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Cord blood-hematopoietic stem cell expansion in 3D fibrin scaffolds with stromal support

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ABSTRACT

Expansion of multipotent, undifferentiated and proliferating cord blood (CB)-hematopoietic stem cells (HSC) *in vitro* is limited and insufficient. Bone marrow (BM) engineering *in vitro* allows mimicking the main components of the hematopoietic niche compared to conventional expansion strategies. In this study, four different 3D biomaterial scaffolds (PCL, PLGA, fibrin and collagen) were tested for freshly isolated cord blood (CB)-$CD34^+$ cell expansion in presence of (i) efficient exogenous cytokine supplementation and (ii) umbilical cord (UC)-mesenchymal stem cells (MSC). Cell morphology, growth and proliferation were analyzed *in vitro* as well as multi-organ engraftment and multilineage differentiation in a murine transplantation model. All scaffolds, except 3D PLGA meshes, supported CB-$CD34^+$ cell expansion, which was additionally stimulated by UC-MSC support. CB-$CD34^+$ cells cultured on human-derived 3D fibrin scaffolds with UC-MSC support i) reached the highest overall growth (5×10^8-fold expansion of total nuclear cells after fourteen days and 3×10^7-fold expansion of $CD34^+$ cells after seven days, $p < 0.001$), ii) maintained a more primitive immunophenotype for more cell divisions, iii) exhibited superior morphological, migratory and adhesive properties, and iv) showed the significantly highest numbers of engraftment and multilineage differentiation (CD45, CD34, CD13, CD3 and CD19) in BM, spleen and peripheral blood in long-term transplanted NSG mice compared to the other 3D biomaterial scaffolds. Thus, the 3D fibrin scaffold based BM-mimicry strategy reveals optimal requirements for translation into clinical protocols for CB expansion and transplantation.

1. Introduction

Cord blood (CB) transplantation, a challenging alternative to traditional hematopoietic stem cell (HSC) transplantation, is so far restricted by delayed engraftment periods and increased transplant related mortalities [1]. Developing an efficient strategy for *ex vivo* expansion of CB–HSC may contribute to overcome those limitations. However, the composition of the bone marrow (BM) niche represents a major challenge for recapitulating these factors *in vitro* [2–5]. *In vivo*, the cross-talk between HSC and their niche modulates HSC function. Typically, a multiplicity of hormones and cytokines, a three dimensional (3D) extracellular matrix and paracrine interactions of several cell types, such as osteoblasts, adipocytes, and fibroblasts within the bone marrow (BM) contribute to the HSC fate [6]. Mesenchymal stem or stromal cells (MSC) are considered to be important constituents of the HSC niche as they are the precursors for typical niche-containing cells [7] and were even shown to play a critical role in HSC function and self-renewal [8,9]. Stroma-dependent cultures using MSC improved *ex vivo* expansion of HSC by direct and indirect cell–cell contacts [10–13]. Indirect contacts occur when HSC interact with the components of the niche, for example, with adhesion proteins such as N-cadherin, CD44, Integrin-β1 (ITGB1) or Notch [14]. Direct cell-contact between HSC and MSC was recently well-described in a study from Mendez-Ferrer et al. [9].

Most of the current hematopoietic culture systems apply two dimensional (2D) expansion strategies that significantly reduce HSC proliferation compared to 3D environments [15]. 3D scaffolds

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increase the cell growth area, provide structural support and approve cell-to-cell contact, thus fulfilling the important requirements of the niche [16]. To date, only a few 3D scaffolds have been applied for the ex vivo expansion of HSC, those include nanofiber meshes, porous matrices, woven and non-woven fabrics and microspheres [17,18]. Ideally, a 3D scaffold for HSC expansion should include sufficient surface area for cell adhesion, increased porosity to allow cell migration and nutrient exchange [16] in addition to superior material compatibility to support cell growth [19]. Interestingly, the development of 3D scaffolds for BM mimicry is nowadays mainly rather empirical than systematic. So microscale structures are unsatisfactorily reproducible while the biomaterial choice results from an iterative process.

We have previously shown that several bio- and synthetic materials were able to support expansion of functional HSC populations in 2D cultures containing sufficient cytokine supplementation [20]. Here, we propose the combined use of four 3D biomaterial scaffolds with defined geometries, pore sizes and fiber diameters, stromal support and efficient cytokine supplementation as a promising strategy for CB-CD34$^+$ cell expansion.

2. Materials and methods

2.1. Materials

2.1.1. Biomaterial scaffolds

3D Insert™-PCL scaffolds were purchased from 3D-Biotek (North-Brunswick, USA) and produced by 3D Precision Microfabrication Technology. 3D OptiMaix™-collagen scaffolds were purchased sterile from Matricel (Herzogenrath, Germany) and produced from porcine collagen using a specific freeze-drying process. 3D PLGA meshes were a gift from the Institute for Textile Technology (ITA, RWTH Aachen University, Germany) and consist of openporous non-woven meshes of 85:15 L-lactide–glycolide copolymer (Purasorb 8523, 2.3 dL/g) (Purac Biomaterials, Gorinchem, Netherlands). 3D fibrin scaffolds were prepared under sterile conditions by mixing 10 μL thrombin (20 units/mL, Sigma, Steinheim, Germany) with 180 μL of a human fibrinogen (Sigma, Steinheim, Germany) homemade suspension consisting of 830 μL fibrinogen (20 mg/mL), 50 μL $CaCl_2$ (50 mM, Roche, Mannheim, Germany) and 20 μL $GBSH_5$ buffer. Period of fibrin polymerization was 20 min at 37 °C (20% O_2, 5% CO_2). Scaffold parameters were different among scaffolds (Fig. 1).

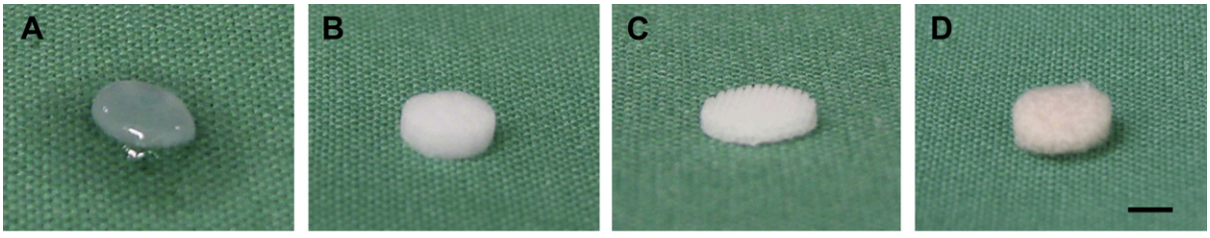
2.2. Methods

2.2.1. Isolation and culture of CB-CD34$^+$ progenitors

Human CB was collected after informed consent approved by the local Ethical Committee of the RWTH Aachen University. CD34$^+$ progenitors were obtained by Ficoll-Hypaque (density 1.077 g/mL) separation of the mononuclear fraction followed by immunomagnetic bead selection using the CD34 progenitor cell isolation kit (Miltenyi Biotec, Bergisch-Gladbach, Germany). Purity of CD34$^+$ progenitors was determined to be 90–98% in flow cytometry, as described before [21]. CD34$^+$ progenitors culture was performed using StemSpan serum-free medium (StemCell Technologies Inc., Vancouver, Canada) supplemented with 1×penicillin and streptomycin (Gibco Invitrogen Corporation, Paisley, UK) and recombinant human stem cell factor (SCF, 10 ng/mL, Peprotech, London, UK), recombinant human thrombopoetin (TPO, 20 ng/mL, Peprotech, London, UK), recombinant human fibroblast growth factor-1 (FGF-1, 10 ng/mL, Peprotech, London, UK), angiopoietin like-5 (Angptl-5, 500 ng/mL, Tebu-Bio, Taipei, Taiwan), insulin-like growth factor binding-protein 2 ($IGFBP_2$, 100 ng/mL, R&D Systems, Minneapolis, USA) and heparin (10 ng/mL, Ratiopharm, *Ulm, Germany*) [22].

2.2.2. Isolation and culture of UC–MSC

Human umbilical cords (UC) were collected after informed consent approved by the local Ethical Committee of the RWTH Aachen University. Human MSC were isolated from Wharton's jelly of the UC, the stromal counterpart for HSC during embryonic hematopoiesis [23]. Isolation was done according to the protocol of Wang and co-workers [24]. Briefly, UC was cut into pieces of 2 cm and opened lengthwise with a scalpel, jelly was scratched out carefully avoiding destruction of blood vessels



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Parameter \ Scaffold	3D Insert™-PCL	3D PLGA mesh	3D fibrin	3D OptiMaix™-collagen
Pore size (μm)	100/300	20	2-50	80
Fiber diameter (μm)	300	20	0.3	10
Diameter (μm)	5500	6000	6500	5000
Height (μm)	1500	2500	2000	1500
Cell growth volume (mm3)	36	70	67	30
Format	96-well	96-well	96-well	96-well

Fig. 1. Macroscopic view and physical properties of the 3D biomaterial scaffolds used. (A) 3D fibrin scaffold, (B) 3D-OptiMaix™-collagen scaffold, (C) 3D-Insert™-PCL scaffold, (D) 3D PLGA mesh. Scale bar = 5 mm. (E) Summary of scaffold properties.