Chapter 4
Alginate Processing Routes to Fabricate Bioinspired Platforms for Tissue Engineering and Drug Delivery

Vincenzo Guarino, Rosaria Altobelli, Francesca della Sala, Assunta Borzacchiello, and Luigi Ambrosio

Abstract Alginate is a water-soluble polymer which has gained much attention in the last 20 years as suitable biomaterial for numerous applications in biomedical science and engineering. The strong biocompatibility in cell microenvironment and the possibility to process alginate solution by safe conditions to reach a stable form after polymer gelation – via ionic, chemical, or thermal route – make them useful to design different types of devices (i.e., injectable gels, porous scaffolds, micro-/nanoparticles) which are attractive for wound healing, cell transplantation, drug delivery, and three-dimensional scaffolds for tissue engineering applications.

In this chapter, current potential applications of alginates in biomedical science, tissue engineering, and drug delivery will be discussed. After a brief overview of general properties of polymer and its hydrogels, we will focus on the processing techniques mainly used for their manufacturing, also suggesting, in the last part, potential uses and future perspectives for their novel applications in biomedical field.

Keywords Phase separation • Electrofluidodynamics • Emulsion • Layer by layer • Porous scaffolds • Micro-/nanoparticles • Drug delivery • Tissue engineering

4.1 Introduction

Alginates are natural polysaccharides typically obtained from brown seaweed which exhibit excellent biocompatibility and biodegradability that can be useful for many applications in the field of biomedicine. They mainly work as ionic polymers derived by the presence of divalent cations such as Ca^{2+}, which confer them

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interesting properties to fabricate micro- and nanostructured devices with improved molecular transport, low cytotoxicity, and relatively low-cost production, suitable for large applications in tissue engineering and drug delivery [1]. They are typically used in the hydrogel form, due to the peculiar organization of linear chains containing hydrophilic/hydrophobic ((1,4)-linked β-$_{D}$-mannuronate (M)/ α-$_{L}$-guluronate (G) blocks) [2] which concur to the assembly of stable three-dimensionally cross-linked networks with high capability of water retention, highly scalable swelling properties, and molecular release kinetics. In particular, only the G-blocks of alginate are mainly involved in the intermolecular cross-linking with divalent cations (e.g., Ca^{2+}) to form hydrogels with different properties [3]. However, relative M/G ratio, M and G sequence and block length, and molecular weight are thus critical factors affecting the physical properties of alginate and, ultimately, its resultant biological properties of hydrogels [4].

Besides, hydrogel-like behavior of alginates mainly contributes to the scaffold biomimesis, being structurally similar to the macromolecular-based components in the body. Their peculiar chemical properties assure a full compatibility in biological microenvironment, by minimizing inflammatory reactions after their administration into the body [5]. However, similarly to other biologically recognized hydrogels, alginate gels have very limited mechanical stiffness. Alginate can be easily modified via chemical and physical reactions to obtain derivatives having various structures, properties, and functions. A fine control of the structure and biological properties such as biodegradability, mechanical strength, gelation property, and cell affinity can be achieved through the combination with other biomaterials, immobilization of specific ligands such as peptide and sugar molecules, and physical or chemical cross-linking [6]. For instance, the mechanical properties of alginate gels typically may be enhanced by modifying the length of G-block and molecular weight. For example, gels prepared from alginate with a high content of G residues exhibit higher stiffness than those with a low amount of G residues [7]. However, an alginate solution formed from high molecular weight polymer generally tends to be too much viscous, showing several limitations in terms of processing [8]. In this case, proteins or cells mixed with an alginate solution of high viscosity may be more easily damaged under the effect of high shear forces generated during mixing and injection into the body [9]. Hence, the elastic modulus of alginate gels can be increased significantly, not drastically changing the solution viscosity, by using a combination of high- and low-molecular-weight alginate polymers [20]. Alternatively, structural properties of the alginates may be varied by different chemical ways. They may include natural treatments based on the use of Azotobacter species for the fabrication of bacterial alginates with higher concentration of G-blocks and, consequently, relatively higher stiffness [10] that confers a better control of the gel stability, drug release rates, and the cell phenotype functions. The most frequently used strategy still is based on the chemical cross-linking, acting directly on chemical groups of hydrophilic blocks [11]. Today, the current challenge is how to match the physical properties of alginate gels to the main requirements of specific applications, by properly setting chemical operational parameters and processing modes generally used in cross-linking strategies – i.e., various chemical

structures of molecules as cross-linking functionality, molecular weights, cross-linking density will often yield gels suitable for each application. In this context, the implementation/revisit of manufacturing processes is a key point to design innovative devices and platforms able to valorize the opportunity to manipulate alginate materials by green processing conditions [12] – for the safe release of molecules and/or cells in vitro and in vivo.

In the last years, alginate has been variously processed to fabricate injectable hydrogels, microspheres, microcapsules, sponges, foams, and fibers to be used as cell or molecular carriers suitable for drug delivery systems or tissue engineering. In this chapter, we aim at overviewing all the main processing techniques used for the manufacturing of alginates and their derivatives, basically remarking the relationships among process conditions, structure, and functions of the final devices.

4.2 Scaffolds for Tissue Engineering

A great challenge of current tissue engineering consists in controlling the delivery of biologics – namely, cells, genes, and proteins – via a degradable scaffold, to promote and support in vitro tissue regeneration. In this context, scaffold design plays a pivotal role to properly address in vitro properties of forming tissue toward an effective use in clinical application. Hence, manufacturing techniques must be sagely optimized to manipulate scaffold properties to trigger selected mechanical, mass transport, and surface functionalities in order to match environmental conditions to efficaciously work as in vivo models. [13]. Indeed, cell-loaded scaffolds have been successfully used as artificial ECM (extracellular matrix) analogue to provide a temporary environment able to support the cell infiltration, adhesion, proliferation, and differentiation [14]. In this case, they have to furnish the required structural support at the beginning of tissue growth and retain cells in the defective area for cell growth, metabolism, and matrix production, thus playing an important role during the development of engineered tissues. In the last years, many polysaccharides including alginates [15], chitosans [16], hyaluronic [17], and cellulose derivatives [18] have been investigated as highly porous, biomimetic scaffolds to overcome the limitations of two-dimensional (2D) culture systems. In particular, hydrophilic alginate hydrogels have raised special interest as a means to provide a temporary support for a variety of cell types including osteoblasts, chondrocytes, fibroblasts, and embryonic stem cells, showing minimal or negligible cytotoxicity and histocompatibility. All these results confirmed the ability of alginate-based scaffolds to properly degrade in vitro, promoting vascularization and elicited low inflammatory responses after transplantation, suggesting them as efficient cell and drug carrier for tissue regeneration.

4.2.1 Phase Separation

Traditional methods for producing porous biopolymer scaffolds include gas foaming, phase separation (freeze-drying), solvent casting, and particulate leaching [19]. In general, a polymer is first dissolved in a suitable solvent and subsequently placed in a mold that will be rapidly cooled until the solvent freezes. The solvent is then removed by freeze-drying, and pores will be left behind in the polymer. Different types of phase separation techniques are available, including thermally induced, solid-liquid, and liquid-liquid separation mechanisms [20–22].

Porous alginate-based scaffolds, foams, and sponges with interconnected porous structures and predictable shapes can be easily manufactured by a regular thermally induced phase separation (Fig. 4.1) [18]. When the temperature is low enough to allow freezing the solution, the phase separation mechanism induces the solid-liquid demixing, therefore forming frozen solvent and concentrated polymer phases. By adjusting the polymer concentration or varying the cooling rate, phase separation could occur via different mechanisms, resulting in the formation of scaffolds with various morphologies. "Freeze-drying" is one of the most extensively used methods that produce matrices with porosity greater than 90%. The pore sizes depend on the growth rate of ice crystals during the freeze-drying process. After the

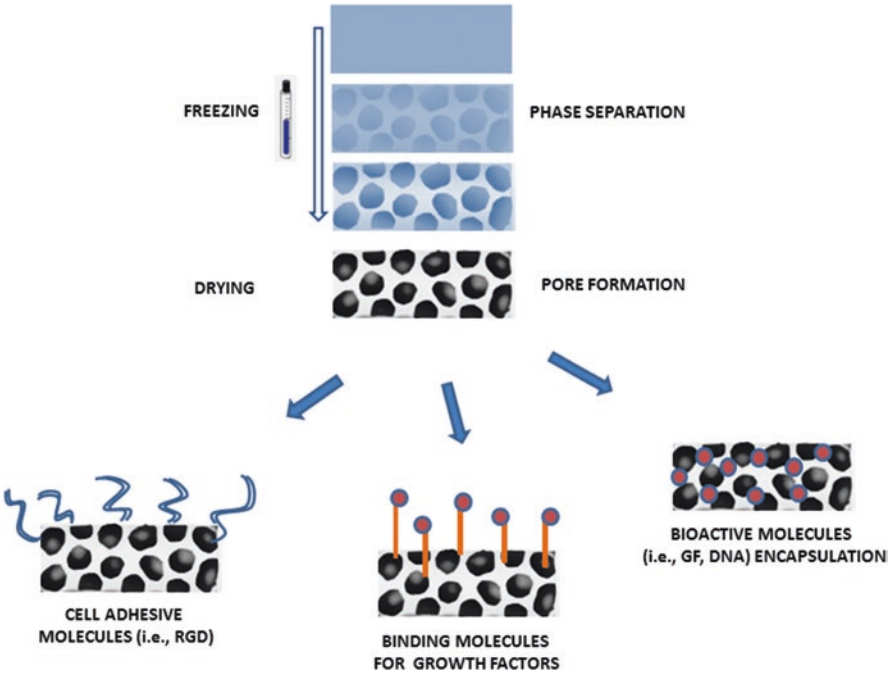

Fig. 4.1 Alginate porous foams fabricated via freeze-drying technique and different bioactivation procedures for improving cell recognition

removal of the liquid or frozen solvent contained in the demixed solution, the space originally occupied by the solvent would become pores in the prepared scaffolds. Obviously, in the stage of solvent removal, the porous structure contained in the solution needs to be carefully retained. Without freeze-drying, a rise in temperature during the drying stage could result in remixing of the phase-separated solution or remelting of the frozen solution, leading to destruction of the porous structure. In the case of hydrogels, the freeze-dry processing does not require additional chemicals, relying on the water already present in hydrogels to form ice crystals that can be sublimated from the polymer, creating a particular micro-architecture. Because the direction of growth and the size of ice crystals are a function of the temperature gradient, linear, radial, and/or random pore directions and sizes can be produced with this methodology [23]. The mechanical properties and biodegradation rate of freeze-dried scaffolds can be simply modulated by changing the relative parameters of the polymers. Porous scaffolds formed by pure alginate are unable to provide enough bioactive properties to support cell metabolism due to the lack of cellular interaction in the molecular structures [8, 18, 24, 25]. Therefore, alginate has been blended with collagen and/or gelatin to enhance cell ligand-specific binding properties to fabricate hybrid scaffolds, which showed better properties for supporting cells [18, 26, 27]. Recently, other efforts were made to enhance the biological properties of alginate porous scaffolds. In this way, alginate was irradiated and oxidized to modify its degradation and covalently grafted with growth factors, lectins, and peptides containing a RGD (arginylglycylaspartic acid) sequence to promote cell adhesion and proliferation [18, 28–30]. As we have seen, the porosity and pore sizes of the scaffolds fabricated through this method are largely dependent on the parameters such as the ratio of water to polymer solution and viscosity of the emulsion. This technique also does not necessitate an extra leaching step, but the addition of organic solvents such inhibits the incorporation of bioactive molecules or cells during scaffold fabrication. In addition, the small pore sizes obtained are another limiting factor of scaffolds fabricated by phase separation [22].

4.2.2 Atomization

The ultimate goal of regenerative medicine is to produce bioinspired scaffolds able to meet specific functionalities of native microenvironment by replying their specific chemical (i.e., composition), physical (i.e., fluid transport), and morphological (i.e., shape, porosity) features [13]. Several investigations have been performed to design alternative systems able to conjugate main advantages and, simultaneously, remove major drawbacks of traditional porous systems. For example, micro- and nanoparticles have been integrated to polymer gels to realize injectable or moldable systems (i.e., gels, cements) able to better support cell activities, i.e., adhesion, proliferation, and mineralization – in the three-dimensional (3D) cavities [31]. These systems offer the possibility of injecting isolated polymeric particles as vehicles to deliver encapsulating bioactive agents or in vitro pre-seeded cells in the defect [32,

33]. Alternatively, different types of micro-sized polymeric particles have been extensively used to assemble porous scaffolds with interconnected porosity by particle agglomeration via sintering or solubilization methods [34], mainly due to relevant benefits associated with high surface area for cell expansion.

More recently, the use of polymeric microgels – i.e., sized from 10 to 1000 μm – is rapidly diffusing for different applications in tissue engineering and nanomedicine [35]. Hydrogels in the form of capsules or particles have been largely used to deliver active molecules or living cells for therapeutic and cell-based disease treatments [36]. Their peculiar capability to generate a highly hydrated microenvironment also allows for protecting sensitive drugs, thus preserving molecular stability prior to the delivery at the site of injury [37]. Meanwhile, they may assure an efficient transport of biological substances, such as nutrients and products from cell metabolism, in and out of the hydrogels, which are fundamental to protect and sustain cell viability during the regeneration processes [38, 39].

In this way, several strategies have been implemented as the in situ formation of scaffolds by cell-induced aggregation of injected or cell-encapsulated hydrogel particles to promote the cell delivery for new scale-up biofabrication methods, such as organ printing [33, 40–43]. The wide versatility of these systems is mainly associated with the variables that can be tuned to obtain an optimal system for a specific application, such as the particle composition, size and shape, existence of porosity, cell culture conditions, or incorporation of bioactive agents.

During the last decades, several techniques have been used to process microparticles for different applications. The preparation of microgels or particles requires several considerations about their manufacturing, modification, and manipulation and should also ideally allow the production of large quantities of particles with a narrow size distribution [33].

Among them, atomization techniques are the most viable way to create systems that have complicated external anatomic shapes and complex internal porous architectures. In this method, an electric field is applied to a polymeric solution extruded from a syringe. The applied high voltage potential forces the polymer to form a jet that, using specific parameters, enables the formation of micro-/nanoparticles [32]. The great advantage of this technique over other commonly used methods is the fact that it is a one-step process that does not make use of organic solvents or cross-linking agents [13]. Their ability is to create devices to achieve both a wide range of effective mechanical and mass transport properties as well as a low variation in those properties. Further the much finer feature resolution that these techniques can generate is beneficial for cell seeding and growth factor delivery [13, 44, 45].

The encapsulation of living cells in a variety of soft polymers or hydrogels is important, particularly, for the rehabilitation of functional tissues capable of repairing or replacing damaged organs (Fig. 4.2). Generally, cells are mixed with the encapsulation material before gelation occurs. Diverse hydrogel membranes have been popularly used as encapsulating materials and permit the diffusion of gas, nutrients, wastes, and therapeutic products smoothly. Microtechnologies have been adopted to precisely control the encapsulated cell number, size, and shape of a cell-laden polymer structure [22, 46]. Many biomaterials have been proposed as

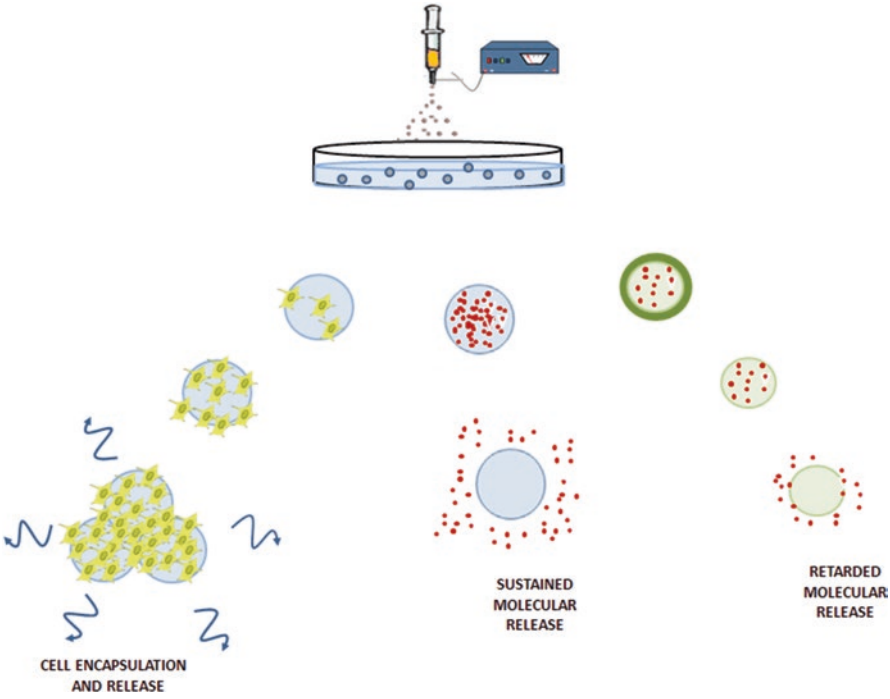

Fig. 4.2 Fabrication of alginate micro-carriers by electrohydrodynamic atomization: schematic approach for cell and molecular release

entrapping matrix to fulfil the specific requirements. Alginate is by far the most studied material for cell encapsulation, and it has been adopted for many biomedical applications. This material has historically been used as a protective barrier to enhance cell therapies, for immunoprotection of pancreatic islets, treatment of brain tumors, treatment of anemia, and cryopreservation [47–49]. The main benefit of using water-absorbable polymers for the preparation of these systems is not only to develop a confined barrier to entrap living xenogeneic or allogeneic cells to be transplanted but also to prohibit the entry of the host antibodies and immune cells. Besides, encapsulated cells generally show limited interactions, and the physical barrier concurs to mask them from the immune surveillance at a local level, thereby assuring a reduction of the inflammatory response after transplantation [50]. Microcapsule and micro-carrier systems provide a larger surface area for cellular attachment, provide cell protection against excessive mechanical stresses, and simulate an in vivo environment [51, 52]. Recent improvements in fabrication technologies allow generating matrices with different conformations – i.e., core shell with liquid or hollow core and/or multicomponent coatings – which may optimally be adapted to co-culture of single/multiple cell types for molecular guided tissue engineering and "bio-organs" manufacturing [52, 53].

Among them, electrofluidodynamic atomization (EFDA) is a promising technology capable of generating micro-sized scaffolds, due to the opportunity to fabricate variously sized particles from micro- to sub-micrometric scale, by controlling a large set of parameters including applied voltage, biomaterial properties (e.g., concentration/viscosity, conductivity), and needle geometry, in order to design structures with growing complexity [54–56]. Recently, electrofluidodynamics has been successfully used as low-cost, high-throughput, controlled technology for the production of full or hollow spheres from a polymer solution, by applying a high-voltage electric field [57]. The principle of the electric field-assisted atomization is based on the ability of electric forces to charge solution droplets by deforming their interface until breaking them into smaller droplets in the micrometric/sub-micrometric range. The jet deforms and disrupts into droplets due mainly to electrical forces by the competition between coulomb forces related to surface charge and cohesive forces inside the droplet, without the administration of additional mechanical energy to reach the liquid atomization [58]. Atomization process can be distinguished in electrodynamic spraying (EDS) and electrohydrodynamic atomization (EHDA), respectively, as a function of the collector used: EDS and EFDA [59, 60].

The former one involves the deposition of charged droplets on a grounded plate, by the breaking of polymer jet into nano-droplets under the solution overcharging conditions to form individual nanoparticles or agglomerates as a function of the local surface charge. The latter one is based on the deposition of charged droplets in a cross-linking agent solution – i.e., calcium chloride ($CaCl_2$) for alginate particles – prior to the solution overcharging, by the perturbation and cutting of polymer jet until the formation of micro-sized particles.

Future directions should veer on the development of technically advanced systems to satisfy several demands as controlled systems, operational rigor, and improved quality control. Cryopreservation, banking, and subsequent culture of cells encapsulated in microcapsule delivery systems represent an alternative to the economically attractive “off-the-shelf” micro-carrier concept. With long-term delivery of therapeutic agents, the cost of encapsulated cells is offset by the cost of short half-life therapeutic peptides and the costs involved in organ transplantation. This favorable pharmaco-economics of electrodynamic atomization-based technologies may impact insurance companies who may favor such an approach over traditional therapies, creating a paradigm shift in regenerative medicine [52].

4.3 Design of Carriers for Drug Delivery

4.3.1 Emulsion

Alginate has many possible applications in the area of drug delivery due to their low cost, low toxicity, biocompatibility, and biodegradability [61, 62]. Alginate microspheres have been widely used as carriers for the controlled release of active agents

due to their low immunogenicity and their muco-adhesive properties [63]. In addition, alginate matrices have the ability to encapsulate protein and DNA while maintaining their biological activities and have shown very strong bioadhesive abilities making alginate a promising candidate for site-specific mucosal delivery [64–66]. Moreover, the alginate polymer also finds application as a surfactant stabilizer in oil-water emulsions in microbead preparation. Alginate surfactants serve to lower the interfacial energy between the phases, thereby increasing the stability and lifetime of the emulsion. In fact, an emulsion is a thermodynamically unstable system consisting of at least two immiscible liquid phases, one of which is dispersed as globules in the other liquid phase, stabilized by the presence of an emulsifying agent, as can be observed in Fig. 4.3. Emulsions fall into a greater class of two-phase systems known as colloids, with the special characteristic that both the dispersed and continuous phases are liquid. Depending on the volume fraction of the phases, both water-in-oil and oil-in-water emulsions can be formed. There is a rule which governs the emulsion formation, known as the Bancroft rule: emulsifiers and emulsifying tend to promote dispersion of the phase in which they do not dissolve well; for example, proteins dissolve better in water than in oil and therefore tend to form oil-in-water emulsions, promoting the dispersion of oil droplets throughout a continuous phase of water. Emulsions can be prepared through various methods of agitation, as the two phases are immiscible and droplets will not form spontaneously, such as sonication, which can produce droplets of 100–400nm. Alginate microbeads for drug delivery can be easily prepared by emulsion-gelation technique. As reported by Putta S.K. et al. [67], an example of alginate microbead preparation consists of sodium alginate dissolution in purified water to form a homogeneous polymer solution. The drug is added to the solution and mixed thoroughly with a stirrer to form a viscous dispersion. The dispersion is then added in an oil phase such as heavy liquid paraffin while stirring at sufficiently high speed to emulsify the added dispersion as fine droplets. Then appropriate amount of calcium chloride solution (15% w/v) is transferred into the emulsion while stirring to complete the gelation reaction and to

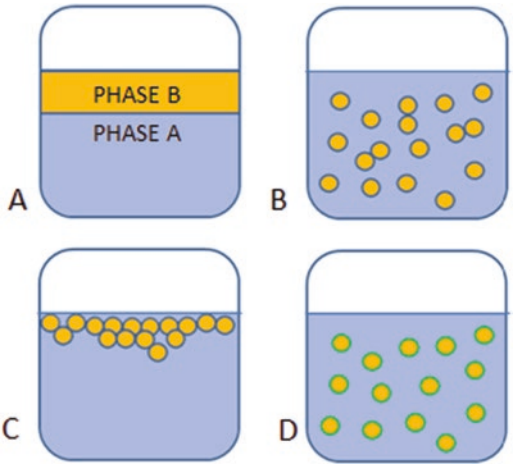

Fig. 4.3 (**a**) Two immiscible liquids not emulsified. (**b**) An emulsion of phase B dispersed in Phase A. (**c**) The unstable emulsion. (**d**) The green surfactant as an emulsion stabilizer

produce spherical rigid microbeads. The microbeads are collected by decantation and washed repeatedly with petroleum ether. The product is then air dried overnight at room temperature to obtain discrete microbeads. This method of preparation shows many advantages [68]; first of all it is easy, mild, and inexpensive preparation technique, it is stable, and it is suitable to encapsulate broad categories of drugs such as macromolecules, protein, and others.

By emulsion technique various alginate microbeads have been prepared with different therapeutic applications, such as the encapsulation of a water-soluble drug as ranitidine hydrochloride (peptic ulcer drug) in sufficient amount and that could be delivered in the stomach for a prolonged period of time without using any organic solvent and any time-consuming step in the preparation, so opening new treatment of gastric acidity and ulcer [69]. Furthermore, for the delivery of acyclovir, antiviral medication, oil entrapped acyclovir floating alginate beads used as floating controlled drug delivery systems have been also realized. The beads showed the excellent sustaining properties as compared to the conventional dosage form. The designed therapeutically efficacious gastro-retentive formulation of acyclovir, combining an excellent buoyant ability and a suitable drug release pattern, could possibly be advantageous in terms of increased bioavailability of acyclovir [70]. Therefore, alginate microspheres appear, technologically, as a promising antigen delivery system; in fact, the alginate microspheres were easy to prepare, and the mean diameter of beads increased with the increase in the amount of the oil phase. The encapsulation efficiency of bovine serum albumin (BSA), chosen as model antigen, was very high. The beads showed excellent sustaining properties as compared to the conventional beads [71]. As regards the treatment of diabetes, insulin, an antidiabetic drug, has been formulated in the alginate microspheres intended for oral administration in order to directly deliver them in the intestinal region without drug degradation in the stomach. Process and formulation variables were of utmost importance for size and encapsulation properties. Encapsulated insulin produced the same bioavailability in diabetic rats as short-acting insulin formulation showing that insulin integrity was maintained during microspheres manufacturing and recovery [72]. Besides, the emulsion-gelation method was successfully utilized for the formulation of floating alginate beads of clarithromycin, antibiotic used to treat various bacterial infections, including strep throat, pneumonia, skin infections, *Helicobacter pylori* infection, and Lyme disease, among others. The adopted method for the estimation of clarithromycin showed good linearity. The formulated floating alginate beads have shown high percentage of drug loading, encapsulation efficiency, particle size, and very low moisture content. In vitro dissolution study showed that, among the formulations, the formulation containing 2 percent sodium alginate solution and 5 percent calcium chloride solution along with 500 mg hydroxypropyl methylcellulose (HPMC) and 5 ml of sunflower oil released clarithromycin for prolonged time (12 h) [73].

4.3.2 Layer by Layer

Alginate microbeads can be also prepared by layer by layer technique, generally to make multilayered beads. An advantage of these multilayered alginate beads can be due to their ability to allow simultaneous encapsulation of cells within the alginate core and delivery of other factors/cells from the outer layer of alginate. Growth factors can be encapsulated and delivered from the outer layer with release kinetics determined by its composition [74, 75].

Coating the alginate core with a perm-selective layer polymer may reduce or possibly completely eliminate the requirements of immunosuppressive drugs when delivering allogeneic or xenogeneic cells. Alginate microcapsules are typically coated with a polycation, such as poly-L-ornithine (PLO) or poly-L-lysine (PLL). The positive cationic chains interact with the negative alginate core to form a polycation-polyanion complex that coats the entire alginate surface. This layer influences the rate of diffusion of solutes and is typically designed to limit the immune system from recognizing the cells contained within the core. PLO and PLL play an important role in transport, but their positive charge results in inflammatory response following implantation, so to avoid this the surface is coated with an outer monolayer of alginate to prevent direct contact of cells from the host with the polycation.

Delivery of fibroblast growth factor-1 (FGF-1) from multilayered alginate beads, made of two alginates layers with in between a PLO layer, has been shown to stimulate blood vessel formation around encapsulated cells [76, 77]. In order to achieve the success in islet trasplantation, the local tissue response, including vascularization and modulation of the foreign body response, is essential. These multilayer structures allow for delivery of factors directly around the encapsulated cells in order to provide both therapeutic cells and local control of tissue development. The procedure for generating multilayer alginate microbeads, shown in Fig. 4.4, involves first the synthesis of uncoated alginate microbeads followed by coating of the polycation layer and, finally, the generation of an outer alginate layer. Briefly, to prepare uncoated alginate microspheres, low and high viscosity alginate solution is extruded in a flask containing the cross-linking solution (such as $CaCl_2$ or Ba) for a defined period of time with continuous stirring. The resulting gelled spherical microbeads to be covered by a polycation layer (e.g., PLL or PLO) are transferred into the polycation solution. After, the polycation solution is removed and washes are performed, an alginate solution of desired concentration is transferred onto the alginate microbeads previously created and incubated for a defined time to allow for alginate to interact with polycation layer. Then, the microbeads are transferred into the cross-linking solution to allow formation of outer layer [78].

Furthermore, multilayer sodium alginate beads have been successfully developed to improve the oral availability of conventional anticancer drug [79, 80]. To improve these, nanoparticles have been immobilized on multilayer alginate beads, by dropping aqueous chitosan/carboxymethylchitosan (CS/CMS) blended with alginate into CaCl2 solution [81]. The cross-linker between $–COO^-$ groups on alginate and Ca^{2+} occurred from external of alginate matrix to form the compact core of

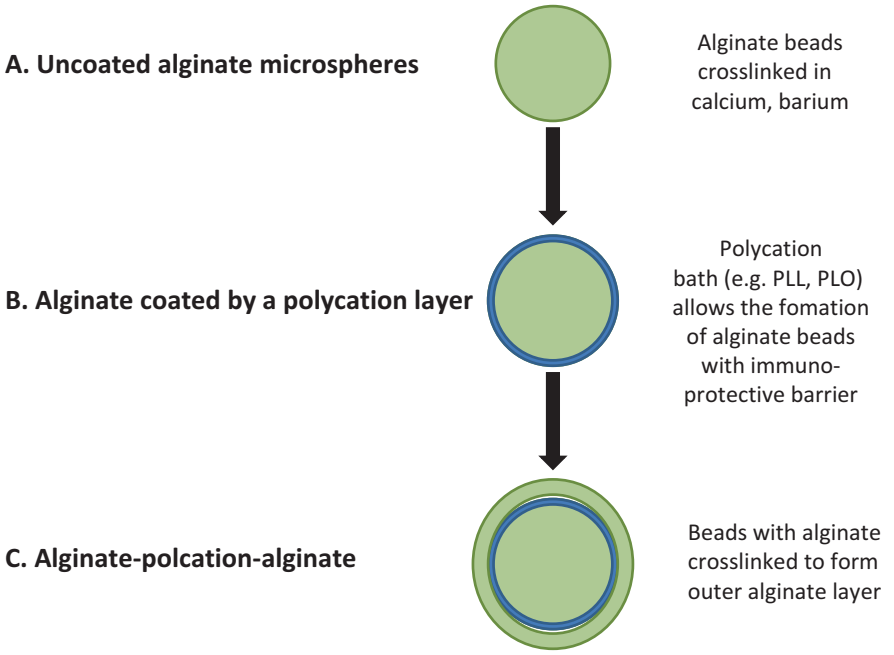

Fig. 4.4 Multilayered alginate microbeads consist of a perm-selective membrane around the alginate core (**a**–**b**) followed by an outer layer alginate (**c**). The outer layer alginate allows for encapsulation of proteins and growth factors while also maintaining biocompatibility

multilayered bead. The alginate matrix could protect encapsulated doxorubicin (DOX) loaded CS/CMS nanoparticles from undesirable drug release in gastric juices but too rapidly releases the intact DOX:CS/CMS nanoparticles in the small intestine. To overcome this limitation, it has been constructed a porous core of multilayer beads by internal gelation method to improve their ability of drug control release. By dropping aqueous CS/CMS nanoparticles blended with alginate and $CaCO_3$ into hydrochloric acid, the cross-linking between Ca^{2+} and $–COO^-$ groups on alginate occurred from interior of alginate matrix to form a compact core with porous structure in multilayer bead. The cross-linking effect is strengthened in the core of porous multilayer beads, and it may overcome undesirable drug release encountered in nanoparticle multilayer beads, to prolong the contact time between the formulation and the small intestinal mucosa, thereby potentially enhancing drug delivery efficacy. Concluding, alginate beads loaded with drug are important in the drug delivery by oral route as well as other routes, as sustained and controlled release formulations. As these microbeads are biocompatible, nontoxic, and biodegradable, so they may be better used and, i.e., they have paved a better way for controlled/sustained release of drug through the use of natural, biodegradable material.

4.4 Applications in Regenerative Medicine and Industrial Pharmacology

Industrial applications of alginates, based on their gelling, viscosifying, and stabilizing properties, account for the quantitatively most important uses of alginates. In comparison, emerging and knowledge-demanding specialty applications within biotechnology and medicine are based on biological effects of the alginate molecule, of alginate molecular building blocks, or of alginate's unique, gentle, and almost temperature-independent sol/gel transition in the presence of multivalent cations (e.g., Ca^{2+}), which makes alginate highly suitable as an immobilization matrix for biocatalysts such as living cells [82]. The conventional role of alginate in pharmaceutics includes serving as thickening, gel forming, and stabilizing agents, as alginate can play a significant role in controlled release drug products. Oral dosage forms are currently the most frequent use of alginate in pharmaceutical applications, but the use of alginate hydrogels as depots for tissue-localized drug delivery is growing [53].

Alginate gels have been investigated for the delivery of a variety of low-molecular-weight drugs and are likely most useful when a primary or secondary bond between the drug and the alginate can be exploited to regulate the kinetics of drug release. Alginate gels are typically nanoporous, leading to rapid diffusion of small molecules through the gel. However, incorporation into beads formed from partially oxidized alginate in the presence of both calcium ions and adipic acid dihydrazide (combination of ionic and covalent cross-linking) led to a prolonged release due to the increased number of cross-links and resultant reduced swelling [83–85]. The controlled and localized delivery of antineoplastic agents has also been achieved using partially oxidized alginate gels. Multiple drugs can be loaded into alginate-based gels for simultaneous or sequential delivery, as the chemical structure of the drug and mode of incorporation will dramatically alter the release kinetics [53, 86–88]. Alginate has also been widely exploited in many drug delivery applications in combination with chitosan, as the combination forms ionic complexes [44, 53, 88].

Alginate gels are increasingly being utilized as a model system for cell culture in biomedical studies. These gels can be readily adapted to serve as either 2D or more physiologically relevant 3D culture systems. RGD-modified alginate gels have been most frequently used as in vitro cell culture substrates to date. Further, the number of cells adherent to the gels, as well as the growth rate, was strongly dependent on the bulk RGD density in the gels. The length of the spacer arm between the RGD peptide and the alginate chain is a key parameter in regulation of cellular responses. Recent studies utilizing alginate gels as 3D cell culture substrates have revealed key insights regarding stem cell and cancer biology. The fate of mesenchymal stem cells was demonstrated to be controlled by the elastic modulus of the RGD-alginate gels in which they were encapsulated, as differentiation down fat and bone pathways was promoted at different values of gel stiffness. Strikingly, and in contrast to 2D culture systems used in previous mechanotransduction studies, the control over stem cell fate was related to the number of adhesive bonds formed between the gel and the cells, as well as alterations in the receptors cells utilized to adhere to the RGD pep-

tides in 3D versus 2D culture. The cells actively reorganized on the nanoscale the adhesion ligands presented from the gels. Alginate gels have also been used to examine how a 3D culture microenvironment influences cancer cell signaling and tumor vascularization [53, 89–92]. Alginate has also been combined with inorganic materials to enhance bone tissue formation. Alginate/hydroxyapatite (HAP) composite scaffolds with interconnected porous structures were prepared by several methods to enhance the adhesion of bone cells. Also cell-encapsulating alginate gel beads were introduced into calcium phosphate cement and demonstrated potential for bone tissue engineering under moderate stress-bearing conditions [53, 93–96].

In this context, alginate has demonstrated great potential for the fabrication of micro-sized devices for many applications in drug delivery, tissue engineering, and cancer therapy. Most attractive features of alginate in biomedical field include biocompatibility, mild gelation conditions, and simple modifications to prepare alginate derivatives with new properties. Alginate has a track record of safe clinical uses as a wound healing dressing material and pharmaceutical component; it has been safely implanted in a variety of applications, including islet cell transplantation [50, 53]. As one looks to the future, the alginate-based materials used in medicine are likely to evolve considerably. In wound healing, and more generally drug delivery applications, precise control over the delivery of single vs. multiple drugs or sustained vs. sequential release in response to external environmental changes is highly desirable. Dynamical control over delivery can potentially improve the safety and effectiveness of drugs, providing new therapies. On-demand drug release from alginate gels in response to external cues such as mechanical signals and magnetic fields can be used to design active depots of many drugs, including therapeutic cells. The introduction of appropriate cell-interactive features to alginate will also be crucial in many tissue engineering applications. The type of adhesion ligands and their spatial organization in gels are key variables, as they can regulate cell phenotype and the resultant function of regenerated tissues. Further, current understating of fundamental properties of alginate and developing of new types of cell and tissue-interactive alginate gels may enable future advances in biomedical science and engineering [53].

4.5 Conclusions and Future Trends

Alginate is one of the most frequently used polysaccharides in biomedical field, to fabricate building blocks or scaffolds for tissue repair and regeneration as well as micro-sized devices for drug delivery applications. The peculiar hydrogel-like structure makes them promising as scaffolds able to integrate either cells or bioactive molecules for tissue engineering. The success of tissue constructs is highly dependent on the design of the alginate-based scaffolds including the physical, chemical, and biological properties. In this context, physical and/or chemical modifications have been carried out to design *ex novo* polymers with the desired properties and functions, despite several limitations still concern the chance to meet all the design parameters simultaneously (e.g., degradation, swelling, and mechanical properties).

In the future perspective, alginate-based materials used in medicine are likely to evolve rapidly. To date, alginate gels played a fairly passive role in the clinical use for wound healing applications. Future dressings will likely play a much more active role of alginates in the controlled delivery of more bioactive agents to facilitate wound healing. Indeed, the use of alginate gels to incorporate bioactive molecules may assure the preservation of local concentrations of biological factors, such as proteins, for extended time periods. Besides, in the current drug delivery applications, a precise control over the delivery of single vs. multiple drugs or sustained vs. sequential release in response to external environmental changes is highly desirable. So, the use of active alginate gels may contribute to dynamically control the delivery mechanisms by improving the safety and effectiveness of drugs, for the discovery of new therapies. On-demand drug release from alginate gels in response to external cues (i.e., mechanical [97] and/or electromagnetic [98] stimuli) can be used to design active depots both for therapeutic drugs and cells.

Moreover, they will allow incorporating cell induction ligands such as growth factors directly to the scaffold surfaces for a controlled delivery of bioactive signals such as functional DNAs or siRNAs by appropriate spatial and temporal way. For this purpose, the introduction of appropriate cell-interactive features to alginate will also be crucial in many tissue engineering applications. The type of adhesion ligands and their spatial organization in gels are key variables, as they can regulate cell phenotype and the resultant function of regenerated tissues. In the place of RGD peptides extensively exploited as a cell adhesion ligand in the last years, multiple ligands and/or a combination of ligands and soluble factors could be alternatively considered to properly address specific biomolecular mechanisms for the replacement of tissues and organs. In this context, a deeper understating of fundamental properties of alginate as well as the development of new tissue-interactive alginate gels with novel chemical and physical moieties could enable future advances in biomedical science and engineering. For instance, it could be possible to design interpenetrating polymer networks by using alginates as building blocks for the design of pharmaceutics and drug delivery vehicles (i.e., drugs, biomolecules, proteins) in the form of films, microspheres, and depot matrices. By their assembly, the peculiar gelling ability with divalent cations offers the unique opportunity to fabricate smart systems able to change and/or adjust their mechanical and drug release properties in response to an external stimulus. For instance, new interpenetrated systems based on the combination of alginates with other natural polymers, mainly including polysaccharides with intrinsic bioactivity such as chitin or cellulose derivatives, have been recently exploited to improve the performances of multiple drug delivery systems. In this case, the synergistic behavior of interacting polysaccharide networks able to exert new features with respect to the single ones will concur to the design of novel in vitro models for fundamental studies to understand and tailor their physicochemical and biological properties, as well as for development of smart systems that fulfil unmet medical and pharmaceutical needs.

Meanwhile, the design and creation of new alginate polymers with better or distinct properties can potentially be achieved using genetic engineering techniques to control bacterial synthesis. Various polypeptides and proteins with improved struc-

tural properties and novel functions have already been prepared by this approach and explored for biomedical applications [99]. The ability to chemically or physically engineer new classes of alginates with precisely controlled functionalities, unlike the limited repertoire available from natural sources, designed for a specific application, paves toward an enormous revolution for the future use of alginate in biomedical field.

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References

1. Gombotz WR, Wee SF (1998) Protein release from alginate matrices. Adv Drug Deliv Rev 31:267–285
2. Rinaudo M (2008) Main properties and current applications of some polysaccharides as biomaterials. Polym Int 57:397–430
3. Fischer FG, Dörfel H (1955) Die polyuronsauren der braunalgen–(kohlenhydrate der algen–I). Z Physiol Chem 302:186–203
4. George M, Abraham TE (2006) Polyionic hydrocolloids for the intestinal delivery of protein drugs. J Control Release 114:1–14
5. SE S–E, Hubbell JA (2001) Functional biomaterials: design of novel biomaterials. Ann Rev Mater Res 31:183–201
6. Augst AD, Kong HJ, Mooney DJ (2006) Alginate hydrogels as biomaterials. Macromol Biosci 6:623–633
7. Kuo CK, Ma PX (2001) Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: part 1. Structure, gelation rate and mechanical properties. Biomaterials 22:511–521
8. Florczyk SJ, Kim DJ, Wood DL et al (2011) Influence of processing parameters on pore structure of 3D porous chitosan–alginate polyelectrolyte complex scaffolds. J Biomed Mater Res A 98:614–620
9. Kong HJ, Smith MK, Mooney DJ (2003) Designing alginate hydrogels to maintain viability of immobilized cells. Biomaterials 24:4023–4029
10. Hay ID, Rehman ZU, Ghafoor A et al (2010) Bacterial biosynthesis of alginates. J Chem Technol Biotechnol 85:752–759
11. Varghese S, Elisseeff JH (2006) Hydrogels for musculoskeletal tissue engineering. Adv Polym Sci 203:95–144
12. Guarino V, D'Albore M, Altobelli R (2016) Polymer bioprocessing to fabricate 3D scaffolds for tissue engineering. Int Polym Process. https://doi.org/10.3139/217.3239
13. Hollister SJ (2009) Scaffold design and manufacturing: from concept to clinic. Adv Mater 21:3330–3342
14. Manferdini C, Guarino V, Zini N (2010) Mineralization occurs faster on a new biomimetic hyaluronic acid–based scaffold. Biomaterials 14:3986–3996
15. Chen CY, Ke CJ, Yen KC et al (2015) 3D porous calcium–alginate scaffolds cell culture system improved human osteoblast cell clusters for cell therapy. Theranostics 5:643–655
16. Rodríguez–Vázquez M, Vega–Ruiz B, Ramos–Zúñiga R et al (2015) Chitosan and its potential use as a scaffold for tissue engineering in regenerative medicine. Biomed Res Int 2015:821279
17. Guarino V, Lewandowska M, Bil M, Polak B, Ambrosio L (2010) Morphology and degradation properties of pcl/hyaff11-based composite scaffolds with multiscale degradation rate. Comp Sc Tech 70(2010):1826–1837

18. Novotna K, Havelka P, Sopuch T et al (2013) Cellulose–based materials as scaffolds for tissue engineering. Cellulose 20:2263–2278
19. Su J, Tan H (2013) Alginate–based biomaterials for regenerative medicine applications. Materials 6:1285–1309
20. Nam YS, Guarino V, Causa F, Salerno A, Ambrosio L, Netti PA (2008) Design and manufacture of microporous polymeric materials with hierarchal complex structure for biomedical application. Mat Sci Tech 24(9):1111–1117
21. Schugens C, Maquet V, Grandfils C et al (1996) Polylactide macroporous biodegradable implants for cell transplantation. II. Preparation of polylactide foams by liquid-liquid phase separation. J Biomed Mater Res A 30:449–461
22. Loh QL, Choong C (2013) Three–dimensional scaffolds for tissue engineering applications: role of porosity and pore size. Tissue Eng Part B Rev 19:485–502
23. Ikada Y (2011) Tissue engineering: fundamentals and applications Vol. 8 in Interface Science and Technology. Academic Press, Cambridge, Elsevier, Uk
24. Yuan NY, Lin YA, Ho MH et al (2009) Effects of the cooling mode on the structure and strength of porous scaffolds made of chitosan, alginate, and carboxymethyl cellulose by the freeze–gelation method. Carbohydr Polym 78:349–356
25. Sapir Y, Kryukov O, Cohen S (2011) Integration of multiple cell–matrix interactions into alginate scaffolds for promoting cardiac tissue regeneration. Biomaterials 3:1838–1847
26. Kim G, Ahn S, Kim Y et al (2011) Coaxial structured collagen–alginate scaffolds: fabrication, physical properties, and biomedical application for skin tissue regeneration. J Mater Chem 21:6165–6172
27. Petrenko YA, Ivanov RV, Petrenko AY et al (2011) Coupling of gelatin to inner surfaces of pore walls in spongy alginate–based scaffolds facilitates the adhesion, growth and differentiation of human bone marrow mesenchymal stromal cells. J Mater Sci Mater Med 22:1529–1540
28. Han J, Zhou Z, Yin R et al (2010) Alginate–chitosan/hydroxyapatite polyelectrolyte complex porous scaffolds: preparation and characterization. Int J Biol Macromol 46:199–205
29. Shachar M, Tsur–Gang O, Dvir T et al (2011) The effect of immobilized RGD peptide in alginate scaffolds on cardiac tissue engineering. Acta Biomater 7:152–162
30. Zhou H, HH X (2011) The fast release of stem cells from alginate–fibrin microbeads in injectable scaffolds for bone tissue engineering. Biomaterials 32:7503–7513
31. Guarino V, Cirillo V, Ambrosio L (2016) Bicomponent electrospun scaffolds to design ECM tissue analogues. Exp Rev Med Dev 13:83–102
32. Guarino V, Cirillo V, Altobelli R et al (2015) Polymer based platforms by electric field assisted techniques for tissue engineering and cancer therapy. Exp Rev Med Dev 12:113–129
33. Oliveira MB, Mano JF (2011) Polymer-based microparticles in tissue engineering and regenerative medicine. Biotechnol Prog 27:897–912
34. Luciani A, Guarino V, Ambrosio L, Netti A Solvent and melting induced microsphere sintering techniques: a comparative study of morphology and mechanical properties. J Mater Sci Mater Med 22:2019–2028
35. Altobelli R, Guarino V, Ambrosio L (2016) Micro- and nanocarriers by electrofludodynamic technologies for cell and molecular therapies. Process Biochem https://doi.org/10.1016/j.procbio.2016.09.002
36. Tabata Y, Horiguchi I, Lutolf MP et al (2014) Development of bioactive hydrogels capsules for the 3D expansion of pluripotent stem cells in bioreactors. Biomater Sci 2:176–183
37. De Koker S, Richard H, de Geest BG (2012) Polymeric multilayer capsules for drug delivery. Chem Soc Rev 41:2867–2884
38. Guarino V, Gloria A, Raucci MG et al (2012) Hydrogel–based platforms for the regeneration of osteochondral tissue and intervertebral disc. Polymers 4:1590–1612
39. Guarino V, Galizia M, al APMA (2015) Improving surface and transport properties of macroporous hydrogels for bone regeneration. J Biomed Mat Res A 103:1095–1105

40. Leferink A, Schipper D, Arts E et al (2014) Engineered micro-objects as scaffolding elements in cellular building blocks for bottom-up tissue engineering approaches. Adv Mat 26:2592–2599
41. Kelm JM, Djonov V, Ittner LM et al (2006) Design of custom-shaped vascularized tissues using microtissue spheroids as minimal building units. Tissue Eng 12:2151–2160
42. Rivron NC, Rouwkema J, Truckenmüller R et al (2009) Tissue assembly and organization: developmental mechanisms in microfabricated tissues. Biomaterials 30:4851–4858
43. Causa F, Netti PA, Ambrosio L (2007) A multi–functional scaffold for tissue regeneration: the need to engineer a tissue analogue. Biomaterials 28:5093–5099
44. Fukui Y, Maruyama T, Iwamatsu Y et al (2010) Preparation of monodispersed polyelectrolyte microcapsules with high encapsulation efficiency by an electrospray technique. Colloids Surf A Physicochem Eng Asp 370:28–34
45. Pankongadisak P, Ruktanonchai UR, Supaphol P et al (2014) Preparation and characterization of silver nanoparticles–loaded calcium alginate beads embedded in gelatin scaffolds. AAPS PharmSciTech 15:1105–1115
46. Kang AR, Park JS, Ju J et al (2014) Cell encapsulation via microtechnologies. Biomaterials 35:2651–2663
47. Gasperini L, Mano JF, Reis RL (2014) Natural polymers for the microencapsulation of cells. J R Soc Interface 11:20140817
48. Hunt NC, Grover LM (2010) Cell encapsulation using biopolymer gels for regenerative medicine. Biotechnol Lett 32:733–742
49. Nicodemus GD, Bryant SJ (2008) Cell encapsulation in biodegradable hydrogels for tissue engineering applications. Tissue Eng Part B Rev 14:149–165
50. Leung A, Nielsen LK, Trau M et al (2010) Tissue transplantation by stealth coherent alginate microcapsules for immunoisolation. Biochem Eng J 48:337–347
51. Malda J, Frondoza CG (2006) Microcarriers in the engineering of cartilage and bone. Trends Biotechnol 24:299–304
52. Naqvi SM, Vedicherla S, Gansau J et al (2016) Living cell factories-electrosprayed microcapsules and microcarriers for minimally invasive delivery. Adv Mater 28:5662–5671
53. Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. Prog Polym Sci 37:106–126
54. Wu H, Liao C, Jiao Q, Wang Z, Cheng W, Wan Y (2012) Fabrication of core-shell microspheres using alginate and chitosan-polycaprolactone for controlled release of vascular endothelial growth factor. React Funct Polym 72:427–437
55. Vasir JK, Tambwekar K, Garg S (2003) Bioadhesive microspheres as a controlled drug delivery systems. Int J Pharm 255:13–32
56. Tanaka H, Matsumura M, Veliky IA (1984) Diffusion characteristics of substrates in Ca-alginate gel beads. Biotechnol Bioeng 26(1):53–58. PubMed PMID:18551586
57. Smith TJ (1994) Calcium algiante hydrogel as a matrix for enteric delivery of nucleic acids. Biopharm 4:54–55
58. Mestecky J (1987) The common mucosal immune system and current strategies for induction of immune responses in external secretions. J Clin Immunol 7:265–276
59. Putta S, Kumar A, Kumar A (2010) Formulation and in-vitro evaluation of mucoadhesive microcapsules of glipizide with gum kondagogu. J Chem Pharm Res 2(5):356–364
60. Sailaja R, Amareshwar P, Chakravarty P (2010) Chitosan nanoparticles as a drug delivery systems. J Pharm Biol Chem Sci Res 1:476
61. Jaiswal D, Bhattacharya A, Yadav I, Singh H, Chandra D, Jain D (2009) Formulation and evaluation of oil entrapped floating alginate beads of ranitidine hydrochloride. Int J Pharm Pharm Sci 1:129–141
62. Singhal P, Kumar K, Pandey M, Shubhini A (2010) Evaluation of acyclovir loaded oil entrapped calcium alginate beads prepared by ionotropic gelation method. Int J ChemTech Res 2:2076–2085

63. Lemoine D, Wauters F, Bouchend'homme S, Pre´at V (1998) Preparation and characterization of alginate microspheres containing a model antigen. Int J Pharm 176:9–19
64. Silva C, Ribeiro A, Figueiredo I, Alves A, Veiga F (2006) Alginate microspheres prepared by internal gelation: development and effect on insulin stability. Int J Pharm 311:1–10
65. Pradeep KN, Vidyasagar G (2010) Preparation and evaluation of floating calsium alginate beads of clarithromycin. Der Pharmacia Sinica 1(1):29–35
66. Khanna O, Moya ML, Opara EC et al (2010) Synthesis of multilayered alginate microcapsules for the sustained release of fi broblast growth factor-1. J Biomed Mater Res A 95:632–640
67. Gao W, Li T, Yu M, Hu X, Duan D, Lin T (2014) Preparation of sustained-release composite coating formed by dexamethasone and oxidated sodium alginate. Int J Clin Exp Med 7(9):3053–3061. PubMed PMID: 25356181; PubMed Central PMCID: PMC4211831
68. Moya ML, Cheng M-H, Huang J-J et al (2010) The effect of FGF-1 loaded alginate microbeads on neovascularization and adipogenesis in a vascular pedicle model of adipose tissue engineering. Biomaterials 31:28162826
69. Mori S, Takada Y (2013) Crosstalk between fibroblast growth factor (FGF) receptor and integrin through direct integrin binding to FGF and resulting integrin-FGF-FGFR ternary complex formation med. Science 1(1):20–36. https://doi.org/10.3390/medsci1010020
70. Somo SI, Khanna O, Brey EM (2017) Alginate microbeads for cell and protein delivery. Methods Mol Biol 1479:217–224. PubMed PMID: 27738939.
71. Li J, Jiang C, Lang X, Kong M, Cheng X, Liu Y, Feng C, Chen X (2016) Multilayer sodium alginate beads with porous core containing chitosan based nanoparticles for oral delivery of anticancer drug. Int J Biol Macromol 85:1–8. https://doi.org/10.1016/j.ijbiomac.2015.12.064. PubMed PMID: 26724684
72. Feng C, Li J, Mu Y, Kong M, Li Y, Raja MA, Cheng XJ, Liu Y, Chen XG (2016) Multilayer micro-dispersing system as oral carriers for co-delivery of doxorubicin hydrochloride and P-gp inhibitor. Int J Biol Macromol 94(Pt A):170–180. https://doi.org/10.1016/j.ijbiomac.2016.10.012. [Epub ahead of print] PubMed PMID: 27720963
73. Feng C, Song R, Sun G, Kong M, Bao Z, Li Y, Cheng X, Cha D, Park H, Chen X (2014) Immobilization of coacervate microcapsules in multilayer sodium alginate beads for efficient oral anticancer drug delivery. Biomacromolecules 15(3):985–996. https://doi.org/10.1021/bm401890x. PubMed PMID: 24502683
74. Murua A, Portero A, Oriv G et al (2008) Cell microencapsulation technology: towards clinical application. J Control Release 132:76–83
75. Xie J, Wang CH (2007) Electrospray in the dripping mode for cell microencapsulation. J Colloid Interface Sci 312:247–255
76. Yao R, Zhang R, Luan J et al (2012) Alginate and alginate/gelatin microspheres for human adipose–derived stem cell encapsulation and differentiation. Biofabrication 4:025007
77. Fonseca KB, Bidarra SJ, Oliveira MJ et al (2011) Molecularly designed alginate hydrogels susceptible to local proteolysis as three–dimensional cellular microenvironments. Acta Biomater 7:1674–1682
78. Guarino V, Ambrosio L (2016) Electrofluidodynamics: exploring new toolbox to design biomaterials for tissue regeneration and degeneration. Nanomed 11:1515–1518
79. Jaworek A (2007) Micro– and nanoparticle production by electrospraying. Powder Technol 176:18–35
80. Guarino V, Altobelli R, Ambrosio L (2016) Chitosan microgels and nanoparticles via electrofluidodynamic techniques for biomedical applications. Gels 2:2
81. Guarino V, Wan Abdul Khodir WK, Ambrosio L (2012) Biodegradable micro and nanoparticles by electrospraying techniques. J Appl Biomat Funct Mat 10:191–196
82. Draget KI, Taylor C (2011) Chemical, physical and biological properties of alginates and their biomedical implications. Food Hydrocoll 25:251–256
83. Maiti S, Singha K, Ray S et al (2009) Adipic acid dihydrazide treated partially oxidized alginate beads for sustained oral delivery of flurbiprofen. Pharm Dev Technol 14:461–470

84. Huebsch N, Kearney CJ, Zhao X et al (2014) Ultrasound–triggered disruption and self–healing of reversibly cross–linked hydrogels for drug delivery and enhanced chemotherapy. Proc Natl Acad Sci U S A 111:9762–9767
85. Wang Y, Zhou J, Qiu L et al (2014) Cisplatin–alginate conjugate liposomes for targeted delivery to EGFR–positive ovarian cancer cells. Biomaterials 35:4297–4309
86. Boekhoven J, Zha RH, Tantakitti F et al (2015) Alginate–peptide amphiphile core–shell microparticles as a targeted drug delivery system. RSC Adv 5:8753–8756
87. Wu JL, Wang CQ, Zhuo RX (2014) Multi–drug delivery system based on alginate/calcium carbonate hybrid nanoparticles for combination chemotherapy. Colloid Surf B 123:498–505
88. Lucinda–Silva RM, Salgado HRN, Evangelista RC (2010) Alginate–chitosan systems: in vitro controlled release of triamcinolone and in vivo gastrointestinal transit. Carbohydr Polym 81:260–268
89. Jeon O, Powell C, Ahmed SM et al (2010) Biodegradable, photocrosslinked alginate hydrogels with independently tailorable physical properties and cell adhesivity. Tissue Eng Part A 16:2915–2925
90. Degala S, Zipfel WR, Bonassar LJ (2011) Chondrocyte calcium signaling in response to fluid flow is regulated by matrix adhesion in 3–D alginate scaffolds. Arch Biochem Biophys 505:112–117
91. Lee JW, Park YJ, Lee SJ et al (2010) The effect of spacer arm length of an adhesion ligand coupled to an alginate gel on the control of fibroblast phenotype. Biomaterials 31:5545–5551
92. Huebsch N, Arany PR, Mao AS et al (2010) Harnessing traction–mediated manipulation of the cell/matrix interface to control stem–cell fate. Nat Mater 9:518–526
93. Jin HH, Kim DH, Kim TW et al (2012) In vitro evaluation of porous hydroxyapatite/chitosan–alginate composite scaffolds for bone tissue engineering. Int J Biol Macromol 51:1079–1085
94. Rubert M, Monjo M, Lyngstadaas SP et al (2012) Effect of alginate hydrogel containing polyproline–rich peptides on osteoblast differentiation. Biomed Mater 7:055003
95. Florczyk SJ, Leung M, Jana S et al (2012) Enhanced bone tissue formation by alginate gel–assisted cell seeding in porous ceramic scaffolds and sustained release of growth factor. J Biomed Mater Res A 100:3408–3415
96. Tang M, Chen W, Weir MD et al (2012) Human embryonic stem cell encapsulation in alginate microbeads in macroporous calcium phosphate cement for bone tissue engineering. Acta Biomater 8:3436–3445
97. Lee KY, Peters MC, Anderson KW et al (2000) Controlled growth factor release from synthetic extracellular matrices. Nature 408:998–1000
98. Zhao XH, Kim J, Cezar CA et al (2011) Active scaffolds for on–demand drug and cell delivery. Proc Natl Acad Sci U S A 108:67–72
99. MacKay JA, Chen MN, McDaniel JR et al (2009) Self–assembling chimeric polypeptide–doxorubicin conjugate nanoparticles that abolish tumours after a single injection. Nat Mater 8:993–999