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Technology of mammalian cell encapsulation[☆]

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Abstract

Entrapment of mammalian cells in physical membranes has been practiced since the early 1950s when it was originally introduced as a basic research tool. The method has since been developed based on the promise of its therapeutic usefulness in tissue transplantation. Encapsulation physically isolates a cell mass from an outside environment and aims to maintain normal cellular physiology within a desired permeability barrier. Numerous encapsulation techniques have been developed over the years. These techniques are generally classified as microencapsulation (involving small spherical vehicles and conformally coated tissues) and macroencapsulation (involving larger flat-sheet and hollow-fiber membranes). This review is intended to summarize techniques of cell encapsulation as well as methods for evaluating the performance of encapsulated cells. The techniques reviewed include microencapsulation with polyelectrolyte complexation emphasizing alginate–polylysine capsules, thermoreversible gelation with agarose as a prototype system, interfacial precipitation and interfacial polymerization, as well as the technology of flat sheet and hollow fiber-based macroencapsulation. Four aspects of encapsulated cells that are critical for the success of the technology, namely the capsule permeability, mechanical properties, immune protection and biocompatibility, have been singled out and methods to evaluate these properties were summarized. Finally, speculations regarding future directions of cell encapsulation research and device development are included from the authors' perspective. © 2000 Published by Elsevier Science B.V.

Keywords: Cells; Encapsulation, micro; Encapsulation, macro; Encapsulation technology; Alginate–polylysine capsules; Thermoreversible gelation

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Abbreviations: M_r , Molecular mass; MWCO, Molecular mass cut-off; PE, Polyelectrolyte; PLL, Poly(L-lysine); M, Mannuronic acid; G, Guluronic acid; CMC, Carboxymethylcellulose; ECM, Extracellular matrix; pMMA, Poly(methyl methacrylate)

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1. Introduction

Cell encapsulation aims to entrap viable cells within the confines of semi-permeable membranes. The scientific literature suggests that membranes are expected to be permeable for transport of molecules essential for cell survival, but not to allow the transport of molecules larger than a desired critical size. Despite its nonphysiological nature, the permselective capsule environment has been shown to support cellular metabolism, proliferation, differentiation and cellular morphogenesis. The primary impetus behind the recent development of cell encapsulation technologies has been the desire to transplant cells across an immunological barrier without the use of immunosuppressant drugs. The latter, due to their systemic 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) [1–3]. These complications cannot justify the ethical use of systemic immunosuppressants for a significant fraction of patients who are candidates for cell

transplants and provide the impetus to develop alternative methods to overcome the immune rejection process. By surrounding a transplant with a membrane barrier, the access of a host's immune system to the transplant can be physically prevented. The feasibility of transplanting cells in immunoprotective membranes is under study for the treatment of a wide variety of endocrine such as anemia [4], dwarfism [5], hemophilia B [6], kidney [7] and liver failure [8], pituitary [9] and central nervous system (CNS) insufficiencies [10], and diabetes mellitus [11] (see other chapters in this Volume). Besides transplantation, encapsulated cells are being pursued for a variety of other applications (Table 1). The common thread for all such applications is the need to isolate a desired cell population from an outside environment. Depending on the choice of membrane material and whether membranes are pre-fabricated or fabricated around viable cells, cells will be subjected to different entrapment conditions. The final capsule properties will be variable to suit the needs of a particular application and it is essential, for any encapsulation technology, to tailor capsule properties in a reproducible fashion by the process variables.

Table 1
Applications of encapsulated cell technology apart from cell transplantation

Application	Ref.
Large-scale production of cell-derived molecules in biotechnology industry	[12–14]
Clonal selection of desired cell phenotypes	[15]
In vitro culture of cells dependent on close cell–cell contact	[16–18]
In vivo cell culture	[19–22]
Reproductive technology	[23–25]
Cytotoxicity testing	[12,26]

An optimal balance has to be maintained among various capsule properties to support cell survival in the context of an expected performance criterion. The mass transport properties of an encapsulation membrane are critical since the influx rate of molecules essential for cell survival and the outflow rate of metabolic end products will ultimately determine the extent of entrapped cell viability (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. Although the role of permeability for particular ele-

ments essential for cell survival has been explored (for example, oxygen [27]), no systematic approach has been taken to determine the permeability requirements of particular cell types. An empirical approach has been typically taken to tailor capsule permeability for cell survival. The upper limit of capsule permeability, i.e., molecular mass cut-off (MWCO), will be application dependent. In the case of transplantation, the MWCO is expected to be different whether xenogeneic or allogeneic tissues are destined for engraftment (see Section 5.3 for more details). For bioreactor cultures, the MWCO will vary

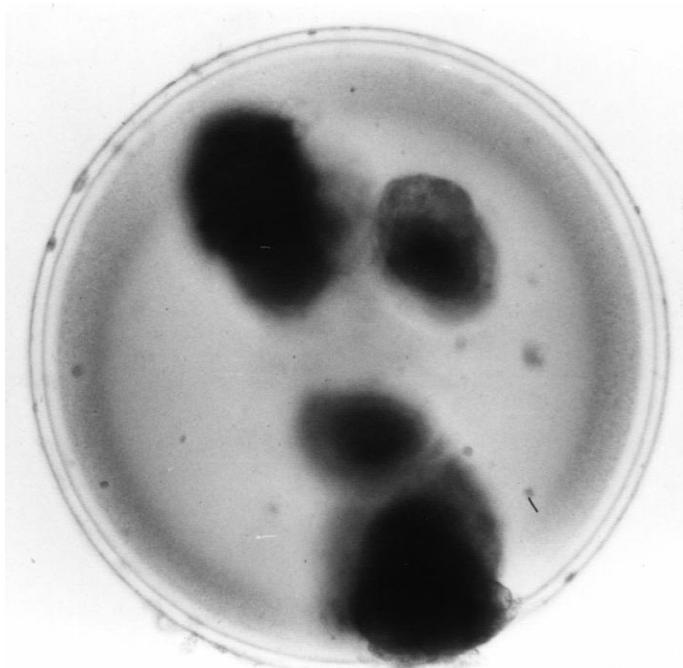


Fig. 1. A micrograph of pancreatic islets encapsulated in an alginate–PLL microcapsule. Note the perfectly spherical configuration. The hazy layer underneath the capsule membrane is due to the accumulation of high M_r waste products that do not pass freely through the capsule membrane.

whether it is desirable to have the cell-derived biomolecules to permeate through the capsule wall. Besides permeability, an important consideration is the availability of an extracellular matrix (ECM) to encapsulated cells [28,29], especially for anchorage-dependent cells. The ECM not only allows the cells to express their differentiated functions but also helps to organize the cell mass within the capsules for optimal viability [28,29]. Capsule biocompatibility is critical when encapsulated cells are intended for transplantation since it is the compatibility of a biomaterial with the host that ultimately determines the nature of host response and graft survival. The physicochemical nature of a biomaterial is important to enhance the capsule biocompatibility and exploration of different materials for this purpose has necessitated development of different encapsulation procedures to accommodate the varying physicochemical properties.

1.1. Scope

This chapter is intended to provide a summary of current cell encapsulation techniques. Since other chapters of this Volume are for specific applications, it was our intention not to emphasize application-specific issues but rather to review the advantages and disadvantages of various cell entrapment processes. A major distinction is in the utilization of ‘macrocapsules’ vs. ‘microcapsules’. In macrocapsules, large groups of cells are enveloped in tube or disc shaped hollow devices while in microcapsules a smaller cell mass is individually entrapped in their own spherical capsule. This manuscript will review techniques of microencapsulation, where cells are entrapped in spherical capsules and beads, or conformally coated. This will be followed by a review of macroencapsulation technology and issues specifically pertinent to macrocapsules. The success of any encapsulation technique will ultimately rely on a systematic evaluation of capsule properties and encapsulated cell performance. Accordingly, various techniques utilized for assessment of capsule properties will be summarized. Where indicated, relationship between the capsule properties and encapsulated cell performance are presented, with a critical emphasis on capsule engineering efforts. Numerous reviews were published over the years on encapsula-

tion technology [30–35], and the reader is referred to these reviews for different perspectives.

2. Microencapsulation

Capsules in the 0.3–1.5 mm range have been traditionally referred to as microcapsules in the cell encapsulation field. Their relatively small size (i.e., large surface area to volume ratio) is considered advantageous from a mass transport perspective. Microcapsules are typically more durable than macrocapsules and difficult to mechanically disrupt. Numerous microencapsulation techniques, fundamentally different in the nature of entrapment mechanism, were developed in independent labs. Besides traditional capsules with a well-defined shell-and-core structure, encapsulation in microbeads without a distinct membrane has been successful for certain applications. The homogenous bead milieu is entrusted to provide the desired permeability barrier in this case. ‘Conformal’ coating where the surface of a cell mass is surrounded with a membrane has also been attempted to minimize the internal mass transfer resistance. Irrespective of the entrapment geometry, a gentle entrapment process is vital for fragile cells incapable of proliferation or of exhibiting a robust regeneration. Relatively mild conditions (e.g., processes carried out in aqueous conditions without the use of reactive species), however, generally yield a less durable microcapsule. Harsher encapsulation conditions might compromise cell viability but the resultant capsule may be more resilient and cells capable of proliferation may be suitable for such conditions. Fragile cells can also be subjected to harsher conditions provided that the cells are compartmentalized from toxic chemicals or conditions. All techniques typically start with a scheme to generate a controlled-size droplet, followed by an interfacial process to stabilize the droplet and obtain a solid microcapsule membrane around the droplet. Although the intricacies in the initial droplet generation may make a significant difference in the success of encapsulation, the readers are referred to the other articles for more information on this subject [36–39].

Microcapsules are almost exclusively produced from hydrogels since they hold a number of ap-

peeling features. Firstly, the mechanical or frictional irritation to surrounding tissue is reduced by the soft and pliable features of the gel. Secondly, as the consequence of the hydrophilic properties of the material, there is virtually no interfacial tension with surrounding fluids and tissues which minimizes the protein adsorption and cell adhesion. Combinations of these two factors result in high biocompatibility (analyzed in more detail in Section 5.4). Thirdly, hydrogels provide a high degree of permeability for low-molecular-mass (M_r) nutrients and metabolites. The complexation of polyanionic alginate with polycationic poly(L-lysine) (PLL), initially developed by Lim and Sun [11], has been the first process utilized for cell microencapsulation. This is a gentle, cell-compatible process and is commonly credited for providing the impetus for the cell encapsulation field. The last two decades have seen adaptation of the initial technique by independent laboratories that naturally led to process improvements and development of superior encapsulation materials. Other techniques have since been attempted, primarily to overcome certain physicochemical limitations of the alginate–PLL capsules. Below is a synopsis of various encapsulation processes.

2.1. Microencapsulation by polyelectrolyte complexation

Interaction of oppositely charged polymers is the simplest way to form a physical membrane barrier around living cells. Being soluble in water, charged polymers offer the feasibility of developing an aqueous encapsulation system that is compatible with cellular milieu. Both natural (alginate being the primary example) and synthetic polymers were utilized for this purpose. The former are typically more cell-compatible but their harvest in large quantities typically results in a product that is heterogeneous in composition and M_r . Both parameters contribute to solution properties (such as viscosity) affecting microcapsule production. Purification schemes for alginates [40–43] and other natural polymers [44] have been reported and these techniques resulted in production of relatively homogeneous preparations. Synthetic polymers, on the other hand, can be conveniently prepared in large quantities but it is critical that such polymers are compatible with the

cells. Below is a synopsis of processes employing natural and synthetic polymers.

2.1.1. Alginate–PLL system

The complexation between polyanionic alginate and polycationic PLL has been widely used for encapsulating a variety of cell phenotypes. A major pursuit has been the encapsulation of pancreatic islets for treatment of Type-I diabetes. Unlike our desire not to focus on specific applications, encapsulation of pancreatic islets in alginate–PLL capsules provides us a unique perspective into the development of encapsulated cell therapeutics, since: (1) a large volume of published data from independent laboratories is available, (2) the alginate–PLL capsules was the first system utilized to fully explore the challenges awaiting the encapsulation technology and, (3) most findings with this system are pertinent to encapsulation systems pursued for other applications.

The technique is based on entrapment of individual cells and tissues in an alginate droplet that is transformed into a rigid bead by gelation in a divalent cation rich solution (mostly calcium, Ca^{2+}). Alginate molecules are composed of mannuronic (M) and guluronic acids (G) and can be connected by Ca^{2+} through binding of consecutive blocks of G-molecules on individual or different molecules. After gelation, the beads are provided with a membrane by simply suspending the beads in a PLL solution. During this step, PLL binds to mixed sequences of G and M in the alginate molecules [45] which establishes the permselective properties of the capsule membrane. By varying the M_r and the concentration of PLL as well as the contact time between alginate and PLL, one can modulate the porosity of the capsule membrane [46,47]. Usually, a 10-min contact time in a solution of 0.1% PLL with a PLL M_r of 22 000 is sufficient for an immunoprotective membrane. However, it should be emphasized that the binding of PLL does not only depend upon the incubation time and the PLL M_r but also on the type and concentration of alginate utilized [48], as well as on the incubation temperature [47]. In a final step, the capsules are suspended in a solution of alginate or other negatively charged molecules [48] to bind all positively charged PLL residues still present at the capsule surface. The last step is to

improve the capsule durability and biocompatibility for in vivo applications. In the following discussion we will use biocompatibility not only as a term to characterize the appropriateness of the host response but also to define the efficacy and survival of cells seeded on or enveloped by a specific biomaterial.

Normoglycemia has been reported after transplantation of alginate–PLL encapsulated allo- and xenogeneic islets in chemically induced and in autoimmune diabetic rodents [49–51], dogs [52], monkeys [53] and even in a human diabetic patient [54]. However, normoglycemia was of limited duration and varied reportedly from several days to several months, and was in one report 2 years in rodents [55]. Usually, the failure to maintain normoglycemia is interpreted as the consequence of insufficient biocompatibility, inducing a non-specific foreign body reaction against the capsules resulting in progressive fibrotic overgrowth of the microcapsules.

2.1.1.1. Modification of alginate–PLL capsules for a reduced host response

A number of modifications of the encapsulation technology have been described to reduce the host response against alginate-based capsules. Basic characteristics of biocompatible alginate–PLL capsules are their smoothness and mechanically stable nature. To produce smooth capsules it is advisable to use high- rather than low-viscosity alginates since the former is associated with much less tails or strains and a more adequate spherical structure of the beads. To improve the mechanical stability, alginates are used with an intermediate rather than low G-concentration, since low-G alginates were observed to swell after implantation with subsequent breakage of the PLL membrane and overgrowth of the capsules [48]. Another important modification in the encapsulation procedure has been the deletion of the so-called liquification of the inner core of the capsule with ethylene glycol-bis(β -aminoethyl ether) *N,N,N',N'*-tetraacetic acid or citrate [56] since many capsules were observed lose their integrity during the treatment. However, the factors related to the production procedure are not the only factors determining the host response against the capsules. Factors with a more chemical and physical nature have been subject of many studies during recent years and have

been shown to be of considerable importance for adequate functioning of microencapsulated tissue.

Alginate is a crude product extracted from alga and contains several inflammatory components. Not surprisingly, the purity of the alginate has been found to be a pertinent factor in the biocompatibility of alginate–PLL capsules. During recent years, a number of purification procedures for alginates has been described [40,42] that do not interfere with the molecular composition of the alginate as such. Virtually all these procedures are composed of filtration, precipitation and extraction steps. Purification substantially reduces the host response, as shown by using purified instead of crude alginate in implantation studies of empty capsules in rats. Crude alginate capsules were found to be adherent to the omentum and the liver at one month after implantation and they were always overgrown with fibrotic tissue. In contrast, 80–100% of the capsules prepared from purified alginate could be routinely retrieved with peritoneal lavage up to 12 months after implantation, and <10% of the retrieved capsules showed fibrotic overgrowth [40]. Not only empty but also islet containing capsules prepared from purified alginate were found to be adequately biocompatible since, similar to the findings with empty capsules from purified alginate, the majority were found freely floating in the peritoneal cavity after both allotransplantation and islet transplantation.

The host response is substantially reduced by applying purified alginate but the studies with purified alginates demonstrate two other pertinent obstacles in the clinical application of microencapsulated cells. First, the duration of graft function is substantially prolonged but still limited to periods of 3 months to a year in spite of a virtually absent host response [40]. Second, fibrotic overgrowth is always found in a small portion of the capsules, in spite of using purified alginate. This latter observation shows that biocompatibility of the microcapsules is influenced not only by systematic inadequacies such as impurities in the materials applied, but also by individual imperfections of a more physical nature.

To study the effect of individual inadequacies on capsule biocompatibility, De Vos et al. have designed an assay to identify islets that have been inadequately encapsulated [57]. In this assay, a lectin (RCA-I) that combines a high affinity for pancreatic

islets with a high M_r (120 000) was utilized. The large size prevents lectin diffusion into the capsule. The lectin allows us to identify imperfect capsules by specifically and individually labeling those islets that have been inadequately encapsulated. By applying this assay, it was found that reducing the capsule's diameter from 800 to 500 μm was associated with an increase of inadequately encapsulated islets from 7 to 25% [57]. This relatively small increase in the number of inadequate capsules has substantial consequences for the functioning of encapsulated islets *in vivo*; the subsequent transplant experiments gave a transplant success (defined as establishment of normoglycemia after transplantation in rats) rate of 5/5 with 800 μm capsules but only 1/7 with 500 μm capsules [58].

Another pertinent factor influencing the physical integrity of capsules is the type of alginate applied. When high-G alginates instead of intermediate-G alginates were applied, a substantial reduction in the percentage of inadequately encapsulated islets was found. For example, when 500 μm capsules were produced from high-G instead of intermediate-G, the percentage of inadequate capsules was decreased from 24 to 12% [57]. The different results with the two types of alginates were caused by difference in the swelling properties of the alginates during the encapsulation steps. The high-G alginates are associated with less swelling and less swelling results in a reduced chance for islet protrusion and, consequently, inadequate encapsulation [57]. Apparently, alginates with a high-G rather than an intermediate-G content should be applied to reduce the frequency of capsules with physical imperfections.

Since most *in vivo* studies with alginate–PLL capsules were restricted to capsules prepared from intermediate-G alginates, it was decided to compare the host response to capsules prepared from high-G alginates to those prepared of intermediate-G alginates. The experiments were performed by implanting empty capsules with varying G-content into the peritoneal cavity of AO/G-rats. The biocompatibility of the capsules was found to strongly depend on the type of alginate applied since capsules prepared from high-G alginates were always overgrown and adherent to the abdominal organs while capsules prepared from intermediate-G alginate remained free of overgrowth. This reaction against high-G alginate was

not caused by the alginate as such since host response was absent when Ca^{2+} -crosslinked alginate beads (i.e., capsules without PLL) were implanted instead of the capsules. Obviously, the different response against high-G and intermediate-G capsules was mainly caused by the inadequate PLL binding to the high-G alginate [59]. Recently, it was found that the high degree of crosslinking of high-G alginate and Ca^{2+} does not allow for adequate binding of PLL onto the capsule surface. Thus, we should search for means to reinforce the interaction of PLL with high-G alginate or search for alternative systems since this type of alginate appears to be advantageous for the process of microencapsulation [58].

One such alternative approach is the use of planar alginate beads, i.e., capsules without PLL and crosslinked with a divalent cation with higher affinity for alginate than Ca^{2+} . Many groups have focused on barium (Ba^{2+}) beads. In contrast to Ca^{2+} , Ba^{2+} not only binds to G molecules in the alginate but also to the M molecules. Consequently, Ba^{2+} beads are characterized by a high degree of crosslinking and a high *in vivo* stability. There is, however, ongoing dispute as to the adequacy of immunoprotection by Ba^{2+} -alginate. In rats, isografts survive for a longer time period than allo- and xenografts which implies that rejection processes cannot be completely prevented [60]. This problem cannot be easily solved since there are virtually no means to modulate the permeability and, thus, the immunoprotective properties of the Ba^{2+} beads.

A very promising approach in the application of planar alginate beads are the so called inhomogeneous alginate beads as recently introduced by Thu et al. [61,62]. Inhomogeneous beads have a higher alginate concentration at the periphery than in the center of the beads, while the commonly applied alginate systems have a homogenous concentration in their core (Fig. 2). These beads are manufactured by gelling alginate-droplets in a solution in which the Ca^{2+} concentration is low and other ionic components are deleted and substituted by iso-osmolites. The alginate molecules in the droplet have a high affinity for the rare Ca^{2+} molecules in the exterior fluid and diffuse towards the surface of the beads. Near the surface the alginate molecules will be connected by the rare Ca^{2+} which diffuses slowly

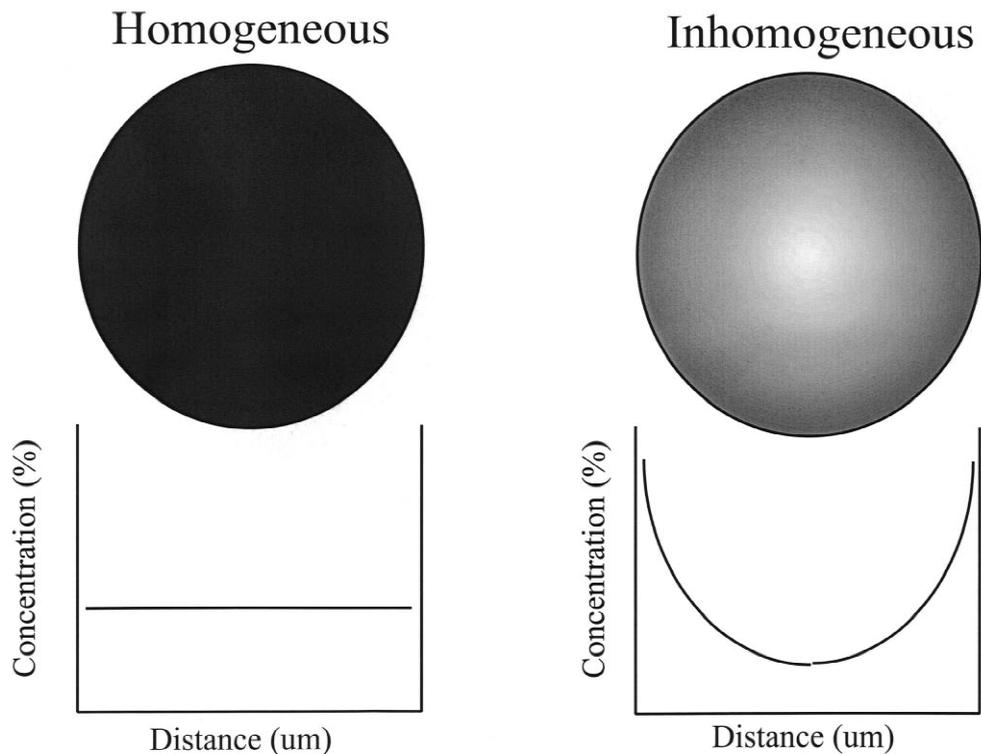


Fig. 2. Comparison of alginate gradient in conventional (left) and inhomogeneous (right) alginate beads. The latter is characterized by a decreasing alginate concentration towards the capsule center.

into the beads. As a consequence of the continuous diffusion of alginate towards the periphery the concentration of the alginate will be higher at the surface than in the center of the beads. The advantage of this system over the Ba^{2+} bead system is that it allows for manipulation of the permeability of the beads in the absence of PLL. By facilitating the diffusion of alginate towards the bead surface we can reinforce the concentration gradient in the bead, and thus decrease the permeability. The concentration gradient can be increased by decreasing the alginate M_w , Ca^{2+} concentration and bead size.

2.1.1.2. The efficacy and survival of encapsulated cells

As illustrated for the alginate–PLL system in the foregoing sections, seemingly minor modifications in the encapsulation procedure may have an important impact on the host response against capsules and thus on the outcome of the graft. In spite of this, the

above mentioned factors are poorly standardized. As a consequence, there are many different alginate–PLL procedures, each resulting in capsules with different permeabilities and with specific chemical and physical characteristics which, regrettably, are rarely documented. Obviously, these differences contribute to the enormous variations in reported success rates of encapsulated islet grafts.

In spite of differences in the procedures applied and new procedures, permanent survival of the graft has never been demonstrated which clearly suggests the presence of a fundamental problem. For many years it has been assumed that indefinite survival of encapsulated islets would be achieved with capsules which elicit a minimal or no host response [51,63,64]. This has been an overestimation of the importance of this response since even when the host response affected only an insignificant portion of not more than 10% of the capsules [40], long term survival of the encapsulated islet graft was not

achieved. This phenomenon of graft failure in the absence of overgrowth of the capsules has been reported before [65,66] and is usually explained by exhaustion of the graft as a consequence of a combination of a too high glycemic load on an insufficient number of transplanted encapsulated islets. In a recent study, however, De Vos et al. [58] implanted a sufficient islet volume of 10 μl , i.e., the equivalent volume of the islets present in the pancreas of a control rat, and found the functional mass of the graft to decrease rapidly not only in diabetic but also in pretransplant normoglycemic recipients. This shows that neither the volume of the graft nor the glycemic load on the islets was the causative factor.

One hypothesis that was tested is that of an imbalance between islet-cell renewal and islet-cell death to be a causative factor in the graft failure. This hypothesis is quite plausible since a β -cell has a life-span of no more than 3 months [67] which implies that in the absence of appropriate replication of cells the encapsulated graft will cease to function after this period. In vitro, we could not demonstrate any effect of the capsules on the replication of islet-cells but in vivo we found an enormous high replication rate of the β -cells (6% per 24 h in the capsules, versus 0.25% in the normal pancreas) [68]. This replication, however, was not sufficient to compensate for the loss of islet-cells since we found a gradual decrease in the functional volume of the grafts and an increase in the number of necrotic islet-cells. This necrosis was mainly found in the center of the islets and suggests insufficient supply of nutrients as the likely cause of this type of cell-death. Our results suggest the long-term graft survival to be limited by interference of the capsule with the blood supply of the encapsulated tissue and, consequently, by interference with optimal nutrition and/or supply of growth factors to the encapsulated tissue. Although this problem is an aspect related to the host response it should be considered to be a true biocompatibility problem, since long-term survival of the tissue is required for this 'specific application' but not compatible with the presence of the biomaterial around the tissue.

2.1.2. Other polyelectrolyte systems

Experience with the alginate system has demonstrated the feasibility of mammalian cell encapsula-

tion since aqueous-based, relatively mild encapsulation conditions did not compromise cell viability. To overcome the problems mentioned in the previous section, some groups have focused on new techniques of encapsulation rather than on improvement of the alginate–PLL system. Other naturally occurring and synthetic PEs have been explored as an alternative to improve capsule strength and better control microcapsule properties. Initial studies on synthetic PEs as an alternative to alginate–PLL were reported by Gharapetian et al. [69]. The polymers were based on either anionic acrylic/methacrylic acid or cationic dimethylaminoethyl methacrylate/diethylaminoethyl acrylate, or other less water-soluble (meth)acrylates. Encapsulation was achieved by extruding liquid droplets of a cell suspension in a charged polymer solution into a aqueous solution of oppositely charged polymer. It was clear in that study that (i) some of the chosen polyacrylates were severely cytotoxic to cells, (ii) there was a complex relationship between the polymer properties and the successful microcapsule fabrication, and (iii) capsules were typically non-spherical, unlike alginate–PLL capsules. The latter observation was due to the absence of a spherical gel capsule core that required PE complexation between two liquid phases (an inherently unstable system). A polyphosphazene-based PE capable of gelling in response to Ca^{2+} in solution has been synthesized [70] and was used to microencapsulate hybridoma cells [71].

A more systematic approach was taken by others for utilization of synthetic PEs for cell encapsulation. Stevenson and co-workers [72–75] have evaluated a wide-spectrum of tailored (meth)acrylate-based PEs with primary emphasis on engineering the 'salt-bridge' between the oppositely charged polymers. In a methodical approach, the influence of strength of charged species, polymer charge density, polymer M_r and hydrophilicity of PE backbone on capsule formation were investigated. Mechanical properties and better resiliency under aqueous conditions were apparently the critical capsule properties that required improvement. Although extensive capsule evaluation remains to be carried out (such as encapsulation of therapeutically-relevant cells and immunoprotective ability of capsules), initial work of this group on the use of other anionic species (such as $-\text{SO}_3^-$) is exciting and may open new avenues for PE engineering. A general concern with PE-based

encapsulation is the excessive osmotic stress created by the polymers. Presumably this issue will be highlighted since the non-coacervated PE will be retained in the core for a prolonged time. Hunkeler and co-workers [76–78] have approached this issue by first evaluating the cytotoxicity of numerous polyanions and polycations. Not surprisingly, synthetic PEs were generally more toxic (using RIN 1036-48 rat insulinoma cells as target) but their toxicity was dependent on the M_r and could be reduced when complexed after coacervation, indicating the possibility of using slightly toxic PEs for capsule formation [76]. Using a combinatorial approach, 1235 PE combinations were screened, out of which 47 were found to yield sufficiently stable capsules. Considering the low success rate (3.8%), one needs to optimize candidate PE screening efforts to arrive at promising combinations. Such a low success rate was also reported by others [79]; an evaluation of six polyanions and six polycations combinations yielded only a single combination [high-viscosity carboxymethylcellulose (CMC)/chondroitin sulfate A-chitosan] with equivalent permeability and mechanical strength to that of alginate–PLL capsules. Hunkeler et al. [76] have proposed certain desired characteristics of PEs both for cell-contacting and outer-capsule compartments and it was interesting to note that some of the PE characteristics were similar to the observations of Stevenson and co-workers (e.g., quaternary polycations form a stronger capsule membrane), but other characteristics (e.g., optimal charge density) differed.

The critical challenge for the use of other PEs remains unequivocal demonstration of *in vivo* survival of the transplanted cells. Although certain combinations of PEs were shown to be superior to alginate–PLL capsules *in vitro* (it must be stressed again that the alginate–PLL capsules prepared in different laboratories are likely to significantly differ), *in vivo* studies are lagging far behind.

2.2. Microencapsulation by agarose

Agarose is a readily available neutral polymer primarily composed of alternating units of β -D-galactopyranosyl and 3,6-anhydro- α -L-galactopyranosyl units [80]. It exhibits a temperature-sensitive water

solubility that can be utilized to entrap mammalian cells. In a typical procedure, an agarose/cell suspension is transformed into liquid microbeads (by extrusion [80] or simply oil-in-water dispersion [81]) which is hardened by a reduction in temperature. Agarose with a gelling temperature range of 15–30°C is commonly utilized (note that gelling temperature is concentration dependent). A homogeneous phase of agarose layer, without a permselective barrier, was shown to be sufficient for cell entrapment. A possible drawback, namely the possibility of cellular protrusion through the agarose membrane, can be eliminated by coating the entrapped cells in agarose with an additional agarose layer (as practiced by alginate–PLL capsules [82]). Despite little information about their physical properties, agarose beads appear to be more successful for allo-transplantation [83] than xeno-transplantation [84,85] of pancreatic islets. The latter could only be achieved under a low dose of a systemic immunosuppressant [86]. This was attributed to sufficient access of xeno-reactive antibodies to entrapped islets. *In vivo* graft survival was improved by producing denser beads (e.g., by increasing the agarose concentration from 5% to 7.5–10%, depending on agarose viscosity) [87] or, alternatively, by introducing an additional permeability barrier around the beads. The additional barrier was made up of oppositely charged PEs [88], the anionic component being poly(styrene-sulfonic acid) (PSSa) and cationic component being polybrene (hexadimethrine · Br) and/or CMC [89]. It appears that the function of PSSa was to prevent the exposure of xeno-antigens to the host's immune system [88], to improve capsule strength and possibly to prevent complement activation [89]. Polybrene is aimed at preventing the leakage of PSSa from the beads, and anionic CMC to improve the biocompatibility *in vivo* (i.e., prevention of fibrotic overgrowth) [89]. The data supporting these modifications remain to be published.

Unlike conventional agarose microcapsules, where the bead size was <0.5 mm, Jain et al. [90] prepared agarose beads of 6–8 mm in diameter (<1000 rat islets per bead) by simply adding a single drop to a sterile mineral oil. Beads were easily formed. Despite a lack of physico/mechanical information on these capsules, they were shown to effectively restore normoglycemia in a xenograft

model. It is surprising that such large beads were able to sustain cellular viability *in vivo*, but their large size is an obvious advantage in terms of scale-up and retrievability. More consistent results were obtained when collagen and gelfoam (gelatin) were present within the agarose, suggesting the importance of an attachment substrate in the capsule core (other agarose capsules were prepared without an ECM additive).

2.3. Encapsulation based on interfacial phase inversion

An interfacial precipitation process was developed by Sefton and co-workers [91–93] to entrap mammalian cells in water-insoluble, thermoplastic polymers. Such polymers offer several advantages over the water-soluble polymers: (i) the thermoplastic polymers can be easily engineered for a desired property, (ii) the polymer synthesis can be achieved in large quantities by conventional polymerization processes, and (iii) a better *in vivo* durability may be achieved because of polymer insolubility in aqueous medium. Encapsulation is performed by first extruding a cell suspension to form a liquid core, then surrounding the cell droplet by a polymer solution to form a liquid shell, followed by the extraction of polymer solvent to precipitate a solid shell. The liquid core-and-shell droplets were generated by a co-axial extrusion assembly, which relied on fluid shear to generate the individual droplets from the liquid phase. Initially an air-stream was utilized to shear-off the droplets [91]. To improve droplet uniformity and lessen shear damage on cells, an oscillating extrusion assembly at a liquid–air interface was then employed for droplet generation [93]. Uniform capsules of $\sim 900 \mu\text{m}$ in diameter were produced in this way [93]. To generate smaller capsules where a higher shear force is needed, a liquid stream immiscible with polymer solution and cell suspension had to be used to generate $\sim 400 \mu\text{m}$ diameter capsules [94]. Irrespective of the droplet generation scheme, maintaining a stable liquid droplet consisting of a liquid core surrounded by a liquid shell is a challenging engineering feat. The viscosity and density of liquid phases are expected to play a critical role to maintain the desired configuration, as

well as to minimize exposure of cells to potentially toxic solvent/nonsolvents. Although initial screens have been implemented to identify reagents with minimal cell toxicity [92], it is unlikely to totally eliminate the cell exposure to such chemicals, but the process can be engineered to reduce loss of cell viability. For example, the entrapment efficiency of viable Chinese Hamster Ovary (CHO) cells can be increased from 25% to 50% of theoretical value when a viscosity/density enhancer (Ficoll-400) was added to cell suspension during extrusion [95]. The entrapment efficiency of viable HepG2 cells, however, was not affected when a gelling matrix (Matrigel) was extruded in the capsule core [96]. Another potential issue, namely long-term solvent exposure to the entrapped cells, needs to be also addressed since solvent removal is expected to be reduced once a solid membrane had been formed. Presumably, the solvent will be preferentially extracted into the capsule exterior but even a small amount of solvent diffusing into the interior might give a high local concentration. As long as the solvent is being extracted, the membrane is likely to undergo microphase transformation and it might be essential to monitor the changes in the capsule physico-chemical properties to ensure that the final microcapsules are sufficiently stable before biological testing. Although PEG-200 was initially used because of its relative nontoxicity (compared to conventional polymer solvents such as dimethylformamide), Morikawa et al. [97] have recently proposed iopamidol as a cell-compatible polymer solvent. A 37% (iodine-equivalent) aqueous solution of iopamidol was capable of dissolving polyacrylates. If cell compatibility could be demonstrated with fragile cells (such as pancreatic islets), iopamidol may enable one to utilize water-insoluble polymers to encapsulate mammalian cells in an aqueous system.

The evolution of the interfacial precipitation process has led to the choice of hydroxyethyl methacrylate–methyl methacrylate (HEMA–MMA, 75:25) as the polymer candidate with optimal balance of hydrophilicity (i.e., permeability) and hydrophobicity (i.e., mechanical strength). This copolymer was successfully employed for the encapsulation of a wide variety of cells, and recently was shown to support intracerebral PC12 cell transplantation for delivery of dopamine [98].

2.4. Encapsulation by *in situ* polymerization

An early technique developed by Dupuy et al. [99] consists of polymerizing a membrane around living cells. Unlike PE and phase-inversion membranes, *in situ* polymerization technique produces a membrane that is held together by a network of covalent bonds. An important consideration, namely toxicity of free-radical species (initiators and monomers), has been addressed by embedding the cells in a gel core [99], so as to restrict the diffusive access of reactive molecules to cells and allow the gel to act as a 'buffer' for reactive species. Alternatively, polymerized particles can be incorporated to the polymerizing milieu [100,101] to reduce the level of monomers and/or reaction time necessary for a stable membrane formation. The early work in this area was concentrated on using acrylamide monomers (which are known to be relatively toxic *in vivo* [102]). Although a modification of this technique has been used for conformal coating of islets (see below), no progress has since been reported on this type of encapsulation method.

2.5. Conformal coating techniques

Conformal coating may be thought of as a special case of microencapsulation where the term is used to describe a method of forming a barrier directly on a small cell mass or a small piece of tissue. The method eliminates unutilized space in a microcapsule core by surrounding the cell mass with the encapsulation membrane. This theoretically provides an improved mass transport between the capsule exterior and the cell mass, and increases the effectiveness of cell packing (hence, minimizes the implant size). The improved mass transport is not only critical for the survival of cells, but also for the rapid delivery of cell-derived therapeutic agents. The latter is particularly appealing in the case of pancreatic islets whose function relies on prompt detection of extracellular glucose levels and secretion of insulin accordingly. Conformal coating is obviously not useful for single cells, since this will result in a large membrane material:cell mass ratio, but ideal for cell clusters up to a certain size where diffusion limitations do not become significant. It is not surprising

that conformal coating was initially developed for pancreatic islets but there is no technological reason why other cell phenotypes cannot be conformally coated as long as the cells are cultured as a solid tissue mass (e.g., hepatocytes cultured as a multicellular aggregates [103]). Conformal coating of islets were achieved by fundamentally different methods.

A method that employed alginate–PLL entrapment consists of centrifuging the islets through a discontinuous gradient of an alginate solution and a crosslinking agent (Ca^{2+} or Ba^{2+}) [104]. If necessary, an additional PLL coating step can be added to the basic protocol. The membrane thickness so formed with alginate– Ba^{2+} coating was $\sim 10 \mu\text{m}$, similar to the membrane thickness formed with conventional alginate–PLL encapsulation. Consistent with the expectation of better mass transport, the insulin secretion kinetics by the conformally-coated islets was similar to the free islets. In a modification of this procedure, May and Sefton [105] have centrifuged pancreatic islets through a solution of polyacrylate in a biocompatible solvent (PEG-200), followed by passage through aqueous phase to precipitate the polymer around the islets. Although the procedure was shown to coat model spheres, results with live cells have not been reported. An interfacial polymerization process was described by Sawhney et al. [106] to conformally polymerize a polyethylene glycol-based membrane around pancreatic islets. The process was carried out in an aqueous phase, was initiated on the islet surface by photoinitiators adsorbed to the cell surface and involved free-radical polymerization of PEG-acrylate monomer into a conformal membrane. The latter was homogenous in structure whose thickness could be varied between 10 and 100 μm in a controlled manner [107]. The choice of the photoinitiator was expected to be significant since it should exhibit a certain degree of hydrophobicity to associate with cellular membranes, but not be too adsorptive to disturb the native structure of cell membrane. Although detailed study of membrane permeability has not been reported yet, the stimulation index of coated islets (i.e., insulin secretion ratio under stimulated vs. basal conditions) were equivalent to that of free islets, indicating free transport of relatively small entities through the PEG membrane [106,107].

Despite its potential, *in vivo* performance of

conformally coated islets remains to be reported in the literature.

3. Macroencapsulation

Macrocapsules are generally much larger devices compared to microcapsules and typically possess a planar or cylindrical geometry, and a smaller surface-to-volume ratio. Living cells are physically isolated from directly interacting with host tissue by enclosure between two or more selectively permeable flat sheet membranes or within the lumen of a semipermeable hollow fiber. Like their smaller counterparts, these devices rely on the host animal's own homeostatic mechanisms for the control of pH, metabolic waste removal, electrolytes, and nutrients. Concentration gradients are used to transfer solutes across the encapsulation membrane that in general confers progressively greater resistance to the diffusive transport of molecules as the encapsulation device increases in size. 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. At least two general categories of macroencapsulation implants can be distinguished based on their association with host vasculature. **Intravascular devices** are connected or anastomosed directly to existing host vasculature, while **extravascular devices** rely on the formation of new blood vessels at the host–device interface following implantation to sustain the viability of the encapsulated cell mass.

Although numerous devices have been described, two designs appear most commonly in the literature. One consists of two planar membranes that are attached to either side of a spacer element to create an internal compartment or encapsulation chamber, while the other design utilizes a preformed hollow fiber membrane in which cells are infused into the lumen and the ends are subsequently sealed. The different device geometries place limitations on: the encapsulation chamber volume and, hence the number of cells that can be transplanted; the number of anatomical locations in which the respective designs can be used; and, the types of clinical applications that can be addressed with each type of device.

Although a method of cell encapsulation has been described in which cells are entrapped with the lumen of a hollow fiber during its formation [108], most macroencapsulation devices described to date make use of one or more preformed semipermeable membranes.

The macroencapsulation approach was originally developed as a basic research tool to understand fundamental issues of immunology and transplantation biology, and is now being developed as a therapeutic device to treat endocrine, metabolic and nervous system disorders [31,34,35]. As basic research tool, the key functional attribute is physical isolation or containment of the transplanted cells or tissue within a selectively permeable membrane. The fact that macroencapsulated cells can be kept alive for prolonged periods following implantation into a variety of tissues and body cavities while being physically isolated from interacting directly with host cells, vasculature, certain size classes of solutes, and the extracellular matrix of host tissues has led to a number of important discoveries in developmental biology, immunology and neuroscience. Although a handful of studies have been performed in amphibians [109] and birds [110–112], the majority of studies have utilized mammalian species. As a therapy, macroencapsulation technology is being developed as a sustained delivery system to provide biologically active agents with a wide range of pharmacological profiles to treat conditions where improvement may be obtained by continuous delivery of a therapeutic substance either systemically or in a site-specific manner. In addition, the macroencapsulation approach is being developed as an implantable homeostatic sensor-release system where the implant responds to changes in solute concentrations of the extracellular space by releasing substances to function in an endocrine or metabolic capacity.

Over the past 50 years, the technology of macroencapsulation has advanced from a simple laboratory tool to a promising bedside therapy [35,113–125]. Several human clinical trials have been conducted or are underway [35,116,121]. Numerous companies have been founded in attempts to develop the immense commercial potential that this technology promises. To deal with the many challenges of clinical application, the technology has evolved from

simple designs involving one to three components to sophisticated multi-component assemblies. Issues related to mass production, safety, and long-term clinical utility have fueled the development of a more sophisticated generation of devices. Technological improvements have taken place in all facets of the technology including: novel device configurations; membrane coatings; core matrix development; retrievable and refillable designs; cell engineering; and surgical technique.

3.1. Early developments in macroencapsulation technology: diffusion chambers

To the best of our knowledge, the development of ‘macroencapsulation technology’ can be traced to experiments conducted in the early 1950s to address basic questions in immunology and transplantation biology. In a series of elegant studies, Alguire et al. introduced a device called the ‘diffusion chamber’ to describe a method for grafting tissue that was physically isolated from host tissue by porous filters [126]. The device permitted the passage of essential metabolites and thus allowed survival of the implanted tissues, but prevented the passage of cells of the host or from the grafted tissue. The authors described two types of encapsulated implants for use in mice. Both designs were planar, disc-shaped devices that employed flat sheet commercial microporous filtration membranes. One type allowed viewing the encapsulated cells following placement between the skin and underlying skeletal muscle. The implant featured a membrane on the ventral or muscle-facing side and a glass cover slip on the dorsal or skin-facing side, each layer was separated by a spacer element. The other design consisted of two flat filtration membranes separated by a spacer element that was placed within the peritoneal cavity. The disc-shaped membranes were punched from flat sheet cellulose ester filters that had an upper pore size of 0.3–0.8 mm. A 30-mm width membrane formulation yielded the best cell viability of a variety of thicker membranes tested by transplantation studies. The cell encapsulation chamber was formed by the inside cavity created by a circular lucite or poly(methyl methacrylate) (pMMA) washer (13.9 mm O.D. × 10.0 mm I.D.) with the membranes being attached with a 1% solution of pMMA in acetone as

an adhesive. The overall size of the implant was approximately 17.5 mm O.D., and 0.5 mm in thickness. Sterilization was achieved by immersion in 70% ethanol. The devices were loaded by placing a droplet of tissue suspension in growth media in the center of the washer placed on top of one of the membranes. The other membrane was placed on top of the washer or spacer element and the three components were sealed together with the adhesive.

The studies of Alguire et al. [126] provided the first published evidence that encapsulated tumor-generating cells could survive for long periods following transplantation within selectively permeable membranes and demonstrated that the approach was able to protect the engrafted host from the risk of tumor formation. More importantly, the study demonstrated that encapsulated, allografted endocrine tissue could survive for weeks to months in animals previously immunized against the grafted tissues, while unencapsulated allografts placed in contact with host tissue were destroyed. This important discovery provided evidence in support of a cell-based mechanism of allograft rejection. Not only did the encapsulated grafts survive in previously immunized mice, but also cell division was observed from both epithelial and mesenchymally-derived encapsulated cell types. These early studies therefore can be viewed as providing the ‘proof of principle’ for what was called ‘tissue culture in vivo’ or later termed ‘macroencapsulation technology’.

By the mid 1970s, the diffusion chamber technique had been established as a unique and useful in vivo method for studying cell growth and differentiation. The results of numerous investigators indicated that several cell intrinsic properties were retained following cell encapsulation and transplantation into host tissues including: long-term survival [127]; cell cycle progression [109]; retention of differentiated function [109]; cell precursors giving rise to more differentiated cells [128–133]; morphogenesis within the device [109]; and the fact that diffusible factors released from the encapsulated transplant could influence function in the transplanted host [111,134–136]. Several early studies suggested the therapeutic potential for the treatment of endocrine insufficiency or immune related disorders [129,135–144]. In addition, it was shown that: device retrieval was possible under light anesthesia [145]; variable cell viability

was common following encapsulation [146]; complete necrosis of the encapsulated tissue almost never occurred but a central zone of necrosis was common in thick specimens; and, macrophages co-transplanted within the diffusion chamber were capable of removing necrotic debris within the capsules [146].

The membranes used during this early period were commercially available macroporous filtration material primarily made of mixed cellulose esters in nominal pore sizes of 0.1, 0.22 or 0.45 μm . Many of the later studies used the 0.1 μm pore size material based upon qualitative evidence that it was better for preventing cell migration into and out of the encapsulation chamber. The original diffusion chambers were fabricated in a series of steps in which one disc-shaped membrane was glued to a pMMA washer as described. Problems with the glue step included its toxicity to cells, its potential to solubilize the membrane changing its permeability, and the fact that it was time consuming to implement [147]. Early refinements in the technology involved the use of newer, commercially available microporous filtration membranes (nucleopore), the introduction of a central washer or spacer element with a predrilled hole that made the introduction of a cell suspension into a preformed device possible and avoided the complication of getting cells on the outside of the device during loading [148–150]. Several methods were introduced to attach the planar membranes to the spacer element including: the use heat sealing methods; the use of adhesive tape to bond the membranes; and the use of solvent bonding of the membranes to the spacer element [109,130,147,149]. To enhance the mechanical robustness of the devices, nylon mesh reinforced mixed cellulose ester membranes were used to reinforcement the membrane [110,112]. The early studies demonstrated that the technology was compatible with of variety of sterilization procedures including 70% ethanol, dry heat 60°C, gamma or cobalt irradiation, ethylene oxide and UV irradiation.

3.2. Introduction of ultrafiltration membranes

One of the biggest technological shifts in macroencapsulation technology was the introduction of ultrafiltration membranes. These newer commercially available filtration materials, fabricated in flat sheet

and hollow fiber forms, were developed in the early 1960s using the phase inversion process. The most commonly used material was made of a poly-(vinylchloride–acrylonitrile) (PAN–PVC) co-polymer referred to as the XM series of membranes. The most commonly used material of the series was the XM-50 hollow fiber membrane with a nominal MWCO of 50 000. This formulation offered considerably greater impedance to the diffusive transport of solutes especially larger M_r species. These newer membranes provided more significant immunoshielding than could be obtained with the use of the macroporous materials and made xenotransplantation without immunoprotection possible.

Some of the earliest cell encapsulation applications utilized the XM-50 hollow fibers in a bioreactor configuration to culture cells [151–153]. These reports inspired the earliest studies using XM-50 hollow fibers as intravascular encapsulation devices that were placed within the abdominal cavity of rodents and loaded with islets of Langerhans [154–156]. Other groups used the hollow fibers as extravascular devices in which insulin-secreting cells were infused into the lumen of XM-50 fibers that were subsequently sealed at the ends with adhesive [157,158]. Most of the early studies were conducted in rats or pigs. The first application of macroencapsulated tissue in the CNS used pituitary cells encapsulated in XM-50 hollow fibers from several species including human implanted into the ventricular system of the brain of hypophysectomized rats. Following the infusion of a cell suspension, individual hollow fibers were heat sealed by crimping with heated forceps. The method was effective in restoring growth to otherwise growth hormone deficient animals for periods up to 3 months. These two early periods of development provided the foundation upon which the present day field of macroencapsulation has been built.

3.3. Loading capacity

The simplest method for loading macroencapsulation devices is the direct injection of a cell suspension into a preformed device. When loading cells as an aqueous suspension, gravitational forces cause cells to settle and can create clumps or aggregates of cells that can complicate the process by making it

difficult to uniformly load a large number of devices at one time. A variety of immobilization matrices have been introduced to overcome this problem [159,160]. In devices that contain only one opening it is necessary to evacuate or displace the air or gaseous phase inside the device. This may be accomplished either through the use of an applied vacuum on the outside of the device or by employing a venting mechanism inside the device. With the vacuum method typically a wetting agent such as ethanol is used to hydrate the hydrophobic membrane to facilitate the removal of the relatively hydrophobic gas phase through the membrane wall. The solvent can then be removed by dilution with an aqueous physiological buffer by repeating several wash steps. In most cases, the translucent nature of the membrane allows the user to observe cell loading under a stereomicroscope at low power as a color change in the membrane as the cell suspension is infused into the encapsulation chamber. The devices are sealed by crimping, thermowelding the material with resistance heating or by use of a biocompatible adhesive [161,162].

Macroencapsulation devices have an encapsulation chamber of variable volume. Device choice must be tailored to the specific application. In some cases, the need to transplant a large number of cells may not be necessary when cells deliver a potent pharmacological agent that is intended for a site specific application such as the delivery of growth factors to specific deep brain sites [108,124,161,163–173]. In other cases, a larger biomass may be necessary for metabolic or endocrine applications such as in chronic liver failure or diabetes. In general, planar devices hold volumes that can range up to several milliliters while hollow fiber devices have an encapsulation chamber that holds from 0.5 to 30 μl . Many studies, however, fail to provide sufficient information to calculate encapsulation chamber volume making it difficult to estimate the upper limit of sustainable biomass that such devices can achieve.

3.4. Immobilization matrices

A common observation of transplantation studies that utilized ultrafiltration type membranes was the appearance of dead or necrotic zones in areas of high cell density. In these studies the cells were typically

loaded as a dilute cell suspension in aqueous growth media. It is presumed that following implantation that the cells settle creating a high-density aggregate that exceeds the transport capacity of the encapsulation barrier. To overcome this problem, a variety of cell immobilization matrices have been examined to more uniformly disperse or distribute the cells within the encapsulation chamber [28,159,160]. For anchorage dependent cells, ECM scaffolding such as collagen or laminin containing gels have been used along with plastic or glass beads [28,162–164,170,173,174]. In the case of microcarriers, cells may be cultivated onto their surface prior to loading the solid supports into the encapsulation device [175]. Many commercially available microcarrier formulations exist and except for references in the patent literature to the best of our knowledge there have been no detailed studies comparing their usefulness with specific macroencapsulation systems.

For cells that do not require anchorage, hydrogels were used to stabilize the position of the cells within the three-dimensional volume of the encapsulation chamber. Anionic PEs, such as alginate and its derivatives as well as several cationic matrices have been employed including chitosan, collagen, Matrigel and laminin [28,176–180]. Uncharged materials such as highly crosslinked polyethylene oxide or polyvinyl alcohol may also be useful. These matrices are employed to prevent cells from aggregating into dense clusters of cells. The matrix material must be of sufficient viscosity to allow dispersion of the cells within the material and to offset gravitational forces. Additionally the internal matrix can be blended with combinations of the aforementioned classes of materials or cells to provide nutrients or trophic support.

3.5. Cell engineering for maximal effectiveness

The choice of cells depends upon the intended application, such as the secretion of a particular naturally occurring bioactive substance like neurotransmitter, cytokine, chemokine, growth factor, growth factor inhibitor, angiogenic factor; or the metabolism of a toxic agent, or the release of an immunizing agent; or based on a sense and release function such as oxygen partial pressure and EPO or glucose and insulin. The use of dividing cells

including stem cells, precursors, progenitors and cell lines to provide the necessary biological functionality to an encapsulation device offers a number of advantages over the use of fully differentiated post-mitotic cells or tissue. These actively dividing cells may be expanded *in vitro*, selected for production, screened for pathogens, cloned, expanded and banked. They therefore present the potential for providing a level of standardization and quality that is difficult to envision with the use of primary sources of cells and tissue. In addition, proliferating cells are easier to genetically modify than primary cells and tissues. Encapsulated genetically engineered cells have been used to release growth factors and hormones and have been used as trophic support for other cell types to enhance the efficacy of implants [162–164,169–171,173,181]. A recent elegant study describes the use of encapsulated cells engineered for regulated delivery of erythropoietin through the use of a hypoxia-responsive promoter and provided evidence for functional effects in a mouse model [182].

One of the goals of macroencapsulation therapy is to maintain a constant population of viable cells within the device at a maximal density in order to minimize the size of the implant. A confounding principle of nature, however, is the fact that cell proliferation and cellular differentiation are generally mutually exclusive endpoints. Therefore, it may be desirable to control cell proliferation during the expansion phase. At some point, it may be desirable to stop cell cycle progression and promote a more differentiated phenotype in the cells to maintain the desired secretory and/or metabolic properties especially during the encapsulation period *in vivo*. It may also be desirable to control the proliferation of cells once inside the device to adjust cell density once the ischemic period is over or to control the dose of a therapeutic agent. Several approaches have been suggested to control both cell division and cellular differentiation. One approach has suggested the use of extracellular matrix or matrix mimetics be used to promote a more differentiated phenotype and prevent cells from proliferating following encapsulation [175]. Alternatively, a soluble factor may be used to influence proliferation or differentiation status including drugs and growth factors [174,183]. Lastly, one could use a cell phenotype whose cell

cycle progression is controlled by a regulatable promoter that controls cell cycle progression.

3.6. Long-term utility

Many clinical situations demand device operation for years or longer. In such situations it may be necessary to replace the device if tissue homeostasis within the encapsulation chamber cannot be achieved. The process of retrieval places different demands on the device. Designs have evolved to impart column strength to the otherwise fragile membranes. One design for use in the subdural space incorporates an adjacent tethering section that can be grasped by the surgeon and pulled to remove the implant [35,116,178]. Included in such designs is an axially oriented strain relief element that provides tensile reinforcement for the device and shields the fragile membrane from excessive loads encountered during explantation [184,185].

An alternative to retrieving and replacing the entire device is to retrieve and replace only the cells contained within the device. This solution has been advanced by several designs that incorporate at least one opening into the device through which cells can be introduced and subsequently removed. In certain cases, such as where the cells are derived from non-autologous sources a risk may be presented to the patient during the unloading and loading procedure. Designs have evolved that minimize this risk by enclosing the cells in a reusable container or chamber consisting of a selectively permeable material or laminate that prevents cell migration but encourages vascularization by the host [186]. These device designs have at least one opening that can be accessed from an exterior surface so that the cells can be placed, removed, or replaced within the luminal cavity of the device without damage to the adjacent tissue and hemorrhage due to the disruption of blood vessels that have colonized the exterior surface. Such a device can be a tube sealed at one end, a U-shaped tubular device, or a rectangular chamber with one or more openings that can be used as a sustained delivery device or used in a metabolic or endocrine capacity [159,165,186–188].

In such devices the cells may be introduced and removed from the encapsulation chamber in a liquid suspension. Recently polymeric matrices have been

developed for such applications that undergo environmentally triggered sol–gel transitions. It is envisioned that cells are blended with these materials in the liquid or sol state, infused into the device where the gel transition occurs and the cells are immobilized inside the device. The opposite procedure is used to remove the cells. One advantage of such designs is that the encapsulated cells are not present during the initial ischemic period immediately following the implantation of the device before neovascularization has been achieved. In addition the devices can be refilled with new cells or varying combinations of cells as needed. An interesting refinement to the aforementioned approach is a method of inserting and removing an immunoprotected insert that can be placed or removed from the vascularized device [189–191]. Another design has been proposed in which the permeability of the membrane is adjusted by the use of hydrogel introduced into the membrane and a cell displacing central core that can be used to adjust the position of the encapsulated cells nearer to the membrane wall at a distance from the nutrient source and at a cell density for optimal cell viability [189].

4. Other encapsulation techniques

A variety of entrapment techniques have been developed to overcome various shortcomings of the micro- and macroencapsulation (Table 2). A silica sol–gel technique has been successfully used to entrap islets in ceramic membranes [192]. This is the first report on the encapsulation of mammalian cells in ceramic membranes. Although detailed investigation of encapsulated cell performance is yet to be performed, this is an intriguing choice of material and it may offer distinct advantages over polymeric materials, such as high mechanical strength (at the

cost of brittleness) and durability in the physiological environment.

Thermosensitive, poly(*N*-isopropylacrylamide) (NiPAM)-based polymers have been utilized to entrap mammalian cells in a gelled medium [193]. The polymers exhibit a temperature-dependent solubility that is analogous to the solubility behavior of agarose: soluble at a relatively low temperature but insoluble beyond a certain temperature limit, so called lower critical solution temperature (LCST). Unlike agarose, the polymers can be conveniently engineered for desired properties, such as LCST. NiPAM as a homopolymer does not undergo gelation when heated beyond its LCST ($\sim 30^{\circ}\text{C}$ in water) and functional polymers for cell entrapment has been obtained by incorporating hydrophobic butyl methacrylate to the polymer backbone [193]. To augment gel stability, amine-reactive *N*-acryloxysuccinimide groups [193], or thiol groups capable of undergoing disulfide formation [194] have been incorporated into the polymer backbone. The gels can be crosslinked with diamino-poly(ethylene glycol) in the former case without affecting the cell viability. Although immunoisolation by NiPAM-based gels remains to be reported, there is no impediment to further engineer the polymer or gel properties for this purpose [195].

Unlike physical isolation barriers, Pollack et al. have developed a biological mechanism to prevent tissue reaction by utilizing the immune privileged properties of a chondrogenic environment [196,197]. This concept is analogous to previous observations where testicular cells co-transplanted with a graft was shown to provide a mechanism of immune suppression [198,199]. Unlike membrane-based devices, a co-transplanted immune suppressive tissue mediates its effect by insoluble and/or soluble molecules derived from the tissue. It will be beneficial to identify the biological factors contributing to the immune suppression. However, if a single factor is found responsible for a broad immune suppression

Table 2
Other techniques of cell entrapment

Technique	Principle	Ref.
Silica sol–gel entrapment	Formation of a ceramic encapsulation membrane from liquid phase	[192]
Thermoreversible gelation	Cell entrapment by synthetic polymers that undergo gelation as the temperature is raised	[193,194]
Chondrocyte membrane	Formation of a chondrocyte-based immunoprivileged site around transplant	[196,197]

(rather than a cocktail of mediators), it might be feasible to incorporate the delivery of such a factor into the conventional membrane-based devices to attain a 'bioartificial' mechanism of immune suppression.

5. Assessment of capsule properties

A critical requirement for the development of a successful cell encapsulation technology is the close relationship between capsule properties and performance of encapsulated cells. The desired capsule properties will obviously depend on a particular application. Even though an expected performance criteria is satisfied (e.g., restoration of normoglycemia in a diabetic model), it is essential to probe the capsule properties that contribute to successful performance. This will facilitate a more rational approach to encapsulated cell technologies and ultimately lead to superior design of encapsulated cell devices. In the case of less than desirable performance, the unsatisfactory performance is likely to be attributed to a particular physicochemical aspect of the capsules, so that identification of this aspect is vital for an improvement in the encapsulated cell performance. A compilation of important capsule properties is provided in recent reviews [30,200]. Among these properties, we have chosen to concentrate on (i) capsule permeability and MWCO, (ii) mechanical properties, (iii) immunoprotection and (iv) biocompatibility. These four criteria, in our opinion, are applicable for most of the current practice.

A pertinent issue in capsule characterization, especially for microcapsules, is the distinction between an 'average' property (e.g., average protein secretion from a capsule population) and the distribution of that property among a population of capsules (e.g., protein secretion from individual capsules). Investigations of microcapsule properties are routinely carried out by an ensemble of capsules, but characterizing the population properties may provide important data on: (i) 'ideal' capsule properties, (ii) interrelation among capsule properties (which might not be obvious because of heterogeneity), and (iii) undesirable capsule properties. The practical application of encapsulated cells,

whether in biotechnology or for medicine, will require a larger cell mass than typically utilized in laboratory investigations. Characterizing the capsule population will be critical to ensure a reproducible production process and will help to establish encapsulated cells as a well characterized tissue-engineered medical product [201].

5.1. Permeability and MWCO

The survival of encapsulated cells will ultimately rely on an optimal balance between capsule permeability (which determines the supply of essential nutrients and removal of toxic metabolites) and MWCO (which determines the upper size limit for molecular transport). The molecular transport through the capsule membranes is expected to be through 'fixed' pores but little information is available about actual pore sizes within capsule membranes. Rather, the permeation rate of different M_r species is determined as a measure of relative pore size. The assessment of capsule transport properties has been relatively straightforward for macrocapsules where the larger volume of membrane devices facilitates characterization. On the other hand, numerous techniques had to be adopted for transport properties of different types of microcapsules.

5.1.1. Microcapsules

The mathematical description of solute transport into a gelled bead [202] or a capsule is well established [203]. A simple experimental system to determine capsule permeability is to equilibrate an ensemble of capsules with a solute of interest and to quantitate either influx of the solute [204] or outflow of capsule-penetrated solute [205] as a function of time. The sensitivity of the solute assessment technique (radiotracer techniques are typically the most sensitive), as well as the capsule permeability, determines the optimum number of capsules required for the test. This method actually yields a mass transfer rate (U) for a particular solute [205], which is then used to calculate the membrane permeability, $P_m = U \cdot d$ (where d = average wall thickness), with the knowledge of capsule physical dimensions (diameter, wall thickness and surface area). The latter are typically taken from an 'average' capsule and a heterogeneity in capsule dimensions can influence

the calculated parameters. Significant differences among the capsule permeabilities (for example, > two-orders of magnitude for PE capsules [206], ~ one-order of magnitude for HEMA–MMA [205] and alginate capsules [207]) have been observed depending on encapsulation process, indicating permeability to be sensitive to process parameters and capable of being engineered. A method to improve the sensitivity of permeability assessment, and even to estimate the permeability of individual capsules, is to use enzymes such as horseradish peroxidase as the diffusing solute [208]. Enzymatic activity can be colorimetrically measured in an automated enzyme-linked immunosorbent assay (ELISA) format so that a large number of capsules may be screened to fully characterize the population characteristics. Enzyme activity per se should be ensured not to change during the measurements.

Assessment of MWCO is almost impossible in this way because of relatively slow permeation of the tracer molecules at the cut-off limit. Given sufficient time, a certain extent of penetration will be observed by almost any solute (at least through ‘defective’ capsules among the population). If this method will be utilized for MWCO determination, sufficient diffusion time should be allowed to determine the extent of penetration. Dextrans with a well controlled polydispersity and size range can serve as suitable solutes for the permeability studies [209]. A novel technique has been recently described by Brissova et al. [210] who utilized inverse size-exclusion chromatography (SEC) with a packed column of microcapsules (a modification of original technique described by Martinsen et al. [202]). This method appears to be amenable for automation and primarily determines the partition coefficient (K_{SEC}) for a solute between the capsule interior and exterior. The K_{SEC} is used to infer the pore size distribution in a population of capsules. A technique to measure the MWCO of an individual capsule is to assess the capsule permeation of fluorescent tracers with confocal microscopy, provided that the capsules are transparent [211]. The latter technique has been used to engineer the MWCO of alginate–PLL capsules [211]. Capsule permeation of IgG can be directly observed in this way [212].

An alternative technique for MWCO is to measure the permeation of proteins derived from encapsulated

cells. All cells typically release a range of proteins varying in M_r . Unlike relatively small proteins, larger proteins experience a sieving effect while permeating through a capsule wall. The secretion rate of a large protein with respect to a smaller protein can be then used to assess the MWCO limit. Halle et al. [213] employed the secretions of arginine esterase (M_r 30 000) and acid phosphatase (M_r 89 000) from encapsulated prostatic epithelial cells and have shown the release of the M_r 30 000 protein without the release of the M_r 89 000 protein. Uludag and Sefton [214] also observed a relatively higher secretion of a smaller protein α_1 -antitrypsin (M_r 52 000) with respect to larger fibrinogen (M_r ~ 340 000). A technical issue is the adsorption of a protein of interest onto the capsule membrane (which gives a lower apparent secretion rate) but this can be prevented by culturing the cells for an extended period to saturate the capsule membrane. A critical issue is the choice of appropriate controls. It is convenient to compare the relative secretion rate of encapsulated cells to unencapsulated cells, but the protein expression level of cells grown in a microcapsule might be different than the cells in culture. Both three-dimensional growth in capsules as well as compositional differences in the cell microenvironment might contribute to the difference in the relative protein secretion. Encapsulating cells without a permeability barrier (e.g., PLL in the case of alginate capsules) might be adequate to address the changes due to three-dimensional culture, but this cannot account of microenvironmental effects. A more representative picture can be obtained by assaying intracapsular concentration of a protein in addition to that of amount secreted extracapsularly. In the case of engineered cells entrapped in HEMA–MMA capsules [215], the relatively small human growth hormone (M_r 27 000) did not accumulate inside the capsules whereas larger proteins β -hexosaminidase (M_r 120 000) and β -glucuronidase (M_r 300 000) did with little permeation through the capsule wall. With HEMA–MMA and alginate–PLL capsules, capsule permeation of even very large proteins (e.g., fibrinogen of M_r 340 000 [214] and β -glucuronidase of M_r 300 000 [215,216]) was observed. The above assessment method typically utilizes an ensemble of capsules that may yield misleading results if a fraction of capsules are

‘defective’ (e.g., cells protruding from the capsule membrane [216]). By using ELISA techniques, it was possible to quantitate secretion from an individual capsule (Fig. 3) [208]. A heterogeneity in protein secretion was typically seen among the capsules. This approach allows one not only to obtain a measure of MWCO for individual capsules but also to determine the capsules that are permeable to high- M_r species, hence making it possible to eliminate undesired capsules. Variations in the MWCO can be used as a design parameter to reduce the fraction of defective capsules from an encapsulation process.

5.1.2. *Macrocapsules*

Unlike the membranes of microcapsules which were developed specifically for cell encapsulation, the membranes utilized in most macroencapsulation studies employ ‘off the shelf’ filtration materials that were developed for a variety of pressure-driven separations applications. As such the materials were engineered to operate under convective processes. The wall architecture of these ‘off the shelf’ membranes, whether flat sheet or hollow fiber, being engineered for sieving type separations have both the appropriate thickness and wall architecture to function under elevated transmembrane pressures. Typically the functional properties of these materials are characterized: by their relative porosity which is

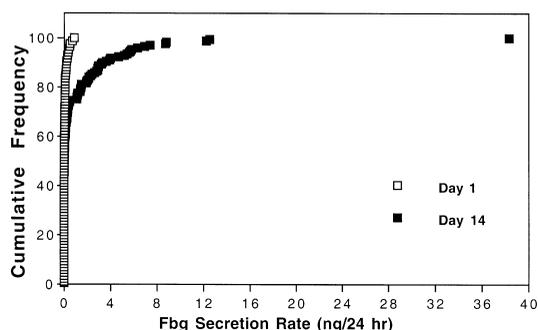


Fig. 3. Fibrinogen secretion from individual microcapsules. The data was obtained from HEMA–MMA microcapsules that were cultured for 1 and 14 days *in vitro*. Note that a significant fraction of capsules does not secrete a detectable amount of fibrinogen over a 24-h incubation period. A few capsules secrete an unproportionally high level of the protein. Such capsules might not be suitable for transplantation where a strict immunoprotective barrier needs to be maintained.

measured in terms of liquid flux rate or hydraulic permeability; and by their ability to retain molecules of particular sizes in convective sieving experiments with results expressed as nominal MWCO (nMWCO).

The term nMWCO has a different meaning depending on the encapsulation technique that can create ambiguity within the field. Within the field of microencapsulation, the term MWCO generally refers to the size of the largest molecule that is not substantially blocked by the semipermeable membrane. Molecules with M_r above the MWCO are substantially prevented from entering or leaving the microcapsule or gel particle. Within the field of macroencapsulation, the term nMWCO is derived from filtration industry terminology and used to describe the M_r where 90% of the molecules of a particular size class are rejected from transport through a membrane under convective or pressure-driven conditions. The nMWCO determination is generally performed at high pressures (typically 50–55 p.s.i.; 1 p.s.i. = 6894.76 Pa), and although this type of characterization can provide an approximate size of the molecules that pass through such membranes, the measurement provides little useful information regarding the rate or selectivity of mass transport under diffusive conditions. Since macroencapsulation devices are diffusion based, it is essential to know the diffusive transport properties of such membranes in order to optimize the engineering of such devices. Interestingly, of 66 macroencapsulation studies reviewed for this manuscript only 14% of the studies include any form of transport characterization [12,30,217–230]. Forty-two of the studies used a PAN–PVC type membrane. Half of the reviewed studies cite only the manufacturers nMWCO to describe membrane transport function, while a third contain no information regarding the transport characteristics of the membranes used in the study. To the best of our knowledge, only one study examined membrane transport characteristics following implantation *in vivo* with no apparent change in convective transport function [220].

Several groups have described methods to characterize transport of macroencapsulation membranes and several theoretical models have been developed to optimize device design [216–220]. One of the limitations in the development of such models is the

lack of quantitative data on the diffusive transport properties of macroencapsulation membranes. It is clear that a detailed understanding of mass transport is necessary to move the field of macroencapsulation from empirical or phenomenological observation to engineering dominated device design.

In general, hydraulic permeability assessment is conducted using high quality water (triple distilled, 0.2 μm filtered). A test system that employs a controllable pump, membrane holder, pressure transducers, and balance to measure flow is generally used. Two flow configurations can be used: (1) an open-ended configuration in which a portion of the flow stream is shunted across the membrane by using a restriction valve to establish a transmembrane pressure, or (2) a dead-ended flow configuration where all of the flow is forced across the membrane at a known pressure. In general, the transmembrane pressure is maintained at low values to minimize membrane deformation. Hydraulic permeability results are reported in units of flow per unit surface area per pressure unit or $[(\text{ml})/(\text{min m}^2 \text{ mmHg})]$.

Typically, convective solute transport (CSF) and diffusive solute transport (DSF) analyses are carried out using either fluorescein isothiocyanate (FITC)-labeled dextrans or labeled proteins with M_r values that range from 4 to $2 \cdot 10^6$ kDa using phosphate-buffered saline (PBS) as the mobile phase [30,218,220]. CSF tests are conducted in an open-ended flow configuration using intraluminal stream for the introduction of labeled test probe solutions. Labeled test probes are applied sequentially from the smallest M_r to the largest M_r probe. Between each test probe run PBS is pumped through the system to clean the feed stream until a baseline level of HP is achieved. CSF results are reported as either a sieving coefficient or as a rejection coefficient.

The DSF tests are performed using either a counter-current flow configuration where the labeled-test probes are introduced in the intraluminal flow path or using a static stirred cell [218]. The probes are dissolved in a PBS solution and either pumped through the lumen of the encapsulation chamber or loaded by injection. A PBS solution is pumped through the extraluminal compartment of the membrane housing in a direction opposite to the direction of the intraluminal flow stream or in the stirred cell method the extraluminal compartment is mixed by

constant stirring. In the counter current method the transmembrane pressure is adjusted to 'zero' condition, to obtain no water transport across the membrane. Solute concentration is monitored by a fluorescent detector and the results for the DSF test is reported in (cm/s). Both of these methods are not without technical difficulties and it is necessary to account for membrane boundary effects, variations in flow rates during characterization tests, and changes in solute concentrations as function of axial and radial position [217]. Refinements in diffusive transport characterization will improve the engineering of such devices.

5.2. Mechanical properties

The assessment of capsule mechanical properties is important, not only to determine the durability of capsules during production and handling, but also as an indication of the capsule membrane integrity. The latter is most informative when time-course studies are performed. Even though mechanical properties of capsules has been recognized as a limiting factor for in vivo applications [231] and numerous studies have been carried out to improve the capsule strength [79,232,237], quantitative values for capsule strength has been reported in only a handful of cases [79]. In some studies, the thickness of the capsule membrane has been engineered and used as a measure of capsule strength [238,239]. Most of the work in this area has been preformed using microencapsulated cells.

A simple way to test the durability of microcapsules is to subject the capsule population to a well-defined shear-flow. The fraction of 'failed' capsules can be used as a simple measure of mechanical durability [232,234]. The mechanical stress experienced by the capsule will depend on the shear rate as well as the viscosity of the fluid in this system. Although a visual failure criteria may be employed, the precision of the method can be improved by using a marker whose extracapsular leakage may be interpreted as a quantitative measure of capsule failure [233]. Despite its simplicity, the described method can be used as a screening tool to implement process improvements or design capsules with improved mechanical properties. For example, coating time [235], extent of surface modification

[236], or the choice of polymeric additives to capsule components [237] were optimized based on this evaluation criteria.

A more sophisticated approach is to measure the failure of capsules under uniaxial loading. It has been possible to perform this on individual capsules [79]. An order of magnitude variations in the capsule rupture force was observed by this method [79]. Two practical aspects that requires special attention are the speed at which the uniaxial loading is performed (since capsules may undergo plastic deformation to accommodate the applied pressure) and the ability to detect failure. Visual observation, although convenient, is likely to detect failure after the fact. The rupture force depends on membrane strength, thickness, as well as the capsule core properties (such as viscosity), but no information is available about the relative contributions of each component to the overall strength of a microcapsule. Anecdotal evidence suggests that the core strength is at least as much important as the membrane strength for alginate–PLL capsules [234].

5.3. Immune protection

The assessment of immune protective ability by a capsule is critical for any cell encapsulation technology designed for tissue transplantation. Unlike permeability and mechanical assessment, techniques to evaluate immune protection are common for both microencapsulated and macroencapsulated tissues. The requirements of a membrane for immune protection are related to its diffusive permeability and will be primary dependent on the host–donor mismatch: allogeneic vs. xenogeneic [240,241]. For an allogeneic tissue, where graft-reactive immunoglobulins are absent and direct pathway of self–nonself discrimination is dominant [31], the access of the host's immune reactive cells to the graft tissue will need to be prevented. In other words, the membrane has to act as a cell barrier. The indirect pathway, where shed antigens from the graft stimulate systemic CD4 T-cells [242], is secondary and less significant for allografts. In practice, however, the host's immune cells are bound to be exposed to the encapsulated cells due to unavoidable failure of a certain fraction of capsules (especially the case for microcapsules). The direct pathway can be activated

in this case, resulting in a clonal expansion of graft-specific T-cells. It is not clear how such an activated cell population will affect the allograft tissue in intact capsules. One concern will be the local production of inflammatory, cytotoxic agents such as IL-1 β , IFN γ and TNF α . One can measure the cytotoxicity of such agents against encapsulated cells using in vitro cell culture techniques [243,244], although two critical parameters, namely the duration and dose of cytokine exposure, will be critical to mimic in vivo conditions. The complement can also be activated by contact with capsules [245] and it is important to assess whether the activated complement results in a significant encapsulated cell lysis [245]. Therefore, if direct pathway of immune recognition or complement is activated by inadequate capsule properties, the capsules designed for allogeneic implants will need to meet more stringent permeability requirements. For xenogeneic tissues, the membrane transport of xenoreactive antibodies (either pre-existing or raised after engraftment) and complement components, which appear to be cytotoxic to xenogeneic tissues even in the absence of immunoglobulins [246], will obviously need to be prevented.

Diffusive permeability to antibodies is straight forward to evaluate, for example, by the methods described in Section 5.1. It is preferable to create an antibody sink within the capsules during this test (e.g., by using protein A beads [247], or tissues that are specific for the chosen antibody [215]) to enhance the driving force for transport. Membrane permeation of large molecules is expected to be slow and assessment of immune protection under facilitated diffusion conditions for cytotoxic agents provides an additional stringency to the test. The permeation of individual complement proteins can be similarly assessed using purified proteins. However, it might be more preferable to assess the permeation of complement 'activity'. This can be easily achieved in culture in the presence of antibodies reactive to encapsulated cells [248,249]. Care should be taken to minimize any changes in the complement activity per se since complement activity is lost within 1–5 days in culture [248]. Protection against cellular components of an immune system can also be performed by using a mixed lymphocyte culture with encapsulated cells [250]. It is possible to

quantitate, for example, cellular proliferation [250] or cytokine production profiles even in the absence of direct cell–cell contact (host response to xenogeneic tissues will be stronger in this test compared to an allogeneic response). A combination of these *in vitro* tests will not only allow the design of appropriate capsules, but also may help to elucidate the relative potency of different immune response pathways against encapsulated cells.

Nevertheless, the ultimate assessment of immune protection will come from *in vivo* studies. A correlation between *in vivo* and *in vitro* results are beneficial to assess the relative importance of a particular *in vitro* technique. For example, although IgG permeation into agarose [248] and alginate [251] capsules was observed (which might lead one to further engineer the capsule MWCO to exclude this protein), these IgG-permeable capsules were xenoprotective *in vivo* [248,251]. Only *in vivo* assessment can shed light on the relative importance of different *in vitro* observations.

5.4. *Biocompatibility assessment: host response*

Biocompatibility is defined as the ability of a biomaterial to perform with an appropriate host response in a ‘specific application’ [252]. This is easy to interpret with the application of conventional artificial organs such as artificial hips, breasts or knees. With bioartificial systems such as the encapsulated cells it is far from simple to interpret. Usually, a fully biocompatible system is considered to be a system manufactured of membranes which elicit no or not more than a minimal foreign body reaction. The host response is a potentially serious problem to the clinical implementation of the technology. The direct consequence of a non-biocompatible membrane is the fibrotic overgrowth on the surface that interferes with diffusive transport of molecules and oxygenated blood supply [48,63,64]. The size of the device, the outer morphology of the capsule, the materials comprising the macroencapsulation device, shed contents of the encapsulation chamber, and the surgical implantation method are believed to be the major parameters that influence the host reaction or biocompatibility to such implanted devices. In almost all cases, host tissue response to the surgical implantation of an encapsulation device is generally thought to limit the performance of the device. The

host reaction is similar to that observed following the implantation of any biomedical device [253,254]. The response varies in degree and in the specific cell types involved depending upon the site of implantation. In general, planar devices are implanted within the abdominal cavity or subcutaneously, while cylindrical or hollow fiber-based devices have been implanted into the brain, the epidural space or under the skin. Upon insertion of the device, the host response is initiated by an acute inflammatory reaction caused by the disruption of host vasculature. Activated platelets, polymorphonuclear leukocytes, humoral components of serum, clot constituents, cell debris, and extracellular matrix are initially present at the host material interface. Tissue macrophages are recruited to the site and mediate the process of clean-up and initial wound healing. Finally, mesenchymally-derived cells mediate matrix production and contracture coupled with a neovascularization response which rounds out the process. The last phase of the reaction is mediated by migratory cells that are primarily fibroblastic and in the case of CNS implants include microglia and astrocytes. In the end, a parameter-sensitive, variable reaction ensheathes the implant within a dense capsule of fibrous matrix components and various glycosaminoglycans which may or may not contain contracted cells specific to the particular implantation site. The inner reactive zone then gives way to a layer of loose connective tissue where new blood vessel formation has occurred. Systematic studies of the host reaction have been conducted with PAN–PVC, PTFE, cellulose acetate, acrylic copolymers, and mixed cellulose esters [255–257]. In the best cases, the host reaction to macroencapsulation membranes is minimal and can be confined to a few layers of reactive cells [255]. However, to the best of our knowledge there has not been a publication that describes a controlled study to determine the relative influence of the aforementioned parameters on the host reaction.

The specific response of the host tissue, including the size of the reactive zone, the arrangement and the availability of vasculature, and the parenchymal/matrix composition determine the clinical utility of such devices including sustainable biomass, therapeutic flux, and retrievability. A number of studies have reported that the size and the extent of the host reaction can be reduced by the local administration of steroidal as well as non-steroidal anti-inflamma-

tory agents (NSAIDs) [258,259]. Agents such as dexamethasone and naproxen (aleve, anaprox or Naprosyn) can inhibit the fibrotic reaction elicited following the implantation of a device. The use of NSAIDs may become more common as their application is not associated with the undesirable effects which accompany the use of other immunosuppressive agents.

In addition to the interaction between the biomaterials and the host tissue, a significant interaction is the one between the biomaterial and the encapsulated donor tissue. The capsule membrane and ECM in the capsule core are two materials with which the encapsulated tissue will interact. Recently, De Vos et al. [57,68] and others [260,261] have shown that encapsulation directly interferes with the function and the viability of the encapsulated tissue. Although, this aspect is not covered by the current definition of biocompatibility, it should be considered a true biocompatibility issue since long-term survival of the tissue is required for almost all applications.

An important aspect of the biocompatibility assessment is the production of cytotoxic entities by the host cells in response to capsule material. The capsule material can present itself as a solid matrix with which host cells directly interact or as diffusible degradation products. Detailed studies on the nature of cytotoxic molecules were carried out in the context of pancreatic islets and they include small reactive species, such as oxygen radicals and aldehydes [262], as well as proteinous cytokines [263]. The small reactive species may not be a major concern since they will be produced at a distance from encapsulated cells and it is likely that they may be dissipated before reaching the cells. It is not known whether such reactive species can alter the membrane structure. Cytokines, on the other hand, are stable and are likely to permeate through the capsule membrane. Recent reviews have addressed the biomaterial-dependent cytokine production in vitro [253,264]. Assessment of cytokine production can be performed by macrophages from peritoneal space [265,266], a commonly used site for capsule implantation, and mononuclear cells from blood [267]. The choice of macrophages is justified by the fact that it is the major cell type involved in the cellular response at peritoneal site [265] and epididymal fat pads [268]. But other cell types, such as

neutrophils and lymphocytes are also activated and they might elevate the local concentration of a unique set of cytokines. Alginates rich in M and G regions can be differentiated based on quantitative difference in proinflammatory cytokine production [267], the latter being less inducive of proinflammatory cytokine production [267]. Polyacrylates, however, with different functional groups exhibited a different cytokine response in the hands of different investigators [266,269]. It is likely that the differences in the cell culture or the cytokine assay (e.g., bioassay vs. ELISA) will give such a difference. The materials response was typically much less than the response elicited by lipopolysaccharide. Several variables are obviously different between a solid polymer and a soluble lipopolysaccharide but, if 'diffusibility' is an important factor in cytokine response, it might be more informative to determine the cytokine response to degradation products of a biomaterial, rather than to the solid biomaterial itself.

To elucidate the relative importance of cytokine response, it is necessary to correlate in vitro observations with the actual in vivo cytokine response. Quantitating the protein concentration at the implant site is difficult and is associated with variations due to extraction process. An alternative approach is to quantify the expression level of the cytokines using an in-situ hybridization method [270], or a reverse transcriptase–polymerase chain reaction assay [271]. The latter assay is attractive because of its semi-quantitative nature. It was used to investigate TGF- β (a potent fibrosis-promoting protein) expression at the capsule implantation site. It will be important to utilize this technique to evaluate the effect of capsule parameters in cytokine production as well as the time-course and spectrum of cytokine production. It will also be important to demonstrate that gene expression results in a functional response; i.e., the levels of cytokine expression is correlated with local cytokine levels or with the observed fibrotic response. Otherwise intracellular levels of mRNA may not be a 'functional' response.

Neovascularization is another critical process determining the success of encapsulation therapy. A number of studies have indicated that the outer microarchitecture of the encapsulation membrane and not necessarily the membrane surface chemistry exerts a profound influence on the neovascularization response [240,256,257,272]. Membranes with sur-

face pores that allowed host cell colonization without inducing significant cell spreading, in general, resulted in the formation of vascular structures very near the host–material interface. Encapsulation devices using such a design were capable of supporting high cell densities. The important discovery was achieved using microporous encapsulation membranes that were ineffective at protecting xenografts from rejection without immunosuppression. The discovery inspired the development of a planar immunoisolation device composed of a bilaminar or composite architecture with an outer shell capable of microarchitecture-driven neovascularization and an inner membrane that was used for either physical isolation or immunoshielding. The use of an inner microporous membrane allowed high-cell density transplantation of allografts, whereas the use of an inner ultrafiltration membrane allowed lower-cell density transplantation of xenografts. The results of the study indicate that the diffusive flux of the barrier and not the closeness of the host vasculature determine the sustainable cellular density within the encapsulation chamber.

The presence of a high degree of blood vessels near the encapsulation membrane may not necessarily be a desirable outcome. For instance, in clinical situations where one seeks to achieve sustained release of a potent bioactive agent for local application, it may be disadvantageous to disperse or dilute the released agent in the systemic circulation. Consider the application of encapsulated growth factor releasing cells for use in regenerating a specific nerve tract or providing trophic support for a deep brain site. In these cases, one may desire to retain the agent at a high local extracellular concentration. In certain applications, the therapy may only be needed for a short period, after which time it may be desirable to remove or retrieve the implant. In such an instance, a high degree of host tissue integration and neovascularization may complicate the retrieval process.

6. Future considerations

Since the early pioneering period, the technology of mammalian cell encapsulation has developed significantly. We reviewed several approaches to

achieve cell encapsulation and possibly to enhance the efficiency and long-term utility of this technology. During recent years, there has been a large increase in the published studies using mammalian encapsulation methods as academic and industrial laboratories drive toward clinical implementation of the technology. In the field of microencapsulation, many groups have enhanced the technology using alginate-based microcapsules by a stepwise analysis of the factors determining success or failure. As a consequence, tremendous advances have been made in the technology of alginate-based cell encapsulation. The choice of the specific alginate and PLL biomaterials, as well as the particulars of the process variables were shown to significantly influence the final microcapsule properties. Present insights emphasize the need not only to focus on the host response towards the biomaterial but also on the interaction between the donor tissue and the biomaterial. It appears mandatory to find or create a transplantation site which offers a more direct contact between the capsule and the recipient's bloodstream in order to improve the functional performance and survival of cells to a duration sufficient for setting up immunoisolated cell transplantation in man. Conceivable approaches include the use of smaller capsules which may enable the use of other sites than the peritoneal cavity, as well as creation of a site which can carry a large volume of an encapsulated graft in the immediate vicinity of the bloodstream. Recently, the concept of a highly vascularized artificial transplantation site for encapsulated islets was introduced in the peritoneal cavity [273] which not only allows for closer contact with the bloodstream but also allows for retrieval of the graft in case of failure. A greater range of optimized cells should be available in the near future as genetic engineering technology improves. Cells could be engineered for better survival in a permselective environment. Regulatable promoters may allow dose adjustment by either changing the gene expression or the number of cells within the devices.

Biomaterials with improved properties are also expected to play a vital role in future progress. The intrinsic biomaterial properties are commonly believed to control the host response for a desired outcome. A better understanding of the host response will be achieved not only by utilizing novel, en-

gineered materials but also by performing a better characterization of the prepared capsules. An urgent need is a thorough capsule characterization from multiple perspectives instead of only focusing on selected aspects. Biomaterials that exhibit a sol–gel transition under a controlled environment are promising candidates to augment the present encapsulation technologies [274]. Such biomaterials may serve as novel carriers of therapeutic cells during encapsulation process or while cell introduction into or retrieval from a macrocapsule. They could be additionally utilized as novel extracellular substrates for cell growth after incorporating moieties specific for cell interactions [275]. Most encapsulation technologies have focused on a single biomaterial to form the permselective membrane since analysis of capsule performance is simplified in this way. However, it might be possible to use blends of different biomaterials to optimize the properties of ‘a biomaterial’ (such as viscosity, density and hydrophobicity) for encapsulation [276]. Blending may be rapid way to identify suitable physicochemical properties and ultimately to formulate such properties into a single biomaterial. Finally, demands on the properties of biomaterials and capsules are likely to be different depending on the particular clinical application. A better understanding of biomaterial effects on capsule properties, combined with a better control of capsule properties during fabrication process will accelerate the transformation of a promising encapsulation technology into clinical reality.

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