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The effects of culture conditions on the functionality of efficiently obtained mesenchymal stromal cells from human cord blood

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Abstract

Background aims. Cord blood (CB) is an attractive source of mesenchymal stromal cells (MSCs) because of its abundant availability and ease of collection. However, the success rate of generating CB-MSCs is low. In this study, our aim was to demonstrate the efficiency of our previously described method to obtain MSCs from CB and further characterize them and to study the effects of different culture conditions on MSCs. *Methods.* CB-MSC cultures were established in low oxygen (3%) conditions on fibronectin in 10% fetal bovine serum containing culture medium supplemented with combinations of growth factors. Cells were characterized for their adipogenic, osteogenic and chondrogenic differentiation capacity; phenotype; and *HOX* gene expression profile. The functionality of the cells cultured in different media was tested *in vitro* with angiogenesis and T-cell proliferation assays. *Results.* We demonstrate 87% efficacy in generating MSCs from CB. The established cells had typical MSC characteristics with reduced adipogenic differentiation potential and a unique *HOX* gene fingerprint. Growth factor–rich medium and a 3% oxygen condition enhanced cell proliferation; however, the growth factor–rich medium had a negative effect on the expression of CD90. Dexamethasone-containing medium improved the capacity of the cells to suppress T-cell proliferation, whereas the cells grown without dexamethasone were more able to support angiogenesis. *Conclusions.* Our results demonstrate that the composition of expansion medium is critical for the functionality of MSCs and should always be appropriately defined for each purpose.

Key Words: *angiogenesis, CD90, cord blood, immunosuppression, low oxygen, mesenchymal stromal cell*

Introduction

Mesenchymal stromal cells (MSCs) regulate many important physiological events in our body and can be isolated and expanded from literally all tissues. Isolated MSCs are studied in regenerative therapy for many indications, such as steroid-resistant graft-versus-host disease [1], Crohn's disease [2], in tissue regeneration of bone [3], cartilage [4] and myocardium repair after infarction [5]. In tissue regeneration, to form functional tissues, it is necessary to have vascularization, a process enhanced by MSCs [6,7]. It is well known that MSCs are not a homogenous population; rather, each preparation contains cells sharing only a few common phenotypic markers: CD73, CD90 and CD105. Characteristically, MSCs have the ability to differentiate into mesodermal linages (bone, fat and cartilage) [8]. It has, however, long been debated that not every generated cell population is capable of differentiating into all lineages; instead, specific cell populations vary in their differentiation potential and gene expression profiles [9].

Generation of MSCs from cord blood (CB) is challenging compared with bone marrow (BM) or fat tissue. Many studies using CB report low yield of MSCs, if any, using standard protocols with 20% oxygen and basic medium with fetal bovine serum (FBS) and no additional growth factors [10–15]. CB-derived stromal cells seem to need higher serum content and/or additional growth factors compared

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with, for example, BM-derived MSCs (BM-MSCs) [16–18]. Generation of different cell populations from CB demonstrates that CB contains many cell types [19], and the generation of different populations may be a result of different culture conditions used during cell harvesting and expansion [20]. Despite the development in culture conditions in recent years, there are still difficulties in deriving clinically relevant amount of cells from CB.

Physiological oxygen pressure is much lower than the 20% that is routinely used in cell culture, and it is known that low oxygen is important in glycolytic energy metabolism of stem cells [21,22]. MSCs use glycolytic pathway in their energy metabolism, and this is known to be further enhanced in low oxygen conditions [23–25]. The oxygen pressure seems to be important factor at the beginning of the cell culture, affecting the subset characteristics of cells with typical *HOX* gene expression pattern [26,27]. Because *HOX* genes are transcription factors coordinately regulating genes involved in differentiation of tissues [28], the finding that *HOX* genes could serve as a specific fingerprint of cell types is intriguing.

Primary cells require specific growth conditions that mimic the original niche of the cells. Stem cells originating from different tissues seem to have their own specific growth factor preferences that are necessary for optimal establishment and growth of the cell lines [29,30]. It is difficult to estimate what characteristic is merely a consequence of different growth factors in the medium and which of the observed characteristics are typical for the original cells [31], as it has been shown how medium changes affect the cell characteristics [31,32].

We previously published a method to obtain CB-MSCs using specific combination of growth factors with 3% oxygen and fibronectin coating [33]. Our results here demonstrate the efficacy of the method to obtain adherent cell populations even from small CB units. In this study, we have further characterized the phenotype, *HOX* gene expression and differentiation capacity of the cells. The generated cell populations seem to vary, and thus further studies are needed to fully understand the nature of different cell populations obtained by our method. For clarity, we refer to all the populations obtained as CB-MSCs. Furthermore, we studied whether modifications in culture conditions affect the proliferation, immunomodulation and angiogenesis supporting potential of MSCs.

Methods

CB-MSC establishment

Human CB units were collected at the Helsinki University Central Hospital, Department of Obstetrics and Gynecology, and Helsinki Maternity Hospital. All donors gave informed consent, and the ethical review board of Helsinki University Central Hospital and the Finnish Red Cross Blood Service approved the study protocol. The CB-MSC cultures were established as described previously [33]. Briefly, human CB units were collected after delivery into blood collection bags and processed within 28 h. The mononuclear cells (MCs) were isolated from diluted CB by density-gradient centrifugation either manually (Ficoll-Paque Plus, GE Healthcare) or by Sepax cell separator (Biosafe). MCs were plated in density of $10^6/cm^2$ on fibronectin (FN, Sigma-Aldrich)-coated plates in standard growth medium (StdM) consisting of alpha-MEM Glutamax (Life Technologies, Thermo Fisher Scientific), 10% FBS (Life Technologies), 50 nmol/L dexamethasone (DX, Sigma-Aldrich), 10 ng/mL epidermal growth factor (EGF, Sigma-Aldrich), 10 ng/mL platelet derived growth factor–BB (PDGF-BB, R&D Systems, Inc.) and 100 U/mL penicillin and 100 µg/mL streptomycin (Life Technologies). The cells were incubated at 37°C in 5% CO_2, 3% O_2 in a humidified atmosphere. The medium was replaced next day and twice a week thereafter until the first passage. The cells were detached with trypsin–ethylenediaminetetraacetic acid (EDTA) solution (0.25%, 1 mmol/L, Life Technologies) and replated for expansion.

Cell culture procedures

To study the effects of culture conditions, the cells were cultured in several media. StdM was modified as follows: Medium 1 (M1) as StdM but FBS level reduced to 5% and excluding DX and EGF and Medium 2 (M2) as StdM but FBS level reduced to 5% and excluding DX. Medium 3 (M3), which was used in conditioning purpose for angiogenesis assay, was as StdM but FBS level reduced to 5% and excluding PDGF-BB (Table I). When the cells were cultured in different conditions for the period of same time, higher seeding densities (1500–3000 cells/cm^2) were used for cells cultured in M1 and M2 compared with seeding density in StdM (700–1500 cells/cm^2) to reach similar confluency (<90%) at the day of harvesting. Studies were performed using at least two donor cell lines.

Table I. Media supplement compositions.

Supplement	StdM	M1	M2	M3
FBS	10%	5 %	5 %	5 %
DX	50 nmol/L	—	—	50 nmol/L
EGF	10 ng/mL	—	10 ng/mL	10 ng/ml
PDGF-BB	10 ng/mL	10 ng/mL	10 ng/mL	—

Each media was alpha-MEM Glutamax–based medium with penicillin-streptomycin and the supplements listed in each column.