Materials Science and Engineering C 55 (2015) 79–87

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Alternative approach of cell encapsulation by Volvox spheres

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ARTICLE INFO

Article history:
Received 6 August 2014
Received in revised form 2 March 2015
Accepted 20 May 2015
Available online 22 May 2015

Keywords:
Volvox sphere
Alginate
Collagen
Mesenchymal stem cell
Cell encapsulation

ABSTRACT

Volvox sphere is a bio-mimicking concept of a biomaterial structure design able to encapsulate chemicals, drugs and/or cells. The aim of this study was to prepare Volvox spheres encapsulating AML12 liver cells and mesenchymal stem cells (MSCs) via a high voltage electrostatic field system. The results demonstrated that AML12 liver cells and MSCs could be successfully encapsulated into the inner spheres and the outer sphere of the Volvox spheres. The improved cell viability of MSCs was achieved by the addition of collagen and polyethylene glycol into the preparation components of the Volvox spheres. Collagen material potentially provides extracellular matrix-like structure for cell adhesion while polyethylene glycol provides a void/loose space for permeability of metabolites. The encapsulated MSCs were able to differentiate into hepatocytes or hepatocyte-like cells and express liver cell markers including albumin, alpha feto-protein and cytokeratin 18. The encapsulated cells secreted albumin to about 140 ng on day 14. Based on these observations, we conclude that Volvox spheres can be used as an alternative approach to encapsulate multiple types of cells, here AML12 hepatocyte cell line and MSCs. Nevertheless, efforts are still needed to improve the viability of the encapsulated cells and increase the differentiation of MSCs into functional liver cells.

1. Introduction

By definition, cell encapsulation means entrapping viable cells within immobilization devices surrounded by a semi-permeable membrane [1]. Culturing cells in such devices (for example, hydrogel or microspheres) offers many advantages over monolayer culturing systems. A three dimensional environment provides a large surface area for cell growth, enabling free exchange of nutrients, oxygen and metabolic products between the entrapped cells and their surroundings and reduces or avoid inflammation evoked by the host's immune system when transplanted [2].

Many studies focus on one type of cell encapsulation used for cell immobilization [3,4], cryopreservation [5,6], transplantation [7,8], artificial organs, etc., where very few have reported on multiple types of cell encapsulation and their potential applications. When more than two different types of cells being cultured together, that enabling cell to cell interactions through direct contact or exchange of soluble factors, which play important roles in regulating the fates and functions of the cells, such as proliferation and differentiation [9,10].

Volvox sphere is a unique structural design consisting of a large vesicle (outer-sphere) containing several smaller vesicles (inner-spheres). Volvox spheres have shown the potential in encapsulating multiple types of drugs in our previous study and we believe that they can also be employed to encapsulate cells for therapeutic applications and tissue engineering. The Volvox sphere structure provides the encapsulated cells with a 3D environment similar to that of the extracellular matrix (ECM). Alginate is a non-toxic material that has demonstrated biocompatibility in a range of applications. Furthermore, alginate is widely employed to form hydrogel for cell encapsulation, due to its mild gelling reaction in the presence of calcium chloride. However, alginate inherently lacks of appropriate ligands that are crucial to promote and regulate cell interactions. To overcome this problem, researchers chemically introduce peptides such as arginine–glycine–aspartic acid (RGD) into alginate to enhance the adhesion and proliferation of encapsulated cells [11]. In this study, we used an alternative biopolymeric material, gelatin or collagen to replace the expensive RGD peptide. Gelatin and collagen could also act as a component of ECM and provide binding sites for cell adhesion to improve cell proliferation or differentiation.

For tissue engineering purposes, it is often desirable to co-culture two or more cell types simultaneously. However, different cell types may proliferate at different rates that may result in the overgrowth of one cell type. Here, we utilized alginate as the Volvox sphere material to isolate the encapsulated cells, reduce the cell-to-cell contact and thus avoid the above-mentioned situation. Volvox sphere is a 3D structure that can encapsulate different types of cells and allows the study of the cell-to-cell interactions in a close vicinity area. In this study, natural polymer alginate was employed to produce Volvox spheres (encapsulate AML 12 hepatocyte cells into the inner spheres and MSCs in the outer spheres), and is the first trial to evaluate the performance of

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http://dx.doi.org/10.1016/j.msec.2015.05.063
0928-4931/

Volvox spheres as cell encapsulation application. The proliferation and differentiation of MSCs were examined. The variation of materials, components, construction of the Volvox spheres, or cell types will be studied further in future studies.

2. Materials and methods

2.1. Materials

Alginate (medium viscosity, 1.5×10^5 Da), gelatin (type A from porcine skin, 300 Bloom), poly-L-lysine (PLL, 1.5×10^4 Da), sodium chloride were purchased from Sigma (St. Louis, MO, USA). Collagen (3 mg/mL) was purchased from Advanced BioMatrix (San Diego, CA). Calcium chloride was purchased from J.T. Baker (Japan). Sodium citrate was purchased from Merck (NJ, USA). An injection pump (74900 series, USA) and a high-voltage power supply (series 230, USA) were supplied by Cole-Parmer and Bertan, respectively. All chemicals used in this study were of reagent grade.

2.2. MSC isolation and culture

Bone marrow was aspirated from the femur bones of 3-week-old Sprague–Dawley rats after dislocation. The marrow was collected and diluted with 4 mL of Dulbecco's modified Eagle's medium (DMEM, Gibco) mixed with an equal volume of 1.073 g/mL Percoll solution (Sigma, USA) and then centrifuged at 1500 g for 15 min. The enriched cells were cultured in a low-glucose DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. After 3 days, nonadherent hematopoietic cells were discarded and the adherent cells were washed with PBS. The culture medium was replenished every 3 days. When the cells reached approximately 90% confluence, they were trypsinized for passage with 0.25% trypsin/EDTA. All experiments were performed using cells from the second passage. A hepatocyte cell line, AML12, was cultured in DMEM/F12 medium.

2.3. Production of MSCs and AML 12 liver cell-encapsulated Volvox spheres

Prior to the production of Volvox spheres, alginate powder was autoclaved first and dissolved in distilled water to reach a concentration of 0.5% (w/v) and 1% (w/v) alginate solution. Gelatin material was sterilized by autoclave and collagen solution was prepared under aseptic condition. The production of Volvox spheres encapsulating cells via high voltage electrostatic field system (HVEFS) was according to our previous settings [12]. Briefly, a final concentration of 1×10^7 cells/mL AML12 cells was mixed into sterilized alginate solution (1 mL), and extruded into 100 mM calcium chloride via HVEFS with parameters of 1 mL/h pump rate, 26 G needle size and 4 kV voltage to prepare the cell-encapsulated inner spheres. These produced microspheres were treated with 0.05% (w/v) PLL for 5 min and washed carefully with saline buffer solution. Following on, these PLL-fabricated microspheres were mixed with 1×10^7 cells/mL of MSCs into 1 mL alginate solution and used a larger diameter 20 G needle to produce cell-encapsulated Volvox spheres. A schematic diagram for the production of cell-encapsulated Volvox spheres is presented in Fig. 1.

2.4. Live/dead staining

The cell viability of the encapsulated cells (AML 12 only or AML12 with MSC cells) inside the Volvox spheres was first examined with a live/dead cell assay (Invitrogen, UK). Briefly, 1 mL of PBS solution containing 2.5 μL/mL of 4 μM ethidium homodimer-1 (EthD-1) assay solution and 1 μL/mL of 2 μM calcein AM solution was prepared. Then, 100 μL of the live/dead assay solution was added to each culture for 15 min, and the mixture was placed in an incubator at 37 °C with a humidified atmosphere and 5% CO_2. The staining solution was removed,

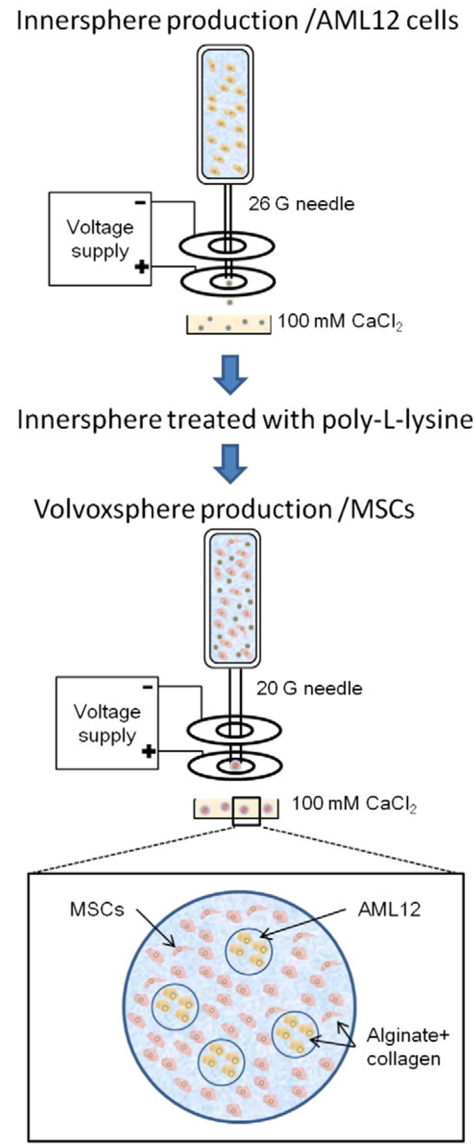

Fig. 1. A schematic representation of the production of Volvox spheres. AML12 cells with a final concentration of 1×10^7 cells/mL were mixed into sterilized alginate solution, and extruded into 100 mM calcium chloride via HVEFS with parameters of 1 mL/h pump rate, 26 G needle size and 4 kV voltage to prepare the cell-encapsulated inner spheres. These produced microspheres were treated with 0.05% (w/v) PLL for 5 min and washed carefully with saline buffer solution. Following on, these PLL-fabricated microspheres were mixed with 1×10^7 cells/mL of MSCs into 1 mL alginate solution and used a larger diameter 20 G needle to produce cell-encapsulated Volvox spheres.

and the samples were viewed under a fluorescence microscope with 494-nm (green, calcein) and 528-nm (red, EthD-1) excitation filters (Olympus IX71, Japan).

2.5. MTT assay for MSC cell viability

In order to evaluate the viability and differentiation of the encapsulated MSCs in the vicinity of liver cells, the Volvox spheres were dissolved by treatment of sodium citrate. The cell viability of the encapsulated MSCs was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. At defined periods of incubation (days 0, 3 and 7), 50 μL of MTT solution (5 mg/mL) was added, and the cell-encapsulated Volvox spheres were incubated for an additional 3 h. The Volvox spheres were then treated with 50 mM sodium citrate to dissolve the outer layer of alginate and collected the encapsulated MSCs by centrifugation. The formazan precipitate was