Chapter

Microencapsulation for Clinical Applications and Transplantation by Using Different Alginates

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Abstract

Microencapsulation has been the most frequently used technique for several different disciplines such as cell-based therapies and/or transplantation. Technology is based on the idea of combining and coating a material or isolating from an external source. Microencapsulation may be performed with different materials and, among natural biocompatible materials, alginate-based microencapsulation technique is the most appropriate material for microencapsulation. The structural components of alginate materials are the derivatives of alginic acid, which is found in brown algae as an intercellular gel matrix. This alginate is preferred for clinical applications due to its safety in human studies. Therefore, the choice and the combined system need to be carefully optimized to achieve biocompatible application through cell microencapsulation especially for long term. Specifications of alginate such as primary source, isolation process, viscosity, and purity contribute to improve its biocompatibility. Clinically, cell microencapsulation is the major contribution to the field of transplantation by its technique and additionally provides local immune isolation. This chapter discusses the potential benefits of clinically suitable alginates and their applications. This promising technology may highlight its considerable potential for patients that require transplantation and/or replacement therapy in the future.

Keywords: microencaspulation, alginate, cell therapy, drug delivery, transplantation

1. Introduction

Cell encapsulation is a process that involves immune protection of the living cell by using different polymers. The polymers can be distinguished into two main groups: natural origin (i.e., polysaccharides, polynucleotides, polypeptides) [1] and synthetic polymers (i.e., polyethylene glycol, polyvinyl alcohol, polyurethane, etc.). Several attempts have been made by scientists to use natural, synthetic, and semi-synthetic polymers in the field of encapsulation. The first approach was made in 1933 by Bisceglie et al. and used enveloped membrane to demonstrate tumor cell survival in the abdominal cavity of guinea pigs [2]. In his report, the cells survived for 12 days by diffusion of the nutrients. However, at that time immunoisolation technology was not known or understood [3]. Later, in 1943 Algire et al. reported a transparent chamber for atherapeutic approach *in vivo* [4]. Since this report, therapeutic demands enhanced this encapsulation technology in a way that combines the polymer source (produced synthetically or isolated from natural sources) and their functionality by using its characterization.

Several advantages and disadvantages have been reported about synthetic polymers [5]. Mechanical specifications can be more easily engineered or modified with the desired characteristics and particularly can be produced with larger amounts [6–8]. The main deficit of synthetic polymers is that they require toxic substances during the capsulation process; therefore, cell viability is a true obstacle after encapsulation [8, 9]. In this regard, synthetic polymers are frequently used in combination with different devices such as macrocapsules. Before accommodation of the encapsules, first, synthetic polymers are manufactured in the absence of living tissue/cells, and second, tissue/cells are combined with the device to preserve direct contact of the toxic solvents [1]. The most common synthetic polymers are poly(ethylene glycol) [10], polyvinyl alcohol [11], polyurethane [12], poly(ether sulfone) [13], polypropylene [14], sodium polystyrene sulfate [15], polyacrylate [16], polyphosphazene [17], AN69 [18], and lastly polytetrafluoroethylene [19].

Natural polymers have been proposed for immunoisolation based on two distinct features. First, there is no interference with the functionality of cells/tissues and second, the stability of the structure they provide during encapsulation [1, 20]. Conventionally, the most frequently used polymers from natural sources are cellulose [21], chitosan [22], collagen [23], agarose [24], and alginate [25]. The experimental success mainly depends on the application and mimicry potential of the natural polymers. Among these, in this chapter, we mainly focused on the alginate-based encapsulation and its clinical application.

2. Alginates

The most versatile biomaterials among natural polymers are alginates, which are used in a wide range of applications including diffusion systems, drug delivery, as a wound dressing, and for encapsulation when the transplantation has to be a substitute [25–27]. Alginates are hydrophilic compounds that are naturally found in the cell wall, extracellular matrix of brown algae and some species of bacteria, for example, *Pseudomonas aeruginosa* and *Azotobacter vinelandii* [26, 27]. The most common algae source is brown seaweed. During alginate extraction, alginic acid is generally obtained and converted to a form of salt [26]. Several forms of alginates are currently approved by the Food and Drug Administration (FDA) for use, particularly in the replacement of missing/nonfunctioning endocrine-related diseases [28].

2.1 Gelling and ionic cross-linking of alginates

Alginates are linear copolymers that include two hexuronic acid residues that become dimeric blocks, which are composed of β-D-mannuronic (M) and α-L-guluronic (G) acids for building the entire molecule [29]. These blocks are known as the building blocks of alginates. Mainly, the ratio of G and M blocks depends on the source of the algae type [9]. The important feature of these building blocks is the sensitivity to binding of multivalent cations. This is the starting point of this water-soluble polysaccharide, which allows alginate to form as hydrogels. This characteristic of the hydrogel equilibrates between the environment and the relatively physiologic internal environment [29]. The divalent cations and hydrogel feature depends on the divalent ion's affinity to alginate. Several studies reported high-rate ion affinity results in a stronger gel structure. In order to decrease the ion binding strength, divalent cations should be chosen from the following order: $Pb^{2+}>Cu^{2+}>Cd^{2+}>Ba^{2+}>Sr^{2+}>Ca^{2+}>Co^{2+}>Ni^{2+}>Zn^{2+}>Mn^{2+}$ [1, 29, 30] (**Figure 1**). Controlling cation addition to maintain a porous alginate structure is a critical step.

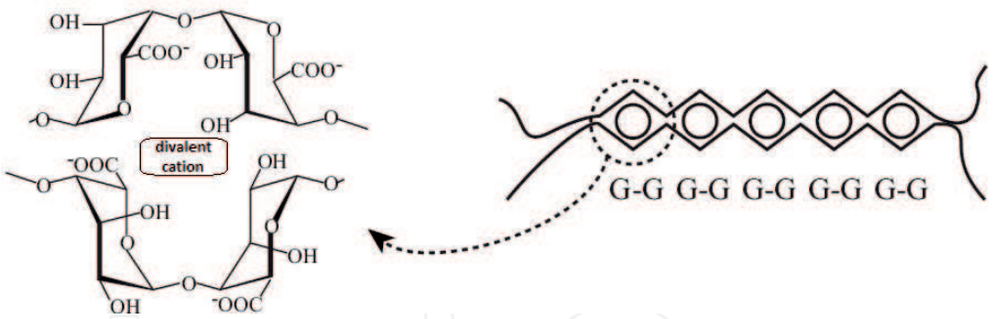

Figure 1.
Representative image for the formation of egg-box ionic cross-links between guluronic acid-rich monomer units (box) and the divalent cations (eggs). Reprinted from Baumberger and Ronsin (2009) [31], an open access article distributed under the terms of the creative commons by attribution 4.0 (CC-BY 4.0).

Successful formation of alginate spheres for delivery purposes requires suitable and selective methods. In 2006, Darrabie et al. identified gelling-cation stability by determining swelling, which contributes to colloid osmotic pressure. They suggested that Ca^{2+} is more hygroscopic and less prominent swelling occurs when compared with Ba^{2+} [32]. Protecting the conformational polymer blocks during preparation of the alginate gels for microencapsulation has been reported using different methods including conjugation of long alkyl chains [33] or dodecylamine [34], temperature (up to 60°C ± 1°C) [35, 36], emulsification by cationic agent [37], and ionotropic gelation of alginate layers [38], etc. Slow gelation utilizes the alginate solution in a more uniform structure in a gradual manner [35].

In the latter case, Lee et al. reported a degree of cross-linking of the alginate can influence a low dose of drug entrapment and unsuitable pore sizes for transplantation. In addition, the efficiency of the microcapsule size or content was found irrelevant, although the preparation step of the water-soluble alginate itself appears to be responsible for the arrangement of the polymer blocks [37]. Cross-linking capacity of the alginates can be modifiable and flexible (**Figure 2**). There are more than 200 types of biocompatible alginates manufactured so far [25, 26]. Moreover, due to the different cross-linking degree of various alginate types, switch in homogenous distribution of the graft/drug during the encapsulation process may occur [36].

Both features of alginates promote several advantages over other polymers such as the stability of the building blocks (-G and/or/both -M repetitively); elasticity of the alginate hydrogel; surface roughness, which is related to elasticity as well, approximately 1% of the alginate can entrap a hundred times more water than its weight; and lastly permitting the ability of oxygen and nutrient permeation inside the spheres.

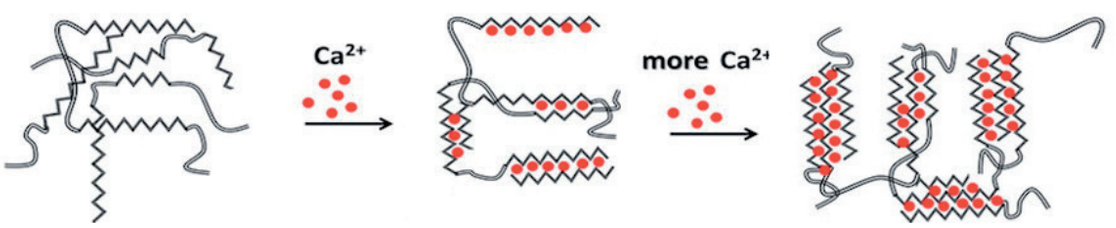

Figure 2.
Representative image of alginate gelation process by continued calcium cations. Reprinted from Dumitru et al. [39], an open access article distributed under the terms of the creative commons by attribution 4.0 (CC-BY 4.0).

2.2 Biotolerability over biocompatibility

Source-dependent impurities may have detrimental effects. Safe and effective delivery of the therapeutic graft/drug with the alginate carrier is frequently mentioned as biocompatible. This naturally derived product provides immunoprotection and most of the studies reported purity of its building block structure preventing a host response when transplanted. Therefore, this makes alginate the most common material for microencapsulation. Higher water-carrying capability of alginates has been shown to directly maintain diffusion and this shows immune-safe characteristics [3]. A decade ago, Kendall et al. focused on the various components of alginate blocks and compared their purity and sphere sizes depending on the cationic agent. They reported that higher purification of alginate prevents imperfections and size/shape properties would affect immunogenicity [40]. Several other reports also demonstrated that -G and -M blocks of the alginate gel need a balance whereas the distribution of these proportions indicates the biocompatibility of the purified alginate is influenced by its viscosity [41–43]. One of the most obvious results from the studies that explains the difference between the building block's balance in alginate gels is higher -M blocks mainly stimulate and induce an immune response [41, 44–46].

There are many immunogenic substances such as endotoxins, proteins, and polyphenols in natural polymers including alginates. Those molecules may diffuse the outer surface from the capsules and then induce an unwanted immune response against the capsules [3]. There are highly conserved molecular motifs that are present in nature and pathogens known as pathogen-associated molecular patterns (PAMPs). PAMPs provoke pattern recognition receptors (PRRs) to enhance inflammatory response [47]. The presence of PAMPs in natural products and alginate as well is not a direct threat; however, complement activation has been reported for encapsulated islets [48]. Therefore, complement activation has a more destructive effect than the inflammatory response; it may activate and produce large quantities of cytokines to induce a stronger response.

Immunoprotective properties still require the exact characterization and preparation of the material to be used as a delivery agent. Despite giving most of the efforts to optimize the encapsulation process, the applicability of this technology has still resulted in an insufficient investigation of graft/drug delivery. In 2014, Rokstad et al. described the duration and the type of host responses and divided the whole process into three categories: acute inflammation, chronic inflammation, and the long-lasting granulation tissue phase [48]. Based on the publications from islet transplantation studies, it was reported that the granulation phase mainly refers to the "vascularized fibrous tissue containing a moderate epithelial histiocytic response" [49–52]. Solely, it is important to observe these responses and that leads to the question: Why do alginate microencapsules contribute to these chain of events even when their purity, stability, and biocompatibility are comparable to most other polymers? Immunocompatibility of the alginate microencapsules should not be determined only by the features of alginate and its preparation process, but should also be evaluated for its protein absorption capability as well.

A profound impact might be introduced with the biotolerability term. Biotolerability is a term for a strategy of making biocompatible encapsulations to induce none/minimal host response. A seemingly minimal cellular overgrowth for graft provides the free diffusion of nutrients, oxygen, and some therapeutic proteins, and controlled drug release from the microcapsules. We should emphasize that the alginate microspheres are not meant to prevent an immune response yet to protect the carrier against an immune response. Therefore, the biocompatibility term not clear enough to explain the biotolerability of the carrier system.

2.3 Vascularization

The vascularization process of the microencapsules is another requirement to increase the survival of the transplanted graft. Sufficient vascularization may be achieved by improving the physical features of the spheres such as the size of the spheres or micropores and the amount/density of the graft/cells. The main argument against vascularization is hypoxia and oxidative stress whereby it develops inside the microspheres [53]. Insufficient oxygenation and nutrition occur particularly in the absence of ideal vascularization. A prerequisite is that the functional performance of the microencapsules often depends on the surface-to-volume ratio. This implies that free diffusion of nutrients and oxygen is necessary and this directly interferes with vascularization.

The majority of researchers developed different strategies to allow a fast exchange of nutrition and demonstrated several boundaries to ensure a low or no inflammatory response while supplying oxygen-nutrients inside [29, 53]. Currently, the accepted limitations of the islet transplantation are defined with three main strategies: first, the cell-to-volume ratio should not exceed 10% even if a large number of cells are required to reach curative treatment. Second, microencapsules should be kept <1 mm, once they reach the spheres they do not maintain their biphasic effect (releasing insulin after a glucose challenge) and also an immune response may be triggered. Third, direct vascular access of the microencapsules to the optimal transplantation site, for instance, glucose must pass and be observed in the transplantation site and then islets release insulin to diffuse through circulation. The whole process requires time and larger amounts to reach an effective dose to lower glucose [1, 53, 54].

3. Clinical applications

A broad range of clinical applications of the microencapsulation process have shown promising results despite encountering some biotolerability issues. Multiple disciplines have been using this alginate encapsulation technology including chemistry, protein science, and cell therapy (mostly transplantation and immunology field). The most studied and reported diseases include Type 1 diabetic patients (T1DM) [55], permanent hypoparathyroidism patients [56], scaffold systems for tissue engineering [57], bone regeneration [58, 59], leukemia (an *in vivo* study that uses alginate to encapsulate specialized hybridoma cells) [60], and even neurodegenerative diseases [61]. Some of the clinical studies about cell therapy are compiled in **Table 1**.

Based on the current experience with alginate, most of the studies have already performed transplantation of beta-cells (islets). The first and well-known clinical trial with islet transplantation was performed in 1994 by Soon-Shiong et al. They reported 9 months of survival of the microencapsules, which were prepared with high guluronic acid containing alginate [49]. Another case reported how islet transplantation was prepared with alginate- poly-l-ornithine and that the patient's need for insulin decreased after transplantation [62]. Following two case reports, Tuch et al. used barium alginate to encapsulate islets and transplanted these into four recipients. In their report, grafts showed various survival rates and did not restore insulin requirements [51]. Considering the last case reports, several companies took the stage and initiated clinical trials to overcome T1DM by using macro- and microencapsulation with alginate and other polymers. In 2014, Scharp and Marchetti evaluated the outcomes of islet encapsulation from companies with larger clinical studies, respectively. The increased interest in islet transplantation reached its most

Alginate type	Graft survival	Graft	Year	Reference
Alginate high in guluronic acid	9 months	Beta-cells (Islets)	1994	[49]
Ultrapure alginate (68% glucuronic acid)	3 months	Beta-cells (Islets)	2013	[50]
Barium alginate	Various duration for four recipients	Beta-cells (Islets)	2009	[51]
poly-l-ornithine-sodium alginate	<1 year	Beta-cells (Islets)	2006	[62]
Ultra-purified sodium alginate	3 years	Beta-cells (Islets)	2011	[63]
Alginate-Poly-L-Ornithine	<8 months	Beta-cells (Islets)	2010	[64]
Collagen/Alginate	n/a	Beta-cells (Islets)	2010	[65]
Sodium alginate	3 months	Parathyroid cells	1997	[66]
Amitogenic alginate	<3 months	Parathyroid cells	2001	[43]
n/a	1 year	Parathyroid tissue particles	2001	[67]
n/a	n/a	Parathyroid cells	2004	[68]
Sodium alginate	<20 months	Parathyroid cells	2009	[69]
Ultrapure-low viscosity high guluronic acid-rich alginate	>1 year	Parathyroid cells	2019	[56]

Table 1.
Some of the examples of alginate derivates used in microencapsulation studies.

popular level between 2010 and 2012 [54]. For the last 2 years, researchers provided detailed *in vivo* experiments and defined an alginate encapsulation strategy in a more enhanced way [52, 60, 70, 71] . The vast majority of attempts have been made to treat T1DM and the critical requirements remain to be elucidated in the future.

Another endocrine replacement therapy performed for hypoparathyroidism by encapsulated parathyroid tissue/cell transplantation (PTX) was described only in seven case reports for 12 recipients [43, 56, 67–69, 72, 73] between 1997 and 2019. Six of these case studies used alginate for encapsulation. In 1997, Hasse et al. performed the first microencapsulated PTX for two recipients and reported 3 months of graft survival [72]. The second one, performed by Zimmerman et al. in 2001 for one recipient showed no trace of parathyroid tissue particles nor microcapsules, after 3 months, from histological samples from the implantation site of the recipient [43]. The third transplantation case reported up to 1 year graft survival by Tibell et al. and they had macroencapsulated the parathyroid tissue particles and transplanted into four recipients [67]. Another case by Ulrich et al. reported two PTX recipients had elevations in PTH levels and reduced the supplementation requirement into half dose [68]. In 2009, Cabane et al. microencapsulated the enzymatically isolated parathyroid cells in one recipient and reported the longest follow-up data with 20 months of graft survival [69]. The last and seventh case performed by Yucesan et al. in 2019 microencapsulated parathyroid cell transplantation for one recipient reported and the results followed for a year with success [56]. Despite these achievements, the necessity of immunoisolation for parathyroid allotransplantation requires more case studies with long-term follow-up data.

A different therapy for using microencapsulation is cell therapy for neurodegenerative diseases. The development of a delivery strategy is limited due to the blood–brain barrier; however, principle studies in animal models may offer new approaches including gene delivery, cell-based delivery, and also biomaterial drug delivery [61]. In the past year, several *in vivo* studies have been reported for neurodegenerative diseases [71, 74–76]. Galli et al. used alginate-poly-L-lysine-alginate (APA) microcapsules and cross-linked the spheres with both Ca^{2+} and Ba^{2+}. They have used this system as a transporter to carry a specialized cell clone for codon optimization of the cerebral dopamine neurotrophic factor gene. According to their recent data, this system has the potential to deliver polymer-encapsulated-drug conjugates for the treatment of Parkinson's and Alzheimer's diseases [76].

4. Conclusion

Immunoisolating construct tuning may be achieved by defining the mechanical properties, molecular weight, cross-linking density of the polymer, and the concentration balance between the therapeutic graft/drug and the biomaterial. These proportions still require optimal decisions even with the known performances of encapsulated cells.

Significant efforts have been made so far by ongoing studies from research laboratories and biotechnology companies, which continue to encounter microencapsulation strategies at every step. The future perspective is strong enough to overcome the current limitations. Nevertheless, alginate is the best natural product to be used by many different disciplines at the same time.

Conflict of interest

The authors declare no conflict of interest.

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