Applications of Alginate microspheres in therapeutics delivery and cell culture: past, present and future

Dinesh Dhamecha[a], **Rachel Movsas**[a], **Ugene Sano**[a], **Jyothi U. Menon**[a,*]

[a]Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA

Abstract

Polymers are the backbone of pharmaceutical drug delivery. There are several polymers with varying properties available today for use in different pharmaceutical applications. Alginate is widely used in biomedical research due to its attractive features such as biocompatibility, biodegradability, inertness, low cost, and ease of production and formulation. Encapsulation of therapeutic agents in alginate/alginate complex microspheres protects them from environmental stresses, including the acidic environment in the gastro-intestinal tract (GIT) and enzymatic degradation, and allows targeted and sustained delivery of the agents. Microencapsulation is playing an increasingly important role in drug delivery as evidenced by the recent surge in research articles on the use of alginate in the delivery of small molecules, cells, bacteria, proteins, vaccines, and for tissue engineering applications. Formulation of these alginate microspheres (AMS) are commonly achieved by conventional external gelation method using various instrumental manipulation such as vortexing, homogenization, ultrasonication or spray drying, and each method affects the overall particle characteristics. In this review, an inclusive summary of the currently available methods for the formulation of AMS, its recent use in the encapsulation and delivery of therapeutics, and future outlook will be discussed.

Keywords

alginate microspheres; delivery; drugs; cells; proteins; vaccines; cell culture

1. Introduction

Conventional methods of drug delivery involving use of tablets and capsules, and simple injections have been widely studied and applied in the biomedical arena for treatment of various human diseases due to the ease of formulation and administration. However, greater

*Corresponding author: Dr. Jyothi U. Menon, Assistant Professor, Nanotherapeutics & Biomaterials Engineering Laboratory, Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, 7 Greenhouse Road, Kingston, RI 02881, Telephone : 401 874 4914, jmenon@uri.edu.

understanding of the complex nature of the pathogenesis in human diseases has led researchers and clinicians to explore the development of novel drug delivery systems for targeted delivery of therapeutics (e.g.: proteins, peptides, nucleic acids, cells and organelles) (Banskota et al., 2019, Cañibano-Hernández et al., 2019). In particular, complex dosage forms such as nanoparticles (liposomes, polymeric nanoparticles, metallic nanoparticles), microspheres, transdermal drug delivery systems, and mucoadhesive drug delivery systems are being studied to deliver therapeutic agents via different routes of administration. These formulations have the advantage of delivering sensitive molecules to the site of action via passive (enhanced permeation and retention effect (Dong et al., 2019)) and/ or active targeting (overexpressed receptors on the diseased cells (Ding et al., 2019)), while protecting the active molecule from degradation. For example, the encapsulated drug can be protected from the reticuloendothelial system by PEGylated liposomes (Yu et al., 2019), and from the acidic gastric environment by alginate - chitosan microspheres (Ling et al., 2019). Based on the intended application, various polymers can be used to design these complex formulations. Polymers can be classified as natural (lipids, alginate, cellulose, chitosan), semisynthetic (lipids, alginate derivatives, cellulose derivatives, chitosan derivatives) and chemically synthesized (poly esters, poly carbonates, lipids like 1,2-dipalmitoyl-sn-glycero-3-phosphocholine).

Alginate polymer has several attractive properties including excellent biocompatibility, low cost, ease of gelation, inert nature, chemically compatible material, derivation from natural sources, ease of availability and feasible method of synthesis, which makes it a polymer of choice for most researchers developing platforms for drug delivery and tissue engineering (Becker et al., 2001). Further, alginate can be easily manipulated to develop different formulations by simple addition of crosslinkers like divalent calcium ions (Fig 1) (Lin et al., 2005).

Commercially available alginate is extracted from brown algae, which undergoes treatment with alkaline solutions, typically sodium hydroxide, and subsequently reacted and purified to yield sodium alginate (Pawar and Edgar, 2012). Bacterial (*Azotobacter* and *Pseudomonas*) synthesis of alginate provides superior chemical and physical properties as compared to alginate extracted from seaweed. Optimization of alginate synthesized by bacterial method is relatively simple and enables manufacture of alginate with the desired properties for easy modification and synthesis of derivatives (Rehm and Valla, 1997). Chemically, alginate is a polymer consisting of units of mannuronic acid (MA) and guluronic acid (GA) organized in an asymmetrical block design with varying proportions of MA and GA units (Augst et al., 2006). These microstructural open functional unit groups are a characteristic feature of alginates, which are derivatized for customized applications such as cell/microbe immobilization (Melvik and Dornish, 2004, Wang et al., 2003), drug delivery (Dalmoro et al., 2017, Mehta et al., 2015), and food industry (F. Liu et al., 2017).

Among the various available formulations of alginate, alginate microspheres (AMS) and alginate microcapsules (AMC) are the most widely explored due to their capacity to encapsulate small and large molecules with diverse chemical properties. Microcapsules consist of a central core containing the active therapeutic agent, and an exterior polymeric shell (Wong et al., 2018). The term 'alginate microspheres' is often used interchangeably in

literature to refer to alginate microgels (Ching et al., 2017), alginate microcapsules (Chandy et al., 1999; Darrabie et al., 2005), alginate microparticles (Jain et al., 2015) or alginate microbeads (Mørch et al., 2006). Therefore, for the purposes of this review, all of the above formulations will be referred to as alginate microspheres (AMS). Most of these formulation methods are based on an external (ionic) gelation method in which an alginate solution turns to gel state in the presence of divalent calcium ion (Lin et al., 2005). Further, due to the presence of open functional MA and GA groups, alginates undergo reactions with other cationic polymers such as chitosan - another natural polymer, which can be used for encapsulation of therapeutics agents (Augst et al., 2006). With this background, the present review aims to discuss in detail the various methods for the development of alginate microparticles, and an update on its application in encapsulation of drugs, vaccines, bacteria, vaccines, cells etc. and in tissue engineering (Fig 2).

2. Fabrication of alginate microspheres

AMS can be formulated through techniques such as single/double emulsion, extrusion, spray drying, or microfluidic methods. Conventionally, water in oil single emulsion is the most common method used for formulation of AMS (Fig 3). This method encapsulates the therapeutic agent in the aqueous sodium alginate polymer, which is then ionically cross-linked by agents such as divalent cations (Liao et al., 2017). Calcium chloride is most commonly used as a crosslinking agent for alginate microsphere formation. Barium chloride (Alizadeh Sardroud et al., 2017), strontium chloride (Lourenço et al., 2017) and zinc chloride (Taha et al., 2005) have also been used as crosslinking agents. Sodium alginate does not form a gel due to its rigid poly l-guluronic acids and solubility in water, but it easily undergoes sol-gel transformation when exposed to a divalent cation such as calcium or zinc (Baimark and Srisuwan, 2014). The alginate matrix specifically forms through the cross-linking and exchange of sodium ions from l-guluronic acid with the divalent ions. Gluconate blocks of alginate forms junctions with the other gluconate blocks of adjacent alginate chains, forming an egg-box model and the gel network results in the formation of sodium AMS (Fig 1). The size of the particles formed by this emulsion dispersion method can be controlled by making changes to the temperature during formulation, the viscosity of aqueous alginate solution, organic solvents or oil phase, the concentration of surfactants, vortexing/homogenization speed, and stir rates applied during the formulation (Patil and Sawant, 2009; Piornos et al., 2017). Emulsification technique is an economical technique and produces AMS with homogeneous morphology. However, this method has several disadvantages like poor industrial scale-up (Reis et al., 2006) and droplet fusion, which can change the characteristics of the particles and may cause random release profiles (Lopes et al., 2017) Also, the extra step of centrifugation done to purify the AMS from the residual oil and surfactants adds to the difficulty for scale-up (Sultana et al., 2000).

Another method used for the fabrication of AMS is extrusion (Ching et al., 2017), which generates AMS with uniform diameters. The extrusion techniques can control particle sizes using electric energy, mechanical energy or jet cutting mechanism. The power of the applied electrical field generates AMS in the range of 500–2000 μm in diameter with spherical morphology (Zhao et al., 2016) Mechanical vibration generates AMS with diameter in the range of 300 μm to 5 mm (Prüsse et al., 2008), while 200 μm to 5 mm diameter AMS with

high uniformity is produced with jet cutting method (Koch et al., 2003). These extrusion techniques however have disadvantages such as requirement of gelling bath, complex nature of formulation, time consuming with poor scale up reproducibility (Ching et al., 2017). An extension of the extrusion method is the formulation of AMS using pneumatic nozzles air atomization method (Ahn and Kim, 2015). In this technique, pressurized sodium alginate solution is passed through a nozzle orifice using a pump. Just after passing through the orifice, the liquid jet of alginate solution is turned into droplets using pressurized gas, which is usually air, leading to atomization and formation of uniform droplets. These sodium alginate droplets (diameter range of 11–80 mm) are finally expelled into a bath/vessel containing crosslinking agent (e.g.: calcium chloride solution). Spray drying is an advanced version of this technique which ensure better uniformity and have an advantage of scalability.

Spray drying is commonly used for the encapsulation of food ingredients such as caffeine (Bagheri et al., 2014). It is also used in the encapsulation of tissue regeneration agents like dermatan sulfate (Martins et al., 2007; Wen et al., 2012), and in pharmaceutical industry to improve drug bioavailability (Ganesh et al., 2015). Spray drying (Fig 4) works on the principle that the drug-polymer dispersion can be transformed to a dry state by atomizing, and then exposed to hot gas to evaporate the solvent by a closed loop operation, to yield microspheres. Spray drying is advantageous for maintaining a stable drug shelf-life, for rapid, precise, scalable development of AMS, and for having high active material encapsulation efficiency (Agüero et al., 2017). Characteristics of AMS formulated by spray drying method are greatly influenced by the flow rate of the feeding solution, concentration of alginate, surfactants and crosslinking agent, atomization time, crosslinking exposure time and drying time (Dalmoro et al., 2014). However, the expensive apparatus and high inlet temperature operating conditions (approx. 100°C or higher), can make it difficult to use spray drying method for encapsulation of heat-sensitive agents (e.g.: proteins) (Anandharamakrishnan et al., 2007). Spray drying coupled with ultra-atomization is a promising technique which can be used for the fabrication of AMS with precise particle characteristics. Ultrasonic atomization can be achieved by high frequency ultrasonic energy or by ultrasonic vibrations. These ultrasonic energies rely on the use of high pressure and velocity motion to convert the fluid into small droplets unlike conventional spray drying atomization. This advanced technique, especially when coupled with ionotropical gelation, is known for its high reproducibility, robust nature, innovativeness, easy scale-up, low cost, faster process, consistent output and single step process. AMS prepared from this technology has high encapsulation efficiency and spherical morphology with uniform size distribution. (Dalmoro et al., 2012).

Another technique used to prepare calcium-linked AMS is the microfluidic method (Fig 5). It is used to produce alginate beads with precise diameters of less than 300 μm with minimal polydispersity. Sugiura et al. developed a new silicon micro-nozzle array-based microfluidic device for the formation of AMS with diameter in the range of 50–200 μm. The aqueous alginate was forced through a 30 x 30 μm construct and short 500 μm micro-nozzle followed by sheared flow of viscous oil to yield alginate droplets. The alginate droplets quickly reacted with calcium chloride to form AMS (Sugiura et al., 2005). Another study used a

chip-based microfluidic system to produce 50–2000 μm uniform calcium alginate beads, and it can be programmed to create many ordered calcium-AMS (Huang et al., 2006).

3. Biomedical applications

3.1 Delivery of drugs

Microencapsulation is a well-known method used to deliver drugs and to control the rate of drug release. Drug-encapsulated microspheres can be used to improve bioavailability and stability of drugs, target the drugs to specific sites in the body, and provide prolonged or controlled drug release. Microencapsulation is particularly advantageous for delivery of low molecular weight or hydrophobic drugs and proteins, as well as to decrease dosing frequency, side effects, and to improve patient compliance.

Various drugs can be microencapsulated into alginate gels for oral administration. This reduces or eliminates the possibility of GI tract irritation. In the acidic gastric fluid, external layer of AMS is hydrated and transformed to an insoluble alginic acid form in which the alginate shrinks and consequently protects the encapsulated drugs. However, at higher intestinal pH, shrunken alginic acid turns to a soluble viscous layer ultimately leading to release of the payloads (Sosnik, 2014). For example, a well know a potent nonsteroidal anti-inflammatory drug (ibuprofen) was used to determine the effects of different parameters such as concentration of sodium alginate, calcium chloride and magnesium stearate on the release profile of ibuprofen encapsulated AMS (Nagpal et al., 2012). Outcomes of the studies showed that AMS were discrete and spherical in shape with a rough outer surface and the mean AMS particle size was 6.89 um when analyzed by scanning electron microscopy (SEM). Percent yields of the different batches was found to be between 66.6 and 96.6 %. Increase in polymer concentration contributed to greater sustained release whereas addition of magnesium stearate improved the drug encapsulation efficiency.

Similarly, AMS were used in the encapsulation of isoniazid for oral sustained drug delivery (Rastogi et al., 2007). Isoniazid is a commonly used anti-mycobacterial agent for first line therapy in tuberculosis but, long term continuous therapy of isoniazid can cause hepatotoxicity and peripheral neuropathy, so it would therefore be advantageous to have a drug formulation with prolonged and controlled enteric release. AMS containing isoniazid were formulated by a single emulsification method in which the particles were found to be smooth and spherical and had a mean particle size of 3.72 μm. Release studies showed release of 26 % of the isoniazid in simulated gastric fluid of pH 1.2 in six hours, and complete release of remaining 71.25% in simulated intestinal fluid of pH 7.4 in 30 hours. Another study demonstrated the formulation of buccal nystatin AMS by the emulsification method for the treatment of oral candidiasis (Martín et al., 2015). The mucoadhesive characteristics of alginate aids in improved targeting to the absorptive sites and increased uptake of the drug. AMS can thus provide closer contact with larger mucosal surface area than traditional buccal tablets or lozenges. In this study, the mean diameter of AMS was found to be 89.03 μm for empty vehicles and 135.21 μm for drug loaded AMS. The microspheres had a clear inhibition effect on *C. albicans* growth. *In vivo* studies showed that the microspheres were retained in the mucosa, with no systemic absorption or tissue damage.

In another study, green tea polyphenol was encapsulated into AMS for the minimally invasive treatment of osteomyelitis (Chen et al., 2018). Osteomyelitis is an infection in the bone which is treated with regular high doses of systemic antibiotics. Polyphenols extracted from green tea are rich in anti-oxidative and antibacterial properties. In this study, green tea extracted polyphenols were encapsulated in porous silica nanospheres which were then encapsulated in AMS. The final formulation was then coated with pH-sensitive calcium carbonate to provide conducive environment for delivery of polyphenols at the site of action killing *Staphylococcus aureus* consequently promoting the proliferation of osteoblasts. Final microspheres were about 3 mm in diameter, had a drug loading efficacy of 92.96 weight percent, and were able to reduce the oxidative stress caused by osteomyelitis. Examples of other drugs delivered using AMS are shown in Table 1.

3.2 Delivery of cells

Microencapsulation of cells involves immobilizing bioactive cells within microparticles. The cells are surrounded by a polymeric membrane to allow free movement of nutrients, oxygen and the therapeutic protein products into and out of membrane. The semi-permeable membrane impedes high molecular weight molecules (>150 kDa), antibodies as well as other immunologic substances from recognizing the encapsulated cells as foreign materials (Orive et al., 2003). Due to the advantageous mild environment of the alginate gel, the cells immobilized in the gel can retain strong viability in long-term culture. AMS can also act as a shield from physical stress and avoid immunologic reactions in host. Hence, there is a lot of research on encapsulation of cells with sodium alginate to form therapeutic products with the aim of treating different diseases.

In one work, the ability of human mesenchymal stem cells (hMSCs) encapsulated in Arginylglycylaspartic acid (RGD) peptide modified AMS to facilitate myocardial repair was studied (Yu et al., 2010). This *in vitro* study found that the RGD modified AMS improved cell attachment and growth, and increased angiogenic growth factor expression. Following intramyocardial delivery, the AMS encapsulated hMSCs were able to induce angiogenesis, which helped maintain left ventricular shape and prevent negative left ventricular remodeling after a myocardial infarction. Another study formed microspheres for hMSCs encapsulation using methacrylated alginate polymer in combination with microfluidic devices (Etter et al., 2018). It was observed that stem cell viability was maintained in the AMS after encapsulation. Grellier et al. encapsulated endothelial cells in alginate to study if these cells can regulate the osteogenic potential of osteoprogenitor cells *in vitro* and *in vivo*, for its use in a long bone defect (Grellier et al., 2009). When human osteoprogenitors in combination with human umbilical vein endothelial cells were encapsulated in RGD-grafted AMS, there was a significant increase in the mineralization of the bone, showing potential for a new injectable bone tissue engineering approach.

AMS are most often studied for the encapsulation of pancreatic islet cells for treatment of diabetes. Limitations of islet transplantation in diabetic patients include a severe shortage of human pancreas donors, and the necessity to use immunosuppressive drugs so as to prevent transplant rejection. Hence, one work developed a bio-artificial pancreas through microencapsulation of perm-selective coating of islets cells with biopolymers for graft

immunoisolation, for the treatment of diabetic patients (Opara et al., 2013). Another study analyzed a multilayer microcapsule for encapsulation of pancreatic islets. Empty and islet containing AMS were coated with the concentric layers of polymers like polythelenemine, polyacrylacid or carboxymethylcellulose along with alginate. This encapsulation procedure and membrane forming step did not have any effect on the stimulatory response of the islet cells which showed improved glucose-induced stimulatory insulin response in three and five weeks of cell culture (Schneider et al., 2001). In another study, human pancreatic islets cells were encapsulated in Ca^{2+}/Ba^{2+} crosslinked AMS and administered intraperitoneally into diabetic Balb/c mice (Qi et al., 2012). It was observed that mice were within normal glycemic levels from day one after transplantation and retained the normal glycemic levels with a significant improvement in the mean graft survival time. Animals transplanted with the same amount of non-encapsulated human islets cells rejected the cells about two to seven days after transplantation. The study concluded that the Ca^{2+}/Ba^{2+} alginate microbeads could potentially be used to prevent human islets from xenogeneic rejection in immunocompetent mice without immunosuppression.

AMS can further play a role in regenerative medicine. Differentiation of stem cells into primordial germ cells have the potential to help treat patients with infertility and produce mature germ cells. One such study demonstrates the application of the mouse embryonic stem cells (ESCs) differentiation capacity to putative primordial germ cells encapsulated within alginate and alginate-collagen IV microspheres (Mansouri et al., 2017). Primordial germ cell gene and protein expression showed the differentiation potential of ESCs to putative primordial germ cell in alginate-collagen IV microspheres, which was significantly increased compared to the control groups. Encapsulation of cells with sodium alginate is being studied actively today to offer therapeutic solutions for a variety of diseases. Other examples of cells delivered using AMS are shown in Table 2

3.3 Delivery of proteins

Research on encapsulation of proteins in AMS dates to more than 30 years ago. Systematic description of the encapsulation of proteins with examples is mentioned in a review done by Gombotz et al. (Gombotz and Wee, 1998) Recent reports on encapsulation of proteins using AMS system as the carrier is described here. Proteins such as enzymes, growth factors, hormones, and interleukins have several biomedical applications and are used in tissue regeneration or as therapeutic agents (Dimitrov, 2012). However, the structure of proteins can easily be denatured or altered in harsh environmental conditions, including changes in pH and temperature, and thus lose their therapeutic qualities (Dimitrov, 2012, Wells and Sheardown, 2007). Therefore, these proteins can be encapsulated in AMS to prevent degradation, and to provide controlled, site-specific release (Wells and Sheardown, 2007). AMS demonstrate improved protein loading compared to formulations prepared using other biomaterials.

Protein-containing AMS are often used in tissue engineering, especially for bone tissue generation, or osteogenesis. In a study done by Quinlan et al, poly lactic-co-glycolic acid (PLGA)-AMS encapsulated recombinant human bone morphogenic protein-2 (rhBMP-2), a pro-osteogenic factor into collagen-hydroxyapatite scaffolds, served as a temporary platform

for improving cell growth (Quinlan et al., 2015). AMS (size range: 1–10 μm) showed sustained release of rhBMP-2 and therefore has huge potential for therapeutic delivery not only for bone regeneration but also to other tissues by regulating the chemical composition of the scaffold frame and the incorporated growth factor. In another study, BMP-2 was encapsulated by AMS (around 30 μm) and delivered at the site of action by using thermosensitive chitosan/dextran-polylactide/glycerophosphate hydrogel for osteogenesis (Zhu et al., 2016). Similarly, carboxymethyl chitosan/poly (vinyl alcohol) gel base was used as a carrier for bovine serum albumin (BSA) loaded AMS (average diameter - 408.6 ± 9.4 μm) which was intended to aid in regeneration after transplantation of components such as bio-interactive implants and artificial skin (Liu et al., 2015). Another study investigated the application of combination of TGF-β3 encapsulated AMS and human mesenchymal stem cells in hyaluronic acid hydrogels base for the fabrication of implantable constructs for cartilage repair (Bian et al., 2011). The TGF-β3 encapsulated AMS were around 38.9 ± 9.2 μm in diameter with high encapsulation efficiency and high biocompatibility, and demonstrated controlled drug release profile (Bian et al., 2011). On similar lines, nanofibrous gelatin scaffolds were also used alongside AMS to encapsulate nerve growth factor for brain tissue engineering (Büyüköz et al., 2018).

Certain proteins will typically have difficulty being absorbed in the body due to their innate properties such as isoelectric points and hydrophilicity (Wells and Sheardown, 2007; Zhai et al., 2015). One study had lysozyme and chymotrypsin encapsulated to test for the rapid loading and sustained released of proteins with high isoelectric points (Wells and Sheardown, 2007). Crosslinking of alginate with the loaded high isoelectric point proteins lead to the sustained release behavior of the system. In another study, PLGA-AMS (around 3 μm) loaded with hydrophilic proteins were used for protein delivery (Zhai et al., 2015). BSA or rabbit anti-laminin antibody protein were used for encapsulation. Higher protein encapsulation efficiency and more controlled protein release rate was observed from the PLGA-AMS compared to PLGA alone, due to the slower degradation rate of the composite microspheres.

AMS have also been used to encapsulate horseradish peroxidase (HRP) and tetramethyl rhodamine conjugated BSA for applications in protein drug delivery and as a biosensor. These AMS (average diameter 700 ± 80 μm) were coated with silk fibroin for improved stability and drug delivery. The silk coating acted as a barrier to help slow the release of the encapsulated agents and limit the amount of degradation of microspheres in comparison to microspheres without a coating. Additionally, the system allowed the attachment of the ligands to the coating to allow for more specific targeting (Wang et al., 2007). Delivery of therapeutic proteins using AMS is being explored in other areas of tissue engineering and regenerative medicine. Some examples of proteins delivered using AMS are shown in Table 3.

3.4 Delivery of vaccines

Vaccines are products that aid in stimulating the production of antibodies in the immune system, either for preventative or therapeutic measures. In this way, the body is able to amass an effective adaptive immune response against foreign pathogens or antigens, and can

therefore develop an immunity to a particular disease (Kim et al., 2002). Vaccines carry a huge commercial value because of their well-known use in contagious diseases such as mumps, rubella, and measles. In most of the cases, vaccines are administered via the parenteral route, and non-parenteral route is used rarely (Giudice and Campbell, 2006). However, scientists are now recognizing the importance of immunization by oral or intranasal route. Effective delivery of vaccines through non-parenteral routes can be successfully achieved by using AMS due to its excellent mucoadhesive property and the ability to protect the vaccine from the GIT acidic environment (Gombotz and Wee, 1998).

Previous reports indicate that AMS allows sustained release of the loaded vaccines and helps maintain the integrity of the encapsulated agents within the microspheres. They have also been tested for long term storage by freeze-drying the AMS loaded vaccines (Hariyadi et al., 2014). For examples, BSA (a model antigen) was encapsulated within AMS protein carriers and freeze-dried for oral delivery (Hariyadi et al., 2014). In this study, Maltodextrin was used as a cryoprotectant and the formulated AMS were approximately 22–65 μm in diameter after lyophilization. Release study experiments revealed that less than 7% of BSA was released from the freeze-dried formulations in simulated gastric fluid in 2 h, whereas 90 % of the BSA was released in simulated intestinal fluid in 10 h. In another study, Tafaghodi et al, had developed AMS loaded with autoclaved *Leishmania major* (ALM) as antigen and CpG-ODN as immunoadjuvant (Tafaghodi et al., 2011). *L. major* is a parasitic species associated with the cutaneous disease leishmaniasis, which is transmissible to humans from an animal vector. CpG-ODN acts as an adjuvant due to its ability to enhance the humoral immune response. AMS loaded with ALM and CpG-ODN had diameter of less than 5 μm and with sustained release profile desirable for vaccine delivery. Similarly, AMS encapsulating the rotavirus promoted an immune response after oral and enteric immunizations in domestic animals (Kim et al., 2002). In another study, AMS were loaded with recombinant adenovirus to protect against any possible vector-specific responses (Sailaja et al., 2002). The most successful routes of delivery for this vaccine were intranasal and intraperitoneal. Similarly, lipopolysaccharide-antigenic fractions from *Klebsiella pneumoniae* were encapsulated within AMS for intratracheal delivery to treat nosocomial infections (Jain et al., 2015). The size of the AMS was less than 5 μm for effective nasal and lung delivery. AMS successfully protected its loaded contents and induced strong systemic and mucosal immune responses within Swiss albino mice (Tafaghodi et al., 2006).

Several studies have also revealed the use of AMS in delivery of vaccine in animals, especially fish. For example, an AMS loaded with plasmid DNA that codes for fish lymphocystis disease virus was used for the treatment of Japanese flounder (Tian et al., 2008). The study showed that the plasmid DNA maintained high transfection efficiencies, implying that AMS effectively preserved the integrity of the plasmid DNA. Another study had AMS encapsulating a DNA vaccine for infectious hematopoietic necrosis virus (pIRF1A-G vaccine), which was then re-suspended in sodium citrate at room temperature for oral delivery (Ballesteros et al., 2015). Results demonstrated that AMS containing DNA vaccine significantly improves fish immune responses and resistance to infectious hematopoietic necrosis virus after the oral delivery. AMS was also found to protect DNA vaccine from the acidic and degradative conditions of the gastrointestinal tract (Ballesteros et al., 2015). Research is ongoing into the development of AMS encapsulating antigens and

antibodies for immunization in humans. Some examples of vaccines delivered using AMS are shown in Table 3.

3.5 Delivery of microbes and vectors

Microbes play a crucial role especially in the GIT of the body (Ma et al., 2008). Microbiota in the lumen of the GIT helps to maintain optimal GIT conditions and outcompete other harmful pathogens and bacteria that could otherwise colonize and infect the GIT. Microbes such as bacteriophages and probiotics can be administered for therapeutic purposes, such as to prevent diarrhea and balance intestinal microbiota. AMS containing bacteriophages and vectors allow for better acid stability and protection of the encapsulated contents from GIT acid degradation as well as controlled, sustained release of the contents. In GIT conditions, alginate acts as a pH buffering agent and prevents excessive temperature changes as the viscosity of the alginates is increased at lower pH (around pH 3) which protects the therapeutic agent from the GI acidic environment. At lower pH carboxylate group of alginates are protonated and form hydrogen bond leading to increase in the viscosity (Lee and Mooney, 2012). AMS also act to prevent encapsulated microbes from being broken down by enzymes and bile in the GIT. A study reports the designing of chitosan – AMS loaded bacteriophage Felix O1 for oral administration (Ma et al., 2008). The free phage is sensitive to acidic environments and could not be detected at pH below 3.7 following a 5-min exposure. Microspheres containing Felix O1, on the other hand, were found to be stable after lyophilization and significantly improved phage survival. Release studies suggested that encapsulated phage was released at the simulated intestinal pH of 6.8 in 6 h.

One study done by Lamas et al, had AMS encapsulating fungal spores and viable cells of *Bacillus subtilis* (Lamas et al., 2001). The results indicated that both types of encapsulated contents were protected from the media due to the biocompatible nature of alginate. Encapsulated spores and cells had a significantly longer germination time than the control (free spores and cells). The growth of encapsulated cells was like the cell growth of free cells, even after the lag-time from encapsulation.

In certain cases, alginate is paired with other biopolymer such as chitosan for enhancement of the protective properties of the microspheres. Complexes of chitosan-AMS were used to protect the bacteriophages from highly acidic conditions in the GIT (Ma et al., 2012). In another study involving chitosan-AMS, the microspheres encapsulated both *Staphylococcus aureus* Phage K with calcium carbonate microparticles for the reduction of pathogen colonization in the intestine (Ma et al., 2012). Calcium carbonate (alkaline compound) was incorporated in the formulation to protect Phage K by gastric fluid. The complex of chitosan-AMS (average diameter 900 ± 28 μm) preserved Phage K and its viability from bile and acidic gastric fluid (Ma et al., 2012).

Based on the requirement and the end application, alginates can be manipulated and derivatized to form modified polymers. In one such study, a complex of protein-AMS was used to encapsulate *Lactobacillus bulgaricus* which served as a wall carrier for probiotic encapsulation (Chen et al., 2014). Efficacy of the formulation was found to be highly dependent on the diameter of AMS. Alginate-milk microspheres were used in another study to encapsulate probiotics (*Lactobaccilus bulgaricus)* to test for the microspheres ability to

protect against simulated GIT conditions (Shi et al., 2013). This complex proved successful at protecting *L. bulgaricus* from highly acidic environments.

In these studies, the microspheres effectively improved the capability of the bacteriophages to provide their beneficial effects to the system. Additionally, AMS allowed for high encapsulation rates of the various payloads. AMS, especially when combined with chitosan, are suitable vectors for probiotic and microbe delivery. Some examples of microbes and vectors delivered using AMS are shown in Table 3

3.6   Applications in cell culture

AMS have been exponentially utilized in cell and tissue engineering. Alginates were initially not widely used for cellular growth due to their hydrophilic surface, which results in poor protein adsorption (Machida-Sano et al., 2014). Alginate also lacks the ability to support cell adhesion due to the absence of cell binding sites (Lee et al., 2016). However, with the increase in demand for cell culture substrates that simulate the natural environment, and technological advances, alginates were successfully coupled with synthetic peptides in a synchronized manner to design/modify the surface to make it conducive for cell adhesion. For example, alginates complexed with peptides such as arginine, glycine and aspartic acid have been shown to augment proliferation and adhesion of osteoblasts (Chen et al., 2015), myoblasts (Sandvig et al., 2015), and bone marrow cells (Guo et al., 2017). Chemical functionalization with such peptides makes alginate an ideal biocompatible scaffold for cellular proliferation *in vitro* and can consequently be explored for translational research in regenerative medicine and tissue engineering.

Although, three-dimensional (3D) tissue systems using alginate hydrogels are not new to the bioengineering field, studies investigating the use of AMS for this application has been scant. Absence of endogenous alginase enzyme in mammals is one of the key reasons that the use of alginate in the clinic has been limited in the past, as alginates are not effectively resorbed and excreted/eliminated from the body. To overcome such limitations, recently researchers have designed alginate hydrogels loaded with alginate lyase-encapsulated PLGA microspheres and the results have demonstrated that these hydrogels were digested in a sustained and tunable fashion to deliver therapeutic stem cells (Ashton et al., 2007).

In another study, alginate core-shell microbeads were incorporated to provide bio-mechanical structural stability to MCF-7 human breast cancer cell lines leading to formation of 3D spheroids model for upto 14 days. These well-structured shells not only resisted the growth of cells as a monolayer but provided long-term protection to cells to facilitate cellular proliferation for formation of multicellular aggregates within the core shell layer. Further, cell encapsulation was done by using one-layer microfluidics method, which generates microspheres with narrow polydispersity and less batch to batch variations (Yu et al., 2015).

4.   Conclusions and future perspectives

AMS are used in a wide range of therapeutic applications including encapsulation, therapeutics delivery, tissue engineering, regenerative medicine and *in vitro* cell culture.

Some of the striking characteristics of alginate are their biocompatible nature, easy gelation and availability of open functional groups with ease of manipulation to synthesize alginate derivatives with advanced desirable properties (Augst et al., 2006). In the present review, we have summarized different methods used for formulation of AMS and discussed the process parameters that controls the characteristics of the microspheres. Further, the key applications of AMS for delivery of therapeutics such as drugs, vaccines, cells, bacteria and proteins, and its use in cell culture techniques are addressed. Encapsulation within AMS is attractive for protection of therapeutic molecules from GIT environment, high drug entrapment and loading, and for drug delivery via a biocompatible and mucoadhesive carrier (Wong et al., 2018). However, AMS can be limited by immediate release of the therapeutic agents and poor long-term stability (Aguirre Calvo and Santagapita, 2016 Halder et al., 2005). Porous nature of alginate polymer matrix is one of the key reasons for this immediate release/ leakage of drugs (Mallikarjuna Setty et al., 2005). Blending alginate with other polymers such as chitosan, dextran sulphate, poly-L-lysine, acrylic polymers or pectin (Silva et al., 2006) not only improves the encapsulation efficiency but also helps control the release profile. For example –the positive charge on chitosan/pectin interacts with the negative charge on alginate to form an intact membrane, having control over the release of encapsulated agents (Jaya et al., 2009). On the other hand, negatively charged dextran sulphate is known to have higher encapsulation efficiency of proteins when used along with alginate (Martins et al., 2007). Further, combination of chitosan and dextran sulphate showed synergistic effect on encapsulation of insulin with better control over the release profile (Martins et al., 2007). Secondary encapsulation of the alginate polymer blends within a scaffold or hydrogel as appropriate for the application, can further modulate the drug release kinetics. In a study by Aguilar et al., poly-caprolactone (PCL)-alginate composite microspheres were placed within a bioscaffold to modulate the release profile of a representative biomolecule. The PCL-alginate composite microspheres demonstrated slower release than AMS alone, and this release could be further modulated by secondary encapsulation within the bioscaffold (Aguilar et al., 2019). However, care needs to be taken during derivatization of alginates to synthesize semisynthetic alginates, as this may lead to decrease in biocompatibility and inertness of the original polymer. Hence an efficient purification method also needs to be developed to ensure complete removal of by-products and unreacted chemicals. Additionally, it is critical to assess the cytotoxicity, blood compatibility, response from immune system and bio-degradation behavior of the synthesized polymer and particles in their potential applications (Sobol et al., 2013)

It is worth noting that AMS are excellent formulations for the microencapsulation and delivery of islet cells, due to their ability to evade antigen reaction, thereby protecting the cells from the host immune response. Due to this property of AMS, advanced clinical trials are now in progress in which the porcine islet cells encapsulated alginate microcapsules were tested in humans for diabetes type I without administration of an immunosuppressive agent. In this clinical trial, the microcapsules (DIABECELL®) were intraperitonially implanted in humans and the outcomes have demonstrated hypoglycemic effect for a long time (U.S. National Library of Medicine, 2017). This clearly demonstrates that the alginate-based microspheres have a huge commercial potential as cell carriers. The increase in research publications on alginate-based cell microencapsulation techniques in recent years

indicate that we can expect to see significant strides in this research area using AMS, in the future.

There has also been considerable advancement in the technology used to develop AMS. Spray drying and microfluidics are available for the large-scale production of monodispersed AMS with improved chemical and mechanical stability. Various strategies like use of other cations (example - Ca^{2+}, Mg^{2+}, K^+, Na^+) for gelation, varying instrumental parameters, chemical modification with other robust polymers, and manipulation of the chains of MA and GA in terms of molecular weight are being considered to design stable and biocompatible AMS for controllable and efficient therapeutics delivery and release. Extensive characterization and optimization studies are required to manipulate and generate new groups of alginate derivatives and polymers with customized and controlled physico-chemical properties for the specific biomedical application which could transform and encourage the use of alginates as a potential carrier for various payloads. Given the various applications that AMS are already being used for due to their attractive physicochemical properties, these new classes of alginates will be significant contributors towards revolutionizing therapeutic research in the future.

Acknowledgements

References

Acarregui A, Ciriza J, Saenz del Burgo L, Gurruchaga Iribar H, Yeste J, Illa X, Orive G, Hernández RM, Villa R, Pedraz JL, 2018 Characterization of an encapsulated insulin secreting human pancreatic beta cell line in a modular microfluidic device. J. Drug Target 26, 36–44. [PubMed: 28535085]

Agüero L, Zaldivar-Silva D, Pena L, Dias ML, 2017 Alginate microparticles as oral colon drug delivery device: A review. Carbohydr. Polym 168, 32–43. [PubMed: 28457455]

Aguilar LMC, Kapsa RM, O'Connell CD, McArthur SL, Stoddart PR, Moulton SE, 2019 Controlled release from PCL–alginate microspheres via secondary encapsulation using GelMA/HAMA hydrogel scaffolds. Soft Matter 15, 3779–3787. [PubMed: 30989161]

Aguirre Calvo T, Santagapita P, 2016 Physicochemical characterization of alginate beads containing sugars and biopolymers. J. Qual. Reliab. Eng 2016.

Ahn S, Kim G, 2015 Cell-encapsulating alginate microsized beads using an air-assisted atomization process to obtain a cell-laden hybrid scaffold. J. Mater. Chem. B 3, 9132–9139.

Alizadeh Sardroud H, Nemati S, Baradar Khoshfetrat A, Nabavinia M, Beygi Khosrowshahi Y, 2017 Barium-cross-linked alginate-gelatine microcapsule as a potential platform for stem cell production and modular tissue formation. J. Microencapsul 34, 488–497. [PubMed: 28699824]

Anandharamakrishnan C, Rielly CD, Stapley AGF, 2007 Effects of process variables on the denaturation of whey proteins during spray drying. Dry. Technol 25, 799–807.

Ashton RS, Banerjee A, Punyani S, Schaffer DV, Kane RS, 2007 Scaffolds based on degradable alginate hydrogels and poly(lactide-co-glycolide) microspheres for stem cell culture. Biomaterials 28, 5518–5525. [PubMed: 17881048]

Augst AD, Kong HJ, Mooney DJ, 2006 Alginate hydrogels as biomaterials. Macromol. Biosci 6, 623–633. [PubMed: 16881042]

Bagheri L, Madadlou A, Yarmand M, Mousavi ME, 2014 Spray-dried alginate microparticles carrying caffeine-loaded and potentially bioactive nanoparticles. Food Res. Int 62, 1113–1119.

Baimark Y, Srisuwan Y, 2014 Preparation of alginate microspheres by water-in-oil emulsion method for drug delivery: Effect of Ca2+ post-cross-linking. Adv. Powder Technol 25, 1541–1546.

Ballesteros NA, Alonso M, Saint-Jean SR, Perez-Prieto SI, 2015 An oral DNA vaccine against infectious haematopoietic necrosis virus (IHNV) encapsulated in alginate microspheres induces dose-dependent immune responses and significant protection in rainbow trout (Oncorrhynchus mykiss). Fish Shellfish Immunol 45, 877–888. [PubMed: 26054788]

Banskota S, Yousefpour P, Kirmani N, Li X, Chilkoti A, 2019 Long circulating genetically encoded intrinsically disordered zwitterionic polypeptides for drug delivery. Biomaterials 192, 475–485. [PubMed: 30504081]

Bayer EA, Jordan J, Roy A, Gottardi R, Fedorchak MV, Kumta PN, Little SR, 2017 Programmed Platelet-Derived Growth Factor-BB and Bone Morphogenetic Protein-2 Delivery from a Hybrid Calcium Phosphate/Alginate Scaffold. Tissue Eng. Part A 23, 1382–1393. [PubMed: 28537482]

Becker TA, Kipke DR, Brandon T, 2001 Calcium alginate gel: a biocompatible and mechanically stable polymer for endovascular embolization. J. Biomed. Mater. Res. An Off. J. Soc. Biomater. Japanese Soc. Biomater 54, 76–86.

Benavides S, Cortés P, Parada J, Franco W, 2016 Development of alginate microspheres containing thyme essential oil using ionic gelation. Food Chem 204, 77–83. [PubMed: 26988478]

Bian L, Zhai DY, Tous E, Rai R, Mauck RL, Burdick JA, 2011 Enhanced MSC chondrogenesis following delivery of TGF-β3 from alginate microspheres within hyaluronic acid hydrogels in vitro and in vivo. Biomaterials 32, 6425–6434. [PubMed: 21652067]

Blemur L, Le TC, Marcocci L, Pietrangeli P, Mateescu MA, 2016 Carboxymethyl starch/alginate microspheres containing diamine oxidase for intestinal targeting. Biotechnol. Appl. Biochem 63, 344–353. [PubMed: 25779356]

Bochenek MA, Veiseh O, Vegas AJ, McGarrigle JJ, Qi M, Marchese E, Omami M, Doloff JC, Mendoza-Elias J, Nourmohammadzadeh M, 2018 Alginate encapsulation as long-term immune protection of allogeneic pancreatic islet cells transplanted into the omental bursa of macaques. Nat. Biomed. Eng 2, 810. [PubMed: 30873298]

Büyüköz M, Erdal E, Alsoy Altinkaya S, 2018 Nanofibrous gelatine scaffolds integrated with nerve growth factor- loaded alginate microspheres for brain tissue engineering. J. Tissue Eng. Regen. Med 12, e707–e719. [PubMed: 27863118]

Cañibano-Hernández A, del Burgo LS, Espona-Noguera A, Orive G, Hernández RM, Ciriza J, Pedraz JL, 2019 Hyaluronic acid enhances cell survival of encapsulated insulin-producing cells in alginate-based microcapsules. Int. J. Pharm 557, 192–198. [PubMed: 30597265]

Chandy T, Mooradian DL, Rao GHR, 1999 Evaluation of modified alginate- chitosan- polyethylene glycol microcapsules for cell encapsulation. Artif. Organs 23, 894–903. [PubMed: 10564287]

Chen C-Y, Ke C-J, Yen K-C, Hsieh H-C, Sun J-S, Lin F-H, 2015 3D porous calcium-alginate scaffolds cell culture system improved human osteoblast cell clusters for cell therapy. Theranostics 5, 643. [PubMed: 25825603]

Chen M-Y, Zheng W, Dong Q-Y, Li Z-H, Shi L-E, Tang Z-X, 2014 Activity of encapsulated Lactobacillus bulgaricus in alginate-whey protein microspheres. Brazilian Arch. Biol. Technol 57, 736–741.

Chen Z, Lv X, Zhao M, Zhang P, Ren X, Mei X, 2018 Encapsulation of green tea polyphenol by pH responsive, antibacterial, alginate microgels used for minimally invasive treatment of bone infection. Colloids Surfaces B Biointerfaces 170, 648–655. [PubMed: 29986260]

Ching SH, Bansal N, Bhandari B, 2017 Alginate gel particles–A review of production techniques and physical properties. Crit. Rev. Food Sci. Nutr 57, 1133–1152. [PubMed: 25976619]

Dalmoro A, Barba AA, d'Amore M, Lamberti G, 2014 Single-pot semicontinuous bench scale apparatus to produce microparticles. Ind. Eng. Chem. Res 53, 2771–2780.

Dalmoro A, Barba AA, Lamberti G, d'Amore M, 2012 Intensifying the microencapsulation process: Ultrasonic atomization as an innovative approach. Eur. J. Pharm. Biopharm 80, 471–477. [PubMed: 22285525]

Dalmoro A, Sitenkov AY, Cascone S, Lamberti G, Barba AA, Moustafine RI, 2017 Hydrophilic drug encapsulation in shell-core microcarriers by two stage polyelectrolyte complexation method. Int. J. Pharm 518, 50–58. [PubMed: 28034735]

Darrabie MD, Kendall WF Jr, Opara EC, 2005 Characteristics of poly-L-ornithine-coated alginate microcapsules. Biomaterials 26, 6846–6852. [PubMed: 15955558]

Dimitrov DS, 2012 Therapeutic proteins, in: Therapeutic Proteins Springer, pp. 1–26.

Ding D, Zhang Y, Sykes EA, Chen L, Chen Z, Tan W, 2019 The influence of physiological environment on the targeting effect of aptamer-guided gold nanoparticles. Nano Res 12, 129–135. 10.1007/s12274-018-2191-9

Ding S-L, Zhang M-Y, Tang S-J, Yang H, Tan W-Q, 2015 Effect of calcium alginate microsphere loaded with vascular endothelial growth factor on adipose tissue transplantation. Ann. Plast. Surg 75, 644–651. [PubMed: 25180953]

Dong X, Liu H-J, Feng H-Y, Yang S-C, Liu X-L, Lai X, Lu Q, Lovell JF, Chen H-Z, Fang C, 2019 Enhanced Drug Delivery by Nanoscale Integration of a Nitric Oxide Donor to Induce Tumor Collagen Depletion. Nano Lett

Dounighi N, Shahcheraghi F, Razzaghi-Abyaneh M, Nofeli M, Zolfagharian H, 2017 A New Vaccine Delivery Vehicle and Adjuvant Candidate: Bordetella pertussis Inactivated Whole Cells Entrapped in Alginate Microspheres. Curr. Pharm. Des 23, 2665–2672. [PubMed: 28079006]

Etter JN, Karasinski M, Ware J, Oldinski RA, 2018 Dual-crosslinked homogeneous alginate microspheres for mesenchymal stem cell encapsulation. J. Mater. Sci. Mater. Med 29, 143. [PubMed: 30151747]

Ganesh M, Jeon UJ, Ubaidulla U, Hemalatha P, Saravanakumar A, Peng MM, Jang HT, 2015 Chitosan cocrystals embedded alginate beads for enhancing the solubility and bioavailability of aceclofenac. Int. J. Biol. Macromol 74, 310–317. [PubMed: 25557368]

Giudice EL, Campbell JD, 2006 Needle-free vaccine delivery. Adv. Drug Deliv. Rev 58, 68–89. [PubMed: 16564111]

Gombotz WR, Wee S, 1998 Protein release from alginate matrices. Adv. Drug Deliv. Rev 31, 267–285. [PubMed: 10837629]

Grellier M, Granja PL, Fricain J-C, Bidarra SJ, Renard M, Bareille R, Bourget C, Amédée J, Barbosa MA, 2009 The effect of the co-immobilization of human osteoprogenitors and endothelial cells within alginate microspheres on mineralization in a bone defect. Biomaterials 30, 3271–3278. [PubMed: 19299013]

Guo Y-H, Zhao S, Du Y-X, Xing Q-J, Chen B-L, Yu C-Q, 2017 Effects of ginsenoside Rg1-loaded alginate-chitosan microspheres on human bone marrow stromal cells. Biosci. Rep 37, BSR20160566.

Halder A, Maiti S, Sa B, 2005 Entrapment efficiency and release characteristics of polyethyleneimine-treated or-untreated calcium alginate beads loaded with propranolol–resin complex. Int. J. Pharm 302, 84–94. [PubMed: 16102927]

Hariyadi DM, Ma Y, Wang Y, Bostrom T, Malouf J, Turner MS, Bhandari B, Coombes AGA, 2014 The potential for production of freeze-dried oral vaccines using alginate hydrogel microspheres as protein carriers. J. Drug Deliv. Sci. Technol 24, 178–184.

Huang Feng-ying, Huang Feng-ru, Chen B, Liu Q, Wang H, Zhou S, Zhao H, Huang Y, Lin Y, Tan G, 2015 Microencapsulation of tumor lysates and live cell engineering with MIP-3α as an effective vaccine. Biomaterials 53, 554–565. [PubMed: 25890751]

Huang K-S, Lai T-H, Lin Y-C, 2006 Manipulating the generation of Ca-alginate microspheres using microfluidic channels as a carrier of gold nanoparticles. Lab Chip 6, 954–957. [PubMed: 16804602]

Hussein N, Omer H, Ismael A, Albed Alhnan M, Elhissi A, Ahmed W, 2019 Spray-Dried Alginate Microparticles for Potential Intranasal Delivery of Ropinirole Hydrochloride: Development, Characterization and Histopathological Evaluation. Pharm. Dev. Technol 1–36.

Jain RR, Mehta MR, Bannalikar AR, Menon MD, 2015 Alginate microparticles loaded with lipopolysaccharide subunit antigen for mucosal vaccination against Klebsiella pneumoniae. Biologicals 43, 195–201. [PubMed: 25737397]

Jaya S, Durance TD, Wang R, 2009 Effect of alginate-pectin composition on drug release characteristics of microcapsules. J. Microencapsul 26, 143–153. 10.1080/02652040802211345 [PubMed: 18615289]

Jung SM, Lee JH, Lee HJ, Jeon JY, Park TH, Yoon JH, Shin HW, 2018 The growth of alginate-encapsulated macroalgal spores. Aquaculture 491, 333–337.

Juri S, ermi E, Topolovec-Pintari S, Bedek M, Vincekovi M, 2019 Physicochemical properties and release characteristics of calcium alginate microspheres loaded with Trichoderma viride spores. J. Integr. Agric

Kanafi MM, Ramesh A, Gupta PK, Bhonde RR, 2014 Dental pulp stem cells immobilized in alginate microspheres for applications in bone tissue engineering. Int. Endod. J 47, 687–697. [PubMed: 24127887]

Kim B, Bowersock T, Griebel P, Kidane A, Babiuk LA, Sanchez M, Attah-Poku S, Kaushik RS, Mutwiri GK, 2002 Mucosal immune responses following oral immunization with rotavirus antigens encapsulated in alginate microspheres. J. Control. release 85, 191–202. [PubMed: 12480324]

Koch S, Schwinger C, Kressler J, Heinzen CH, Rainov NG, 2003 Alginate encapsulation of genetically engineered mammalian cells: comparison of production devices, methods and microcapsule characteristics. J. Microencapsul 20, 303–316. [PubMed: 12881112]

Lamas MC, Bregni C, D'Aquino M, Degrossi J, Firenstein R, 2001 Calcium alginate microspheres of Bacillus subtilis. Drug Dev. Ind. Pharm 27, 825–829. [PubMed: 11699834]

Lee JW, Kim H, Lee KY, 2016 Effect of spacer arm length between adhesion ligand and alginate hydrogel on stem cell differentiation. Carbohydr. Polym 139, 82–89. [PubMed: 26794950]

Lee KY, Mooney DJ, 2012 Alginate: properties and biomedical applications. Prog. Polym. Sci 37, 106–126. [PubMed: 22125349]

Liao J, Wang B, Huang Y, Qu Y, Peng J, Qian Z, 2017 Injectable alginate hydrogel cross-linked by calcium gluconate-loaded porous microspheres for cartilage tissue engineering. ACS omega 2, 443–454. [PubMed: 30023607]

Lin Y-H, Liang H-F, Chung C-K, Chen M-C, Sung H-W, 2005 Physically crosslinked alginate/N, O-carboxymethyl chitosan hydrogels with calcium for oral delivery of protein drugs. Biomaterials 26, 2105–2113. [PubMed: 15576185]

Ling K, Wu H, Neish AS, Champion JA, 2019 Alginate/chitosan microparticles for gastric passage and intestinal release of therapeutic protein nanoparticles. J. Control. Release 295, 174–186. [PubMed: 30557649]

Liu F, Ma C, Gao Y, McClements DJ, 2017 Food- Grade Covalent Complexes and Their Application as Nutraceutical Delivery Systems: A Review. Compr. Rev. Food Sci. Food Saf 16, 76–95.

Liu Q, Huang Y, Lan Y, Zuo Q, Li C, Zhang Y, Guo R, Xue W, 2017 Acceleration of skin regeneration in full- thickness burns by incorporation of bFGF- loaded alginate microspheres into a CMCS–PVA hydrogel. J. Tissue Eng. Regen. Med 11, 1562–1573. [PubMed: 26118827]

Liu Q, Zuo Q, Guo R, Hong A, Li C, Zhang Y, He L, Xue W, 2015 Fabrication and characterization of carboxymethyl chitosan/poly (vinyl alcohol) hydrogels containing alginate microspheres for protein delivery. J. Bioact. Compat. Polym 30, 397–411.

Lopes M, Abrahim B, Veiga F, Seiça R, Cabral LM, Arnaud P, Andrade JC, Ribeiro AJ, 2017 Preparation methods and applications behind alginate-based particles. Expert Opin. Drug Deliv 14, 769–782. 10.1080/17425247.2016.1214564 [PubMed: 27492462]

Lourenço AH, Neves N, Ribeiro-Machado C, Sousa SR, Lamghari M, Barrias CC, Cabral AT, Barbosa MA, Ribeiro CC, 2017 Injectable hybrid system for strontium local delivery promotes bone regeneration in a rat critical-sized defect model. Sci. Rep 7, 5098. [PubMed: 28698571]

Lupo B, Maestro A, Porras M, Gutiérrez JM, González C, 2014 Preparation of alginate microspheres by emulsification/internal gelation to encapsulate cocoa polyphenols. Food Hydrocoll 38, 56–65.

Ma Y, Pacan JC, Wang Q, Sabour PM, Huang X, Xu Y, 2012 Enhanced alginate microspheres as means of oral delivery of bacteriophage for reducing Staphylococcus aureus intestinal carriage. Food Hydrocoll 26, 434–440.

Ma Y, Pacan JC, Wang Q, Xu Y, Huang X, Korenevsky A, Sabour PM, 2008 Microencapsulation of bacteriophage Felix O1 into chitosan-alginate microspheres for oral delivery. Appl. Environ. Microbiol 74, 4799–4805. [PubMed: 18515488]

Machida-Sano I, Hirakawa M, Matsumoto H, Kamada M, Ogawa S, Satoh N, Namiki H, 2014 Surface characteristics determining the cell compatibility of ionically cross-linked alginate gels. Biomed. Mater 9, 25007.

Maestrelli F, Mura P, González-Rodríguez ML, Cózar-Bernal MJ, Rabasco AM, Mannelli LDC, Ghelardini C, 2017 Calcium alginate microspheres containing metformin hydrochloride niosomes and chitosomes aimed for oral therapy of type 2 diabetes mellitus. Int. J. Pharm 530, 430–439. [PubMed: 28778628]

Mallikarjuna Setty C, Sahoo SS, Sa B, 2005 Alginate-coated alginate-polyethyleneimine beads for prolonged release of furosemide in simulated intestinal fluid. Drug Dev. Ind. Pharm 31, 435–446. [PubMed: 16093209]

Mansouri V, Salehi M, davood Omrani M, Niknam Z, Ardeshirylajimi A, 2017 Collagen-alginate microspheres as a 3D culture system for mouse embryonic stem cells differentiation to primordial germ cells. Biologicals 48, 114–120. [PubMed: 28483511]

Martín MJ, Calpena AC, Fernández F, Mallandrich M, Gálvez P, Clares B, 2015 Development of alginate microspheres as nystatin carriers for oral mucosa drug delivery. Carbohydr. Polym 117, 140–149. [PubMed: 25498619]

Martins S, Sarmento B, Souto EB, Ferreira DC, 2007 Insulin-loaded alginate microspheres for oral delivery–effect of polysaccharide reinforcement on physicochemical properties and release profile. Carbohydr. Polym 69, 725–731.

Md S, Ahuja A, Khar RK, Baboota S, Chuttani K, Mishra AK, Ali J, 2011 Gastroretentive drug delivery system of acyclovir-loaded alginate mucoadhesive microspheres: formulation and evaluation. Drug Deliv 18, 255–264. [PubMed: 21110695]

Mehta AS, Singh BK, Singh N, Archana D, Snigdha K, Harniman R, Rahatekar SS, Tewari RP, Dutta PK, 2015 Chitosan silk-based three-dimensional scaffolds containing gentamicin-encapsuled calcium alginate beads for drug administration and blood compatibility. J. Biomater. Appl 29, 1314–1325. [PubMed: 25492055]

Meier RPH, Mahou R, Morel P, Meyer J, Montanari E, Muller YD, Christofilopoulos P, Wandrey C, Gonelle-Gispert C, Bühler LH, 2015 Microencapsulated human mesenchymal stem cells decrease liver fibrosis in mice. J. Hepatol 62, 634–641. [PubMed: 25450712]

Melvik JE, Dornish M, 2004 Alginate as a carrier for cell immobilisation, in: Fundamentals of Cell Immobilisation Biotechnology Springer, pp. 33–51.

Mennini N, Furlanetto S, Cirri M, Mura P, 2012 Quality by design approach for developing chitosan-Ca-alginate microspheres for colon delivery of celecoxib-hydroxypropyl-β-cyclodextrin-PVP complex. Eur. J. Pharm. Biopharm 80, 67–75. [PubMed: 21864681]

Mørch ÝA, Donati I, Strand BL, Skjåk-Bræk G, 2006 Effect of Ca2+, Ba2+, and Sr2+ on alginate microbeads. Biomacromolecules 7, 1471–1480. [PubMed: 16677028]

Nagpal M, Maheshwari DK, Rakha P, Dureja H, Goyal S, Dhingra G, 2012 Formulation development and evaluation of alginate microspheres of ibuprofen. J. Young Pharm 4, 13–16. [PubMed: 22523454]

Opara EC, McQuilling JP, Farney AC, 2013 Microencapsulation of pancreatic islets for use in a bioartificial pancreas, in: Organ Regeneration Springer, pp. 261–266.

Orive G, Hernández RM, Gascón AR, Calafiore R, Chang TMS, De Vos P, Hortelano G, Hunkeler D, Lacík I, Shapiro AMJ, 2003 Cell encapsulation: promise and progress. Nat. Med 9, 104. [PubMed: 12514721]

Patil SB, Sawant KK, 2009 Development, optimization and in vitro evaluation of alginate mucoadhesive microspheres of carvedilol for nasal delivery. J. Microencapsul 26, 432–443. [PubMed: 18932060]

Pawar SN, Edgar KJ, 2012 Alginate derivatization: a review of chemistry, properties and applications. Biomaterials 33, 3279–3305. [PubMed: 22281421]

Piornos JA, Burgos-Díaz C, Morales E, Rubilar M, Acevedo F, 2017 Highly efficient encapsulation of linseed oil into alginate/lupin protein beads: Optimization of the emulsion formulation. Food Hydrocoll 63, 139–148.

Pitaktamrong P, Kingkaew J, Yooyongwech S, Cha-um S, Phisalaphong M, 2018 Development of Arbuscular Mycorrhizal Fungi-Organic Fertilizer Pellets Encapsulated with Alginate Film. Eng. J 22, 65–79.

Prüsse U, Bilancetti L, Bu ko M, Bugarski B, Bukowski J, Gemeiner P, Lewi ska D, Manojlovic V, Massart B, Nastruzzi C, 2008 Comparison of different technologies for alginate beads production. Chem. Pap 62, 364–374.

Qi M, Mørch Y, Lacík I, Formo K, Marchese E, Wang Y, Danielson KK, Kinzer K, Wang S, Barbaro B, 2012 Survival of human islets in microbeads containing high guluronic acid alginate crosslinked with Ca2+ and Ba2+. Xenotransplantation 19, 355–364. [PubMed: 23198731]

Qi X, Qin X, Yang R, Qin J, Li W, Luan K, Wu Z, Song L, 2016 Intra-articular Administration of Chitosan Thermosensitive In Situ Hydrogels Combined With Diclofenac Sodium–Loaded Alginate Microspheres. J. Pharm. Sci 105, 122–130. [PubMed: 26852847]

Quinlan E, López-Noriega A, Thompson E, Kelly HM, Cryan SA, O'brien FJ, 2015 Development of collagen–hydroxyapatite scaffolds incorporating PLGA and alginate microparticles for the controlled delivery of rhBMP-2 for bone tissue engineering. J. Control. Release 198, 71–79. [PubMed: 25481441]

Rastogi R, Sultana Y, Aqil M, Ali A, Kumar S, Chuttani K, Mishra AK, 2007 Alginate microspheres of isoniazid for oral sustained drug delivery. Int. J. Pharm 334, 71–77. [PubMed: 17113732]

Rehm BHA, Valla S, 1997 Bacterial alginates: biosynthesis and applications. Appl. Microbiol. Biotechnol 48, 281–288. [PubMed: 9352672]

Reis CP, Neufeld RJ, Vilela S, Ribeiro AJ, Veiga F, 2006 Review and current status of emulsion/ dispersion technology using an internal gelation process for the design of alginate particles. J. Microencapsul 23, 245–257. [PubMed: 16801237]

Sailaja G, HogenEsch H, North A, Hays J, Mittal SK, 2002 Encapsulation of recombinant adenovirus into alginate microspheres circumvents vector-specific immune response. Gene Ther 9, 1722. [PubMed: 12457287]

Sandvig I, Karstensen K, Rokstad AM, Aachmann FL, Formo K, Sandvig A, Skjåk-Bræk G, Strand BL, 2015 RGD- peptide modified alginate by a chemoenzymatic strategy for tissue engineering applications. J. Biomed. Mater. Res. Part A 103, 896–906.

Schneider S, Feilen PJ, Slotty V, Kampfner D, Preuss S, Berger S, Beyer J, Pommersheim R, 2001 Multilayer capsules: a promising microencapsulation system for transplantation of pancreatic islets. Biomaterials 22, 1961–1970. [PubMed: 11426874]

Shi L-E, Li Z-H, Li D-T, Xu M, Chen H-Y, Zhang Z-L, Tang Z-X, 2013 Encapsulation of probiotic Lactobacillus bulgaricus in alginate–milk microspheres and evaluation of the survival in simulated gastrointestinal conditions. J. Food Eng 117, 99–104.

Shi L-E, Zheng W, Zhang Y, Tang Z-X, 2016 Milk-alginate microspheres: Protection and delivery of Enterococcus faecalis HZNU P2. LWT-Food Sci. Technol 65, 840–844.

Shinde UA, Shete JN, Nair HA, Singh KH, 2014 Design and characterization of chitosan-alginate microspheres for ocular delivery of azelastine. Pharm. Dev. Technol 19, 813–823. [PubMed: 24032373]

Silva CM, Ribeiro AJ, Ferreira D, Veiga F, 2006 Insulin encapsulation in reinforced alginate microspheres prepared by internal gelation. Eur. J. Pharm. Sci 29, 148–159. [PubMed: 16952452]

Sobol M, Bartkowiak A, de Haan B, de Vos P, 2013 Cytotoxicity study of novel water- soluble chitosan derivatives applied as membrane material of alginate microcapsules. J. Biomed. Mater. Res. Part A 101, 1907–1914.

Sosnik A, 2014 Alginate particles as platform for drug delivery by the oral route: state-of-the-art. ISRN Pharm 2014.

Sugiura S, Oda T, Izumida Y, Aoyagi Y, Satake M, Ochiai A, Ohkohchi N, Nakajima M, 2005 Size control of calcium alginate beads containing living cells using micro-nozzle array. Biomaterials 26, 3327–3331. [PubMed: 15603828]

Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K, 2000 Encapsulation of probiotic bacteria with alginate–starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. Int. J. Food Microbiol 62, 47–55. [PubMed: 11139021]

Szekalska M, Amelian A, Winnicka K, 2015 Alginate microspheres obtained by the spray drying technique as mucoadhesive carriers of ranitidine. Acta Pharm 65, 15–27. [PubMed: 25781701]

Tafaghodi M, Eskandari M, Khamesipour A, Jaafari MR, 2011 Alginate microspheres encapsulated with autoclaved Leishmania major (ALM) and CpG-ODN induced partial protection and enhanced immune response against murine model of leishmaniasis. Exp. Parasitol 129, 107–114. [PubMed: 21767536]

Tafaghodi M, Tabassi SAS, Jaafari MR, 2006 Induction of systemic and mucosal immune responses by intranasal administration of alginate microspheres encapsulated with tetanus toxoid and CpG-ODN. Int. J. Pharm 319, 37–43. [PubMed: 16701972]

Taha MO, Aiedeh KM, Al-Hiari Y, Al-Khatib H, 2005 Synthesis of zinc-crosslinked thiolated alginic acid beads and their in vitro evaluation as potential enteric delivery system with folic acid as model drug. Die Pharm. Int. J. Pharm. Sci 60, 736–742.

Tian J-Y, Sun X-Q, Chen X-G, 2008 Formation and oral administration of alginate microspheres loaded with pDNA coding for lymphocystis disease virus (LCDV) to Japanese flounder. Fish Shellfish Immunol 24, 592–599. [PubMed: 18328731]

U.S. National Library of Medicine, 2017 Open-label Investigation of the Safety and Effectiveness of DIABECELL(R) in Patients With Type I Diabetes Mellitus [WWW Document] URL https://clinicaltrials.gov/ct2/show/NCT00940173

Utech S, Prodanovic R, Mao AS, Ostafe R, Mooney DJ, Weitz DA, 2015 Microfluidic generation of monodisperse, structurally homogeneous alginate microgels for cell encapsulation and 3D cell culture. Adv. Healthc. Mater 4, 1628–1633. [PubMed: 26039892]

van Elk M, Ozbakir B, Barten-Rijbroek AD, Storm G, Nijsen F, Hennink WE, Vermonden T, Deckers R, 2015 Alginate microspheres containing temperature sensitive liposomes (TSL) for MR-guided embolization and triggered release of doxorubicin. PLoS One 10, e0141626. [PubMed: 26561370]

Wang L, Shelton RM, Cooper PR, Lawson M, Triffitt JT, Barralet JE, 2003 Evaluation of sodium alginate for bone marrow cell tissue engineering. Biomaterials 24, 3475–3481. [PubMed: 12809776]

Wang Xiaoqin, Wenk E, Hu X, Castro GR, Meinel L, Wang Xianyan, Li C, Merkle H, Kaplan DL, 2007 Silk coatings on PLGA and alginate microspheres for protein delivery. Biomaterials 28, 4161–4169. [PubMed: 17583788]

Wells LA, Sheardown H, 2007 Extended release of high pI proteins from alginate microspheres via a novel encapsulation technique. Eur. J. Pharm. Biopharm 65, 329–335. [PubMed: 17156984]

Wen Y, Grøndahl L, Gallego MR, Jorgensen L, Møller EH, Nielsen HM, 2012 Delivery of dermatan sulfate from polyelectrolyte complex-containing alginate composite microspheres for tissue regeneration. Biomacromolecules 13, 905–917. [PubMed: 22296594]

Wong CY, Al-Salami H, Dass CR, 2018 Microparticles, microcapsules and microspheres: a review of recent developments and prospects for oral delivery of insulin. Int. J. Pharm 537, 223–244. [PubMed: 29288095]

Wu J, Kong T, Yeung KWK, Shum HC, Cheung KMC, Wang L, To MKT, 2013 Fabrication and characterization of monodisperse PLGA–alginate core–shell microspheres with monodisperse size and homogeneous shells for controlled drug release. Acta Biomater 9, 7410–7419. [PubMed: 23535235]

Yang M, Yang T, Jia J, Lu T, Wang H, Yan X, Wang L, Yu L, Zhao Y, 2018 Fabrication and characterization of DDAB/PLA-alginate composite microcapsules as single-shot vaccine. RSC Adv 8, 13612–13624.

Yu J, Chen H, Jiang L, Wang Jianhong, Dai J, Wang Jie, 2019 Codelivery of Adriamycin and P-gp Inhibitor Quercetin Using PEGylated Liposomes to Overcome Cancer Drug Resistance. J. Pharm. Sci

Yu J, Du KT, Fang Q, Gu Y, Mihardja SS, Sievers RE, Wu JC, Lee RJ, 2010 The use of human mesenchymal stem cells encapsulated in RGD modified alginate microspheres in the repair of myocardial infarction in the rat. Biomaterials 31, 7012–7020. [PubMed: 20566215]

Yu L, Ni C, Grist SM, Bayly C, Cheung KC, 2015 Alginate core-shell beads for simplified three-dimensional tumor spheroid culture and drug screening. Biomed. Microdevices 17, 33. [PubMed: 25681969]

Zhai P, Chen XB, Schreyer DJ, 2015 PLGA/alginate composite microspheres for hydrophilic protein delivery. Mater. Sci. Eng C 56, 251–259.

Zhang Y, Wang X, Su Y, Chen D, Zhong W, 2016 A doxorubicin delivery system: samarium/mesoporous bioactive glass/alginate composite microspheres. Mater. Sci. Eng. C 67, 205–213.

Zhao D, Li J-S, Suen W, Chang M-W, Huang J, 2016 Preparation and characterization of Ganoderma lucidum spores-loaded alginate microspheres by electrospraying. Mater. Sci. Eng. C 62, 835–842.

Zheng X, Yang X, Li X, Qiu G-H, Dai A, Huang Q, Huang C, Guo X, 2017 Omp16-based vaccine encapsulated by alginate-chitosan microspheres provides significant protection against Haemophilus parasuis in mice. Vaccine 35, 1417–1423. [PubMed: 28187951]

Zhu Y, Wang J, Wu J, Zhang J, Wan Y, Wu H, 2016 Injectable hydrogels embedded with alginate microspheres for controlled delivery of bone morphogenetic protein-2. Biomed. Mater 11, 25010.

Zuo Q, Guo R, Liu Q, Hong A, Shi Y, Kong Q, Huang Y, He L, Xue W, 2015 Heparin-conjugated alginate multilayered microspheres for controlled release of bFGF. Biomed. Mater 10, 35008.

Ca²⁺

Aqueous Alginate solution

Calcium Crosslinked Alginate gel microspheres
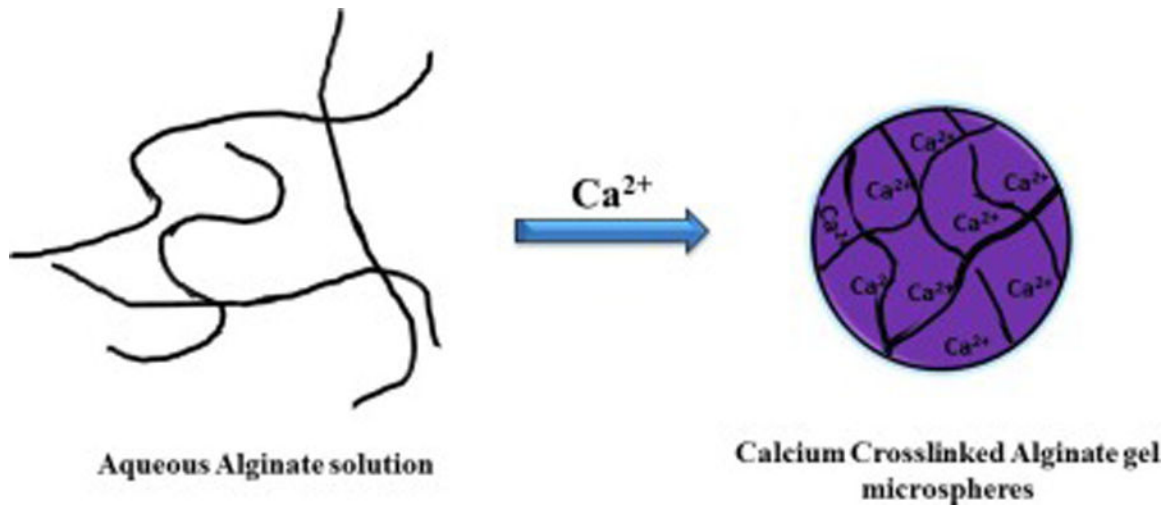
Figure 1:
Representation of the standard calcium-alginate gel egg-box structure formation on addition of Ca^{2+} ions
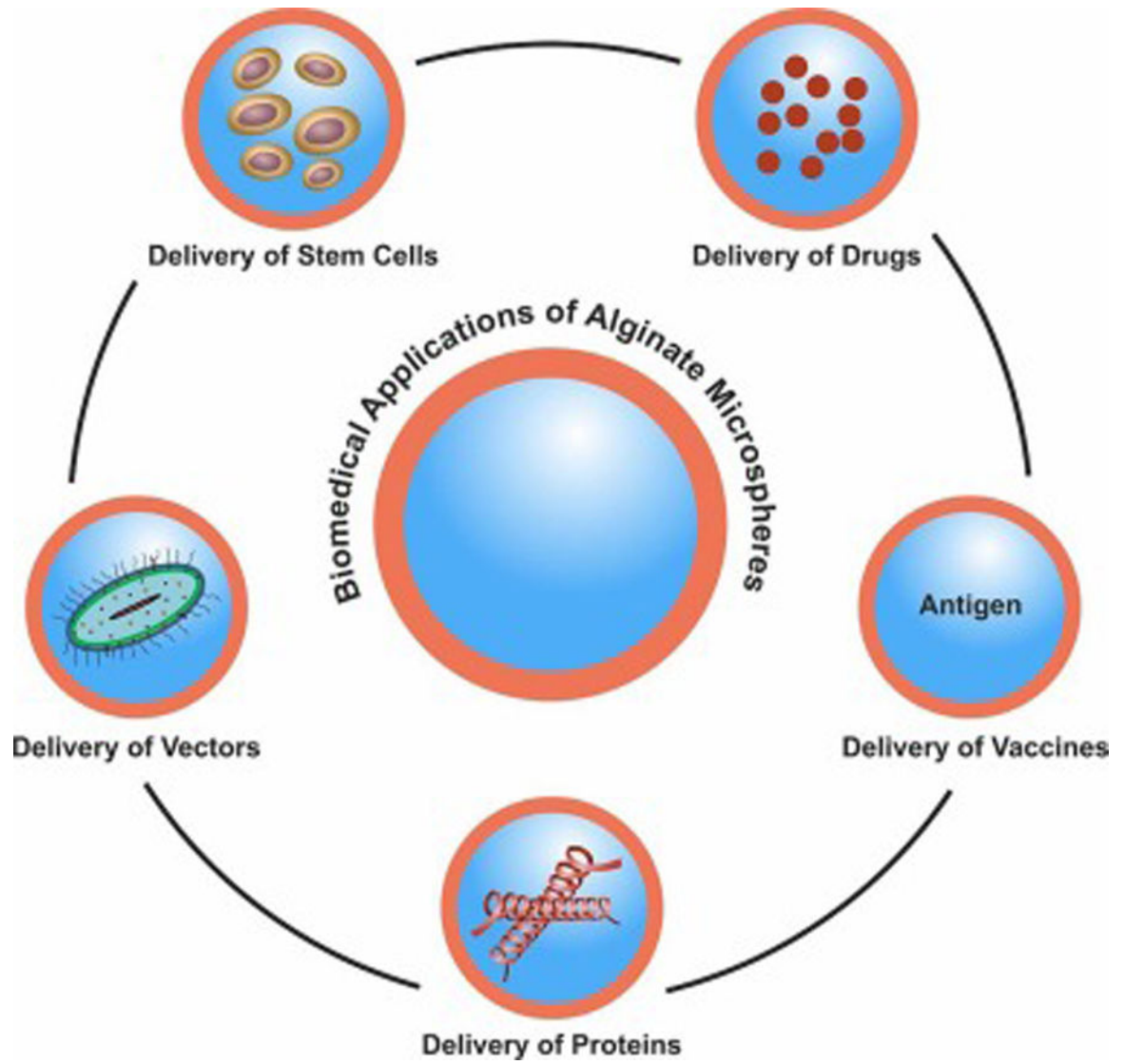
Figure 2:
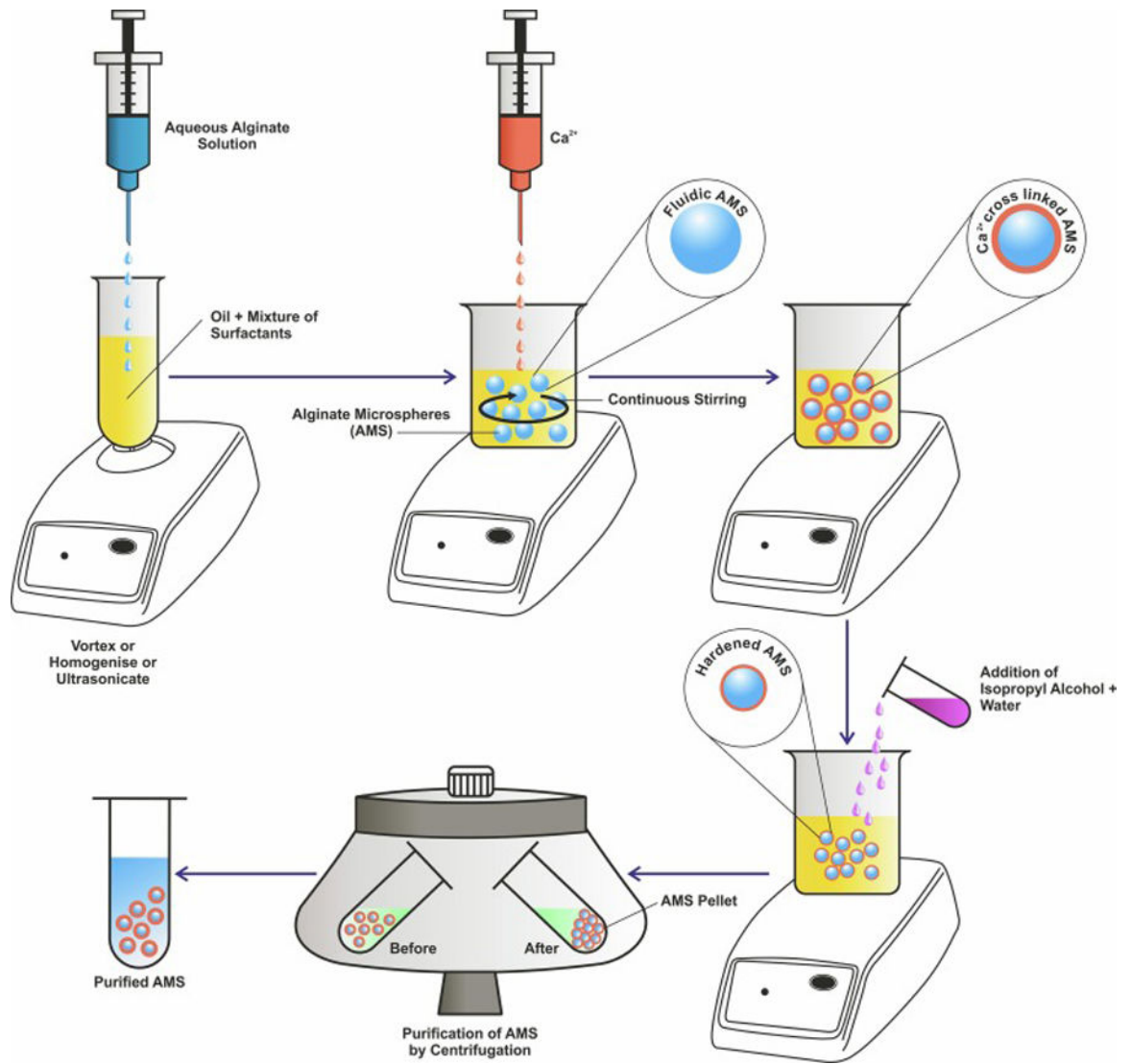The various biomedical applications of alginate microspheres

Figure 3:
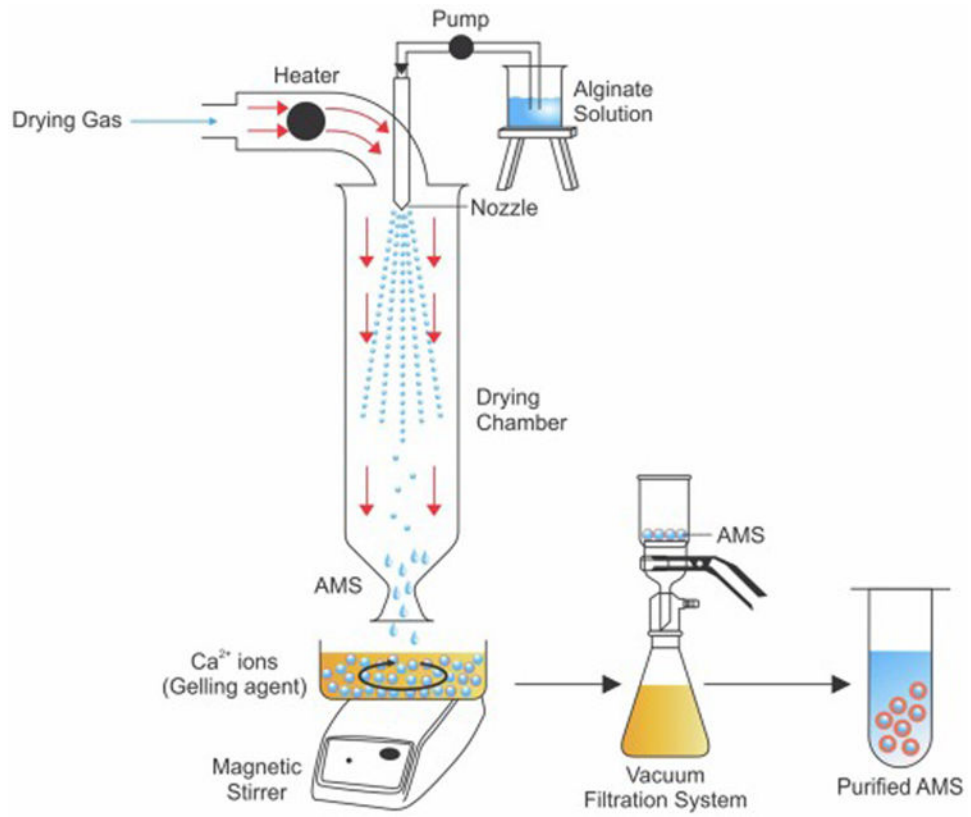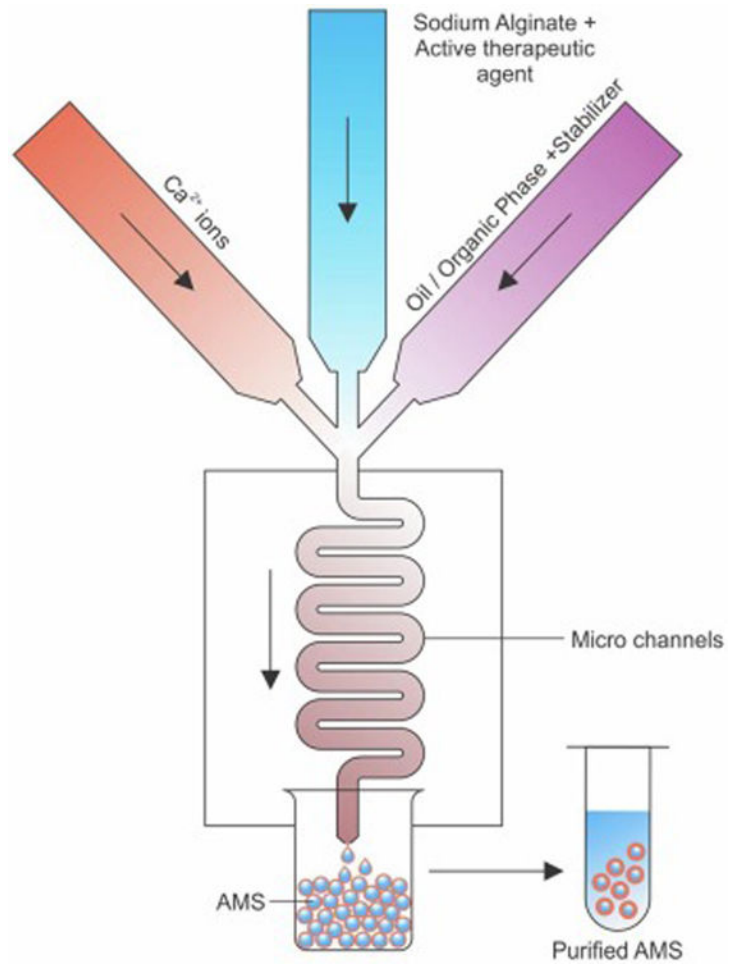Schematic representation of the formulation of AMS by single emulsion technique

Figure 4:
Schematic representation of the formulation of AMS by spray drying method

Figure 5:
Schematic representation of the formulation of AMS by microfluidics method

Table 1:

Examples of AMS used in drug delivery applications (arranged according for formulation method)

Therapeutic agent	Formulation method	Materials used	Findings	Reference
Metformin	Emulsification	Metformin encapsulated chitosomes and niosomes loaded in AMS	Significant improvement of metformin hypoglycemic effect when administered orally as chitosomal-AMS. AMS-chitosan protected metformin from gastric acidic environment.	(Maestrelli et al., 2017)
Thyme essential oils	Emulsification ionic gelation	Calcium crosslinked AMS	AMS protected essentials oils from volatilization or oxidation. High encapsulation efficiency of 85%	(Benavides et al., 2016)
Cocoa polyphenols	Emulsification/ internal gelation	Calcium/citrate, alginate, spans	Optimized formulation showed 60% retention of cocoa extract encapsulated into AMS.	(Lupo et al., 2014)
Celecoxib	Co-grinding micro-mill for HPBCD and PVP composite with celecoxib and emulsion method for AMS	Hydroxypropyl-β-cyclodextrin (HPBCD) and PVP (polyvinylpyrrolidone) Chitosan-Ca-AMS	HPBCD and PVP improved solubility of celecoxib, which significantly shortened pain alleviation onset *in vivo*	(Mennini et al., 2012)
Acyclovir	Simple emulsification phase separation technique	Alginate mucoadhesive microspheres	Entrapment efficiency = 51.42–80.46%. Optimized AMS possess good mucoadhesion and *in vivo* gastroretention for > 4h	(Md et al., 2011)
Doxorubicin	Emulsification	Samarium (Sm)/ mesoporous bioactive glass (MBG)/ alginate composite microspheres	Sm/MBG/AMSs exhibit sustained doxorubicin delivery, and release by Fickian diffusion according the Higuchi model. The release is dependent on Sm doping and pH	(Zhang et al., 2016)
Azelastine	Ionotropic gelation	Chitosan, sodium alginate	Entrapment efficiency = 73.05%, 65% mucin binding efficiency, controlled release for 8h.	(Shinde et al., 2014)
Diclofenac sodium	Emulsification and/or gelation method	Thermosensitive hydrogels of chitosan and β-glycerophosphate combined with AMS	Sol-gel transition at $31.72 \pm 0.42°C$. Gel formation within 5 min following intraarticular injection. Sustained release for upto 5 days.	(Qi et al., 2016)
Doxorubicin + T1 and T2 MRI contrast agents	Spraying device	Barium crosslinked AMS loaded with temperature sensitive liposomes (TSL)	TSL incorporation had slight effect on temperature-triggered drug release. Particles detected by MRI before and after hyperthermia *in vitro* and *in vivo*	(van Elk et al., 2015)
Ropinirole hydrochloride	Spray drying	Sodium alginate	Spherical morphology (2.5 – 4.37 μm), yield = 70%, encapsulation efficiency = 100% when the inlet temperature of spray drying was 140 °C.	(Hussein et al., 2019)
Ranitidine	Spray drying	Sodium alginate	Smooth surface with narrow size distribution. Loading efficiency = 70.9 %, prolonged drug release observed	(Szekalska et al., 2015)
Caffeine	Spray drying	Antioxidant peptides, Sodium alginate	Peptidic nanoparticles encapsulating caffeine protected from gastric pH and caffeine leakage by spray drying and alginate encapsulation. 7.4 μm diameter, encapsulation efficiency = 85%	(Bagheri et al., 2014)
Rifampicin	Microfluidics	PLGA, Sodium alginate, dichloromethane, polyvinyl alcohol, toluene and span 80	Monodispersed PLGA–alginate core–shell microspheres, controlled release profile	(Wu et al., 2013)

Table 2:

Examples of cells encapsulated AMS (arranged according for formulation method)

Therapeutic agent	Formulation method	Materials used	Findings	Reference
Dental pulp stem cells (DPSC)	Emulsification	Calcium-AMS	Enhanced osteogenic potential of cells, maintained high cell viability for bone tissue regeneration	(Kanafi et al., 2014)
Enterococcus faecalis	Emulsification	Milk, alginate	Good tolerance to stimulated gastric fluid and bile salt solution (1.0 or 2.0%), improved storage stability of *E. faecalis*	(Shi et al., 2016)
Allogeneic pancreatic islet cells	Emulsification	Chemically modified alginate derivative Z1-Y15	Three of the several chemically modified AMS elicited a reduced foreign body response, are now in clinical trials.	(Bochenek et al., 2018)
Mesenchyma l stem cells (MSC)	Microfluidics with calcium crosslinking	Sodium alginate, calcium chloride-ethylenediam ine tetra acetic acid.	MSC encapsulated within homogenous AMS, stable cell growth and proliferation for 15 days.	(Utech et al., 2015)
Modified human pancreatic islets cells (1.1B4)	Microfluidics	Alginatepoly-L-lysine-copolymer	Microspheres formulated as microcapsules in the static state and using microfluidics in dynamic state. Cell viability and glucose simulated insulin secretion better in microcapsules formulated by dynamic microfluidics method	(Acarregui et al., 2018)
MSC	Co-axial air-flow droplet generator	Alginate-polyethylene glycol microspheres	Encapsulated MSC displayed anti-fibrotic and anti-inflammatory effects on liver fibrosis in mice due to secretion of soluble factors	(Meier et al., 2015)

Table 3:

Examples of proteins, microbes and vaccines encapsulated AMS (arranged according for formulation method)

Category	Therapeutic agent	Formulation method	Materials used	Findings	Reference
Proteins	Bone morphogenetic protein 2 (BMP- 2)	Emulsification	Calcium phosph ate cement with PLGA microsph eres incorporated in AMS	BMP-2 release from AMS led to the programmed delive ry to cell cultures representing fracture healing	(Bayer et al., 2017)
	Basic fibroblast growth factor (bFGF)	Emulsification	bFGF alginate, carboxymethyl chitosan (CMCS), poly (vinyl alcohol) (PVA)	bFGF encapsulated AMS were formulated and delivered using CMCS-PVA hydrogel base for wound healing in burns.	(Q. Liu et al., 2017)
	Diamine oxidase enzyme	Ionotropic gelation	Carboxymethyl starch (CMS)/alginate by complexation with Ca^{2+}	CMS and alginate were used as a novel matrix system microsphere for the encapsulation of diamine oxidase for protection against gastrointestinal degradation.	(Blemur et al., 2016)
	Basic fibroblast growth factor (bFGF)	Electrostatic droplet generation technique followed by a layer-by-layer self-assembly technique	Microsphere, heparin-conjugated alginate	Polyelectrolyte multilayer-coated heparin-conjugated AMS demonstrated controlled and sustained release of heparin-binding growth factors.	(Zuo et al., 2015)
	Vascular Endothelial Growth Factor (VEGF)	high-pressure sterilizer, dried and preserved	Calcium AMS	VEGF-loaded calcium AMS significantly promoted the neovascularization of adipose graft, improved survival rate of adipocytes.	(Ding et al., 2015)
Vaccines	*Bordetella pertussis*	Emulsification	Alginate microparticles	The microparticles showed mean size of 151.1 μm, loading efficiency of 89.6%, and constant release	(Dounighi et al., 2017)
	Omp16-based vaccine	Emulsion internal gelation method	Alginate-chitosan microspheres	Vaccines provided significant protection against *Haemophilus parasuis* in mice	(Zheng et al., 2017)
	Hepatitis B surface antigen	Modified spray-solidification technique	Antigen-loaded DDAB/PLA (didodecyldimet hylammonium bromide/poly (lactic acid)) nanoparticles (NPs)-alginate composite microcapsules	Single-shot microcapsule vaccine formulation induced high enough antigen-specific IgG antibody responses and cytokine secretion levels	(Yang et al., 2018)
	Tumor vaccine	Spray drying	AMS (named PaLtTcAdMIP3 α) that encapsulated tumor lysates, live tumor cells engineering with a recombinant MIP-3α adenovirus and BCG	AMS expressed and excreted macrophage inflammatory protein 3α, which effectively attracted dendritic cells *ex vivo* and *in vivo*	(Huang et al., 2015)
Vectors and microbes	*Trichoderma viride* spores	Emulsification / cross-linking	Calcium AMS	*Trichoderma viride* germination inside microsphere lowered the amount of released calcium ions and slowed release kinetics than microspheres without *Tricoderma viride*	(Juri et al., 2019)
	Recombinant human adenovirus type 5 (HAd5)	Emulsification	Alginate	Had5 encapsulation efficiently avoided vector-specific immune response.	(Sailaja et al., 2002)

Category	Therapeutic agent	Formulation method	Materials used	Findings	Reference
	Macroalgal spores of a green alga (*Ulva intestinalis*) and brown algae (*Undaria pinnatifida* and Ecklonia cava)	Emulsification	Alginate	Encapsulated spores of the three algal species tended to grow faster than non-encapsulated spores	(Jung et al., 2018)
	Arbuscular Mycorrhizal Fungi Organic Fertilizer Pellets	Emulsification	Calcium-Alginate Complex	Pellets were fully degraded within 30 days	(Pitaktamrong et al., 2018)
	Ganoderma lucidum spores (GLS)	Electrospraying	GLS–Alginate (GLS/A) micro beads	No release of GLS encapsulated beads in the simulated gastric fluid (pH of 1.8), a rapid, size dependent release was found in the simulated intestinal solution (pH of 7.5)	(Zhao et al., 2016)