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Polymer science for macroencapsulation of cells for central nervous system transplantation

Frank T. Gentile *, Edward J. Doherty, David H. Rein, Molly S. Shoichet, Shelley R. Winn

CytoTherapeutics, Inc., 2 Richmond Square, Providence, RI 02906, USA

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Abstract

The goal of encapsulated cell therapy research is to develop implants containing living xenogeneic cells to treat serious and disabling human conditions. The enabling concept is straightforward: cells or small clusters of tissue are surrounded by a selective membrane barrier which admits oxygen and required metabolites, releases bioactive cell secretions but restricts the transport of the larger cytotoxic agents of the body's immune defense system. Use of a selective membrane both eliminates the need for chronic immunosuppression in the host and allows cells to be obtained from non-human sources, thus avoiding the cell-sourcing constraints which have limited the clinical application of general successful investigative trials of *unencapsulated* cell transplantation for chronic pain, Parkinson's disease, and type I diabetes. Target applications for encapsulated cell therapy include these same disorders as well as other disabilities caused by loss of secretory cell function which cannot be adequately treated by current organ transplantation or drug therapies and conditions potentially capable of responding to local sustained delivery of growth factors and other biologic response modifiers. Several types of device configurations are possible. Here we focus on easily retrieved, non-vascularized, macrocapsules. Such devices have four basic components: a hollow fiber or flat sheet membrane (usually thermoplastic based), cells (primary or dividing), and extracellular matrix (natural or synthetic) to promote cell viability and function, and other device components such as seals, tethers and radio-opaque markers. Choice of membrane and extracellular matrix polymers as well as issues surrounding implantation and biocompatibility evaluation are complex, inter-related, and ultimately driven by implantation site and delivery requirements. Cross species immunoisolated cell therapy has been validated small and large animal models of chronic pain, Parkinson's disease, and type 1 diabetes and is under active investigation by several groups in animal models of Huntington's, Hemophilia, Alzheimer's, ALS, and other CNS disorders.

Keywords: Biomaterials; Cell encapsulation; Extracellular matrices; Membranes; Transplantation

1. Introduction

The term *biomaterials* encompasses all materials used for medical applications. This term includes materials that are in direct contact with living systems (intracorporeal) as well as systems whose function is outside the body (extracorporeal). Biomedical materials include metals, ceramics, natural polymers (biopolymers), and synthetic polymers [1]. The terms biodegradable, bioerodible, bioacceptable and biocompatible are associated with many of these materials. For the purpose of this review, the term biocompatible will deal with the quality of the response

* Corresponding author.

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of the host tissue to an implanted material while the terms biodegradable or bioerodible will deal with materials which are designed to last only a specific time after implantation.

Much of biomaterials research stems from major efforts to investigate clotting phenomena related to the response of blood in contact with polymeric surfaces (originally for dialysis membranes and other extracorporeal circuits), and to develop systems with non-thrombogenic behavior in short- and long-term applications. These systems can be used as implants or replacements, and they include artificial hearts, lung oxygenators, hemodialysis systems, artificial blood vessels, small ($\leq$4 mm in diameter) and large (>4 mm) vascular grafts, artificial skin, wound healing agents, catheters, and some drug-delivery systems. These uses encompass the majority of research into the areas of biomaterials.

Recently, more attention has been focused on a new class of biomaterials used in tissue engineering: cell-containing artificial organs, based upon diffusion of key substances, which are either directly connected to the vascular system or implanted in tissue (e.g. subcutaneous or interparenchymal) or non-blood fluid (e.g. cerebral spinal fluid (CSF) or intraperitoneal fluid).

Much of the general biomaterial criteria developed over the last two decades [1,2] can be applied to this new area. Issues involved when choosing biomaterials for cell transplantation are: (1) chemical composition and chemical modification; (2) physical properties such as surface morphology, mechanical strength, and stability; (3) toxicology testing such as local tissue response, systemic responses, pyrogenicity, carcinogenicity and teratogenicity (see also Food and Drug Administration guidelines such as Tripartite testing [3]); (4) manufacturing; and (5) sterilization. These issues, as they relate to bio-artificial organs, are illustrated in Table 1.

Table 1
Major tests of physical properties for materials to be used for bio-artificial organs

General in vivo material properties
Non-toxic
Non-carcinogenic
Non-teratological
Sterilizable
Non-biodegradable (unless desired)
Not induce inflammatory reactions
Not cause thrombosis
Not alter the stability of biological fluids
Membrane properties
Biocompatibility
Protein adsorption
Stability of transport properties
Satisfactory mechanical properties in tension, compression, and shear
Processable in a wide range of geometries and morphologies
Matrix properties
Chemical stability
Lack of toxicity to the cells
Increases cell product output
Stabilizes cells
Allows easy refilling
Device material properties
Provide reproducible seals and easy closure to the device
Seals are no more permeable than the membrane material and testable prior to loading with cells
Materials are non-toxic to cells or host
Materials do not cause damage to the membrane when attached
Satisfactory mechanical properties in tension, compression, and shear
Allows the incorporation of a radio-opaque marker

1.1. Delivery of active substances to specific sites

A growing area of interest for implantable biomaterials is the ability to deliver active substances to specific sites in vivo. Traditionally this area has been dominated by degradable and non-degradable polymer capsules containing one or more drugs. The substances are mixed in with a polymer matrix during fabrication and then released over time through the material or as the material degrades. Proper control of the release kinetics is important. One example is the zero order release kinetics achieved in ethylene–vinyl acetate copolymer (EVAC) rods or patches [4,5].

Another category of delivery systems encompasses indwelling catheters or pumps. In most cases, the active agent is stored ex vivo and delivered to the in vivo location through the implanted system. In other cases, a depot or

reservoir of the agent is implanted in the subcutaneous site for easy access.

The type of delivery system that researchers have most recently focused on for delivery of active substances to specific sites are bio-artificial organs comprised of a membrane and a cellular component encapsulated within the membrane.

The goal of encapsulated cell therapy research is to develop implants containing living xenogeneic or allogeneic cells to treat serious and disabling human conditions. The enabling concept is straightforward: cells or small clusters of tissue are surrounded by a selective membrane barrier which allows unhindered passage of oxygen and other required metabolites, releases bioactive cell secretions, but restricts the transport of the larger cytotoxic agents of the body's immune defense system. The use of selectively permeable membranes both eliminates the need for chronic immunosuppression in the host and allows cells to be obtained from non-human sources, thus avoiding the cell-sourcing constraints which have limited the clinical application of successful investigative trials of *unencapsulated* cell transplantation.

Target applications for encapsulated cell therapy include chronic pain, Parkinson's disease and type I diabetes as well as other disabilities caused by loss of secretory cell function which cannot be adequately treated by current organ transplantation or drug therapies. In addition, conditions potentially capable of responding to locally sustained delivery of growth factors and other biologic response modifiers can be treated with this approach. Cross-species immuno-isolated cell therapy has been validated in small and large animal models of chronic pain [6], Parkinson's disease [7], type I diabetes [8–13] and acute liver failure (extracorporeally) and is under active investigation by several groups in animal models of Huntington's disease [14], hemophilia [15], Alzheimer's disease [16–18], amyotrophic lateral sclerosis (ALS) [19] and epilepsy. Of these, chronic pain and acute liver failure are the closest to human clinical products. Chronic pain has been treated in humans using intrathecal implantation of xenogeneic adrenal chromaffin cells which secrete catecholamines and enkephalins in humans. This trial represents the record for xenograft function in humans (40–160 days). It is important to note that no immunosuppressive agents were administered [20].

Much attention has been focused on cell transplantation in the central nervous system (CNS). The use of this approach in both the parenchymal and intrathecal spaces (lateral ventricle and lumbar intrathecal) has particular strong advantages. First, it provides site-specific delivery of various agents. Many of the factors of interest do not cross the blood–brain barrier (e.g. dopamine for Parkinson's patients) or have a relatively short half-life (e.g. dopamine and ciliary neurotrophic factor (CNTF), for ALS patients). Second, encapsulation provides the added safety benefit of being, in some forms, retrievable. Table 2 shows the patient population for some of the CNS disorders for which delivery of various factors may be possible through encapsulated cells).

Encapsulation of tissues has generally taken two forms: microencapsulation, where one or several cells are encapsulated in many spherical dispersions (100–300 μm in diameter) and macroencapsulation (intravascular and extravas-

Table 2
Prevalence of central nervous system disorders which may be treated via encapsulated cells [a]

Indication	Prevalence (U.S. only) [b]
Dementias	4,000,000–5,000,000
(Alzheimer's disease)	(3,000,000–4,000,000)
Parkinson's disease	800,000
Amyotrophic lateral sclerosis (ALS)	25,000
Huntington's disease	12,000–25,000
Brain tumor (all types)	1,000,000
Chronic pain	600,000
Multiple sclerosis	200,000–500,000
Epilepsy	2,000,000

[a] Adapted from Swen et al. [21].
[b] Sources: National Parkinson Foundation, Parkinson's Disease Foundation, National Information Center for Orphan Drugs and Rare Diseases (ODPHP), National Health Information Center, and National Institute of Neurological Disorders and Statistics.

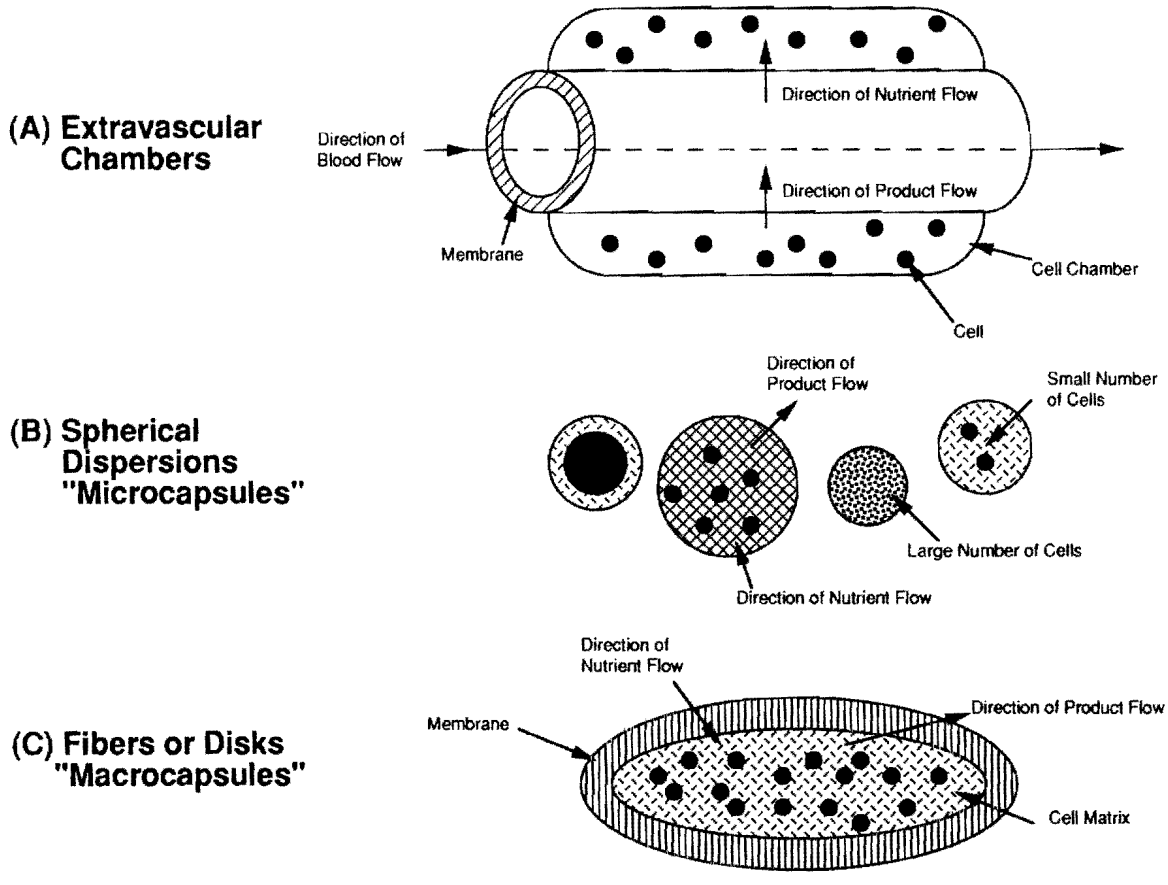

Fig. 1. Classes of immuno-isolatory vehicles. A: extravascular chambers. These chambers are directly connected into the vascular system. Flow is through the hollow fiber(s) and the cells are located in a sealed compartment around the fibers. One or more chambers are required. B: spherical dispersions (microcapsules). One cell cluster or a small number of cells or cell clusters are surrounded by hydrogel forming polymers (crosslinked or not). Selectivity is achieved by tightly crosslinking the hydrogel or by binding other polymers to the hydrogel surface. C: macrocapsules. Large numbers of cells or cell clusters are encapsulated within the lumens of hollow fibers or between two flat-sheet membranes. Disks or rods are implanted directly into tissue or into non-blood fluids.

cular), where large numbers of cells or cell clusters are transplanted in one or several relatively large capsules (for hollow fibers, typical dimensions are 0.5–6 mm in diameter with a total length of 0.5–10 cm). Advantages of the latter approach include better mechanical and chemical stability and ease of retrievability if warranted or desired. See Fig. 1 for a schematic view of these devices.

Table 3 summarizes the advantages and disadvantages of these systems along with other delivery technologies. The main advantages of erodible drug-delivery systems or depot systems such as catheters or pumps, are the relative ease of the surgical procedure and the high control over dose, especially over the short term. Where cell delivery systems, especially macroencapsulation systems, seem to have an advantage are in 4 basic areas, namely: (1) when the factor of interest needs to be delivered over long periods of time and has a very short half-life; (2) when steady-state release of this factor over the time period is important; (3) when the factor needs to be delivered in the CNS, especially beyond the blood–brain barrier; and (4) when quick termination of treatment or easy retrieval of the

Table 3
Advantages and disadvantages of implantable delivery systems

System	Main advantages	Main disadvantages
Non-cell based delivery		
Erodible drug Delivery systems	Single surgical procedure Relatively good release in if needed in short term	Not easily retrieved Poor long-term release kinetics Factor may degrade
Non-erodible drug Delivery systems	Retrievable Relatively good release in short term	Poor long-term release kinetics Factor may degrade
Catheters/pumps	Quick delivery of factor Retrievable	Prone to infections External depot not very comfortable to patient Factor may degrade
Cell-based delivery		
General	Cells constitutively produce active factor(s) Xenogeneic or engineered cells available	More complex regulatory issues
Microcapsules	Large number of cells transplanted with small volume Relatively good biocompatibility Easy implantation	Fragile (degrade) Not easily retrieved Problematic in CSF fluid
Macrocapsules	Large number of cells in single device Mechanically strong Easily retrieved Easily implanted Good biocompatibility	All cells in one device (a problem in device failure — but can be overcome using more than one device)

device is warranted or desired. Based upon these observations, it seems clear that the use of encapsulated cells has promise for the treatment of severe disorders due to secretory cell dysfunction. Each disease state may require different macroencapsulation systems. Choice of proper materials and cells for each disease is critical for the success of each application.

2. Polymeric membrane properties and fabrication

2.1. Structure–property relationships for polymers used in macroencapsulation

Three basic classes of materials are required for the production of membrane-based artificial organs: (1) the membrane material; (2) device components, i.e. materials used to create the 'device' such as seals, radio-opaque markers, tethers, sutures or other fasteners; and (3) materials used within the membrane as a scaffold or immobilizing agent for the cells (i.e. a cell matrix).

It is important that the membrane material and selective membrane properties stay within an appropriate range over time. Membrane degradation involves the changing of both the physical and transport properties over time due to its interaction with the in vivo environment. The main properties that can be changed are the pore sizes (what can get through and what cannot) and the diffusive mass transfer coefficients (how fast things can get through the membrane).

A wide variety of membrane materials can be used for these types of artificial organs. One of the more extensively used materials for this application is poly(acrylonitrile–co-vinyl chloride) (P(AN–VC)), a statistical copolymer made from acrylonitrile and vinyl chloride monomers, used in artificial organs for the following reasons:

- history in medical applications (used for hemofiltration);

- long history in research application for artificial organs;
- in vivo stability;
- no inflammatory tissue response (when properly prepared) in multiple implant sites;
- can be manufactured by a phase-inversion process;
- membranes with appropriate transport processes have sufficient mechanical strength for most implantation and explantation procedures;
- the acrylonitrile (AN) monomeric units on the chain give P(AN–VC) membranes a degree of hydrophilicity not found in most thermoplastic materials (thus, P(AN–VC) membranes do not adsorb as much protein as other materials.

Other candidate materials include poly (acrylonitrile) (PAN), poly(sulfone) (PS), poly (ether sulfone) (PES), poly(vinylidine difluoride) (PVDF), poly(amides), poly(carbonate) (PC), poly(ether imides), poly(propylene) and poly (ethylene). The following sections will cover in more detail the issues involved in the use of membranes, extracellular matrices, and in vivo evaluation of these materials.

2.2. Membrane preparation for macroencapsulation

The physical make-up of membranes used in macroencapsulation is determined by: (1) the metabolic requirements of the cells to be encapsulated; (2) the size of the therapeutic substance(s) to be released; (3) the degree of required immunoprotection; and (4) adequate tissue biocompatibility. The most critical transport properties of membrane are determined by the metabolic requirements of the encapsulated cell. The membrane must have sufficient passage of nutrients for encapsulated cells to remain viable and functional. While maintaining cell function, the membrane must also have pores that are large enough to allow the therapeutic agent free passage to the target site. If the encapsulated cells require immunoprotection the membranes must reject the entrance of immunological elements into the capsule. In addition, membrane morphology has a strong impact on the biocompatibility at the host/membrane interface. The transport properties and external morphologies can be manipulated using techniques described later.

Semipermeable membranes have been used extensively in studies of macroencapsulated insulin-secreting cells for the treatment of type I diabetes. Semipermeable phase-inversion membranes fabricated using a P(AN–VC) polymer have been used to encapsulate insulin secreting cells by Scharp et al. [11,13], Hegre et al. [22], and Altman et al. [23]. The technique employed by these groups involves placing the islets of Langerhans containing insulin-secreting β-cells in a sealed hollow fiber membrane which allows the insulin to be secreted via a glucose stimulus while maintaining cell viability by oxygen and nutrients diffusing into the implant.

Similar approaches to the encapsulation of insulin-secreting cells have been undertaken using microporous membranes fabricated with poly(urethane) [24] and poly(2-hydroxyethyl methacrylate) (PHEMA) [25]. The poly(urethane) membrane was formed by dissolution of an entrapped pore-former, whereas the PHEMA membrane was formed by a crosslinking agent.

Macroencapsulation of islets has also been studied in devices that are intravascular, whether implanted or extracorporeal. Although still diffusion based, a connective component may also be present in this system (e.g. Colton et al. [26] and Catapano et al. [27] have argued that Starling flow is possible in these devices). The design of these devices places the cells around the membrane and blood is passed through the membrane lumen. The P(AN–VC) copolymer have been used in studies involving multiple fiber cartridges, primarily by Chick et al. [8]. Other membrane materials such as semipermeable PS membranes have been evaluated by Sun et al. [28,29]. Segawa et al. [30] have also used multiple fiber devices made with poly(vinyl alcohol) to macroencapsulate insulin-secreting cells.

Sullivan et al. [31] have used a similar approach with a single large-bore membrane in an implantable artificial pancreas through which blood is passed. Single-fiber devices made of poly (amide) semipermeable membranes have also been used by Catapano et al. [27] in this system.

Other endocrine cells besides the islets of Langerhans have been studied to treat hypoparathyroidism [32] using immunoprotective P(AN–VC) membranes. Immunodeficiencies of the thymus by Christenson et al. [33] have been evaluated with a similar membrane.

Microporous PS fiber bundles have been used as extracorporeal devices when seeded with hepatocytes for use as a liver assist device [34]. Heparinized blood was pumped through the cartridge to increase the removal of harmful metabolites. Macrocapsules have also been used for the treatment of growth deficiencies [35] and for chronic pain [6,19,20].

Macroencapsulation of cells for the treatment of neurodegenerative diseases such as Parkinson's disease in rodents and non-human primates has been studied by Aebischer et al. [7,36] who used P(AN–VC) as the membrane material. Encapsulation and transplantation of cells that secrete nerve growth factor (NGF) by Hoffman et al. [16] and Winn et al. [18] in the fimbria-fornix lesioned rat have demonstrated survival of basal forebrain cholinergic neurons whose loss is associated with learning and memory disabilities in Alzheimer's disease.

2.3. Membrane manufacturing techniques

Phase inversion

The majority of thermoplastic ultrafiltration (UF) and microfiltration (MF) membranes used to encapsulate cells are manufactured from homogenous polymer solutions by phase inversion. UF membranes have pore sizes ranging from 5 nm to 0.1 μm, while MF (or microporous) membranes have pores ranging from 0.5 to 3 μm. Phase inversion is a very versatile technique allowing for the formation of membranes with a wide variety of nominal molecular weight cut-offs, permeabilities and morphologies [37]. The morphology and membrane properties depend on thermodynamic parameters and kinetics of the process. The polymer is dissolved in an appropriate solvent. The solution is then cast as a flat sheet or extruded as a hollow fiber. As part of the casting or extrusion procedure, the polymer solution is precipitated by a phase transition which can be brought about by a change in temperature or solution composition. This process involves the transfer of a single-phase liquid-polymer solution into a two-phase system that consists of a polymer-rich phase that forms the membrane structure and a second liquid-polymer-poor phase that forms the membrane pores. Any polymer that will form a homogenous solution which, under certain temperatures and compositions will separate into two phases, can be used. Thermodynamic and kinetic parameters such as chemical potential of the components and the free energy of mixing of the components determine the manner in which the phase separation takes place [37]. The process can be described by polymer/solvent/non-solvent ternary phase diagrams.

Thermal gelation phase inversion

The thermally induced phase-inversion process utilizes a polymer dissolved at an elevated temperature in a latent solvent (one that shows a lower solvency for a particular polymer at lower temperatures) which will produce a solution that will form a gel when cooled due to the loss of solvent power by heat removal [38]. The non-volatile latent solvents must then be extracted from the gel using another liquid which is a solvent for the latent solvent and a non-solvent for the polymer. The thermal gelation process is capable of yielding asymmetric and isotropic microporous and UF structures.

Diffusion-induced precipitation

The diffusion-based precipitation requires solvent removal which results in the insolubility of the polymer. In one method, the solvent in which the polymer is dissolved is removed by

evaporation, contact with a non-solvent vapor or total immersion in a non-solvent bath. The evaporation of a volatile solvent as the membrane is cast creates a dense homogenous structure. The vapor or immersion techniques rely on the diffusion of the non-solvent into the solution precipitating the polymer due to the decreased solubility.

Post-treatments of dense films

Certain types of MF membranes are prepared by either mechanical stretching or chemical etching of dense films. For example, Teflon and PTFE membranes are prepared by subjecting the films to a tensile stress. Another example are PC membranes prepared by track etching process [39].

The membranes that have been evaluated for macroencapsulation have generally been UF or MF types. A UF membrane is defined as retaining species in the molecular range 300–300,000 MW depending on the membrane [40]. Most xenograft cell transplantations require a UF membrane while allografts may be successfully encapsulated in MF grade membranes which retain species in the range of 300,000 MW to 0.4 μm and solely inhibit host cell/transplanted cell contact.

2.4. Membrane strength

The resilience of a medical device in the in vivo environment ultimately limits its success. The design of the final device configuration will determine the extent of strength required from the membrane. Note that membrane strength is in general inversely proportional to the diffusive transport in a homologous series. The membrane must also exhibit some degree of flexibility to remain intact during implantation and retrieval. If some other device component is used as a strength-bearing member, the choice of membrane structure, dimensions, composition and materials may be limited to those that optimize transport properties.

If membrane strength is limiting for the overall device strength, then the membrane must be manufactured with certain considerations in mind. For example, the membrane dimensions, composition and structure may have to be altered to increase the strength. Choosing a material that is inherently stronger (i.e. more ordered), or has a higher molecular weight with which to cast the membrane should increase the overall mechanical properties. UF or MF membranes can be fabricated with macrovoids within the wall or as an open cell foam where the microvoids are small and interconnected. By incorporating techniques that increase this isoreticulated structure within the membrane wall, the tensile strength can be increased. The strength can also be improved by increasing the cross-sectional area of the membrane by thickening the walls. Decreasing the overall membrane porosity will also serve to increase the overall membrane strength. Examples of both macrovoid-containing and isoreticulated structures are shown in Fig. 2A and B, respectively.

The outer morphology of the membranes can be altered during fabrication or by a post-treatment to improve the reaction required for a successful implant. Using various phase-inversion techniques, the outer surface of the membrane can range from a rejecting skin to a structure that is large enough to allow cells to enter into the wall itself (approximately 10 μm in diameter). The combination of proper membrane transport and outer morphologies may also be achieved using composite membranes. Brauker [15], and Boggs et al. [41] have used such membranes for the treatment of type I diabetes.

3. Membrane characterization

The polymer membrane used for cell encapsulation serves as a selectively permeable (permselective) barrier. This barrier must provide the appropriate metabolic flux to ensure cell viability, yet inhibit the transport of host immunological species immunoglobulin G (IgG, MW $\approx$ 150k), immunoglobulin M (IgM, MW $\approx$ 900k), and the various complement fractions

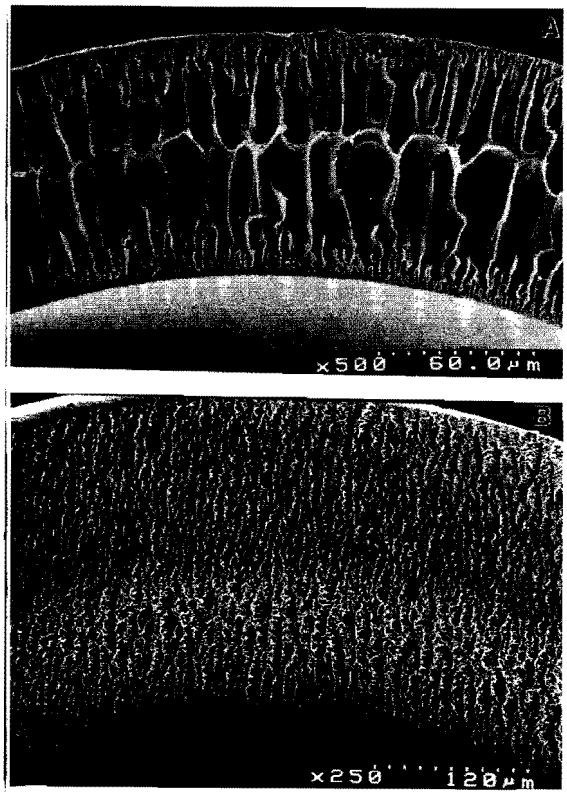

Fig. 2. (A) Scanning electron micrograph of macrovoid containing membrane. (B) Scanning electron micrograph of isoreticulated membrane structure.

(MW ≈ 150–500k)). A membrane of insufficiently high molecular weight selectivity will expose the encapsulated cells to host immunological attack, while one of low flux will deprive cells of necessary nutrients. A thorough understanding of the membrane transport characteristics as well as the specific cell requirements is essential for optimizing an encapsulation membrane for cell viability and function.

3.1. Characterization of membrane morphology

An understanding of the transport performance of a membrane must be linked to the chemical and physical structure of the membrane. These structure–property relations are complex [42] and demand a combination of morphological characterization and transport measurements. Morphological parameters influencing transport behavior include gross dimensions (inner diameter, wall thickness, concentricity), surface structures (porosity and roughness), and transmembrane structures (pore size, pore size distribution, bulk porosity, and internal surface area).

Inner diameter and wall thickness are easily characterized and documented using an ocular micrometer equipped with a video scanner. The transmembrane pore size distribution can be estimated for UF and microporous membranes using liquid–liquid and gas–liquid displacement measurements, respectively. Surface and transmembrane morphologies are routinely analyzed qualitatively using scanning electron microscopy (SEM) and, to a lesser extent, transmission electron microscopy (TEM) and atomic force microscopy (AFM) [42]. Although estimation of the pore size and pore size distribution using microscopy is difficult due to structure deformations during sample preparation and image distortion at high magnifications, these techniques are indispensable for membrane characterization.

3.2. Characterization of transport through membranes

The phenomenological transport characteristics of an encapsulation membrane can be determined using many different transport measurements including hydraulic permeability (HP), solute rejection and diffusive coefficient. HP and solute rejection relate to convective processes in which bulk fluid motion is driven by a transmembrane pressure difference. Solute rejection here is defined by the ratio of concentrations of a particular molecular weight species on either side of a membrane in a convective process (see below). These convective resistances for water and solute flow are captured in HP and solute rejection measurements, respectively. Diffusion is the process by which molecules move from a region of high concentration to low concentration via Brownian-like motion. Measurements of the convective and diffusive properties of an en-

capsulation membrane will indicate the capacity to maintain immuno-isolation.

Convective techniques

The HP of a membrane is determined from the convective water flux at a set transmembrane pressure. HP is normalized with respect to the exposed surface area and the transmembrane pressure, resulting in the units of flux/area/pressure. Contemporary hemodialysis membranes range in HP from 2–6 ml/h m^2 mmHg for low-flux membranes to 10–200 ml/h m^2 mmHg for high-flux membranes [43]. This performance parameter is proportional to the percentage of surface pores which are continuous through the membrane wall, and averages the pore size, pore size distribution and tortuosity into a single parameter. Experimental measurements can be compared to theoretical fluxes calculated from the Hagen–Pouseille equation [42]:

$$Q_f = \Delta p n \pi r^4 / 8 l \eta \tau \quad (1)$$

where: Q_f = volumetric water flux (m^3/s), n = number of pores (dimensionless), η = viscosity (kg/m/s), r = pore radius (m), and τ = tortuosity (dimensionless), l = membrane thickness (m), and Δp = pressure difference across the membrane (N/m^2).

Skin-layer thicknesses for asymmetric UF poly(phenylene oxide) (PPO) membranes were measured using a solution of colloidal gold particles, and the size distribution of open pores was determined by permporometry. Assuming a tortuosity of 1, Eq. 1 was then used to calculate water fluxes for the PPO membranes, which were in good agreement with the measured values. This comparison of measured and theoretical pure water fluxes indicated that the membrane morphological parameters found with permporometry and the colloidal gold particle method were relevant for the membrane transport properties [42].

In another study, pore radii were calculated for Nucleopore™ membranes based on water flux measurements [44]. These calculations were used to estimate the hydrodynamic thickness of adsorbed dextran (negligible), poly(ethylene oxide) (PEO), (6–7 nm) and poly(vinyl pyrolidone) (PVP) (3–6 nm) used as tracers in diffusion experiments.

Membrane pore size can also be assessed by determining the ultrafiltrate flow rate. The ultrafiltrate flux, J_f, is defined as the volumetric flow, Q_f, per unit membrane area, A, according to [45]:

$$J_f = Q_f / A \quad (2)$$

The rate of solute removal, M, is given by:

$$M = Q_f C_f \quad (3)$$

where C_f is the ultrafiltrate solute concentration, which is related to the bulk concentration in the retentate, C_{wb}, by the observed sieving coefficient, S, according to:

$$S = C_f / C_{wb} \quad (4)$$

The solute rejection coefficient, R, often appears in the literature, and is defined as:

$$R = 1 - S \quad (5)$$

Eqs. 2–5 can be combined to define the solute flux, J_s, in terms of the ultrafiltrate flux and the observed rejection coefficient as:

$$J_s = J_f C_{wb} (1 - R) \quad (6)$$

Therefore, a solute flux through a membrane can be calculated given the ultrafiltrate flux, the bulk solute concentration and the membrane rejection coefficient for the solute.

A range of solute sizes is used to generate a rejection coefficient profile representative of the membrane. This profile can be characterized by the nominal molecular weight cut-off (nMWCO), defined as the solute MW reduced in concentration by a log order upon convective transport through the membrane (i.e. 90% rejection). The position of a rejection profile (nMWCO) represents an average pore size, while the shape of the curve represents the pore size distribution.

Concentration polarization caused by boundary layer formation during the rejection measurement can have a tremendous effect on the measured rejection curves. To obtain rejection profiles which represent the properties of the membrane instead of hydrodynamic effects, operating flow conditions must be controlled based on the fiber geometry. This involves setting lumenal flow rates to establish a set wall shear rate and setting the permeate flux to minimize concentration polarization.

Solutes commonly used to generate rejection curves include globular proteins such as IgG (MW ≈ 150k), bovine serum albumin (BSA) (MW ≈ 67k), ovalbumin (MW ≈ 59k), and myoglobin (MW ≈ 16k) and polysaccharides such as dextran and ficoll. These solutions can be run singularly or as a mixture of different size tracers. Protein detection systems include UV spectrophotometry for single component solutions and size exclusion chromatography coupled with UV spectrophotometry for protein mixtures. For enhanced sensitivity, enzymes such as lactate dehydrogenase or pyruvate kinase can be used with their respective enzymatic assay, or a fluorescein-tagged protein coupled with fluorescence detection.

Polydisperse dextran solutions (2000–2,000,000 g/mol) are commonly used to generate membrane rejection curves. Size exclusion chromatography with refractive index detection is used to analyze reservoir and filtrate concentrations as a function of molecular weight, and fluorescein tagged dextrans with fluorescence detection can be used for enhanced sensitivity. Membrane fouling issues inherent in protein rejection curves are minimized using dextran solutions due to their low binding capacity to many polymeric membrane structures [46].

Protein and dextran rejection curves representative of a P(AN–VC) UF membrane are shown in Fig. 3. Three types of rejection curves are displayed: protein rejection, dextran rejection of a clean membrane, and dextran rejection for a membrane ultrafiltrated with a BSA solution at 4 mg/ml for 30 min. Recent work

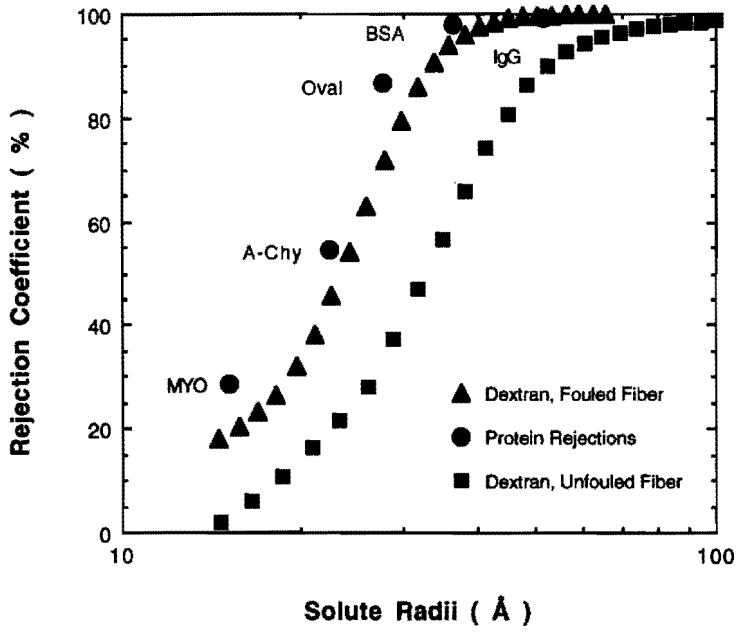

Fig. 3. Rejection curve of a typical immuno-isolatory hollow fiber using polydisperse dextrans and individual proteins on clean and BSA-treated membrane.

[47–49] has demonstrated the effect of protein adsorption on pore shut-down for PS and PES membranes. Fig. 5 shows a decrease in nMWCO from 40k to 14k upon exposure to a BSA solution, which is consistent with a decrease in pore size with protein adsorption. A comparison between the protein and dextran rejection curves based on solute size shows good agreement for the BSA-treated dextran curve.

Diffusive techniques

The convective MWCO characterization test provides information on the membrane sieving properties, and is dominated by pressure-driven convective transport processes. Molecular transport in most immuno-isolation devices is governed by diffusive processes, which are driven by concentration gradients instead of pressure gradients. The diffusive flux can be described according to Fick's law [50]:

$$F = -D_{\text{eff}} \Delta C / \Delta x \tag{7}$$

where F = diffusive flux per unit area (g/cm^2/s), D_{eff} = effective diffusion coefficient (cm^2/s), and $\Delta c/\Delta x$ = solute concentration gradient across the membrane thickness (g/cm^2). Eq. 7 can be simplified to the expression:

$$F = k_{\text{m}} \Delta C \tag{8}$$

where the membrane thickness, diffusivity and partition coefficient are incorporated into the overall membrane mass-transfer coefficient k_{m} with the units cm/s.

Eq. 7 states that the diffusive flux is inversely proportional to the membrane thickness, but the selectivity is independent of the thickness [43]. This has led to the development of extremely thin membranes which meet the separation requirements at an acceptable transmembrane diffusive flux (note that the same is true for membranes used in convective applications). Commonly used asymmetric membranes incorporate a thin separating layer in series with a support substrate. The support substrate provides minimal transport resistance, and a majority of the mechanical strength. Experimental determination of the device diffusive properties in both low- and high-range molecular weight is necessary to understand the encapsulated cellular environment.

Molecular transport across an immuno-isolation device is influenced by the steric repulsion supplied by the pores of the membrane as well as the bulk porosity. Diffusive properties of smaller molecular weight species are governed by the overall membrane porosity, while larger molecular weight diffusive properties are governed by the membrane pore size. Techniques have been developed [51] to measure the diffusive mass transfer for high-flux low-molecular-weight species (180–1000 MW) as well as low-flux high-molecular-weight species (60,000–150,000 MW) for P(AN–VC) hollow fiber membranes.

The experimental apparatus for small molecular weight diffusion measurements involves a dialysis set-up in which a tracer solution flows around the outside of the fiber, while a sampling solution flows down the fiber lumen. These flow rates are adjusted to minimize boundary layer formation on both the inside and outside of the fiber. Diffusion coefficients are calculated from the concentration difference between lumen and bath at a set lumen flow rate. This test was developed to measure the diffusion coefficients for glucose (MW = 186), vitamin B_{12} (MW = 1.3k), and cytochrome c (MW = 13.4k) through relatively higher water flux membranes.

For the large molecular species membrane resistances are far greater than the boundary layer resistances, and thus the experimental design does not involve flow [52]. The measurement can be made with the tracer diffusing across the membrane in either direction, both directions resulting in the same value for the diffusion coefficient. This test has been developed to measure the protein species myoglobin, BSA, and IgG and dextran species with molecular weights from 50,000 to 300,000 MW. To enhance the sensitivity of the measurement, fluorescently tagged species were used. Fig. 4 shows a graph for the diffusion

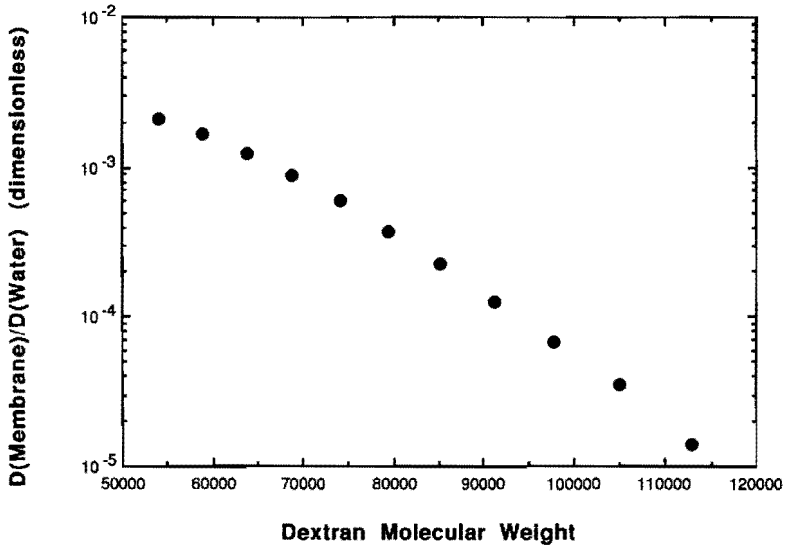

Fig. 4. Large molecular weight diffusion curve for a typical immuno-isolatory hollow fiber. Diffusion coefficient of dextran through the membrane relative to that of water.

of dextran across a P(AN–VC) membrane with respect to diffusion through water as a function of dextran molecular weight.

4. Materials for cell encapsulation

The extracellular matrix (ECM) that is produced by cells has been shown to affect both cell growth and differentiation [53] as well as tissue strength and structure [54]. In addition, specific binding sites have been identified at the cell–ECM interface which allow cell–matrix adhesion [55]. The matrix materials chosen for cell encapsulation aim to mimic at least some of the properties of the extracellular matrix.

In cell encapsulation, a matrix material may be provided for the cell to sustain its viability, function, and growth or differentiation. The matrix material is meant to provide primary or mitotically active cells (cell lines) with the appropriate environment. For example, anchorage-dependent cells may require a substrate on which to grow, whereas suspension cells may not. Both synthetic and naturally occurring matrix materials have been used to mimic the ECM. The configuration of the matrix material determines its effectiveness and usefulness in a given application. Micro- and macroencapsulation techniques are discussed below with emphasis on the different materials used.

4.1. Microencapsulation

A number of academic and industrial researchers have developed polymer microbead technology for cell encapsulation. Microencapsulation involves the formation of a semipermeable membrane around a given substance, in this case viable cells. The microencapsulated material is bead-shaped on the order of 200–2000 μm. In microencapsulation, as in macroencapsulation (described in more detail below), the long-term stability of the material under physiological conditions is essential. The microcapsule must be permselective with a cut-off less than approximately 100,000 MW to allow the passage of nutrients and oxygen without allowing that of immunoglobulins. Additionally, the material must be biocompatible such that a fibrous reaction of the host tissue is not evoked. Such a reaction will not only further decrease the diffusive flux through the semipermeable membrane and thereby deny the cells within of essential nutrients, but also the cells in such a layer

will compete for nutrition with the transplanted cells.

Microcapsules were prepared for the delivery of proteins [56] and later for cell encapsulation [57]. The latter researchers prepared microbeads of islet cells in sodium alginate crosslinked in calcium chloride, coated with poly(L-lysine) (PLL) and then poly(ethylene imine). The beads were prepared by co-extruding a slurry of cells and alginate through a syringe needle, into an aqueous solution containing calcium chloride which crosslinked the alginate, thereby causing it to gel upon immersion. Further research was done with cell-encapsulated alginate microbeads for xenogeneic transplantation of islets [58], pheocromocytoma (PC12) cells [59] and bovine adrenal chromaffin cells [60]. The biocompatibility of the alginate microcapsules was reportedly improved by exposing crosslinked alginate microspheres to PEG-grafted PLL [61] or by grafting hydroxyethyl methacrylate (HEMA) to alginate prior to crosslinking it in calcium chloride [62].

In addition to alginate, other materials have been used for microencapsulation of cells. For example, agar, agarose, carrageenan, chitosan, polyacrylamide, gelatin, fibrinogen, and collagen were used to encapsulate both suspension and anchorage-dependent animal cells, plant cells, bacteria, algae, and fungi [63]. The polymer beads were prepared by dispersing an aqueous solution of polymer and cells in an organic phase. In a similar study, islet cells were suspended in agarose which was extruded from a syringe into a cooled bath of paraffin oil [64]. These beads were then further coated with polyacrylamide by polymerizing acrylamide photochemically in situ and in the presence of bisacrylamide. Agarose-encapsulated islet cells have shown a favorable insulin response to glucose [65]. While the agarose displayed good biocompatibility and diffusion results, it failed to provide the cells with the immuno-isolation required in allogeneic and xenogeneic transplantation. Conversely, PEG microbeads were found to be both biocompatible and immunoprotective [66]. Cells were suspended in multi-armed PEG-acrylate with ethyl eosin and triethanolamine which was then polymerized in situ by exposure to UV irradiation [66].

The microcapsules offer the cells an optimal geometry (i.e. high surface to volume ratio) for diffusion of nutrients and oxygen which enhances cell survivability. However, the implanted microcapsules are subject to degradation, difficult to retrieve and may block the flow of CSF when implanted in the ventricles of the brain [67].

4.2. Macroencapsulation

Macrocapsules, like microcapsules, provide cells with immuno-isolation, nutrient diffusion, and an optimum environment for cell survivability and functioning. A macrocapsule consists of a membrane sealed after the injection of cells which are often suspended in a matrix material that ensures their even distribution within the device.

In recent studies using P(AN–VC) hollow fiber membranes (or macrocapsules), cells were suspended in a matrix material prior to injection into the membrane. The matrix material provided an even distribution of cells within the membrane, thereby optimizing transport of nutrients to the cells and thus their viability. In addition, the matrix material prevented aggregation of primary tissue as was shown with bovine adrenal chromaffin cells suspended in sodium alginate and crosslinked within the capsule with calcium chloride [68]. A slurry of chitosan and PC12 cells was prepared and injected into a hollow fiber immunoisolatory membrane after which the pH of the external environment was increased to deprotonate and precipitate the chitosan within the capsule [69]. Chitosan was shown to enhance both cell survival and distribution within the capsule in vivo with respect to capsules without chitosan. Fig. 5A and B, respectively show examples of the use of alginate (with bovine adrenal chromaffin cells) and chitosan (with PC12 cells) as extracellular matrix materials.

Andreatta-van Leyen et al. [70] used a microporous membrane for the delivery of growth factors to wounds. Keratinocytes (SCC-13) that

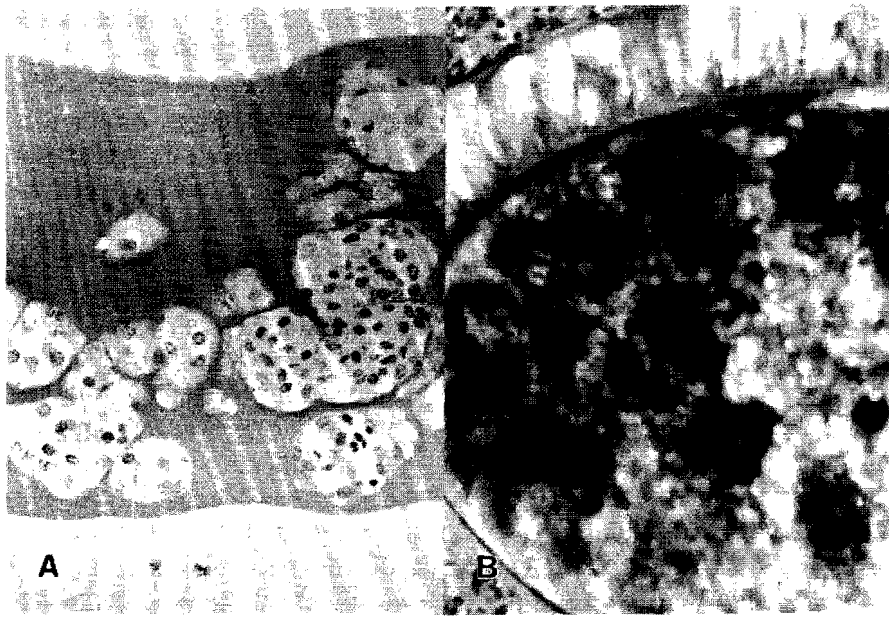

Fig. 5. (A) Bovine adrenal chromaffin cell-loaded (alginate) macrocapsule processed for a glycol methacrylate-embedded technique stained with hematoxylin and eosin (HE) after 6 weeks in rat striatum shows abundant viable cells. ×80. (B) PC12 cell-loaded capsule after 12 weeks in chitosan from a guinea pig striatum stained with an antibody against the Thy-1.1 cell surface marker and counterstained with HE. ×160.

release bovine growth hormone were maintained in a microporous PS membrane which allowed the passage of the growth hormone while inhibiting that of the cells. The device was applied to a wound with a hydrogel adhesive bandage consisting of either a hydrocolloid adhesive or a poly(urethane) foam.

Macrocapsules, unlike microcapsules, separate the function of the matrix and the membrane. The matrix function is simplified to optimizing the environment for cell survivability and functioning. This is accomplished most simply by providing an even distribution of cells within the device. Other improvements involve modifying the matrix to interact with cells in specific ways.

4.3. Chemical and mechanical manipulations of the matrix material

The matrix material can be manipulated chemically to provide specific functions. For example, spherical ferromagnetic beads were coated with specific receptor ligands that mediate cell attachment, but not cell spreading [71]. The oligopeptide, arginine–glycine–aspartic acid (RGD), which is a known ligand for fibronectin receptors, was used to modify the beads for adherent endothelial cells. The cell's sensitivity to a mechanical stimulus was shown to be altered by changing ECM receptor number, location, or adhesion strength or by modulating focal formation. It has also been shown that glass microbeads modified with RGD or tyrosine–isoleucine–glycine–serine–arginine (YIGSR) provide sites for cell adhesion [72].

More recently, Cima et al. [73] have synthesized PEO–star copolymers as a potential synthetic ECM. The star copolymers provide many hydroxyl groups where various synthetic oligopeptides can be attached.

The matrix material can also be used for reconstructive processes to rebuild injured tissue. The shape and pore structure of the material affect its performance. For example, both chitosan [74] and copolymers of lactic and glycolic

acids [75] have been used in tissue engineering applications. In addition, numerous scientists are working to regenerate skin with selective re-endothelialization within a specific material [76].

5. Biocompatibility issues for cellular encapsulation

The interactions at a host tissue/biomaterial interface ultimately determine the bioacceptance (or biocompatibility) of an implanted device. A fibrous reaction around a cell-containing polymer capsule (the implant) may impede the diffusive properties of a permselective membrane that is vital for successful applications. Identifying the cellular elements and mediators at a material interface provides insight into understanding the events that determine material biocompatibility [77]. With this information the host cellular response to an implant may be manipulated to enhance the success of encapsulated cell-loaded devices.

5.1. Biocompatibility evaluation in the CNS

The composition and types of cellular elements observed around an implant influence long-term compatibility. When a host reacts to an invasive procedure or material, an inflammatory response results, the extent of which is dependent upon the content of the reactive components. In addition, the constituents of the implant must be analyzed through rigorous testing procedures, in vitro and in vivo, in order to evaluate device biocompatibility. Recent emphasis has been on in vivo methods for evaluating biomaterial compatibility, especially with respect to the CNS tissue reaction to thermoplastics and polyelectrolytes.

The extent of a host tissue reaction to implants is influenced by the implantation method [78], site [79–81], and material properties. Material variables that may affect the reaction include material composition [82,83], surface charges [84], morphology [85,86], the size [87] and shape [79] of an implant, sterility issues [88], and material degradation [89,90].

Implant biocompatibility is dependent on the cellular and humoral events that transpire at a tissue/biomaterial interface. Macrophages are a major cellular constituent of the reactive tissue, and the various inhibitors and effectors of macrophage activity, as well as products of macrophage activation, may have different levels of expression between species. The mediation of macrophage chemotaxis, i.e. mobility within the tissue toward the area of insult along a chemical gradient [91,92] is controlled by various agents that are intimately involved in the inflammatory process. These agents attract blood monocytes through endothelial cells lining the vessel walls and move into the tissue producing the reactive stimulus. The monocytes differentiate into macrophages and, in some cases, foreign body giant cells in response to stimulating factors. The complex interrelated mechanisms of mediators, effectors, inhibitors and products of activated macrophages may be influenced by altering biomaterial properties, but it also appears that species differences in the expression and regulation of cellular and humoral pathways of inflammation and immune complexes affect the degree of compatibility. Lastly, the general health and nutritional status of experimental animals should be consistent with control animals in a controlled environment. Increased activity or excitation of animals may contribute to changes in humoral factors that could lead to differences in a foreign body reaction.

5.2. Implantation issues

The objective of the implantation procedure, regardless of the site, is to minimize the extent of inflammation elicited by the insertion technique. The surgical procedure induces an acute inflammatory response, resulting in localized vascular dilatation, which in turn increases local blood flow. Swelling and pain, due to increases in local vascular permeability and accumulation of chemical mediators, respectively, stimulate the

migration of blood monocytes and permit localized access for monocytes to differentiate into macrophages. The surgery must be performed under aseptic conditions, with minimal vascular disturbances and without the introduction of any potential contaminants, such as foreign chemicals or pyrogens. To isolate the inflammation induced by the implant itself from that associated with the surgery, one should study the implant site at 3 days postimplantation. Besides the chemical composition of the implants another important issue for implant compatibility is the device geometry. Below is an evaluation of two types of cell-loaded selectively permeable polymer capsules based on their geometry (reviewed in Aebischer et al. [93]). Microcapsules are formed with water-soluble polyelectrolytes into a spherical shape, while macrocapsules are fabricated from thermoplastic hollow fiber material into a tube or U-shaped configuration.

The CNS, especially brain tissue, provides a unique environment in which to analyze a biomaterial/host interface. The primary reactive cells in the brain, as well as others, can be labeled immunocytochemically by using cell-specific markers. Tagging cell types permits following the cells that encounter a foreign body. Furthermore, provided that host trauma is minimized during the implantation procedure, the CNS, in contrast to the peripheral system, is essentially devoid of a fibroblastic scar reaction. The ability to conserve the host tissue/material interface, while maintaining a minimal host reactive tissue layer, may enhance encapsulated cell survival and allow the secreted neuroactive compounds quicker access into the host brain.

5.3. CNS compatibility of micro- and macrocapsules

The brain tissue reaction to both polyelectrolytic microcapsules and thermoplastic hollow fiber macrocapsules has been characterized within several sites in rat, guinea pig and non-human primates. Evaluation of the host tissue/capsule interface has been accomplished utilizing general histologic stains such as hematoxylin and eosin (HE) or a Nissl stain, such as cresyl violet, together with immunospecific markers against many of the brain cell types. For example, the glial cells can be immunolabeled to identify astrocytes (antibodies to glial fibrillary acidic protein (GFAP)), oligodendrocytes (antibodies to galactocerebroside (GalC)) and microglia (antibodies to rat Ia determinants, OX-42). Evaluation of the neuronal population adjacent to the cell-loaded polymer implants has been performed with antibodies to neuron-specific enolase, NSE, or the phosphorylated and non-phosphorylated neuronal neurofilament population with the antibodies SMI-31 and SMI-32, respectively.

A primary host site for CNS implantation has been the region of innervation for the nigrostriatal dopaminergic pathway, the striatum, which in Parkinson's disease is characterized by deficiencies in striatal dopamine. Cell-loaded polymer capsules have been evaluated in the striatum for delivering neuroactive factors, e.g. dopamine and neurotrophic molecules, in animal models of Parkinson's disease [7,94,95]. Previous studies have indicated that the host brain tissue adjacent to macrocapsule implants is highly preserved and no differences in oligodendrocytic density or proliferation were observed [94]. Additionally, with the NSE antisera, host neurons were immunopositive within 50 μm from the implant site for both thermoplastic macrocapsules [94] and polyelectrolytic microcapsules.

Perturbations to brain tissue will result in an increase in the expression of the astrocytic intermediate filament GFAP in adjacent astrocytes, especially prevalent in the white matter fibrous astrocytes as compared to protoplasmic astrocytes found in the tissue parenchyma. Thus, antibodies to GFAP will label the reactive astrocytes more readily since their expression of GFAP is increased. As was shown in a previous study [94], an increase in reactive astrocytes was observed approximately 500 μm from thermoplastic polymer capsules at 2 weeks in rat brain tissue. However, with time the distance of re-

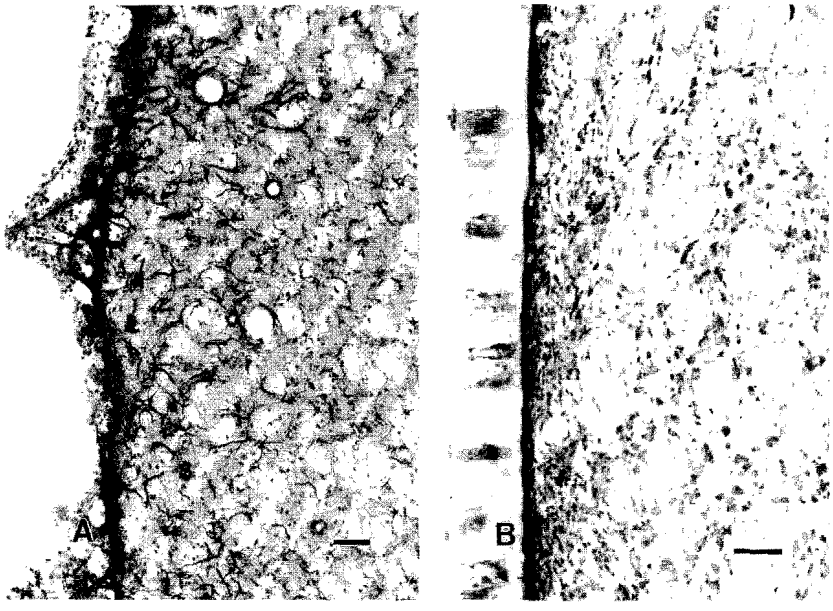

Fig. 6. Light micrographs of the host tissue/biomaterial interface in the rat striatum showing reactive astrocytes identified by glial fibrillary acidic protein (GFAP) immunostaining. In (A), GFAP immunopositive astrocytes can be observed approximately 200 μm from the material interface of a polyelectrolytic microcapsule after 4 weeks postimplantation. However, by 12 weeks (B) immunoreactive astrocytes can be observed only at the host tissue/material interface of a thermoplastic polymer capsule. Scale bar = 50 μm.

active astrocytes observed away from the host tissue/implant interface diminishes. As can be observed in Fig. 6A, by 4 weeks this distance decreased to 200–250 μm for polyelectrolytic microcapsules. In general, by 8 weeks, only the astrocytes located at the host tissue/implant interface remain reactive (Fig. 6B) and may do so for the duration of the implant. These observations are consistent for both types of polymer capsules. Provided implantation trauma is minimized, host reactions described herein do not adversely affect encapsulated cell survival.

Researchers have used polyelectrolyte-based microcapsule and thermoplastic hollow fiber capsule cell-loaded devices as neuroactive factor delivery systems in the CNS. The polyelectrolyte microcapsules are transparent and offer an optimal geometry to facilitate diffusion (enhanced cell survival). However, the polyelectrolyte membranes can be mechanically unstable: they are relatively fragile, difficult to retrieve, and may block the flow of CSF when implanted within the ventricles [93]. The thermoplastic opaque macrocapsules, in contrast, are cylindrical and therefore have less than optimal geometry for diffusion, but are mechanically more durable and easy to retrieve. Both systems have been effective for the release of neuroactive factors from the encapsulated cells while maintaining adequate bioacceptance of the implants within the host.

6. Current challenges facing encapsulated cell therapy

The use of encapsulated cell therapy for the treatment of a wide variety of diseases has come a long way. Perhaps the most important milestone is the recent survival of encapsulated calf adrenal chromaffin cells in humans without the use of any immunosuppression therapy [19,20]. These data far exceed what any contemporary investigator has been able to achieve with whole organ or unencapsulated cellular xenografts, even with the use of nearly toxic levels of immunosuppression.

The key points in the use of materials to create these devices are: (1) biocompatible membranes, device components and matrix materials; (2) strong implants; (3) materials that can be made into membranes with the proper transport properties; (4) long-term maintenance of those transport properties in the presence of protein which may foul the membrane; (5) using the proper geometries, morphologies, and host-cell/device interaction for the specific cell, site and application; and (6) a method to deal with possible systemic responses relating from shed antigens.

How can polymer science address some of the concerns discussed above? As mentioned earlier, stronger members can be achieved by changing membrane wall morphology by variation of precipitation conditions. The proper host-cell/membrane interaction can be achieved by varying outer morphology to form a vascularized or non-vascularized implant [15]. This morphology may be achieved by changing polymers or processing conditions. Transport properties are a direct result of the membrane polymer and formulation conditions. The membrane literature contains examples of how membrane nMWCO can be varied along the entire UF and MF ranges [39]. Membranes have also been shown to be immunoprotective and still deliver large molecules such as NGF (MW $\approx$ 20k) in both small [18] and large [17] animals. Maintenance of these transport properties in vivo may be possible by the addition of a non-fouling coating. One recent example is the chemical grafting of large chain PEO molecules on the surface of P(AN–VC) hollow fibers. Shoichet et al. [96] showed that protein adsorption could be decreased by this method.

The greatest advances for the use of encapsulation strategies may lie in the optimizing cell–polymer interactions within the device, and outside the device, in a manner similar to that described by Cima et al. [73] and Peppas and Langer [2]. As was noted, these groups are attempting to optimize the cell–polymer interaction by synthesis of 'smart-polymers' which contain natural occurring cell-adhesion molecules.

We have presented a brief overview of some of the methods that various researchers have used to develop these devices. Possible 'best uses' for polymer science may be to both optimize and properly integrate the disciplines of membrane science, implant construction, immunology, cell and molecular biology and surgery. This is the challenge faced to move bio-artificial organ devices from the level of rodent and some large animal studies to commonplace for treating human diseases.

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