Review Article

Cell microencapsulation with synthetic polymers

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Abstract: The encapsulation of cells into polymeric microspheres or microcapsules has permitted the transplantation of cells into human and animal subjects without the need for immunosuppressants. Cell-based therapies use donor cells to provide sustained release of a therapeutic product, such as insulin, and have shown promise in treating a variety of diseases. Immunoisolation of these cells via microencapsulation is a hotly investigated field, and the preferred material of choice has been alginate, a natural polymer derived from seaweed due to its gelling conditions. Although many natural polymers tend to gel in conditions favorable to mammalian cell encapsulation, there remain challenges such as batch to batch variability and residual components from the original source that can lead to an immune response when implanted into a recipient. Synthetic materials have the potential to avoid these issues; however, historically they have required harsh polymerization conditions that are not favorable to mammalian cells. As research into microencapsulation grows, more investigators are exploring methods to microencapsulate cells into synthetic polymers. This review describes a variety of synthetic polymers used to microencapsulate cells. © 2014 The Authors. Journal of Biomedical Materials Research Part A Published by Wiley Periodicals, Inc.: 103A: 846–859, 2015.

Key Words: microencapsulation, microsphere, microcapsule, synthetic polymer, cell therapy

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INTRODUCTION

Cells are considered microencapsulated when entrapped within a semipermeable polymer matrix (microsphere, microbead) or membrane (microcapsule) at the micrometer scale (Fig. 1). These microparticles have also been referred to as artificial cells and when implanted into a living host, the encapsulating polymer prevents both migration of the entrapped cells and invasion of host immune responders. The micrometer scale of microencapsulated cell implants is within the diffusion limits of many small molecules such as nutrients, oxygen, and electrolytes, while the pores of the encapsulating polymer are large enough to permit their ingress (Fig. 2). Simultaneously, small molecules produced by entrapped cells are permitted egress, such as hormones, metabolites, and waste. The larger host immune responders comprise cells, immunoglobulins, antibodies, and complement, which at 160–900 kDa cannot penetrate the microparticle walls and are, therefore, hindered from interacting with surface antigens on microencapsulated cells (Fig. 3).[1,2] Thus, microencapsulated cells are immunoisolated, which has benefits over immunosuppression.

Although immunosuppression can prevent host rejection of donor tissues, nearly 50 significant side effects have been identified as a result of immunosuppressant drug use, not including the additional consequences of multiple drug interactions that can result in from attempts to ameliorate the adverse effects.[3] Furthermore, the 5-year graft survival rate while on immunosuppressants is only 50%.[1] In addition to their various side effects, immunosuppressants only allow allogeneic transplantation, whereas immunoisolation permits the transplantation of both xenogeneic and allogeneic grafts.[1,4–6] Thus, with protection from the host immune response, microencapsulated donor cells can deliver therapeutic factors to augment or replace impaired function of native tissues.

Eukaryotic cells have been microencapsulated within polymers for more than half a century, when in 1966 Chang et al.[7] first reported encapsulating human erythrocytes in nylon microspheres. A therapeutic application of microencapsulated cells was first demonstrated in 1980 when Lim and Sun transplanted into diabetic rats microencapsulated pancreatic islets of Langerhan cells, which responded to glucose levels with insulin release and returned the rats to normoglycemia for 2–3 weeks.[8,9] Over a decade later, this application was first investigated clinically, when in 1994 Soon-Shiong et al.[10] implanted microencapsulated islets into

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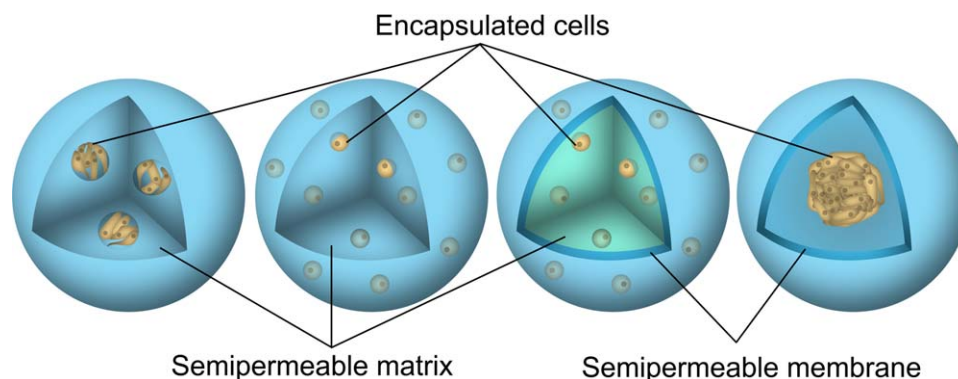
FIGURE 1. Common microencapsulation strategies. From left to right: dual core microsphere, polymer matrix microsphere, coated matrix microsphere, microcapsule. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

a diabetic patient who maintained normoglycemia for 9 months. Microencapsulation materials and methods have since evolved and microencapsulated islet cells have retained their function in the transplant site for over 2 years.[9,11] Allogeneic and xenogeneic cells have been successfully microencapsulated and transplanted into mice, rats, dogs, monkeys, and humans without the use of immunosuppressants.[4–6,12,13] Microencapsulated pancreatic islets within alginate/poly-L-lysine (PLL) microspheres are the most highly investigated microencapsulated cell system, and are currently in multiple clinical trials to treat diabetes.[13–16] In addition, many different microencapsulated cell systems are now being investigated clinically, including: parathyroid hormone released by microencapsulated parathyroid cells to treat hypothyroidism; ciliary neurotrophic factor (CNTF) released by microencapsulated retinal pigment epithelium cells to treat atrophic macular degeneration or retinitis pigmentosa; microencapsulated baby hamster kidney (BHK) cells genetically modified to release CNTF to treat Huntington's disease; microencapsulated cells genetically modified to release nerve growth factor (NGF) to treat Alzheimer's disease; microencapsulated cells genetically modified to release cytochromes for pancreatic cancer therapy; and microencapsulated cells genetically modified to release glucagon-like peptide-1 to treat stroke.[16–21]

As different cell types and applications are being explored, different materials for microencapsulation are being investigated. Microencapsulation materials have comprised natural or synthetic polymers or blends, including collagen, gelatin, fibrin, polyphosphazenes, poly(acrylic acids), poly(methacrylic acids), copolymers of acrylic acid and methacrylic acid, poly(alkylene oxides), poly(vinyl acetate), polyvinylpyrrolidone, polyethylene glycol (PEG), polyethersulfone, polysaccharides such as agarose, cellulose sulfate, chondroitin sulfate, chitosan, hyaluronan, and copolymers, and blends of each.[22,23] Each has advantages and drawbacks. In general, natural polymers have properties that cannot be changed. For instance, alginate-PLL microcapsules have a 50–80 kDa molecular weight cutoff (MWCO), which renders it ineffective in applications where the product to be released is greater than 80 K, as is the case in the liver.[24] Also, because natural polymers come from a living source, they must undergo

harsh chemical processes to isolate them from their native origins. Often, the process is not absolute and remnant proteins remain. Additionally, these chemical processes themselves often leave residual toxins, which along with any remnant proteins can elicit an immune response, hence the resulting polymer properties and immunogenicity can vary with purification and processing.[25,26] For instance, a high mannuronic acid content in the seaweed extract, alginate, often results in fibrosis when alginate microspheres are delivered *in vivo*.[27] Nevertheless, alginate has historically been the hydrogel material of choice for cell microencapsulation due to the ease of forming microspheres under gentle conditions.[28,29] Despite the ease of use, alginate-PLL capsule membranes have additional drawbacks that drive research toward more clinically and economically feasible materials.[30] For instance, alginate-PLL capsules have poor long-term durability in chelating agents typically present in physiologic solutions,[31] PLL is expensive and cytotoxic,[32,33] and capsules under strain are more likely to rupture than to deform.[34]

Alternatively, synthetic polymers such as covalently crosslinked PEG and polyacrylates have many advantages including both greater mechanical and chemical stability,
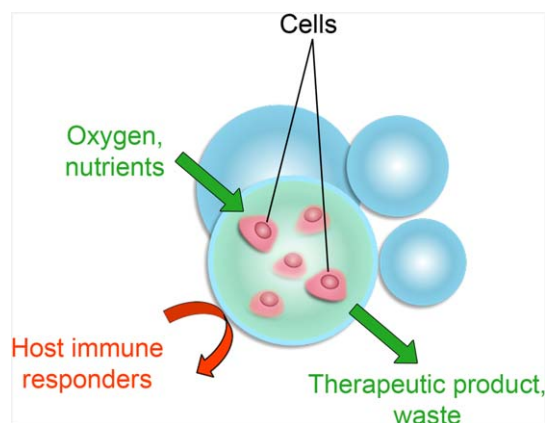
FIGURE 2. Microencapsulation permits the free exchange of nutrients and waste while excluding agents of the immune system, thereby promoting transplanted cell survival. The microsphere also permits release of therapeutic cell products. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
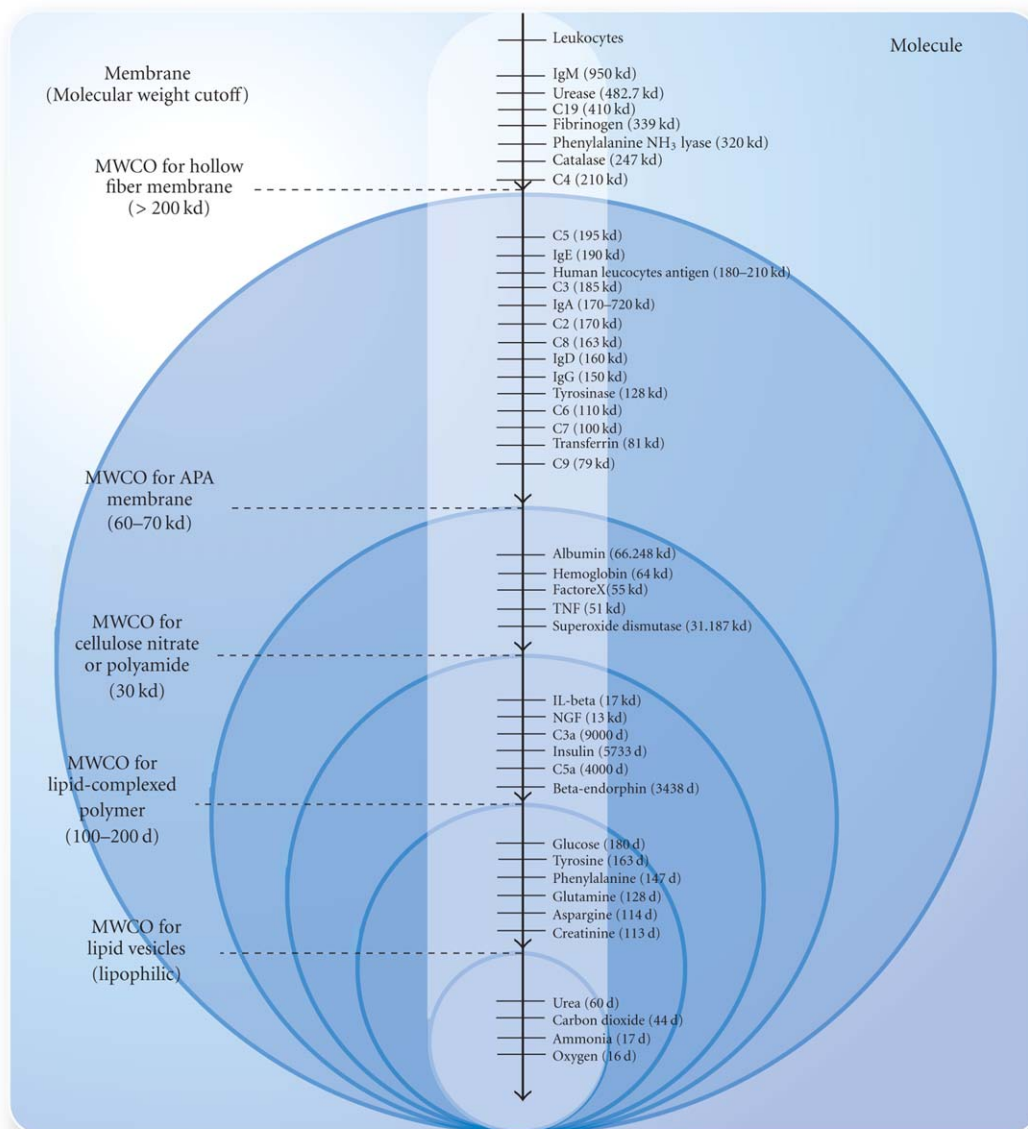
FIGURE 3. The membrane MWCOs of different materials used to make microcapsules are listed on the left and the molecular weights of various cells, enzymes, antibodies, etc. are listed on the right. Reprinted from Prakash and Jones,[2] with permission from Wiley. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

increased reproducibility due to the minimized batch to batch variation, reduced nonspecific protein binding, ease of modification, and tunable properties.[29,30] In the past, these advantages were outweighed by the disadvantages of using synthetic polymers to microencapsulate cells: primarily, microsphere fabrication using synthetic polymers often requires harsh conditions such as nonphysiological pH or temperature, or organic solvents toxic to cells.[29,35,36] As a result, there are few reports in the literature describing cell microencapsulation using synthetic materials. Traditionally, efforts have focused on increased purification of alginate or coating alginate microspheres with bioinert synthetic materials and despite advances, there remains variation in alginate microsphere chemistry.[28] Eliminating the natural polymer all together may prove necessary to advance the field of immunoisolation further. Investigators exploring microencapsulation with purely synthetic materials have had varying successes (Tables I and II), but there is no single source that documents these. Although there are abundant reviews of cell microencapsulation, few delve into microencapsulation with synthetic materials. The few that do conflate microencapsulation with macroencapsulation. Microencapsulation involves the encapsulation of cells with at least one dimension of the microparticle below 1000 μm, whereas macroencapsulation geometries are above 1 mm. Generally, microparticles are spherical, but can have multiple simple geometries (cube, prism, cylinder, etc.), whereas macrocapsules are essentially either tubular or planar. The difference in geometries and sizes leads to differences in specifications and challenges. The goal of this review is to

TABLE I. An Overview of Microencapsulation Materials and Encapsulated Cells

Polymer	Microstructure	Method	Encapsulated Cell	Application	Reference
Aliphatic Polyesters					
PLGA	Microcapsules	Interfacial polymerization	Porcine islets	Diabetes	37
Polyacrylates					
AN69	Microcapsules	Coextrusion	NIH 3T3, Porcine, rat hepatocytes	Macular degeneration, liver failure	24,38
HEMA-MMA, HEMA-MMA-MAA	Microcapsules	Interfacial precipitation	CHO, PC12, L929, human erythrocytes, human fibroblasts, H4IIEC3, HepG2, rat islets, hepatocytes	Parkinson's, angiogenesis, diabetes, immune suppression	1,39–60
PAN/PVC	Hollow fibers	Dry-jet wet spinning, proprietary	PC12, embryonic mesencephalon tissue, BHK, thymic epithelial cells, adrenal chromaffin cells, islets, R208F	Alzheimer's, Huntington's, diabetes	19, 61–68
Polyphosphazines					
Ca-PCPP Al-PCPP	Microspheres, microcapsules	Interfacial ionic crosslinking	Hybridoma cells	Immunoisolation	35,69
Polyepoxides					
SU-8	Microcontainer (box with lid)	Photolithography	Breast cancer cells, islets, rat glioma cells	Diabetes	71–73
PEGDA	Microcylinders, microcapsules, microspheres, conformal coats	Photolithography, microfluidics, emulsion photopolymerization	β cells, bEnd.3, CHO HUVEC, mouse ESCs, erythrocytes, MRC5, MC3T3, NIH 3T3, hepatocytes, Leydig cells, leukocytes, islets, macrophages, MHP36, C3H10T1, splenocytes	Blood replacement, diabetes, tissue engineering, fracture repair, hormone replacement	30,38,73–107
PVA	Microspheres	Electrospray photopolymerization	L929 cells	Immunoisolation	29

present a comprehensive examination of the synthetic polymers used for microencapsulation purposes; macroencapsulation materials will not be discussed.

ALIPHATIC POLYESTERS

Aliphatic polyesters are biodegradable polymers that have been used for some time in biomedical applications and comprise resorbable sutures, drug delivery systems, bone screws, and tissue engineering scaffolds.[111–113] Aliphatic linear polyesters are based on either the $[—CO—(CH_2)_x—O—]_n$ or the $[—CO—(CH_2)_x—CO—O—(CH_2)_y—O—]_n$ repeat groups, where x and y, the density of the ester groups, determine their physical properties.[114] Six aliphatic polyesters and their copolymers are currently approved by the US Food and Drug Administration for wound closure and orthopedic applications: poly(ε-caprolactone) (PCL), poly(glycolic acid) (PGA), poly(lactic acid), poly(lactic-co-glycolic acid) (PLGA), polydioxanone, and poly(trimethylene carbonate).[115] They degrade by hydrolysis of main chain ester bonds in a bulk or surface erosion manner, and the degradation rate and extent depends on polymer characteristics such as structure,

initial molecular weight, exposed surface area and size, degree of crystallinity, level of hydrophobicity, applied stresses, amount of residual monomer and, in the case of copolymers, the ratio of the hydroxyacid monomers.[111,116,117] Hence these polymers are often copolymerized to adjust material properties such as degradation rate.[118] One such copolymer, PLGA, has been extensively investigated for the microencapsulation of therapeutic agents to effect sustained and controlled delivery. In the last two decades, particular attention has been given to the development of protein-loaded PLGA microspheres or microcapsules.[119] Only within the past 15 years has PLGA been investigated for cell microencapsulation and challenges remain. During degradation, the microspheres undergo a pH drop that can result in instability, chemical modification, and aggregation of loaded proteins, which can elicit an immune response.[120–127] Although these issues are more relevant to controlled release of proteins than microencapsulated cells, it may also affect proteins that are released by entrapped cells. Abalovich et al.[37] encapsulated porcine islets of Langerhans into PLGA microcapsules and implanted them into wild type Wistar rats. Over a period of 35 days,

TABLE II. An Overview of Microencapsulation Technique, Material, Cell Type and *In Vivo* Application

Polymer	Microstructure/Method	Microencapsulation Technology	Cell Type → Animal	Secreted Product/Application	Reference
Aliphatic polyesters					
PLGA	Microcapsules/ interfacial polymerization	Extrusion and turbulence	Porcine islets → rat	Insulin/Diabetes	38
Polyacrylates					
AN69	Microcapsules	Coextrusion	Porcine, rat hepatocytes → rat, NIH3T3→ rat	Urea, albumin/ liver failure hFGF-2/macular degeneration	24,38
HEMA-MMA, HEMA-MMA-MAA	Microcapsules/ interfacial precipitation	Coaxial fluid extrusion, air jet coaxial extrusion	CHO → mice,	−/*In vivo* test IL-10/immunosuppression VEGF/angiogenesis	59,108,109
PAN/PVC	Preformed hollow fibers	Dry-jet wet spinning, proprietary	R208F → rat, BHK → human patients	NGF/Alzheimer's, CNTF/Huntington's	19,61–68
PEG/POE/PEO PEGDA	Microspheres/ photo- polymerization, Conformal coat	Water-in-oil agitation-induced emulsion, covalent surface binding via cyanuric chloride coupling, interfacial photopolymerization, hydrophobic interaction, self assembly, photo-polymerization following coextrusion, surface pegylation, selective withdrawal	bEnd3 → rat; Sheep or mouse erythrocytes → mice; MRC-5 → mice; MC3T3 → mice; rat fibroblasts → rat; MHP36 → rat; rat, porcine, human islets → mice, rats, human; mouse splenocytes → mice	Oxygen/blood replacement BMP2/bone tissue engineering Insulin/diabetes	16,73,92,95,104,106,110

CHO, Chinese Hamster Ovary; BHK, Baby Hamster Kidney; VEGF, Vascular Endothelial Growth Factor; NGF, Nerve Growth Factor; CNTF, Ciliary Neurotrophic Factor; BMP2, Bone Morphogenetic Protein type 2.

they achieved significantly reduced blood glucose levels, but the rats did not return to normoglycemia. In their *in vitro* studies, they noted that equivalent numbers of islets within diffusion chambers released more insulin than the PLGA microencapsulated islets. This result may indicate that the pH drop during PLGA microsphere degradation also affects proteins released from encapsulated cells. Nevertheless, the authors concluded that PLGA was a suitable material for islet microencapsulation, and suggested that further investigation would improve insulin yields. Despite this assertion, a more recent publication by Abalovich investigates pig islet transplantation into spontaneously diabetic dogs using PLL-alginate microspheres, rather than the PLGA microcapsules they developed.[128] In fact, none of the original eight authors who participated in the PLGA-encapsulated islet study have published further investigations of PLGA as a microencapsulation material.

This abandonment by the developing authors suggests that encapsulating mammalian cells within PLGA was wrought with too many difficulties to further develop. Encapsulated plasmid DNA is thought to be damaged by organic solvents and shear forces arising during PLGA particle formation in addition to the low pH environment of the degrading PLGA particle.[129] This phenomenon has been observed repeatedly,[130–133] and may also have an adverse effect on entrapped cells. Although PCL has been successfully used to macroencapsulate human atrial natriuretic peptide-releasing Chinese hamster ovary (CHO) cells for implantation into hypertensive rats,[134] microencapsulation using PCL has not yet been described, which may indicate that PCL also suffers a pH drop that is toxic to encapsulated cells.

POLYACRYLATES

Polyacrylates are bioinert nondegradable polymers that vary in their hydrophilicity based on the crosslinking agent used. These polymers are based on the $[\!-\!CH_2\!-\!C(R_1)COOR_2\!-\!]_n$ repeat unit where if $R_2 = CH_3$, $R_1 = H$ results in poly(methyl acrylate), which is soft and rubbery while $R_1 = CH_3$ results in poly(methyl methacrylate), which is a hard plastic. When $R_1 = CH_3$, $R_2 = CH_2CH_2OH$ corresponds to poly(2-hydroxyethyl methacrylate), and $R_2 = CH_2CH_2N(CH_3)_2$ corresponds to poly(2-dimethylaminoethyl methacrylate). These chemical substituents cause a wide variety in the chemical and physical properties of polyacrylates. For instance, poly(methyl methacrylate) (polyMMA) is a stiff, transparent glass-like material that has been used to produce intraocular lenses, bone cement, dentures, and middle ear prostheses.[135,136] Conversely, poly(2-hydroxyethyl methacrylate) (polyHEMA) is a compliant hydrogel that has been used in soft contact lenses, burn dressings, artificial cartilage, and as a matrix in drug delivery systems.[136] This wide range in mechanical and chemical properties enables the design of polymers with physical properties tuned to a specific application, simply by blending two or more polyacrylates. For instance, the hydrogel polyHEMA is often blended with the glassy polyMMA to produce the copolymer hydrox-

yethyl methacrylate–methyl methacrylate (HEMA–MMA), which is a hydrogel with elasticity suited to forming microcapsules.

Polyacrylates containing HEMA, MMA, methacrylic acid (MAA), and dimethylaminoethyl methacrylate (DMAEMA) have been used successfully to microencapsulate mammalian cells.[1,39–44] Sefton and Broughton[45] developed a method to use polyacrylates to microencapsulate mammalian cells. Their group and others furthered investigations into polyacrylates, using Eudragit RL (a commercially available acrylic methacrylic acid copolymer), HEMA–MMA, HEMA–MAA, DMAEMA–MMA, and DMAEMA–MAA–MMA to microencapsulate a variety of cells: CHO cells, human fibroblasts, human erythrocytes, rat islet cells, hepatocytes, PC-12 cells, rat hepatoma H4IIEC3 cells, and HepG2 cells have been encapsulated within microcapsules with membranes 200–300 Å thick.[42,45–60] Of these polyacrylates, HEMA–MMA proved superior in terms of mechanical strength, permeability, cell viability, and biocompatability.[22,137] Encapsulated cells demonstrated long-term *in vitro* viability,[138] but similar *in vivo* results have not been attained. The Sefton group found that HEMA–MMA microcapsules were capable of postponing xenogeneic graft destruction, but not preventing it.[59] Having determined that the MWCO of their microcapsules was approximately 100 kDa,[48] they postulated that shed antigens freely escape the microspheres and activate T cells.[59] The group implanted microencapsulated luciferase-expressing CHO cells into the peritoneal cavity of Balb/c mice. The luciferin expression enabled live-animal imaging of the implanted cells. The authors were able to demonstrate that despite microencapsulation, the majority of cells had been destroyed between 4 and 10 days. When donor cells were transduced to express the immunosuppressant IL-10, the survival of these cells was extended beyond 21 days.[59] The group further investigated whether microencapsulated cells would fare better when transfected to produce vascular endothelial growth factor (VEGF; Fig. 4).[108]

Their rationale was that VEGF would promote an angiogenic response and provide an improved blood supply to their microencapsulated cells. They found that VEGF did not improve the survival of their cells.

Despite the increased viability with IL-10, the use of an immunosuppressant in concert with immunoisolation techniques circumvents the motivation behind immunoisolation as a means to avoid immunosuppressants. It has been suggested that when using xenogeneic versus allogeneic grafts, different MWCO criteria may be needed.[139] In fact, when HEMA–MMA microencapsulated cells were xenogeneic, they survived less than 7 days *in vivo*; when these cells were allogeneic, they survived 7–14 days.[109] The immune reaction to transplanted cells can be classified as direct or indirect recognition of antigens of the major histocompatibility complex by host T-helper cells. Direct recognition requires cell-to-cell contact between the antigen-presenting cells of the graft and the T-helper cells of the host. Indirect recognition occurs when shed antigens from the graft are processed by the host's own antigen-presenting cells and then presented to host T-helper cells. The dominant immune
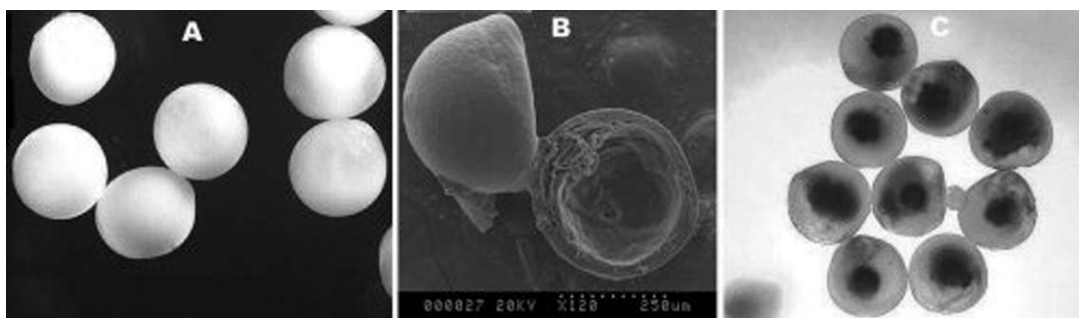
FIGURE 4. Microcapsules are uniform in size (light microscope, A) and about 400 μm in diameter (SEM, B). 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT)-stained viable cells (dark core) at the center of the microcapsules are seen in panel C. From Cheng et al.[108] J Biomed Mater Res A, 2008, 87, 321–331, reproduced with permission.

reaction to allogeneic grafts involves direct allorecognition.[140] Thus, in the case of allogeneic grafts, simply preventing direct contact between transplanted cells and host immune responders may be sufficient. In contrast, xenogeneic grafts trigger both the direct and indirect immune pathways.[140] The antigens of the major histocompatibility complex are shed from entrapped donor cells and are small enough to escape the microparticle membrane where they can be processed by host antigen-presenting cells that then present them to host T-helper cells. In essence, microcapsules can prevent activating the direct pathway, but not the indirect pathway: the antigens shed by xenogeneic grafts diffuse through the membrane activating systemic CD4 T-cells and other complement components, which are cytotoxic to the xenogeneic cells.[141] As complement components include proteins ranging in size from 74 to 460 kDa,[142] it is likely that the 100 kDa MWCO HEMA–MMA microcapsules fail to restrict certain complement proteins, resulting in the destruction of xenogeneic cells.[143] Thus, rather than adding immunosuppressants, another approach to increasing xenogeneic graft viability should include reducing the MWCO of the HEMA–MMA membranes.

In addition to HEMA–MMA, other polyacrylates have been explored for mammalian cell microencapsulation. Polyacrylonitrile (PAN) and polyacrylamide are polyacrylate derivatives containing nitrogen and are based on the $[-CH_2-C(H)CN-]_n$ and $[-CH_2-C(H)CONH_2-]_n$ repeat units, respectively. Multiple groups have used PAN copolymers to encapsulate cells within hollow fibers with micrometer-scale diameters (Fig. 2).[21,24,38,61–63] Honiger et al.[24] used AN69, a PAN-sodium methallylsulfonate copolymer, to encapsulate hepatocytes because of its demonstrated biocompatibility as a hemodialysis membrane. They demonstrated that the microfibers were permeable to 150 kDa human immunoglobulin G, but impermeable to 170 immunoglobulins A.[24] The investigators found that AN69 did not activate the complement system and its ~160 kDa MWCO was desirable to permit efflux of important liver proteins. Through histological examination, the group demonstrated a low inflammatory response to encapsulated hepatocytes 6 weeks postimplantation in the peritoneum of syngenic rats. Sections showed connective tissue around the implants, with no giant multinucleate cells. Recovered hepatocytes demonstrated an 85%

viability rate at 45 days postimplantation and albumin release was sustained during this period.

Successful results using AN69 were also achieved in a degenerative photoreceptor model. Uteza et al.[38] genetically engineered mouse fibroblasts (NIH 3T3) to express human fibroblast growth factor (hFGF-2) and then encapsulated the cells within AN69 hollow microfibers. The loaded microfibers were implanted into the vitreous cavity of the eyes of Royal College of Surgeons rats. Encapsulated fibroblasts survived at least 90 days both in vitro and in vivo, and continued to secrete hFGF-2, delaying photoreceptor cell degeneration. The group found no evidence of immune response nor hFGF-mediated tumor formation. Despite such promising results, there have been few publications using AN69 to microencapsulate cells since the late 90s.[144] Several groups began macroencapsulating cells using AN69 with a 65 or 80 kDa MWCO.[145–148] Kessler et al.[145] demonstrated a reduced permeability to glucose and insulin over time, which they correlated to increasing protein
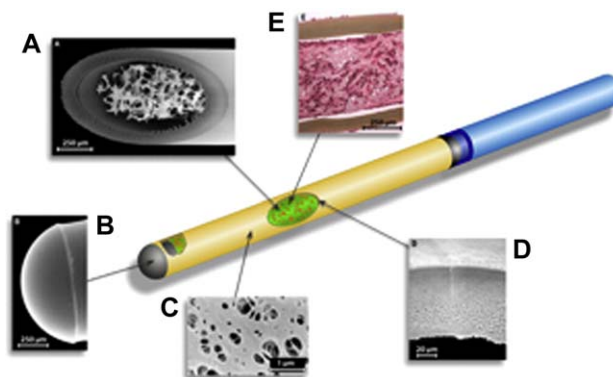
FIGURE 5. Typical hollow microfiber structure. (A)–(D) Scanning electron micrograph of polyethersulfone hollow microfibers containing a PVA matrix used to encapsulate human fibroblasts secreting glial cell derived neurotrophic factor for implantation into the corpus striatum of the forebrain. (A) A cross section showing the PVA matrix and the microfiber walls; (B) the glued-end; (C) the microfiber membrane pores (C); (D) a high power cross section showing the microfiber wall; and (E) a photomicrograph of encapsulated cells implanted for 1 month in the rat striatum. Reprinted from Zanin et al.,[64] with permission from Elsevier. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 6. Ca-PCPP microspheres containing hybridoma liver cells via phase contrast microscopy. (magnification ×1540). Reprinted with permission from Cohen et al.[35] J Am Chem Soc, 1990, 112, 7832–7833 ©American Chemical Society.

adsorption.[149] This may have been a contributing factor to abandoning AN69 as a microencapsulation material.

Copolymers containing PAN have also been used to microencapsulate cells for treatment in models of neurodegenerative disorders (Fig. 5).[64] Hoffman et al.[61] used the acrylonitrile/vinyl chloride copolymer (PAN/PVC) to form hollow microfibers with a 50 kDa MWCO. They microencapsulated a rat fibroblast line (R208F) genetically engineered to release NGF and implanted these into Sprague-Dawley rat brain lesions. The implants successfully prevented a lesion-induced reduction in acetyltransferase expression by neurons and did not elicit an immune response. Such successes paved the way for clinical trials to treat neurodegenerative disorders in humans. Bloch et al.[62] transduced BHK cells to secrete CNTF, a protein identified to have neuroprotective effects in the adult brain. The researchers encapsulated these cells into PAN/PVC hollow microfibers and implanted them into the right lateral ventricle of the brains of patients with Huntington's disease. The implants were exchanged every 6 months for a total of 2 years. Retrieved capsules were intact, but the numbers of surviving cells varied, resulting in a concomitant variation in CNTF release. Increased release corresponded to better response in patients: the research group observed improvements in patients' electrophysiological results that correlated to implants releasing the highest amounts of CNTF. Improved electrophysiological results indicated better function of intracerebral neural circuits. The group reported that no adverse events were noted, and touted the procedure as a feasible treatment for the disease, with future improvements planned to increase cell survival.

In addition to Huntington's disease, PAN microfiber-encapsulated cells have been examined for treating Alzheimer's disease. In a two-prong approach, researchers led by Jönhagen and Linderoth[63] transduced BHK cells to express NGF, encapsulated them within PAN/PVC microfibers, then implanted them into Alzheimer's patients. The microfibers have a 280 kDa MWCO and are macroscale in terms of length (11 mm), but remain within the microscale in terms of diame-

ter (720 μm). The 2010 phase I clinical trial with these microfibers demonstrated the implantability, retrievability, 12-month NGF secretion, long-term safety, and tolerability of the device within Alzheimer's patients.[65] The investigators intend to repeat the study with NGF levels increased to therapeutic doses. These devices arguably straddle the boundary between microencapsulation and macroencapsulation; nevertheless, the diffusion kinetics across the diameter dimension retain the benefits of microscale devices. The microfiber/cell combination releasing NGF is a commercial product in development dubbed NsG0202, which is currently produced by NsGene. Of the synthetic methods used to microencapsulate cells, it is one of the few that continues to be investigated in humans on both the macro- and microscale.[66–68]

POLYAMIDES

Polyamides or poly(amino acids) are biodegradable polymers that like proteins are linked by amides, but polyamides are composed of only one type of amino acid. There are many potential polyamides that can be synthesized, but only three are known to occur naturally: poly(ε-L-lysine) (ε-PLL), poly(γ-glutamic acid) (γ-PGA), and multi-L-arginyl-poly(L-aspartic acid) (cyanophycin granule polypeptide, CGP).[150] Nylons are examples of synthetic polyamides and though not degradable by mammalian enzymes, are now degradable by microbial systems that have evolved the capability since the introduction of nylons.[151] Nylons are common in many household items and were the first materials used to microencapsulate cells.[7] Since Park and Chang's[152] original encapsulation of erythrocytes, polyamides were briefly explored for microencapsulating bacterial cells but eventually abandoned because conventional monomers were shown to be very toxic to live cells.

POLYEPOXIDES

Polyepoxides form by reacting polyamines with epoxides to add epoxy functional groups to the polymers and increase their crosslinking ability. One such polyepoxide, SU-8, has an average epoxide group functionality of 8, from whence it derives its name. SU-8 was originally developed at the International Business Machines corporation (IBM) as an epoxy resin photoresist for semiconductor applications,[153] but has more recently been used in cell microencapsulation applications.[70–72,154,155] Applying sequential steps involving spin coating, baking, and ultraviolet exposure, Gimi et al.[70–72] used SU-8 to fabricate micrometer scale boxes complete with removable lids to microencapsulate a variety of cells. The authors first established the viability cells of encapsulated within their microcontainers by transducing 9L rat glioma cells to express luciferase when in hypoxic conditions.[72] Cells were loaded with automatic pipettes, lids were placed with microforceps and secured with biocement. They determined that when microcontainer lids had nanopores, encapsulated cell viability was improved. The group next encapsulated single islets within their microcontainers and performed viability and glucose challenge assays.[70] The group observed similar results with dynamic imaging of Ca^{2+} fluxes for both
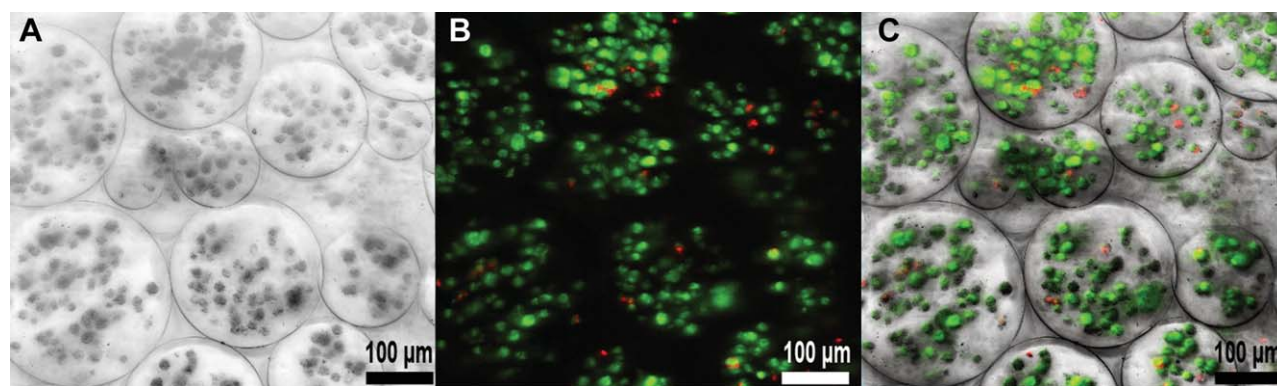
FIGURE 7. Viability of AdBMP2-transduced cells (2500 VP/cell) within microspheres was assessed at Day 7 using a LIVE/ DEAD© Viability/Cytotoxicity Kit for mammalian cells (Invitrogen, Molecular Probes, Eugene, OR). (A) Minimum intensity projection of a differential interference contrast Z-stack. (B) Maximum intensity projection of fluorescent Z-stack merge of red and green channels. The red channel was thresholded to eliminate diffuse virus staining. Dead cells appear red and live cells appear green. (C) Overlay of panels (A) and (B). Living cells accounted for 95.08 ± 0.47% of total cells encapsulated. BMP, bone morphogenetic protein; VP, viral particle. From Olabisi et al.[73] Tissue Eng Part A, 2010, 16, 3727–3736, reproduced with permission. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

encapsulated and free islets in response to elevated glucose. They concluded that encapsulation in the microcontainers did not impair the islet function. They have recently completed animal studies in BALB/c mice to evaluate the *in vivo* compatibility of SU-8,[155] but have yet to deliver cells encapsulated with their microcontainers into an animal model.

POLYPHOSPHAZENES

Over 700 different polyphosphazene polymers have been identified and these polymers are based on the $[-N=PR_1R_2-]_n$ repeat group, where R_1 and R_2 are generally organic or organometallic chemical substituents.[156] These side chain substituents are responsible for the high number of polyphosphazene polymers and also determine the wide range of physical, chemical, and biomaterial properties of the resulting polymers.[157] As such, polyphosphazenes have highly tailorable properties, and are biocompatible and biodegradable through hydrolysis that results in nontoxic and neutral pH degradation products, which lend these polymers well to tissue engineering applications. Led by the Langer group, Bañó et al.[35,69] used calcium or aluminum crosslinked poly[-bis(carboxylatophenoxy)phosphazene] (Ca-PCPP or Al-PCPP, respectively) microspheres to microencapsulate hybridoma cells (HFN 7.1 and CC9C10, Fig. 6). The group found that Ca-PCPP had better viability results than Al-PCPP (70 vs. 50%) and that antibody production of the cells was significantly decreased when encapsulated within Al-PCPP microspheres. The researchers then used Ca-PCPP to form both solid microspheres and liquid-core microcapsules. Microspheres were coated with PLL then exposed to a potassium chloride solution to liquefy the core, resulting in PCPP–PLL microcapsules. The group found that liquefying the core permitted increased cell growth compared to cells entrapped within solid microspheres. The increased cell growth resulted in a concomitant increase in antibody production by intracapsular cells.

Despite this promise, much of the research into polyphosphazenes has shifted from cell microencapsulation since the early 90s. Recent publications of these materials for use to microencapsulate mammalian cells include only reviews and patent applications. At present, the primary focus of these polymers is now as a tissue engineering scaffold or as an immunoadjuvant (an enhancer of vaccine activity). In fact, the investigators who developed the approach have stated that their successes with cell microencapsulation paved the way for their investigations toward using polyphosphazenes as a delivery vehicle for vaccine molecules in oral vaccinations, for which they established a company.[158]

POLY(ETHYLENE GLYCOL)

PEG is a polyether compound based on the $(-O-CH_2-CH_2-)$ repeating unit. PEG and its derivatives can be functionalized with groups such as acrylates and methacrylates, enabling their ability to form crosslinked hydrogel networks. Hydrogels formed from PEG polymers are bioinert and their biomechanical properties can be tuned to mimic those of most soft tissues.[73,159] PEG-based hydrogels have been used to microencapsulate a variety of cell types in a medley of micrometer-scaled containers, including cylinders, spheres, stars, cubes, conformal coats, and capsules.[73–80] The Hubbell group immunoisolated porcine pancreatic islet cells by encasing them in a conformal coat of PEG diacrylate (PEGDA), then evaluated their performance *in vitro* and *in vivo*.[81] Encapsulated islets released insulin when subjected to glucose challenges, and were able to return diabetic immunocompromised mice to normoglycemia for 110 days. In their immune competent xenotransplantation model, encapsulated cells remained viable and continued to produce insulin after 30 days in the peritoneal cavities of Sprague-Dawley rats. Their work led to further research into PEG-encapsulating islet cells,[81–89] and has resulted in a clinical trial in human diabetic patients.[16] Although the trial was terminated with no published results,

such a trial shows promise for the potential of PEG in this application.

Since the Hubbell group's work, other researchers have attempted using PEG to immunoisolate islet cells through a variety of methods. Many of these groups focus on attaining thinner and thinner layers of PEG. Miura et al.[89] bound PEG to lipids (PEG-lipid), and these PEG-lipids spontaneously formed thin coatings around islets when mixed with islet suspensions. Kizilel et al.[87] also developed a layer-by-layer self-assembly process to encapsulate islets, using heterobifunctional PEGs (biotin-PEG-N-hydroxysuccinimide [biotin-PEG-NHS] and biotin-PEG-peptides) with streptavidin to generate nanothin covalently bound PEG layers around islets. Wyman et al.[90] encapsulated islets in a single layer of PEGDA by adapting a method of selective withdrawal, whereby islets are soaked in liquid PEG, which is selectively withdrawn until the desired thickness of the polymer is achieved, followed by polymerization of the PEG. Teramura and Iwata[160] used PEG-lipid or PEG-urokinase to form a surface layer around islets, and demonstrated that these coatings reduced the instant blood-mediated inflammatory reaction to transplanted cells. The group further improved islet survival by immobilizing into the PEG layer soluble complement receptor 1 and heparin, which functioned against complement activation and coagulation, respectively.[161] An even greater number of researchers are exploring combining PEG with natural materials,[162–167] but the focus of this review is microencapsulation using purely synthetic approaches.

Although the microencapsulation of islet cells dominate the research, PEG has also been used to microencapsulate a variety of other cell types for both tissue engineering and immunoisolation applications. The Hubbell group also pioneered this work, encapsulating into microspheres human foreskin fibroblasts, CHO cells and β-cell insuloma cells.[91] They monitored diffusion profiles of proteins from 10 kDa PEGDA microspheres, demonstrating the effusion of BSA (67 kDa), but not IgG or fibrinogen (150 and 350 kDa, respectively). Research led by Fisher and coworkers, Meiselman and coworkers, and Pourpak and coworkers have investigated methods to conformally coat erythrocytes with PEG to use them as universal blood replacements.[92–94] The West group microencapsulated neural stem cells within PEG microspheres and implanted them into a rat model of stroke where they successfully released neurotrophic factors.[95,96] The group also used PEG to microencapsulate fibroblasts genetically modified to release bone morphogenetic protein, type 2 (BMP2, Fig. 7).[73] The immunoisolated cells formed heterotopic bone and were able to repair critical size defects in rat femurs.[73,110] Histological evaluation showed normal bone healing without infiltration of inflammatory cells at time points from as early as 2 weeks to as late as 1 year postimplantation.

CONCLUSIONS

Challenges still remain when microencapsulating cells within synthetic polymers. The conditions required for gel-ling many synthetic hydrogels have traditionally not been compatible with mammalian cells and must be modified. Further, successful *in vitro* results do not always correspond to biocompatibility when microspheres are placed *in vivo*. Nevertheless, synthetic polymers avoid the batch to batch variability posed by many natural polymers and circumvent the toxic residues left by the purification processes natural polymers must undergo. An increasing body of research is demonstrating synthetic polymers with terminal reactive groups that allow chemical crosslinking in conditions mild enough to support mammalian cell microencapsulation. As one process or polymer is abandoned, another emerges with improved viability and simpler protocols. Synthetic polymers offer greater flexibility in molecular design, permitting the fine tuning of mechanical, chemical, and transport properties. Currently, the most promising systems appear to be the PAN/PVC microfibers and PEG microparticles, with ongoing research into each material. Polyphosphazenes' abandonment appeared to be more a result of changing interests of the inventors than a failure of the material. As this review has attempted to demonstrate, synthetic polymers are a promising means to achieve future cell-encapsulation systems as immunoisolated cell therapies are increasingly emerging into the clinical arena.

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