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The effect of medium composition on deposition of collagen type 1 and expression of osteogenic genes in mesenchymal stem cells derived from human adipose tissue and bone marrow

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ABSTRACT

The two mesenchymal stem cell (MSC) populations that have gained most attention in relation to bone tissue engineering are adipose tissue (AT) MSCs and bone marrow (BM) MSCs. The purpose of this study was to investigate the ability of human BM-MSCs and AT-MSCs to survive, proliferate and deposit collagen type 1 when cultured on polycaprolactone nanofiber scaffolds and to ascertain the effect of medium composition on collagen type 1 formation and expression of osteogenic genes. The cells were seeded on polycaprolactone nanofiber scaffolds and cultured in three different types of media that differed by the presence of ascorbic acid, β-glycerophosphate and dexamethasone, that are typical components used for osteogenic differentiation of MSCs *in vitro*.

In summary, AT-MSCs were proliferating significantly faster than BM-MSCs. AT-MSCs also showed better ability to deposit collagen type 1 and had a higher expression of early osteogenic markers, whereas BM-MSCs had higher expression of late osteogenic markers. This suggests that MSCs from diverse sources have different attributes and with respect to osteogenic differentiation, AT-MSCs are more immature compared to BM-MSCs. Collagen formation was depending on medium composition and the organization of collagen type 1 appeared to be influenced by the presence of dexamethasone.

1. Introduction

Bone possesses the ability to undergo complete regeneration rather than to heal with scar formation as seen in most other tissues. However, large bone defects, e.g. following trauma, infections and tumor resections, require bone formation beyond the intrinsic capacity of the bone [1,2]. Bone tissue engineering (BTE) has emerged as a promising alternative to the current strategies for bone reconstruction. It aims to combine biomaterials, cells and osteogenic factors to form a functional bone implant. Mesenchymal stem cells (MSCs), which can be isolated from several tissue sources, are favoured cell sources for use in BTE. Common for all MSCs is the ability to differentiate into bone, cartilage and fat, when cultured under appropriate conditions [3]. However, there are differences in differentiation and proliferation potential between MSCs of different origin [4,5]. The two MSC populations that have gained most attention in relation to BTE are adipose tissue-derived (AT) MSCs and bone marrow-derived (BM) MSCs. AT-MSCs are abundantly available and easily expandable, whereas BM-MSCs are considered to have a greater osteogenic potential. During osteoblast differentiation, the cells synthesize a bone extracellular matrix (ECM) that serves as a scaffold upon which mineral is deposited. Several ECM components are involved in the binding of minerals, but the ECM scaffold mainly consists of collagen type I (COL1) [6]. Hence, for a synthetic scaffold to be suitable for use in BTE applications it is not only required to facilitate adhesion, proliferation and osteogenic differentiation of MSCs but

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also to support bone matrix formation. The biodegradable microporous, non-wowen polycaprolactone (PCL) nanofiber scaffold is a potential candidate scaffold for BTE [7,8] It has morphology and mechanical properties that mimic the ECM and support cell adhesion [9]. Furthermore, PCL is a biocompatible and biodegradable polyester, which is approved by the US Food and Drug Administration, and is widely used in different tissue engineering applications [10]. Our previous research showed that PCL nanofiber scaffolds are suitable for culture of porcine BM-MSCs and supports differentiation towards the osteogenic lineage [11]. The biomimicking structure of PCL nanofibers enables adhesion and proliferation of MSCs and supports osteogenic differentiation.

Osteogenic differentiation of MSCs is typically induced *in vitro* by supplementing the culture medium with a cocktail of L-ascorbic acid-2-phosphate (Asc), β-Glycerophosphate (β-Gly), and dexamethasone (Dex). Asc and β-Gly are necessary for collagen synthesis and mineralization, respectively [12,13]. Dex is among others known to induce expression of the osteoblast related genes via activation of RUNX2 [14,15]. However, it has been reported that Dex is not required for osteogenic differentiation [16]. Furthermore, it was recently published that the structure of a surface or scaffold alone can induce BM-MSCs to differentiate into the osteogenic lineage without the addition of soluble osteogenic inducers [17].

The purpose of this study was to investigate the ability of human BM-MSCs and AT-MSCs to survive, proliferate and deposit COL1 in the ECM when cultured on PCL nanofiber scaffolds and to ascertain the effect of medium composition on COL1 formation and expression of osteogenic genes by the two cell types.

2. Materials and methods

2.1. Preparation and characterization of poly-ε-caprolactone nanofiber scaffold

The nanofiber scaffold was prepared by electrospinning. Electrospinning was performed from a 24 wt% poly-ε-caprolactone (PCL) with a molecular weight of 45 kDa (Sigma–Aldrich, St Louis, MO, USA) using chloroform-ethanol dissolving system in a ratio of 9:1. The electrospinning electrode was based on a polytetrafluorethylen needleless electrode connected to a high-voltage source. The electrospun nanofibers were deposited in the form of a non-woven mesh on a grounded metal plate collector covered with a spun bond textile. The morphology of the nanofiber mesh was characterized using VEGA3 scanning electron microscope (Tescan, Brno, Czech Republic) and the fiber diameter was measured using ImageJ software and every value was calculated by taking at least 100 dimensions [18]. In addition, surface properties were analyzed by contact angle and surface zeta-potential measurement. The surface zeta-potential analysis was performed by indirect method using trace particles (latex).

2.2. Isolation and culture of MSCs

Adipose tissue (AT) was obtained from liposuction material from abdominal regions of three healthy female donors undergoing cosmetic surgery. The donors provided informed consent, and the collection and storage of AT and AT-MSCs were approved by the regional committee for ethics in medical research. The stromal vascular fraction (SVF) was separated from AT as described previously [19]. Briefly, lipoaspirate was washed repeatedly to remove erythrocytes and leukocytes. This material was then digested using 0.1% collagenase A type 1. Following centrifugation the pellet was re-suspended in HBSS and the cells were filtered through 100 μm and then 40 μm cell sieves (Becton Dickinson, San Jose, CA). SVF cells were obtained from the interface after Lymphoprep gradient separation (Axis Shield, Oslo, Norway), washed and re-suspended in HBSS.

The isolation and *in vitro* expansion of AT-MSCs were carried out as previously described [20,21]. Briefly, $CD44^+$ cells were removed using Dynabeads (Dynabeads Pan Mouse IgG, Invitrogen Dynal AS, Oslo, Norway) according to the manufacturer's description. Dynabeads were washed, then pre-coated with monoclonal anti-CD44 antibody (Southern Biotech, Birmingham, AL) and incubated for 2 h with gentle tilting and rotation. Dynabeads were then washed again and approximately 400×10^6 magnetic CD44 beads were added to 100×10^6 SVF. The CD44+ cells, which were positively selected from the SVF were expanded in a medium consisting of Dulbecco's modified Eagle's medium/F12 (DMEM/F12) (Gibco) containing 10% human platelet lysate plasma (hPLP), 1% penicillin/streptomycin and 1000 IU/ml heparin (Wockhardt, Wrexham, UK) to avoid fibrin clot formation due to the hPLP.

BM-MSCs were obtained from BM aspirate from the superior posterior iliac crest of three voluntary donors after informed consent, as described previously [22]. The aspirate was immediately diluted 1:3 in DMEM-F12 following density gradient centrifugation (Lymphoprep, Axis Shield, Oslo, Norway), the mononuclear cell layer was collected, washed and allowed to adhere overnight in culture flaks (Nunc, Roskilde, Denmark) at 37 °C. Both cell types were cultured in DMEM/F12, supplemented with 10% hPLP, 1000 IU/ml heparin and 1% penicillin and streptomycin (growth medium = GM). Cells were subcultured at confluence and expanded for 3–4 passages.

For osteogenic differentiation, culture medium was supplemented with 150 μM Asc, 50 ng/ml β-Gly and 10 nM Dex (osteogenic medium supplemented with Dex = dOM) or only with Asc and β-Gly without Dex (osteogenic medium = OM).

2.3. Cell seeding

The nanofiber mesh was cut into round-shaped patches (diameter 6 mm) and sterilized by ethylene-oxide at 37 °C. BM-MSCs and AT-MSCs were seeded on PCL scaffolds at passage 3 and 4, respectively. The cells were seeded on scaffolds placed in 96-well plates, by pipetting a suspension of cells onto the scaffolds to achieve a final density of 89 000 cells/cm^2. The cells were allowed to attach to the scaffold for 24 h before beginning of the experiment. To test the effect of culture medium the cells were then cultured in 200 μl of GM, OM or dOM for 21 days. The medium was changed every 3 days. Samples for analyses of cell proliferation and gene expression were collected at day 0, 10 and 21, and samples for immunnohistochemistry and live/dead staining were collected at day 21. Cells from different donors were cultured and evaluated independently.

2.4. Cell proliferation analysis and cell viability assay

A PicoGreen assay kit (Quant-iT™ PicoGreen® dsDNA Assay Kit, Life Technologies, Paisley, UK) was used to determine the DNA concentration. Samples for PicoGreen assay were collected during RNA isolation as it was established as an efficient method combining RNA extraction and DNA measurement [23]. Scaffold samples were collected and stored at −80 °C until RNA isolation. Thereafter, cell lysis RLT solution in volume of 140 μl per scaffold was added to achieve a lysis of cells. Subsequently, samples were processed through 2 freeze/thaw cycles, so scaffold samples were frozen deeply and then thawed at room temperature. Between each cycle, samples were roughly vortexed. Samples were collected during the process of RNA isolation and stored at −80 °C until analysis. Before performing the assay, samples were thawed and roughly vortexed again. The DNA amount was determined by mixing of 40 μl of PicoGreen reagent and 40 μl of sample. Samples were loaded in duplicates and the fluorescence intensity was measured