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Short communication

Shape-controlled high cell-density microcapsules by electrodeposition

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ABSTRACT

Cell encapsulation within alginate-poly-L-lysine (PLL) microcapsules has been developed to provide a miniaturized three-dimensional (3D) microenvironment with an aqueous core while promoting development of encapsulated cells into high cell-density structures. In this paper, a novel method for fabricating shape-controlled alginate-PLL microcapsules to construct 3D cell structures based on electrodeposition method is provided. Two-dimensional Ca-alginate cell-laden gel membranes were electrodeposited onto a micro-patterned electrode and further detached from the electrode. The PLL was coated onto the gel structures to form alginate-PLL complex as an outer shell and sodium citric solution was utilized to melt the internal alginate to achieve miniaturized 3D microcapsules (sphere, cuboid, and rod shape). By this proposed method, rat liver cells (RLC-18) formed multi-cellular aggregates with high cell-density after cultivation for 2 weeks.

Statement of Significance

The use of alginate-poly-L-lysine (PLL) microcapsules has shown great potential in fabricating 3D cell structures with high cell density. Despite their success related to their ability to provide a miniaturized microenvironment with an aqueous core, alginate-PLL microcapsules has drawback such as a limited shape-control ability. Because of the mechanism of Ca-induced alginate gel formation, it is still difficult to precisely control the gelation process to produce alginate-PLL microcapsules with specific shape. The present study provides an electrodeposition-based method to generate shape-controlled microcapsules for 3D cell structures. Sphere, cuboid, and rod shaped microcapsules of RLC-18 cells were produced for long-term culture to obtain desired morphologies of cell aggregates.

1. Introduction

Recent advances in tissue engineering have relied upon development of methods to place spatially selective biological components at specific three-dimensional (3D) locations [1–3]. There is similar interest in developing methods to assemble cells within bio-scaffolds for fabrication of 3D cell structures. Current methods to assemble cells into two-dimensional (2D) or 3D structures include non-spherical polymeric microparticle *in situ* photo-polymerization [4,5], cell patterning on 2D surfaces by using dielectrophoresis technique [6,7], 3D bio-printing [8,9], cell sheet engineering [10], and cell encapsulation units [11,12]. Thus, many fabrication methods have been developed to immobilize and culture cells in 3D formats.

The use of alginate-poly-L-lysine (PLL) microcapsules has shown great potential in fabricating 3D cell structures with high cell density ever since Lim et al. first reported this approach for fabrication of microencapsulated islets for implantation in 1980 [11]. Lately, cell-laden Ca-alginate fibres or droplets have been transformed into 3D microcapsules to form tissue-like cell spheroids and cylindroids after long-term cultivation [13,14]. These microcapsules provide a soft and "liquid-like" platform that mimics the embryonic microenvironment for self-assembly of cells [15]. Small molecular weight substances like nutrient and oxygen molecules can pass through the alginate-PLL membrane of microcapsules, while cells are blocked by the membrane [12,16]. However, because of the mechanism of Ca-induced alginate gel formation,

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it is still difficult to precisely control the gelation process to produce alginate-PLL microcapsules with specific shape. Therefore, despite the commonly used alginate fibres or droplets generated microfluidic devices, there is still a lack of an efficient approach to achieve shape-controlled alginate-PLL microcapsules for 3D cell structure fabrication.

For production of shape-controlled alginate-PLL microcapsules, the electrodeposition method is a promising technique. Electrodeposition of Ca-alginate hydrogels on specific device plays an important role in entrapment and immobilization of biological components, such as cells and bacteria, for studying cell-cell signalling and 3D cell culture [3,17]. Cheng et al. demonstrated a novel approach for fabrication of alginate gels inside a microfluidic system [17,18]. In their work, a Ca-alginate composite hydrogel biofilm entrapping bacterial cells was fabricated on gold electrodes inside the microfluidic system, with its shape controlled by using electrical signals. By electrodeposition, Ca-alginate gelation can be triggered by an electrical signal, which enables us to fabricate an *in situ* Ca-alginate gel membrane with controllable size and shape on microelectrodes. Consequently, other researchers have also focused on the fabrication of cell structures by depositing cell-laden alginate gel on electrodes [19,20]. Their results show that cell viability can be maintained during culture, but cells did not spread because of the lack of cell adhesion molecules and spaces within the Ca-alginate gel. Thus, promoting cell proliferation has become the main challenge concerning the electrodeposition method for 3D cell structure fabrication.

Therefore, we introduced a new approach to solve the issue of cell proliferation in the electrodeposition method. Our approach particularly aims at the fabrication of shape-controlled alginate-PLL microcapsules for 3D cell structures based on electrodeposition. In this study, we applied the electrodeposition method to alginate-PLL microcapsule fabrication by transforming the 2D gel membrane into 3D microcapsules. A Ca-alginate gel membrane was formed on the micro-patterned fluorine-doped tin oxide (FTO) electrode, thus forming a microfabricated conductive array. The electrodeposition-based gel-membrane formation process was applied to cell encapsulation into alginate-PLL microcapsules with liquid cores (sphere, cuboid, and rod), where cells were cultivated for 2 weeks.

The contribution of this paper includes: (1) This report describes the technique of electrodeposition in generating 3D microtissue as a product of shape control. (2) Using the common electrodeposition approach, deposition of calcium alginate gel structures has previously been restricted to a 2D surface; however, this work demonstrates overcoming this limitation to fabricate 3D microtissue. (3) Compared with other methods for fabrication of 3D tissue architecture [21,22], micro-scale tissue can be fabricated by our approach owing to the resolution of the electrodeposition method (~200 μm). Additionally these microstructures can be easily transferred by a 1-ml pipette for further applications. Therefore, our method provides a suitable platform for construction of 3D high cell-density structures using shape-controlled alginate-PLL microcapsules. This platform has potential to stimulate new uses for microencapsulation technology in various applications such as in tissue engineering.

2. Materials and methods

2.1. Materials and solution preparation

Sodium alginate (A2033, Sigma-Aldrich, St Louis, MO), sodium citrate tribasic dihydrate (S4641, Sigma-Aldrich, St Louis, MO), PLL hydrobromide (molecular weight 30,000–70,000 Da, Sigma-Aldrich, St Louis, MO), fluorine-doped tin oxide coated glass slide (surface resistivity ~7 Ω/sq, 735140, Sigma-Aldrich, St Louis, MO), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 346-01373, Wako Pure Chemical Industries, Osaka, Japan) were used. Calcium carbonate ($CaCO_3$, ϕ 0.97 μm, #2300) was supplied by Sankyo Seifun Ltd (Japan). Photoresist (AZ 5214 E) was purchased from AZ Electronic Materials (Germany) GmbH. Water used to prepare the solution was deionized with a Millipore Direct-Q3 water purification system (Millipore, Worcester, MA).

2.1.1. Deposition solution

The deposition solution was prepared by dissolving 1% (w/v) sodium alginate in solution containing NaCl (126 mM), KCl (2.7 mM), $Na_2HPO_4{\cdot}12H_2O$ (8.1 mM), KH_2PO_4 (1.47 mM), and HEPES (21 mM). The pH was adjusted to 7.3 by adding 0.5 M NaOH solution. $CaCO_3$ (0.5%, w/v) was uniformly dispersed in the solution by using a magnetic stirrer for 24 h.

2.1.2. HEPES buffer solution

The HEPES buffer solution was prepared by dissolving HEPES (5 g/L) in a solution containing NaCl (8 g/L), KCl (0.37 g/L), Na_2HPO_4 (1.076 g/L), and glucose (1 g/L). The pH was adjusted to 7.3 by adding 0.5 M NaOH solution.

2.1.3. Calcium chloride solution

To prepare 1.1% (w/v) calcium chloride solution, 0.55 g of $CaCl_2$ (anhydrous) was dissolved in 50 mL distilled water.

2.1.4. Sodium citrate solution

Sodium citrate tribasic dihydrate (1.62 g) was dissolved in 100 ml of 0.45% (w/v) NaCl solution to prepare 55 mM sodium citrate solution.

2.1.5. Cell viability test solution

The cell viability solution was prepared by mixing 0.8 μL calcein AM (1 mg/mL, Wako Pure Chemical Industries), 2.8 μL propidium iodide (PI, 1 mg/mL, Wako Pure Chemical Industries), and 1 mL HEPES buffer solution.

2.2. Fabrication of the micro-patterned electrode

The photolithographic technique was used to construct the electrode used in this experiment. Briefly, the FTO glass slide was washed with acetone, isopropyl alcohol, and Milli-Q water by using an ultrasonic cleaner. The patterned electrode was fabricated by coating 1.4-μm thick photoresist (AZ 5214 E) on the surface of the FTO glass. The photoresist was exposed to UV light (power, 19 mW/cm^2; 4 s) through a specially designed chrome mask. To demonstrate the feasibility of this method for electrodeposition of Ca-alginate hydrogels with controllable shape and size, micropatterns of different geometry, including circle, square, and rectangle, were prepared as shown in Fig. 1(1). Diameter of the circular pattern was 1 mm, length of the square pattern was 1 mm, and length and width of the rectangular pattern were 2.5 mm and 700 μm, respectively.

These geometries were chosen in the current work to eventually fabricate 3D cell structures in sphere, cuboid, and rod shapes because of the following reasons. (1) Cell aggregations in spheroid forms are among the most widely used models for 3D cell culture. Liver-specific gene expression has been observed to increase for 3D cultured spheroids compared to 2D monoculture [20]. (2) Cuboid structures indicate the capacity of the current method for fabricating predefined structures accurately because cells aggregations tend to form spheroids rather than cuboids during regular 3D cell culture. (3) Cell-encapsulated rod structures can be potentially applied to vascular tissue engineering [13]. Hepatocyte cylindroids