An integrated biodegradable, immunoprotective, dual nanoporous capsule for cell-based therapies

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1. Introduction

Cell-based therapeutic strategy has been around for two decades. Primary cells or cell line with specific secretion can be used as "seed" cells for therapeutic function, such as pancreatic B cell secreting insulin for diabetes therapy [1]. Immunoprotection of transplanted cells and tissue by size-based semipermeable membranes is an attractive approach for cell-based therapy to treat patients with such diseases as Alzheimer's, diabetes mellitus, hepatic disease, etc. [2-5]. Such cell-based devices are thought to hold great promise in applications requiring site-specific and sustainable drug delivery of cell-synthesized molecules. To design the immunoprotection devices, the semipermeable membrane plays a critical role. Typically, its pore size must be in a few nanometers to allow diffusion of small nutrient molecules but to prevent the passage of large immune molecules to achieve high immunoisolation. Diffusion chamber is a device used in such applications. In medical and pharmaceutical fields, the drug-loaded chambers are capable of controlling the release rate and keeping the concentration within the therapeutic

windows for a long time [6]. Micromachined silicon membranes containing arrays of channel-like nanopores and alumina nanopore membranes produced by anodization have been used in biocapsules for the immunoisolation of transplanted pancreatic islet cells to treat diabetes [7-9]. The precisely controlled pore size of ~10 nm effectively prevents cellular and humoral immune species from penetrating through the membranes. These pores, on the other hand, allow sufficient passage of oxygen and nutrients to maintain cell viability and permit the reasonable release of insulin. However, if implanted, these devices must be surgically removed after use. Inexpensive polymers such as poly (ε-caprolactone) (PCL), on the other hand, can be fully biodegradable. A variety of low-cost techniques are available for the fabrication of polymeric micro/nanostructures, including nanopore membranes [10].

In this work, an immunoprotective and biodegradable delivery system combining two nanoporous capsules was developed for releasing cell-secreted product (Figure 1(a)). Figure 1(b) shows the schematic of microcapsules for cell encapsulation. Microencapsulation is a technology that encloses chemicals, drugs, enzymes, and cells within a membrane. A microcapsule is a hollow sphere with a diameter that can be controlled in the range of 200-1500 μm and a biocompatible semipermeable membrane with a surface pore size of 5-200 nm. These pores allow the bidirectional diffusion of nutrients, oxygen, secreted therapeutic products, and waste, but can prevent the penetration of high molecular weight substances from the microcapsule. Due to the 3-D microenvironment in microcapsules, transplanted cells, can grow well and provide therapeutic effect to treat a wide range of diseases from endocrine disorders and central nervous system diseases [11, 12]. Figure 1(c) describes the fabrication protocol for the

large nanoporous capsule including the formation of a chamber, design of the release gate, and device assembly. As a cell-based release system, the chamber and the membrane need to provide sufficient space for good cell viability and proper nanopores for nutrient and oxygen diffusion and immunoprotection. The outer surface of the chamber membrane also needs to be biocompatible to prevent fibrosis and other inflammatory responses for in vivo applications. In this study, we investigate the cell growth profile and the secreted product release in the integrated device. For comparison, the same measurements were also carried out in individual capsules.

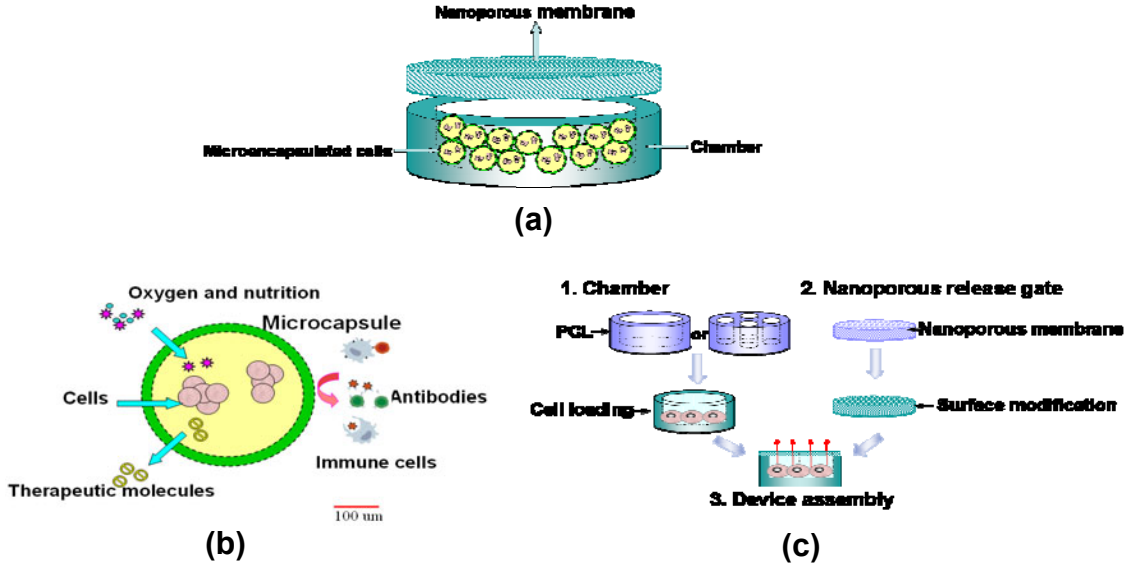

Figure 1. Schematic diagram of biodegradable nanoporous membrane systems. (a) integrated device; (b) microcapsule; (c) PCL large capsule with nanoporous gate.

2. Materials and Methods

2.1 Chemicals and reagents

Alginate sodium (low viscosity), poly-$_L$-lysine (MW15,000~30,000), PCL (MW 70,000), lysozyme (MW 14,700), poly(ethylene glycol) diacrylate (PEGDA), and monothioglycerol were purchased from Aldrich Chemicals (Milwaukee, WI). 1,4-

dioxane was obtained from Mallinckrodt Chemicals (Philipsburg, NJ). 2-methoxyethanol (ACS reagent, ≧93%) was purchased from Sigma-Aldrich (St. Louis, MO). Citrate sodium was provided by Fluka Biochemika. EnzChek@ Lysozyme assay kit, Phosphate Buffered Saline (PBS).

2.2 Device design and fabrication

Figure 1(c) describes the fabrication protocol for the PCL nanoporous capsule. This miniature device consists of two parts: a nanoporous membrane gate and a chamber for loading mES cells or microcapsules. PCL nanoporous membranes were prepared via the combination of thermally and nonsolvent induced phase separations [13]. PCL was first dissolved and stirred well in a diluent to form a 20 wt. % solution at 50°C for 2 hr. The diluent was a mixture of dioxane as solvent and 2-methoxyethanol as nonsolvent with a ratio of 15 to 65 by weight. After complete dissolution, the polymer solution was cast over a Teflon plate and immersed in a coagulation bath, i.e. water, at 5°C. After 6 hr, the resulting membrane was peeled from the Teflon plate and dried in a vacuum oven for 24 hr at room temperature. To prevent the fibrosis formation, hydrophilic PEG molecules were grafted on the PCL surface by using plasma technique [14].

An array of wells, each 4.5 mm in diameter and 1.8 mm in height, was first patterned on an aluminum plate using CNC machining. Then, a mixture of poly (dimethyl siloxane) (PDMS) resin and its curing agent (SYLGARD 184, Dow Corning) was poured onto the patterned mold. After 2 hr of curing at 70°C, the PDMS mold with an inverse pattern (i.e. pillars) was peeled off for hot-embossing of the PCL chamber. The PCL powders were first added into another aluminum mold patterned with slightly larger wells.

The mold was placed on a hotplate and set at 140°C for melting PCL. The PDMS mold was then aligned with the aluminum mold and the PDMS pillars were pushed into the molten PCL in the wells. After 5 min, the setup was cooled down to below 40°C and the embossed chambers were taken out from the mold. After the PCL chambers were grafted with PEG molecules using the same procedure, the inside chamber was coated with 1.0 wt. % gelatin solution for mES cell culture.

Figure 1(b) shows the schematic of microcapsules. AP microcapsules were prepared as described previously with modification [15]. Briefly, mES cells (2×10^6 cells/mL) were suspended in 2 % w/v sterilized sodium alginate and extruded through a 27-gage needle into 100 mM $CaCl_2$ using an electrostatic droplet generator (NISCO, Sweden) to form calcium alginate gel beads. The voltage used was 10 KV and the distance between the needle tip and the solution level was 1.5 cm. The gel beads were incubated with 0.05 % w/v poly-$_L$-lysine to form alginate-poly-$_L$-lysine membrane around the surface. The membrane-enclosed gel beads were further suspended in 55 mM sodium citrate to liquefy the alginate gel core. Resulted AP microcapsules were 300-400 μm in diameter. Microcapsules with mES cells were cultured in a 24-well plate at 37°C in 5% CO_2 in the mES maintenance medium. The integrated device was formed by loading microcapsules containing cell in the PCL capsule before bonding with nanoporous membrane (shown in Figure 1(a)).

2.3 In vitro release performance

To investigate the release performance through nanoporous membranes, a hydrophilic protein, lysozyme, was selected as a model drug and lysozyme diffusion

through PCL membranes was conducted in a Costar Transwell Test Setup. A modified PCL membrane was cut into a disc shape of 1.4 cm in diameter and bonded to the bottom of the Transwell insert by the bioadhesive to separate the two chambers. After the adhesive was completely solidified, 0.3 mL of a 1.0 mg/mL lysozyme solution was pippeted into the donor chamber and allowed to diffuse into the receptor chamber containing 3.7 mL PBS (Phosphate Buffered Saline) solution with 0.1% sodium azide. These diffusion experiments were subjected to constant shaking at 100 rpm. At various time intervals, the donor chamber was placed in a new receptor chamber containing 3.7 mL fresh buffer. Samples containing diffused lysozyme were analyzed using EnzChek@ Lysozyme assay kit. To investigate lysozyme diffusion through microcapsule surface, empty microcapsules were placed in 1.0 mg/mL lysozyme solution. The remaining lysozyme concentration in the solution was measured at different time intervals.

2.4 Scanning Electron Microscopy (SEM) for membrane morphology

To visually examine the surface morphology of nanoporous PCL membranes, a Hitachi Model S-4300 SEM was used to analyze the porous structure. The air-dried samples were loaded on the surface of an aluminum SEM specimen holder and sputter coated with gold for 40 s (Pelco Model 3 Sputter Coater) before observation. A working distance about 8-10 mm, an accelerating voltage of 10 KV, and a chamber pressure of

10^{-8} Torr were found to be suitable for obtaining high-resolution images. The magnification used in this study varied from 2000× to 120,000× depending on the pore structure.

3. Results and Discussion

3.1 SEM results of PCL membrane

Figure 2 shows the SEM images of the PCL membranes with or without surface treatment. PCL membrane has an asymmetric structure consisting of a thick porous support layer (bottom) and a thin and dense layer (top). The thick support layer contributes to the mechanical stability of the membranes. During the casting step, the solvent molecules located at the bottom of the cast film had difficulty for evaporation and

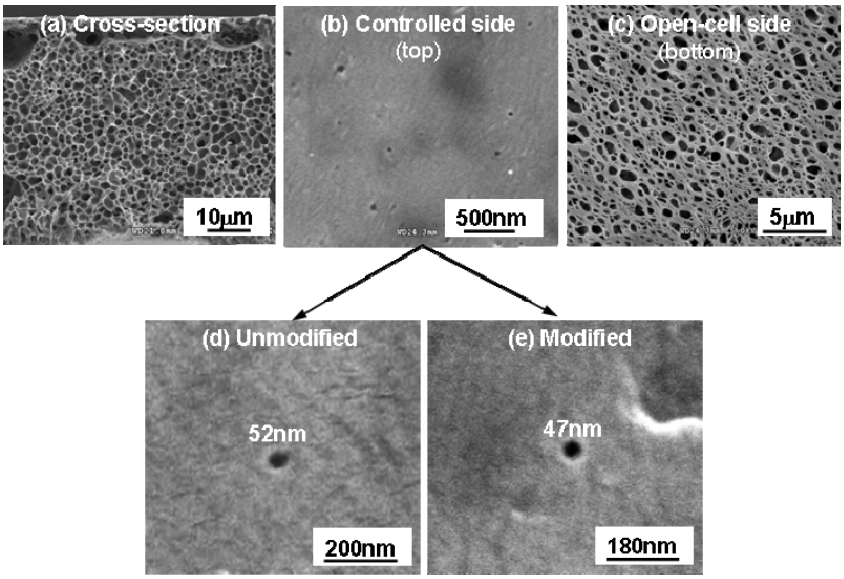

Figure 2. SEM images of PCL membrane formed with 20 wt% in mixture of dioxane and 2-Methoxyethanol with a ratio of 15 to 65 by weight (evaporation time: 60 sec, temperature of water bath: 5 ^{o}C): (a) cross-section, (b) controlled side from top view, (c) open-cell side from top view, (d) controlled side of unmodified PCL membrane using higher magnification, and (e) controlled side after PEG grafting.

could easily diffuse into the water bath for liquid-liquid diffusion, leading to micro-sized open cells (shown in Figure 2(c)). On the other hand, the thin and dense layer has nano-sized pores and is used for rate controlling in membrane separation processes and controlled drug delivery. On the dense side image in Figure 2(b), the average pore size is

around 50nm before PEG grafting (shown in Figure 2 (d)). After PEG molecules grafted on the PCL surface using plasma technique, the membrane showed a slightly lower pore size. This can be clearly seen from SEM image in Figure 2(e). For microcapsule alginate and poly-$_L$-lysine membrane, the cut-off molecular weight is around 72,000 Da (data not shown).

3.2 In vitro diffusion through nanoporous membranes

Lysozyme was selected as a model drug to demonstrate the releasing profile with molecular weight of 14,700 Da. Figures 3 compared the release pattern in the two types of capsules. For microcapsules, lysozyme released very fast in the first 3-4 hr because the surface area to volume ratio is very large and the diffusion distance is very short implying that microcapsules were not suitable for controllable and sustained release of drug. On the other hand, the lysozyme diffusion through the PCL membrane increased very slowly.

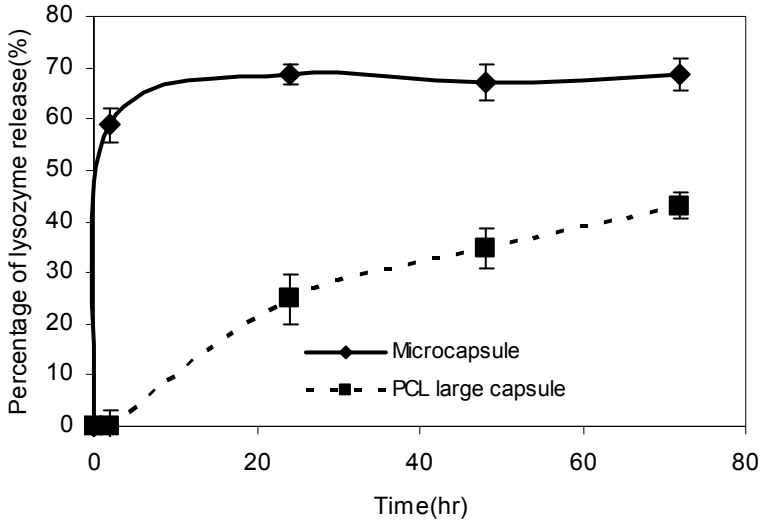

Figure 3. Lysozyme diffusion through the nanoporous membrane in microcapsule and PCL large capsule systems.

4. Summary

In summary, a new biodegradable cell-based device has been developed based on the integration of two techniques: microcapsules and surface coated PCL capsule. Microcapsules provide a 3-D microenvironment for spatial cell growth with good viability and proliferation. Coating biocompatible and hydrophilic PEG gelatin on the PCL surface could mediate the inflammatory response, minimize the fibrosis formation, and maintain the release performance. Our ongoing work is to test AP and PCL nanoporous membrane immunoprotection and controlled release functions for therapeutic cell secreting products. Hopefully, the dual nanoporous construct provides a unique way to allow superior cell growth, immuoprotection, fibrosis prevention and controllable release of secreted product in a biodegradable device.

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References:

[1] Kofman AV, Kharazova AD, Pinaev GP. Gene- and cell-based therapy of diabetes: between bench and bedside. Tsitologiia 2002; 44:1157-1177.

[2] Aebischer P, Winn SR, Galletti PM. Transplantation of neural tissue in polymer capsules. Brain Res 1988; 448:364–368.

[3] Colton CK. Implantable biohybrid artificial organs. Cell Transplant 1995;4:415–436.

[4] Desai TA, Hansford DJ, Ferrari M. Micromachined interfaces: new approaches in cell immunoisolation and biomolecular separation. Biomol Eng 2000; 17: 23-36.

[5] Robert PL, William LC, Willem M. Microcapsules and composite microreactors for immunoisolation of cells, US Patent No: 402209, 1995.

[6] LaVan DA, McGuire T, Langer R. Small-scale systems for *in vivo* drug delivery. Nat. Biotech. 2003; 21:1184-1191.

[7] Desai TA, Chu WH, Rasi G. Microfabricated biocapsules provide short-term immunoisolation of insulinoma xenografts. Biomed microdevices 1999;1:131-138.

[8] Walczak RJ, Boiarski A, West T, Shapiro J, Sharma S, Ferrari M. Long-term biocompatibility of NanoGATE drug delivery implant. NanoBiotech 2005;1:35-42.

[9] LaFlamme KE, Mor G, Gong D, Tempa T, Fusaro VA, Grimes CA, et al. Nanoporous alumina capsules for cellular macroencapsulation: transport and biocompatibility. Diabetes Technol Ther 2005;7: 684-694.

[10] Lin WJ, Lu CH. Characterization and permeation of microporous poly(ε-caprolactone) films. J Mem Sci 2002; 198:109-118.

[11] Orive G, Hernandez RM, Gascon AR, Igartua M, Pedraz JL. Survival of different cell lines in alginate-agarose microcapsules. Eur J Pharm Sci 2003;18:23-30.

[12] Jeon Y, Kwak K, Kim S, Kim Y, Lim J, Baek W. Intrathecal implants of microencapsulated xenogenic chromaffin cells provide a long-term source of analgesic substances. Transplant Proc 2006;38: 3061-3065.

[13] Yen C., He H., Lee L.J., Ho W. Synthesis and characterization of polycaprolactone nanoporous membranes for liquid-phase controlled release. J Mem Sci. (submitted).

[14] Carlisle ES, Mariappan MR, Nelson KD, Thomes BE, Timmons RB, Constantinescu A, et al. Enhancing hepatocyte adhesion by pulsed plasma deposition and polyethylene glycol coupling. Tissue Eng 2000; 6: 45-52.

[15] Ma X, Vacek I, Sun A. Generation of alginate-poly-Llysine-alginate (APA) biomicrocapsules: the relationship between the membrane strength and the reaction conditions. Artif Cells Blood Subtit Immobil Biotechnol 1994; 22: 43-69.