Cytotherapy, 2016; 18: 344–359

International Society for Cellular Therapy
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Choice of xenogenic-free expansion media significantly influences the myogenic differentiation potential of human bone marrow–derived mesenchymal stromal cells

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Abstract

Background aims. Mesenchymal stromal cells (MSCs) have great potential for use in cell-based therapies for restoration of structure and function of many tissue types including smooth muscle. *Methods.* We compared proliferation, *immunophenotype*, differentiation capability and gene expression of bone marrow–derived MSCs expanded in different media containing human serum, plasma and platelet lysate in combination with commonly used protocols for myogenic, osteogenic, chondrogenic and adipogenic differentiation. Moreover, we developed a xenogenic-free protocol for myogenic differentiation of MSCs. *Results.* Expansion of MSCs in media complemented with serum, serum + platelet lysate or plasma + platelet lysate were multipotent because they differentiated toward four mesenchymal (myogenic, osteogenic, chondrogenic, adipogenic) lineages. Addition of platelet lysate to expansion media increased the proliferation of MSCs and their expression of CD146. Incubation of MSCs in medium containing human serum or plasma plus 5% human platelet lysate in combination with smooth muscle cell (SMC)-inducing growth factors TGFβ1, PDGF and ascorbic acid induced high expression of *ACTA2, TAGLN, CNN1* and/or *MYH11* contractile SMC markers. Osteogenic, adipogenic and chondrogenic differentiations served as controls. *Discussion.* Our study provides novel data on the myogenic differentiation potential of human MSCs toward the SMC lineage using different xenogenic-free cell culture expansion media in combination with distinct differentiation medium compositions. We show that the choice of expansion medium significantly influences the differentiation potential of human MSCs toward the smooth muscle cell, as well as osteogenic, adipogenic and chondrogenic lineages. These results can aid in designing studies using MSCs for tissue-specific therapeutic applications.

Key Words: *GMP, mesenchymal stromal cell, myogenic differentiation, myogenesis, plasma, platelet lysate, serum, smooth muscle cells*

Introduction

Human mesenchymal stromal cells (MSC) have great potential for use in cell-based therapies and represent a potential resource in regenerative medicine and tissue engineering for restoration of the structure and function of many tissue types. Yet despite the first clinical trial using MSCs being reported 20 years ago [1], transfer of MSCs into the clinic has been a slow process in some disciplines and is complicated by several issues, especially in the context of good manufacturing practice (GMP) preparations of these cells [2]. Because of the limited availability of MSCs in most tissues, *ex vivo* amplification before clinical application is essential to obtain therapeutic cell doses [3].

Fetal bovine serum (FBS) is commonly used to supplement cell culture media in the expansion of MSCs because of its high content of growth factors. Because of the safety, ethical and regulatory concerns raised by FBS [4,5] and the fact that regulatory authorities in an increasing number of countries now encourage the use of xenogenic-free media for generating cells for clinical use, many laboratories have been or considering switching to xenogenic-free culture conditions [2]. To minimize these concerns, autologous or allogeneic human blood derived products including human serum (S), plasma (P) or platelet derivatives such as platelet lysate (PL) are being investigated as alternative growth supplements in MSC expansion medium [4]. Compared with other human

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(Received 6 October 2015; accepted 25 November 2015)

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http://dx.doi.org/10.1016/j.jcyt.2015.11.019

supplements, PL was reported to be a more preferred human product supplement for expanding bone marrow–derived MSCs (bmMSCs) in recent clinical trials [6]. However many studies have shown that expansion of MSCs in different types of media can lead to increased heterogeneity and skew toward certain subpopulations, which can increase or decrease specific cellular phenotypes [7–9]. For example several studies have shown that addition of PL to the medium yields MSCs with a reduced osteogenic or adipogenic differentiation potential [10–12], whereas other studies have shown that it favors chondrogenesis [13].

Hence, changes in expansion conditions have an impact on the quality and differentiation potential of MSCs. This is an important consideration in tissue-specific engineering and regenerative studies. Moreover, MSC heterogeneity has been linked to the enrichment of MSC subsets driven by small differences in culture conditions [7–9], which may alter the differentiation potential of those cells as well. Because heterogeneity of MSCs due to differences in culture conditions is a well-known phenomenon that may explain the success or failure of MSCs in tissue regeneration [14], in this study we examined the effects of four MSC expansion media containing human S or P, with or without PL in combination with commonly used protocols for myogenic, osteogenic, chondrogenic and adipogenic differentiation. Although many studies have shown that changes in medium can modulate cellular fate, to the best of our knowledge, a comparison on the effects of these components on the differentiation toward the smooth muscle cell (SMC) lineage has not been examined. Detailed knowledge on effects of medium changes on SMC differentiation is essential for clinical application of the MSCs because, unlike skeletal or cardiac muscle cells that are terminally differentiated, SMCs do not terminally differentiate but rather display remarkable plasticity *in vitro* as well as *in vivo* [15–18].

Population doubling levels and MSC surface markers related to their stemness were first analyzed after expansion in the different media, including CD146 expression because it is associated with a commitment of bmMSCs to the vascular SMC phenotype [19]. To assess the differentiation state of MSCs after expansion in the different media, the myogenic differentiation potential of bmMSCs toward the SMC lineage was investigated by quantitative reverse transcription polymerase chain reaction (qRT-PCR) to determine the gene expression levels of smooth muscle markers *ACTA2, TAGLN, CNN1* and *MYH11* after being placed into myogenic, osteogenic or adipogenic media for 1 week. These contractile SMC genes were chosen because they are progressively expressed at different stages of myogenesis [15–18]. Western blot was performed to confirm expression of the calponin protein and to verify myogenic differentiation. We also examined the gene expression levels of typical osteogenic differentiation markers *RUNX2* and *OPN*, the chondrogenic differentiation marker *CDRAP* and classical adipogenic specific markers *PPARγ2* and *LPL* by qRT-PCR and histochemical staining after induction of differentiation, respectively. Finally, to comply with GMP guidelines for cell therapies [20], we adapted standard myogenic differentiation medium commonly used by many laboratories [21–28] to a xenogenic-free expansion-differentiation protocol.

Our study provides novel data on the myogenic differentiation potential of human bmMSCs toward the SMC lineage using different cell culture expansion media in combination with various differentiation medium compositions and shows that the choice of expansion media can significantly influence the differentiation potential of human bmMSCs toward smooth muscle cells, and osteogenic, adipogenic and chondrogenic lineages.

Methods

Isolation and expansion of human MSCs

Bone marrow from nine donors was obtained from the BG Trauma Clinic (Tübingen, Germany) and obtained according to institutional approval from the University of Tübingen ethics committee (623/2013BO2) and with written informed donor consent. Bone marrow was washed with phosphate-buffered saline (PBS), centrifuged at 150*g* (10 min at room temperature), the supernatant was discarded and cells were resuspended in PBS. MSCs were isolated using a Ficoll density gradient fractionation (density 1.077 g/mL, GE Healthcare Life Sciences, 400*g*, 30 min, room temperature). The mononuclear cell layer was harvested, washed with PBS and seeded in T75 flasks (BD Falcon) at a density of 1.5×10^5 cells per flask in 10 mL of xenogenic-free expansion media (Dulbecco's Modified Eagle's Medium [DMEM] low glucose, Sigma-Aldrich; 1000 IU heparin, Carl Roth; 25 mmol/L HEPES, Sigma; 1% penicillin-streptomycin, Life Technologies; 2 mmol/L L-glutamine, Lonza; and 5% human serum, Blood Donation Center, Tübingen, Germany, or 5% human plasma, TCS Biosciences, with or without 5% human pooled platelet lysate (PL, Blood Donation Center, Tübingen, Germany). After 24 h of incubation, the medium was discarded and replaced to remove unattached cells, and medium was changed twice a week. When bmMSCs reached approximately 70–80% confluence in primary culture, the cells were split into the next passage (passage 1) at a density of 1.5×10^5 cells/flask in the same xenogenic-free expansion medium. At passage 2, when the cells reached a cell density of approximately 70%, myogenic,

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