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Microfabrication of homogenous, asymmetric cell-laden hydrogel capsules

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ABSTRACT

Cell encapsulation has been broadly investigated as a technology to provide immunoprotection for transplanted endocrine cells. Here we develop a new fabrication method that allows for rapid, homogenous microencapsulation of insulin-secreting cells with varying microscale geometries and asymmetrically modified surfaces. Micromolding systems were developed using polypropylene mesh, and the material/surface properties associated with efficient encapsulation were identified. Cells encapsulated using these methods maintain desirable viability and preserve their ability to proliferate and secrete insulin in a glucose-responsive manner. This new cell encapsulation approach enables a practical route to an inexpensive and convenient process for the generation of cell-laden microcapsules without requiring any specialized equipment or microfabrication process.

1. Introduction

Cell encapsulation is a strategy to allow for the transplantation of non-autologous cells without the use of immunosuppressive drugs, which have potentially severe side-effects [1–4]. Transplanted living cells are protected from the host immune system because they are encapsulated in a semi-permeable hydrogel membrane which allows the diffusion of nutrients and cellular metabolic products while excluding antibodies and immune cells [5]. This technique has potential applications as therapies for many diseases such as diabetes, hormone deficiencies or hepatic failures [4].

Encapsulated islets have been explored as a method to allow transplantation of allogenic or xenogeneic insulin-secreting cells into diabetic hosts [6–11]. Electrostatic droplet generation is the most widely used method in the production of microcapsules containing islets or other insulin-secreting cells [12]. Typically, a laminar liquid jet is broken into droplets by a harmonically vibrating nozzle combined with an electrostatic dispersion mechanism which prevents droplet aggregation [13]. Currently, droplet generator encapsulation systems are commercially available from several manufacturers such as Inotech Biosystem (Rockville, MD) and Nisco Engineering AG (Zürich, Switzerland). The electrostatic droplet generator system is appropriate for the continuous production of hydrogel microcapsules (200–600 μm in diameter) from a polymer/cell mixture of an unchanged composition [13]. However, this apparatus, which requires sterilization of the bioreactor chamber after each use, is not convenient for studies which involve screening a large number of different material formulations [14]. Furthermore, this approach does not allow for generation of non-spherical capsules. Therefore, it is desirable to develop new and convenient methods for fast generation of geometrically controlled, cell-laden microcapsules.

PDMS molds made via soft-lithography have been used to fabricate alginate hydrogel microcapsules in 2D arrays as well as discrete single modules [15–18]. Many living cells, including bacteria and mammalian cells, have been encapsulated in hydrogels with this approach [15–18]. However, a potential challenge for this technique is the difficulty of releasing the microcapsules from the PDMS mold without damaging the cells. Even though methods such as mechanical stretching of the mold, swelling the hydrogel capsules in organic solvent [15] or microtransfer molding [17,19] have been applied to facilitate capsule release, these processes decrease cell viability and capsules yield [17,18]. Whitesides and coworkers recently demonstrated the production of cell-laden

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microcapsules using a PDMS membrane with fully penetrating pores to improve the ease of capsule retrieval [20]. However, this method is still limited by the necessity of a microfabrication process to generate the PDMS membranes and the difficulty of obtaining membranes of large area.

Here, we describe an inexpensive and convenient approach utilizing commercially available polypropylene meshes for the fabrication of cell-laden hydrogel microcapsules without using any specialized equipment or microfabrication processes. The *in vitro* viability, proliferation and insulin-secreting function of the encapsulated cells were characterized. We also demonstrated the fabrication of capsules with different geometries and selective modification of the microcapsule surface using this mesh-based fabrication approach.

2. Materials and methods

2.1. Fabrication of alginate hydrogel capsules

Thermo-molded plastic meshes were purchased from McMaster-Carr and Industrial Netting. The plastic meshes were cut into pieces of 4×3 cm^2 and placed in a Chex-all® II instant sealing pouch. These meshes were autoclaved at 121 °C and kept sterile until use. Sodium alginate (FMC BioPolymer, LF 10/60 LS), with or without materials to be encapsulated, was dissolved in 0.9 wt% NaCl solution at a concentration of 1.5% w/v. When encapsulation of microspheres or cells was desired, 1 wt% solid solution of 1 μm red fluorescent microspheres (Invitrogen, Eugene, OR) were diluted 500-fold with this 1.5% w/v alginate solution while INS-1 cells were suspended in alginate at a density of 1.5×10^6 cells/mL. The resulting mixture was vortexed to obtain a homogeneous mixture which was then centrifuged at 200*g* for three minutes. The air bubbles at the top of the mixture were removed by aspiration before this suspension was poured into a sterile Petri dish. A piece of sterile mesh was lightly dipped onto the cell/alginate mixture with the less reflective surface of the mesh in contact with the suspension. Excess alginate was removed by gently wiping the mesh against the edge of a sterile Petri dish. The mesh was immediately immersed in a solution of 100 mM $CaCl_2$ and subsequently transferred to HEPES buffer for mild washing. The hydrogel capsules were finally released by holding the mesh with a pair of tweezers and gently agitating it in a collecting solution.

2.2. Asymmetric surface modification of hydrogel capsules

Before the capsules were released from the mesh template into the collecting solution, one side of the mesh containing cross-linked hydrogels were gently placed on the liquid surface of a 4 mg/mL solution of 40–70 kDa FITC-labeled poly-L-lysine (Sigma Aldrich, St Louis, MO) for 3 min. The mesh was then gently washed in HEPES buffer before the capsules were released into a collection medium.

2.3. Cell culture

The rat insulinoma cell line (INS-1) was a gift from the Joslin Diabetes Center (Boston, MA). INS-1 cells at passages of 7–20 were cultured according to a protocol previously described [21]. The cells were cultured in complete medium composed of RPM1 1640 supplemented with 10 mM HEPES, 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate, and 50 μM 2-mercaptoethanol. Cultures were incubated at 37 °C in a humidified 95% air–5% CO_2 atmosphere and manipulated under sterile tissue culture hoods. The confluent dish of INS-1 cells was passaged every week at a subculture ratio of 1:3 and fed every 2–3 days. For trypsinization, the adherent cells were exposed to 0.025% trypsin–EDTA solution to yield a cell suspension which was centrifuged at 150*g* and 4 °C for 3 min. For preparation of frozen cell stock, freshly trypsinized cells were suspended in complete medium containing 10% of dimethyl sulfoxide and kept at −80 °C overnight in a Nalgene® Cryo Freezing container before being transferred to liquid nitrogen for long-term storage.

2.4. Viability analysis of encapsulated cells

The viability of INS-1 cells in alginate microcapsules was characterized using the Live/Dead® Viability/Cytotoxicity Assay Kit (Molecular Probe, Carlsbad, CA). Microcapsules were incubated in HEPES buffer containing fluorescent dyes at the concentrations of 2 μM Calcein-AM and 12 μM ethidium homodimer-1 for 45 min. The capsules were washed with HEPES buffer and visualized with a Nikon TE 300 inverted microscope under either phase contrast or fluorescent microscopy settings. Green fluorescence was viewed under a FITC filter and red fluorescence under a TRITC filter. Images were photographed using a CCD camera and Metamorph imaging software (Molecular Devices, Sunnyvale, CA). Confocal images were taken using a Zeiss LSM 510 confocal microscope with an argon excitation source. For live cell imaging, excitation wavelength of 488 nm and a filter set of 505–537 nm were used. Dead cells were imaged using an excitation wavelength of 543 nm and an emission filter set of 559–623 nm.

2.5. Static glucose-stimulated insulin secretion

Five 500 μl aliquots of microcapsules containing INS-1 cells were cultured over a period of 10 days. At the desired time points, the capsules were subjected to a static glucose stimulation study following a procedure slightly modified from a protocol elsewhere [22]. Briefly, the capsule samples were pre-incubated for one hour in Krebs Ringer buffer Hepes (KRBH) (137 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4 \cdot 7H_20$, 2.5 mM $CaCl_2 \cdot 2H_20$, 5 mM $NaHCO_3$, 16 mM HEPES, 0.1% (w/v) BSA, pH 7.4) containing 2.8 mM glucose. The *in vitro* insulin secretion was then assessed by consecutive incubations of the capsules in 4 mL of KRBH containing 2.8 mM glucose or 16.8 mM glucose for one hour. The samples were kept at 37 °C in humidified air and 5% CO2 for the duration of the pre-incubation and stimulation. The capsules were washed with HEPES buffer in between two incubations to remove residual insulin. At the end of each incubation, 1000 μl of the KRBH was removed and frozen at −20 °C for storage before insulin assay. Insulin concentrations were determined using Ultrasensive EIA assay kits (ALPCO Diagnostics, Salem, NH).

3. Results and discussion

3.1. Properties of template meshes for successful capsule fabrication

We fabricated the alginate hydrogel microcapsules using a process illustrated in Fig. 1. Briefly, the sterile polymer mesh with an array of uniform pores is brought into gentle contact with the alginate solution (1.5% w/v) with or without materials to be encapsulated. After capillary force completely fills the pores with the alginate solution, the mesh is held vertically so that most of the excess alginate is removed by gravity. The mesh surface is subsequently wiped against the flat edge of a sterile Petri dish to further remove any excess alginate. This polymer mesh containing uniform pores filled with alginate is immediately dipped into an aqueous solution of calcium chloride which cross-links the alginate polymer chains into a hydrogel. After mild washing in HEPES buffer, the hydrogel microcapsules are released by holding the mesh with a pair of tweezers and gently agitating it in a collection solution of culture medium. Fig. 2A shows large sheets of thermo-molded polypropylene mesh and Fig. 2B is a mesh template with an array of uniform pores typically used in this fabrication process. Fig. 2C and D shows the different pore shapes of the polypropylene meshes from McMaster-Carr and Industrial Netting respectively.

After testing several types of commercial polymer meshes from different suppliers, we determined that the surface properties of the polymer mesh are critical for successful fabrication of individual alginate capsules. Table 1 shows the feasibility of capsule formation and retrieval for a variety of thermoplastic meshes manufactured from nylon, polypropylene and Teflon. Filling of mesh pores by alginate solution is classified as easy (+) for instant pore wetting and liquid retention or as difficult (−) if the alginate fails to remain in the pores. Capsule retrieval is considered easy (+) if more than 80% of the free-standing microcapsules are released from the mesh pores instantly during the agitation step. If the capsules formed are connected by a thin film of residual alginate, the retrieval process is considered difficult (−). We observed that meshes made from Teflon are too hydrophobic and the alginate solution is unable to fill the pores. This is similar to observations of trapped air bubbles in PDMS molds [15] and membranes [20] due to the hydrophobic nature of PDMS before surface treatment by oxygen plasma. On the other hand, nylon meshes are too hydrophilic and the alginate solution forms interconnected capsules easily, even after the excess alginate is removed by wiping against the edge of the Petri dish. Only polypropylene mesh appears to possess appropriate surface property which enables fabrication of individual free-standing capsules. Furthermore, polypropylene has a high heat-resistant ability