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Microencapsulated feeder cells as a source of soluble factors for expansion of CD34$^+$ hematopoietic stem cells

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Abstract

Expansion of hematopoietic stem cells (HSCs) from cord blood is highly desired for treatment and transplantation of adult patients for hematologic diseases. For efficient proliferation of HSCs, CD34$^+$ cells from cord blood were co-cultured with microencapsulated murine stromal cells (HESS-5) or immortalized human mesenchymal stem cells (MSCs) in their conditioned media (CM). Bioactive substances for HSC proliferation in CM at the onset of culture are likely consumed by HSCs with time, and co-culturing with microencapsulated feeder cells ensures a continuous supply. The cell number of CD34$^+$ cell progeny efficiently increased under these culture conditions, and progeny were analyzed by flow cytometry, the colony assay and the cobblestone area-forming cell (CAFC) assay. Total nucleated cells and CD34$^+$ cell number increased 194- and 7.4-fold, respectively, in the presence of microencapsulated HESS-5 in CM. Colony forming cells and CAFCs were well maintained. The effective expansion of total cells and maintenance of primitive progenitor cells suggest that transfusion of the progeny obtained from CD34$^+$ cell culture with microencapsulated HESS-5 in CM could shorten the time to engraftment by bridging the pancytopenic period and support functional hematopoietic repopulation.

Keywords: Cell encapsulation; Microcapsule; Hematopoietic stem cell; Stromal cell; Co-culture; Expansion

1. Introduction

Umbilical cord blood (UCB) collected from a newborn baby is accepted as one of the promising sources of hematopoietic stem cells (HSCs) for transplantation [1]. The first UCB cell transplantation was performed by Gluckman et al. in 1989 [2]. UCB cell recipients have been limited to children with an average weight of 20 kg, as the major disadvantage of UCB cell transplantation is the low cell dose, which results in slower time to engraftment and higher rates of engraftment failure than those that occur with bone marrow transplantation. Numerous efforts have been made to expand HSCs *ex vivo* to improve engraftment time and reduce the graft failure rate in the recipients, particularly in developing this therapy for adult patients [3–5]. *Ex vivo* manipulation of HSCs dates back to the development of Dexter-type culture techniques, which identified the benefits of stroma for the maintenance of hematopoietic colony-forming units [6]. At the moment, the co-culture of UCB cells with murine stromal cells, such as M2-10B4 [7], MS-5 [8], OP9 [9,10], or HESS-5 [11,12], is the currently accepted method for expansion of HSCs.

In these co-culture systems, the stromal cells are generally considered to provide signal transductions

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through two major mechanisms to promote the expansion of HSCs. First, stromal cells secrete various soluble factors, such as growth factors and cytokines, that stimulate the proliferation and differentiation of HSCs, and provide niche as an appropriate environment for the proliferation and maintenance of HSCs. Secondly, co-culturing with stromal cells provides direct cell–cell contact, which plays an important role in HSC proliferation and differentiation.

Use of the xenogeneic stromal cells to expand HSCs in the clinical setting, however, has encountered several obstacles. Public heath officials are concerned about zoonosis and other unknown infectious diseases caused by contamination of xenogeneic feeder cells and concerned about pathogens which they carry, such as bovine spongiform encephalopathy (BSE) and porcine endogenous retrovirus (PERV) [13]. Furthermore, rejection or unexpected reactions against transplanted HSCs are shown to be caused by xenogeneic cell debris or proteins and polysaccharide derived from stromal cells [14]. Thus, the development of a co-culture system free from contamination of feeder cells or their debris is highly desired.

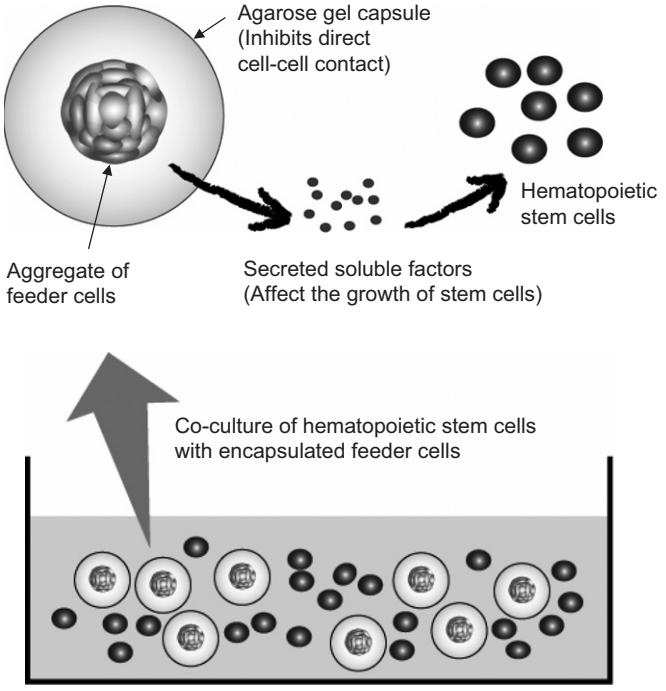
Conditioned media (CM) of the feeder cells has been examined to avoid the risks pointed above [15–17]. Although sufficient amounts of essential bioactive substances exist in CM for HSCs proliferation at the start of the culture, the cultured HSCs eventually consume the nutrients, and thus the efficacy of CM decreases with time. For more efficient proliferation of HSCs, the culture medium supplemented with CM should be frequently changed or a continuous supply of bioactive substances should be included in the UCB cell culture system.

In this study, efficacy of microencapsulated feeder cells was examined as a continuous supply unit of bioactive substances as shown in Scheme 1. Spherical aggregates of feeder cells were enclosed into agarose gel microcapsules and then added to the culture flask to co-culture with UCB cells. Agarose gel is permeable to various chemicals and low molecular proteins, such as amino acids, glucose and growth factors [18–20]. Agarose gel also can inhibit contact between feeder cells and UCB cells [21,22]. We proposed a novel culture system to expand HSC using the encapsulated feeder cells.

2. Materials and methods

2.1. Cytokines and monoclonal antibodies

A mixture of recombinant human (rh) stem cell factor (SCF), rh-thrombopoietin (TPO) and rh-Flt3 ligand (StemSpan CC110) was purchased from StemCell Technologies Inc. (Vancouver, Canada). Monoclonal antibodies used for cell surface markers analyses by the flow cytometry are as follows: fluorescein isothiocyanate (FITC)-conjugated anti-human CD34 (clone 581) and allophycocyanin (APC)-conjugated anti-human CD45 (clone HI30), purchased from BD Pharmingen (CA, USA), and phycoerythrin (PE)-conjugated anti-human CD38 (clone HB7), purchased from Becton Dickinson (CA, USA).



Scheme 1. Schematic representation of a co-culture system of $CD34^+$ cells with microencapsulated feeder cells. Cell aggregates, which are formed by the hanging drop method, are enclosed into agarose gel microcapsules. Soluble factors, such as growth factors and cytokines, are secreted from feeder cells and permeate through the agarose gel into the culture medium. $CD34^+$ cells are expected to proliferate and differentiate in response to those factors.

2.2. Culture of murine stromal cell line and human mesenchymal stem cell line

Two cell lines, HESS-5 and MSC-3, were used as feeder cells. HESS-5 was established from murine bone marrow as hematopoiesis-supportive stromal cells [11,12]. HESS-5 cells were maintained in minimal essential medium alpha (MEM-alpha; Invitrogen Corporation, CA, USA) supplemented with 10% horse serum (JRH Biosciences, CA, USA), 100 U/mL of penicillin and 100 μg/mL of streptomycin (Invitrogen) at 37 °C under 5% CO_2 in a humidified atmosphere, and subcultured at $5–8 \times 10^3$ per cm^2 every 3–4 days.

MSC-3 was a human bone marrow-derived immortalized mesenchymal stem cells (MSC) clone established by Okamoto et al. [23] that supports the proliferation of HSCs (S. Fujita et al., publication in preparation). MSC-3 was kindly donated by Dr. Aoyama of the Institute for Frontier Medical Sciences, Kyoto University. MSC-3 cells were maintained in Dulbecco's Modified Essential Medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (BIOWEST, France), 100 U/mL of penicillin and 100 μg/mL of streptomycin (Invitrogen) at 37 °C under 5% CO_2 in a humidified atmosphere, and subcultured at $5–8 \times 10^3/cm^2$ every 3–4 days.

2.3. Microencapsulation of feeder cells

Prior to microencapsulation of feeder cells, spherical cell aggregates were prepared by the hanging drop method as reported previously [24]. In brief, HESS-5 or MSC-3 cells were suspended in the respective maintaining media at a certain cell density, and 20 μL of the cell suspension were spotted on a lid of a multi-tray (Asahi Techno Glass Corp., Tokyo, Japan). The lid was turned upside down to hang drops and statically cultured at 37 °C under 5% CO_2 in a humidified atmosphere for 3 days. After hanging, the cells formed a spherical aggregate in a drop.