Acta Biomaterialia 8 (2012) 1749–1758

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

# Effect of the surface density of nanosegments immobilized on culture dishes on ex vivo expansion of hematopoietic stem and progenitor cells from umbilical cord blood

Li-Ying Chen [a,1], Yung Chang [b,1], Jui-Shiang Shiao [a], Qing-Dong Ling [c,d], Yu Chang [e], Yung Hung Chen [e], Da-Chung Chen [f], Shih-Tien Hsu [f], Henry Hsin-chung Lee [g], Akon Higuchi [a,c,h,*]

[a] Department of Chemical and Materials Engineering, National Central University, Jhongli, Taoyuan 32001, Taiwan
[b] Department of Chemical Engineering, R&D Center for Membrane Technology, Chung Yuan Christian University, 200, Chung-Bei Road, Chungli, Taoyuan 320, Taiwan
[c] Cathay Medical Research Institute, Cathay General Hospital, No. 32, Ln 160, Jian-Cheng Road, Hsi-Chi City, Taipei 221, Taiwan
[d] Institute of Systems Biology and Bioinformatics, National Central University, No. 300, Jhongda Road, Jhongli, Taoyuan 32001, Taiwan
[e] Department of Obstetrics and Gynecology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, No. 100, Tzyou 1st Road, Kaohsiung 807, Taiwan
[f] Li Shin Hospital, 77, Kuangtai Road, Pingjen City, Tao-Yuan County 32405, Taiwan
[g] Cathay General Hospital, No. 280, Sec. 4, Jen-Ai Road, Taipei 106, Taiwan
[h] Department of Reproductive Biology, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

ARTICLE INFO

*Article history:*
Received 5 September 2011
Received in revised form 25 November 2011
Accepted 4 January 2012
Available online 10 January 2012

*Keywords:*
Hematopoietic stem cells
Surface modification
Extracellular matrix
RGD peptide
Cell culture

ABSTRACT

Umbilical cord blood (UCB) is an attractive source of hematopoietic stem and progenitor cells for hematopoietic stem cell (HSC) transplantation. However, the low number of HSCs obtainable from a single donor of UCB limits direct transplantation of UCB to the treatment of pediatric patients. In this study, we investigated the ex vivo expansion of HSCs cultured on biomaterials grafted with several nanosegments, i.e. polyamine, fibronectin, RGDS, and CS1 (EILDVPST), at several surface densities. No direct correlation was found between fold expansion of HSCs and physical parameters of the culture dishes, i.e. surface roughness and water contact angle of the culture dishes. However, a small amount of grafted amino groups, less than 0.8 residual μmol $cm^{-2}$, on the dishes was effective for the ex vivo expansion of HSCs. A high amount of grafted amino groups hindered the ex vivo expansion of HSCs on the dishes. HSCs cultured on dishes with a high concentration of CS1 (2.40 residual μmol $cm^{-2}$) showed greater expansion of HSCs and more pluripotent colony-forming units (i.e. colony-forming unit–granulocyte, erythroid, macrophage, and megakaryocyte (CFU-GEMM)) than those on fibronectin-grafted and polyamine-grafted dishes. These data suggest that the specific interaction between HSCs and CS1 helps to maintain the pluripotency of HSCs during the ex vivo expansion of HSCs.



## 1. Introduction

Hematopoietic stem and progenitor cells (HSCs) are multipotent cells that have the specific capacity to self-renew and differentiate into all types of mature blood cells [1–4]. Intravenous infusion of HSCs has been commonly performed to treat patients suffering from hematological disorders and malignant diseases after radiation and/or chemotherapy [5–8]. Bone marrow transplantation has frequently been used for HSC transplantation [6]. However, HSCs from peripheral blood and umbilical cord blood (UCB) have recently been used for HSC transplantation [9,10]. In particular, UCB is an attractive source of HSCs because there is lower risk of graft-vs.-host disease (GVHD) for UCB transplantation compared to bone marrow or peripheral blood transplantation [11–15]. Furthermore, there is no risk of side-effects for donors when HSCs are collected from UCB, whereas some side-effects for donors have been reported when HSCs are collected from peripheral blood or bone marrow. For example, a rate of two deaths out of 8000 bone marrow donors has been reported [6]. However, the low number of HSCs and small volume (50–150 ml) obtainable from a single donor of UCB limits direct transplantation of UCB to the treatment of pediatric patients [4]. A density of $\sim 1.7 \times 10^7$ $CD34^+$ cells $kg^{-1}$ is necessary for the transplantation of UCB into patients [11]. Therefore, UCB transplantation has been limited to children with an average weight of 20 kg [16–18]. The major disadvantage of such transplantation is the low cell dose, which results in slower time to engraftment and higher rates of engraftment failure than with bone marrow transplantation. Numerous efforts have been made

* Corresponding author at: Department of Chemical and Materials Engineering, National Central University, Jhongli, Taoyuan 32001, Taiwan. Tel.: +886 3 4227151x34253; fax: +866 3 280 4271.
*E-mail address:* higuchi@ncu.edu.tw (A. Higuchi).
[1] These authors contributed equally to this work.

1742-7061/$ - see front matter © 2012 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2012.01.002

to expand HSCs ex vivo to improve engraftment time and reduce the graft failure rate, particularly in developing this therapy for adult patients [16–18].

The success of HSC transplantation depends on both the dose of HSCs and the pluripotency of the HSCs transplanted [19,20]. The goal of ex vivo HSC expansion is to produce a sufficient number of cells for engrafting and to sustain long-term hematopoiesis by mimicking bone marrow niche [2,3,21,22]. HSCs are located in the bone marrow niche in vivo, which provides (a) cell–cell interaction between stromal cells and HSCs; (b) cytokines (growth factors) such as FLt3, stem cell factor (SCF), interleukin-3 (IL-3), interleukin-6 (IL-6), erythropoietin (EPO), thrombopoietin (TPO), and granulocyte-macrophage colony stimulating factor (GM-CSF) [20]; and (c) interaction between extracellular matrix and HSCs. In this study, we focus on biomaterials for the ex vivo expansion of HSCs from UCB. Several researchers have attempted to develop biomaterials for the ex vivo expansion of HSCs by mimicking the bone marrow niche [1,21–28].

One strategy is to use surface-modified nanofibers prepared by electrospinning. Chua et al. analyzed the ex vivo expansion of HSCs on unmodified, carboxylated, hydroxylated, or aminated nanofibers and films [23]. Aminated nanofibers and films were the most efficient at supporting the expansion of HSCs (195- and 178-fold, respectively). Although the aminated nanofibers resulted in a higher degree of expansion of HSCs than the aminated films, the difference appears insignificant. The effect of amino group surface density on the expansion of HSCs was not reported [23].

Extracellular matrix (ECM) elements are crucial in the bone marrow niche for the support of HSCs. They mediate the adhesive interactions between HSCs and cell adhesion molecules (CAMs), which are of critical importance in the regulation of hematopoiesis by interacting with HSCs in the bone marrow niche, in close contact with osteoblasts and stromal cells, thus exposing HSCs to the signaling molecules the osteoblasts and stromal cells secrete [4,21,29,30]. Feng et al. investigated the ex vivo expansion of HSCs on fibronectin (FN)-immobilized and collagen (COL)-immobilized polyethylene terephthalate (PET) mesh [21]. HSCs cultured on FN-immobilized PET mesh yielded the highest expansion of HSCs and long-term culture-initiating cells. FN appears to be preferable to other ECMs for the ex vivo expansion of HSCs [21].

Two of the most important adhesion domains in fibronectin are the connecting segment-1 (CS1, EILDVPST) and RGD (arginine-glycine-aspartic acid) motifs, both of which are recognized by surface receptors on early hematopoietic progenitors. Jiang et al. investigated the ex vivo expansion of HSCs from UCB on PET films with immobilized CS1 and RGD (GRGDSPC) motifs [24]. The highest cell expansion was observed on the CS1 peptide-modified films, and the cells cultured on the CS1-immobilized film could generate positive engraftment after 10 d of ex vivo expansion from 600 $CD34^+$ cells. The PET film immobilized with the RGD peptide was less efficient than the corresponding CS1 peptide film. These results suggested that covalently immobilized adhesion peptides could significantly influence the proliferation characteristics of HSCs cultured from UCB [4,24].

No systematic research has investigated the effect of nanosegment species such as FN, CS1, RGDS, and segments with amino groups immobilized on culture dishes on the ex vivo expansion of HSCs. Furthermore, no studies have examined the effect of the surface density of the nanosegments immobilized on culture dishes on the ex vivo expansion of HSCs. In this study, polymers with amino groups were grafted onto polystyrene dishes at various surface densities, and the effects of the surface densities of the amino groups on the ex vivo expansion of HSCs were investigated. Furthermore, polystyrene dishes with FN, CS1, CS1i, RGDS, RGES, or segments with amino groups were prepared systematically, and the optimal nanosegments for the ex vivo expansion of HSCs on the biomaterials were examined.

## 2. Materials and methods

### 2.1. Materials

Fibronectin (human, F2006) was purchased from Sigma–Aldrich (St Louis, MO, USA). The oligopeptides CS1 (EILDVPST), CS1i (EILEVPST), RGDS (GRGDSPC), and RGES (GRGESPC), were obtained from MDBio Inc., (Piscataway, NJ, USA). Non-treated 35-mm polystyrene dishes (PS, 35-1008) and tissue culture polystyrene dishes (TCPS, 35-3001) were purchased from Becton Dickinson and Company (Franklin Lakes, NJ, USA). 1,6-Dimethyl suberimidate dihydrochloride (DMS, 74835) and 2-aminoethyl methacrylate hydrochloride (AMA, 516155) were obtained from Sigma–Aldrich. Serum-free medium (StemSpan™ SFEM), StemSpan™ CC110 cytokine cocktail, and MethoCult™ GF H4434 were purchased from StemCell Technology, Inc., (British Columbia, Canada). Low-density lipoprotein (LDL, L8292) was purchased from Sigma–Aldrich. Anti-CD34 antibody conjugated with PE (A07776) and anti-CD45 antibody conjugated with FITC (IM2653K) were obtained from Beckman Coulter Inc. (Brea, CA, USA). 7-AAD (A07704), Optilyse C (IM1401), and flow-count bead solutions (7547053) were purchased from Beckman Coulter. Other chemicals, which were purchased from Sigma–Aldrich, were of reagent grade and used without further purification. Ultrapure water produced by a Milli-Q system (Millipore Corporation, Billerica, MA) was used throughout the experiments.

### 2.2. Preparation of PS dishes grafted with polymerized AMA

PS dishes were pretreated with a continuous stream of an $O_3/O_2$ mixture in a reactor at 25 °C. In this reactor, ozone generated using a custom-built ozone generator (Model OG-10PWA, Ray-E Creative Co. Ltd, Taipei, Taiwan) was bubbled through ultrapure water at a flow rate of 360 l $h^{-1}$. After the ozone treatment, the reactor was cooled quickly to 4 °C, and the ozone-treated PS dishes were dried under vacuum for 30 min at 25 °C to remove residual water. Subsequently, the ozone-treated PS (PS-ozone) dishes were immersed in 0.3–320 mM (0.005–5% w/w) aqueous AMA solution under argon gas with purging at 60 °C and constant stirring for 48 h. A schematic illustration of the reaction is shown in Fig. 1. After the reaction, PS dishes grafted with polyAMA (PS-AMA) were washed carefully three times with ethanol to remove unreacted monomers and homopolymers, then washed with ultrapure water three times. Finally, the PS-AMA dishes were dried under vacuum at 25 °C. The term PS-AMA-X (e.g. PS-AMA-0.3) dishes refers to dishes prepared with X mM (e.g. 0.3 mM) of aqueous AMA solution.

### 2.3. Preparation of PS dishes grafted with ECM and oligopeptides

The fibronectin solution was diluted to 10 μg $ml^{-1}$ with phosphate-buffered saline (PBS), pH 7.4. The CS1 (EILDVPST), CS1i (EILEVPST), RGDS (GRGDSPC), and RGES (GRGESPC) oligopeptides were diluted to 10 or 100 μg $ml^{-1}$ with PBS. CS1i is the control oligopeptide of CS1, which does not have biological activity due to the change of aspartic acid (D) on CS1 into glutamic acid (E) on CS1i. RGES is the control oligopeptide of RGDS, which does not have biological activity.

Polyepoxy segments were grafted onto PS dishes by plasma polymerization with glycidyl methacrylate (GMA) after the PS dishes had received plasma discharge treatment at 20 W for 5 min under argon gas at 0.2 torr using the same procedures and apparatus as reported in the literature for the modification of polyurethane foaming membranes [4,31,32]. Fig. 1 shows the reaction scheme and procedures for grafting ECM and oligopeptides onto PS dishes. An amino group ($NH_2$) was introduced by reaction