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Bio-encapsulation for the Immune-Protection of Therapeutic Cells

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Abstract: The design of new technologies for treatment of human disorders is a complex and difficult task. The aim of this article is to explore state of art discussion of various techniques and materials involve in cell encapsulations. Encapsulation of cells within semi-permeable polymer shells or beads is a potentially powerful tool, and has long been explored as a promising approach for the treatment of several human diseases such as lysosomal storage disease (LSD), neurological disorders, Parkinson's disease, dwarfism, hemophilia, cancer and diabetes using immune-isolation gene therapy.

Introduction

Encapsulated cells offer enormous potential for the treatment of human disease. Encapsulation of non-autologous cells can provide mechanical protection and immuno-isolation, permitting the use of standard cell lines that are genetically modified to express key enzymes or other actives missing in patients. The capsule wall constructed from polymer matrix that serves as a semi-permeable membrane, allows the exchange of oxygen, metabolites and release of therapeutic proteins, while obscuring the encapsulated cells from the host's immune system. These capsules need to be

compatible with both host and implanted cells. The main motive of cell encapsulation technology is to overcome the problem of graft rejection in tissue engineering applications and thus reduce the need for long-term use of immunosuppressive drugs after an organ transplant to control side effects. This article summarizes the recent advances of cell encapsulation technology with an emphasis in designing materials and methods for their potential applications in the field of biology and medicine for the possible treatment of enzyme deficiency disorders.

Gene Therapy

Principle of Gene Therapy. Genes are specific sequences of bases, is the protein that carries out everyday life functions and even make up the majority of cellular structures. When genes are unable to carry out their normal functions, genetic disorders can results. The development of genetic technology has made it possible for man to engineer proteins- from the genetic code to the expression of protein product, and can be introduced into a patient as a means to alleviate disease. Alternatively, it was understood that instead of introducing the gene product, one might treat the disease at a genetic level, by gene therapy. Gene therapy is a technique for correcting defective genes responsible for disease development. It involves the introduction of a nucleic acid sequences into a patient's cell to compensate for abnormal genes or to make a beneficial protein.

The earliest references to gene therapy were by Joshua Lederberg [1] and shortly thereafter by Edward Tatum [2]. Lederberg postulated that one could transfer DNA from one cell line into the chromosome of another cell and that technology could in turn be used to repair genetic-metabolic disease. Later on, Tatum proposed that the new genes can be introduce from healthy donor cells into defective cells or particular organs would be done by an *ex vivo* mechanism. The resulting cells with the desired change could then be selected, grown in culture and re-implanted into the patient [2]. Just a year later, in 1968, French Anderson proposed that for gene therapy the desired gene from a donor chromosome would have to be isolated, amplified and then incorporated into the genome of the defective cell [3]. Today, gene therapy encompasses many different strategies and the material

transferred into a patient cells may be genes, gene segments or oligonucleotides. The genetic material may be transferred directly into cells within a patient (*in vivo* gene therapy), or cells may be removed from the patient and the genetic material inserted into them in culture, prior to transplanting the modified cells back into the patient (*ex vivo* gene therapy).

A major motivation for gene therapy research has been the need to develop novel treatments for diseases for which there is no effective conventional treatment. Although many gene therapy protocols have been tested in clinical trials, none has become the standard treatment for a given disease. Successful gene therapy of any disease will likely be an extraordinary task with the first successes involving gene therapy effectively combined with other conventional treatments. Therefore, the relative merits of any gene delivery system have to be evaluated carefully to achieve the final target.

***In Vivo* vs. *Ex Vivo* Gene Therapy.** *In vivo* gene therapy involves those methods of gene therapy whereby the genetic material is transferred directly into the patient. This may be the only option allowing for the introduction of genetic material into tissues whereby individual cells cannot be extracted and cultured from the patient (such as neuronal cells). Genetic material is introduced into a patient by a virally derived vector or by DNA complexes in a liposome medium. As there is no way to select and amplify the cells that have incorporated the foreign genetic material, the success of this approach is dependent on the general efficacy of the gene transfer and expression [4,5].

Ex vivo gene therapy involves the transfer of cloned genes into patient-derived cells grown in culture. Those cells that have been modified successfully and expanded by cell culture *in vitro*, then introduced into the patient. As the implanted cells were originally derived from the same patient, therefore it would not be rejected by the host immune system. This approach clearly would only be applicable to tissues that can be removed from the body, altered genetically and returned to the patient where they will engraft and survive for a long period of time. Blood or bone marrow cells are often used for *ex vivo* gene therapy because they are easy to collect and return to the patient [6,7].

Cell Encapsulation

Immobilized cell/bioactive agent systems have found applications in a variety of areas, including encapsulated cell therapy [8-11], immobilized biocatalysts [12,13], and polymeric drug delivery systems [14,15]. The concept of using encapsulation for the immune-protection of transplanted cells was introduced [16] and described the principle of bio-encapsulation for the first time in the early 1960s [17] by quoting "Microencapsulated cells might be protected from destruction and from participation in immunological processes, while the enclosing membrane would be permeable to small molecules of specific cellular product which could then enter the general extra cellular compartment of the recipient. For instance, encapsulated endocrine cells might survive and maintain an effective supply of hormone".

The encapsulation of cells instead of therapeutic products allows the delivery of the product for a longer period of time as cells release the products continuously. It has a great potential to reduce the lack of human donors for tissue and organ transplantations. Immunoprotection may allow transplantation of cells without the need for immunosuppression and transplantation of cells from non-human species (xenograft) [18]. In addition, genetically modified cells can be immobilized to express any desired protein *in vivo* without the modification of the host's genome. Immobilization of cells shows an important advantage compared with encapsulation of proteins because it allows a sustained and controlled delivery of produced therapeutic products at a constant rate-giving rise to more physiological concentrations, which is shown in Fig. 1. Furthermore, if the encapsulation device is broken or cells manage to exit the encapsulation device the host's immune system might attack them compromising their survival.

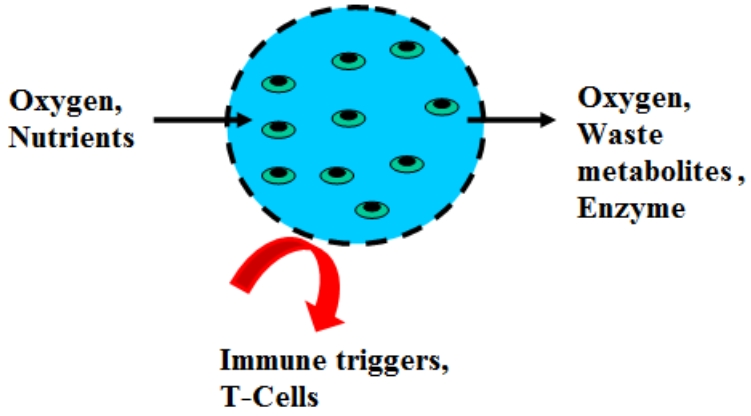

Fig. 1. A schematic representation of the basic principle of artificial cells.

Hormone or protein secreting cells could be enclosed into a wide range of bioactive materials within polymeric matrices. In this way disease caused by failure of secretory cell function could be treated. The concept is based on the protection of allo- and xenogenic transplanted cells within the confines of semi-permeable matrices or membranes that are ultimately designed to be implanted into the intraperitoneal cavity of patients. The cells are genetically engineered to express certain enzymes missing in the patient, such as insulin for diabetic patients, and lysozymes for patients suffering from one of about 40 identified lysosomal storage disorders (LSD's). To allow the use of standard laboratory cell lines rather than autologous cells (derived from each patient), they must be isolated from the host's immune system within semi-permeable membranes. In general, these membranes have to be designed to keep high molecular weight molecules, antibodies and other immune system's immunoglobulins (~250 kDa) out, while allowing exchange of oxygen, nutrients and metabolites including the specific enzymes needed by the patient (Fig. 1). The metabolic requirements of various cell types are different and, hence, optimal membrane permeability is expected to depend on the choice of cells. The upper limit of membrane permeability, i. e., molecular weight cut- off (MWCO), will be an application dependent. The membrane must be biocompatible with regards to the host and to the cells it surrounds. It must be physically strong enough to protect cells during handling,

transplantation, and to shield them from mechanical and biochemical stresses inside the host for about one year. Current cell encapsulation membranes have been shown to break down after about two weeks of implantation in mammals.

Many groups have studied microencapsulated genetically- engineered cells, and so far the method has been applied in small or large animal models of pancreas dysfunction [19], liver failure [20], Parkinson disease [21], hemophilia [22], dwarfism [23], Alzheimer disease [24] and some other cases. Other than transplantation, encapsulated cells are being pursued for a variety of other applications such as large-scale production of cell-derived molecules in biotechnology industry [25], Clonal selection of desired cell phenotypes [26], *In vitro* culture of cells dependent on close cell-cell contact [27,28], *In vivo* cell culture [29], Reproductive technology [30], and Cytotoxicity testing [31].

Brief History of Cell Encapsulation

Bisceglie gave the earliest demonstration of cell encapsulation in 1933, which enclosed tumor cells in a polymer membrane and transplanted them into a pig's abdominal cavity. The results showed that the cells survived for at least 21 days, and were not destroyed by the immune system [32]. Thirty years later, Chang introduced the idea of using encapsulation for the immunoprotection of transplanted cells, and coined the term 'artificial cells' for this concept [8]. The breakthrough in applying chang's principles of bioencapsulation came with the work of Lim and Sun [33]. In 1980, they successfully implemented Chang's idea to immobilize xenograft islet cells to aid in glucose control for diabetes in mice and other small animals. Since then, enormous efforts have been made to understand the biology, genetics, polymer and pharmaceutical science for the development of novel microencapsulations. As a consequence, Chang applied cell encapsulation for the therapeutic treatment of renal failure [34], and Sun used it for the treatment of diabetes [35]. Those results have provided the scientific basis for several clinical trials. The Hasse group encapsulated allogenic islets

for the treatment of diabetes [36], and Lohr's group immobilized cells expressing cytochrome P450 in attempts to treat pancreatic cancer [37].

Cell microencapsulation is a technology with enormous clinical potential for the treatment of a wide range of diseases [38]. The last three decades have seen tremendous advances in the field of cell encapsulation [39,40]. However, despite very promising and encouraging results it appears that we are still far from routine clinical application of therapy tailored, cell based constructs due to many difficulties, some of which will certainly challenge our scientific ingenuity. One of the main reasons is the lack of reproducibility. Moreover, due to their systematic administration, are associated with unwanted side effects due to non- specific suppression of the immune system that leads to a variety of undesired complications (e.g., opportunistic infections, failure of tumor surveillance, as well as adverse effects on the encapsulated tissues). However, in the last few years, the strategy in the field has been changed, applying a stepwise analysis of the essential obstacles instead of an approach via trial and error, coupled with increased international collaboration, should move the technology forward in a careful and controlled way and bring it much closer to clinical reality. This has renewed the excitement and hopes surrounding this cell-based technology.

Encapsulation Technique

The general cell encapsulation processes can be broadly divided into two groups:

Macroencapsulation. Macroencapsulation allows the implantation of large groups of living cells into the host without immune rejection (Fig. 2a). These systems can be in the form of a flat disk, hollow fibre or tube, and follow planar or cylindrical geometry that isolate the cells from the body, thereby avoiding the immune responses that the foreign cells could initiate. They are much larger in size compared to microcapsules. Unlike the hydrogel membranes of microcapsules, the membranes of macrocapsules are typically composed of thermoplastic materials that vary in their structural, functional, and mechanical properties. The main advantage of macrocapsules is that they can be

implanted and retrieved with minimal risk. However, the main drawback is the limitation of oxygen diffusion and nutrient transport, which dampens the cell functions, including viability.

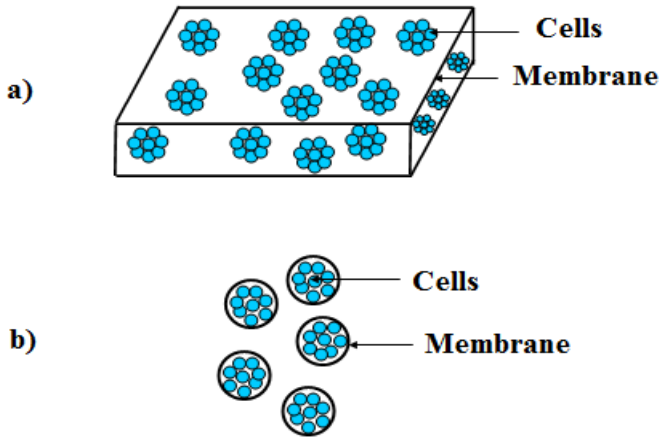

Fig. 2. Schematic representation of cell encapsulation process: a) Maroencapsulation, b) Microencapsulation

Microencapsulation. The objective of microencapsulation is to entrap smaller groups of typically 100 cells into a semi permeable membrane (Fig. 2b). These microcapsules are usually spherical in shape [16], and capsule diameter is usually 50 μm to 1.5 mm in range [41], with the upper limit determined by passive oxygen diffusion. Microcapsules can be stronger and more durable than macrocapsules and difficult to mechanically disrupt, and their spherical shape results in large surface/volume ratios, which increased oxygen, nutrient and metabolic exchange. However, the main drawback of microencapsulation is the difficulty in removing the implants if necessary. To construct the spherical geometry, microcapsules are almost exclusively produced from hydrogels. Being a hydrogel, they hold a number of appealing features. Firstly, the hydrogel reduced the mechanical or frictional irritation to surrounding tissue. Secondly, hydrogel by nature is hydrophilic, which virtually have no interfacial tension with surrounding fluids and tissues that helps to minimize the protein adsorption and cell adhesion. Thirdly, hydrogels provide a high degree of permeability for low-molecular-mass nutrients and metabolites. Some of the most popular biomaterials, which have

been applied to microencapsulations, are alginate, carboxymethyl cellulose, carrageenan, chitosan, and agarose.

The debate between macro versus microencapsulation is an ongoing dispute, and neither technique has demonstrated clear superiority over the other. Above all, the success of any encapsulation technique will ultimately rely on a systematic evaluation of capsule properties and encapsulated cell performance.

Present Methods of Microcapsule Preparation

In cell encapsulation system, cells are usually surrounded by liquid. One of the most important general goals for coating cells or tissues is to achieve isolation from their environment. Microcapsules can be formulated by many different methods.

Dropping Method. The most often described microencapsulation system is based on the calcium gellation of polyanions, as was shown in the following Fig. 3 in which cell containing alginate polyanions was extruded through a needle with concentric air flow dropping into the gellation bath which contains a cross-linking agent solution of calcium chloride, and calcium ionically cross links the alginate chains.

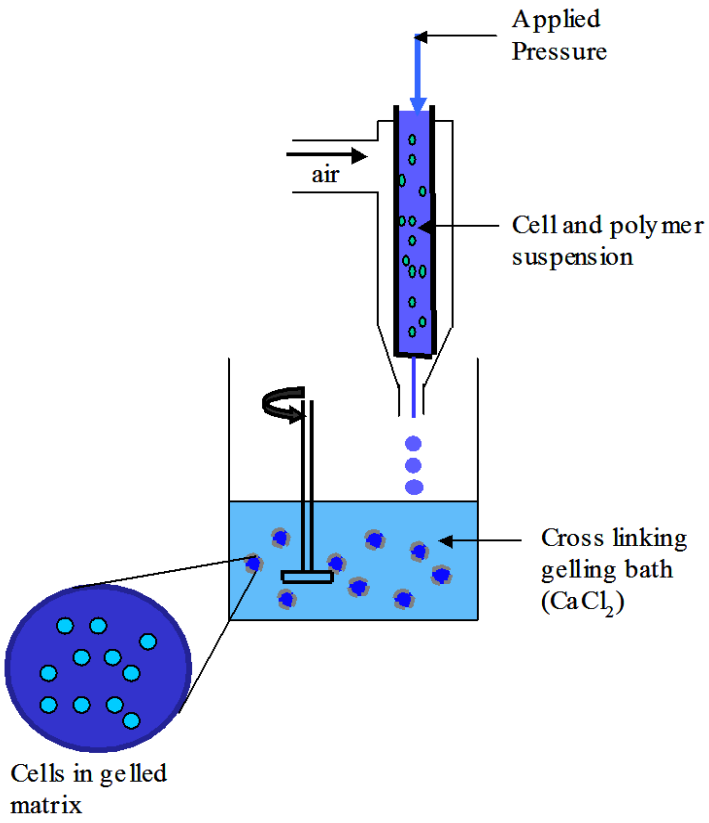

Fig. 3. Schematic representation of entrapment of cells in gel beads using the dropping method.

The major advantage of this method is the rapid gelation at ambient temperature. Since the spontaneous electrostatic reaction of the polymer and cross-linking agent is fast (a fraction of second). This method can be regarded as having high yield and efficiency. However, in order to control the droplet size, the viscosity, the wettability, the molecular weight and concentration properties of the polymer solution, as well as the diameter and geometry of the nozzle need to be considered.

Simple Coacervation Method. The coacervation (often called co extrusion) method is generally used as an encapsulation process. This method is based on dispensing a suspension of the cells through the inner needle and the polymeric wall-forming solution through the outer needle of a concentric needle assembly, into a precipitation bath. The polymer solution gels upon entering the precipitation bath. This polymer gels can be brought by changes in temperature or solvent

composition (pH, ionic strength, non-solvents). For example, the addition of alcohol to an aqueous solution of gelatin causes dehydration of the gelatin, leading to the formation of a simple coacervate consisting of a separate phase rich in gelatin [42]. A schematic representation of encapsulation of cells using the coacervation method is shown in Fig. 4. The major advantage of the final product is that it enables the cells to have some degree of movement freedom, and the gel matrix is present only as external shell.

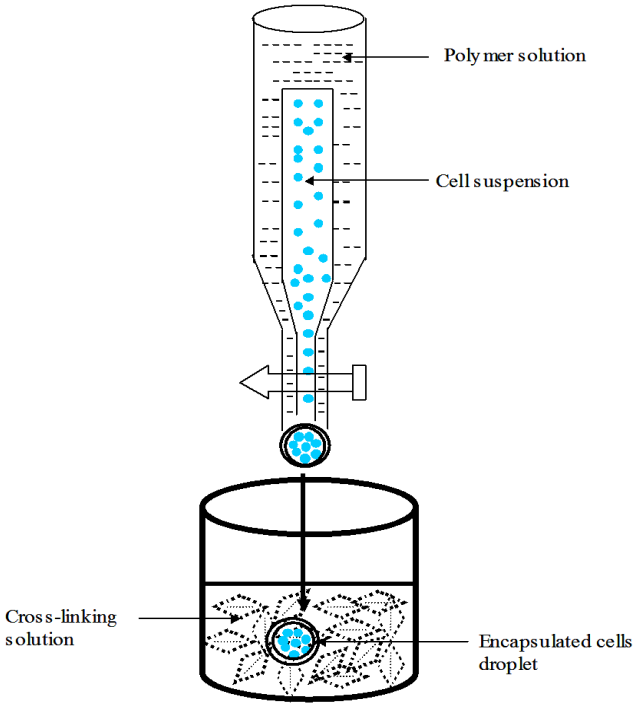

Fig. 4. Schematic representation of encapsulation of cells using the coacervation technique.

Complex Coacervation Method. There is a similar technique as described in simple coacervation method, called complex coacervation, which involves the interaction between two oppositely charged polyelectrolytes. The interaction between the two can form a film. By using this technique, it was possible to encapsulate somatic embryos for producing artificial vegetative seeds, although they could not survive after the process [43]. This method mainly used for coating oil droplets in i.e.

perfume industry. Coating consists typically of gum acacia/gelatin, and is cross-linked using small reactive molecules (i.e., formaldehyde or glutaraldehyde).

Spraying Method. Spraying also can be used for producing matrix type microcapsules [44]. During the free falling of cells- polymer suspension droplet, a rapid interfacial reaction can occur by spraying an aerosol cross-linking solution towards it, which is shown in Fig. 5. The cross-linking spontaneous reaction then stops when the drop is washed with reactant free solution. An inverted method can involve spraying a charged polymer solution on drops of a suspension of cells in a cross-linking solution. The advantage of this method is that it can be used to form relatively thin films around cell containing droplets, and it also enables the production of dry encapsulates using hot-air blowers. However, gelation depends upon the droplet exposure time and nature to the cross-linking aerosol along the spraying path.

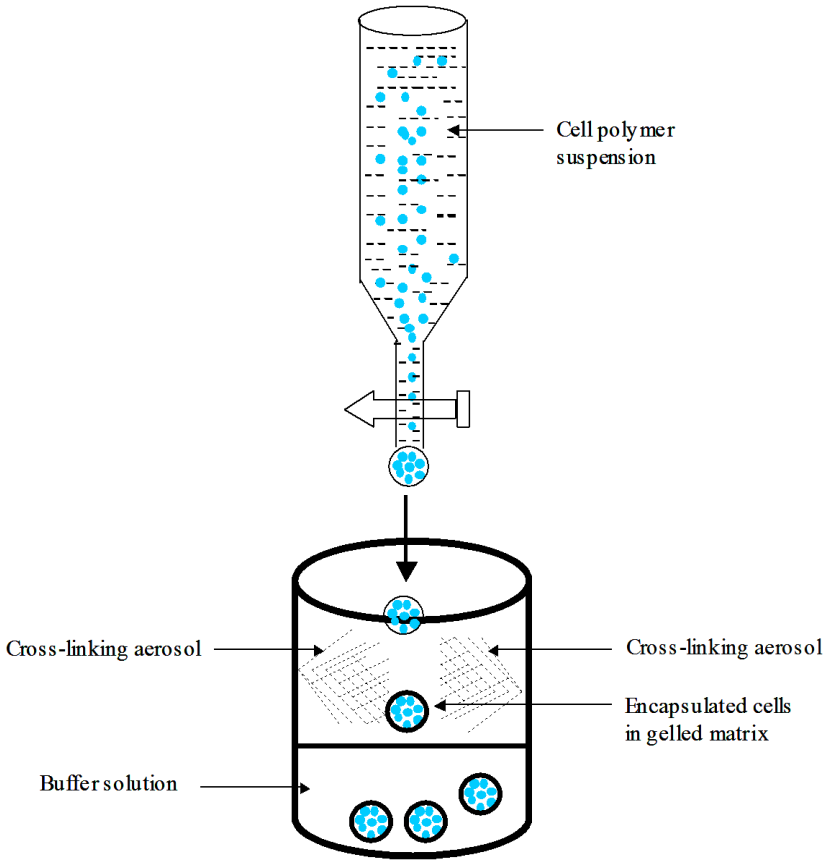

Fig. 5. Schematic representation of encapsulation of cells using the spraying method.

Interfacial Reaction. Surface reaction between polymers can be used for cell encapsulation. The mechanism of this reaction is based on interfacial polymerization. The Dautzenberg et al [45] described a method of microcapsules preparation by interfacial reaction that can be potentially used for microencapsulation of biological material. In this system, the aqueous solution of anionic polyelectrolyte is interfacially reacted with the aqueous solution of cationic polyelectrolytes. The principle of microcapsules preparation by interfacial reaction is demonstrated in the following Fig. 6.

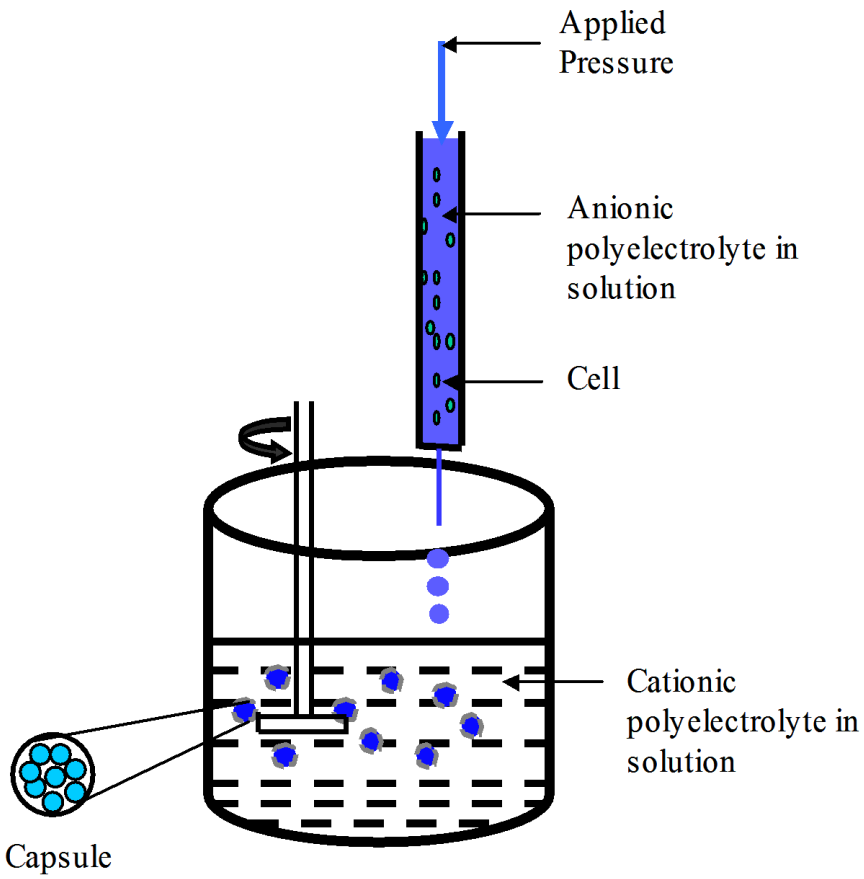

Fig. 6. Schematic representation of microcapsule preparation by interfacial reaction.

This process often involves extruding the anionic polyelectrolyte/cell solution through a needle, and forms droplets. These droplets are then reacted in a precipitation bath with a suitable cationic polyelectrolyte, and form a microcapsule with a liquid core containing the cell. Several research groups successfully immobilized living cells and enzymes by this method without losing their

biological activity [46,47]. Other methods can involve photosensitive polymers whereby cross-linking occurs by exposure to UV light [48].

Layer- by- layer Method. The layer-by-layer method, introduced by Decher [49] involves several sequential deposition steps of polyanions and polycations, and each optimized on a certain property [50]. In this process, the cell suspensions are dipped into an oppositely charged polyelectrolyte for a certain period, and then wash with detergent (saline) solution to remove untreated polyelectrolyte, followed by the oppositely charged polyelectrolyte. The process is continued until the specific property (capsules with well-controlled size and shape, finely tuned capsule wall thickness and variable wall compositions) is achieved. The schematic representation of the preparation process is shown in Fig. 7. During the process oppositely charged polyelectrolytes served as the counter ion to build up the multilayer polyelectrolyte membrane.

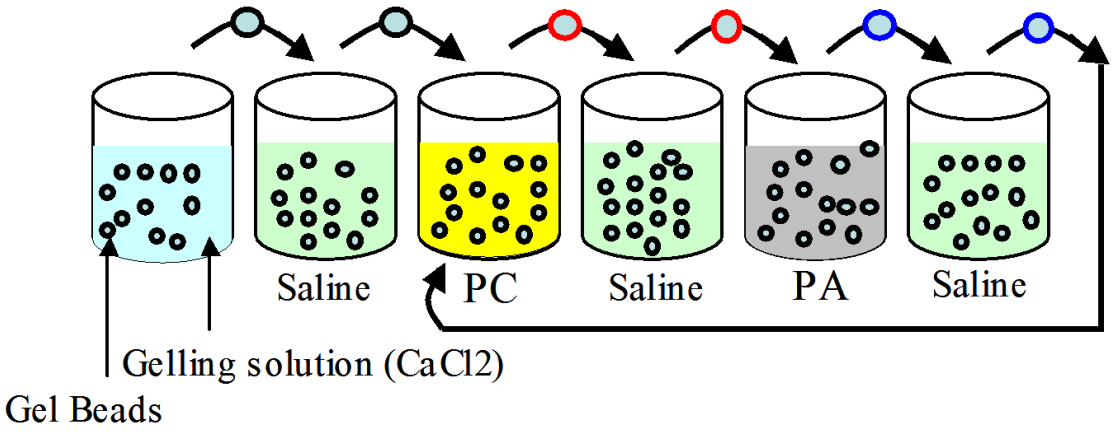

Fig. 7. Schematic representation of the preparation process of layer-by-layer microcapsules. PC represents polycation; PA represents polyanion.

Moulding Method. In the moulding method, a hot solution of cells and polymer mixture is cast into moulds with the desired shape, which is shown in Fig. 8. After cooling, the gels can be removed and transferred to an appropriate buffer solution. The advantage of this method is the possibility of

producing other shapes of gels than round beads, but it suffers from low efficiency and temperature limitations. However, in order to get a suitable cell containing gel beads, the sensitivity of cells to heat should be taken into account.

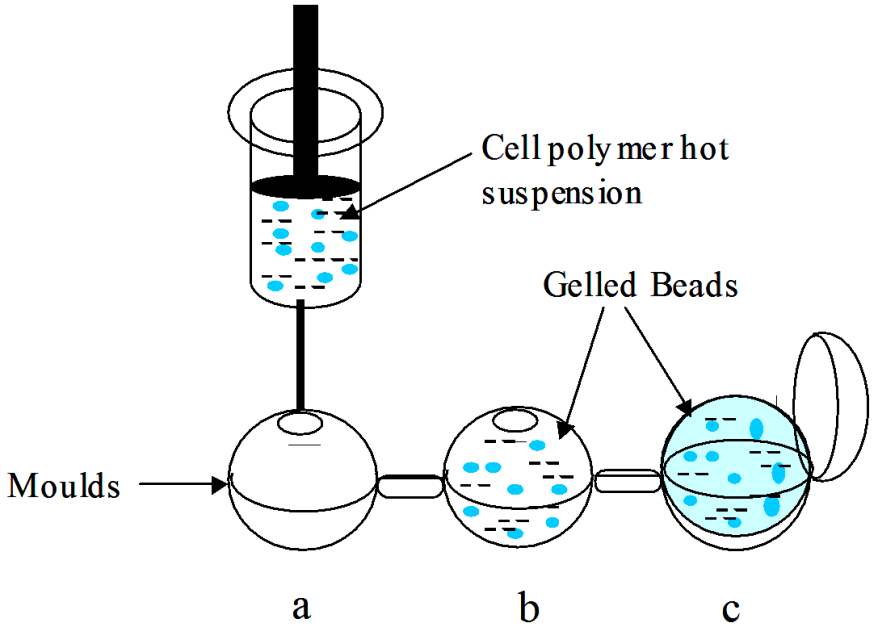

Fig. 8. Schematic representation of entrapment of cells using moulds. a) The polymer-cell suspension solution (usually hot) into moulds, b) after the gel setting time, c) entrapped cells are carefully taken out of the moulds, and then stored in a buffer solution.

Polyelectrolyte Complex

Polyelectrolytes are macromolecules that have charged or chargeable groups when dissolved in polar solvents like water. Basically they dissociate into a macromolecular ion and small counter ions, similar to their cross linked analogs that have found much uses as ion exchange resins. Combining solutions of oppositely charged polyelectrolytes can lead to formation of layer-by-layer assemblies [41,51-53], soluble [54,55], liquid coacervate [56], gel-like [57] and even solid [58,59] polyelectrolyte complexes (PEC's) that in the latter three cases phase-separate from solution. Polyelectrolyte complexes have been widely used for a number of applications such as separation

membranes, immobilization of enzymes or cells, drug carriers, gene delivery tools and protein purification.

This complexation is driven by the gain of entropy of the small counter ions upon PEC formation. The resulting complex is insoluble in water, organic or common solvents. In general, the stoichiometric composition of these polyelectrolyte complexes depends on the degree of dissociation of the two polyelectrolytes. The equilibrium complex water content can be varied from 30 to 90% by weight [60]. Possible combinations of polyelectrolyte complexes are strong polyacids-strong polybases, strong polyacids-weak polybases, weak polyacids-strong polybases, and weak polyacids-weak polybases. In general, the nature of the interactions/ complexes, the properties of the polyelectrolyte complexes and the efficiency of complexation formed depend on variables such as charge ratio, ionic strength, polymer concentration, pH, charge density, molecular weight, temperature and polymer structure [43,61]. The layer-by-layer assembly, introduced by Decher [41], involves sequential deposition of polyanions and polycations, and allows excellent control of the thickness and composition of the layers [42,43]. During this process oppositely charged polyelectrolytes served as the counter ions to build up the multilayer polyelectrolyte membrane.

When the interaction is strong, the complex can precipitate. On the other hand, if the interaction is weak, the complexes often remain soluble. The formation of polyelectrolyte complexes is depicted in Fig. 9. In between these two interactions, the solution can separate into two immiscible liquid phases, in a process called liquid coacervation (from latin, coacere: heaping together). One of the phases, the polymer rich phase is called the coacervate phase, and the second, polymer lean phase, is called the supernatant or equilibrium phase [62].

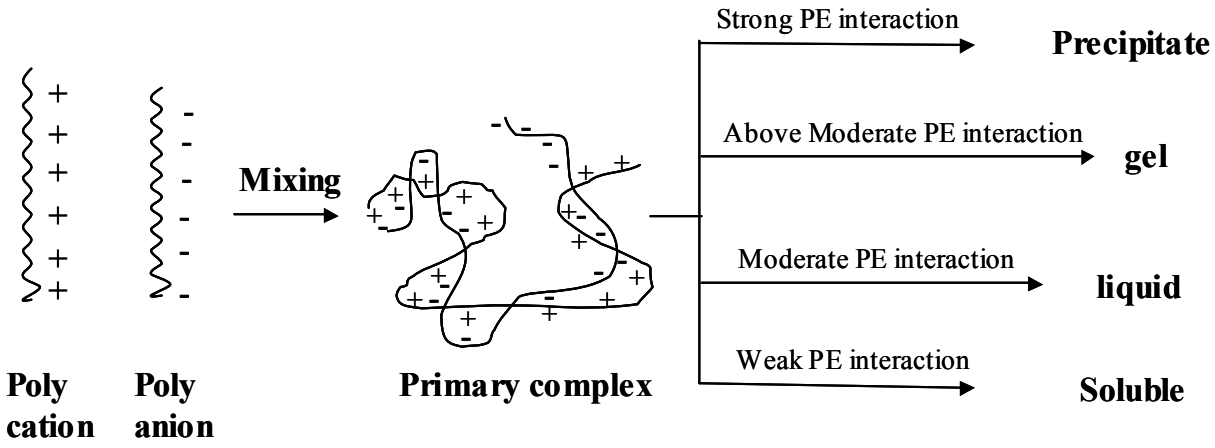

Fig. 9. Schematic representation of formation of polyelectrolyte complexes.

Coacervation can occur with a single polymeric species, and is then called simple coacervation. This is a liquid-liquid phase separation to form a condensed droplet rich in a single polymer species. It involves only one macromolecule and may result from the addition of a dehydrating agent that promotes polymer- polymer interactions over polymer solvent interactions. This polymer solvent interaction can be brought by changes in temperature or solvent composition (pH, ionic strength, non-solvents). For example, the addition of alcohol to an aqueous solution of gelatin causes dehydration of the gelatin, leading to the formation of a simple coacervate consisting of a separate phase rich in gelatin [42]. Complex coacervation involves the interactions of two oppositely charged polyelectrolytes in aqueous media, where the charges are sufficiently large to activate interaction, but not large enough to cause precipitation [54]. For this case, Piculill and Lindman [63] have recommended the term “associative phase separation” to replace “complex coacervation”. One of the best-studied examples of complex coacervation is the gelatin-gum arabic system [64]. At pH around 4.6, gelatin has a net cationic charge due to its high lysine contents, and can hence form a PEC with the anionic gum acacia, and are typically covalently cross linked with formaldehyde or glutaraldehyde. Complex coacervation is dependent on the molecular weight, charge density, concentration, hydrophobic/hydrophilic content of the polymer, and on the nature of the medium such as temperature, pH, ionic strength or the presence of non-solvents. Both coacervation processes have been extensively applied in the field of encapsulation, as well as for protein separations [65-68].

Several examples of synthetic and natural coacervate systems are illustrated below:

Synthetic Polyelectrolytes. Some non-ionic thermo-responsive polyacrylamides can show thermal coacervation when heated, and potentially they can be used in protein separation processes [69]. Anionic polyphosphazenes can form micro droplets by NaCl induced simple aqueous coacervation. These can be stabilized by the addition of $CaCl_2$ to form hydrogel microspheres. These hydrogel microspheres were however not stable at very high or very low salt concentrations [70]. Cationic polyacrylamide copolymers (cPAM) above a certain molecular weight (MW) can form coacervate complexes with anionic sulfonated kraft lignin (SKL). Coacervation efficiency increases with increasing MW of cPAM. Below the cPAM critical molar mass, colloidal complexes form instead [71].

Natural and Mixed Polyelectrolytes. Gelatin and gum arabic form a complex coacervate that can be covalently cross-linked with formaldehyde or glutaraldehyde to make stable microcapsules [72]. Ca-alginate gel beads can be coated with positively charged polymers such as poly-L-lysine, chitosan and others. The resulting PEC's are usually only stable at low salt concentrations [73]. Bovine serum albumin and poly (diallyldimethyl ammonium chloride) (PDADMAC) can form coacervate complexes in specific pH region (6.5-7), depending on their stoichiometry as well as on the salinity[60].

Materials in Cell Encapsulation

The materials used in cell encapsulation must be of constant quality and be biocompatible with the host and the enclosed cells. The capsules membrane must have reasonable mechanical strength to survive handling, implantation, and the mechanical, biochemical and biological stresses imposed by the host, and allow the exchange of oxygen, nutrients and metabolites, while obscuring the encapsulated cells from the host's immune system (Fig. 1). Moreover, rough surfaces of the microcapsules must also be avoided because they can elicit immunological reaction.

In the search for a better microencapsulation design (single and multilayer), many types of natural and synthetic polymers are being explored since polymers are playing an important role in the encapsulation of cells. The main reason for using them in these systems is their ability to exist at different phases as liquids, gels or solids, which enable them to meet a large range of mechanical and physical demands. There are large varieties of polymers that can be used in these systems are synthetic, semi- synthetic (chemically modified natural polymers) and natural polymer. Among them, alginates are nowadays the most widely studied and characterized material for cell encapsulation. Alginate is a naturally produced anionic polysaccharide obtained by extraction from marine brown algae. It is a linear binary copolymer composed of β-D- mannuronic acid (M) and α-L- guluronic acid (G) residues (Fig. 10).

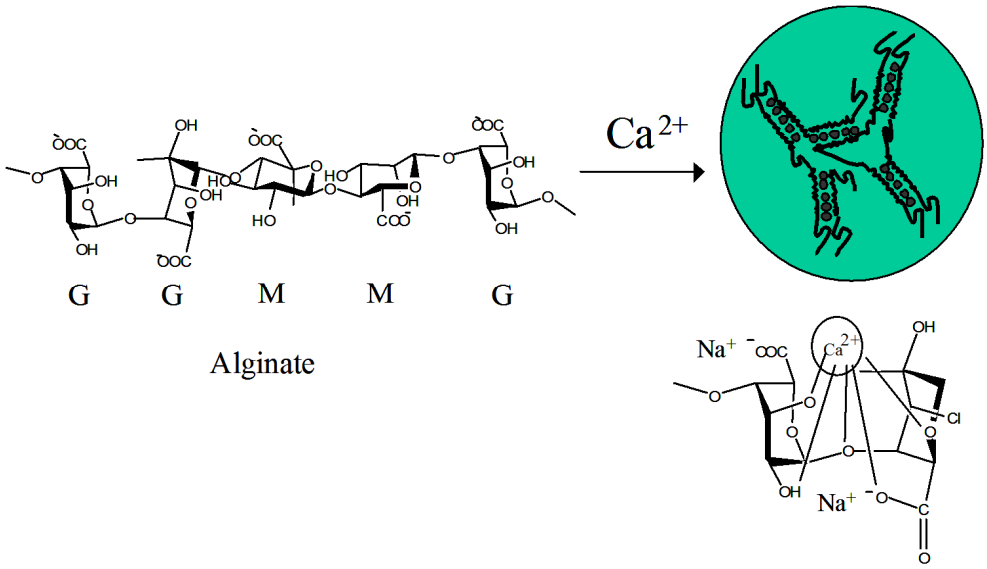

Fig. 10. Calcium cross-linking of Alginate bead formation in Calcium Chloride bath.

Conceptually, non-autologous recombinant cells are encapsulated within a biocompatible polymer, and microcapsules can be formed by many different methods. One of the most widely studied microencapsulation system involves the alginate-poly-L-lysine-alginate (APA) microcapsules derived from the protocol of Lim and Sun [25]. This encapsulation process involves three different

steps. The first step involves dispersing an alginate solution containing cells in droplet form into a calcium chloride gelation bath. When these droplets are immersed in the calcium gelation bath, calcium ionically cross-links the guluronic residue of the alginate chains (Fig. 10), which leads to the formation of firm calcium alginate hydrogel beads. This process often involves extruding the alginate/cell solution through a needle with continuous annular airflow, and is illustrated in Fig. 3. The second step in encapsulation is the coating of the alginate hydrogel beads with poly-L-lysine (PLL) in order to strengthen the outer bead surface. PLL interacts with the alginate through ionic interactions and the resulting alginate/PLL polyelectrolyte complex on the bead surface serves to increase the structural stability of the microcapsules, and defines permeability. The third step involves coating the outside of the resulting capsule with a layer of alginate, in order to hide the inflammatory PLL from the host and make the final capsules biocompatible. While these APA capsules meet many of the requirements for immuno-isolation of cells implanted into mice (intact for 6 months), they still show insufficient strength when implanted into larger animals such as dogs, where they collapse within 2 weeks [74]. This may be due to the slow exchange of calcium ions with other physiological ions and/or the loss of the polyelectrolyte overcoats.

In addition to the standard APA microcapsules, several other alginate-based microcapsules have been used for the purpose of immuno-isolation. Since barium ions have a higher affinity for alginate than calcium ions, barium has been used instead of calcium, and found to create mechanically stronger microcapsules [75]. Despite promising results, the clinical use of barium cross-linked microcapsules will likely be limited by the fact that barium ions are neurotoxic [76]. A number of studies have attempted to address the challenge of long-term mechanical stability by varying the molecular weight (ultra high viscosity) [77] or G/M ratio of the alginate [69,78], uncoated alginate microcapsules [79], the cross-linking ion [80] and/or use of polyelectrolytes to coat the alginate based capsule that includes Alg- chitosan-Alg [81,82], Alg-PLL-poly(acrylic acid) [83], Alg- PLL-poly(ethylene glycol), Alg-chitosan-poly(ethylene glycol), and Alg-PLL-poly(ethylene glycol)-Alg

[84], Alg-poly-L-ornithine-Alg [85,86], Alg-poly(allylamine)-Alg [87], Alg-PEI- poly(acrylic acid)-PEI-Alg, and Alg-PEI- carboxymethylcellulose-PEI-Alg [88]. Some of alginate-based microcapsules have also been tried for targeted application based on immune isolation, which are listed in the following Table 1.

Table 1. Cell encapsulation approaches based on alginate matrices.

Material	Modification	Application
Alginate	-	Bone and cartilage engineering, diabetes and cancer [89-92]
Alginate	With RGD	Bone regeneration and muscle regeneration [93,94]
Alginate	Enzymatic modification	Increased stability [95]
Alginate	Photo reactive liposomes	Substrates containing cells immobilized in precise locations [96]
Alginate	Phenol moieties	Increased stability [97]
Alginate-PLL-alginate	-	Bone repair and regeneration, chronic neuropathic pain and anemia [98-100]
Alginate-PLL-alginate	Covalent cross links between membrane	Increased stability [101,102]
Alginate-PLL-alginate	Polymerization	Increased stability [103]
Alginate-Agarose	-	Sub sieve- size capsules [104]
Alginate-chitosan	Lactose modified chitosan	Increased mechanical properties [105,106]
Alginate-PLO-alginate	-	Diabetes and neuroprotection [107,108]

While alginate microcapsules are appealing because they are well characterized and reasonably biocompatible, their usefulness may be limited by the presently limited stability. Consequently, new types of microcapsules have been made from synthetic polymers that will potentially yield stronger microcapsules. Till to date few synthetic system, such as poly(hydroxyethyl methacrylate-*co*-methyl methacrylate), p(HEMA-*co*-MMA) [109], poly(hydroxyethyl methacrylate-*co*-ethyl methacrylate), p(HEMA-*co*-EMA) [110] and polyphosphazene (PPP)[111] have been studied as biomaterials for microencapsulation. The major difference between water-soluble polymers such as alginate and water insoluble polymers such as poly(HEMA-*co*-MMA) is that water insoluble polymers are assumed to be more stable than water-soluble polymers after implantation, but do require the use of somewhat cytotoxic solvents during their formation [112]. The p(HEMA-*co*-MMA) microcapsules were predicted to be biocompatible since poly(MMA) and poly(HEMA) constituents have been successfully used for biomedical purposes [113]. To date, there are no published results available for *in vivo* delivery of therapeutic proteins from cells in p(HEMA-*co*-MMA) microcapsules. On the other hand, p(HEMA-co-EMA) has shown to elicit an immune reaction to the host [103]. Polyphosphazene is a synthetic polymer composed of alternating nitrogen and phosphorus atoms. While there are relatively few studies on the delivery of protein using polyphosphazene microcapsules, cells remain viable when encapsulated in an ionically cross linkable polyphosphazene and it has been shown that secretion of protein does occur *in vitro* [104]. However, *in vivo* biocompatibility of polyphosphazene microcapsules may be limited since the use of polyphosphazene elicited an immune response [114]. Finally, Agarose (natural polymer) has been investigated for the microencapsulation of pancreatic islets and treatment of diabetes. *In vivo* the microcapsule mechanical strength was increased [115]. However, when pancreatic islets were encapsulated in agarose and implanted into mice, a cellular immune response was observed [116].

Thus calcium alginate remains a promising matrix for cell encapsulations, but it would be desirable to improve the mechanical strength through biocompatible cross-linking. Another approach has been to examine the use of alternate hydrogel cores [2,117], including those made of composite materials.

Reinforcement of the alginate core through the formation of an interpenetrating network or composite may lead to improved mechanical properties while maintaining most of the desirable properties of alginate. A number of alginate composite materials have also been explored for cell encapsulation [118]. Compounds added to the alginate forming the bead core were designed to be thermally (agarose [97]), ionically (carrageenan [119]) or photochemically gelled [120], or designed to modify viscosity or water content (carboxymethylcellulose [121]), act as wall forming materials (heparin [113], cellulose sulphate [122]), control permeability or provide an improved environment for cell growth (chitlac - lactitol-functionalized chitosan [99]). For example, capsules designed for longer-term cell implantation have been prepared with alginate-cellulose sulfate composite cores where the cellulose sulfate acted as a viscosity modifier and was thought to be a better wall builder than alginate when forming polyelectrolyte complexes with the polycations used to coat the capsules [114].

Still other composite cores have been formed by the in-diffusion of polymeric or monomeric species followed, in some cases, by cross-linking or polymerization. To study the properties like controlled release and swelling, the physical interpenetrating networks were reported using alginate and chitosan [123] or acrylic acid [124]. Gaserod et al [125] found that the in-diffusion of low MW chitosan into CaAlg beads resulted in the formation of an alginate-chitosan gel in the core of the capsule that was able to withstand the loss of calcium. Smeds et al [126] introduced methacrylate groups to alginate to study the degree of methacrylate modification and covalent affect mechanical properties like swelling, compression, and creep compliance of alginate based hydrogel.

Several groups have tried to improve the strength of alginate-based materials via the photo polymerization of (meth)acrylate-functionalized alginate [118,127-130] or of other monomers within the alginate core [131,132]. Childs and coworkers formed a capsule composed of alginate and poly(sodium acrylate-*co*-N-vinylpyrrolidone) formed by UV-photo polymerization of vinyl monomers allowed to diffuse into the CaAlg beads [123]. This approach worked reasonably well; through the presence of photoinitiator and added sodium acrylate did pose some toxicity challenge to

the encapsulated cells. Recently, Hallé and coworkers [133,134] studied APA microcapsules in which a photoactivatable cross-linker was used to covalently link the PLL layer with the adjacent alginate in both the core and the outer coating. These capsules displayed greatly improved mechanical stability while maintaining the cell viability and permeability of standard APA capsules. In addition, these covalently cross-linked capsules prevented the escape of malignant cells into the body of the recipient [135]. We recently described a pair of polyelectrolytes bearing complementary reactive groups that underwent spontaneous mutual cross-linking to produce a covalently cross-linked polyelectrolyte shell on CaAlg beads [136]. In contrast to photochemical cross-linking approaches, this cross-linking reaction is based on condensation reactions that occur instantly upon contact. The resulting coated alginate capsules showed enhanced resistance to mechanical and chemical stress, attributed to the cross-linked outer coat. The improvements were however limited, and there was evidence of fibrotic overcoats after implantation, indicating an adverse immune reaction. Analogous four-layer coats that included outer layers of PLL and alginate, led to improved biocompatibility, and further enhanced mechanical properties [137]. Recently we investigated some amine-bearing synthetic acrylic polycations as materials that might be used in place of, or in addition to, PLL in the preparation of capsules for cell encapsulation. One of the synthetic polycations, poly(N-(3-aminopropyl)methacrylamide) (PAPM), was found to be a promising alternative to PLL [138]. We also explored the feasibility of using these synthetic materials and their cross-linking reaction to reinforce the core of the CaAlg capsules. This reinforcement process involves incorporating the reactive polyanion in the CaAlg gel core by ionic or physical cross-links, where it subsequently becomes covalently cross-linked by in-diffusion of PLL of suitable MW. This approach is shown to lead to different morphologies, depending on the relative ability of the two reactive polyelectrolytes to diffuse through the CaAlg matrix [139].

Conclusion

Cell immobilization and encapsulation has a wide range of applications. It appears likely that by the end of the decade clinical trials of encapsulated cells to treat many of these diseases will become a reality. Although simple and biocompatible conditions are required for cell encapsulation, technological development including the sourcing of raw materials, the design and building of manufacturing facilities, the scale-up and optimization process, storage and distribution of the product, and quality control thus far is time-consuming, which require collaboration between scientist and engineers from many disciplines. A discussion about the application of encapsulated cells is a discussion about the future. It represents an important objective for scientist and industry for the benefit of all.

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