



Alginate and Its Versatile Application in Drug Delivery

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Abstract

Alginates are established among the most versatile biopolymers used in a wide range of applications. Alginate is a biomaterial that has found ample applications in drug delivery due to its favorable properties, including thickening, gel forming, stabilizing and biocompatibility properties. The molecule can be tailor-made for a number of applications apart from the ordinary use as excipients. This review article gives a comprehensive overview on its present use in various fields of controlled drug delivery, biomedical applications and future possibilities.

Keywords: Alginate, Drug delivery, Pharmaceutical application, Biomedical application, Controlled drug delivery.

1. INTRODUCTION

Alginates are unbranched polysaccharides consisting of 1 →4 linked β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G). The natural copolymer is an important component of algae such as kelp, and is also an exopolysaccharide of bacteria including *Pseudomonas aeruginosa*. It is comprised of sequences of M (M-blocks) and G (G-blocks) residues interspersed with MG sequences (MG-blocks). While it is possible to obtain alginates from both algal and bacterial sources, commercially available alginates currently come only from algae. Industrial alginate production is approximately 38,000 metric tons annually, and is estimated to comprise less than 10% of the biosynthesized alginate material¹ and 30% of this is utilized by the food industry, the rest being used in industrial and pharmaceutical application².

The interest in formulated dosage forms, where the drug release can be controlled, has increased steadily during the last 60 years³. The amount of the drug delivered to the site of action will depend on the administration dose, the rate and extent of absorption, and the distribution throughout the body³. The clinical effect will last until the drug concentration falls below a minimum level due to excretion and metabolism. Drugs are almost never administered to a patient in an unformulated state. A dosage form generally consists of one or more active principles together with a varying number of other substances (excipients) that have been added to the formulation in order to facilitate the preparation and administration, promote the consistent release and bioavailability of the drug, and protect it from degradation. In this context it should be mentioned that the use of polymers as a formulation aid in controlled drug delivery systems has over the years become an important area of research and development. The present trend points to an increasing interest in the use of natural ingredients in food, drugs, and cosmetics. The naturally occurring alginate polymers have a wide potential in drug formulation due to their extensive application as food additives and their recognized lack of toxicity⁴. Alginates can be tailor-made to suit the demands of applicants in both the pharmaceutical and biomedical areas. This group of polymers possesses a number of characteristics that makes

it useful as a formulation aid, both as a conventional excipient and more specifically as a tool in polymeric-controlled drug delivery. Alginate is a naturally occurring anionic polymer typically obtained from brown seaweed, and has been extensively investigated and used for many biomedical applications due to its biocompatibility, low toxicity, relatively low cost, and mild gelation by addition of divalent cations such as Ca²⁺⁴. Alginate hydrogels can be prepared by various cross-linking methods, and their structural similarity to extracellular matrices of living tissues allows wide applications in wound healing, delivery of bioactive agents such as small chemical drugs and proteins, and cell transplantation. Alginate wound dressings maintain a physiologically moist microenvironment, minimize bacterial infection at the wound site, and facilitate wound healing. Drug molecules, from small chemical drugs to macromolecular proteins, can be released from alginate gels in a controlled manner, depending on the cross-linker types and cross-linking methods⁵. In addition, alginate gels can be orally administered or injected into the body in a minimally invasive manner, which allows extensive applications in the pharmaceutical arena.

This review article represents extensive focus on pharmaceutical and biomedical application of alginates.

2.0 APPLICATIONS IN DRUG DELIVERY

The conventional role of alginate in pharmaceutical delivery includes serving as thickening, gel forming, and stabilizing agents, can play a significant role in controlled release drug products. Oral dosage forms are currently the most frequent use of alginate in pharmaceutical applications, but the use of alginate hydrogels for biomedical application is growing. Tablets and capsules are by far the most frequently used oral dosage forms. The products are often designed as immediate-release type, i.e., immediate release of drug for rapid absorption. Coating of the units can lead to sustained-release products, i.e., the release of the therapeutic agent is controlled. Controlled-release drug delivery systems are designed to give a reproducible and kinetically predictable release of drug substance. Alginates may be utilized in dosage forms designed for either type of drug release. Traditionally,

sodium alginate has been used as a tablet binding agent, while alginate acid is used as a tablet disintegrant in compressed tablets designed for immediate drug release⁴. The effect of sodium alginate on tablet properties is, however, dependent on the amount incorporated in the formulation and in some cases the alginate salt can promote disintegration. Here, emphasis is given to describe the application of alginate and/or its derivatives in pharmaceutical and biomedical applications.

2.1. PHARMACEUTICAL APPLICATIONS

2.1.1 Oral Drug Delivery

Oral dosage forms are currently the most frequent use of alginate in pharmaceutical applications. The application of alginates in oral drug delivery is discussed under the following headings:

2.1.2 Controlled release delivery

The design of oral dosage forms are often made according to one of the following principles: (i) the entire drug dose is in the same physical unit or (ii) the dose is contained in an assembly of small sub-units. In the latter case the sub-units are filled into a capsule or compressed into a tablet. The formulations employ a chemical or physical "barrier" to provide a controlled release of the drug. Many formulation techniques have been used to build the barrier into the peroral dosage form, e.g., the coating of a core containing the active ingredient or the embedding of the active ingredient in a polymer matrix⁶. Hydrocolloids like alginate can play a significant role in the design of a controlled-release product. The alginate molecule will undergo an almost immediate hydration to create a hydrocolloidal layer of high viscosity. This makes up a diffusion barrier decreasing the migration of small molecules (e.g., drugs). So far, alginate has mainly been applied in systems based on diffusion³.

Diffusion systems based on alginate can be divided into two main categories. In the polymer membrane system the drug formulation is encapsulated within a drug reservoir compartment. The drug formulation may exist as a solid or suspension, or in a solution. The drug release is controlled by the polymeric encapsulating membrane having a specific permeability. The encapsulation of drug is accomplished by various techniques, e.g., spray coating or microencapsulation. Alginate has been applied in the preparation of gel capsules. In one study, the compound theophylline was encapsulated and the drug release rate was significantly reduced compared to the matrix-type alginate gel beads³. The release rate became lower as the coat thickness increased. The release followed zero-order kinetics as expected. A further decrease in release rate can be obtained by incorporating additives such as carnauba wax into the drug reservoir. This is demonstrated for indomethacin, a non-steroidal anti-inflammatory drug which is highly irritating to the mucosa in the upper gastrointestinal (GI) tract⁶.

In the polymer matrix system the drug is homogeneously dispersed in a rate-controlling polymer matrix. The final product may be in the form of swellable microspheres or conventional tablets. When such systems are exposed to the dissolution medium, drug release is modulated by diffusion

through matrix swelling and dissolution/erosion at the matrix periphery⁷. The "swelling-dissolution-erosion" process is highly complex. In systems based on sodium alginate cross-linked with calcium chloride, the osmotic pressure gradient that exists between the alginate gel and the environment comprises an important factor in the swelling process. Under acidic conditions (e.g., in the stomach) swelling of the calcium alginate beads scarcely occurs. A drug is likely to be released by diffusion through the insoluble matrix. Under neutral conditions (e.g., intestine) the beads will swell and the drug release depend on the swelling and erosion process. The swelling behavior of calcium alginate has been thoroughly exploited for the development of a multiple-unit, controlled-release drug delivery system⁸. Alginate gel beads seem to be most effective in retarding drugs at higher alginate concentrations^{7,8} and when the alginates are rich in guluronic acid⁸. The guluronic acid conformation gives a high degree of coordination of the calcium, and thereby forms more rigid gels that are less prone to swelling and erosion. By increasing the mannuronic acid content the gels become softer, more elastic, but less porous and they dissolve more easily. The situation may be different for drug molecules that strongly interact with alginate. Gentamicin sulfate was found to interact selectively on the mannuronic residues of alginate without competition with calcium ions involved in the polymer gelation. In this case a higher mannuronic acid content would lead to a higher binding capacity for drug molecules, and mannuronic rich alginates may be preferred⁸. The molecular weight and viscosity of the alginates did not affect the drug release of nicardipine HCl in neutral medium⁹. Interestingly, the release of the basic drug pindolol was, however, demonstrated to be dependent on the alginate molecular size¹⁰. The slowest in vitro release rate of pindolol (at neutral pH) was observed for the beads prepared by alginate of low molecular weight, although this showed the fastest in vivo absorption rate. The drug: alginate ratio and calcium chloride concentration affect the drug release.

The release of nicardipine from alginate particles prepared in a ratio of 1:1 was delayed more than that from 1:2 particles⁹. A high calcium content is favorable¹¹. The curing time seems to be of minor importance in some systems⁹ (Rosenberg and Lee 2004), while a long gelling time is favorable under other experimental conditions¹². The cross-linker type and concentration seem to have a pronounced influence on the drug release. Calcium alginate beads displayed prolonged release profiles when compared to alginate beads prepared from other cross-link agents like Ba2p and Sr2p¹³. It is interesting to notice that even calcium bound with the G block was displaced by monovalent cations at high salt concentration (>0.2 M), resulting in an increased swelling¹⁴. Variation in bead size may be used as a formulation principle to change the release time onset. Release of dextran in a pulsative fashion is obtained by mixing different bead sizes³. This may be of importance in the design of drug delivery systems intended to follow the circadian rhythm in the body. Drug release from polymer matrix systems can be modulated by varying the material for encapsulation. Cross-linking with

glutaraldehyde decreased the swelling of alginate microspheres while the loading of the water-soluble drug nimesulide increased.

An enteric protection of a preparation with the drug diclofenac was demonstrated by mixing the alginate with chitosan¹⁵. The resulting alginate–chitosan beads showed a release behavior dependent on pH. The chitosan polymer is poorly soluble in water. In acidic medium, protonation of the amine groups improves solubility. The interpolymeric complex between alginate and chitosan exists in a gel form at low pH. At neutral pH the viscous complex will swell and the gel formed will slowly disintegrate, releasing the drug. The release rate is a function of the degree of cross-linking between both polymers. An interaction between chitosan and the drug molecule was also observed¹⁵. Other coatings like Eudragit will also modify the release of drugs from alginate beads¹⁶.

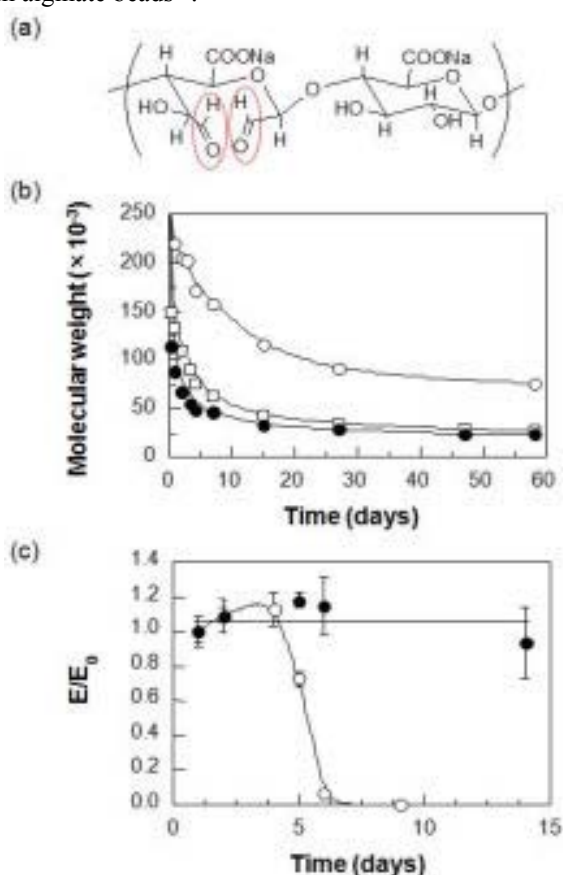


Fig. 1 (a) A microsphere/hydrogel combination system can be prepared by ionic cross-linking of a suspension of poly(d,l-lactide-co-glycolide) (PLGA) microspheres containing protein drugs in an alginate solution. (b) Scanning electron microscopic image clearly shows even dispersion of PLGA microspheres in an alginate hydrogel

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Combinations of liposomes and alginate have been investigated in order to modify the release of drugs from such phospholipid vehicles and to stabilize the products. Alginate either served as a vehicle for the liposomes or formed a gel inside the liposome¹⁷ leading to stabilization and a delayed drug release respectively. Alginates are

demonstrated to have antioxidative activity and will thereby further stabilize the liposome preparation¹⁸.

Alginate-based polymeric matrix systems can also be prepared as compressed tablets. Drug release from hydrophilic matrix tablets is controlled by the formation of a hydrated viscous layer around the tablet, which acts as a barrier to drug release by opposing penetration of water into the tablet, and also the movement of dissolved solutes out of the matrix. A compressed alginate tablet will have a very closed structure compared to a gel bead, and the degree of sustained release effect will therefore be higher in the tablet. Water-soluble drugs are released primarily by diffusion of dissolved drug molecules across the gel layer, whilst poorly water-soluble drugs are released predominantly by erosion mechanisms. In a preparation made by direct compression of drug–alginate blends it has been demonstrated that drugs of high water solubility are released significantly faster in simulated gastric fluid than in simulated intestinal fluid, whereas the opposite effect is observed for drugs of poor solubility¹⁹. This is explained in terms of the different hydration kinetics in these two media. Cationic drugs (e.g., lidocaine HCl) seem to be released more slowly than anionic drugs (e.g., sodium salicylate), possibly because of the negative charge of the matrices³. By incorporating a pH independent hydrocolloid gelling agent (e.g., cellulose polymers) in the tablet the release rate of a basic drug can be made independent of pH²⁰. Spray-dried composite particles of lactose and sodium alginate used as a filler of tablets can also modify the release properties. The release of acetaminophen from tablets containing spray-dried lactose–alginate particles was found to be slower in an acidic solution and more rapid in a neutral solution than release from a “conventional” sodium alginate matrix tablet²¹. One explanation could be that the spray-dried particles had a much smaller particle size than an average sodium alginate particle, leading to a more effective gel network and thereby a more effective barrier. As the pH increases the lactose was more rapidly dissolved than the sodium alginate and a faster erosion occurred. In order to obtain specific delivery to the colon, lactose–sodium alginate particles were applied as the coating filler of dry-coated tablets. Addition of chitosan to the mixture further prolonged the induction period in the drug release process²². Dry-coated tablets with alginate in the coating or alternatively alginate in the tablet core and a calcium-containing salt in the coating also showed a slow release rate²³. Gelatin capsules coated with alginate are demonstrated to remain intact as long as they are retained in the stomach, allowing for drug delivery selectively to the intestine. In one study acrylic polymer microspheres containing a highly water-soluble drug (acebutolol hydrochloride) were powder-coated with sodium alginate and formulated into capsules or tablets^{24,25}. This caused a prolonged release because of the gelled matrix structures formed during dissolution. The same effect has also been demonstrated when the drug substance (indomethacin) is crosslinked with sodium alginate and compressed into tablets²⁶. An oral sustained-delivery formulation based on in situ gelation of sodium alginate has been reported²⁷. The formulation depends for its action on in situ gelling induced

by the sequential administration of two solutions, the first containing sodium alginate immediately followed by administration of a solution containing calcium ions in a complexed form. The acidic environment in the stomach causes the calcium ions to be released, allowing the gellation to take place. This formulation principle leads to a significant increase in the bioavailability of theophylline compared to a proprietary oral sustained-release formulation, although no increase in the mean residence time could be observed. Drug molecules with low water solubility can show poor bioavailability when formulated into solid dosage forms. Low-molecular alginate, i.e., hydrolysis products of the polymer, may be used as a carrier to enhance the dissolution rate of acidic, basic, or neutral drugs. Kneaded mixtures of various drug substances and low-molecular alginate were investigated, and a significant increase in dissolution rate was observed^{28,29}. This may be due to an improvement of wettability and to changes of the crystallinity, microcrystal size, and shape. Alginate coating of the drug substance prior to compression is another approach to make the particles more hydrophilic³⁰. Microcrystalline cellulose co-processed and partly surface-coated with an alginate calcium/sodium salt complex (Avicel AC- 815) provides excellent suspending agents for water insoluble drugs³¹. Highly lipophilic drugs can be incorporated into alginate microspheres by use of a multiple-phase emulsion technique³². The drug is dissolved in oil (e.g., soybean oil) and the resulting alginate microspheres contain immobilized drug containing oil micro droplets. The microspheres can be coated further, allowing a pH-dependent release.

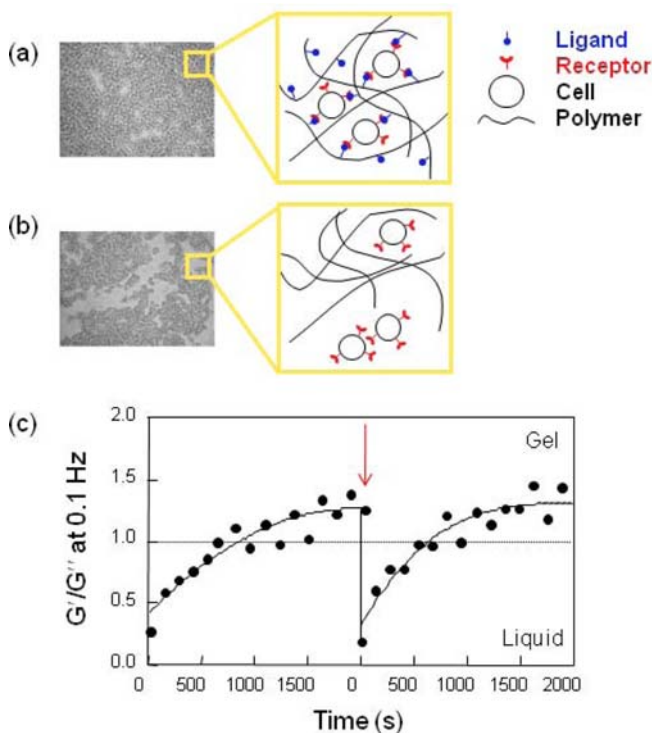


Fig. 2 *In vitro* TAT-HSP27 release from microsphere/hydrogel combination delivery systems prepared at different mixing ratios (■, 0; ●, 1.0; ▲, 1.5; PLGA/alginate = w/w)¹¹⁴.

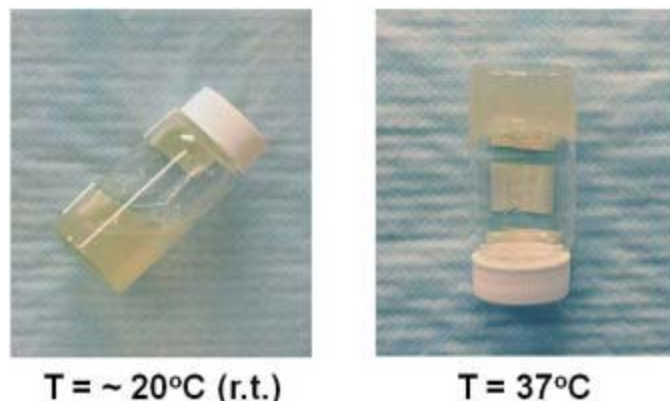


Fig. 3 Optical microscope images of C2C12 myoblasts adhered to the surface of (a) non-peptidemodified alginate gels and (b) RGD-modified alginate gels. No cells attach and spread on the unmodified gels, while large numbers of well spread cells are found on the RGDmodified alginate. Images were taken after 24 hr culture at 100 \times magnification¹¹⁵.

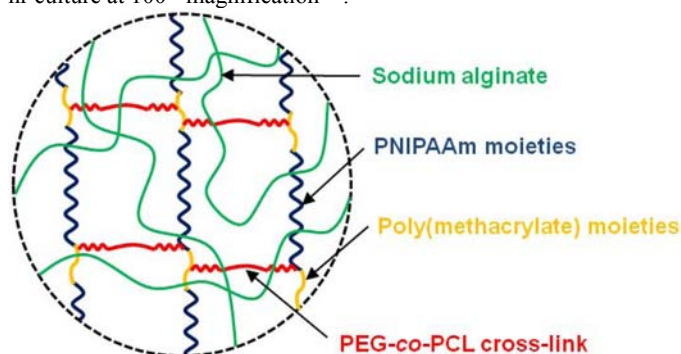


Fig. 4 Confocal microscopic images of primary human fibroblasts cultured on alginate gels (2-D) modified with either (a) RGDSP or (b) G12RGDSP, and cells encapsulated within the same two types of gels (3-D). Images were taken after cells were treated with anti-vinculin antibody, followed by visualization with rhodamine-conjugated donkey anti-mouse IgG (scale bar, 20 μm)¹¹⁶. Cells cultured with G12RGDSP-alginate gels, both in 2-D and 3-D, clearly display focal contact formation, which is a sign of strong cell adhesion and regulates cell spreading and migration, as demonstrated by positive immunostaining for vinculin.

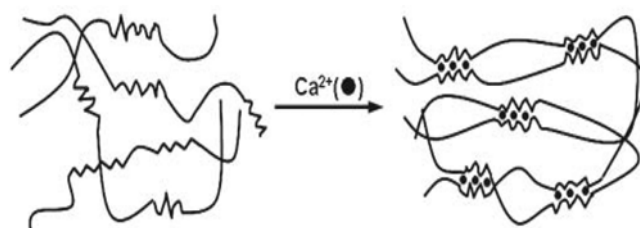


Fig. 5 Directly visualizing and quantifying cell-gel adhesions, and their re relation to cell phenotype. (a) A strong green emission of fluorescein in the cell membrane within unmodified gels was observed. (b) The color intensity of fluorescein in the cell membrane was greatly decreased and the color intensity of rhodamine at the interface between cells and gels was increased when cells were encapsulated in alginate gels presenting rhodamine-G4RGDASSKY, due to fluorescence resonance energy transfer (FRET). (c) The relationship between the amount of [3H]thymidine uptake by cells (indication of cell multiplication) and $N_{\text{bond}}/\text{cell}$ for two cell types: muscle cells (C2C12) and bone cells (MC3T3-E1). The number of cell receptor– ligand bonds (N_{bond}) was determined using the FRET measurements¹¹⁷.

2.1.3 Modulation of Gastrointestinal Transit Time

A variation in gastric emptying time may strongly influence the absorption of drugs, especially of compounds with an "absorption window" or with poor bioavailability from the lower regions of the digestive tract. A buoyant capsule formulation has been prepared for the pH-independent controlled release of a basic drug. This is obtained by the preparation of a powder consisting of the drug substance in combination with alginate and a pH-independent hydrocolloid gelling agent (e.g., hydroxypropylmethyl cellulose). The powder is filled into hard gelatin capsules³³. The preparation does not contain calcium ions. Neither does it involve gas generation. In the stomach, water penetrates the capsule shell, initiating surface hydration of the pH-independent polymer, leading to the formation of a gel layer. Air is trapped inside the less dense powder bulk to account for the buoyant behavior of the capsule. At this low pH, alginic acid is formed from alginate and this further modifies the gel layer. Erosion of the gel layer gradually exposes more dry matrix that hydrates at the same time as drug dissolves in the gel and diffuses out to the surrounding aqueous environment. After buoyancy is lost the dosage form is emptied from the stomach followed by an increase in pH. The gelled powder plug changes structure and becomes more porous as the alginic acid turns into a more soluble salt. The drug can diffuse more readily through the matrix and this compensates for the lower dissolution of basic substances at higher pH. Another potential approach to extend the gastrointestinal residence time is to prepare a bio(muco)- adhesive drug delivery system. Specialized cells located in the stomach, duodenum, and transverse colon continuously secrete a large amount of mucin-containing mucus to protect the surface epithelium against the acidic environment and protein-splitting enzymes present in the GI tracts. Mucin consists of an oligosaccharide chain with terminal sialic acid (pKa 2.6). The drug delivery systems should contain a mucoadhesive component that binds to mucin. It appears that polyanions, especially polymers containing carboxylic groups and with a high charge density, are highly active. Alginate is demonstrated to have excellent bioadhesive properties. The addition of a chitosan coating to alginate beads increased the adhesive properties significantly. Both the coated and the uncoated microcapsules showed the strongest affinity for the stomach mucosa. The adhesive properties increased with an increase in homogeneity of the beads. Alginate/chitosan tablets have been prepared for adhesion to human cheek mucosa^{34,35}.

2.1.4 Gastric Reflux Control by Raft formation

Alginate-based raft-forming formulations have a wide application in the treatment of heartburn and esophagitis³⁶. The raft consists of discontinuous alginate gel with bubbles of carbon dioxide entrapped in the gel network. The raft is created when a colloidal gel of alginic acid is formed after sodium alginate enters the stomach and reacts with the gastric acid. The resulting gel floats on the surface of the gastric contents, impeding the flux of acid into the esophagus. The raft-forming formulations can be used in

combination with antacids or cimetidin (H₂ blocker). Recently, a liquid preparation of sodium alginate in combination with a calcium-containing solution has been evaluated for eradication of *Helicobacter pylori* (HP), a bacterium closely associated with chronic gastritis and peptic ulcers. Although HP is highly sensitive to most antibiotics, it is difficult to obtain minimum inhibitory concentrations in the gastric mucus, in which HP colonizes, by systemic treatment. The alginate preparation will spread in the stomach and release the incorporated drugs to the gastric wall rather than the gastric lumen by gel formation on the surface of the preparation³⁷.

2.1.5 Film Strips

The production of edible films from natural polymers has received much attention due to the excellent biodegradability, biocompatibility and edibility of the films. In recent years, films that dissolve in the mouth have increased in popularity as a dosage form for delivery of pharmaceutical actives and dietary supplement ingredient. There are many polysaccharide sources for the production of edible films, such as root and cereal starches, plant cell pectin, alginate and carrageenan from seaweed and cellulose³⁸. Alginate has potential use in films or as a coating component because of its unique colloidal and gel-forming properties³⁹. Sodium alginate films are typically cast from aqueous solution containing 5 to 10 % sodium alginate. The selection of sodium alginate will depend on the desired solids level and viscosity of the solution. The resulting films are water soluble, clear and transparent. The addition of a plasticizer, such as glycerin, provides greater flexibility to the film strips.

2.2 IN NASAL DELIVERY

The nasal route for systemic drug delivery is of interest because it provides several advantages over other routes of drug administrations. These have been suggested as follows: rapid absorption, avoidance of the intestinal and hepatic presystemic disposition, fast onset of therapeutic action, avoidance of irritation of the gastrointestinal membrane, noninvasive administration, ease of convenience, and selfmedication, improved patient compliance^{40,41}.

Nasal mucociliary clearance is one of the most important limiting factors for nasal drug delivery. It severely limits the time allowed for drug absorption to occur and effectively prevents sustained nasal drug administration. Thus, to retain the drug in the nasal cavity, a particle formulation would be preferable to solution⁴². In addition, mucoadhesive polymers have been introduced to prepare microparticles, which could further overcome problems of poor bioavailability by increasing the residence time at the applied site. Mucoadhesion requires a highly expanded and hydrated polymer network, which could promote an intimate contact between microspheres and the mucus layer⁴³. Thus, mucoadhesive microspheres have been developed to decrease the effect of mucociliary clearance⁴⁴. The microparticles form a gel-like layer, which is cleared slowly from the nasal cavity, resulting in a prolonged residence time of the drug formulation. Mucoadhesive

microspheres significantly increase the systemic absorption of conventional drugs as well as polypeptides across the nasal membrane without the use of absorption enhancing agents that have the potential for irritation or damage⁴⁵. Alginate is an anionic mucoadhesive polymer, which is known for its ability to create hydrogen bonds with mucin-type glycoproteins through carboxyl-hydroxyl interactions. This anionic biopolymer is used in many pharmaceutical and biotechnological applications⁴⁶. Sodium alginate is a water-soluble, natural, linear polysaccharide that is most widely used as a polymer matrix due to its nontoxicity, biocompatibility, and gel formation ability. It has been reported that polyanion polymers are more effective bioadhesives than polycation polymers or nonionic polymers⁴⁶. Sodium alginate develops a simple and rapid gelation with divalent metal ions such as Ca²⁺. Therefore, it has been used frequently as the matrix to prepare microparticles. It can be cross-linked with divalent or polyvalent cations to form an insoluble meshwork. Ca²⁺ and Zn²⁺ have been reported for cross-linking of acid groups of alginate⁴⁷.

2.3. BIOMEDICAL APPLICATION

Alginate got new dimension in the application of biomedical field due to significant number of research has been done on alginate properties over the last decade. Alginate has several unique properties that have enabled it to be used as a matrix for the entrapment and/or delivery of biomolecules like DNA, proteins, and cells. A relatively mild gelation process free of organic solvents enables biomolecules and cells to be incorporated into the matrices with the retention of the three-dimensional structure (i.e., full biological activity)⁴⁸. The aqueous environment within the matrix is quite inert, and may consist of distilled water or sucrose solutions⁴⁹. The porosity of the gel allows for acceptable diffusion rates of macromolecules or low molecular weight drugs bound to macromolecules. The diffusion rate can be further controlled by coating of the beads⁵⁰. Alginate gels are stable in the temperature range 0–100°C and may be autoclaved under special conditions⁵¹. Freeze-drying of alginate beads containing cells or biomolecules is a possibility to keep their secondary structure and to ensure their stability during storage. A snapshot of recent application of alginate in biomedical field has been discussed in this article.

2.3.1 Protein delivery

The protein drug market is rapidly growing, and various protein drugs are now available owing to the development of recombinant DNA technology. Alginate is an excellent candidate for delivery of protein drugs, since proteins can be incorporated into alginate-based formulations under relatively mild conditions that minimize their denaturation, and the gels can protect them from degradation until their release. A variety of strategies have been investigated to control the rate of protein release from alginate gels. In general, the release rate of proteins from alginate gels is rapid, due to the inherent porosity and hydrophilic nature of the gels. However, heparin binding growth factors such as vascular endothelial growth factor (VEGF) or basic

fibroblast growth factor (bFGF) exhibit similar, reversible binding to alginate hydrogels, enabling a sustained and localized release⁵². The release in this scenario can be readily manipulated by altering the degradation rate of the gels (e.g., use of partially oxidized alginate), in order to make protein release at least partially dependent on the degradation reaction⁵³. Many attempts have been made to further control the release of angiogenic molecules from alginate gels, particularly for factors that are not heparin binding. Ionically cross-linked alginate microspheres efficiently encapsulated high pI proteins such as lysozyme and chymotrypsin; these proteins appear to physically cross-link the sodium alginate, allowing for more sustained release⁵⁴. Amino group-terminated poly((2-dimethylamino) ethyl methacrylate) has also been reacted with oxidized alginate without using a catalyst, and gel beads were prepared by dropping the aqueous solution of the alginate derivative into an aqueous CaCl₂ solution to form particles for oral delivery of proteins⁵⁵. Alginate was also used as a building block in the synthesis of a tetra-functional acetal-linked polymer network for stimuli-responsive gels with adjustable pore sizes. The gels protected acid-labile proteins such as insulin from denaturation in the gastric environment (pH 1.2), while releasing the loaded protein at near zero-order kinetics in neutral pH⁵⁶. The low encapsulation efficiency and fast release from alginate gels exhibited by many proteins can also be addressed with various cross-linking or encapsulation techniques, and/or by enhancing protein-hydrogel interactions. For example, insulin-loaded alginate microspheres were prepared by blending alginate with anionic polymers (e.g., cellulose acetate phthalate, polyphosphate, dextran sulfate), followed by chitosan-coating in order to protect insulin at gastric pH and obtain its sustained release at intestinal pH⁵⁸. Alginate microspheres have also been coated with Bombyx mori silk fibroin using layer-by-layer deposition techniques, which provided mechanically stable shells as well as a diffusion barrier to the encapsulated proteins⁵⁹. A combination of microspheres that serve as a depot for proteins and alginate hydrogels also enables sustained protein release. Hydrogels loaded with microspheres were prepared by encapsulation of a suspension of poly (d,l-lactide-co-glycolide) (PLGA) microspheres in alginate prior to ionic cross-linking. A homogeneous dispersion of PLGA microspheres was observed by SEM (Fig.1), and the release of bovine serum albumin (BSA), a model protein, from this combination delivery system was primarily controlled by the mixing ratio between PLGA microspheres and alginate hydrogels, independent of total BSA content and the size of PLGA microspheres used⁶⁰. The release behavior of TAT-HSP27 (heat shock protein 27 fused with transcriptional activator) was also regulated by varying the mixing ratio between microspheres and gels (Fig.2)⁶⁰. Alginate gels releasing proteins are being widely explored in tissue engineering and regeneration, as described in the following sections on blood vessel, bone, and muscle regeneration.

2.3.2 Wound dressings

The treatment of acute and chronic wounds is a pressing need in many facets of medicine, and alginate-based wound dressings offer many advantageous features. Traditional wound dressings (e.g., gauze) have provided mainly a barrier function – keeping the wound dry by allowing evaporation of wound exudates while preventing entry of pathogen into the wound⁶¹. In contrast, modern dressings (e.g., alginate dressings) provide a moist wound environment and facilitate wound healing⁶². Alginate dressings are typically produced by ionic cross-linking of an alginate solution with calcium ions to form a gel, followed by processing to form freeze-dried porous sheets (i.e., foam), and fibrous non-woven dressings. Alginate dressings in the dry form absorb wound fluid to re-gel, and the gels then can supply water to a dry wound, maintaining a physiologically moist microenvironment and minimizing bacterial infection at the wound site. These functions can also promote granulation tissue formation, rapid epithelialization, and healing. Various alginate dressings including Algicell™ (Derma Sciences) AlgiSite MTM (Smith & Nephew), Comfeel Plus™ (Coloplast), Kaltostat™ (ConvaTec), Sorbsan™ (UDL Laboratories), and Tegagen™ (3M Healthcare) are commercially available. A variety of more functional and bioactive alginate based wound dressings have also been studied to date. The sustained release of dibutyl cyclic adenosine monophosphate, a regulator of human keratinocyte proliferation, from partially oxidized alginate gels accelerated wound healing, leading to complete re-epithelialization of full thickness wounds within 10 days in a rat model⁶³. Alginate gels releasing stromal cell-derived factor-1 were also effective in accelerating wound closure rates and reducing scar formation in pigs with acute surgical wounds⁶⁴. Incorporation of silver into alginate dressings increased antimicrobial activity and improved the binding affinity for elastase, matrix metalloproteinases-2 (MMP-2), and pro inflammatory cytokines (e.g., TNF- α , IL-8). The addition of silver into alginate dressings also enhanced the antioxidant capacity⁶⁵. Alginate fibers cross-linked with zinc ions have also been proposed for wound dressings, as zinc ions may generate immunomodulatory and anti-microbial effects, as well as enhanced keratinocyte migration and increased levels of endogenous growth factors⁶⁶. Blends of alginate, chitin/chitosan, and fucoidan gels have been reported to provide a moist healing environment in rats, with an ease of application and removal⁶⁷.

2.3.3 Cell delivery

Alginate gels are increasingly being utilized as a model system for mammalian cell culture in biomedical studies. These gels can be readily adapted to serve as either 2-D or more physiologically relevant 3-D culture systems. The lack of mammalian cell receptors for alginate, combined with the low protein adsorption to alginate gels allows these materials to serve in many ways as an ideal blank slate, upon which highly specific and quantitative modes for cell adhesion can be incorporated (e.g., coupling of synthetic peptides specific for cellular adhesion receptors).

Further, basic findings uncovered with in vitro studies can be readily translated in vivo, due to the biocompatibility and easy introduction of alginate into the body. RGD-modified alginate gels have been most frequently used as in vitro cell culture substrates to date. The presence of RGD peptides in alginate gels allows one to control the phenotype of interacting myoblasts, chondrocytes⁶⁸, osteoblasts⁶⁹, ovarian follicle⁷⁰, as well as bone marrow stromal cells (BMSCs)⁷¹. For example, the adhesion and proliferation of myoblasts cultured on alginate gels were dramatically enhanced by chemical conjugation of RGD peptides to the alginate backbone, compared with non-modified alginate gels (Fig.3)⁷². Further, the number of cells adherent to the gels, as well as the growth rate, were strongly dependent on the bulk RGD density in the gels. The length of the spacer arm between the RGD peptide and the alginate chain is a key parameter in regulation of cellular responses. The adhesion and growth of primary human fibroblasts cultured on alginate gels modified with a peptide with the sequence of (glycine)_n-arginine-glycine-aspartic acid-serine-proline (GnRGDSP) was dramatically influenced by the spacer arm length, irrespective of the same total concentration of the peptides in the gels (Fig.4). At least four glycine units as a spacer arm allowed proper binding to the cellular receptors, but using more than 12 glycine units led to no further improvement in cell adhesion and growth⁷³. The number of RGD peptides per alginate chain, and the spacing between clusters of RGD peptides, even independently of the overall density of RGD ligands, dramatically impact the response of cells to RGD-modified alginate gels⁷⁴, likely due to the ability of these variables to affect the clustering of integrin receptors⁷⁵. While the presence of the RGD ligands typically enhances cell adhesion and differentiation, chondrogenic gene expression and matrix accumulation of BMSCs encapsulated in RGD-alginate gels (3-D) was inhibited with an increase of the RGD density in vitro⁷⁶. Interestingly, alginate gels have recently been formed in a microfluidic device through light-triggered release of caged calcium using DM-nitrophen™ compounds, and used as a 3-D cell culture substrate. Preosteoblasts (MC3T3-E1) and human umbilical vein endothelial cells were co-cultured in the microfluidic device using photo-patterning of alginate hydrogels, and this system may provide a useful means for integrating 3-D culture microenvironments into microfluidic systems. Recent studies utilizing alginate gels as 3-D cell culture substrates have revealed key insights regarding stem cell and cancer biology. The fate of mesenchymal stem cells was demonstrated to be controlled by the elastic modulus of the RGD-alginate gels in which they were encapsulated, as differentiation down fat and bone pathways was promoted at different values of gel stiffness. Strikingly, and in contrast to 2-D culture systems used in previous mechanotransduction studies, the control over stem cell fate was related to the number of adhesive bonds formed between the gel and the cells, as well as alterations in the receptors cells utilized to adhere to the RGD peptides in 3-D versus 2-D culture. The cells actively reorganized on the nanoscale the adhesion ligands presented from the gels⁷⁷. Alginate gels have also been used to examine how a 3-D

culture microenvironment influences cancer cell signaling and tumor vascularization. Integrin engagement within a 3-D tumor microenvironment (i.e., encapsulation in RGD-alginate gels) dramatically altered how cancer cells signal to recruit blood vessels, and this finding may lead to the development of new anti-angiogenic cancer therapies⁷⁸.

A crucial limitation of most 3-D cell culture systems is the difficulty in analyzing and quantifying cell-matrix interactions, particularly in a non-invasive, real time manner. However the development of several FRET techniques has recently enabled a previously unprecedented ability to quantitatively probe the relation between cell adhesion and decision-making. In one FRET technique, cell membranes are pre-stained with fluorescent molecules (i.e., acceptor), and a different fluorophore (i.e., donor) can be coupled to the cell adhesion peptides conjugated to the polymer chains. This FRET technique allows one to quantify cell receptor-ligand binding, and a similar FRET technique provides information on cell mediated rearrangements, at the nanometer size scale, of the adhesion ligands attached to gels⁷⁹. The relationship between cell behavior and the number of receptor–ligand bonds was investigated by encapsulating either preosteoblasts (MC3T3-E1) or myoblasts (C2C12) in alginate gels presenting RGD peptides using a FRET technique. The adhesive interactions can be directly visualized, as the green emission of fluorescein in the cell membrane was greatly decreased and the red emission of rhodamine at the interface between cells and gels was increased when the cells were encapsulated in rhodamine- G4RGDASSKY-alginate gels, due to FRET (Fig.5). The proliferation and differentiation of both cell types were significantly dependent on the number of receptor–ligand bonds calculated using the FRET signal. This type of analysis may allow one to predict cell behavior, particularly in 3-D culture, and to design proper 3-D cell culture substrates for many applications.

2.3.4. Bone

Despite recent progress, treatment of bone injuries is still often limited due to poor healing, and alginate gels have found potential in bone regeneration by delivery of osteoinductive factors, bone-forming cells, or combination of both. Alginate gels have advantages for bone and cartilage regeneration, as compared to other materials, due to their ability to be introduced into the body in a minimally invasive manner, their ability to fill irregularly shaped defects, and the ease of chemical modification with adhesion ligands (e.g., RGD) and controlled release of tissue induction factors (e.g., BMP, TGF). However, alginate gels do not have sufficient mechanical properties to allow load bearing in the initial stages of regeneration without fixation. They are also not inherently degradable in physiological conditions, as reviewed earlier, highlighting the need to control their degradation in order that residual gels do not interfere with regeneration. Alginate gels have proven useful in animal models for the delivery of growth factors that can effectively drive bone regeneration (e.g., bone morphogenetic proteins). The use of RGD-alginate gels allows complete regeneration of critical-sized femoral

defects in rodents with a low dose of BMP⁸⁰. Alginate gels that deliver DNA encoding bone morphogenetic proteins (BMPs) have also demonstrated significant bone tissue can be regenerated⁸¹. The delivery of multiple factors, either in combination or sequence, is also being explored, in a similar manner as described for angiogenesis. Sequential delivery of BMP-2 and BMP-7 using alginate gels enhanced osteogenic differentiation of bone marrow derived stem cells in vitro⁸², and co-delivery of BMP-2 and VEGF releasing from alginate gels enhanced the repair and regeneration of critical sized bone defects⁸³.

The transplantation of stem cells using alginate hydrogels has been widely explored in bone tissue engineering. The thickness of calcium cross-linked alginate gels was demonstrated to alter the behavior of rat bone marrow cells; however, different geometries did not influence cell differentiation. Bone marrow stromal cells, after being induced down the osteoblast pathway in vitro and mixed with calcium cross-linked alginate gels, repaired horizontal alveolar bone defects in dogs. Alginate/ chitosan gels entrapping mesenchymal stem cells and bone morphogenetic protein-2 also showed potential for trabecular bone formation in mice⁸⁴.

Alginate has also been combined with inorganic materials to enhance bone tissue formation. Alginate/ hydroxyapatite (HAP) composite scaffolds with interconnected porous structures were prepared by a phase separation method, which enhanced the adhesion of osteosarcoma cells⁸⁵. Cell-encapsulating alginate gel beads were introduced into calcium phosphate cement, and demonstrated potential for bone tissue engineering under moderate stress-bearing conditions⁸⁶. In addition, alginate gels containing collagen type I and -tricalcium phosphate enhanced adhesion and proliferation of human bone marrow stromal cells that do not readily attach or proliferate on pure alginate gels⁸⁷.

2.3.5 Cartilage

Repair of damaged or degraded cartilage is still one of the major challenges facing the orthopedics field, but tissue engineering approaches have recently shown potential in cartilage regeneration. Alginate gels have proved to be useful for transplanting chondrogenic cells to restore damaged cartilage in animal models. Early studies utilized a suspension of chondrocytes in an alginate solution mixed with calcium sulfate, and injected into molds of facial implants in order to produce pre-shaped cartilage. These constructs formed cartilage with three-dimensional shape retention after 30 weeks of subcutaneous implantation into mice and sheep, and the contents of proteoglycan and collagen as well as the elastic modulus of the engineered cartilage reached about 80% of those found in native cartilage⁸⁸. Shape-memory alginate gels were subsequently developed to engineer cartilage with desired shape and size in vivo following minimally-invasive delivery. In brief, macroporous alginate gels with predefined geometries were compressed into a significantly smaller form (dry state) and introduced into mice through a small catheter. The gels were then rehydrated in situ with a suspension of primary bovine articular chondrocytes, and recovered their original

shape and size within 1 h, which allowed cartilage formation in mice with the desired geometry⁸⁹.

The use of stem cells in cartilage regeneration is very attractive, due to the invasive and destructive processes required to obtain primary chondrocytes from tissues. Encapsulation in alginate can regulate differentiation of stem cells, and in particular chondrogenesis may be enhanced. It has been demonstrated that chondrogenic lineage of adult stem cells could be regulated via the introduction of soluble factors and biophysical cues in 3D cell culture systems⁹⁰. In addition, it has been hypothesized that chondrogenesis of stem cells relates to the morphology of the encapsulated cells (i.e., rounded cell shape)⁹¹, and alginate gels promote a rounded morphology that may promote the cellular differentiation process⁹².

Human mesenchymal stem cells (MSCs) encapsulated in alginate gel beads have been cultured in serum-free medium with the addition of transforming growth factor (TGF)-1, dexamethasone, and ascorbate 2-phosphate for more than one week, and demonstrated to form cartilage in large osteochondral defects⁹³. Rabbit bone marrow stromal cells cultured in alginate gels have also been injected into osteochondral defects in rabbit knees without the use of a periosteal patch, which significantly enhanced the cellular proliferation and chondrogenic differentiation of BMSCs. This resulted in histologically and mechanically improved tissues in osteochondral defects⁹⁴. The chondrogenic potential of human adipose derived stem cells (hASCs) suggests these cells as a possible cell source for cartilage regeneration, and chondrogenic differentiation of hASCs seeded in alginate gels was greatly improved in the presence of TGF- β 1⁹⁵. Pre-differentiated hASCs induced by transduction with an adenovirus carrying a TGF- β 2 plasmid maintained the chondrogenic phenotype *in vivo* and led to new cartilage formation when the cells were encapsulated into alginate gel beads and subcutaneously transplanted into mice⁹⁶.

2.3.6 Miscellaneous tissues and organ (Muscle, nerve, pancreas, and liver)

The potentiality of alginate gels are also being actively investigated for their ability to mediate the regeneration and engineering of a variety of other tissues and organs, including skeletal muscle, nerve, pancreas, and liver. Recent strategies for skeletal muscle regeneration include cell transplantation, growth factor delivery, or a combination of both approaches⁹⁷, and alginate gels have found potential in these strategies. A combined delivery of VEGF and insulin-like growth factor-1 (IGF-1) from alginate gels was used to modulate both angiogenesis and myogenesis. The localized and sustained delivery of both growth factors led to significant muscle regeneration and functional muscle formation, due to satellite cell activation and proliferation, and cellular protection from apoptosis by the released factors⁹⁸. Long-term survival and outward migration of primary myoblasts into damaged muscle tissue *in vivo* from RGD-alginate gels were dramatically enhanced by the sustained delivery of hepatocyte growth factor (HGF) and fibroblast growth factor 2 (FGF 2) from the gels⁹⁹. This led to extensive repopulation of host

muscle tissues and increased the regeneration of muscle fibers at the wound site⁹⁹. Alginate gels have also been investigated for the repair of the central and peripheral nerve systems. Alginate-based highly anisotropic capillary gels, introduced into acute cervical spinal cord lesions in adult rats, were integrated into the spinal cord parenchyma without major inflammatory responses and directed axonal regrowth¹⁰⁰. Alginate gels, covalently cross-linked with ethylenediamine, were useful to restore a 50-mm gap in cat sciatic nerves¹⁰¹, and promoted the outgrowth of regenerating axons and astrocyte reactions at the stump of transected spinal cords in young rats¹⁰². Alginate gels were also used as glue for repair of peripheral nerve gaps that could not be sutured¹⁰³. Alginate gels may be useful for cell-based neural therapies, as mouse-derived neural stem cells cultured in calcium alginate beads maintained their capacity for multi lineage differentiation into neurons and glial cells. Alginate gels modified with a peptide containing the YIGSR (Tyr-Ile-Gly-Ser-Arg) sequence promoted adhesion of NB2a neuroblastoma cells and neurite outgrowth from the cells, depending on the peptide density in the gels¹⁰⁴.

The alginate gel has a potential scope to provide hepatic tissues for replacement of a failing liver, and alginate gels encapsulating hepatocytes may offer a suitable platform for developing a bio artificial liver as they are easily manipulated and can be cryopreserved¹⁰⁵. The hydrophilic nature of alginate gels processed to exhibit an interconnected porous structure allows efficient seeding of hepatocytes into the gels, while maintaining high hepatocellular functions¹⁰⁶. Primary rat hepatocytes maintained viability in alginate gels and appeared to synthesize fibronectin, which was deposited on the spheroids and promoted their functional expression¹⁰⁷. Hepatocyte engraftment was improved when hepatocytes were transplanted into the liver lobe of Lewis rats using VEGF releasing porous alginate gels¹⁰⁸.

One of the first applications of alginate gels in tissue engineering involved the transplantation of encapsulated pancreatic islet allografts and xenografts in an effort to cure Type I diabetes. In this approach, the gel was used to provide protection from the host immune system, in order to avoid the use of immunosuppressive drugs that would otherwise be required to prevent graft rejection. This approach has been successfully used to treat animal models of Type I diabetes without the use of immunosuppressive drugs¹⁰⁹. These alginate beads encapsulating islets are generally coated with poly (amino acids), such as poly-L-lysine, to decrease the outer pore size, while keeping a liquid core structure¹¹⁰. The transplant volume of microencapsulated islet cells can be reduced by choosing an appropriate alginate composition and purity¹¹¹. However, the mechanical and chemical instability of alginate beads is believed to be a limiting factor for long term survival of the transplanted islets *in vivo*, prompting continued investigations into the use of different poly (amino acids) as a coating material and the use of various micro fabrications methods¹¹².

CONCLUSION

Alginate has proved itself as an important pharmaceutical and biomedical drug delivery tool due to its versatile favorable characteristics. Especially in the field of controlled released delivery, wound healing and tissue engineering. The most important features of alginate for these applications include non toxicity, biocompatibility, mild gelation and easy modification technique. Alginate has a very good track record of clinical use both in biomedical and pharmaceutical field. Due to extensive research of alginate properties such as chemical modification, cross linking strategy, establish the structure activity relationship, improving the physical properties of alginate for particular use has increased the potentiality of alginate use in many purposes. The engineering of new classes alginates with precisely designed physical and chemical properties designed for the particular application could revolutionize the use of these materials and so far this is the demand of time.

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