Chapter 2

Applications of Cell Microencapsulation

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Abstract

The goal of this chapter is to provide an overview of the different purposes for which the cell microencapsulation technology can be used. These include immunoisolation of non-autologous cells used for cell therapy; immobilization of cells for localized (targeted) delivery of therapeutic products to ablate, repair, or regenerate tissue; simultaneous delivery of multiple therapeutic agents in cell therapy; spatial compartmentalization of cells in complex tissue engineering; expansion of cells in culture; and production of different probiotics and metabolites for industrial applications. For each of these applications, specific examples are provided to illustrate how the microencapsulation technology can be utilized to achieve the purpose. However, successful use of the cell microencapsulation technology for whatever purpose will ultimately depend upon careful consideration for the choice of the encapsulating polymers, the method of fabrication (cross-linking) of the microbeads, which affects the permselectivity, the biocompatibility and the mechanical strength of the microbeads as well as environmental parameters such as temperature, humidity, osmotic pressure, and storage solutions.

The various applications discussed in this chapter are illustrated in the different chapters of this book and where appropriate relevant images of the microencapsulation products are provided. It is hoped that this outline of the different applications of cell microencapsulation would provide a good platform for tissue engineers, scientists, and clinicians to design novel tissue constructs and products for therapeutic and industrial applications.

Key words Microencapsulation, Applications, Goals, Cell therapy, Immunoisolation, Tissue engineering, Drug delivery, Cancer treatment, Probiotics

1 Introduction

Cell microencapsulation is an aspect of the broader field of biencapsulation which involves the immobilization of therapeutic cells using polymer scaffolds or semi permeable hydrogel capsules that provide the cells with a favorable protective environment for a variety of purposes [1–4], which is the focus of this chapter. Historically, an experiment designed for a different purpose provided a model platform for the takeoff of this technology that dates back to 1933 when Bisceglie enclosed tumor cells in a permselective polymeric membrane and transplanted them in the abdominal cavity of a pig to determine the effect of loss of vasculature on the survival of

implanted tissues [5]. The results from this early study showed that the cells survived long enough for it to be concluded that they were shielded from destruction by the immune system. However, as recently proposed by David Scharp and Piero Marchetti the credit for the development of extravascular diffusion device should go to Algire and his colleagues who developed the technology in order to study both cellular mechanisms of tissue rejection and tumor growth in a series of publications that spanned over a decade [6].

For some applications of cell microencapsulation, the required properties of the microbead matrix may be quite different. For example applications directed towards the repair or regeneration of tissue, biodegradability of the matrix may be desirable. When the matrix degrades, the entrapped cells may proliferate and create their own extracellular matrix in place of the artificial one used to entrap them [7]. Since there are many different applications of cell encapsulation technology, the specific requirements to achieve the desired goal in each case will include the choice of the biomaterials used as the entrapping matrix. It has been suggested that suitable materials for cell encapsulation should mimic the extracellular matrix and should be processed under conditions compatible with the presence of cells [7]. A classic example of cell encapsulation application utilizing injectable and degradable hydrogel formulations is cartilage and bone tissue engineering, where it has been shown that the swelling and degradation properties of the hydrogels influence the chondrogenic and osteogenic differentiation of encapsulated bone-marrow derived mesenchymal stromal cells [8, 9]. In addition, it has been shown that the implantation of biodegradable oligo(poly(ethylene glycol) fumarate) hydrogel microbeads encapsulating the pigment epithelial cells (PECs) of the dorsal iris in lentectomized newts resulted in a regenerated lens 30 days after explantation, thus demonstrating that the degrading hydrogel did not adversely affect regeneration [10].
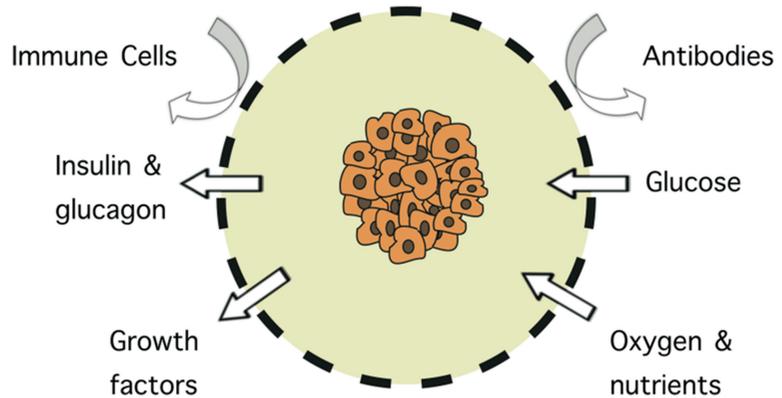
2   Cell Immunoisolation

Following the pioneering work of Algire and his colleagues, Chang subsequently introduced the idea of using encapsulation for the immunoisolation (immunoprotection) of transplanted cells and coined the term 'artificial cells' in describing this concept [11], which was successfully put into practice when it was used to immobilize non-autologous islet cells in a construct termed a bioartificial endocrine pancreas for glycemic control in a diabetic rat model [12]. Generally, this construct shown in Fig. 1 is now simply referred to as a bioartificial pancreas [4, 13]. The principle of immunoisolation entails the enclosure of cells in semi permeable hydrogel microcapsules that provide the cells with an appropriate environment that allows the exchange of nutrients and oxygen
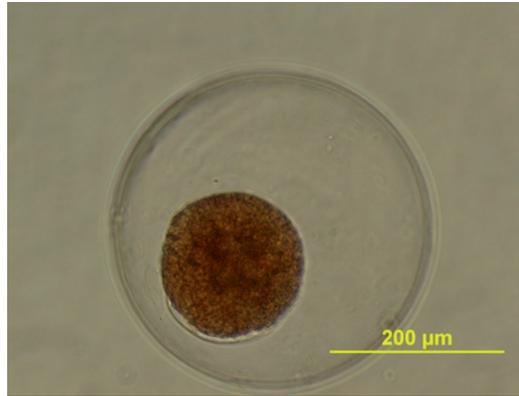
Fig. 1 Picture of a rat islet in alginate–PLO–alginate microcapsule

Fig. 2 Illustration of the principle of immunoisolation of islets encapsulated in a semipermeable microcapsule that allows entry and exit of molecules with permissible sizes

while protecting the cells from the host's immune system by blocking the entry of cytotoxic antibodies and cells, as illustrated in Fig. 2.

The report by Lim and Sum on the successful use of microencapsulation for the immunoisolation of transplanted islet cells generated enormous enthusiasm and provided the impetus for the belief that microencapsulation has significant potential to solve two major barriers to islet cell transplantation, namely the severe shortage of human pancreas, and the need to use immunosuppression to prevent transplant rejection [4, 6, 13, 14]. Eventually, this concept has been tested successfully with allografts in numerous studies performed in diabetic rodents [6, 12, 15], but has only had limited success in human studies [16–20]. There are numerous reasons for the limited success of microencapsulated islet cells in human studies [4, 6, 14]. However, there is room for improvement, which is why there is still significant enthusiasm for further

development of this technology [4, 6, 10] that many believe has the promise of a cure for Type 1 diabetes. It is noteworthy that the immunoisolation of xenogeneic islet cells by microencapsulation has been shown to be successful in both small and large animal studies [21–24]. Interestingly, de Vos et al. have shown that encapsulated islet xenografts in humans may not be prone to cytotoxic cytokine destruction, in contrast to islet allografts, perhaps at least in part because xenogeneic islet cells are resistant to the binding of human cytokines [25], which may explain why encapsulated porcine islet xenografts have been shown to survive for nearly a decade in a diabetic patient [26]. Another study with microencapsulated porcine islets has also reported long-term function >6 years after transplantation in many Type 1 diabetic patients [27]. In addition, a recent report has also shown significant efficacy of microencapsulated neonatal porcine islets in Type 1 diabetic patients many years after transplantation [28], thus suggesting that the bioartificial pancreas in the form of xenografts may indeed hold significant promise as a future treatment option for patients.

While microencapsulation of islet cells has been the most researched topic on the concept of cell immunoisolation, cell microencapsulation has been applied to a wide variety of endocrine diseases and other cell replacement therapies either as allografts or xenografts, as illustrated in this book. Thus, its application has been extended to other cell types including hepatocytes [29, 30]; parathyroid cells [31], myoblasts [32, 33], stem cells and others [34, 35]. It is noteworthy that although a few studies on cell immunoisolation by microencapsulation have been performed with other polymeric materials, the overwhelming majority of studies in the literature on this subject have utilized the complex polysaccharide, alginate, as the encapsulating material [36]. Uncoated non-permselective alginate microbeads have been reported to have high permeability for molecules with molecular sizes >600 kD. Uptake studies with IgG (150 kD) and thyroglobulin (669 kD) have suggested these molecules are able to get into these uncoated microbeads. Similarly, uncoated alginate microbeads implanted in the peritoneum were positive for both IgG and C3 component after only 1 week [37]. Therefore, molecules with a range of sizes from macrophages and T-cells to smaller cytokine molecules such as IL-1β, TNF-α, and IFN-ɣ can easily penetrate into the alginate microcapsules and cause damage or destruction of the encapsulated islets [38]. To provide immunoisolation for the microcapsules, it is essential to apply a permeability barrier between the encapsulated islets and host immune system. Coating the alginate microcapsules with a polyamino acid layer, followed by an additional outer coating with alginate, typically creates this barrier. The positively charged polyamino acid molecules will readily bind to the negatively charged alginate molecules forming a complex

membrane [39], which significantly reduces the pore size of the microcapsule, and prevents immune cells from entering the microcapsule. In order to prevent interactions of non-bound polyamines to host tissue, a thin second layer of alginate is added [4, 14, 40]. The polyamino barrier acts as a shell, providing mechanical stability to the microcapsule, allowing for the liquefaction of the inner alginate [41], if desired. The thickness of this barrier can be varied through manipulations of incubation time and polymer concentration. The most routinely used perm-selective biomaterial is poly-L-lysine (PLL), which was the first material used to create this barrier [12]; however more recent research has shown that poly-L-ornithine (PLO) coating provides stronger mechanical support to the microcapsules with markedly reduced immune response [42, 43].

3   Immobilization of Cells for Localized Delivery of Therapeutic Products

The second area in which cell microencapsulation plays a role is the immobilization of cells for therapeutic applications [44]. As is the case in cell immunoisolation, in this application the rationale is to protect the cells from destruction by the immune system while they deliver products for different therapeutic purposes. One prominent application of encapsulated cells is in blood vessel engineering. Properly structured microvascular networks are essential for normal tissue function. Physiologically, blood vessels are required for transport of nutrients and oxygen to cells and tissues. When blood perfusion of tissue is altered, cells undergo starvation, which can lead to a pathologic state. Examples include some of the leading causes of death in the USA such as chronic heart failure, myocardial infarction, and stroke. In addition, altered vascular supply plays a role in peripheral vascular disease, can contribute to poor outcomes in reconstructive and transplant surgery, and limits the broad clinical application of engineered tissues [44].

Neovascularization, the formation of new blood vessels, can occur via either vasculogenesis or angiogenesis. While vasculogenesis is the assembly of precursor cells into vascular networks angiogenesis is the formation of new blood vessels sprouting from existing vessels and is the primary mechanism of neovascularization in adults [44]. While vascular networks are relatively stable, angiogenesis occurs during embryogenesis, tissue healing and regeneration, the menstrual cycle, and in some pathological situations. It is a highly complex process that involves a complex series of steps: including endothelial cell (EC) activation, degradation of the extracellular matrix (ECM), EC migration, alignment, proliferation, lumen formation, branching, and anastomosis. Vessel stabilization is then achieved, in part, by

mural cell recruitment and proliferation followed by basement membrane production [44].

In angiogenesis as a therapy for pathological conditions a typical approach is to investigate the delivery of cells (mature, progenitor, etc.) and/or growth factors (in the form of proteins or genes) to the target tissue. Therapeutic stimulation of vessel growth to improve tissue perfusion is an area of significant interest in many regenerative medicine and tissue engineering applications, and the delivery of multiple factors may improve outcomes. Cell delivery approaches focus on stimulating vascularization either via cell release of soluble factors, cell proliferation and incorporation into new vessels or prevascularization of tissue construct prior to implantation. Our group has successfully used the approach of co-encapsulating islet cells with angiogenic protein in alginate microcapsules to enhance tissue vascularization after implantation [4, 15, 45]. Another group has reported that the co-encapsulation of pancreatic islets with bioengineered IGF-II-producing cells promotes islet cell survival [46]. Other groups have also shown that mesenchymal stromal cells (MSCs) can be co-encapsulated with islets to improve microencapsulated islet cell function in vitro and in vivo [47, 48].

Another illustration of the utilization of microencapsulation to immobilize cells for delivery of therapeutic molecules is the microencapsulation of stem cells. For instance, mesenchymal stromal cell (MSC) therapy has emerged as a potential treatment option for a variety of medical conditions [49–58]. While MSC's innate migratory ability is invaluable for treating diseases involving multiple tissues, it has been shown that systemic infusion of MSC results in lung entrapment and consequent decreased numbers at the injury site [59]. Unfortunately this migratory property also results in transient effect of MSC therapy. In addition, despite possessing immunomodulatory properties, it has been shown that MSC are not immuno-inert, and allogeneic MSC are likely to be recognized by the recipient's immune system in a non-myeloablative setting [60–64]. Moreover, even in an autologous setting, once MSC arrive at sites of injury/inflammation within the body, they encounter a relatively hostile environment, which is often pro-apoptotic, resulting in rapid loss of the transplanted MSC. Preliminary studies in our laboratory indicate that MSC microencapsulation in alginate microbeads results in long-term viability when maintained in in vitro cultures. One can hypothesize then that MSC microencapsulation prior to transplantation will restrict cells to the site of injury, avoid their immune destruction when delivered as allografts, as well as protect MSC from apoptotic signals, thus maximizing MSC's therapeutic effect. Indeed, it has been shown that encapsulated GLP-1-producing MSC have a beneficial effect on failing pig hearts [65].

4   Compartmentalization of Cells in Multilayered Tissue Engineering

In 2010, our group described a multilayer alginate microencapsulation system suitable for coencapsulation of islet cells with angiogenic proteins [4, 66]. We have subsequently used this multilayer microbead approach to fabricate an ovarian tissue construct (Fig. 3) for hormone replacement [67]. This tissue construct is a bioinspired mimic of the follicular architecture of theca cells in an intermediate layer that surrounds granulosa cells in an inner layer of alginate microbeads. The two layers are separated by a semipermeable membrane made of poly-L-ornithine (PLO) for the immunoisolation of the encapsulated granulosa cells while acting as a basement membrane for the theca cells on top. The theca cells in the intermediate alginate layer are also immunoisolated by a PLO membrane that precedes a final coating with alginate.

To test the hypothesis that this natural arrangement of the follicular cells in our multilayer microcapsules was necessary for optimal construct function, we examined two other encapsulated cell culture schemes as controls. In these two other culture schemes, theca cells and granulosa cells were either encapsulated in separate
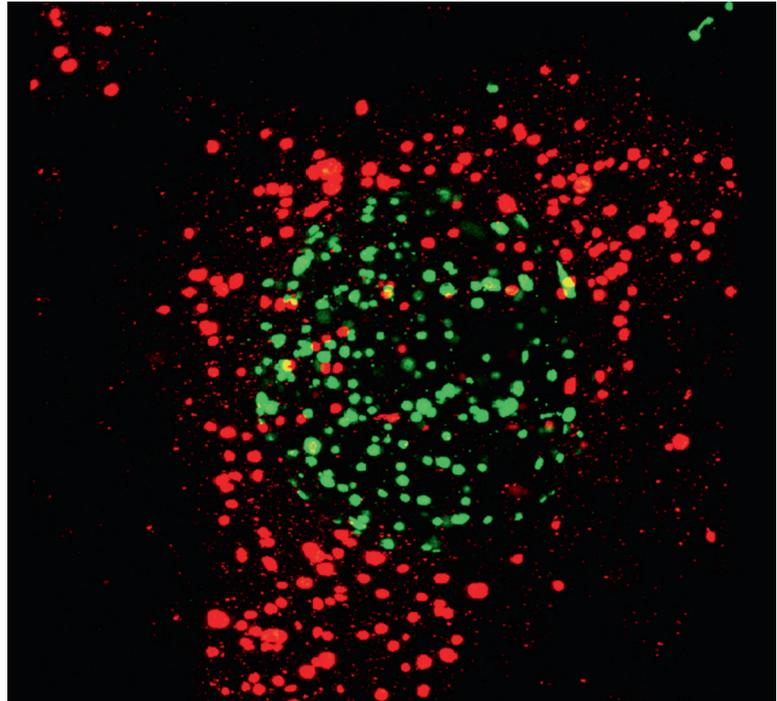
Fig. 3 Confocal Image (Composite of Z-stack) of compartmentalized ovarian cells in a multilayer alginate microbead (*red external layer* = theca cells; *green inner layer* = granulosa cells)

microcapsules and cocultured or encapsulated randomly together (non-layered) in the same microbeads and maintained in culture for 30 days. We found that encapsulated cells showed sustained viability during long-term in vitro culture with those encapsulated in the multilayered microcapsules secreting significantly higher and sustained concentrations of 17 β-estradiol (E_2) than the two other encapsulation schemes in response to follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In addition, the cells in the multilayer microcapsules also secreted activin and inhibin in vitro. In contrast, when granulosa and theca cells were cultured in 2D culture, progesterone (P_4) secretion increased while E_2 secretion decreased over a 30-day period, thus demonstrating the importance of the 3D culture provided by the microcapsules in the maintenance of cell phenotype [67]. Furthermore, this study represents an elegant example of the application of cell encapsulation technique for spatial compartmentalization of cells in the engineering of complex tissues.

5   Expansion of Cells in Culture

Induced pluripotent stem cells (iPSCs) represent an emerging cell source for biomedical applications, and such applications usually require a large number of cells. Suspension culture of iPSC aggregates can augment cell yields but may result in excess aggregation or cell death by shear stress. Hydrogel-based microencapsulation may solve such problems observed in cell suspension culture, albeit, this remains to be determined [68]. In general, for cell therapies, donor primary cells are often difficult to obtain and expand to appropriate numbers, rendering stem cells an attractive alternative because of their capacities for self-renewal, differentiation, and trophic factor secretion. Microencapsulation of stem cells offers several benefits, namely the creation of a defined microenvironment which can be designed to modulate stem cell phenotype, protection from hydrodynamic forces and prevention of agglomeration during expansion in suspension bioreactors, and a means to transplant cells behind a semipermeable barrier, allowing for molecular secretion while avoiding immune reaction [69].

Prior to the application of microencapsulation, successful methods for culturing human hematopoietic cells employed some form of perfused bioreactor system, which does not permit the clonal outgrowth of single progenitor cells. Levee et al. successfully used alginate–poly-L-lysine-alginate microencapsulation of human bone marrow, combined with rapid medium exchange, as a mechanism to achieve the clonal outgrowth of single progenitor cells for the purpose of studying the kinetics of progenitor cell growth [70]. They reported that a 12 to 24-fold multilineage expansion of adult human bone marrow cells was achieved in about 16–19 days with this system and that visually identifiable colonies within the

capsules were responsible for the increase in cell number. The colonies that represented the majority of cell growth originated from cells that appeared to be present in a frequency of about 1 in 4000 in the encapsulated cell population. These colonies were predominantly granulocytic and contained greater than 40,000 cells each. Large erythroid colonies were also present in the capsules, and they often contained over 10,000 cells each. Time profiles of the erythroid progenitor cell density over time were obtained [70].

Microencapsulation is also a very useful tool for therapeutic and industrial technologies that require the production of sufficient numbers of well-characterized cells and their efficient long-term storage. In order to establish a scalable bioprocess, human embryonic stem cells (hESC)-microcapsules were cultured in stirred tank bioreactors. The combination of microencapsulation and microcarrier technology resulted in a highly efficient protocol for the production and storage of pluripotent hESCs. This strategy ensured high expansion ratios (an approximately 20-fold increase in cell concentration) and high cell recovery yields (>70%) after cryopreservation. When compared with non-encapsulated cells, cell survival post-thawing demonstrated a threefold improvement without compromising hESC characteristics. Microencapsulation also improved the culture of hESC aggregates by protecting cells from hydrodynamic shear stress, controlling aggregate size and maintaining cell pluripotency for 2 weeks [71].

6   Cell Microencapsulation in Cancer Treatment

In cancer treatment, cell microencapsulation has been used as a technique to deliver therapeutic molecules to destroy (ablate) cancer cells. In this application, the cell microencapsulation approach is used as a strategy to protect the cells delivering the therapeutic products to cancer cells. An example of such therapeutic molecules is immunostimulatory monoclonal antibodies directed toward surface proteins of immune cells where they enhance immune response against cancer. Exogenous administration of the recombinant humanized immunoglobulins is being tested in clinical trials using this approach [72]. To illustrate a role for microencapsulation in this treatment modality, encapsulated antibody-producing hybridoma cells have been tested and compared with systemic administration of monoclonal antibodies. Hybridomas producing anti-CD137 and anti-OX40 mAb were encapsulated in alginate to generate microcapsules containing viable cells that secrete antibody. Immobilized cells in vitro were able to release the rat immunoglobulin produced by the hybridomas into the incubation medium. When the hybridoma-loaded microcapsules were implanted by injection into the subcutaneous tissue of mice, they provided a platform for viable secreting cells, which lasted for more than 1 week [73].

Also, it is known that tumors may develop resistance to specific angiogenic inhibitors via activation of alternative pathways. Therefore, multiple angiogenic pathways may be targeted to achieve significant angiogenic blockade [74, 75]. In a study investigating the effects of a combined delivery of the angiogenic inhibitors, endostatin and tumstatin, in a model of human glioblastoma multiforme, microencapsulated transfected porcine aortic endothelial (PAE) cells producing these inhibitors were applied as localized therapy in a subcutaneous glioblastoma model. When endostatin (ES) or tumstatin (Tum) were delivered separately, in vivo tumor growth was inhibited by 58% and 50%, respectively. However, the combined application of ES + Tum resulted in a significantly more pronounced inhibition of tumor growth (83%). cDNA microarrays of tumors treated with ES + Tum showed an upregulation of prolactin receptor (PRLR). ES + Tum-induced upregulation of PRLR in glioma cells was also observed in vitro [76].

Microencapsulated cells have also been studied for the treatment of bone cancer pain. Cancer-induced bone pain (CIP) is the most common cause of cancer pain [77, 78], and approximately 70% of patients with terminal breast or prostate cancer have evidence of bone metastases [79]. It has been suggested that human pheochromocytoma cells, which are known to contain and release met-enkephalin and norepinephrine, may be a promising resource for cell therapy in cancer-induced intractable pain [80]. In a recent study by Li et al.; microencapsulated human pheochromocytoma cells (micro-HPC) were intrathecally implanted to determine if this was a viable procedure to reduce pain in the rat model of bone cancer. The results showed that human pheochromocytoma cell implant-induced antinociception, which was mediated by met-enkephalin and norepinephrine secreted from the cell implants and acting on spinal receptors [80].

7   Microencapsulation of Bacteria Cells for Probiotic Applications

There is also an emerging interest in microencapsulation to deliver probiotic bacteria in controlled or targeted release in the gastrointestinal tract. With the explosion of probiotic health-based products, many reports indicate that there is poor survival of probiotic bacteria in these products. Further, the survival of these bacteria in the human gastrointestinal system is presently unknown. Providing probiotic living cells with a physical barrier against adverse environmental conditions is therefore an approach currently receiving considerable attention [81, 82]. In this application, microencapsulation of probiotics is associated with the protection of the probiotic cells in food products [83]. Functional foods provide a health benefit that goes beyond general nutritional content [84, 85]. In particular, foods containing probiotics are a

natural way of enhancing the functionality of food products [86]. The viability of probiotic cells must be maintained throughout a food product's shelf-life and under gastrointestinal conditions after consumption. Maintenance of their survival until they reach the gastrointestinal tract is one of the key requirements for health benefit. The International Dairy Federation (IDF) has suggested that a minimum of 10^7 CFU probiotic bacterial cells should be alive at the time of consumption per gram of the product [85].

In a recent study microencapsulation of *Bifidobacterium longum* (B. longum) with *Eleutherine americana* extract, oligosaccharides extract, and commercial fructo-oligosaccharides was assessed for bacterial survival after sequential exposure to simulated gastric and intestinal juices, and refrigeration storage. Microencapsulated B. longum with the extract and oligosaccharides extract in the food products showed better survival than free cells under adverse conditions. Sensory analysis demonstrated that the products containing co-encapsulated bacterial cells were more acceptable by consumers than free cells. In particular, pineapple juice prepared with co-encapsulated cells had lower values for over acidification, compared with the juice with free cells added. These observations suggested that microencapsulated *B. longum* with *E. americana* could enhance functional properties of fresh milk tofu and pineapple juice [85]. It has also been recently shown that incorporation of maize starch (2 %) in alginate microbeads followed by coating of the beads with stearic acid (2 %) led to better protection and the complete release of entrapped lactobacilli in simulated colonic pH solution. Thus, the resulting encapsulated probiotics can be exploited in the development of probiotic functional foods with better survival of sensitive probiotic organisms [87].

8 Other Industrial Applications

Microencapsulation has also been applied to other industrial purposes as a means to improve the efficiency of production of different metabolites [3]. For instance, in fermentation it has been applied to enhancing cell density, aroma, and capacity of systems [3, 88, 89]. It has also been used to prevent the washout of biological catalysts from fermentation reactors [3]. Recently, microencapsulation using an emulsification method to immobilize *Clostridium acetobutylicum* ATCC 824 spores has been utilized for biobutanol production. The encapsulated spores were revived using heat shock treatment and the fermentation efficiency of the resultant encapsulated cells was compared with that of the free (non-encapsulated) cells. The microspheres were easily recovered from the fermentation medium by filtration and reused up to five cycles of fermentation. In contrast, the free (non-encapsulated) cells could be reused for two cycles only. The microspheres

remained intact throughout repeated use demonstrating their capability as microbial cell nurseries in fermentation [90]. In an earlier study it had been shown that the production of lactic acid from glucose was enhanced by microencapsulation of *Lactococcus lactis* IO-1 cells [91].

9   Summary

It is apparent from the above review that there are many different applications of the cell microencapsulation technology that range from biomedicine to bio-industry. In biomedicine, cell microencapsulation has been mostly applied in the creation of bioartificial organs that specifically target a variety of endocrine disorders as well as kidney and liver failures [3]. However, it has also been applied in industry for a variety of purposes ranging from increasing cell manufacturing efficiency to enhancement of food aroma.

Each application may require peculiar specifications for the polymeric material used for encapsulation. In particular, adequate chemical characterization of the material to be used is necessary as it is critically important for the quality and function of the resultant product. One peculiarity required of polymers used for immunoisolation of encapsulated cells is adequate permselectivity for optimal protection of cells from immune destruction when such cells are implanted for therapeutic purposes in non-autologous setting. Currently, it does not appear that a naturally occurring biopolymer with adequate endogenous permselectivity for perfect immunoisolation is available. As discussed earlier, alginate is currently the most widely used material for encapsulation of cells designated for cell therapy, and we have noted that this polymer does not have appreciable permselectivity for immunoisolation. Hence polyamino acid polymeric (PLL or PLO) coatings of alginate microbeads have generally been utilized to enhance the pore-size exclusion of toxic immune molecules. Another polymer that has been utilized to enhance the permselectivity of alginate microcapsules is chitosan (β-[1–4]-linked-D-glucosamine) obtained by the deacetylation of chitin (β-[1–4]-linked *N*-acetyl-D-glucosamine), which is one of the most abundant naturally occurring polysaccharides [92]. Chitosan coating of alginate microbeads has been performed with or without modifications utilizing other materials and cross-linking agents to improve the mechanical stability and immunoisolation of the microbeads [93].

Obviously, for sustained performance of the encapsulated cells, it is necessary to fabricate microcapsules that possess long-term stability during storage or after implantation. There are various ways to manipulate the mechanical strength of microcapsules without adversely affecting the desired characteristics of other capsule parameters including permeability properties. It has been

suggested that successful achievement of this goal will depend on the following factors: the type of biomaterials used for fabricating the capsule matrix and membrane [42, 94, 95], the type of gelling ion [96, 97], the type of cell, and the selected encapsulation procedure [3].

Another critically important consideration for efficient use of microencapsulated cells is the storage of microcapsules, a requirement that appears to be presently underappreciated [3]. Optimal storage conditions for microcapsules are necessary for transportation between manufacturing and implantation sites as well as during the period of product manufacture. Invariably the method of fabrication (cross-linking) of the microbeads comes into play in this matter. Several studies have shown that microcapsule characteristics and performance are often very sensitive to environmental parameters such as temperature, humidity, osmotic pressure, storage solution, or solvent [3, 98–101]. Successful use of the cell microencapsulation technology for whatever purpose will ultimately depend upon careful consideration of these parameters.

Acknowledgement

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