Chapter

# Current Perspective and Advancements of Alginate-Based Transplantation Technologies

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## Abstract

Versatile yet biocompatible bio-materials are in high demand in nearly every industry, with biological and biomedical engineering relying heavily on common biomaterials like alginate polymers. Alginate is a very common substance found in various marine plants which can easily be extracted and purified through cheap nonhazardous methods. A key characteristic of alginate polymers includes easily manipulatable physical properties due to its inert but functional chemical composition. Factors including its functional versatility, long-term polymer stability and biocompatibility have caused alginate-based technologies to draw major attention from both the scientific and industrial communities alike. While also used in food industry manufacturing and standard dental procedures, this chapter will focus on a discussion of the both clinical and nonclinical use of alginate-based technologies in transplantation for regenerative cell and drug delivery systems. In addition, we overview the immune system response prompted following implantation of alginate hydrogels. Consequences of immune cell reactivity to foreign materials, such as inflammation and the foreign body response (FBR), are also analyzed and current and future strategies for potential circumvention of severe immune responses toward alginate-based devices are reviewed and suggested.

**Keywords:** alginate, biomaterial, foreign body response, inflammation, transplantation, pancreatic islet, stem cell, microencapsulation

## 1. Introduction

Alginate was first described in the 1880s by the British chemist E.C.C Stanford after extracting it from seaweed using a rudimentary ion exchange method [1]. After the commencement of commercial production in the late 1920s, alginate became a widely used substance in several industries due to it numerous useful and nontoxic attributes. By the middle of the twentieth century, alginate was a major contributor to the food industry as a food-stabilizer. Today, Alginate-derived products contribute to the seaweed industry which had an annual production value of over $4.1 billion USD in 2017 [2].

## 2. Sources of alginate

### 2.1 Brown algae source

Contributing up to 40% of the dry matter, a major raw source of alginic acid is derived from marine algae and kelp-like brown seaweed [3, 4]. There are several species of brown algae from which alginate can be derived; however, two species, namely *Macrocystis porifera* and *Ascophyllum nodosum*, account for most of the world's supply of alginate [5]. These species of seaweed are members of a class of seaweed that are both large and lengthy, making these plants ideal for alginic acid harvesting [5]. Unlike eukaryotic animal cells, plant cells are known to contain a rigid cell wall composed mainly of carbohydrate polysaccharide like cellulose and pectin [6]. Marine plants, such as algae, contain cell walls which are maintained by hydrocolloid polysaccharides such as alginate, carrageen, and agar which differentiate aquatic vegetation from land plants [6, 7]. These precursors can be used to cultivate alginic acid for market production.

### 2.2 Bacterial exopolysaccharide alginate source

Two types of gram-negative bacteria genera, namely *Pseudomonas* and *Azotobacter*, can produce alginate in the form of exopolysaccharides which constitute bacterial biofilm [8, 9]. For example, *Pseudomonas aeruginosa* can synthesize alginate which contributes to the mucosal buildup of biofilms along the respiratory tract of patients with the disease cystic fibrosis [10–12]. The biosynthesis of alginate in these bacteria genera are conducted through genetic expression of alginate substrate producing genes (alg), the products of which are exported to the cell exterior [9]. The isolation of marginally pure alginate from immobilized bacterial cell extracts has been reported, but the progress of advanced bacterial alginate isolation techniques has been slow overall [13, 14]. Although these bacteria can be utilized as a source of alginate, the main commercially available form of alginate is derived from brown algae sources.

### 2.3 Alginate extraction

Alginate exists *in vivo* as a mixture of different alginate salts including magnesium, strontium, or calcium within the intracellular matrix of brown algae tissue [4, 15]. Alginate is the conjugate base of the alginic acid and is formed upon treatment with alkaline medium [4, 16]. The primary extraction of alginic acids from brown seaweed is performed using an alkaline ion exchange treatment method which can extract and separate each of the major carbohydrate constituents of the brown algae [8, 17]. The initial extraction of algal particles from brown algae results in semi-pure fractions of differing polysaccharides which include the salt form of alginate [18, 19]. A chemical purification process, usually $CaCl_2$ purification, involves treatment of algal particles with a mineral acid followed by neutralization with strong base (**Figure 1**). Alginate precipitates are formed via further ion transfer acid-base reaction which results in a product of mainly sodium alginate [4, 8, 18]. The sodium alginate form of alginic acid is the most favored form produced after extraction which is mainly due to cold water solubility [16]. Current techniques examine the quality and purity of extracted alginate during extraction and purification processes through several chemistry-based analytical assays including nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy [16, 20]. Recent advances in computational modeling have allowed

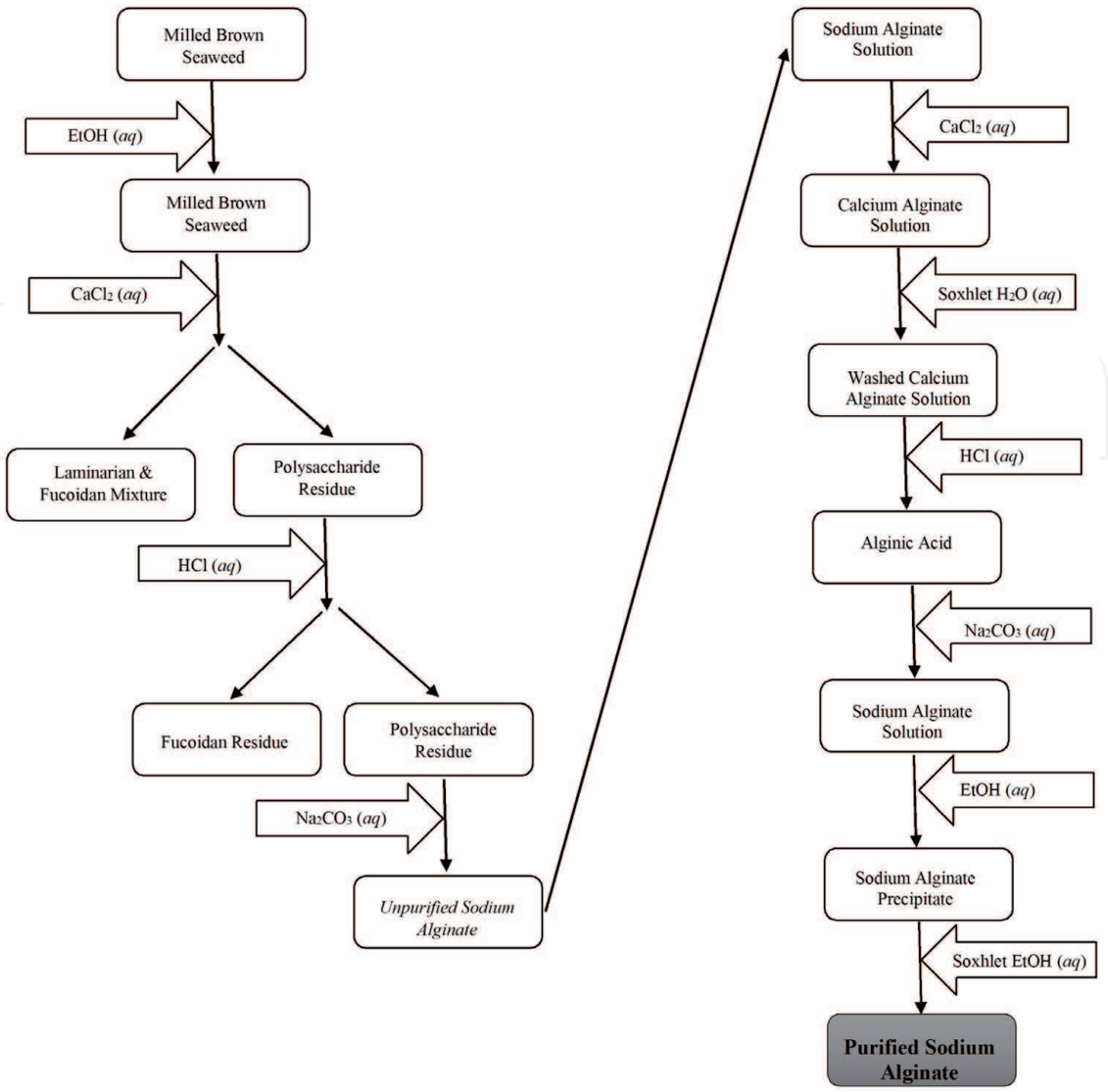


**Figure 1.**
*Extraction of sodium alginate from brown seaweed followed by sodium alginate purification via $CaCl_2$ purification.*

researchers to construct models of precipitation stages which has allowed for optimization of the extraction and purification process overall [20].

## 3. Composition of alginate

### 3.1 Types of alginate polymers

Alginate is an unbranched copolymer of β-D-mannuronic acid (M group) and α-L-guluronic acid (G group) commercially derived from algae [8]. Hydroxyl and carboxyl groups are abundantly distributed across the polymer, giving rise to a plethora of chemically modifiable sites [21]. Commercially, alginate is blocked in either consecutive M, consecutive G, or alternating M & G [22]. Given that it is a polymer with versatile properties, alginate can be easily changed to exhibit a variety of properties tailored to the individual needs of an implant. Pre-existing properties of alginate are able to be chemically enhanced through manipulating the percentage of M and G in the material, adding immunoprotective layers that impede diffusion, as well as divalent cation crosslinking. Under dicationic conditions, viscous alginate transforms

into a gel, which can then be used as an encapsulation medium for islet cells [23–25]. M groups lie with the main ring in an equatorial conformation, with each mer connected covalently through an ether (**Figure 2**). With dications situating on top and on bottom of each layer, a "swelling" effect is noted as there is no discrete pocket between mers resulting in each linear polymer stacking on top of the crosslinking dication. In contrast, G groups lie with the main ring axial (**Figure 3**). These mers are also attached through an ether. However, these ethers create pockets large enough for dications to situate without creating a swollen stacking effect. Manipulation of molecular weight and composition (%G or %M) leads to varying strength and porosity of the gel [26, 27]. Generally, alginates with higher %G have higher comparative levels of elasticity and stability [22].

Along with *Macrocystis porifera* and *Ascophyllum nodosum*, another type of brown seaweed that is a main source of alginate is called *Sargassum polycystum* C. Agardh. One study extracted alginate from *S. polycystum* to examine its chemical makeup and viscosity in the leaf, stem and thallus regions [28]. Thallus of seaweed refers to undifferentiated tissues where nutrient uptake and gas exchange occurs [29]. Of the structures studied for *S. polycystum*, the leaf region was found to have the highest alginate yield followed by the stem region and the thallus [28]. In contrast, the viscosity of alginate was highest in the stem region, followed by the leaf region and thallus [28]. Of the M/G ratios observed, the stem region had the lowest M/G ratio and the highest level of G-block content, optimal properties of forming a rigid and stable gel [28]. Similarly, other commercial brown seaweeds such as the stems of *Laminaria hyperborea* have a high G-block content (70%) and a low M/G ratio (0.43) [30].

### 3.2 UPLVM

Purified alginate containing almost entirely M blocks with little to no G blocks will typically not yield the ideal type of hydrogel desired through dication crosslinking. The lack of pockets available for crosslinking will result in a form of layers of alternating alginate polymers and dications. In terms of structural integrity, UPLVM alginate will be less porous and strong, allowing higher malleability. In addition, although there has been no clear consensus, studies have suggested that a greater percentage of M blocks in alginate cause an increased immune response compared to UPLVG alginate, as indicated by increased production of TNF-α, IL-1 and IL-6 [31].

### 3.3 UPLVG

Alginate with a higher %G blocks (>60% G) will result in a material a higher polymer strength and available crosslinking sites for barium atoms [27]. Since there are sites for divalent cation network crosslinking, the cations will situate themselves in the "egg carton" that the G blocks form. The cations in this position

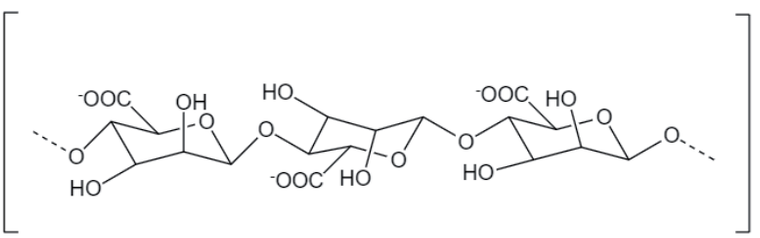


**Figure 2.**
*Chemical structure of M-block monomer.*

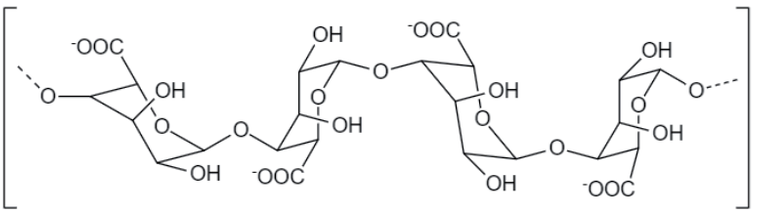


**Figure 3.**
*Chemical structure of G-block monomer.*

create a resistance to shear and frictional forces that the layers undergo as alternating molecular weight increases [32]. Therefore, this orientation allows for higher density, strength and porosity. The carboxylate anions that lie equatorial in G blocks are usually protonated under acidic conditions, which are deprotonated as the basicity of the solution increases [33]. The alcohol groups also present in the polymer provide a hydrogen that is capable of being deprotonated in basic conditions. Although this pH change resistance results in an increased stability of G-rich polymers against depolymerization, pH changes in solution will result in swelling of alginate capsules [34].

### 3.4 Mixtures

Alginate is commercially sold as a mixture of M & G blocks with varying purification. NovaMatrix in Norway is a widely used company that manufactures and distributes purified alginates [34]. Their UP LVG (ultrapure low viscosity, high G) alginate has at least a 1.5:1 ratio of G:M blocks, with UP LVM (ultrapure low viscosity, high M) alginate having a ratio of G:M of less than 1. NovaMatrix recommends a shelf life of 5 years for their UP LVG alginate while only 3 years for UP LVM alginate. Both types of alginate should ideally be stored in a fridge and maintained at a relatively neutral pH (between 5 and 8) so as to decrease the rate of depolymerization. Higher temperatures will result in an increased rate of polymerization, which is expanded even further in the presence of an acid or a base. A neutral pH will prevent acid or base catalysts from recruiting oxygen to initiate oxidative-reductive depolymerization. Both M-rich and G-rich alginates react with no significant difference when base catalyzed, but M-rich alginates are hypothesized to be more vulnerable to acid catalyzed depolymerization, as supported by a catalytic constant that is twice as large for M-rich alginates than that of G-rich alginates [35]. In addition, in the presence of oxygen and an acidic or alkaline environment, the phenol and carboxylate groups will react in undesired ways, resulting in free radical depolymerization.

### 3.5 Alginate derivatives

Alginate derivatives have also been created to encompass certain properties. One example of this is amphiphilic alginate derivatives that utilize the alginate backbone and synthesize it with hydrophobic moieties [8, 36]. An example of this is sodium alginate being conjugated with long alkyl chains via ester bonds [36]. The microparticles for the derivative are prepared using dispersion in a sodium chloride solution; this allows for encapsulation of proteins and their subsequent release by adding surfactants to disrupt intermolecular hydrophobic junctions or esterases that hydrolyze the ester bond between the alginate backbone and alkyl chains [36]. Other types of derivatives include dodecylamine conjugated to an alginate backbone via amide linkage which leads to long-term stability in aqueous media compared to other alginate derivatives composed of dodecyl esters which have

a risk of hydrolysis [36]. Amphiphilic alginate derivatives that are water soluble have also been developed using cholesteryl groups which are synthesized using N,N′-dicyclohexylcarbodiimide as a coupling agent and 4-(N,N′-dimethylamino) pyridine as a catalyst at room temperature [37]. Other derivatives include cell-interactive alginates which are composed of cell-adhesive peptides. To prepare these, carbodiimide chemistry is used to combine peptide side-chains with carboxylic groups on sugar molecules [36]. This is important because alginate must acquire the proper ligands to participate in cell-to-cell interactions since it does not innately have cell-adhesivity to mammalian cells [36]. The peptide sequence arginine-glycine-aspartic acid (RGD) has been studied widely due to the copious integrin receptors for this ligand on a variety of cell types [38]. This, among other types of cell-interactive alginates, has been used for scaffolds for tissue engineering and 2-D and 3-D cell culture matrices.

## 4. Current biotechnological uses

### 4.1 Food industry applications

The alginate industry saw increases in production in response to the scientific advancements in the study of alginate properties and potential uses [39]. Currently, the FDA classifies alginate as meeting the guidelines of the Food Chemical Codex and lists over seven different uses of it in the food industry [40]. The primary market for alginate in the processed food market where they serve as texturing agents and food stabilizers [41]. The gel-like chemical properties, which are safe for consumption, also allow for several other uses such as increasing storage life of potatoes, immobilization of banana enzymes, immobilization of lactic acid bacteria, and fillers in meat and fish products [39, 42]. The ability of alginate hydrogel to be polymerized, or gelled, into a microcapsule with a hollow core has led to the development of food encapsulation technologies aimed at food preservation [42]. Recently, researchers have shown that application of an alginate coating on egg shells has led to the elimination of cross-contamination from *Salmonella enteritidis* for up to 42 days [43]. Currently in the beverage industry, alginate is used as an emulsifying agent to maintain the stability of foam within beverages like beer [44, 45]. Continued use in the food industry will likely increase the commercial demand of alginate in the future.

### 4.2 Pancreatic islet cell-delivery system

In 1933, Biscegeli implanted polymer matrix coated murine-derived tumor cells in to the abdomen of guinea pigs which remained viable without rejection, thus discovery prompted entire fields to research into microencapsulation technologies [46]. Several advances in biotechnological approaches to both cell microencapsulation and the development of drug delivery systems using alginate have been achieved for nearly 50 years [47]. When reconstituted in aqueous solution, alginate forms a hydrogel which in the presence of polyvalent cation (e.g., $Ba^{2+}$, $Ca^{2+}$, $Sr^{2+}$) reorganizes into a rigid biomaterial [4]. Alginate hydrogels can be used to encapsulate cellular tissue and nonliving substances for therapeutic cellular-delivery as a treatment to many diseases such as Type 1 Diabetes [48]. Xenograft transplantations for type 1 diabetes heavily rely on alginate hydrogels as a source biomaterial for encapsulation of porcine pancreatic islets. Key obstacles observed with porcine islet xenograft include possible transmission of hemagglutinating

and lymphocytotoxic antigens, such as carbohydrate α-(1,3)-galactose, which are a major contributor to acute host immune rejection [49–51]. Alginate encapsulation, in contrast to other biomaterials, reduces transmission of humoral immune response elements while allowing for the transport of nutrients and export of insulin across the semipermeable barrier [52]. Recently, alginate-encapsulated APIs were transplanted intraperitoneally into STZ-induced IDDM nonhuman primates. Nonhuman primates exhibited a marked decrease in exogenous insulin demands and lower %HbA1C in addition to normalized blood glucose values for 20–70 days. However, the glycemic control was reduced over time, which they concluded was due to decreased graft function and viability [53].

### 4.3 Stem cell-delivery system

With the application of FDA regulations and recent advances in stem cell-related technologies, there has been an increase in need for viable targeted delivery system for therapeutics [54–57]. The biocompatibility and relative ease of production of alginate hydrogels makes alginate an important candidate for production of immune-isolated stem cell delivery systems [8, 58, 59]. A major disease model currently researching stem cell-related therapies is for the treatment of type 1 diabetes, among insulin-dependent conditions. Recently, insulin-producing human SC-derived β-cells encapsulated with alginate polymers were implanted in immune-competent mice. Multiple studies report glycemic control with immuno-isolated β-cells in mice, some resulting in up to 174 days of glycemic correction, in addition to detection of human C-peptide in mouse serum [60, 61]. A 2019 study showed similar results after transplanting human pluripotent stem cell-derived (iPSCs) β-cells into immunocompetent mice which resulted in glycemic control for more than 150 days [62]. Other disease models have also used alginate microencapsulation for therapeutic use of xenogeneic stem cell-delivery. Researchers have reported functional alginate encapsulated adrenal SCs for use in adrenal hormone insufficiency diseases [63, 64]. Successful alginate encapsulation of neural embryonic stem cells was also reported for targeted cell-delivery serving possible treatment for neural tissue repair in several neurological disorders [65]. Microencapsulation of stem cells is a very active field of research and advances with this technology have a wide range of clinical applications.

### 4.4 Cryoprotective use of alginate microencapsulation

Transplantations can sometimes rely heavily on transportation or storage of tissues before the procedure. Cryopreservation, known as a viable option for tissue preservation and storage, can have severe effects on the viability and function of tissues during the freezing/thawing processes and from use of cryoprotective agents [66]. Alginate microcapsules have demonstrated the ability to maintain structure, slow cooling process, reduce effects of ice crystal formation on tissue, and regulate influx of cryoprotective agents during cryopreservation [67, 68]. First used in preservation of plant cells, use of an alginate coating has been observed to have cryoprotective properties [69–72]. Success with plant cell preservation catalyzed the research into alginate cryoprotective potential with other tissue types. Alginate coatings of beneficial probiotic bacteria have demonstrated maintenance of viability and function through the cryopreservation process which has potential applications in yogurt and probiotic preservation [73–75]. An augmented necessity of tissue preservation methods has caused a rise in research related to cryopreservation for storage and distribution of animal cells. Alginate microencapsulation of cells offers significant

degree of cryoprotection during cryo processing [76, 77]. Transplantation research has particularly benefited from use of alginate technologies during cryopreservation. Recent studies in pancreatic islet transplantation have demonstrated that use of alginate microencapsulation helps maintain islet viability during preservation and improves islet secretory function during transplantation [67, 78, 79]. A 10 years study using encapsulated rat, pig, human islets showed that alginate encapsulated islets maintained significantly higher viability, secretory function, and yield compared to nonencapsulated islets after cryopreservation for 10 years [80]. Like pancreatic islets grafts, cryopreservation is also used for preservation of stem cells before transplantation. Alginate encapsulation has been shown repeatedly to improve both yield and function of multiple stem cell lines, including human stem cells [81, 82]. Recently, the use of alginate microcapsules improved viable recovery of human adipose-derived stem-cells after 72-h storage in hypothermic conditions [83]. The overall benefits of cryoprotection via alginate encapsulation will ensure relevance in future research of cell preservation and transplantation.

### 4.5 Conjugated alginate potential as drug-delivery system

Due to the relative feasibility of conjugation and semi-permeable wall structure of alginate microcapsules shows promising secretory capabilities for robust nanoparticle drug-delivery systems [8, 84–86]. Recently researchers have been able to conjugate alginate microcapsules with anti-HIV zidovudine nanoparticles. Results showed significant improvements in internalization of the nanoparticle into glioma cells during *in vitro* experiments marking for potential use as a targeted viral drug delivery system [87]. Another study demonstrated the conjugation of sodium alginate with graphene oxide which is known to create functional groups for synthesis. GO-conjugated alginate hydrogels readily loaded the anticancer drug oxorubicin hydrochloride (DOX·HCl) and caused high cytotoxicity when exposed to immortal HeLa cell lines [88]. When islet containing alginate microcapsules were conjugated with VEGF, increases in angiogenesis, islet viability, and islet function were observed [89]. More recently, similar benefits were observed through increases in bone formation and blood vessel growth after biomineral-conjugated alginate microcapsules containing MSCs were transplanted into sheep with ovine iliac crest bone defects [90]. The versatile conjugation behavior of alginate hydrogels has impressive potential for a variety of future medical applications in transplantation.

## 5. Immune response

### 5.1 Alginate purity effect on immunogenicity

Crude unpurified alginate has been shown to provoke the immune system which then causes fibrosis around the microcapsule [91]. This overgrowth of immune-cells and fibroblasts interferes with the transfer of nutrients, hormones, and other proteins via the semi-permeable membrane of the capsule [92]. This is a difficult problem to avoid especially since the industrial processing used for extraction can leave contaminates in the alginate like polyphenols, endotoxins and algal proteins [92, 93]. Purification is a technique utilized to minimize the amount of contaminations in the alginate gels. Commercially available sodium alginate can be purchased in several different purified states including “ultra-purified” form from companies like Novamatrix® for use in hydrogel production [94–96]. Although this is highly purified there are still small concentrations of contaminants in the alginate that can prompt a severe foreign body response [91, 97]. If the alginate biomaterials

hold living tissue within a drug-delivery system, the foreign body reaction tends to reduce the survivability of those tissues [92, 98].

### 5.2 Inflammation and the foreign body response

The human immune system is broken into two parts, the innate and adaptive immune systems, which both work in tandem to defend and destroy external bacteria, viruses and various other microbes that may infiltrate a host's body [99]. The first aspect of the foreign body response (FBR) is the innate immune system which has no flexibility or memory to foreign antigens. This is followed by the adaptive immune system which generates a custom response to each specific pathogen while simultaneously building an immunological memory [92]. Alginate hydrogels used in implantations are highly purified so as to not present antigens on their surface and thus should not cause T cell activation due to antigen-presentation [100]. Despite lacking these immunoreactive properties, within nanoseconds of entering a host, biomaterials initiate an inflammatory FBR due to biomaterial surface interactions with tissue, proteins, and blood [100, 101]. The foreign body response results in fibrotic overgrowth surrounding the implanted alginate hydrogels which essentially isolates the biomaterial from host's body [102]. This fibrotic overgrowth is initiated by a sequence of cascading events including injury formation, recruitment of immune cells and fibroblasts, and cell adhesion which leads to fibrous tissue deposition [103]. Two stages of inflammation, namely acute followed by chronic, facilitate the foreign body response once alginate biomaterials are implanted [102].

It was determined that the immunogenic properties of alginate polymers were not directly responsible for the overgrowth around capsules but do contribute to is formation. In order for the immune cells to adhere to the cell surface, there must be a significant amount of protein adsorption or anchor sites for immune cell adhesion [92, 102]. Instead, the immune response occurs in the tissue immediately surrounding the alginate structure [92]. Studies have demonstrated that within a few days after alginate biomaterial implantation into animals, immune cells and differentially activated macrophages (i.e., stadia, granulocytes, and basophils) collect around, but not on, the alginate surface [92, 104]. This acute inflammatory period is characterized by the recruitment of macrophages and neutrophils in addition to the release of histamine and fibrinogen adsorption from mast cells to surrounding tissue [92, 102]. This is all done without significant immune cell adhesion to alginate biomaterials. A key player in immune cell recruitment are pattern recognizing receptors (PRRs) which act as sensors on cells of the innate immune system by responding to evolutionary conserved molecules with can contaminate alginate biomaterials [92]. Even after purification, lipoteichoic acid and LPS, the most commonly known endotoxin, can remain and are recognized by Toll-like Receptors (TLRs) [52]. It has been demonstrated that recognition of PAMPs by TLRs causes the activation of macrophages through the NF-κB pathway [105]. NF-κB results in the transcriptional upregulation of genes most associated with cytokine and chemokine production which serve to intensify humoral response [105]. Within a week, the initial acute inflammatory period transitions to a chronic inflammatory period.

The chronic inflammatory stage is characterized by the accumulation of lymphocytes and monocytes, among other mononuclear cells, in the tissue surrounding the alginate biomaterial [106, 107]. It was discovered *in vitro* that lymphocytes can adhere to alginate biomaterial surfaces, but when placed in co-cultures with macrophages, they appeared to attach predominantly to the macrophage rather than the alginate microcapsule surface [100]. Further research has suggested that macrophage adhesion and fusion to biomaterial surface is

assisted by T lymphocytes [100]. Both macrophages and lymphocytes secrete inflammatory mediators. Examples of these include cytokines IL-1β, IL-6, and TNFα and chemokines IL-8, MCP, MIP-1β, and ENA-78, all of which are known to activate and recruit inflammatory effector cells such as neutrophils, monocytes, T lymphocytes, and natural killer cells [100, 105]. Similarly, it was discovered that certain cytokine, chemokine levels, matrix metalloproteinases (MMPs) and tissue inhibitor MMPs (TIMP), fluctuated over time which suggests that T lymphocyte-macrophage interactions have the ability to facilitate the chronic inflammatory phase of the FBR [100, 108]. There have been *in vivo* studies that have shown active T cells in response to synthetic materials during an inflammatory response; however, *in vitro* studies have shown a lack of lymphocyte response to alginate biomaterials [100, 109]. A novel focus of study regarding mitogens, which are functional groups on the surface of biomaterials, led to the hypothesis that they can possibly trigger lymphocytes via cross-linking of glycoproteins, but these mitogenic properties have not been observed [100]. The chronic inflammatory period usually subsides 2 weeks after implantation and any longer periods of inflammation are usually the result of infection [100, 102]. A combination of granulation, fibroblast infiltration, and macrophage/fibroblast surface adhesion result in the final fibrotic encapsulation of alginate biomaterials.

### 5.3 Immuno-modulation of alginate hydrogels

To overcome the challenges of inflammation and fibrosis associated with the foreign body response after alginate hydrogels are implanted, researchers are currently trying to establish technologies for potential immune modulation. Research into alginate immune-modulation methods currently involves the use of bioactive nanoparticles, immune regulatory cells, hydrogel surface topography, and passive non-fouling agents [110]. In the past bioactive nanoparticles, like ketoprofen, have shown potential to modulate the degree of inflammation during FBR [111]. A recent study was able to load monoclonal antibody IgM nanoparticles during alginate encapsulation of pancreatic islets. This resulted in localized controlled release of the monoclonal antibody after implantation which decreased the immune response against transplants [112]. An islet transplantation study using the anti-inflammatory nanoparticle rapamycin, demonstrated reduced fibrosis and sustained glycemic control improvement [113]. Mesenchymal-derived stem cells have been shown to have a wide range of regenerative effects on several tissue injury types because of their multipotent capacity [114]. Several recent studies have demonstrated immune modulation of alginate hydrogels through co-delivery of MSCs with cell-delivery systems. This can help reduces inflammation and can modulate T-cell toxicity while also boosting wound healing properties and blood vessel formation of the graft site [115–117]. A better understanding of the mechanism of FBR in the future will help improve immune modulation technologies for transplantations involving alginate hydrogels.

## 6. Clinical relevance of alginate-based biomaterials

### 6.1 Type 1 diabetes mellitus treatment

While extensively performed in animal models for several decades, islet xenotransplantations have taken more time in advancing to clinical human

studies around the world. This is because the area of xenografts in general is still a highly regulated area in the transplantation field and is being studied heavily in animal models [118, 119]. In the United States, the stringent FDA regulations for all xenotransplantations has provided certain standards which must be achieved pre-clinically before moving to clinical trials [120]. This has made initiation of clinical trials involving alginate xenografts very slow in the United States. Nonetheless, here have recently been a small amount of clinical islet xenotransplantation trials that have been conducted outside of the US using porcine islet xenografts to treat IDDM in human subjects (**Table 1**). The first phase I/IIa clinical trial saw 7 T1DM patients receive up to 3 implantations of encapsulated neonatal porcine islets which resulted in 5 patients with improved blood glucose levels and two patients which achieved insulin independence for up to 32 weeks post-transplantation [121, 122]. From 2000 to 2003 in a clinical trial in Mexico, two cohorts totaling 21 IDDM patients underwent xenograft implantation of a collagen-covered pre-vascularized scaffold which housed 250,000 islets with 30,000–100,000 Sertoli cells. After the first transplantation HbA1C showed significant reductions, with decreases in exogenous insulin demands observed for up to 4 years, and glucose stimulated C-peptide detected in urine for up to 4 years; in addition, zoonotic transmission of porcine microorganisms was not detected for the duration of the study [123–125]. Unfortunately, the human clinical trial in Mexico did not include a control population under exogenous insulin therapy that could further validate the results [121]. In patients who received a steroid-based regimen 33–62% decrease in insulin requirements for up to 1 year with detectable presence of porcine C-peptide compared to two patients, which received steroid-free regimen, who saw change in IDDM conditions [126]. A comprehensive 2011 study, 14 IDDM patients were given 5000–20,000 IE/kg intraperitoneally via laparoscopy using wild-type pre-weaned juvenile porcine islets without immunosuppressive therapy for 52 weeks [127]. Overall, 8 of the 14 patients showed a moderate decrease in insulin administration and HbA1C levels. A nonsignificant reduction in severe hypoglycemic events, or unaware hypoglycemic episodes, was also observed in the same patients [127, 128]. Alginate-based macrodevices have been the topic of heavy research since the late 1940s and recently have been tested clinically as a scaffold for therapeutic islet-delivery [115]. Patients with T1D have been subcutaneously implanted with macroencapsulated, bioartificial pancreatic βAir (BetaO2 Technologies Ltd., Israel) device; the study designed to monitor for 180 days post-transplantation followed by removal of the device, and 6 months of follow-up afterwards. The device involved oxygen pumps which allowed for daily refueling of oxygen to devices while in-vivo. For the duration of the study, islets in the device did not face any immune rejection and survived to time of explant. Although there were no rejections in the body, the overall efficacy of the islets was low and metabolic control was absent; C-peptide levels were detected on day 1 of post-transplantation in all patients (range 0.028–0.093 nmol/L) and remained detectable up until 2–4 weeks but for one patient detectable up to 3 months post-transplantation [129, 130]. Continued research into alginate immune-isolation of pancreatic islet xenografts will continue to develop and will be tested clinically in the future.

### 6.2 Wound healing treatment

Alginate has shown effectiveness as a constituent of acellular matrix skin grafts to reduce wound healing duration, which is especially poor with diabetic patients

| Number of subjects (n) | Porcine islet types used | Number of islets transplanted/ kg of body weight | Transplantation site | Study outcome | Reference |
|---|---|---|---|---|---|
| 12 | Neonatal + Sertoli cells encapsulated in scaffold | 14,000–21,000 | Scaffold in subcutaneous space | • Deceased exog- enous insulin requirement for up to 4 years (50%)<br>• No serum C-peptide detected | [124, 125] |
| 7 | Encapsulated neonatal | 5000–1000 | Intraperitoneal space | • Two patients achieve independence for 32 weeks<br>• 6 Patients demonstrate lower HBA1c | [154] |
| 1 | Encapsulated fetal | 15,000 | Intraperitoneal space | • -12-week period of 30% reduction in insulin requirement<br>• Improvement in glycemic control for 14 weeks<br>• Detectable C-peptide for 11 months<br>• Live Islet detected for 9.5 years | [127] |
| 14 | Encapsulated pre-weaned | 5000–20,000 | Intraperitoneal space | • Moderate decrease in insulin requirement in 57% of patients<br>• Major reduction in hypoglycemic episodes | [127] |
| 8 | Encapsulated pre-weaned | 5000–20,000 | Intraperitoneal space | Patients that received 20,000/kg maintain 7% HbA1c for 600 days. Reduced Hypoglycemic events | [123] |

**Table 1.**
*Selected clinical encapsulated islet xenotransplantations in humans. IE is used to denote islet equivalents that make up the graft which are implanted during transplantation.*

[131–133]. A large multicenter study involving 75 patients with 100 wounds (50% UT grade 3 wounds) was conducted using an alginate containing GraftJacket® matrix skin graft. The study resulted in 91% of complete wound closure averaging around 9.6 weeks indicating the graft was a safe option for complex lower extremity wounds [134]. Recent clinical studies with an alginate containing skin graft known as AlloPatch® have shown the effectiveness in wound healing even when elevated HbA1C levels witnessed in one patient [135]. Several alginate-based skin grafts are commercially available for advanced wound healing which include Nu-Gel® (Systagenix), Tegagel® (3M GmbH)), Curasorb® Alginate (Medtronic), Sorbsan® (B. Braun Melsungen AG), and Kendall™ Hydrocolloid Dressing (Medtronic) [136]. Advances in the development of alginate matrices will continue to contribute to skin regeneration therapy improvements in the future.

### 6.3 Clinical uses in dentistry

The hydrophilic nature of alginate along with hydrogel forming and biocompatible nature has made it a staple hydrocolloid for application in the field of dentistry [137]. Alginate-based gels have traditionally been used in dentistry to provide the dentist with impressions of teeth and bitemarks which can then be used as a template for dental implants [138–140]. Alginate is frequently used in clinical trials to obtain impressions when comparing various dental procedures [141–144]. In a recent clinical trial, silicone was found to be favored among patients in terms of comfortability although alginate impressions were found to be a cheaper procedure option [145, 146]. Alginate-containing matrices have also been tested for advanced periodontitis treatment, when compared to the normal 0.2% chlorhexidine treatment alginate-containing Emdogain® saw significant reduction in plaque viability [147]. These alginate-containing enamel matrices have also shown significant clinical regenerative capabilities for periodontal disease symptoms such as intrabony defects and gingival recession [148, 149].

## 7. Future direction for alginate-based biotechnologies

The development and use of alginate macro/micro devices for implantation have met both improvements and challenges. Specifically, alginate has been used in the production of microcapsules as well as scaffolds as a way to implant encapsulated islet cells in *vivo*. The use of natural polymers such as alginate introduces immunogenic and foreign body responses, which impact the functionality of the device over time [150]. For example, the mechanical integrity or structure of the device could be compromised, or it can decompose while inside the recipient. As a potential solution, alginate can be chemically modified so its properties can be better controlled against inflammatory response and mechanical stress [151]. This chemical modification includes both ionic and covalent crosslinks within alginate polymers which will increase the mechanical stability of both scaffolds and microcapsules [151]. This method of using two types of crosslinking to create the microbeads compared to using only covalent crosslinking has shown results of greater stability of these microcapsules within cells after weeks following implantation [151]. Furthermore, in the long run, purification techniques of alginate hydrogels can be improved to decrease PAMP concentration which would help reduce FBR [92]. Purifying alginate gels of PAMPs can be difficult for those with a high G content, which are stronger and more viscous. However, the use of chemical extraction or dialysis methods as well as specific purification methods would be required to remove these molecules. Another potential

solution to overcome graft failure is to use smaller islet cell graft volumes with vascularized membranes to produce stable neovascularization near the grafted tissue [152]. Using a macroencapsulation device such as TheraCyte and loading it with islet cells of smaller volume can increase blood supply near the site of encapsulation [152].

Given the molecular composition of alginate and its ability to crosslink and form hydrogels, this biomaterial can be used in an array of medical and clinical applications. Unfortunately, the implantation of alginate induces an immune response of the host, producing setbacks that current labs are attempting to solve. Because of both macrophages mediated and T-cell mediated immune response, the alginate macro/micro device loses its viability and long-term function once it is implanted in the host. Strategies aiming to reduce or prevent fibrosis on alginate encapsulated cells present conflicting results. Newly developed capsules contain alginate without a polycation layer shown improvement potential because the pro-inflammatory characteristic of polycations have been connected to fibrotic growth around capsules [153]. For alginate beads, removing the polycation layer can lead to less stability and more permeability to cytokines and growth factors [153].

Currently, the main focus regarding implantable alginate hydrogels is to address problems such as immune response, adequate sources of oxygen, and fibrosis. As more alginate-based devices are being brought into clinical application, these strategies among others should be considered and pursued for current and future alginate-based device production.

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## Conflict of interest

The authors declare that there is no “conflict of interest” with respect to this review.

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