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Surface modified alginate microcapsules for 3D cell culture

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ABSTRACT

Culture as three dimensional cell aggregates or spheroids can offer an ideal platform for tissue engineering applications and for pharmaceutical screening. Such 3D culture models, however, may suffer from the problems such as immune response and ineffective and cumbersome culture. This paper describes a simple method for producing microcapsules with alginate cores and a thin shell of poly(L-lysine)-graft-poly(ethylene glycol) (PLL-g-PEG) to encapsulate mouse induced pluripotent stem (miPS) cells, generating a non-fouling surface as an effective immunoisolation barrier. We demonstrated the trapping of the alginate microcapsules in a microwell array for the continuous observation and culture of a large number of encapsulated miPS cells in parallel. miPS cells cultured in the microcapsules survived well and proliferated to form a single cell aggregate. Droplet formation of monodisperse microcapsules with controlled size combined with flow cytometry provided an efficient way to quantitatively analyze the growth of encapsulated cells in a high-throughput manner. The simple and cost-effective coating technique employed to produce the core–shell microcapsules could be used in the emerging field of cell therapy. The microwell array would provide a convenient, user friendly and high-throughput platform for long-term cell culture and monitoring.

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1. Introduction

Cells in the body live in three-dimensional (3D) environments composed of complex fibrous networks of extracellular matrix (ECM) that provide both chemical and physical signals to cells. To understand the behavior of cells, it is vital to study cells in 3D environments. Unfortunately, it is very challenging to reproduce or mimic the 3D microenvironment around cells in living organs, including tissue–tissue interfaces, spatiotemporal gradients of chemicals and of oxygen, and other physical and chemical parameters [1–3]. Recently, there have been many attempts to develop a 3D cell culture system to better reproduce the microenvironment surrounding cells. These techniques include free scaffolds (spinner flask cultures, rotary well vessel reactors, cell liquid overlay on non-adherent surfaces and hanging drop method) for spheroid formation [4,5], scaffolds [6], gels [7], bioreactors [8] and microfluidic chips [9,10]. Because 3D cell matrixes better represent the physical and chemical signaling environment in natural ECM, they are often used in clinical applications, such as tissue engineering and regenerative medicine, as well as in model studies, such as drug screening.

Among the various 3D cell culture systems, cell encapsulation is a promising alternative for forming a cell spheroid and has potential significance for tissue engineering applications or drug screening models. Encapsulated cells in a selectively permeable membrane are isolated from the host immune system without a need for immunosuppression in allogeneic or xenogeneic transplantation while the membrane allows the exchange of nutrients and metabolites and the release of therapeutic agents. Research has been shown that cell encapsulation provided added possibilities for the transplantation of insulin-producing beta cells to help treat type 1 diabetes [11]. Currently, the major challenge in cell encapsulation is to reproducibly generate microcapsules with control over the capsule size and shape. Recently, microfluidic devices have been demonstrated to prepare monodisperse alginate capsules for live cell encapsulation [12–15]. Cells encapsulated in alginate microcapsules are able to survive and proliferate in cell media. The highly porous alginates, however, may not effectively block humoral immune responses to achieve immunoisolation.

In addition, to better predict the clinical response to drug compounds, 3D cell culture platforms are considered appropriate drug screening models because they are faithful to *in vivo* behavior. In drug screening and analysis, it is crucial to efficiently screen and validate potential drug candidates in the initial stages of drug discovery. Although the advantages of 3D cell culture platforms have been widely recognized, it has been difficult to scale up the 3D cell culture in a high-throughput manner for screening and testing due to involvement in cumbersome and ineffective handling procedures.

In this paper, we address the above-mentioned issues: (1) restrict immune responses and (2) scale up 3D cell cultures. We present a simple method of producing microcapsules with alginate cores and copolymer poly(L-lysine)-graft-poly(ethylene glycol) (PLL-g-PEG) shells for the encapsulation and immunoisolation of mouse induced pluripotent stem (miPS) cells. miPS cells cultured in the microcapsules survived well and proliferated to form a single cell aggregate. We demonstrated

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the trapping of the alginate microcapsules in a microwell array for enabling long-term culture and continuous monitoring of a large number of immobilized encapsulated cells at a desired location. Utilizing a droplet microfluidic platform, we show that cell-growth in microcapsules was quantitatively analyzed at different time points in a high-throughput fashion using flow cytometry.

2. Experimental

2.1. Reagents and materials

All chemicals and reagents were obtained from Sigma-Aldrich unless otherwise specified and were used without further purification. Water obtained from a Millipore system with a resistance of 18.0 MΩ-cm was used in the experiments.

2.2. Formation of alginate microcapsules

Alginate microcapsules were generated using a five-branch flow-focusing microfluidic device (Dolomite Microfluidics). The layout of the microfluidic device was depicted in Fig. S1. Fluidic channels were 300 μm in width and 100 μm in depth. Oleic acid was used as the oil phase, and sodium alginate in water was used as the aqueous phase. For the encapsulation of miPS cells, 2×10^6 cells/mL miPS cells were suspended in 1.2% (*w*/*v*) sodium alginate solution. Calcium salts (a cross-linking agent) dissolved in oleic acid was prepared by mixing $CaCl_2$ in 2-methyl-1-propanol at a 3% (*w*/*v*) ratio [16]. After mixing, the mixture was added to an equal volume of oleic acid and was placed on a 120 °C hot plate to evaporate the organic solvent. Prior to microcapsule production, the device was flushed with water for 30 min. Sodium alginate, mineral oil and oleic acid with calcium salts were individually loaded into inlets 1, 2 and 3, respectively, of the microfluidic device (Fig. S1) via polyethylene tubing and plastic syringes equipped with syringe pumps. The sample solution was flowed through the device using a flow rate of 0.6 μL/min for the aqueous phase and 7.0 μL/min for the oil phase. The alginate solution was focused and broken into alginate droplets at the orifice. The calcium ion gelified the alginate droplets downstream of the microchannel. Lastly, the resultant microcapsules were collected in a glass vial. The process of droplet formation was monitored and recorded using a microscope equipped with an i-speed LT high-speed video camera (Olympus). Optical and fluorescence micrographs of all of the resulting alginate microcapsules and the encapsulated cells were obtained using a TCS SP5 confocal laser scanning microscope (Leica).

2.3. Modification of alginate microcapsules with a polymer mixture

To coat the surface of the negatively charged alginate microcapsules with the copolymer PLL-g-PEG, the alginate microcapsules were first washed with 10 mM HEPES buffer and suspended in a solution of 1 mg/mL PLL-g-PEG conjugated to FITC (pH 7.4) (SuSoS) for 3 min after they settled to the bottom of the vessel. This process resulted in the formation of a monolayer of PLL-g-PEG on the surface of the alginate microcapsules. The microcapsules were then collected after 5 min of centrifugation at 1000 rpm and were rinsed 3 times in HEPES buffer. Lastly, the PLL-g-PEG-coated alginate microcapsules were re-suspended in HEPES buffer. The coating of cell-encapsulating microcapsules was also performed by the same method except with the use of PLL-g-PEG conjugated to TRITC (SuSoS).

2.4. Cell culture and analysis of encapsulated miPS cells

miPS cells transfected with an Oct4-GFP reporter were acquired from the laboratory of Dr. Chia-Ning Shen. miPS cells were cultured in DMEM (Gibco) supplemented with 10% fetal bovine serum, 0.1 mM 2-mercaptoethanol (Gibco), 0.01 mg/mL leukemia inhibitory factor (Millipore), and 100 U/mL and 100 mg/mL penicillin/streptomycin. miPS cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2. Encapsulated cells were cultured in dishes or in microwell arrays using the same method.

Encapsulated miPS cells were analyzed using a BD FACSCalibur (Becton, Dickinson and Co., Franklin Lakes, NJ) flow cytometer equipped with a 488-nm laser as the illumination source and six detectors: forward scatter, side scatter and four fluorescence channels FL1 to FL4. FL1 with the corresponding 515–545 nm bandpass filter was used to detect the fluorescence signals of the miPS cells in the microcapsules. Flow cytometry data were acquired after measuring 5000 events for each sample (Fig. S2).

2.5. Fabrication of a microwell array

First, standard photolithography and ICP reactive ion etching techniques similar to those described in our previous works produced a silicon master with depressions in the desired pattern [17]. The micropatterned silicon master was salinized with trichloro(1H,1H,2H,2H-perfluorooctyl)silane vapor to passivate the surface and to allow the detachment of PDMS in step 2. Second, a PDMS stamp with a square pillar ($100 \times 100 \times 100$ μm^3) pattern was fabricated by pouring a 10:1 ratio of elastomer prepolymer (Sylgard 184 Silicon Elastomer, Ellsworth Adhesives) to curing agent over the master. The mixture was degassed under vacuum and thermally cured at 70 °C for 3–4 h. After curing the PDMS, the stamp was peeled off of the master. Lastly, using a transferring procedure, the salinized PDMS stamp was exposed to vacuum to aid the proper filling of the micropatterned PDMS with liquid PDMS, and the filled stamp was placed on a glass support. After polymerization, the stamp was lifted off of the stamped structure, yielding patterned microwells. Once the microwell array was fabricated, the patterned substrate and the PDMS cover were plasma-cleaned for 90 s (Nordson MARCH Co.). The patterned substrate was coated with PLL solution, and the rims of the microwells were subsequently microcontact-printed with diluted alginate. This process resulted in negatively charged rims and positively charged wells. The assembly of the patterned substrate and the PDMS cover was achieved by bringing the substrate into contact with the cover and firmly pressing to form an irreversible seal.

3. Results and discussion

3.1. Formation of alginate microcapsules

Compared to conventional methods, microfluidic methods offer precise control over the size, shape and internal structure of alginate hydrogels. Two existing microfluidic routes to preparing alginate microgels that employ post-emulsification are internal and external gelation [18]. Internal gelation produces soft and unstable microgels in oil, whereas external gelation creates stable microgels with tunable mechanical properties in both oil and aqueous phases. With external gelation, the internal structure of alginate microgels can be conveniently controlled from capsules to microgels with a uniformly gelled structure. In this work, external gelation was used to prepare alginate microcapsules.

We adopted a modified process to implement continuous alginate droplet formation using a flow-focusing microfluidic device that consisted of a cross-junction with five inputs and two outlet channels. To generate alginate microcapsules, sodium alginate solution, mineral oil and oleic acid containing calcium chloride were pumped from inlet 1, inlet 2 and inlet 3, respectively (Fig. S1). The dispersed phase, the alginate solution, was broken up into droplets by the continuous phase, oleic acid, and gelation of the alginate droplet was achieved downstream of the microchannel following the diffusion of cross-linked calcium ions from the continuous phase. A major advantage of our design is the on-chip gelation of alginate microgels, which involves controlling the extent of gelation in the channel and results in the facile production

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