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Review

Alginates as a useful natural polymer for microencapsulation and therapeutic applications

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ABSTRACT

This review outlines the role of alginates in microencapsulation and therapeutic applications. It focuses on the physicochemical properties of alginates (e.g. viscosity, thermo-stability, sol–gel transformation and drug release) to gain better insight into their potential medical applications, particularly for wound care and therapeutics. In order to understand how alginates can be optimized as a useful delivery system for therapeutic applications, various factors that impact drug release from alginate matrices (e.g. types of cations used in cross-linking, porosity of alginate matrices, pH effect, alginate composition, molecular weight of encapsulated drugs and modification of the functional groups in alginates) are also discussed. More specifically, practical applications of the cross-linking mechanism and sol–gel transformation property of alginates are explored to assess their potential to improve the mechanical properties of alginate dressings, to impart anti-microbial properties for treating wound infections and to develop products for tissue repair and wound healing. Innovative processes of developing alginate carriers and delivery systems and their recent applications are also discussed. Strategies employed to improve gelation of alginates commonly target the formulation by the inclusion of non-gelling cations or sequestrants during cross-linking. The application of other strategies, such as hot-made alginate gel method, *in situ* gelation method, crystal gun method, acoustic excitation method, and the use of extrusion devices with improved design are reviewed.

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1. Introduction

Alginates are generally referred to as a family of polyanionic copolymers derived from marine kelp, mainly the brown sea algae. As a commercial entity, Stanford patented an impure alginate salt in the early 1880s and this natural anionic polysaccharide subsequently received great attention. Its interesting chemical and physical properties resulted in many commercial applications, as described in several reviews (Chapman & Chapman, 1980; Gombotz & Wee, 1998). Table 1 summarizes the functional role of alginates in some common applications. In the food and beverage industry, alginates remain as one of the most important ingredients in food products. Alginates are used as stabilizers and thickeners for a wide range of products that include jelly, drinks such as chocolate milk and desserts such as ice cream. Yeast cells are encapsulated with alginates for use in ethanol production. In other industries, alginates are used as a thickening agent for colour pigments in fabric printing, adhesive agent and filler in paper industries and stabilizer cum suspending agent in paints to give a better paint flow and surface coverage (Chapman & Chapman, 1980). Some interesting innovations also explore alginates as a novel way of ceramic shaping in gelcasting and biocatalysts in water treatments (Tramper & De Man, 1986; Wang, Xie, Huang, & Cheng, 2002). Alginates were found to be a good metal-sequestrant (Aderhold, Williams, & Edyvean, 1996). Fungus-entrapped alginate pellets can selectively react with toxic metallic ions (e.g. Cd^{2+}) in sewage and potentially useful in waste water-treatment systems (Arıca, Kacar, & Genc, 2001).

Alginates are of growing importance in the healthcare and pharmaceutical industry. Since the first successful encapsulation of islet cells by Lim and Sun (1980), alginate matrices were extensively employed for cell culture and transplantation (Stevens Molly, Qanadilo Hala, Langer, & Prasad Shastri, 2004). Other workers further explored the use of hybridoma cells in monoclonal antibodies production and immobilize drugs for sustained release systems (Dubrot et al., 2010; Wang, Zhou, Sun, & Huang, 2010) and patents for alginate coating of physiologically active cells/enzymes had been reported (see Table 2). Alginates were also included in tablet making to improve on bioadhesive property for buccal adhesive tablets (Choi & Kim, 2000). Oral administration of low molecular weight sodium alginate (10–100 kDa) could potentially combat hypercholesterolemia and diabetes mellitus, by encapsulation of cholesterol and blood glucose following physiological gelation of alginic acid in the stomach (Kimura, Watanabe, & Okuda, 1996).

Given the broad range of applications and immense information available for alginates, this overview intends to provide a summary of alginates as potentially useful encapsulating matrices in the pharmaceutical industry. The scope of the overview includes fundamental discussion on alginate composition and biosynthesis, common detection techniques to novel methods of producing alginate carriers and delivery systems. The physicochemical properties of alginates are examined and related to various research studies on alginate formulations and strategies in wound care management.

Table 1
Functional role of alginates in some common applications.

Application	Function
Food and beverage industry	
Drinks	Stabilizers, thickeners
Ice-cream	Stabilizers, thickeners
Jelly	Stabilizers, thickeners
Ethanol production	Encapsulation material of yeast cells
Pharmaceutical industry	
Cell culture and transplantation	Encapsulation material
Dental impression material	Mould
Tablets	Adhesive agent, sustained-release
Wound dressing	Haemostatic and absorbent
Other industries	
Fabrics	Thickeners
Paper	Adhesive agent, filler
Paint	Stabilizer and suspending agent
Toothpaste	Stabilizers, thickeners

2. Alginate overview

The overview of alginates constructs the interrelation between the nature of alginates (sources or composition) and their physicochemical properties while understanding the various methods of analysis. The references included (see Table 3) are by no means exhaustive, and intended as a basis to appreciate the science of alginates in pharmaceutical applications.

2.1. Sources and production of alginates

Alginates are produced from two sources, algae and bacteria. The commercially available alginates are derived primarily from brown algae. Common algae species that are commercially important include *Laminaria hyperborea*, *Ascophyllum nodosum* and *Macrocystis pyrifera* (Sutherland, 1991). Alginates isolated from bacteria such as *Azotobacter* and *Pseudomonas* species are usually not economically viable for commercial applications and confined to small-scale research studies (Skjaak-Braek, Grasdalen, & Larsen, 1986).

The commercial production of alginates involves mainly alkaline extraction processes. Brown algae collected are dried and undergo various chemical treatments to remove impurities (e.g. heavy metals, endotoxin, proteins, other carbohydrates and polyphenols) normally present, before being processed into finished raw material as a powder in the acid or salt form (Sutherland, 1991).

2.2. Composition of alginates and synthesis pathway

Alginates consist of two basic building blocks, α-L-guluronic acid (G) and β-D-mannuronic acid (M) residues, linearly linked together by 1–4 linkages (Fig. 1) (Chapman & Chapman, 1980; Gaserod, Smidsrod, & Skjak-Braek, 1998). These conformational isomer residues, which differ in structural arrangement of the hexopyranose rings (Chandrasekaran, 1999), form at least three different forms of polymer segments: the MM and GG containing segments being interdispersed with regions of alternating MG segments (Gaserod et al., 1998; Haug & Larsen, 1962). A polydisperse size distribution of alginates was observed using gel permeation chromatographic methods (Berth, 1992).

The formation of uronates involves a mannuronan C-5 epimerase interconversion of synthesized mannuronic acid to guluronic acid. Several statistical models (Markov's models and

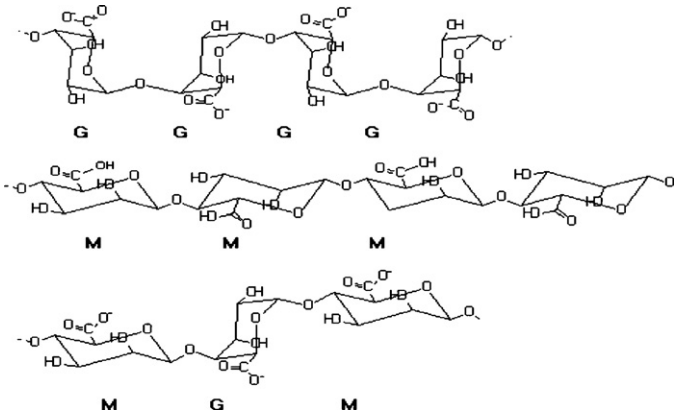

Fig. 1. Components of alginates: G–G, M–M and M–G blocks.

Table 2
Some alginate formulation patents for cell transplant.

Patent no.	Patent title	Inventor(s)	Published date	Field of invention
US 5,639,467	Electrostatic process for manufacturing coated transplants and product	Dorian and Cochrum	June 17, 1997	Pancreatic islet cell transplants
WO 9,713,474	Retrievable bioartificial implants	Antanavich and Dorian	April 17, 1997	Bioartificial pancrease implants
WO 2,000,040,252	Novel polymer formulations containing perfluorinated compounds for the engineering of cells and tissues for transplantation that improves cell metabolism and survival, and methods for making same	Fraker, Inverardi, Mares-Guia, and Ricordi	July 13, 2000	Cell transplant matrix modification
WO 2,000,047,236	Matrices for drug delivery and methods for making and using the same	Babich, Zubieta, and Bonavia	August 17, 2000	Pro-drug converting enzymes encapsulation
WO 2,010,032,242	Optimization of alginate encapsulation of islets for transplantation	Barkai, Rotem, Azarov, Neufeld, and Gendler	March 25, 2010	Bioartificial pancrease implants
US 20,100,143,464	Cross-linked alginate–polyalkylene glycol polymer coatings for encapsulation and methods of making	Stabler, Gattas-Asfura, Finn, Ricordi, and Rengifo	June 10, 2010	Cell transplant matrix modification

Bernoullian model) which ascribe bacterial composition to a post-polymerization epimerization pathway appeared less fitting for monomer algal sequences (Panikkar & Brasch, 1996). Contrary to belief of enzymatic isomerisation at the polymeric level, formation of homopolymeric blocks in high M and G algal alginates could be the sequential 5-epimerization of some mannuronate residues at the monomer level and an additional copolymerization process (Panikkar & Brasch, 1997). The kinetics and activities of different epimerases were also studied in *Azotobacter vinelandii* to understand the biosynthesis mechanism (Hartmann et al., 2002).

The proportion and arrangement of the uronic blocks vary with the source and are related to the physical strength and other physical properties of the alginate structure (BeMiller, 1999; Gaserod et al., 1998; Gombotz & Wee, 1998). The properties of alginates produced by bacteria are further influenced by the presence of acetylated groups (absent in algal alginates) at O-2, O-3 positions and residues resulting from epimerization (Rehm & Valla, 1997). The presence of acetylated mannuronate segments from normal strains of *Pseudomonas aeruginosa* was positively correlated with a triad composition of lower homopolymeric M block contents and an absence of GGG sequences (Schurks, Wingender, Flemming, & Mayer, 2002).

2.3. Physicochemical characteristics

The structural–property relationships of alginates are better understood, owing to extensive research and wider pharmaceutical applications in recent years. Physical properties of alginates are largely governed by the composition and arrangement of the uronate residues, molecular weight of the polymer and concentration of the cross-linking cation solution used (Draget, Oestgaard, & Smidsroed, 1990; Martinsen, Skjaak-Braek, & Smidsroed, 1989).

2.3.1. Viscosity

Although sodium alginate rich in guluronic acid is more soluble in water than mannuronic acid-rich sodium alginate (Jimenez-Escrig & Sanchez-Muniz, 2000), the viscosity of an alginate solution is related to the concentration of alginates and the length or number of monomer units in the alginate segments (Gombotz & Wee, 1998). At similar concentrations, alginates with longer segments were found to have higher viscosities (Berth, 1992). This also implies that viscosity of alginate solution can be affected by the molecular weight of the constituent alginate polymeric segments. It is interesting to note, however, that alginates from bacteria do not show such correlations (Rehm & Valla, 1997).

Table 3
Alginate overview: scope and references.

Scope	Reference(s)
Sources and production of alginates	Sutherland (1991) and Skjaak-Braek et al. (1986)
Composition of alginates and synthesis pathway	BeMiller (1999), Berth (1992), Chapman and Chapman (1980); Chandrasekaran (1999), Gombotz and Wee (1998), Gaserod et al. (1998), Hartmann et al. (2002), Haug and Larsen (1962), Panikkar and Brasch (1996), Panikkar and Brasch (1997), Rehm and Valla (1997), and Schurks et al. (2002)
Physicochemical characteristics	Draget et al. (1990) and Martinsen et al. (1989)
Viscosity	Berth (1992), Gombotz and Wee (1998), and Rehm and Valla (1997)
Thermo-stability	Andresen and Smidsroed (1977), Gacesa (1988), Miura et al. (1999), and Zheng (1997)
Sol–gel transformation	Braccini et al. (1999), Chan et al. (2006), Chapman and Chapman (1980), Cheetham (1979), Chen et al. (1994), Clark and Ross-Murphy (1987), DeRamos et al. (1997), Elder et al. (2006), Draget et al. (1994), Gaumann et al. (2000), Gombotz and Wee (1998), Hassan, El-Shatoury, et al. (1988), Hassan, Wahdan, et al. (1988), Haug (1961a, 1961b), Martinsen et al. (1989), Nestle and Kimmich (1996), Papageorgiou et al. (1994), Potter and McFarland (1996), Rees and Welsh (1977), Rees (1981), Sherys et al. (1989), Smidsroed and Skjaak-Braek (1990), Smidsroed et al. (1973), Stockwell et al. (1986), Sutherland (1991), Zheng (1997), and Zheng et al. (1998)
Drug release	Andresen and Smidsroed (1977), Badwan et al. (1985), Chan and Heng (2002), Choi et al. (1999), De Vos et al. (1996), Fundueanu et al. (1999), Gaserod et al. (1998), Gray and Dowsett (1988), Hannoun and Stephanopoulos (1986), Klein et al. (1983), Klock et al. (1997), Martinsen et al. (1989), Mi et al. (2002), Mumper et al. (1994), Rastello De Boisseson et al. (2004), Segi et al. (1989), Smidsroed et al. (1973), Sriamornsak et al. (2007), Stabler et al. (2001), Stockwell et al. (1986), and Yotsuyanagi et al. (1987)
Analytical characterization	Donati et al. (2003), Gacesa (1988), Grasdalen (1983), Larsen et al. (1985), Mammarella and Rubiolo (2003), Morris et al. (1980), Potter et al. (1994), Thom et al. (1982), Rastello De Boisseson et al. (2004), Reisenhofer et al. (1984), Yang et al. (2000), and Zimmermann et al. (2003)

2.3.2. Thermo-stability

The studies to date suggest a temperature-dependent nature of alginates. Differential scanning calorimetry had been employed to study the thermal and viscoelastic properties of alginate films (Miura, Kimura, Suzuki, Miyashita, & Nishio, 1999). Although thermostable alginate gels were formed in the temperature range between 0 and 100 °C (Gacesa, 1988), there was a report of decreased gel rigidity with increased temperature (Andresen & Smidsroed, 1977). It appeared that at temperatures below the boiling point of water, non-covalent bonding between contiguous polymeric segments kept the alginates intact under small-deformation oscillatory conditions but this equilibrium was disrupted by thermal agitation from a steady shear (Zheng, 1997).

2.3.3. Sol–gel transformation

Aqueous sodium alginate solution can undergo sol-to-gel transformation in the presence of cross-linking cations. Generally, the formation of cross-linked alginate gel matrices can occur by three mechanisms, namely external gelation, internal gelation, and gelation by cooling. In the formation of large pellets or micropellets by external gelation, the drug-containing alginate solution is delivered to the cross-linking solution as extruded or atomized droplets and gel formation occurs as cross-linking cations diffuse into the alginate solution. In the internal gelation method, an insoluble calcium salt (e.g. calcium carbonate) is first added to the alginate-drug solution and free calcium ions are subsequently liberated by pH adjustment with glacial acetic acid (Chan, Lee, & Heng, 2006). Sequestrant such as trisodium citrate can also be employed to further control the reaction rate by competing with the alginates for the free calcium ion. The pH condition, amount and particle size of the insoluble calcium salt have been reported to affect the releasing rate of cations (Chan et al., 2006). For alginates formed by cooling, the alginate, calcium salt, and calcium sequestrant are dissolved in a hot medium of 90 °C and then allowed to set through cooling (Papageorgiou, Kasapis, & Gothard, 1994). At high temperature, the high thermal energy of the alginate chains prevented polymeric alignment and irreversibly destabilized any non-covalent intra-molecular bonding between neighbouring chains (Papageorgiou et al., 1994). Upon cooling at a lower temperature, the reestablishment of the inter-molecular bonds between the polymer chains facilitated the formation of an ordered tertiary structure and resultant homogeneous matrix (Zheng, 1997).

Polyvalent cations can replace and be preferentially bound to the binding sites of the residing sodium ions in the polyguluronate segments and produce a cross-linked "egg-box" model (Gombotz & Wee, 1998; Rees, 1981). In general, divalent cations (Cd^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) are suitable cross-linking agents but not monovalent cations or Mg^{2+} (Sutherland, 1991). The chelation at the G-residue of the alginate molecules results in ionic interaction between the guluronic acid groups while the van der Waal forces between alginate segments result in a three-dimensional gel network (Gaumann et al., 2000; Rees & Welsh, 1977). This inner sphere ion–alginate complex formation during cross-linking was absent for Mg^{2+} (DeRamos, Irwin, Nauss, & Stout, 1997). Braccini, Grasso, and Perez (1999) used a modeling simulation study to demonstrate high specificity of polyguluronate residues (compared to polymannuronate residues) in calcium binding, hence reaffirming the role of guluronate residues in gel formation. The efficient axial–equatorial–axial arrangement in guluronate segments facilitated cross-linking by complementary fitting of oxygen atoms from neighbouring residues into the coordination sphere of the cation (Braccini et al., 1999).

Cross-linking metal ions show different affinities for alginates although the extent of cross-linking may be influenced by the chemical composition of alginates that vary between seasons of harvest, age and component of the plant being used (Haug, 1961a). Divalent alkaline ions (Ca^{2+}, Ba^{2+}, and Sr^{2+}) bound mainly to GG segments while trivalent lanthanide ions (La^{3+}, Pr^{3+}, and Nd^{3+}) showed affinity for both GG and MM segments (DeRamos et al., 1997). The differences in binding affinity were related to the ionic radius, coordination number of cross-linking ions and the presence of water of hydration surrounding trivalent ions (DeRamos et al., 1997). Divalent ions of larger ionic radii (Ba^{2+} and Sr^{2+}) produced stronger alginate pellets than Ca^{2+} (Clark & Ross-Murphy, 1987). In contrast, the presence of water molecules surrounding Eu^{3+} may disrupt coordination binding in alginate cavities, thereby affecting effective cross-linking (DeRamos et al., 1997). Alginates derived from *Laminaria digitata* are high in mannuronic acid and show lower affinity to calcium than the high guluronate-containing alginates in a sodium–calcium ion exchange process (Haug, 1961b).

The kinetic studies on various divalent cations (Ni^{2+} and Cu^{2+}) by Hassan, El-Shatoury, Mousa, and Hassan (1988) and Hassan, Wahdan, and Hassan (1988) provided insight into the gelating mechanism of cross-linking cations. Investigation on cupric alginate (Hassan, El-Shatoury, et al., 1988) demonstrated the non-selective mechanism of copper, which was postulated to chelate inter- or intra-molecularly with the carboxylate and hydroxyl groups of different homopolymeric segments in alginates. Other workers related the gelating mechanism of calcium to the functional groups in the guluronate segments (Zheng et al., 1998).

It was observed that positively charged proteins can potentially compete with the divalent cations for the available carboxylic acid sites on alginate segments at pH values above the protein isoelectric point (Sherys, Gurov, & Tolstoguzov, 1989; Stockwell, Davis, & Walker, 1986). As alginates can potentially form coacervates with cationic proteins, additives may be included to protect the active agent from the alginate polymer (Gombotz & Wee, 1998). The mechanism of copper ion absorption within alginate matrices was also described by spatial and temporal data from NMR microscopy (Nestle & Kimmich, 1996). In the presence of excess calcium ions, the movement of copper ions into the calcium alginate matrix appeared to be largely affected by the physical hindrance of the charged matrix (Potter & McFarland, 1996). Sequestrants, such as trisodium citrate, are often employed during cross-linking to moderate the alginate gelation process. The added trisodium citrate acts as a retarding agent by chelating free calcium ions that are introduced into the cross-linking solution. This complex formation delays the availability of free calcium ions during cross-linking and the slow release of calcium into the alginate solution facilitates the formation of a homogeneous alginate matrix (Papageorgiou et al., 1994).

It is known that calcium ions cross-link alginates preferably at the binding sites in the guluronic segments. The composition of the guluronic segments (molecular weight and M/G ratio) and hence the extent of cross-linking will largely affect the quality of the matrices formed. In particular, the gel strength, which is an indicator of the mechanical stability of cross-linked matrices, has been studied by compression testing of alginate pellets (Martinsen et al., 1989). Alginate matrices made from a high content of guluronic acid tend to be rigid and brittle, while more elastic gels were produced from alginates of low α-L-guluronic acid content (Chapman & Chapman, 1980). Alginate pellets containing a guluronic acid content greater than 70% and an average length of guluronic blocks higher than 15 were reported to exhibit less shrinkage, good mechanical strength and better stability but greater porosity (Martinsen et al., 1989) Matrices that are physically strong and associated with greater porosity to facilitate release activity are potentially useful for immobilization of living cells (Martinsen et al., 1989). These factors are also discussed in relation to drug release from alginate matrices in the next section. However, gel strength appeared to be unaffected by molecular weight beyond 200 kDa

(Draget, Braek, & Smidsrod, 1994). In addition, increased flexibility of alginate matrices was affected by the polymer segments according to the following order: MG > MM > GG (Smidsroed, Glover, & Whittington, 1973). Earlier studies have also identified the concentration of alginates and amounts of entrapped yeast as factors that affected the mechanical stability of the pellets produced and their performance in ethanol production (Cheetham, 1979). Elder et al. (2006) also observed differential response between chondrocytes in pellet culture and those suspended in alginate hydrogel, further highlighting the impact of the mechanical stability of alginates on various applications.

The ionotropic gelation of alginates is inherently a reversible process. The alginate pellets formed can be easily destabilized in a reacting solution containing citrate or phosphate buffer at pH 7 (Smidsroed & Skjaak-Braek, 1990). An increase in buffer concentration causes increased swelling and rapid disintegration of the alginate pellets, with dire consequences to the encapsulated materials. Improved bioactivity and mechanical stability were reported for alginate-encapsulated cells surface modified with polyacrylamide although the cell performance was affected by the type of cross-linking cations (Chen, Yin, Tiu, & Houng, 1994). Iron species (Fe^{2+} and Fe^{3+}) were less effective as a gelating cation in comparison with Ca^{2+} and showed increased steroid transformation activity with immobilized *Aspergillus* species.

2.3.4. Drug release from alginate matrices

Alginate is useful as a matrix for cell immobilization, as well as entrapment of bioactive compounds and drugs. The material encapsulated within the inert alginate environment could be delivered at a desired rate in a controlled release system. Encapsulated drugs are released from alginate pellets by diffusional processes through pores and the release is facilitated by the degradation of the polymeric network. In general, the release of water-soluble drugs is controlled predominantly by diffusion while that of water-insoluble drugs is largely dependent on gel erosion (Mi, Sung, & Shyu, 2002; Sriamornsak, Thirawong, & Korkerd, 2007). The movement of encapsulated drug in an alginate matrix is governed by the property of the drug and chemical composition of the alginate polymer.

The pore size of alginate matrix directly affects diffusion and is controlled by various factors. It was found that the matrix porosity was significantly reduced by drying (Smidsroed et al., 1973). Compared with other techniques, the emulsification method produced micropellets with much smaller pore size (Fundueanu, Nastruzzi, Carpov, Desbrieres, & Rinaudo, 1999). Although a low pH environment did not cause significant drug release (Segi, Yotsuyanagi, & Ikeda, 1989), acid treatment during preparation increased matrix swelling and drug release (Mumper, Hoffman, Puolakkainen, Bouchard, & Gombotz, 1994; Yotsuyanagi, Ohkubo, Ohhashi, & Ikeda, 1987). Proton hydrolysis at low pH may have resulted in faster degradation and drug release upon re-equilibration at neutral pH.

Alginate composition plays an important role in the porosity of gel matrices. It was found that an increase in alginate content increased porosity, enhancing water uptake ability and drug release (Choi et al., 1999). Aside from their ability to maintain gel integrity for longer periods of time, alginates containing a high content of guluronic acid have a more open pore structure and exhibited high diffusion rates for the encapsulated material. Conversely, high mannuronic acid alginate pellets were softer, less porous and tend to disintegrate easily with time. The smaller pore sizes created in the alginate matrices may have resulted in higher degree of swelling and shrinkage during cross-linking (De Vos, De Haan, Wolters, & Van Schilfgaarde, 1996; Klock et al., 1997). It was also observed from Fourier transform infrared (FT-IR) spectroscopy that alginates with high mannuronic acid content bound more strongly to poly-L-lysine layer in multiple coating (Badwan, Abumalooh, Sallam, Abukalaf, & Jawan, 1985), which produced stable sustained-release matrices (Stabler, Wilks, Sambanis, & Constantinidis, 2001).

The effect of divalent cations on the gel porosity was also investigated using various concentrations of calcium chloride (Gaserod et al., 1998). The results showed higher drug release rates with increased sodium or calcium contents and high matrix porosities. However, drug release was limited by ionic strength effect at very high concentrations of calcium while high sodium contents (molar ratio of $Na^+/Ca^{2+} > 30$) may compete with the gelating calcium cations in the alginate carboxyl groups, promoting weak gel formation (Martinsen et al., 1989).

The differences in sustaining the release of drugs (chlorpheniramine, sodium salicylate and caffeine) from alginate matrices appeared to be related to the molecular weight of entrapped drug (Gray & Dowsett, 1988) and ionic interaction between drug and negatively charged alginates (Mumper et al., 1994; Stockwell et al., 1986). Although high drug content should promote drug diffusion, Segi et al. (1989) reported significant reduction in matrix diameter and precipitation of propranolol in alginate matrices as drug content was increased, thus reducing drug release. Earlier studies using electron microscopy and column analysis found smaller pores on the pellet surface than in the core (Andresen & Smidsroed, 1977; Klein, Stock, & Vorlop, 1983). Diffusion of large protein molecules was reported to be influenced by their molecular weights although smaller molecules like glucose and ethanol did not show similar trends (Gray & Dowsett, 1988; Hannoun & Stephanopoulos, 1986).

Functional groups in alginates may affect the rate of drug release from the microspheres. This was observed when different types of aldehydes were cross-linked with the hydroxyl groups of the alginate polymer. Bulkier aldehydes (ocatanal and ocatadecanal) were less capable of sustaining drug release when compared with methanal and pentanedial (Chan & Heng, 2002). The steric effects of these bulkier groups may result in weaker bonding between the drug and the alginate matrices. A separate study attributed the lower affinity of calcium ions for polyguluronic residues to the non-uniform network of loose microstructures (Rastello De Boisseson et al., 2004).

2.4. Analytical characterization

Chemical analysis of alginates presents difficulties as the commonly employed acid hydrolysis step can lead to destruction of the uronic acids. Circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy provide quick and non-destructive methods to determine the composition and arrangement of alginate uronic residues.

CD uses the principle of optical activity of chromophores to match the linear spectra of the alginates with model samples of well-characterized homopolymeric blocks (Donati et al., 2003; Morris, Rees, & Thom, 1980; Thom, Grant, Morris, & Rees, 1982). Besides unraveling the alginate structure, CD also enables a better understanding on the mechanism of cross-linking. For example, the wider range of wavelengths and opposite sign in the spectra of Cu^{2+} suggested a less-specific binding mechanism and demonstrated the lack of selectivity of copper to the binding sites in alginate matrices.

NMR spectroscopy is a widely accepted method for routine analysis and molecular structure determination of alginates using the triad frequencies of the uronate residues (Grasdalen, 1983). The diffusional behaviour of the cross-linking cations during gelation was also studied by NMR (Mammarella & Rubiolo, 2003; Potter, Balcom, Carpenter, & Hall, 1994).

The selection of analytical techniques is often decided by the inherent preparatory requirements. Samples of high molecular weights must be partially hydrolyzed prior to NMR analysis. CD analysis is therefore preferentially used for modified alginates

which contain hydrolysable ester groups (Rastello De Boisseson et al., 2004). However, both NMR and CD techniques are only suitable for analysis of alginate samples of high purity (Gacesa, 1988). Larsen, Vreeland, and Laetsch (1985) attempted to analyze complex mixture or natural forms of alginates using standard immunological methodology. The specificity of the antibodies for the alginate substrates was found to be significantly affected by the concentrations of the various ions.

Other instruments used in alginate characterization included confocal laser scanning microscopy and atomic force microscopy for studying the crystalline homogeneity of the alginate pellets formed (Zimmermann et al., 2003). Differential pulse polarography was also used to investigate the binding ability of alginates to polyacrylate (Reisenhofer, Cesaro, Delben, Manzini, & Paoletti, 1984) while X-ray diffraction patterns was used to study the cross-linking effects of alginates with cellulose in alginate membranes (Yang, Zhang, Peng, & Zhong, 2000).

3. Alginate microspheres: method(s), factors affecting synthesis and research development

Alginates can readily form pellets or microspheres in the presence of suitable cross-linking cations and can be made as delivery systems. Various methods for producing pellets of different sizes were reviewed by Gombotz and Wee (1998).

Large pellets (greater than 1.0 mm in diameter) are conventionally prepared using a simple syringe or pipette (Arıca, Arpa, Ergene, Bayramoglu, & Genç, 2003; Arıca et al., 2001; Bodmeier & Paeratakul, 1991; Elcin, 1995). Droplets of sodium alginate solution are extruded into a divalent cross-linking solution bath usually of Ba^{2+}, Ca^{2+} or Sr^{2+} salts. Pellets formed are then allowed to cure in the cross-linking solution (varying from a few minutes to hours), rinsed with water and air-dried. The mean diameter of the pellets is affected by the size of the extrusion nozzle, viscosity and concentration of the alginate solution used. The use of a larger diameter nozzle and higher viscosity solution resulted in bigger pellets (Badwan et al., 1985). Increased sphericity was also observed as the concentration of sodium alginate increased but solutions with more than 5% (w/v) sodium alginate were difficult to prepare (Gombotz & Wee, 1998).

Micropellets (less than 0.2 mm in diameter) can be prepared using atomization, emulsification and coacervation methods (Abraham, Vieth, & Burgess, 1996; Gombotz & Wee, 1998). The atomization or spray method uses a syringe pump and a loading syringe. The alginate solution is delivered through an orifice of about 1 mm in diameter. It is possible that the smaller diameter orifice may be more easily clogged by a high viscosity alginate solution. Pellet size can be controlled either by pressure of the infusing nitrogen gas, syringe pump rate or distance between orifice tip and surface of the cross-linking solution. The co-axial air/gas used may be filtered for sterility (Hardikar, Risbud, & Bhonde, 1999). An oil-in-water emulsion is used for encapsulation of peptides, proteins or synthetic drugs of low molecular weight (Fundueanu et al., 1999). Microsphere size is affected by the stirring speed and rate of addition of the cross-linking solution. The coacervation method uses oppositely charged polyelectrolytes to form bi-layer equilibrium phases under specific pH and ionic conditions, causing the dense coacervate phase to form microspheres (Bungenberg de Jong, 1949; Daly & Knorr, 1988; Singh & Burgess, 1989).

The extrusion method may be a simple process, but it is less efficient in forming spherical pellets. Rapid cross-linking and hardening at surfaces of the pellets retard movement of cross-linking cations into the inner core and less stable pellets are formed. The diffusion of calcium ions through the gel network is dependent on the initial concentration of cross-linking ions, ionic strength of alginate solutions for cross-linking and size of pores in the gel matrices (Skjaak-Braek, Grasdalen, & Smidsroed, 1989). The addition of excessive cationic cross-linkers overwhelmed the non-specific binding sites and resulted in a near-neutral gel matrix (Potter et al., 1994).

Table 4
Strategies to improve gelation of alginates.

Strategies	References
Inclusion of non-gelling cations	Skjaak-Braek et al. (1989)
Hot-made alginate gel method	Papageorgiou et al. (1994)
In situ gelation method	Draget et al. (1990)
Crystal gun method	Zimmermann et al. (2003)
Acoustic excitation method	Berkland et al. (2001)
Improved design of extrusion orifice	Yang et al. (2005)

Various studies have reported improved gelation mechanism by modulating various critical parameters necessary for homogeneous alginate matrix formation (Table 4). The formation of homogeneous calcium pellets in the presence of other non-gelling cations, such as sodium and magnesium ions, has been reported (Skjaak-Braek et al., 1989). In another study, Papageorgiou et al. (1994) described an external gelation method of synthesizing homogeneous gels by adding dry blends of alginates and trisodium citrate to calcium chloride at 90 °C. This hot-made alginate gel method was reported to promote slow formation of intermolecular junctions in the alginate gel, giving rise to a homogeneous network rather than a precipitate. The effects of saturated levels of the cross-linking salt was also investigated and found to affect the homogeneity of the gel (Papageorgiou et al., 1994). Homogeneous pellets were also prepared by an *in situ* gelation method where the calcium ions were liberated within the alginate solution (Draget et al., 1990). The properties of the pellets were found to be influenced by the viscosity and the concentration of alginate solution used.

Other innovative strategies were attempted to further improve the gelation of alginates. Stable homogeneous pellets were reported to form by employing $BaCl_2$ crystals with alginate solution (Zimmermann et al., 2003). Known as the crystal gun method,

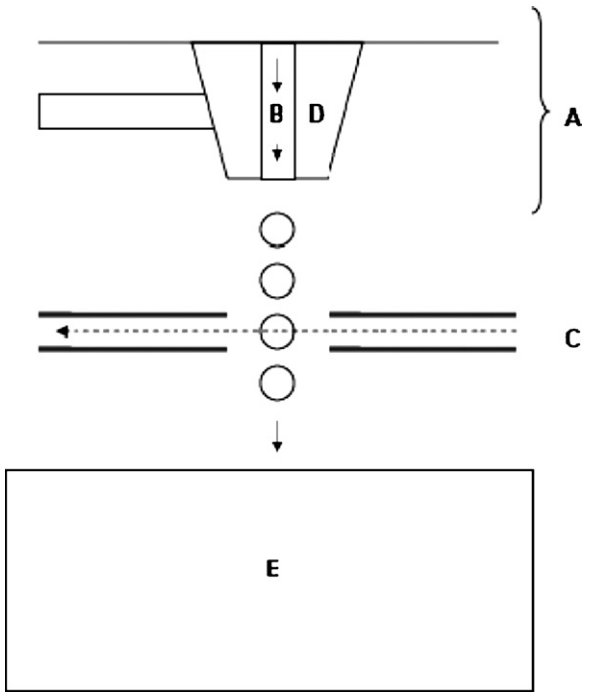

Fig. 2. Set up of the crystal gun method. (A) Two-channel droplet generator, (B) inner channel, (C) air-regulated jet, (D) second channel of co-axial air, and (E) cross-linking solution.

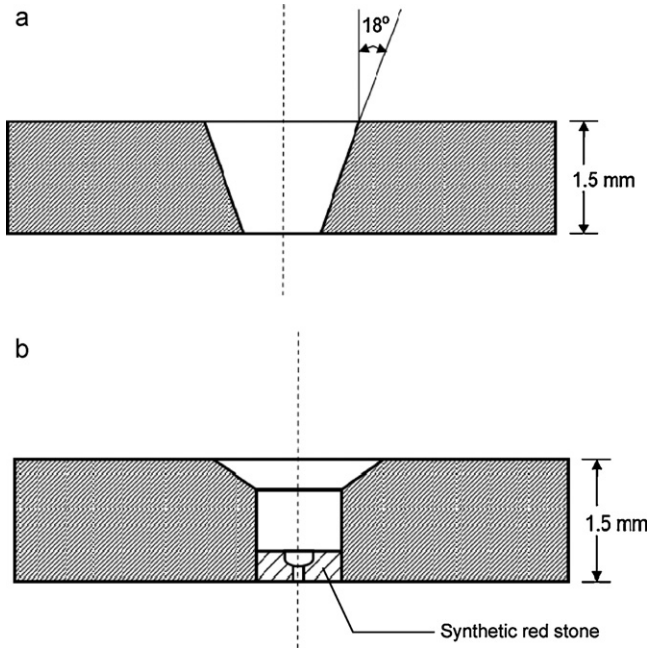

Fig. 3. (a) Cross-section of laser-drilled nozzle plate. (b) Cross-section of synthetic red stone nozzle plate.

this is essentially an improvement over the conventional air-jet method (Fig. 2). Alginate solution present in the inner channel (B) of the droplet generator (A) was extruded with the aid of co-axial air eluted from a second channel (D). Internal gelation was improved by injecting barium crystals through an air-regulated jet (C) mounted between the nozzle of the droplet generator and the cross-linking bath, perpendicular to the flow of alginate droplet stream. The internal cross-linking of alginates was not shown to affect the viability and activity of the encapsulated rat islets. In an earlier study, Berkland, Kim, and Pack (2001) discussed the use of acoustic excitation to produce spherical poly(D,L-lactide-co-glycolide) (PLG) spheres. Like the air jet method, the PLG solution was pumped through a circular orifice. An ultrasonic transducer (controlled by a frequency generator) provided mechanical agitations that broke the stream into uniform-sized droplets.

The design of the spray nozzle was recently reported to improve the gelation method (Yang, Wang, & He, 2005). An angled nozzle (18° gradient, 0.03 mm outer diameter) was laser-drilled on plates (Fig. 3a). The differential diameter at both ends of the nozzle minimized surface tension, improved alginate flow and formed smaller microspheres (Yang et al., 2005). A synthetic red stone may be incorporated to the tip of the plate nozzle to create a more uniform and round orifice (Fig. 3b). The precision of this method (as seen by the narrow size distribution of the microspheres) is promising and potentially useful for microencapsulation using alginates.

4. Alginate in wound care and therapeutics

4.1. Status of alginates in wound care

Since the first clinical use by Major George Blaine in the 1940s (Blaine, 1947), many useful applications of alginates are included in wound care management. A summary of alginates in wound care is given in Table 5. The popularity of alginate products over conventional cotton gauzes relates much to the better understanding of wound healing. One important aspect of wound healing process involves wound exudate management. A moist environment is known to promote wound healing (Lloyd, Kennedy, Methacanon, Paterson, & Knill, 1998) by facilitating epithelialization (Bishop, Walker, Rogers, & Chen, 2003). The sodium ions in wound fluids also slowly convert the cross-linked alginate dressing into a viscous sodium alginate liquid, which soothes and protects the wound. Alginate dressings are capable of absorbing large volumes of exudates while providing an adequately moist environment for wound healing (Thomas, Harding, & Moore, 2000).

Table 5
Summary of alginates in wound care.

Scope	References
Status of alginates in wound care: desirable properties	
Absorbent property	Bishop et al. (2003), Choi et al. (1999), Lloyd et al. (1998), Miraftab et al. (2003), Thomas et al. (2000), Walker et al. (2003), and Waring and Parsons (2001)
Haemostatic property	Groves et al. (1995), Lloyd et al. (1998), and Segal et al. (1998)
Immunological effects	Espevik et al. (1993), Klock et al. (1997), Orive et al. (2002), Otterlei et al. (1991, 1993), and Soon-Shiong et al. (1991)
Good clinical outcomes in wounds	Agren (1996), Attwood (1989), Bale et al. (2001), Fraser and Gilchrist (1983), Gilchrist and Martin (1983), Gilchrist et al. (1985), Kneafsey et al. (1996), Lalau et al. (2002), Lim and Tan (1992), and Sayag et al. (1996)
Alginate products and research developments	
Mechanical strength	Miraftab et al. (2003), Knill et al. (2004), and Goh et al. (2012)
Microbial containment	Choi et al. (1999), Goh et al. (2008), Iannuccelli et al. (1996), Karandikar et al. (2006), Kong (1998), and Yang et al. (2002)
Tissue repair and regeneration	
Incorporation of wound healing peptides	Bak et al. (2002) and Hashimoto et al. (2004)
Incorporation of cells	Mellor et al. (2001), Paige et al. (1996), Pannunzio Michael et al. (2005), and Shapiro and Cohen (1997)
In situ gelation of alginates	Balakrishnan et al. (2005), Fragonas et al. (2000), and Stevens Molly et al. (2004)

The absorbent ability of the dressing can be affected by the constitution of the fibers, relative proportions of mannuronic and guluronic acid residues, and content of calcium and sodium ions (Thomas et al., 2000; Walker, Hobot, Newman, & Bowler, 2003). Dressing with high mannuronate content is capable of greater uptake of moisture but the fibers are characteristically weaker (Miraftab, Qiao, Kennedy, Anand, & Groocock, 2003). Studies using scanning electron microscopy demonstrated that the fluid-retention properties of alginate wound dressing could also encapsulate pathogenic bacteria, such as *P. aeruginosa* and *Staphylococcus aureus*, under the gelled surface (Walker et al., 2003). The exudates were retained in the dressing matrices and minimized bacterial migration. The weak ion-exchange mechanism, however, resulted in uneven hydration on the contact surface of dressing. This could limit its effectiveness as an exudate retention system to reduce microbial load. Patients may also experience transient burning sensation in less exudating wounds (Thomas & Leigh, 1998). The water-retaining capability of alginate dressings had also been investigated by determining their water uptake ability, gel blocking and free swell absorbency (Choi et al., 1999; Waring & Parsons, 2001).

The haemostatic and absorbent properties make alginates suitable for wound dressing management (Lloyd et al., 1998). A recent study also suggested that the uronate content of alginates might activate prothrombic coagulation in the formation of blood clot. Tumor necrosis factor (TNF) production induced by alginates is believed to recruit fresh leukocytes from the blood and re-initiate the cascade of events leading to wound healing (Groves, Allen, Ross, Barker, & Macdonald, 1995). Zinc-containing alginate dressings were also shown to possess greater potentiating effect on

prothrombotic coagulation and platelet activation than calcium (Segal, Hunt, & Gilding, 1998). This may be related to the role of zinc ions as a cofactor for the coagulation factor XII and the intrinsic pathway of coagulation (Schousboe, 1993). Further support was found in the study by Kowalska, Juliano, Trybulec, Lu, and Niewiarowski (1994), reporting enhanced zinc ion aggregation to adenosine diphosphate (ADP) during wound healing. ADP is released from the wound tissues, which in turn activates platelets and other processes of the clotting cascade.

It was previously suggested that the immunological properties of alginates are related to the sequential structures and molecular size of the polymer (Espevik et al., 1993; Otterlei et al., 1991; Soon-Shiong et al., 1991). High mannuronic-containing alginate residues appeared to be involved in a receptor-mediated mechanism that stimulated and released cytokine in wounds while a high content of guluronic acid suppressed this activity (Otterlei et al., 1993). However, Klock et al. (1997) demonstrated that purified alginates did not show any immunological responses in animal studies and attributed this to the presence of bioactive impurities in the alginates. Alginates with high mannuronic content were reported to show greater biocompatibility and recommended as suitable materials for implants (Orive et al., 2002).

Currently, the use of alginates in wound care relies largely on the clinical knowledge that alginates promote healing through a moist environment. The wide acceptance of alginates in wound healing is also related to the positive clinical advantages shown in various studies. A prospective, randomized, controlled trial involving patients with full-thickness pressure ulcers reported better clinical outcome using alginate wound dressing when compared to topical treatment with dextranomer paste (Sayag, Meaume, & Bohbot, 1996). Treatments with calcium alginate dressings had shown good healing outcomes in various types of skin wounds (Attwood, 1989; Bale et al., 2001; Fraser & Gilchrist, 1983; Gilchrist & Martin, 1983; Gilchrist, Mitchell, & Burrows, 1985; Kneafsey, O'Shaughnessy, & Condon, 1996; Lalau et al., 2002; Lim & Tan, 1992) and animal model (Agren, 1996).

4.2. Alginate products and research developments

Alginates can be made into various forms such as sponges, films and extrudates (woven or nonwoven dressing) depending on the process of cross-linking and commercial products of alginate-based dressings are available (BNF, 2011). The present interest in alginates for wound care management involves an understanding of the advantages of alginates and the strategies are largely targeted at improving their functionality as a wound healing promoter.

One important consideration of a wound dressing will be its mechanical integrity. The dressing should be sufficiently strong during the intended use. It was reported that the composition of the alginates (mannuronate to guluronate or M/G ratio) influenced the physical attributes of the fibers formed. The effect of uronate content on the mechanical strength and tenacity of alginate fibers had been investigated (Miraftab et al., 2003). Knill et al. (2004) explored the feasibility of improving the alginate fibers with chitosan. Structurally, the cationic nature of chitosan is expected to interact strongly by ionic bonds with the anionic alginates. At the same time, the smaller molecules of hydrolysed chitosan could better penetrate into the base alginate fiber structure and reinforce the mechanical properties. The study found that the inclusion of suitable contents of hydrolysed chitosan (e.g. lowering molecular weight of hydrolysed contents) was able to enhance the tensile strength of the fibers formed. In a recent study, the effects of divalent Ca^{2+}, Cu^{2+} and Zn^{2+}, as well as added Na^{+} during cross-linking, on the mechanical properties of cross-linked alginates were investigated (Goh, Heng, & Chan, 2012). The study found that the added Na^{+} during cross-linking increased the tensile strength of the films cross-linked by Ca^{2+} but it exerted the opposite effect on those cross-linked by Cu^{2+} or Zn^{2+}. Hence, careful selection of cross-linking cations is important to ensure that the functions of the alginate dressings are not compromised.

Researchers had focused on microbial containment in wound care. Since burn wounds are ideal environment for microbial growth (Edwards-Jones & Greenwood John, 2003), some investigators had attempted to incorporate a drug onto alginate fiber/dressing surfaces to treat secondary wound infection. Testing using silver sulfadiazine-loaded alginate sponges showed good wound healing effect on Wistar rat (Choi et al., 1999). The encapsulation of gentamicin in alginate matrices for the treatment of osteomyelitis was reported to be promising (Iannuccelli et al., 1996). Implanted alginates showed biocompatibility with a complete bioabsorption and no observed signs of rejection or allergic reactions in the surrounding tissues (Gilchrist & Martin, 1983). In another strategy, anti-microbial metals were incorporated into alginate dressings or used them in combination with other anti-microbial agents to enhance the overall anti-microbial activity (Karandikar, Gibbins, & Cornell, 2006; Kong, 1998; Yang, Han, Bu, Ma, & Pan, 2002). The compatibility between cross-linking cations and commonly used topical anti-microbial agents was further studied (Goh, Heng, Huang, Li, & Chan, 2008). Divalent cations such as Cu^{2+} and Zn^{2+} were found to exhibit higher anti-microbial activities than Ca^{2+} against the skin pathogens *S. aureus* and *P. aeruginosa* and were generally compatible with common topical anti-microbial agents (Goh et al., 2008).

The hallmarks of wound management are tissue repair and regeneration. With the advent of tissue engineering, extensive work has been done to integrate wound healing components/cells with biocompatible polymers to provide an environment capable of sustaining viable cells for tissue repair. Hashimoto, Suzuki, Tanihara, Kakimaru, and Suzuki (2004) incorporated hybrid peptides (derived from laminin and elastin) into a commercially available non-woven fibrous dressing (Kaltostat®, ConvaTec). The animal studies reported epithelization and regeneration of tissues. In another study by Bak et al. (2002), the rheology of human fibronectin–fibrinogen solution was found to affect both the mechanical integrity of the fibers formed. The orientation of these fibers was important in inducing cellular response. Results from animal models suggested that transplant cells could be delivered in alginate matrices for tissue or vital organ repair (Paige et al., 1996; Pannunzio Michael et al., 2005). Human hepatocytes encapsulated in alginate matrices remained viable and are promising in liver transplant (Mellor, Chowdhury, Selden, & Hodgson, 2001). The alginate matrix could be modified by preparation process to produce a better cell delivery system. For instance, the porous alginate sponge obtained by freezing and lyophilization was better in supporting the fibroblast culture than the conventional collagen matrices (Shapiro & Cohen, 1997).

The *in situ* gelation of alginates has been mentioned in various wound management applications (Table 5). Typically, cross-linking solutions and cell-containing alginate component can be loaded separately into a dual syringe applicator. Concurrent cross-linking and gelation occur as the contents are extruded through a common orifice. The gradual gelation of alginate matrix moulds better at the wound site, making application easy and economical. The *in situ* injectable alginate hydrogel is a potentially useful wound dressing (Balakrishnan, Mohanty, Umashankar, & Jayakrishnan, 2005; Stevens Molly et al., 2004). An *in vivo* gelation technology was also reported for cartilage repair (Fragonas et al., 2000). A chondrocyte–alginate suspension was delivered directly to the injured tissue, which was then overlaid with cross-linking calcium chloride to seal the wound. No fibrosis was observed and the repaired cartilage showed good structural continuity (Fragonas et al., 2000).

5. Other pharmaceutical applications and potential issues

Most of the reported research work on alginates attempted to formulate alginates as a matrix to retard drug release. Drugs, such as ibuprofen, omeprazole and mefenamic acid, had been studied and other polymeric excipients such as hydroxypropylmethylcellulose (HPMC), lactose and chitosan included to improve the quality of the matrix tablets prepared (Choi & Kim, 2000; Gungor, Yildiz, Ozsoy, Cevher, & Araman, 2003; Miyazaki, Nakayama, Oda, Takada, & Attwood, 1995; Sirkiae, Salonen, Veski, Juerjenson, & Marvola, 1994). Release-retarding polymers, such as HPMC, could form highly viscous gel upon contact with aqueous fluids and encourage controlled release of the drug from the alginate matrix (Tadros, 2010). Structurally, the neutral cellulose groups in HPMC do not encourage any electrostatic interaction with the hydroxyl groups (OH) of alginates, but probably provided entanglements with alginates due to their long molecular structures. Hence, HPMC could also moderate and optimize the bioadhesive properties of sodium alginate for use as an effective buccal adhesive tablet (Tadros, 2010). From FT-IR studies, it was further suggested that the smaller lactose molecules could fill the interstitial spaces between the polymeric chains of alginates, interact and deprotonate the carboxylic groups of alginates (Bajdik et al., 2009). This effect would increase the repulsive forces and affect the conformation of alginates. Hence, lactose can be used to facilitate drug release from alginate-based matrix tablets. Oral controlled-release tablets, prepared by compressing the drug and a dry mixture of sodium alginate and calcium, showed gradual dissolution and progressive drug release in the gastrointestinal fluid. Another alginate formulation for oral administration included sodium bicarbonate which released carbon dioxide in an acidic medium. The gas produced increased the buoyancy of the gel network, enabling sustained release of drug (Stockwell et al., 1986). The formation of floating raft-like alginate structures on the stomach contents could mechanically inhibit gastric reflux (Davies, Farr, Kellaway, Taylor, & Thomas, 1994). The formation of alginate gels on eye surfaces had also been suggested for prolonged drug delivery (Cohen, Lobel, Trevgoda, & Peled, 1997). Alginates had also been investigated in the formulation of nanoparticles for mucosal vaccination (Borges, Borchard, Verhoef, de Sousa, & Junginger, 2005).

The therapeutic applications of alginates are not without inherent problems. Despite many years of extensive research and established information on their physical properties, the exact mechanism of alginates in wound healing is not clearly understood. Moreover, long-term applications in animal studies reported graft rejection owing to the overgrowth of fibroblasts (Lim & Sun, 1980). Fibrosis may affect the efficiency in delivering nutrients and oxygen and thus affect the viability of the encapsulated cells that form the graft (Yang & Wright, 1999). Attempts had been made to optimize the performance of alginate capsules and pellets (Fan et al., 1990; Goosen, O'Shea, Gharapetian, Chou, & Sun, 1985) but the issue of tissue rejection of implanted alginates in the long term remains to be resolved. Alginates may leach out of the dressing over time and exert an immunological effect on the body. This has practical importance in biomedical applications. Indeed, the different alginate segments were implicated in the inhibition mechanism of pepsin, a detrimental factor to the esophageal mucosa in gastric reflux (Strugala, Kennington, Campbell, Skjak-Braek, & Dettmar, 2005). The molecular weight and chemical composition of the implicated alginate segments were determined (Stokke, Smidsrod, Zanetti, Strand, & Skjaak-Braek, 1993). The C-6 carboxyl group in polymannuronate residues could be part of a conformational mechanism related to its antigenicity (Gan et al., 2005). More recently, the weight-average molecular weight (M_W 30,000–690,000) of alginates and the mole fraction (F_M 0.69–0.86) of mannuronate residues present in alginate chains were also identified as important factors relating to the immunological activity of alginates (Suzuki, Christensen, & Kitamura, 2011). However, the current research has not clearly evaluated the actual immunological impact of alginates on wound management. Perhaps, more *in vivo* studies and molecular models may help to establish the immuno-regulating mechanism of alginates and add value to the use of current alginate products in wound management.

6. Conclusion

This review has shown that the unique physicochemical properties of alginates can be utilized successfully in various medical applications. The physicochemical properties of the alginate matrix are significantly affected by the composition of the alginate. By controlling the preparation conditions, pH, type and concentration of available ions and temperature, alginate matrix of desirable characteristics can be produced for encapsulating cells and drugs. Alginate-based wound products with good stability, biocompatibility, exudate-retaining ability and antimicrobial properties can be developed for wound management.

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