sachan et al. 2009). The alginates possess high G blocks forms high-strength alginate gels whereas large M block form medium strength alginate gels with higher resistance to syneresis. The capability of creating viscous solution may vary not only with molecular weight but also with concentration, solvent pH (maximum pH is reached around 3.0–3.5), temperature and presence of divalent ions. Alginate solutions can lower the pH as the dissociation constant of mannuronic and guluronic acid monomers are 3.38 and 3.65 respectively. Alginates have a shelf life up to several months and if we put it in freezer than shelf life increases to several years without any drastic decrease in molecular weight. The optical rotation $[\alpha]^D$ value of sodium alginate varies in between -113° and -148° (Hasnain et al. 2020).

Due to the outstanding properties of alginate in terms of biocompatibility, biodegradability, non-antigenicity and chelating ability, it has been widely used in variety of biomedical applications. Alginate is a pH dependent anionic polysaccharide (Cattelan et al. 2020). It can interact with polyelectrolytes and proteoglycans through simple electrostatic interaction so that cationic molecules and drug delivery system can be obtained. Attractiveness of alginate towards biomedical applications enhance by some properties such as bio-adhesivity, antibacterial/viral activity. Being an anionic polymer with carboxyl end, alginate can be a good mucoadhesive agent which allows interaction with mucin by hydrogen and electrostatic bonding (Cattelan et al. 2020).

2.2 Synthesis and Characterization

The extraction process of alginates from sea weeds is a multistage process. First algae are usually washed with organic solvent and water, dried and milled. After

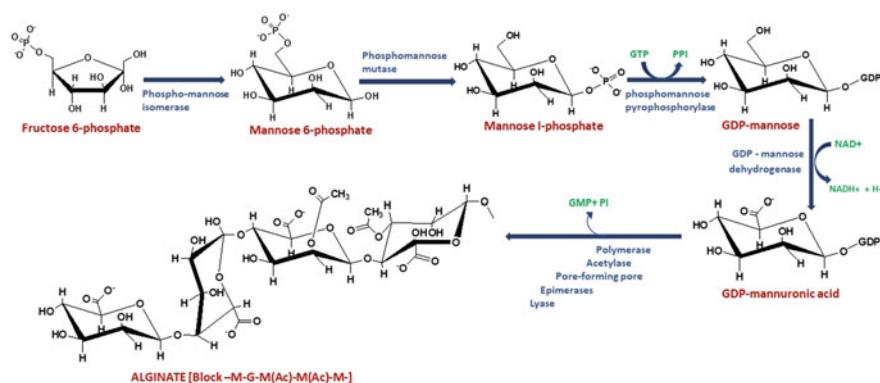


Fig. 3 Synthesis of alginate

mineral acid pretreatment, alginate is solubilized with Na₂CO₃. The crude extract is concentrated, dialyzed against water and freeze-dried or precipitated with ethanol. After the purification process, conversion into water soluble sodium salt in presence of calcium carbonate, and further transforming back into acid, or its expected salt (Łabowska et al. 2019).

The biosynthetic route of alginate was first established by Lin. and Hassid in 1966. A similar pathway in *Azotobacter vinelandii* was proposed by Pinder and Bucke (1975) in which the starting precursor is fructose 6-phosphate as shown in Fig. 3 (Núñez et al. 2022).

To analyze block copolymeric compositions and structural features, some standard chemical methodologies are followed. Partial and complete acid hydrolysis of alginates are used as chemical method for the analysis of M/G ratios in the alginate chain structure. The molecular weight of the alginate samples can be determined by size exclusion chromatography with refractive index in line viscometer. Average G/M ratio for the alginates can be determined by measuring monomer concentrations in fully hydrolyzed sample by HPLC. The spectroscopic methods like ¹H and ¹³C nuclear magnetic resonance have been used to analyze the molecular structure of alginate. Some limitations of ¹³C NMR for quantification analysis as because all the carbon atoms do not possess the similar nuclear over Hauser enhancement hence ¹H NMR is used for quantitative analysis even if the lesser amount of alginate sample is available for the analysis. Using peak ratios obtained from ¹H NMR spectrum can be determined the M/G ratio in alginate and the data obtained are the yield of G-G, M-M, G-M and M-G fractions. Alginate solution viscosity can also be determined by using viscometer (Jeoh et al. 2021). The viscoelasticity of the alginate gel can be determined by measuring the storage modulus, loss modulus and if the gel contains divalent cation young modulus of elasticity as a function of cation and alginate concentration. Swelling degree is inversely dependent on gelling time hence swelling properties can also act as modulators of mechanical strength (bt Ibrahim et al. 2019).

Negatively charged alginate interacts with the outer bacterial cellular surface of the leading to its disruption. The formation of viscous layer around the bacterial cell prevents nutrient transport, decrease membrane function, and inhibit microbial growth and other crucial factors for micro-organisms stability reporting antibacterial activity against a wide variety of species, including *Pseudomonas*, *Escherichia*, *Proteus*, and *Acinetobacter*. The strong anionic charges of sulfated alginates interact with the positively charged host cell and prevent virus contact with host cell. Anti-viral efficacy has been reported against *Flaviviridae*, *Togaviridae*, *Rhabdoviridae*, and *Herpesviridae* viruses' families. Additional advantages of alginate are that from in vitro test it is easy to switch to in vivo test as they are biocompatible and do not create problems with introduction to living organisms.

2.3 Molecular Weight and Solubility of Alginate

2.3.1 Molecular Weight

It has been revealed that low-molecular-weight sodium alginate (LMWSA) possesses distinct physicochemical characteristics and bioactivities. Investigation was done into how ozonation affected the chemical makeup, color change, M/G ratio, and molecular weight of sodium alginate. During the 80 min ozonation period at 25 °C, the molecular weight of sodium alginate fell from 972.3 to 76.7 kDa. Two different degradation-rate constants were determined. The LMWSA's molecular weight distribution significantly changed. LMWSA's color cannot change because of ozonation. Comparing LMWSA to the original alginate, the M/G ratio did not considerably change. The chemical structure of LMWSA produced by ozonation was not significantly affected, according to the FT-IR and ¹³C NMR spectra. The molecular chain of sodium alginate has a variety of glycosidic linkages. Ozonation has the ability to break each of these types of glycosidic linkages (Yue et al. 2021). Alginate hydrogels are frequently employed for cell encapsulation and transplantation, and to increase their mechanical rigidity and stability, they are frequently surface reinforced with secondary polymers. The hypothesis says that Alginate's durability would be significantly influenced by the polymer's molecular weight (MW), especially if the gel network is uniformly supplemented with the polymer. Alginate hydrogels reinforces with poly (ethyleneimine) (PEI) with various MWs are used to test this idea. The gels are crosslinked with Ca²⁺ and used to test this hypothesis. Following gelation, significant interactions between the two polymers lead to larger elastic moduli (E) than gels without PEI. An increase in the MW of the PEI helps to counteract the gradual decline in E of gels incubated in isotonic salt solutions, which is used as a sign of gel breakdown. Additionally, the usage of high MW PEI reduces the dependence of the moduli and visco-elasticity on temperature. This most likely result from the high MW PEI's limited mobility, which raises the dissociation energy. Even with constant agitation in a bioreactor, the stable associations between the alginate and PEI kept the pore structure in the gels from changing

and reduced the degradation of gel characteristics. The findings of this research show that the MW of the reinforcing PEI is crucial for preserving the physical characteristics of alginate gels in both static and dynamic situations. The energy of the polymer chains that makes up the gel is controlled by the MW of PEI, and as a result, their resistance to de-cross-linking and opening of the pore structure (Kong and Mooney 2003). New tissue creation has been linked closely to the mechanical stiffness and breakdown rate of hydrogels used as cell transplantation vehicles. However conventional methods for accelerating gel deterioration also make them less effective as mechanical supports. We postulated that modifying the molecular weight distribution of gel-forming, hydrolytically labile polymers would enable one to change the rate of gel disintegration over a wide range while reducing the range of their elastic moduli (E). This idea is tested using binary alginate hydrogels made from ionically and covalently cross-linked low [molecular weight (MW) 60,000 g/mol] and high MW alginates (MW 120,000 g/mol), partially oxidized (1% uronic acid residues), in order to assess the usefulness of this approach with various cross-linking strategies. Regardless of the cross-linking strategy, increasing the fraction of low MW alginates to 0.50 maintained a value of E like that for the high MW alginate gels but caused faster deterioration. This outcome is attributable to the low MW alginate's quicker chain scission than the high MW alginates, which is accompanied by a quicker separation of cross-linked domains upon chain breakages. Compared to non-oxidized high MW hydrogels, the more quickly disintegrating oxidized binary hydrogels promotes the development of new bone tissues from transplanted bone marrow stromal cells (Kong et al. 2004). Low molecular weight alginate is prepared by performing oxidation reactions of alginate solution with H_2O_2 under various conditions, and its potential use for tissue engineering was examined. Alginate can be depolymerized by H_2O_2 oxidation, and the rate of depolymerization depends on the reaction duration, temperature, pH of the solution, and H_2O_2 concentration. The fundamental mechanism during H_2O_2 depolymerization is the breaking of glucoside bonds in alginate chains into low molecular weight polymers, according to the structural investigations by FT-IR, elemental analysis, and ^{13}C NMR. The creation of ionic connections with calcium ions is not considerably hampered by the oxidation of alginate under weak oxidation conditions (H_2O_2 0.6% (w/v), 50 °C, 1 h). Due to the oxidized alginate's reduced molecular weight and the generated aldehyde groups' susceptibility to hydrolysis, the cross-linked alginate scaffold prepared from this alginate shows a faster deterioration rate than that from unmodified alginate. By shortening the end of C-1, H_2O_2 treatment of alginate causes the chain length to be reduced and aldehyde groups to be formed. Alginate that had been oxidized produces a calcium cross-linked scaffold that degrades more quickly than alginate that hadn't been altered (Li et al. 2010).

2.3.2 Solubility of Alginate

Alginates are C-5 epimers of β -D- mannuronic acid (M) and α -L- guluronic acid (G) which are (1–4)-linked linear polysaccharides. There are several ways to make

organically modified alginate derivatives, although practically all the chemistries are done in aqueous or aqueous-organic media. Alginates could react homogeneously in organic solvents, which would allow for a vast array of novel chemistries and derivatives. However, the lack of techniques for alginate dissolution in organic medium has limited earlier attempts. Therefore, a method for dissolving alginic acid's tetrabutylammonium (TBA) salts is described in polar aprotic liquids that also include tetrabutylammonium fluoride (TBAF). Under homogenous circumstances, TBA-alginate is acetylated until both the M and G residues reached a total degree of substitution (DS) of 1.0. The first four solvent systems (DMSO/TBAF, DMF/TBAF, DMAc/TBAF, and DMI/TBAF) that completely dissolve alginic acid as its TBA salt and that give favorable circumstances for organic reactions such as esterification are reported in the current work. The results show the importance of identifying solvents systems in which alginate is fully soluble, in order to provide equal access of the reagents to alginates independent of factors like molecular weight, M/G ratio and copolymer sequence. The fully soluble systems (TBA alginate in TBAF/DMSO) and partially soluble systems (TBA alginate in DMSO not containing any TBAF) systems were compared (Pawar and Edgar 2011). Due to sodium alginate's (Na-Alg) insolubility and tetrabutylammonium alginate's (TBA-Alg) insufficient solubility in organic solvents, alginates can only be adapted to food and pharmaceutical standards in aqueous chemistry. In the current study, these limitations are overcome by increasing the solubility of TBA-Alg, which is then neutralized with tetrabutylammonium hydroxide after being heterogeneously acidified with hydrochloric and formic acid during its production from Na-Alg. Formic acid produces the best acidification outcomes because it has a better reaction controlling the byproduct's solubility in the acidic solvent than hydrochloric acid. The degree of TBA substitution (DS_{TBA}), the molecular weight of TBA-Alg, and the relative permittivity of the solvent all worked together to increase the solubility of TBA-Alg in polar aprotic organic solvents. Chemical reactions like esterification or amidation are influenced by the solubility of TBA-Alg, including its efficacy and selectivity. It is established that the solvent's relative permittivity, the DS_{TBA} , and the molecular weight of the TBA-Alg all have an impact on the substance's solubility in polar aprotic organic solvents. To dissolve TBA-Algs with relatively high molecular weights uniformly in polar aprotic organic solvents with a relative permittivity of at least that of acetonitrile, a DS_{TBA} bigger than 0.9 is required (Schleeh et al. 2014). This study's goal is to examine the *in vitro* drug release characteristics of pH-sensitive hydrogels made of sodium alginate and hydroxyethylacryl chitosan (HC)(SA). Different ionic crosslinkers, such as Ca^{2+} , Zn^{2+} and Cu^{2+} are used to crosslink the hydrogels utilizing the dipping approach. The FT-IR confirms the crosslinking process. In simulated digestive medium, the hydrogels' behavior during swelling and stability are examined. The outcome suggests that HC and SA together could postpone the hydrogels' rate of deterioration. Compared to copper or zinc crosslinking systems, the calcium crosslinking system demonstrates greater stability. The *in vitro* drug release characteristics of paracetamol, a soluble model medication, are investigated. In simulated gastric fluid (SGF), just around 20% of the

paracetamol is released. By increasing the HC content and/or using crosslinker, the burst release of paracetamol in simulated intestinal fluid (SIF) was suppressed. The linearity of the drug release profile was established by the HC75SA25 formulation. Additionally, less than 20% of the medication is released from the 0.5 M calcium HC50SA50 hydrogel in SIF (Treenate and Monvisade 2017).

2.4 Biocompatibility and Biodegradability of Alginate

2.4.1 Biocompatibility of Alginate

The treatment of numerous diseases through the continuous supply of therapeutic agents is made possible by cell microencapsulation. The long-term effectiveness of this technology depends critically on the biocompatibility of the microcapsules and the biomaterials that make up their components. It provides comprehensive information on the key elements influencing the biocompatibility of alginates and alginate-poly-L-lysine microcapsules, the two most used encapsulation tools for cell immobilization and biomaterials, respectively. The composition and purity of the alginate, the choice of the polycation, the interactions between the alginates and the polycation, the creation of the microcapsules, the uniformity of the devices, and the implantation process are a few of these variables. The development of standardized, transplantation-grade biomaterials and biocompatible microcapsules will result from increased knowledge (Orive et al. 2006). Based on their physicochemical properties, alginate-polycation microcapsules need to be better understood in terms of their biocompatibility. Alginate with 44% (IntG) or 71% (HiG) glucuronate, calcium (Ca) or barium (Ba) gel, poly L-lysine (PLL) or poly-L-ornithine (PLO) coating and IntG alginate were used to make the microcapsules. After 2 days, PLO instead of PLL reduced immune cell adherence to microcapsules containing an IntG(Ca) gel core in C57BL/6 J mice. Additionally, the PLO microcapsules displayed increased hydrophilicity and had higher resistance to swelling and deterioration under osmotic stress. The biocompatibility and physicochemical characteristics of the microcapsules were not significantly affected by alginate coatings. Therefore, the amount of polycation at the surface or the alginate coating is less crucial for biocompatibility than the interactions between alginate and polycation in membrane formation (Tam et al. 2011). Alginate dialdehyde, gelatin and nano-scaled bioactive glass (45S5) are said to combine excellent cellular adhesion, proliferation and differentiation properties, good biocompatibility, and predictable degradation rates in addition to the good mechanical properties required for three-dimensional tissue engineering. In order to use it as a scaffold in bone tissue engineering, the aim of this work to access the in vitro and in vivo biocompatibility. Mesenchymal stem cells generated from marrow that were seeded on covalently crosslinked alginate dialdehyde-gelatin (ADA-GEL) hydrogel sheets both with and without 0.1% nano-Bioglass demonstrated good cell adhesion and proliferation in an in vitro evaluation. Comparing the ADA-GEL and ADA-GEL-nBG groups to the alginate group, lactate dehydrogenase

(LDH)- and mitochondrial activity considerably increased in both groups. In contrast to ADA-GEL, however, the addition of 0.1% nBG appeared to have a minor cytotoxic impact. In vivo implantation did not result in a substantial inflammatory response, and after 4 weeks, continuing deterioration was visible. After 4 weeks, ongoing vascularization was found. Future research using ADA-GEL and nBG for bone tissue engineering applications is encouraged by the materials' good biocompatibility (Rottensteiner et al. 2014).

2.4.2 Biodegradability of Alginate

The creation of extremely porous, biodegradable aerogel with a large surface area is made possible by the supercritical extraction of the solvent from organic gels like polysaccharides. The preparation techniques and the gel phase's composition have a significant impact on the structural characteristics of the final aerogel. In this study, a novel emulsion approach is used to create biodegradable micro spherical alginate aerogel particles. A Na-alginate solution (dispersed phase) and oil (continuous phase) were combined to create a water in oil (W/O) emulsion, which was then cross-linked to create gel particles. To regulate the shape and structural characteristics of the produced alginate aerogel, the parameters of the gelation and emulsion processes were examined (Alnaief et al. 2011). In this work, there presents a unique technique for creating calcium alginate gel microparticles with controllable shapes using a microfluidic device. The microfluidic device might be used to produce hydrogel microparticles and shape-controlled microparticles simultaneously. In order to create gel microparticles with precise control over size and form, a unique microfluidic device is made up of two separate flow-focusing channels and a synthesizing channel is successfully used as a continuous microfluidic reactor. Successfully creates monodisperse sodium alginate microparticles with a variety of shapes (such as plugs, discs, microspheres, rods and threads) in the flow-focusing channels of the microfluidic device by passive control based on the microchannel geometric confinement and liquid-phase flow rates. By changing the flow rates of the various streams, it was possible to customize the size and shape of the sodium alginate microparticles. By combining calcium chloride (CaCl_2) solution with sodium alginate microparticles in the synthesizing channel, other phases of the chemical reaction might be started. The production of calcium alginate gel microparticles allows for the long-term preservation of the sodium alginate microparticles' morphologies (Liu et al. 2006). Solution casting is used to create an alginate-based nanocomposite film reinforced with nanocrystalline cellulose (NCC). NCC concentrations in the matrix ranged from 1% to 8% (w/w%) of dry matrix 1. The best tensile strength, which rose by 37% in comparison to the control, was reported in the nanocomposite reinforced with 5 wt% NCC content. The water vapor permeability (WVP) of the nanocomposite was greatly enhanced by the incorporation of NCC and shows a 31% decrease because of the 5-weight percent NCC loading. Fourier Transform Infrared Spectroscopy validated the molecular interactions of alginate and NCC. The appearance of crystalline peaks caused by the presence of NCC inside

the films was further supported by the X-ray diffraction experiments. The addition of NCC increases the thermal stability of nanocomposite films based on alginate (Huq et al. 2012). The ability of photocross linkable biomaterials to be injected and create hydrogels in situ with little invasiveness makes them intrigue for tissue engineering applications. The creation of photocrosslinked alginate hydrogels with regulated biodegradation rates, mechanical characteristics, and cell adhesion qualities is reported. Heparin is added to photocross linkable alginate hydrogels (HP-ALG) in this study to create an affinity-based growth factor delivery system that enables the slow, steady release of therapeutic proteins. The biodegradation patterns, swelling ratios, and elastic moduli of the hydrogels in media are barely affected by heparin alteration. Without an initial burst release, the growth factor release profiles from this affinity-based platform are sustained for 3 weeks, and the released growth factors kept their biological activity. The periphery of the implant was somewhat boned when bone morphogenetic protein-2 (BMP-2) laden photocrosslinked alginate hydrogels were implanted. Importantly, after 8 weeks of implantation, BMP-2-loaded photocrosslinked HP-ALG hydrogels significantly increased osteogenesis compared to BMP-2-loaded photocrosslinked unmodified alginate hydrogels, with 1.9-fold greater peripheral bone formation and 1.3-fold greater calcium content in the BMP-2-loaded photocrosslinked HP-ALG hydrogels. With independently adjustable physical and cell adhesive qualities, this prolonged and controllable growth factor delivery method may offer a potent mechanism for a range of therapeutic applications (Jeon et al. 2011).

2.5 Derivatives and Derivatization

2.5.1 Amphiphilic Alginate

Various alginates derivatives have been reported which are used in wide range of biomedical applications. Alginate derivatives which possess both hydrophilic and lipophilic properties, i.e., amphiphilic nature have been synthesized by introducing hydrophobic moieties (alkyl chains, hydrophobic polymers) to the alginate backbone. These derivatives can form self-assembled structure and have potential in many drug delivery applications.

By conjugation of long alkyl chains to the alginate backbone via ester bond formation amphiphilic derivative of sodium alginate have been prepared with typical rheological properties of physical cross-linked, gel-like networks useful for cartilage repair and regeneration. Dodecyl amine is conjugated to the alginate backbone via amide linkage formation using coupling agent and hydrogel prepared from this derivative exhibit long-term stability in aqueous medium compared to those prepared from alginate derivative with dodecyl ester. Water soluble, amphiphilic alginate derivatives grafted with cholesteryl groups formed self-aggregates with a mean diameter of 136 nm in an aqueous sodium chloride solution. Hydrophobically

modified sodium alginate with poly (butyl methacrylate) leading to prolong release of model drugs as compared with unmodified alginate gels (Lee and Mooney 2012).

2.5.2 Cell-Interactive Alginate

Alginate derivative containing cell-adhesive properties are typically prepared by chemically introducing peptides as sidechains via carboxylic groups of the sugar residues. Peptide including the sequence arginine-glycine-aspartic acid (RGD) has been extensively used as model of adhesion ligand because these can be chemically coupled to the alginate backbone using water soluble carbodiimide chemistry. A minimum concentration of RGD peptides in alginate gel is needed for the adhesion and growth of cell and cyclic RGD peptide have been more potent at low concentrations than linear peptides. Though majority work has reported with RGD alginate, various peptides containing DGEA (Asp-Gly-Glu-Ala) and YIGSR (Tyr-Ile-Gly-Ser-Arg) sequences derived from other extracellular matrix enhance the adhesive interaction with different cell types. Cell interactive alginates have been widely used in both 2-D and 3-D cell culture as cell culture substrates and as scaffold in tissue engineering application (Lee and Mooney 2012).

The derivatization of alginates depends on three important parameters: solubility, reactivity, and characterization which are summarized in Fig. 4. Alginates may be dissolved in aqueous, organic or mixed aqueous organic media for derivatization and the degree of solubility in the solvent can impact derivative substitution pattern. Modification of alginate structure basically occurs at the two secondary -OH position (C-2 and C-3) or the one -COOH (C-6) position. The selective reactivity between two functional group types can be easily differentiate but selective modification of either the C-2 or C-3 hydroxyl group is challenging. The reactivity of alginates toward acids, bases and reducing agents cannot be ignored during derivatization reactions. To understand the alginate derivative substitution patterns, quite essential to have multiple alginate samples with a range of M/G ratios. Due to the complex nature of co-polysaccharide backbone, advanced analytical techniques are often necessary for characterization.

The carboxylic groups in the alginate backbone dictate the solubility so these groups are deprotonated to set the pH above a particular critical value in an aqueous environment. Carbodiimide method is utilized to convert carboxylates into esters and amide derivatives. Ugi multicomponent reaction is used to explore the possibility of creating alginate hydrogel with varied cross-linkers which provides variety of controlled mechanical features. The method is used to synthesize amphiphilic alginates which displays tunable organic solubility and thermal properties (Rosiak et al. 2021).

Some different modification could perform alginate derivatization through the hydroxyl groups providing distinct character of alginate derivatives and open up the possibilities for a wide range of applications. Synthesis of acetylated alginate derivatives by a series of acetylation reactions increases the swelling ability of alginate calcium gels and decreases their viscosity with the increasing acetylation

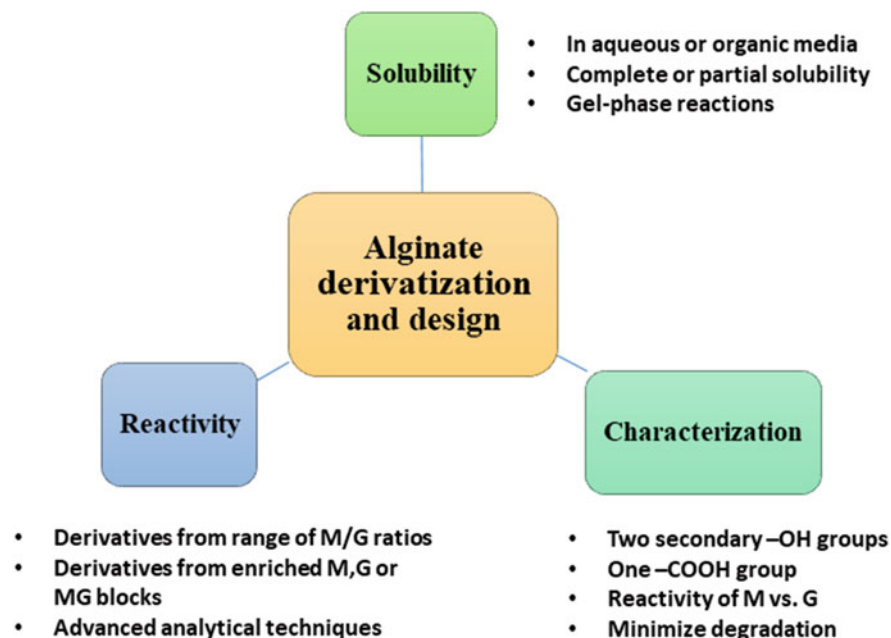


Fig. 4 Important parameters for alginate derivatization

degree. 1,3 axial interactions between C₂-OH groups with C₄ protons or C₃-OH groups with C₅ protons within the alginate chain structure are responsible for the acetylation (Pawar and Edgar 2012). Though both the carboxylic acid and hydroxyl group can be modified by esterification, but ester alginates can be formed through hydroxyl group modifying with dodecyl chain at 12% mol/mol saccharide units. This chemo-selective reaction is suitable to synthesize benzyl, butyl, ethyl and methyl alginates. By epoxide ring opening mechanism ester formation can be explored to prepared hydrophobically modified alginates resulting self-assembled amphiphilic alginate derivative which are water-insoluble substance carrier. The phosphorylation of alginate hydroxyl groups generated alginate derivatives that possessed lower molecular weight than their origin and decreases gelation ability. To synthesize heparin like molecules sulfation reaction is used by the formation of C-O-S bond. The microspheres of the sulfated alginate derivative are used to promote a drug-controlled release behavior in human induced pluripotent stem cell (hiPSC)-derived endothelial cells. The oxidation of alginate can be performed in pure water or ethanol/water dispersion to form alginate di-aldehyde (ADA) by ring opening mechanism. Depending on the degree of oxidation a decrease of molecular weight and gelation ability is expected of the alginate di-aldehyde (ADA). ATR-FTIR spectroscopy is used to characterize the newly formed aldehyde and NMR spectroscopy for confirming the newly group formation. The resulting alginate di-aldehyde from oxidation of alginate derivative is highly reactive compared to the original sodium alginate. A single electron transfer living radical polymerization

(SET-LRP) is resulted a self-assembled amphiphilic alginate derivative by grafting method. Free radical polymerization has been performed through atom transfer radical polymerization (ATRP) to synthesized a macrophage-targeted carrier from sodium alginate to be grafted with allylamine with the presence of ammonium persulfate (APS) (Putri et al. 2021).

2.6 Alginate as Cross-Linker

Alginate hydrogels that have been ionically crosslinked are desirable scaffolds because to their biocompatibility and mild gelation response, which permits gentle cell integration. Ionically crosslinked hydrogels have a limited range of applications due to their instability in aquatic environments. The ionic concentration of the medium can be controlled in this study in order to alter the size and mechanical characteristics of ionically crosslinked hydrogels. In physiological saline baths that were set to particular calcium ion concentrations, homogeneous calcium alginate gels were incubated. Low and high ionic concentrations of the medium caused swelling and shrinkage, respectively, while an “optimal” intermediate calcium ion concentration of the medium was discovered to keep the hydrogel’s original size and shape. This ideal calcium ion concentration is discovered to depend on the crosslinking density, polymer concentration, and chemical makeup of the alginate in the hydrogel. On the swelling behavior, calcium content, dry weight, and mechanical properties of the immersed hydrogels, the impacts of optimal and high calcium ion concentrations of the medium are examined. It is discovered that the composition and mechanical characteristics of the resulting hydrogel were dependent on the gel’s crosslinking density and polymer concentration in addition to the medium’s calcium concentration (Kuo and Ma 2008). Alginate-gelatin crosslinked (ADA-GEL) hydrogel is created through the covalent crosslinking of alginate di-aldehyde (ADA) and gelatin in response to the alginate hydrogel’s relatively poor cell-material interaction. This hydrogel supports cell attachment, spreading and proliferation. This work focuses in comparing the physic-chemical characteristics of synthetic ADA-GEL hydrogels of various compositions to films made of alginate. Additionally, utilizing regular human dermal fibroblasts, the in vitro cell-material interaction on ADA-GEL hydrogels of various compositions in comparison to alginate was studied. In comparison to alginate, fibroblast viability, adhesion, spreading and proliferation were much higher on ADA-GEL hydrogels. Additionally, it was discovered that the in vitro cytocompatibility of ADA-GEL hydrogels enhanced with increasing gelatin concentration. These results suggest that the ADA-GEL hydrogel is an effective material for biomedical applications in tissue engineering and regeneration (Sarker et al. 2014). The ability to encourage cellular migration and proliferation for tissue regeneration is greatly enhanced by biopolymeric hydrogels that mirror the characteristics of extracellular matrix. Previously it’s stated that periodate-oxidized alginate and gelatin may be crosslinked by themselves in the presence of borax to create quickly gelling, biodegradable,

injectable hydrogels without the need of any harmful cross-linking agents. The goal of the current study is to determine whether this hydrogel is suitable for use as a cell-attractive, injectable, adhesive scaffold for cartilage tissue engineering in the treatment of osteoarthritis. Gel formation was confirmed by time and frequency sweep rheology measurements to occur within 20s. With a burst pressure of 70 ± 3 mmHg, hydrogel integrated nicely with cartilage tissue, demonstrating its adhesive nature. An essential component of managing and treating osteoarthritis is reducing inflammatory and oxidative stress responses, which hydrogel does not significantly increase. The adhesion of cells to the hydrogel matrix was seen in SEM pictures of primary murine chondrocytes enclosed within the matrix (Balakrishnan et al. 2014).

3 Biomedical Application

3.1 *Alginate for Regenerative Medicine*

Over the years tissue regeneration has been widely used in the field of regenerative medicine. Due to biodegradability and biocompatibility natural polysaccharide alginate-based biomaterials has been used as three-dimensional (3-D) scaffold. Alginate based biomaterials has been utilized as cell carriers and in drug delivery system (Sun and Tan 2013). Improved mechanical strength, gelation properties, cell affinity, biodegradability was achieved via chemical and physical modification for desired alginate. Globa et al. discussed about stem cells cultivation and proliferation in 3-D scaffold which is a very innovative and highly effective strategy for regenerative medicine (Katsen-Globa et al. 2014). They explored Mesenchymal stem cells with their ability to differentiate in various cell types and cryo preserved successfully adhesion based in synthesized scaffolds of alginate-gelatin based biomaterials. Their application has been utilized as transplantation unit for tissue repair as pores allow a direct contact of graft cells and recipient tissue. The success of cryopreservation relies on some factor like cell viability, cell spreading area, cell migration ability, morphology, adherent viable cells. They performed adhesion- based cryopreservation of human mesenchymal stem cells within alginate–gelatin cryogel scaffolds varying the cell cultivation times for 0.5, 2 and 24 h. Cell viability, functionality was checked from 0 to 48 h recovery times in comparison to non-frozen control. It is evident from confocal laser scanning microscopy and scanning electron microscopy lesser the cultivation times (0.5 and 2 h) greater is the adherent hMSCs cryopreservation than long (24 h) cultivation time. Due to cell's expansion and migration a short cultivation time produced better viability. hMSCs cultivated for 2 h before cryopreservation is recovered more effectively than 0.5 h cultivated cells from freezing. Therefore, this system can be highly useful in cryogenic temperatures for storage purpose in biobanks. Naturally available polymers like alginate, carrageenan has been utilized into making hydrogel beads and fibers for tissue engineering application (Popa et al. 2011). Different ratios of alginate and carrageenan are

used while maintaining some crucial parameters like cell viability, mechanical stability, functionality of the developed structure. Apart from morphology, degradation behavior, size the encapsulation efficiency of chondrocytes by the beads/fibers were estimated. They used ATDC5 cells for encapsulation in 2.5% alginate – carrageenan with various polymeric ratio and metabolic activity was checked. Cell culture is done for 1, 7, 14 and 21 days and based on that test was performed. Two types of carrageenan with different concentration of the precipitation bath were utilized and ATDC5 cell proliferation was checked in hydrogel bend after entrapment. They also did the DNA quantification study and cell encapsulation assays showed that alginate/ κ -carrageenan hydrogels can be used as a promising cell carrier system because of their positive cellular response and capability to encapsulate bioactive agents. Alginate based polymer has been encapsulated with multiple anti-cancer drug for a designing a smart drug delivery system to overcome chronic diseases like tuberculosis (Ahmad and Khuller 2008). Their release studies have been checked over control dosage time. During cation induced gelation of alginate ATDs were encapsulated to form alginate microspheres as antitubercular drugs carriers. Later as a stabilizer chitosan was added maintaining fixed polymer drug ratio and reducing gelling time to improve the encapsulation efficiency of modified microspheres. They also discussed about the alginate nanoparticles as drug carrier. Alginate can work as a drug carrier for numerous molecules like insulin, indomethacin, sodium diclofenac, nicardipine, dicoumarol, gentamicin, vitamin C, ketoconazole, amoxicillin and ATDs. Generally, those drug delivery system are mainly two types of matrix system and membrane system. Interactions between anionic alginates and multivalent inorganic cations by simple ionotropic gelation lead to the formation of hydrogel. Applications of alginate based hydrogel as cell carriers and drug delivery carriers has been extensively discussed in the review by Giri et al. (2012). Major concerns associated with hydrogel beads are low encapsulation efficiency, mainly water insoluble drug has better efficiency than its soluble counterpart. Sometime fast drug release may also cause problem because of the leakage from the beads having large porosity. To expand their application this, review focused on the modification aspects where they developed hydrolytically polymers via alginate oxidation. They synthesized a control oral drug delivery system on chitosan-grafted- poly (acrylic acid)/attapulgit/SA composite (CTS-g-PAA/APT/SA) hydrogel beads and diclofenac sodium (DS) was loaded. They performed the drug release study at different pH of 2.1, 6.8 and 7.4. It was found that at lower acidic pH the hydrogel beads shrinks and due to shrinkage loaded drug could not migrate out of the beads properly and they show only 5% cumulative drug release after 24 h while at pH 6.8 it showed complete drug release after 24 h and at pH 7.4 it showed entire drug was released after 2 h. At higher pH due to swelling of the beads the space for migration within the polymer increases that favors the release. Colon specific drug delivery that involves systemic peptide drug and protein delivery demands sustained release of drug for longer time after oral administration and their application is gaining massive importance day by day. Here they mentioned about a hydrogel system made of AL and chitosan and cross linked by calcium chloride and/or sodium sulphate and checked the release of bovine serum albumin

(BSA) for colon specific drug delivery. It was found that In vitro release of BSA from the Ca^{++} single cross linked hydrogel beads showed 80% release after 5 h at SGF (pH = 1.0) than simulated colonic fluid (SCF) (pH = 7.4) and release data changes proportionally with the mass ratio of chitosan. But different result was obtained for dual cross linked gel blend in gastrointestinal tract conditions where BSA release remains unaltered even after increasing the equivalent ratio of chitosan. Cumulative release pattern was 2.35% (in SGF-4 h), 82.86% (in SIF-3 h) and 97.84% (in SCF-3 h) respectively. The reason that was estimated that in small intestinal and colonic conditions swelling degree decreased and therefore release of BSA was sustained in SIF and SCF. Interpenetrating network hydrogel membranes has been utilized for transdermal drug delivery system where anti-hypertensive drug, prazosin hydrochloride was loaded (Kulkarni et al. 2010a). An IPN hydrogel membrane was synthesized using sodium alginate (SA) and polyvinyl alcohol (PVA) via solvent casting method. The release of drug through skin depends on the concentrations of PVA and glutaraldehyde. Release percentage is inversely proportional to the concentration of glutaraldehyde and PVA and it was studied upto 24 h. Thus prepared IPN hydrogel membranes were safe and can be applied for transdermal drug delivery application. Giovagnoli et al. recently reported about intradermal sustained delivery system using alginate (ALG)–doxycycline (DOX) hydrogel microparticles (MPs) (Giovagnoli et al. 2010). Then it was loaded into PF127, PF127-HPMC, and PF127-Methocel thermogels. Among this sustained release of 7 days were achieved by PF127-methocel thermogel and PF127-HPMC thermogel showed an almost linear release, reaching 80% after 3 days and 90% up to 6 days. In other study an electrically responsive hydrolyzed polyacrylamide-grafted-sodium alginate (H-PAAm-g-SA)-based membrane were considered to be a promising platform for controlled transdermal drug delivery (Kulkarni et al. 2010b). In presence of electric stimulus, the structure of the synthesized membrane changes that causes faster release of drug as per skin histopathology. The administration of ophthalmic drugs in hydrogels has shown larger contact time of the drugs and their ocular bioavailability. Alginate microsphere-collagen composite hydrogel was synthesized for ocular drug delivery (Liu et al. 2008). Bovine serum albumin (BSA) was loaded within hydrogel and release study was performed in phosphate buffer. It showed controlled release of BSA for 11-days. The composite hydrogel helps in human corneal epithelial cell growth and has potential mechanical strength and excellent optical clarity for possible use as therapeutic lens for drug delivery. A novel in situ-forming ophthalmic drug delivery system was reported by Cohen et al. (Cohen et al. 1997). This ophthalmic drug delivery system contains aqueous solution of SA. In vitro release study showed that such drug delivery system could provide sustained release of pilocarpine over a period of 24 h where rabbit eyes were treated with 2% (w/v) pilocarpine nitrate in solution and the drug release could be monitored via diffusion via gels. A novel pH-sensitive hydrogel based on dual crosslinked alginate/N- α -glutaric acid chitosan (GAC) was developed for protein drug delivery (Gong et al. 2011). Calcium chloride solution was cross linked with homogeneous alginate/GAC solution that was suspended in sodium sulfate solution. They reported encapsulation efficiency of BSA increased with increasing the amount of GAC. High

loading efficiency of BSA due to an electrostatic attraction between the negatively charged BSA and the positively charged amino groups of GAC chains. Sustained release data of BSA was checked in different physiological pH and swelling study of dual crosslinked beads were performed in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and simulated colonic fluid (SCF). The amount of BSA released from the beads at pH 1.2 was relatively low (below 18%), while almost 100% of BSA could be released at pH 7.4 and this innovative result showed this dual crosslinked hydrogel can be a potential polymeric carrier of protein delivery.

3.2 Alginate in Tissue Engineering (TE)

Alginate possesses some interesting features like gelation, film formation and thickening ability of solutions. Because of these properties mainly gentle gel formation, it can be used in new tissue growth which helps to take part in TE (including different kind of scaffold formation, 3D bioprinting or plotting). Despite such unique characteristics, alginate has some limitations to develop the scaffold system in TE. Such major drawbacks include (1) undetermined and uncontrolled degradability for its high molecular weight. Practically alginate remains non-degraded in mammals (2) its low cell material interaction which further results (3) lower cell attachment, differentiation, and proliferation. Hence scaffolding only with alginate can't be applied in tissue regeneration and repairing. Alginate blending with other natural polymers (collagen, chitosan, gelatin etc.) or synthetic substances or any cellular materials overcomes its difficulties. However, oxidized alginate is also able to reduce the problem regarding alginate forming hydrogel cross-linking with Ca^{2+} ion and becomes suitable for TE purposes. Mainly the oxidation is done by H_2O_2 or sodium periodate to yield Alginate di aldehyde ADA. Because of oxidation, low molecular weights results facilitate easy degradation of those. The partially Oxidized alginate can develop hydrogel through ionic crosslinking with calcium ion as like unmodified alginate. Polyamino acids, different class of polymer has its biomedical application for providing cytocompatibility and mimicking ECM protein. It may serve in TE while mixing with alginate having better mechanical, hydration properties and cellular behavior. Thus, these hybrid scaffold materials formed via combination with other natural and chemical substituents are enriched with several beneficial properties and participate in different fields of TE (Sahoo and Biswal 2021; Reakasame and Boccaccini 2018; Wang et al. 2016).

3.2.1 Alginate in Bone Tissue Engineering

Bone tissue is considered as vital connective tissue with complex composition of both organic and inorganic substances in macro scale, microscale and even nanoscale. This multifaceted biocomposite mainly contains hydroxyapatite (HAp, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) (70%) and collagen I (30%). This perfectly harmonized major

tissue of human tissue system not only helps in the storage of essential components like minerals etc. but also takes part in blood pH regulation and homeostasis. Bone damage because of some injury, trauma and any congenital purpose becomes a serious health issue nowadays all over the world. Often the treatment of bone defect involves grafting processes including autograft, synthetic graft, allograft and xenograft. Among these, 'autologous' bone is still known as 'gold standard' in clinical terms., but these surgical procedures have a restriction due to poor number of tissues, immune rejection and some other reasons. Hence, bone tissue engineering (BTE) becomes more attentive field where the main purpose is to design a construct having similar physiochemical and biological behavior of the natural bone. Along with the common features of scaffolds, the 3D porous scaffold for BTE should possess some distinct characteristics, i.e., it should be mechanically robust as comparable to the cortical bone, should show excellent osteoinductivity and osteoconductivity, as well as should have increased surface area. Alginate can serve this area of TE. But, owing to poor mechanical strength and modulus, it's often blended with other important materials to be a good construct in BTE application. The scaffold composed of CS-alginate- fucoidan is found to be better responsive material compared to normal CS-alginate scaffold. Fucoidan, a polysaccharide containing L-fucose and sulfate linkage, helps in increment of alkaline phosphate level as well as osteocalcin and BMP-2 level with depositing mineral for bone reformation. Also increasing the collagen expression level and osteopontin in human adipose-derived stem cell, this negatively charged fucoidan can be one of the best potentially active candidates using for BTE. Strong ionic interaction among the substances makes this ternary scaffold highly stable and porous in nature. The hydrogel made up of Alginate-CS-fucoidan shows almost equivalent water uptake and retention capability rate with CS-Alginate scaffold. But, promoting higher cell viability along with higher cell proliferation towards MG-63 cell, the former could be more promising towards bone regeneration technique (Venkatesan et al. 2014). A novel scaffold of alginate coated with required amount of metal ions like (wt.%) 65 SiO₂, 15 CaO, 18.4 Na₂O, 0.1 MgO and 1.5 B₂O₃ is designed for BTE. Alginate having the ability of gel formation with metal ions is crosslinked with the above-mentioned ions to induce excellent mechanical strength and biological properties. This scaffold shows release of Cu²⁺ ion in controlled rate, which is also non crystalline, highly biodegradable, advantageous to provide the angiogenic potential for bone regeneration. This boron induced bioactive glass scaffold made up from foam replica method hence can act as a potential candidate for BTE (Erol et al. 2012). Using 3D printing method, a porous but hard scaffold of alginate can be fabricated via partially crosslinked with TEMPO-Oxidized cellulose nanofibril in order to replacing the bone damages. To promote prolong stability and fidelity this newly prepared scaffold undergoes complete crosslinking with Ca²⁺ ion. As a result, this material shows superiority not only in mechanical and structural behavior, but also can promote greater viscosity recovery rate compared to pure alginate. Moreover, mimicking the inorganic composition of blood plasma, this mineralized printed scaffold can play an important role in BTE (Abouzeid et al. 2018). A new composite bio ink made up with Alginate and bone tissue derived with methacrylated (Ma)-decellularized extracellular matrix

(dECM) is developed. Low biological activity of alginate is improved here due to combination with Ma-dECM. Here normal dECM is not used for its poor printing ability hence this ECM is modified via methacrylation. High cell viability, cell proliferation along with better osteogenic properties (ALP activity, calcium deposition, and osteogenic gene expression) is observed providing suitable microenvironment by this bio ink. This polysaccharide-based composite can be a promising candidate for BTE application (Lee et al. 2020). Another composite system of alginate modified with nano HAP is designed with 3D plotting process using in situ mineralization in mild environment. Direct mixing of nano HAP with alginate scaffold is not suitable due to inhomogeneous distribution of particles. This appropriate technique of surface mineralization where a smooth layer of nano HAP is coated fully on Alginate helps to induce improved mechanical as well as better cellular activity with controlled protein release. Being processed in mild condition, BSF loading can be easier for it during plotting with good loading efficiency. This scaffold with uniform coating of apatite both in outward and inward portion is an interesting option for BTE (Luo et al. 2015). The composite of chitosan and alginate further modified with introduction of nano-HAP at pH 10. This HAP induced scaffold shows optimized porosity and mechanical strength due to homogeneous dispersion of nano-HAP into it. Hence this material, compared to normal CS-alginate composite provides excellent cell proliferation and osteoblastic differentiation and mineralization against MC3T3-E1 cell (Kim et al. 2015). The nano scaled silica, nSiO_2 can also be incorporated in the scaffold of alginate for BTE purposes. A bio composite is designed where the composition is alginate with chitosan and nSiO_2 . The strong ion-ion interaction between the amine groups of chitosan and carboxyl groups of Alginate results the stability of this porous hydrogel. This silica particle for its high surface area takes part to make tight structural network with polymeric materials. Here nSiO_2 induction helps for better protein absorption along with good swelling property and cytocompatibility for osteolineage cells. High biodegradability and enhancing apatite decomposition rate of this CS/Alginate/ nSiO_2 scaffolds increases bone formation (Sowjanya et al. 2013). Having Arg-Gly-Asp (RGD) sequences (like ECM) in Gelatin, when incorporated into the scaffold of alginate induces better hydrophilicity, swelling property, biodegradability. Due to presence of several oxygenated groups GO (graphene oxide) has open surface for different types of interaction involving covalent, electrostatic and hydrogen bonding with various kind of biomolecules. Introduction of GO in the nanocomposite of alginate and gelatin enhances physiochemical and mechanical properties which helps to increase regeneration of bone tissue. This positive effect of containing GO in the composite also reflects the cellular behavior of the scaffold in its in vitro study (with MG-63 cells). Cell differentiation study disclosing greater osteoblast transcription expression makes this material a good osteoconductive scaffold (Purohit et al. 2019).

3.2.2 Alginate in Cartilage Tissue Engineering

Articular cartilage (AC) is a special type of tissue having viscoelastic nature appearing at the coverage of bone surface in the articulating joints. The lubricating surface materials secreted by it helps to tolerate high loading force at a long-term effect. And this further helps to absorb shock, reduces friction articulation, and transfer low pressure to the subchondral bone beneath it. Damage in this AC, mainly comes from any joint related injury and diseases and the treatment includes autologous chondrocyte implantation (ACI, matrix-induced autologous chondrocyte implantation (MACI), marrow stimulation techniques, cell-based therapies etc. But the recovery of an injured AC is not only long-term process, but also quite costly. However, it results osteoarthritis (OA) when expanding the injury in subchondral parts. In this respect cartilage tissue engineering (CTE) is a promising way to cure damaged AC and OA restoring its native functions. The choice of cell sources and scaffold formation are the two important aspects for CTE as like other filed of TE. Cell sources such as chondrocyte cell lines, adult mesenchymal stem cells, and embryonic stem cells are suitable here. And the biomaterials chosen for scaffold generation for CTE should possess perfect mechanical and physiological properties resembling the nature of native ECM of AC 3D printed bio ink, porous sponges, hydrogel etc. can be used as scaffold system here to design a construct for improved performance. Alginate though is a good candidate for this purpose, but for having few difficulties like poor cell attachment and cell proliferation and inhomogeneous neo-tissue deposition inside the construct only alginate hydrogel is not much effective for CTE purpose. So, mixing with some other natural or synthetic substances this anomalies of Alginate is overcome and also the 3D material is more enriched to serve as a better candidate in CTE (Liao et al. 2017; Farokhi et al. 2020). Alginate scaffold when mixed with collagen type II can form an anisotropic shape memory scaffold having more beneficial towards CTE. Due to blending with this ECM component the limitations of Alginate are overcome and the new system shows incremented cell migration, proliferation and chondrogenesis towards stem cell. This functionalized scaffold helps supporting hyaline-like cartilage formation over fibrocartilage development which may be resulted when mixed with Collagen type I. Moreover the shape-persistent ability of the former device makes it suitable for using in invasive surgeries to promote mechanical and physical support to the challenging area (Almeida et al. 2017). However oxidized Alginate mixed with gelatin can be used as CTE scaffold. Moreover, incorporation of ZrO₂ nanoparticle enhances the material characteristics of the hydrogel. Zr having the ability to make prosthetic groups for its good biocompatibility and mechanical strength, is used in bone grafting methods. This Alginate/gelatin/Zr-NP hydrogel shows perfect pore size and good chemical stability so, cells are well attached to the surface of this scaffold. And also for its controlled swelling and degradation rate of it, this trio-hydrogel can be a good nominee of CTE (Ghanbari et al. 2021). 3D bioprinting is an excellent method in the field of articular cartilage treatment. Here, alginate is combined with collagen I and agarose (AGR) to build up to different scaffold system

applying in CTE. These highly stiffed materials COL and AGR being attached with alginate, two bio inks show improved mechanical properties and resistant towards deformation along with lower water uptake compared to only alginate scaffold. SA/COL bio ink promotes better cell adhesion and proliferation as well as more suitable chondrocytes integration than SA/AGR and SA system. For this kind of bioactivity of the former bio ink cartilage specific genes like are excellently accelerated by it providing better application in CTE (Yang et al. 2018). Another bio ink can be developed by alginate di aldehyde (ADA) and gelatin mixture. With high printability and hierarchical property, this hydrogel can mimic the intrinsic structure of original cartilage tissue. Having more fidelity and stability compared to normal Alginate-GEL scaffold, this ADA-GEL can be a prerequisite for CTE field (Kreller et al. 2021). A novel thermosensitive hydrogel is fabricated for cartilage repair process using a copolymer Alginate-Poloxamer (POL) and silk fibroin (SF). POL when grafted into Alginate hydrogel results thermos-sensitivity into it triggering sol-gel transition. But due to brittle network this Alginate-POL gel is easily collapsed and loose its mechanical stability under physiological environment. But, being stimulated by body temperature the system should survive under this condition. Hence here the introduction of SF is performed within the gel structure to induce outstanding elasticity and strength. This crosslinked Alginate-POL/SF solution now easily forms gel under body temperature and pH. And the dual gel for its good porous nature and high swelling index is capable to support chondrocytic growth preserving their development in chondrogenic phenotype (Liu et al. 2020). Porous micro sphere can be developed from block copolymer like as poly (D,L-lactic acid) (PDLLA) and poly(D,L-lactic-co-glycolic acid) (PLGA), by solvent evaporation method. But owing to several limitations of these synthetic polymer components such as decrement of potential pH and chronic it should be crosslinked with natural polymer like alginate. Moreover, this MPs releases calcium gluconate within an Alginate solution. This injectable hydrogel of alginate/MPs has profound modified characteristics including shape memory property, high mechanical strength, good bioactivity, and stability hence can be useful scaffold in CTE. For these synergistic effects as well as enhanced pore size and biocompatibility, these crosslinked carriers can attach and proliferate cell well when encapsulated by chondrocytes and repairs cartilage defects by supplying calcium in rabbit model (Liao et al. 2017). Another injectable hydrogel is developed by self-crosslinking method when PLGA is combined with oxidized alginate the polypeptide PLGA, having L-glutamic acid, contains plenty of -COOH linkage which can be modified further. Here modification is done by H_2O_2 . PLGA with its non-immunogenic and non-toxic nature can be applied in biomedical field. But only PLGA based scaffold is non-injectable. To recover this disadvantage, PLGA derivative undergoes Schiff base reaction with oxidized alginate. These two-component polymers have similarity of ECM and the resulting highly biodegradable hydrogel is cell viable after rabbit chondrocyte induction in it. Having perfect gelation time, good swelling rate and biocompatibility, this binary hydrogel system cell growth and chondrogenesis (Yan et al. 2014).

3.2.3 Alginate in Skin Tissue Engineering

Skin is the largest principal organ of human body shielding the internal parts of our body from several external environmental insults. This protective part mainly contains two layers: (a) keratinized epidermis; (b) collagen enriched thick layer of dermal connective tissue beneath it. Skin tissue frequently faces several injuries by physical and chemical means including trauma, burns and diseases. These wounds resulted must follow many complicated steps to re-establish the functions and integrity of it. A full thickness skin damage in many acute and chronic cases needs long period of time to recover and is a critical clinical problem. Autogenous skin graft is the only standard treatment of such wounds. But due to various shortcoming (including high cost, donor site shortage, scarring at the affected site, addition pain and risk) of this grafting method shifted the attention in search of more promising technique for wound recovery. Skin tissue engineering (STE) is an interesting approach restoring defected skin tissue and their function. An ideal STE scaffold must have perfect biocompatibility, liquid absorbing capacity, antimicrobial nature and proper gas permeation to protect the soft skin tissue against inflammation, infection, dehydration and tissue damage. There are numerous benefits using alginate-based composite in wound healing. It assists the fast evaporation of exudates of wound with prevention of coming of pathogenic bacteria in the area, finally the healing process is accelerated. Epithelialization and new tissue formation consisting of macrophages are done in the time of wound propagation covering the surface with creating novel ECM. Alginate containing scaffold easily mimics the ECM and helps in wound repairing. Povidone iodine (PVPI), a complex of iodine and polyvinylpyrrolidone is a broad-spectrum antiseptic agent effective against wide area of microbes such as bacteria, fungi, viruses, yeast and Protozoa. This water-soluble material when applied in film formation with alginate shows enhanced re-epithelialization deducting the wound area. Curcumin (chemical name-diferuloylmethane) is the chief components of turmeric (*Curcuma longa*). Owing to anti-oxidant, anti-inflammatory, anti-infective and anti-carcinogenic properties it is used as natural wound healer and used in recovery of several diseases (Sahoo and Biswal 2021). This curcumin while blending with alginate and Carboxymethylcellulose (CMC) forms a special kind of nanoparticles helps in tissue formation and remodeling and wound shrinkage in better way. So may act as a healing agent for diabetic wound (Yuvarani et al. 2012). Another work includes formation of a biomaterial involving Alginate/CS and curcumin which improves the properties at the wounded area. It decreases the natural response to the affected area of wound against oxidation and inflammation due to incorporation of curcumin and acts as a rapid therapeutic agent for healing (Karri et al. 2016). Being crosslinked with Alginate/Collagen scaffold, curcumin provides a composite which can be used in STE for wound healing purpose (Naghshineh et al. 2019). Honey, a naturally occurring substance, can also use in healing process. Honey is highly enriched with various types of carbohydrates like glucose, fructose and fructo-oligosaccharides and has application in traditional medicine. Honey has been

mixed with numerous polymers (both synthetic and natural) including CS, PVC, gelatin, PET, Alginate to design scaffold in STE and tissue regeneration in wound. It can be blended with Alginate at low concentration to fabricate a bio ink using 3D bioprinting technique. Being a quite viscous solution, honey while combined with Alginate, results an improved bio ink with better cell viability and cell proliferation without any reduction in printability. As it has also an anti-bacterial, anti-microbial properties, the scaffold shows excellent cellular activity for wound healing mechanism (Datta et al. 2018). Gelatin combined with Alginate is widely used in wound dressing treatment because of their renowned properties. The former hydrogel is famous for its non-antigenicity, hemostatic effect assists the activation of macrophages to faster healing process. So, while mixing with Alginate the combined effect of both hydrogels appears into the resulting material making it more suitable for dermal substitutes. Now, crosslinking are performed of these epidermis grafts with chemical substances such as calcium chloride solution (for Alginate) and mixed solution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxy succinimide (NHS) (for gelatin). Three step cross linking are carried out with Alginate/Gelatin hydrogel (10% wt. for each) via extrusion free forming method involving one physical gelation at low temperature and two chemical gelation by CaCl_2 or EDC/NHS. And it is found that the construct showing better stability and bioactivity proliferate cell well, but, the system crosslinked with CaCl_2 -EDC being more biocompatible and mechanically (compared with the other crosslinkers, i.e., CaCl_2 only and EDC- CaCl_2) adequate shows better promising agent in STE (Shi et al. 2018). Chitosan is also an important biopolymer for STE due to its very good biocompatibility, non-toxicity, desirable would be healing and sterilizing nature. A CS/Alginate scaffold is designed followed by modification with fucoidan for wound repairing effectivity. The controlled release of bFSF along with well cell viability this scaffold provides L929 fibroblast migration. But here the modification is performed based on electrostatic interaction between -COOH group and free amine group (Zeng and Huang 2018). Another modification is done by amidation where the scaffold system is built with alginate/CS grafting with Flurbiprofen (FLU). This Flu also has its application in biomedical area for its Analgesic and anti-inflammatory behavior. The 3D bio composite is prepared by freeze drying method and shows excellent compressive strength and hydrophilicity. The synergistic effect of FLU and the natural polymer helps to promote increased L929 cell attachment and proliferation. Having sufficient effective modulus for STE, this material shows rapid cell growth and cell migration to reduce local inflammation (Zhu et al. 2019). Collagen is an essential component of mammalian tissue and has its own application in TE field. Fish collagen (FC), having almost identical properties with conventional Collagens (like bovine and porcine collagen) and no risk in transmission of diseases is used in TE. The limitations of poor tensile integrity and fast degradability are overcome if crosslinked with different biopolymers. Fabrication of FC/Alginate sponge scaffold which is further modified with Chitoooligosaccharides (COSs) using nontoxic crosslinker EDC is performed. COSs extracted from collagen is a $-\text{NH}_2$ containing compound undergoing crosslinking with Alginate and FC via EDC without any extra linking moieties. And as an effect of this mixing, the resulting material turns

out better biodegradable, mechanically strong and cytocompatibility, its non-allergenic and non-toxic nature makes it more cell viable enhancing its response towards cell proliferation of the NHDF-neo cells. Thus, mimicking the physiochemical and biological activity of skin tissue, this composite acts as a superior candidate in STE (Chandika et al. 2015). In another work, Scaffold strand made up with collagen in the shell and Alginate in the core part is developed. This Alginate/collagen scaffold shows improved mechanical strength and biological activity with perfect pore size and high porosity. And with respect to pure collagen scaffold, it is able to provide fast vascularization better tissue formation in its in vivo study. This hybrid biomaterial prepared by core-shell cryogenic method can mimic the ECM of original skin tissue by expanding with several growth factors and peptide in the core (Kim et al. 2011). Alginate may also be crosslinked with bio ceramics like Akermanite (Aker) and the corresponding composite may be used in STE. Aker is a different kind of biomolecules that accelerates the release of minerals such as Ca, Mg, Si under physiological condition. This ion donor Aker when crosslinked with Alginate by the help of acidic amino acids (glutamic acid & Aspartic acid) gives rise to a novel injective hydrogel having used in situ for STE purposes. This bioactive material stimulates cell migration and proliferation, recruits stem cell against human umbilical vein endothelial cells (HUVECs) in vitro. Moreover it also takes part in incrementation of angiogenesis, collagen deposition, and regeneration of neo epidermis & neo dermis in its in vivo study, hence behaves as a promising candidate for chronic wound healing process (Han et al. 2017).

3.2.4 Alginate in Liver Tissue Engineering

Liver is an essential metabolic organ in our body and plays vital role in metabolism of glucose, lipid and also participates in detoxification and protein synthesis. Chronic liver disease affects a large amount of people in all over the world. Additionally, liver related deaths have also increased. The only treatment of this problem is liver transplant. But, due to lower availability of the organ to fulfill the requirement of increasing number of patients the search of an alternative pathway acting as bridging method to liver regeneration or transplantation is needful. Liver tissue engineering (LTE) then has come to the picture with its own importance. An appropriate scaffold for LTE should mimic the ECM of hepatocyte culture and accelerate cell attachment and differentiation and proliferation functions. Fabrication a suitable scaffold is very important factor for LTE. Hydrogel can serve this purpose for its excellent properties. Alginate hydrogel with combination of some other natural ingredients or cell line assists in scaffold formation. Calcium Alginate sponge can be used in hepatocytes culture, but it faces some difficulties owing to have mechanical instability and lack of cell adhesive signals important for long-term hepatocyte functions. Hence novel Alginate/galactosylated chitosan (GC) sponge is synthesized via insertion of galactose residues in Alginate by coupling lactobionic acid (LA) with chitosan which increases the scaffold-hepatocyte interaction with inducing specific hepatocyte recognition signal. Moreover, GC being good water-

solubility while introduced in Alginate shows improved the cell adhesion rate and better retains the differentiated functions along with better stability of this scaffold (Yang et al. 2001). Now, an LTE scaffold had been successfully developed via Schiff base cross-linking reaction between GC and ADA, which is mainly functionalized alginate further prepared by oxidation of Alginate with periodate. The scaffold having the beneficial characteristics of both ADA and GC, is highly porous interconnected network, controlled degradation rate and better biocompatibility with approving swelling properties (Chen et al. 2012). Fibrin can be incorporated in ADA-GC hydrogel to prepare a good scaffold in LTE. Fibrin for its high porosity, good cytocompatibility and mechanical properties can promote cell proliferation and growth along with cell adhesion with its various cell attachment pockets. It is observed that, cells (HepG2 cell lines) on ADA-G-FIB hydrogels enhances hepatic cell function (like ICG uptake, albumin synthesis, CYP-P450) being metabolically active in nature (Rajalekshmi et al. 2021). Nowadays, bioartificial liver system (BALs) is becoming an interesting method which has its own success to provide fulfillment the liver functions. The major factors of BALs are the bioreactor and the scaffold system. Recently the human cell lines are found better potential for this process. The beneficial property of hepatocyte cell line is their almost unlimited proliferative ability and the relatively easy culture process. In this regard, hepatic cell microencapsulation of hepatic cell into the porous bead of Alginate is an outstanding alternative to cell immobilization in hollow fiber. Being inert, non-toxic and cytocompatibility in nature, this HepaRG based biomaterial can be a good construct to LTE. Moreover this system can encapsulate hepatocyte in enhanced order for its high gelation rate by divalent cation like calcium ion (Pasqua et al. 2020). Injectable hydrogel made up of GL and Alginate-Ca can be used as a good BAL device in LTE. GL, extracted from *Glycyrrhiza uralensis* Fisher (Fabaceae) being a member of terpene glycoside family, promotes the inhibition of liver damage due to carbon tetrachloride, liver fibrosis and many other liver diseases. In this hydrogel, the role of GL is a modifier and regulator for which it provides stability, good viscoelasticity, and better biocompatibility over HepG2 cell. Moreover, for its cell proliferation and Liver specific activities GL-Alginate-Ca hydrogel can be a promising candidate for BAL (Tong et al. 2018).

3.2.5 Alginate in Cardiac Tissue Engineering

Myocardial infarction, commonly known as heart attack is the fatal consequences of cardiovascular diseases, which is one of the greatest threats of death in worldwide. This heart attack happens due to immediate blockage of coronary arteries which leads to heart failure and finally death. The only recoveries of post MI heart failure are either heart transplantation or mechanical left ventricular assist device. But, having several challenges, like shortage of donor organs, immune rejection for cardiac transplantation along with infection, thrombosis and device breakdown for LVAD development of new technique for regenerating of the features of myocardium becomes really needful. The cell based remedial technique which involves the direct

injection of cells to the affected zone is also not effective due to the disappearance of most of the cells by extrusion and death of the rest of cells within 1 week of injection. In this circumstances, Cardiac tissue engineering is a promising approach that gets renowned attention for repairing the damaged cardiac tissue. In this particular field of TE, the main aim is to develop biomimetic scaffolds made up with biomolecules as well as the cell or signaling materials for which it can imitate the functions of the native micro environment of extracellular matrix (ECM) to recover the damaged heart (Sepantafar et al. 2016). Hence, the choice of cell source and matrix plays an important role improve the cardiac cellular function. Hydrogels can be used as the scaffold for Cardiac tissue engineering with its 3D crosslinked structure. Being biocompatible and biodegradable, hydrogels are very much advantageous as it promotes appropriate environment for the growth of cell with its high swelling rate and good water content. The nature of hydrogel can be greatly controlled by the choice of the biomaterials and the synthesis processes (Sun and Nunes 2015). Among several natural hydrogel alginate has its own advantage due to have excellent viscoelastic properties, good hydrophilicity and hydration capacity and low cytotoxicity so that it can resemble the original ECM. To enhancing its mechanical and physiochemical behavior, copolymerization of Alginate is performed with a new polyester made up of three the biomolecules fumaric acid, mannitol and sebacic acid to prepare pFMSA. Further the Alginate part and the unsaturation segment of the esters is crosslinked with calcium ions with different vinyl monomers such as acrylic acid (AA), methacrylic acid (MA) and N,N0-methylene bisacrylamide (NMBA) respectively. All these three bimodal scaffolds, i.e., pFMSA-AA, pFMSA-MA and pFMSA-NMBA having chemical identity (except the vinyl part), expresses good cell viability, biocompatibility and stability. In spite of exhibiting normal cell cycle response, pFMSA-AA shows superiority of its physiochemical performance in non-freezable water content and water absorption rate per unit mass and pore length with respect to the other two hydrogels. The biological properties of this hydrogel are highly influenced with its incremented non-freezable water content & water absorption, and it exhibits long term viability assay and cell cycle proliferation. Hence, for these favorable water status pFMSA-AA as a very good candidate for scaffold applicable can be for Cardiac tissue engineering (Thankam and Muthu 2015). A novel porous scaffold is designed using Alginate accompanied with chitosan and the decellularized ECM of bovine myocardium. Chitosan which is a is a marine –based polymer gained by the deacetylation of chitin, has similar to glycosaminoglycans (GAGs), one of components of ECM. But, being positively charged polysaccharide, cardiomyocytes is not allowed to survive on the scaffold made up of for its non-cell adhesive and proliferative nature. Combined with Alginate a negative charged polymer, CS-Alginate can be a good scaffold material overcoming the insufficiency of the former. Here with fabrication of the CS-Alginate pair with ECM, the porosity (more than 96%), swelling rate and stability (in PBS) has improved interestingly. The mechanical properties of ECM have also increased for blending with these two natural polysaccharide with increasing amount of polysaccharides in ECM (Tamimi et al. 2020). Another new double network hydrogel is designed from Oxidized alginate (OA) and decellularized myocardial ECM, incorporated with Amine-rGO

for Cardiac Tissue Engineering application. Decellularized cardiac ECM is a bioactive interconnected network consisting of various protein (like collagen, elastin), GAGs and growth factors which helps to provide an essential microenvironment for better cell adhesion & proliferation and an improved cell function. But due to its poor mechanical strength and faster degradation rate, alginate is used for supporting biopolymer. Here, partially oxidized alginate (OA) is selected for exposing aldehyde linkage in the polymer chain and having good biodegradability for in vivo systems. In this system 3-(2-aminoethyl amino) propyltrimethoxysilane (APTMS)-functionalized reduced graphene oxide (Amine-rGO) is inserted in various ratio to improve the electrochemical nature of the desired hydrogel. And it is observed that, the sample of 4% (w/w) OA incorporated with 25 ppm of Amine-rGO, owing to better mechanical and physiochemical properties, which reflects on its biological behavior, is the correct hydrogel over other concentration and promotes a new platform to be used as scaffold for cardiac tissue engineering in future (Mousavi et al. 2021).

3.2.6 Alginate in Muscle Tissue Engineering

Alginate hydrogel is also used as a good scaffold to cure muscle damages. This muscle damage which happens due to exercise (Owens et al. 2019; Mckee and Daneshvar 2015). In most of the cases, the traumatic injury with external force, resulting rupture or tear of muscle fibers is occurred on muscle and tendons. Breakage of blood vessels with bleeding brushing and tissue necrosis is potentially able to make the patients suffer from prolongs disability. Most common treatment is auto/allo-grafting that involves the transfer of self-tissue from another muscle site. But this method has many risks in short duration, donor site morbidity, viral infection, graft rejection etc. Hence focus is on the muscle tissue engineering (MTE). The aim of this field of tissue engineering is to design tissue construct that can replace the damaged muscle and can imitate the highly organized cellular arrangement of muscular system. These scaffold systems must understand the growth and mechanism of native muscle tissue. A natural porous scaffold composing alginate and starch is designed for muscle tissue engineering (MTE) purpose. Starch is most common carbohydrate in diet and easily available biomaterial in nature. For its lower price, hydrating nature, good water absorption rate and swelling property, starch can be combined with the polysaccharides to help in construction of native microenvironment promoting equivalent physiochemical behavior of protein. Here, tapioca, as a source of starch is incorporated into the network of alginate to provide a 3D material which can mimic the real ECM functions. This crosslinked tapioca-alginate (TA) scaffold shows high biocompatibility biodegradability along with providing support to cell proliferation and differentiation against C₂C₁₂ myoblast cell. Because of better interconnected porosity, water retention level and irregular pore geometry this TA scaffold gives extra influence to the cellular functions to that particular cell, hence promoting suitable environment for 3D cell culture and myogenic differentiation process (Lin et al. 2021). Self-crosslinked alginate-Gelatin

hydrogel may be used as TE scaffold in MTE field. Gelatin, for its poor mechanical characteristics, is combined with Oxidized alginate rather than original alginate due to the free aldehyde groups present in the latter which undergoes reaction with the available amine group of the former. The characteristics of this hydrogel can be tuned with changing the degree of oxidation (OD) and it is observed that the crosslinking density and tensile strength increasing with enhancing the aldehyde group in the OA. This transparent, non-toxic OA-GEL hydrogel shows high biodegradability and biocompatibility, and the biological properties are confirmed by the cell adhesion and proliferation behavior on by MTT assay. Moreover, the OA-GEL sheet with the OA/GEL weight ratio of 30/70 is better candidate to be a scaffold owing to higher cell viability and mechanical strength for MTE application (Baniasadi et al. 2016). 3D bio printed tissue scaffold is a promising tool to imitate the original tissue function. With the help of this bioprinting model gelatin methacryloyl (GelMA)-Alginate bio inks is used as suitable niche to mimic muscle tissue framework. GelMA though familiar hydrogel with its several properties in mimicking ECM of muscle tissue, but due to low viscosity is not alone appropriate in bioprinting model. So, the presence of large number of alginates within GelMA induces more strength of it as bio ink material to fabricate skeletal muscle tissue. More denser and aligned muscle fibers formed by the printed cells provides good performance skill compared to 2D cultured cell. And studying the mechanical, rheological and finally biological nature of these bio inks formed by varying the Alginate concentration in GelMA, 8% (w/v) Alginate with 10% (w/v) GelMA prepared by dual crosslinking methods with UV and CaCl_2 shows an optimized construct for muscle tissue environment (Sayedmahmoud et al. 2019).

3.2.7 Alginate in Nerve Tissue Engineering

Treatment of peripheral nerve damage remains a challenging platform for decades. Salehi et al. reported in their study used alginate/chitosan (alg/chit) hydrogel for nerve generation (Salehi et al. 2019) using olfactory ectomesenchymal stem cells (OE-MSCs). Sciatic nerve injury in a rat model was created and OE-MSCs were embedded to the injured area with alg/chit hydrogel. The developed hydrogel can be an alternative substrate for cell survival confirmed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, 4',6-diamidino-2-phenylindole, and LIVE/DEAD staining assay. A comparative study was done using alg/chit hydrogel with and without OE- MSCs was injected into a 3-mm sciatic nerve defect of Wistar rats. Nerve generation was more prominent in case of alg/chit hydrogel with OE-MSCs to the sciatic nerve defect. Peripheral nerve regeneration study was done by Bagher et al. using alginate/chitosan hydrogel (Bagher et al. 2021). In their study, different concentration (0.1%, 1%, and 10% (w/v)) of Hesperidin was loaded into cross-linked alginate/chitosan hydrogel. They revealed this system showed low toxicity and very good blood compatibility and from the MTT assay they concluded they have ability to increase the proliferation rate of Olfactory Ecto-

mesenchymal stem cells (OEMSCs). This could be a potential material for peripheral nerve generation which is promoted by hesperidin.

4 Conclusion

In this book chapter, we have discussed about the recent development of alginate-based materials, their structure and general properties, synthesis and characterization, molecular weight and solubility. To overcome the barrier associated with toxicity their structure has been modified by physical and chemical crosslinking. Advantages of alginate-based hydrogel beads/fibers are that they are biocompatible, easily biodegradable, nontoxic. Their wide range of application has been utilized in tissue engineering as bone tissue engineering, skin tissue engineering, cartilage, and nerve tissue regeneration. Also, they were extensively studied as drug delivery such as controlled oral drug delivery, colon specific drug delivery, transdermal drug delivery, ocular drug delivery and protein drug delivery. Their potential has been explored as cell carriers as alginate-based hydrogel, microspheres etc. are capable of cell encapsulation. Alginate based hydrogel could be an innovative and promising platform in tissue repairing and regenerative medicine.

Acknowledgements This work is financially supported by DST-SERB funding agency through three sanctioned projects *EEQ/2020/000496*, *EEQ/2016/000712*, and *ECR/2016/002018*. K.S. also acknowledges UGC for UGC-BSR Research Start-Up Grant (F.30-363/2017(BSR), Dt- 08/08/2017) and CU for UPE-II Nanofabrication project fund (UGC/166/UPE-II, Dt- 03/04/2017) for financial support.

Conflicts of Interest The authors declare no conflicts of interest.

References

- Abouzeid RE et al (2018) Biomimetic mineralization of three-dimensional printed alginate/TEMPO-oxidized cellulose nanofibril scaffolds for bone tissue engineering. *Biomacromolecules* 19(11):4442–4452
- Ahmad Z, Khuller G (2008) Alginate-based sustained release drug delivery systems for tuberculosis. *Expert Opin Drug Deliv* 5(12):1323–1334
- Almeida HV et al (2017) Anisotropic shape-memory alginate scaffolds functionalized with either type I or type II collagen for cartilage tissue engineering. *Tissue Eng A* 23(1–2):55–68
- Alnaief M et al (2011) Preparation of biodegradable nanoporous microspherical aerogel based on alginate. *Carbohydr Polym* 84(3):1011–1018
- Bagher Z et al (2021) Hesperidin promotes peripheral nerve regeneration based on tissue engineering strategy using alginate/chitosan hydrogel: in vitro and in vivo study. *Int J Polym Mater Polym Biomater* 70(5):299–308
- Balakrishnan B et al (2014) Self-crosslinked oxidized alginate/gelatin hydrogel as injectable, adhesive biomimetic scaffolds for cartilage regeneration. *Acta Biomater* 10(8):3650–3663
- Baniasadi H et al (2016) Design, fabrication and characterization of oxidized alginate–gelatin hydrogels for muscle tissue engineering applications. *J Biomater Appl* 31(1):152–161

- Berthiaume F, Maguire TJ, Yarmush ML (2011) Tissue engineering and regenerative medicine: history, progress, and challenges. *Annu Rev Chem Biomol Eng* 2:403–430
- bt Ibrahim SF, Azam NANM, Amin KAM (2019) Sodium alginate film: the effect of crosslinker on physical and mechanical properties. *IOP Conference Series: Materials Science and Engineering* 509(1):012063
- Cattelan G et al (2020) Alginate formulations: current developments in the race for hydrogel-based cardiac regeneration. *Front Bioeng Biotechnol* 8:414
- Chandika P et al (2015) Fish collagen/alginate/chitooligosaccharides integrated scaffold for skin tissue regeneration application. *Int J Biol Macromol* 81:504–513
- Chen F et al (2012) Preparation and characterization of oxidized alginate covalently cross-linked galactosylated chitosan scaffold for liver tissue engineering. *Mater Sci Eng C* 32(2):310–320
- Cohen S et al (1997) A novel in situ-forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. *J Control Release* 44(2–3):201–208
- Datta S et al (2018) Alginate-honey bioinks with improved cell responses for applications as bioprinted tissue engineered constructs. *J Mater Res* 33(14):2029–2039
- Dzobo K et al (2018) Advances in regenerative medicine and tissue engineering: innovation and transformation of medicine. *Stem Cells Int* 2018:2495848
- Erol M et al (2012) Copper-releasing, boron-containing bioactive glass-based scaffolds coated with alginate for bone tissue engineering. *Acta Biomater* 8(2):792–801
- Farokhi M et al (2020) Alginate based scaffolds for cartilage tissue engineering: a review. *Int J Polym Mater Polym Biomater* 69(4):230–247
- Ghanbari M et al (2021) The impact of zirconium oxide nanoparticles content on alginate dialdehyde-gelatin scaffolds in cartilage tissue engineering. *J Mol Liq* 335:116531
- Giovagnoli S, Tsai T, DeLuca PP (2010) Formulation and release behavior of doxycycline–alginate hydrogel microparticles embedded into Pluronic F127 thermogels as a potential new vehicle for doxycycline intradermal sustained delivery. *AAPS PharmSciTech* 11(1):212–220
- Giri T et al (2012) Alginate based hydrogel as a potential biopolymeric carrier for drug delivery and cell delivery systems: present status and applications. *Curr Drug Deliv* 9(6):539–555
- Gong R et al (2011) A novel pH-sensitive hydrogel based on dual crosslinked alginate/N- α -glutaric acid chitosan for oral delivery of protein. *Carbohydr Polym* 85(4):869–874
- Han Y et al (2017) Injectable bioactive akermanite/alginate composite hydrogels for in situ skin tissue engineering. *J Mater Chem B* 5(18):3315–3326
- Hasnain MS et al (2020) Alginates: sources, structure, and properties. In: Nayak AK, Hasnain MS (eds) *Alginates in drug delivery*. Elsevier, London, pp 1–17
- Huq T et al (2012) Nanocrystalline cellulose (NCC) reinforced alginate based biodegradable nanocomposite film. *Carbohydr Polym* 90(4):1757–1763
- Jeoh T et al (2021) How alginate properties influence in situ internal gelation in crosslinked alginate microcapsules (CLAMs) formed by spray drying. *PLoS One* 16(2):e0247171
- Jeon O et al (2011) Affinity-based growth factor delivery using biodegradable, photocrosslinked heparin–alginate hydrogels. *J Control Release* 154(3):258–266
- Karri VVSR et al (2016) Curcumin loaded chitosan nanoparticles impregnated into collagen–alginate scaffolds for diabetic wound healing. *Int J Biol Macromol* 93:1519–1529
- Katsen-Globa A et al (2014) Towards ready-to-use 3-D scaffolds for regenerative medicine: adhesion-based cryopreservation of human mesenchymal stem cells attached and spread within alginate–gelatin cryogel scaffolds. *J Mater Sci Mater Med* 25(3):857–871
- Kim G et al (2011) Coaxial structured collagen–alginate scaffolds: fabrication, physical properties, and biomedical application for skin tissue regeneration. *J Mater Chem* 21(17):6165–6172
- Kim H-L et al (2015) Preparation and characterization of nano-sized hydroxyapatite/alginate/chitosan composite scaffolds for bone tissue engineering. *Mater Sci Eng C* 54:20–25
- Kong HJ, Mooney DJ (2003) The effects of poly (ethyleneimine)(PEI) molecular weight on reinforcement of alginate hydrogels. *Cell Transplant* 12(7):779–785
- Kong HJ et al (2004) Controlling rigidity and degradation of alginate hydrogels via molecular weight distribution. *Biomacromolecules* 5(5):1720–1727

- Kreller T et al (2021) Physico-chemical modification of gelatine for the improvement of 3D printability of oxidized alginate-gelatine hydrogels towards cartilage tissue engineering. *Mater Des* 208:109877
- Kulkarni RV, Setty CM, Sa B (2010b) Polyacrylamide-g-alginate-based electrically responsive hydrogel for drug delivery application: synthesis, characterization, and formulation development. *J Appl Polym Sci* 115(2):1180–1188
- Kulkarni RV et al (2010a) Interpenetrating network hydrogel membranes of sodium alginate and poly (vinyl alcohol) for controlled release of prazosin hydrochloride through skin. *Int J Biol Macromol* 47(4):520–527
- Kuo CK, Ma PX (2008) Maintaining dimensions and mechanical properties of ionically crosslinked alginate hydrogel scaffolds in vitro. *J Biomed Mater Res A* 84(4):899–907
- Łabowska MB, Michalak I, Detyna J (2019) Methods of extraction, physicochemical properties of alginates and their applications in biomedical field—a review. *Open Chem* 17(1):738–762
- Lee J et al (2020) Bone-derived dECM/alginate bioink for fabricating a 3D cell-laden mesh structure for bone tissue engineering. *Carbohydr Polym* 250:116914
- Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. *Prog Polym Sci* 37: 106–126
- Li X et al (2010) Preparation of low molecular weight alginate by hydrogen peroxide depolymerization for tissue engineering. *Carbohydr Polym* 79(3):660–664
- Liao J et al (2017) Injectable alginate hydrogel cross-linked by calcium gluconate-loaded porous microspheres for cartilage tissue engineering. *ACS Omega* 2(2):443–454
- Lin C-W et al (2021) An environmental friendly tapioca starch-alginate cultured scaffold as biomimetic muscle tissue. *Polymers* 13(17):2882
- Liu J et al (2020) Alginate-poloxamer/silk fibroin hydrogels with covalently and physically cross-linked networks for cartilage tissue engineering. *Carbohydr Polym* 247:116593
- Liu K et al (2006) Shape-controlled production of biodegradable calcium alginate gel microparticles using a novel microfluidic device. *Langmuir* 22(22):9453–9457
- Liu W, Griffith M, Li F (2008) Alginate microsphere-collagen composite hydrogel for ocular drug delivery and implantation. *J Mater Sci Mater Med* 19(11):3365–3371
- Luo Y et al (2015) Alginate/nanohydroxyapatite scaffolds with designed core/shell structures fabricated by 3D plotting and in situ mineralization for bone tissue engineering. *ACS Appl Mater Interfaces* 7(12):6541–6549
- McKee AC, Daneshvar DH (2015) The neuropathology of traumatic brain injury. *Handb Clin Neurol* 127:45–66
- Mousavi A et al (2021) Biohybrid oxidized alginate/myocardial extracellular matrix injectable hydrogels with improved electromechanical properties for cardiac tissue engineering. *Int J Biol Macromol* 180:692–708
- Naghshineh N, Tahvildari K, Nozari M (2019) Preparation of chitosan, sodium alginate, gelatin and collagen biodegradable sponge composites and their application in wound healing and curcumin delivery. *J Polym Environ* 27(12):2819–2830
- Núñez C et al (2022) Genetic regulation of alginate production in *azotobacter vinelandii* a bacterium of biotechnological interest: a mini-review. *Front Microbiol* 13:845473–845473
- Orive G et al (2006) Biocompatibility of alginate–poly-L-lysine microcapsules for cell therapy. *Biomaterials* 27(20):3691–3700
- Owens DJ et al (2019) Exercise-induced muscle damage: what is it, what causes it and what are the nutritional solutions? *Eur J Sport Sci* 19(1):71–85
- Pasqua M et al (2020) HepaRG self-assembled spheroids in alginate beads meet the clinical needs for bioartificial liver. *Tissue Eng A* 26(11–12):613–622
- Pawar SN, Edgar KJ (2011) Chemical modification of alginates in organic solvent systems. *Biomacromolecules* 12(11):4095–4103
- Pawar SN, Edgar KJ (2012) Alginate derivatization: a review of chemistry, properties and applications. *Biomaterials* 33(11):3279–3305

- Popa EG, Gomes ME, Reis RL (2011) Cell delivery systems using alginate–carrageenan hydrogel beads and fibers for regenerative medicine applications. *Biomacromolecules* 12(11):3952–3961
- Purohit SD et al (2019) Development of a nanocomposite scaffold of gelatin–alginate–graphene oxide for bone tissue engineering. *Int J Biol Macromol* 133:592–602
- Putri AP et al (2021) Alginate modification and lectin-conjugation approach to synthesize the mucoadhesive matrix. *Appl Sci* 11(24):11818
- Rajalekshmi R et al (2021) Scaffold for liver tissue engineering: exploring the potential of fibrin incorporated alginate dialdehyde–gelatin hydrogel. *Int J Biol Macromol* 166:999–1008
- Reakasame S, Boccaccini AR (2018) Oxidized alginate-based hydrogels for tissue engineering applications: a review. *Biomacromolecules* 19(1):3–21
- Rosiak P et al (2021) Modification of alginates to modulate their physic-chemical properties and obtain biomaterials with different functional properties. *Molecules* 26(23):7264
- Rottensteiner U et al (2014) In vitro and in vivo biocompatibility of alginate dialdehyde/gelatin hydrogels with and without nanoscaled bioactive glass for bone tissue engineering applications. *Materials* 7(3):1957–1974
- Sachan NK et al (2009) Sodium alginate: the wonder polymer for controlled drug delivery. *J Pharm Res* 2(8):1191–1199
- Sahoo DR, Biswal T (2021) Alginate and its application to tissue engineering. *SN Appl Sci* 3(1):1–19
- Salehi M et al (2019) Alginate/chitosan hydrogel containing olfactory ectomesenchymal stem cells for sciatic nerve tissue engineering. *J Cell Physiol* 234(9):15357–15368
- Sarker B et al (2014) Evaluation of fibroblasts adhesion and proliferation on alginate-gelatin crosslinked hydrogel. *PLoS One* 9(9):e107952
- Schleeh T, Madau M, Roessner D (2014) Synthesis enhancements for generating highly soluble tetrabutylammonium alginates in organic solvents. *Carbohydr Polym* 114:493–499
- Sepantafar M et al (2016) Stem cells and injectable hydrogels: synergistic therapeutics in myocardial repair. *Biotechnol Adv* 34(4):362–379
- Seyedmahmoud R et al (2019) Three-dimensional bioprinting of functional skeletal muscle tissue using gelatin methacryloyl–alginate bioinks. *Micromachines* 10(10):679
- Shi L et al (2018) Three-dimensional printing alginate/gelatin scaffolds as dermal substitutes for skin tissue engineering. *Polym Eng Sci* 58(10):1782–1790
- Sowjanya J et al (2013) Biocomposite scaffolds containing chitosan/alginate/nano-silica for bone tissue engineering. *Colloids Surf B: Biointerfaces* 109:294–300
- Sun J, Tan H (2013) Alginate-based biomaterials for regenerative medicine applications. *Materials* 6:1285–1309
- Sun X, Nunes SS (2015) Overview of hydrogel-based strategies for application in cardiac tissue regeneration. *Biomed Mater* 10(3):034005
- Szekalska M et al (2016) Alginate: current use and future perspectives in pharmaceutical and biomedical applications. *Int J Polym Sci* 2016:7697031
- Tam S et al (2011) Biocompatibility and physicochemical characteristics of alginate–polycation microcapsules. *Acta Biomater* 7(4):1683–1692
- Tamimi M, Rajabi S, Pezeshki-Modaress M (2020) Cardiac ECM/chitosan/alginate ternary scaffolds for cardiac tissue engineering application. *Int J Biol Macromol* 164:389–402
- Thankam FG, Muthu J (2015) Alginate–polyester comonomer based hydrogels as physiochemically and biologically favorable entities for cardiac tissue engineering. *J Colloid Interface Sci* 457:52–61
- Tong XF et al (2018) Injectable hydrogels based on glycyrrhizin, alginate, and calcium for three-dimensional cell culture in liver tissue engineering. *J Biomed Mater Res A* 106(12):3292–3302
- Treenate P, Monvisade P (2017) In vitro drug release profiles of pH-sensitive hydroxyethylacryl chitosan/sodium alginate hydrogels using paracetamol as a soluble model drug. *Int J Biol Macromol* 99:71–78
- Venkatesan J, Bhatnagar I, Kim S-K (2014) Chitosan–alginate biocomposite containing fucoidan for bone tissue engineering. *Mar Drugs* 12(1):300–316

- Wang Y et al (2016) A biomimetic silk fibroin/sodium alginate composite scaffold for soft tissue engineering. *Sci Rep* 6(1):1–13
- Yan S et al (2014) Injectable in situ self-cross-linking hydrogels based on poly (L-glutamic acid) and alginate for cartilage tissue engineering. *Biomacromolecules* 15(12):4495–4508
- Yang J et al (2001) Hepatocyte-specific porous polymer-scaffolds of alginate/galactosylated chitosan sponge for liver-tissue engineering. *Biotechnol Lett* 23(17):1385–1389
- Yang X et al (2018) Collagen-alginate as bioink for three-dimensional (3D) cell printing based cartilage tissue engineering. *Mater Sci Eng C* 83:195–201
- Yue W et al (2021) Preparation of low-molecular-weight sodium alginate by ozonation. *Carbohydr Polym* 251:117104
- Yuvarani I et al (2012) Preparation and characterization of curcumin coated chitosan-alginate blend for wound dressing application. *J Biomater Tissue Eng* 2(1):54–60
- Zeng H-Y, Huang Y-C (2018) Basic fibroblast growth factor released from fucoidan-modified chitosan/alginate scaffolds for promoting fibroblasts migration. *J Polym Res* 25(3):1–9
- Zhu T et al (2019) Regulating preparation of functional alginate-chitosan three-dimensional scaffold for skin tissue engineering. *Int J Nanomedicine* 14:8891