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Encapsulation of Human Hematopoietic Stem Cells with a Biocompatible Polymer

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Abstract. Hematopoietic stem cells (HSCs) are the progenitor for all blood types including leukocytes, erythrocytes and platelets. Normally, the human body is programmed to maintain sufficient level of hematopoietic stem cells or derivatives. However, in certain conditions, including repeated chemotherapy, leukemic cancer, or genetic mutation, bone marrow disorders; the HSC transplantation becomes necessary. HSC transplantation deals with two major obstacles: human leukocyte antigen (HLA) matching and high cell dose. Non-match HSC transplantation activates immune reaction and induces graft versus host diseases, which can be fatal to the recipient. Hydrogel encapsulation technology offers a promising method to solve these problems. In this study, we introduced a feasible HSC encapsulation technique with a biocompatible polymer. The cells were isolated from umbilical cord blood with a density gradient method and continued by a magnetic bead separation based on CD34 expression. HSCs, identified as CD34+ cells, were encapsulated with collagen type 1 by a hanging drop method. On day three, the capsule was degraded with collagenase treatment and the cells were analyzed to evaluate the effect of encapsulation on cell viability and stemness. It was found that the cell viability remained high on encapsulated cells. However, the ratio of CD34+ cells was slightly decreased on encapsulated cells than non-encapsulated cells. As a conclusion, the employed method is suitable to encapsulate HSCs without compromising its viability and the modification of coating material is needed to maintain the stemness of HSCs.

Keywords: Biocompatible, encapsulation, hematopoietic, stem cells, polymer

INTRODUCTION

Hematopoietic stem cells (HSCs) are the progenitor for all blood components including leukocytes, erythrocytes and platelets [1]. HSCs can be isolated from various sources such as bone marrow, umbilical cord blood (UCB), and peripheral blood, with unique proliferating capacity [2]. Normally, the human body is programmed to maintain sufficient level of hematopoietic stem cells or derivatives. However, in certain conditions, including repeated
chemotherapy, leukemic cancer, or genetic mutation, bone marrow disorders, and the HSC transplantation becomes necessary. Human isolated HSCs is readily available source for HSC transplantation as long as good manufacture practices/clinical standards are applied [3]. While mesenchymal stem cells transplantation is widely reported, the success of HSC transplantation is limited. This is because HSC transplantation has to deal with two major problems: human leukocyte antigen (HLA) matching and low therapeutic efficacy. Modification of delivery system, i.e. encapsulation technology, is expected to improve the HSC therapy.

Hydrogel encapsulation technology offers a promising method for stem cell therapy. Unlike injection of free-suspended stem cells, the use of encapsulation technology can provide longer protection against immune cells and desirable microenvironment for transplanted cells. The current study is attempted to develop a feasible technique for HSC encapsulation intended for clinical application. In brief, mononuclear cells were isolated from human umbilical cord blood based on gradient density principle. Subsequently, the HSCs were purified from mononuclear cells with a magnetic separation technique based on the expression of CD34, a specific marker for hematopoietic progenitor. Finally, the cells were encapsulated with a biocompatible material, collagen, by hanging drop method.

Despite the prospective future for HSC therapy, the study on HSC encapsulation/three-dimensional structure is limited for ex vivo applications [4,5]. Leeve et al. (1991) reported microencapsulation of mononuclear cells without investigating the stem cell potency [4]. In other studies, Kim et al. (2003) and Avitabile et al. (2012) utilized collagen-based surface for HSC expansion [6,7]. To our knowledge, our study is the first report on the HSC encapsulation intended for multipotent stem cell therapy. The current study is expected to promote the HSC transplantation for regenerative medicine.

MATERIALS AND METHODS

HSC Isolation

UCB was collected from healthy donors and all participants were given an informed consent prior to the UCB collection. The protocols of this study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia – Dr. Cipto Mangunkusumo General Hospital and the studies were conducted in compliance with the Helsinki Declaration. The mononuclear cells were isolated from human UCB by a density gradient method. Briefly, each 20 ml umbilical cord blood was transferred carefully on 20 mL Ficoll-Paque (GE Healthcare, USA). The mixture was centrifuged at 400 × g 22 °C for 10 min (no break). Buffy coat layer was transferred to a new tube containing 20 mL 0.9 % NaCl solution and then centrifuged at 650 × g 22 °C for 10 min. Subsequently, HSCs were purified from mononuclear cells with EasySep Human Cord Blood CD34 Positive Kit II (Stem Cell Technologies, USA) according to the manufacturer’s instruction. The positive CD34 selected cells were re-suspended in complete medium composed of RPMI (Biovet, USA) supplemented with 10% UCB serum, antibiotic-antimycotic (Life Technologies), and heparin (10 IU/mL).

Flow Cytometry Analysis

The surface markers analysis was conducted to measure the purity of isolated HSCs. Briefly, the collected cells were washed twice with phosphate-buffered saline (PBS). Cells were then stained with Stem Cell Enumeration antibody cocktail containing FITC-CD45, PE-CD34 and 7-AAD (BD Biosciences, USA) for 30 min at 4 °C and washed twice by PBS. The stained cells were then analyzed on a FACS Aria II flow cytometer (BD Biosciences, USA).

Encapsulation

The solutions and mixing process were kept at 4 °C to prevent premature gelling. Collagen I Bovine (10 mg/ml; Life Technologies, USA) was neutralized with 1N NaOH. The solution was mixed with cell suspension (6×10^6 cells/mL) gently to avoid bubble formation. The mixture was pipetted onto plastic dish lid (20 or 50 μL/drop) and immediately put on hanging position. The dish was incubated at 37 °C for 30 minutes.
Culture Assay

The non-encapsulated and encapsulated HSCs were cultured on a complete medium. The culture was incubated at 37 °C in a fully humidified incubator with 5% CO₂. The gel was dissolved with one volume of collagenase (1 mg/mL; GID Group, USA) and incubated for 30 min at 37 °C. The dissolved gel was removed by centrifugation at 650 ×g 22 °C for 10 min and then the cells were washed twice with PBS. The cells were then analyzed with flow cytometer as described above.

Cell Loading Capacity

The encapsulated cells (20 µL/drop) were cultured as previously described. On day one, the gel was dissolved by collagenase treatment and the total cell number was counted by a dye exclusion method with trypan blue. The data of cell loading capacity were presented as mean ± standard error.

RESULTS

The mononuclear cells were harvested from fresh UCB based on density gradient method with Ficoll. Approximately 9.4 × 10⁸ cells/mL mononuclear cells were collected from 120 mL UCB. The HSCs were purified from mononuclear cells with a magnetic separation with CD34 as a specific protein marker. After magnetic separation, it was collected 1.9 × 10⁶ cells/mL as positive CD34 selected cells, indicating that ~0.2% of UCB-derived mononuclear cells were CD34⁺ cells.

Flow cytometry analysis was conducted to evaluate cell viability and specific protein expressions in positive CD34 selected cells. The results of flow cytometry analysis are depicted in Figure 1. The 7-AAD staining was used to exclude non-viable cells. It was shown that ~97.4% selected cells were viable (Figure 1(a)). CD34 is the universal protein marker for hematopoietic lineage [8]. Figure 1(b) shows that ~89.3% of selected cells were identified as CD34 expressing cells suggesting high purity of HSCs. CD45 is an important specific marker for hematopoietic lineage with expression level corresponding to developmental stages. The earliest embryonic HSCs lack CD45 expression and more mature HSCs have higher CD45 expressions [9]. Figure 1(c) shows that positive CD34 selected cells consisted of several sub-populations based on CD45 expression.

![FIGURE 1](image-url) Flow cytometry analysis results of CD34 positive selected cells isolated from human umbilical cord blood: (a) Population of non-viable and viable cells; (b) Population of CD34⁺ and CD34⁻ cells; (c) Population of CD45⁺ and CD45⁻ cells.

Collagen is the most abundant protein in human body [10]. Biodegradability and biocompatibility make collagen as an attractive material for tissue engineering. In the present study, we successfully fabricated collagen-encapsulated HSCs with hanging drop method. The images of encapsulated cells are shown in Figure 2. The 3D collagen gel was formed after 30 minutes of incubation at 37 °C (Figure 2(b)). The cells were homogenously distributed in the solid-core capsules (Figure 2(c)). At 24 hours after encapsulation, ~80% of cells remained viable. It was expected that gas exchange may be critical for cell survival in solid-core capsule. By hanging drop method, the minimum volume to maintain spherical shape of capsule was 20 µL. If the volume is less than 20 µL, it causes
the shape to be flat and capsules could not be formed. The loading capacity of a capsule was counted on day one after encapsulation, and it was about $1.5\pm0.4 \times 10^4$ cells per capsule (20 µL).

![Figure 2](image)

**FIGURE 2.** HSC encapsulation with hanging drop method: (a) Gel-cells solution just after hanging; (b) Gel formation after 30 minutes incubation at 37 °C; (c) Microscopic image of encapsulated cells (bar indicates 100 µm).

The encapsulated HSCs were cultured on a complete medium to evaluate the effect of encapsulation for cell stemness and viability. On day three, cell viability remained high (>90%) for both encapsulated and non-encapsulated cells (Figure 3(a)). This result suggested that the material and encapsulation method were relatively non-toxic for HSCs. However, it was found that the ratio of CD34+ cells of encapsulated HSCs was lower than that of non-encapsulated cells. The reduced ratio of CD34+ cells with uncompromised viability indicates that a part of cells differentiated into a specific lineage. As reported by Celebi et al., high concentration of collagen type I coating during HSC culture reduced the CD34 frequencies [11]. In addition, the study found that collagen coating increased the ratio of myeloid progenitor, further suggesting that collagen type I might promote cell differentiation.
FIGURE 3. Evaluation of encapsulated and non-encapsulated (No) HSCs on day 3 after culturing: (a) Cell viability; (b) Ratio of CD34+ cells.

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