Journal of Biotechnology 164 (2012) 211–219

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

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

Optimization of SCF feeding regimen for *ex vivo* expansion of cord blood hematopoietic stem cells

Zheng Du, Haibo Cai*, Zhaoyang Ye, Wen-Song Tan

State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, PR China

ARTICLE INFO

Article history:
Received 17 April 2012
Received in revised form 11 July 2012
Accepted 20 August 2012
Available online 29 August 2012

Keywords:
Hematopoietic stem cells
Cord blood
Stem cell factor
Ex vivo expansion
Feeding regimen

ABSTRACT

Stem cell factor (SCF) plays important roles in *ex vivo* expansion of hematopoietic stem cells (HSCs). In this study, the effects of dose and feeding time of SCF on *ex vivo* expansion of $CD34^+$ cells were investigated in serum-free medium supplemented with a cytokine cocktail composed of SCF, thrombopoietin (TPO) and flt3-ligand (FL). Among the four tested doses (0, 5, 50 and 500 ng/mL), a SCF dose of 50 ng/mL was demonstrated to be most favorable for *ex vivo* expansion of $CD34^+$ cells, which resulted in 34.22 ± 10.80 and 8.89 ± 1.25 folds of expansion regarding total cells and $CD34^+$ cells, respectively. Meanwhile, the specific growth rate of cells, the consumption rate of SCF and the percentage of $CD34^+c\text{-}kit^+$ cells during the 21-day culture process were analyzed. The results indicated that initial 4-day period was a critical stage for SCF functioning on $CD34^+$ cells during *ex vivo* expansion. Based on this, a modified SCF feeding regimen was proposed, in which SCF (50 ng/mL) was only supplemented on day 0 in the cytokine cocktail and cells were then fed with TPO and FL till the end of culture. It was found that this SCF feeding regimen could expand $CD34^+$ cells efficiently, thus providing a cost–effect expansion protocol for HSCs.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Hematopoietic stem cells (HSCs) are capable of self-renewal and giving rise to all blood cell types, such as myeloid and lymphoid cells under certain physiological or experimental conditions, and represent a very good cell source for cell-based therapy. As a novel therapeutic approach, transplantation of HSCs has already been practiced for a long time in treating severe diseases including malignant solid tumor, congenital immunodeficiency and hematological diseases, as well as organ injuries (Broxmeyer et al., 1989). HSCs can be derived from bone marrow, mobilized peripheral blood and cord blood (CB). Among these, HSCs derived from CB (CB-HSCs) hold several advantages than others such as reduced incidence and severity of acute and chronic graft-*versus*-host disease, longer telomere length, higher telomerase activity, larger hematopoietic colonies and superior *ex vivo* expansion capability (Gluckman, 2000; Hows et al., 1992; Yvonne et al., 2004). To date, CB-HSCs have been successfully transplanted into hundreds of pediatric patients (Goussetis et al., 2010; Sozos and Schenker, 2000). However, the very low frequency of HSCs in CB preparations remains a big barrier to their wider clinical applications, since both efficient engraftment and fast blood reconstitution largely rely on the number of infused HSCs (Ann et al., 2011; Hofmeister et al., 2007; Juliet and Wagner, 2003).

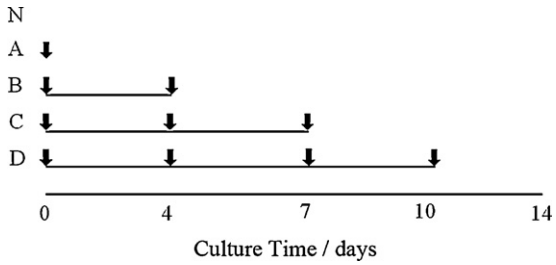
To address such a challenging issue, *ex vivo* expansion of HSCs represents the most promising strategy. So far, various methods have been explored to expand HSCs *ex vivo*, while maintaining their self-renewal potential by following a general principle of mimicking the *in vivo* microenvironment of HSCs (Martinez-Agosto et al., 2007; Susan et al., 2005; Taichman, 2005). Such a microenvironment, also called niche, is essentially composed of stromal cells, soluble factors and extracellular matrix and tightly regulates maintenance and differentiation of HSCs. On one hand, stromal cells can secrete cytokines and other factors to support the growth of HSCs in a paracrine manner (Alakel et al., 2009; Walenda et al., 2011). On the other hand, cytokines themselves are a large family of soluble protein factors, such as stem cell factor (SCF), thrombopoietin (TPO), flt3-ligand (FL), interleukin (IL) and fusion protein (FP) and have proven to be extensively involved in regulating the *in vivo* fate of HSCs (Hofmeister et al., 2007; Hsun et al., 1996; Virginia et al., 1996; Young et al., 2007). Nonetheless, studies have shown that by co-culturing with stromal cells or directly supplementing cytokines, *ex vivo* expansion of HSCs could be improved (Wagner et al., 2007; Walenda et al., 2011; Zhang et al., 2004). In addition, due to the low possibility of immuno-rejection in CB-HSCs, allogenetic transplantation of *ex vivo* expanded CB-HSCs has been tested in clinical trial, demonstrating good safety and efficacy in clinically relevant indexes (Ann et al., 2011).

* Corresponding author at: The State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Mei-Long Road, P.O. Box 309#, Shanghai 200237, PR China. Tel.: +86 21 64251962; fax: +86 21 64252099.
E-mail address: caihaibo@ecust.edu.cn (H. Cai).

0168-1656/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jbiotec.2012.08.007

However, due to the undefined factors of generally used serum in culture medium and the risk of animal pathogens, which might render *ex vivo* expanded cells susceptible to immunorejection upon *in vivo* transplantation, current research efforts aim at developing serum-free and xeno-free culture systems for HSCs expansion, especially based on recombinant human cytokine supplementation (Graça et al., 2000; Luc, 2001). A variety of different combinations of cytokines have been investigated concerning the viability, functionality and proliferation of CB-HSCs during the *ex vivo* culture. Kiyoshi et al. (2006) combined three different cytokines of SCF (50 ng/mL), TPO (50 ng/mL) and FL (50 ng/mL) and achieved 20.1 ± 2.7 folds of expansion regarding total cells after a 5-day co-culture with stromal cells. In contrast, Kishimoto et al. (2009b) used a similar combination of SCF (5 ng/mL), TPO (10 ng/mL) and FL (10 ng/mL) but with a much lower dose for each individual cytokine to expand $CD34^+$ cells on heparin/protamine microparticle-coated culture plates and after a 6-day culture the total cell number was increased by 16.3 folds. In another study, Alakel et al. (2009) tested the cytokine cocktail of SCF (150 ng/mL), FL (50 ng/mL) and IL-3 (50 ng/mL), demonstrating a 19 ± 3 fold expansion of total cells within 7 days. However, the exact relationship between cytokine combination (including cytokine type and dose) and *ex vivo* expansion efficiency remains largely unknown. Among widely used cytokines for *ex vivo* expansion of HSCs, SCF is a 30 kDa glycoprotein and represents an essential one (Reber et al., 2006). It has been established that SCF is able to promote proliferation as well as early differentiation of HSCs (Ding et al., 2012; Kishimoto et al., 2009a; Michelle et al., 2007a). By binding to its cell-surface receptor c-kit, SCF causes c-kit to homodimerize and auto-phosphorylate at tyrosine residues, which subsequently leads to the activation of multiple signaling cascades, including RAS/ERK, PI3-Kinase, Src kinase and JAK/STAT pathways (Rönnstrand, 2004; Kent et al., 2008). It has been reported that SCF has the ability to regulate *ex vivo* expansion of HSCs and prevent human $CD34^+$ cells from apoptosis when applied along with other cytokines (Kawada et al., 1999; Kiyoshi et al., 2006; Maurizio et al., 1993). However, it is noticed that there are large variations among the SCF doses tested for *ex vivo* expansion of HSCs (Daniela et al., 2009; Madlambayan et al., 2005; Vichalkovski et al., 2005; Walenda et al., 2011). For example, there was a 15-fold difference between SCF doses in two reports (Kishimoto et al., 2009b; Yvonne et al., 2004). It should be emphasized that while an insufficient amount of SCF may obviously result in low cells expansion, the overdose of SCF can instead cause the loss of stem cells properties of HSCs (Annika et al., 2008). It is therefore very urgent to gain a clear understanding of the dose effect of SCF on the expansion of HSCs.

In the present study, four different doses of SCF ranging from 0 to 500 ng/mL were chosen to stimulate *ex vivo* expansion of CB-derived $CD34^+$ cells, along with fixed doses of TPO and FL in the cytokine cocktail. It is well known that HSCs are regulated dynamically within the niche and cytokines may play distinct roles at different time points during the development of HSCs (Martinez-Agosto et al., 2007). Moreover, the sensitivity of HSCs to cytokines has been demonstrated to decrease markedly during the process of cell development (Michelle et al., 2007b). Therefore, cell growth, the consumption of SCF by cells and the proportion of $CD34^+$c-kit^+ cells were also analyzed to investigate the relationship between SCF dose and cell expansion. Additionally, it was hypothesized that the timing that cytokines exert influence on cells would also be critical for HSCs expansion. Accordingly, the feeding timing of SCF was explored to further optimize the *ex vivo* expansion process of HSCs. It is envisioned that this study would provide a rational guidance regarding cytokine application including cytokine types, doses and timing to eventually achieve favorable *ex vivo* expansion of HSCs and expand their therapeutic applications.



Scheme 1. Different SCF feeding regimens. SCF was fed at the dose of 50 ng/mL in different stages during the culture process, with TPO and FL fed on day 0, 4, 7 and 10. Five experimental groups including the control were set as the following: N: control, no SCF was fed during the *ex vivo* culture process; A: SCF fed on day 0 only; B: SCF fed on day 0 and 4; C: SCF fed on day 0, 4 and 7; D: SCF fed on day 0, 4, 7 and 10.

2. Materials and methods

2.1. Cell preparation

CB samples were obtained from full-term healthy delivery with informed consent. Low-density mononuclear cells were enriched using density gradient centrifugation on Ficoll/Histopaque (density: 1.077 g/mL). Fresh $CD34^+$ cells or expanded $CD34^+$ cells were isolated by positive selection using CD34 antibody-conjugated paramagnetic microbeads and MiniMACS columns (Miltenyi Biotech). The purity of acquired $CD34^+$ cells was >95% as assessed by flow cytometric analysis (FACS Calibur, Becton Dickinson).

2.2. Cell culture

$CD34^+$ cells were seeded at a density of 5×10^4 cells/mL in 24-well plates with 2 mL of serum-free medium (Stem Pro®-34 SFM, Gibco) in each well, which was supplemented with a combination of recombinant human cytokines (Peprotech) including SCF, TPO and FL. While the doses of TPO and FL were fixed at 20 and 50 ng/mL, respectively, the SCF dose varied (0, 5, 50 or 500 ng/mL) to test the dose effect of SCF on the expansion of HSCs. Cell cultures were maintained for 21 days at 37 °C in a humidified incubator with 5% CO_2 and 21% O_2 in nitrogen. Cells were demi-depopulated on day 0, 4, 7, 10, 14, 17 and 21 and fed with an equal volume of fresh medium containing the cytokine cocktail at the specified doses. At the same time points, total cell numbers were counted. In addition, for secondary expansion studies, $CD34^+$ cells isolated from *ex vivo* expanded cells under the four SCF feeding regimens (A–D as defined in Scheme 1) were cultivated for 14 days in the serum-free medium supplemented with a cytokine cocktail of SCF (50 ng/mL), TPO (20 ng/mL) and FL (50 ng/mL) with medium refreshment on day 4, 7 and 10 similarly as described above. Total cell counting was performed on day 0, 4, 7, 10 and 14.

2.3. Determination of the proportions of $CD34^+$ cells, $CD34^+CD38^-$ cells and $CD34^+$c-kit^+ cells

A total of 1×10^6 cells was collected, rinsed with phosphate-buffered saline (PBS) and resuspended in 50 μL of PBS. Cells were then stained with PE-conjugated anti-human CD34 antibody (Becton Dickinson) at 4 °C for 30 min in dark. For the detection of $CD34^+CD38^-$ cells and $CD34^+$c-kit^+ cells, cells pretreated with the CD34 antibody were further stained with FITC-conjugated anti-human CD38 antibody (Becton Dickinson) and PE-CY5-conjugated anti-human c-kit antibody (Becton Dickinson), respectively. Cell preparations were analyzed on a flow cytometer (FACS Calibur, Becton Dickinson) to determine the proportions of $CD34^+$ cells, $CD34^+CD38^-$ cells and $CD34^+$c-kit^+ cells in total cell population.

Download English Version:

https://daneshyari.com/en/article/23312

Download Persian Version:

https://daneshyari.com/article/23312

Daneshyari.com