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Regenerative potential of human adipose-derived stromal cells of various origins

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ABSTRACT

In regenerative concepts, the potential of adult stem cells holds great promise concerning an individualized therapeutic approach. These cells provide renewable progenitor cells to replace aged tissue, and play a significant role in tissue repair and regeneration.

In this investigation, the characteristics of different types of adipose tissue are analysed systematically with special attention to their proliferation and differentiation potential concerning the angiogenic and osteogenic lineage. Tissue samples from subcutaneous, visceral, and omental fat were processed according to standard procedures. The cells were characterized and cultivated under suitable conditions for osteogenic and angiogenic cell culture. The development of the different cell cultures as well as their differentiation were analysed morphologically and immunohistochemically from cell passages P1 to P12. Harvesting and isolation of multipotent cells from all three tissue types could be performed reproducibly. The cultivation of these cells under osteogenic conditions led to a morphological and immunohistochemical differentiation; mineralization could be detected. The most stable results were observed for the cells of subcutaneous origin. An osteogenic differentiation from adipose-derived cells from all analysed fatty tissues can be achieved easily and reproducibly. In therapeutic concepts including angiogenic regeneration, adipose-derived cells from subcutaneous tissue provide the optimal cellular base.

1. Introduction

Adult stem cells are detected in the human organism in different regions such as the dental pulp, the liver, or various types of adipose tissue (Gronthos et al., 2000; Palmer et al., 1997). The advantage of these cells is their capacity to differentiate into various cells and tissue types. Mesenchymal stem cells from the bone marrow are able to develop into different cell types within the limits of their original blastodermic layer, i.e. osteoblasts or chondrocytes. Multilineage developmental plasticity describes the stem cells' ability to overcome the histological limitations of their initial germ layer and their capacity to differentiate into progenitor cells and ultimately into cell types of another origin (Keller, 2002). Cell-based concepts are of increasing interest, especially in personalized regenerative strategies. In different interventional approaches to guided tissue regeneration, adult mesenchymal stem cells with their characteristic properties have been under examination. In animal models of myocardial and cerebral ischaemic infarction, the implantation of adipose-derived cells led to improved tissue repair and functional regeneration (Schenke-Layland et al., 2009; Lee and Yoon, 2008). For the regeneration of critical bone defects or bony non-union after trauma, a combination of tissue engineering and cell therapy including the use of mesenchymal stromal cells in an autologous graft represents a promising possibility (Schubert et al., 2013).

The growth of the nutrient vasculature parallel to the developing muscle or bone structures is an important issue in tissue regeneration. An ideal cellular base for a personalized therapeutic theory of tissue replacement or regeneration therefore should be able to support the development not only of the severed tissue but also the initiation of the committing vessel structures.

In personalized regenerative concepts, the multipotent cells have to satisfy several conditions:

(1) The cells have to be able to support the regeneration of the severed tissue, including its vasculature, effectively after autologous transplantation.

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(2) The cells have to be reliably expandable, and their processing and differentiation must be reproducible.
(3) The cells have to be easily accessible in certain amounts without donor site morbidity.

For many years, bone marrow-derived mesenchymal stromal cells (MSCs) have been considered the optimal cells for regenerative purposes. A fatty tissue is another source of tissue-specific stromal cells that comes with several advantages: compared to other multipotent cells or endothelial progenitor cells, adipose-derived cells occur in abundance and can be harvested in greater volume. In addition, the cells proliferate without difficulty, expand quickly in culture, and express their characteristic surface markers over several passages, which indicate their stability and lasting multipotency (Zuk et al., 2001, 2002). Human adipose-derived stromal cells (hADSCs) are spindle-shaped cells localized in the fatty tissues. Their harvesting and isolation can be easily performed with a minimally invasive approach without tissue damage or excessive scarring, for example by liposuction. These cells are distinguished by their easy handling and their pronounced plasticity. They are able to differentiate within their embryotic germ layer to chondrocytes or myoblasts and across the cytological restrictions of the mesoderm to neuronal and endothelial cells (Ashjian et al., 2003; Ning et al., 2009). These characteristics make adipose-derived cells an ideal material for personalized regenerative concepts including bone or chondral tissue. Adipose-derived tissue represents an enormous reservoir of potentially angiogenic cells. Their potential in therapeutic (neo-)vascularization is not yet fully elucidated (Ouma et al., 2012; Szöke and Brinchmann, 2012).

During this investigation, we were able to isolate and cultivate hADSC from three different types of adipose tissue samples. In all three types, characteristic stem cell markers were expressed. It was possible to induce osteogenic differentiation with expression of osteoblast markers and mineralisation. It was possible also to induce a differentiation across the original germ layers to endothelial cells.

2. Material and Methods

2.1. Cell isolation and cultivation

2.1.1. Adipose-derived stromal cells (ADSC)

Human omental, visceral, and subcutaneous fat tissue was collected under sterile conditions during elective abdominal surgery, excluding oncological surgery, from the General and Visceral Surgery, University Hospital, Münster (Germany). The samples were collected anonymously from leftover tissue after patients provided informed consent. The Ethics Committee of the medical faculty approved the study.

The isolation and culture technique of Zuk et al. (2001) was adopted. The tissue was minced and washed with phosphate-buffered saline (PBS) (Sigma–Aldrich Chemie GmbH, Germany) for 10 min to reduce cellular debris and blood particles. This procedure was repeated until no cellular debris or blood particles were visible. The pieces were then digested with 0.1% collagenase (Biochrom AG Seromed, Germany) in PBS for 60 min with gentle agitation at 37 °C. The suspension was filtered through two nylon meshes (Sefar AG, Switzerland) and centrifuged at 1200 *g* for 5 min. The pellet was washed with 10 mL of PBS following centrifugation and resuspended in minimum essential medium (Alpha Eagle [α-MEM]; Lonza Walkersville, MD, USA) containing 10% foetal bovine serum, 1% amphotericin B, 1% glutamine, 1% penicillin [10,000 U/mL]/streptomycin [10,000 μg/mL] (Biochrom AG Seromed, Germany) following centrifugation. For cell cultivation, cells were cultured in a 90-mm culture dish for a period of 2 days at 37 °C with 5% CO_2, then washed with PBS and fed with α-MEM. The cells were fed every 2–3 days and passaged with 10,000 cells/cm^2 every 5–7 days after reaching 90% of confluence.

For estimation of the diameter of adherent cells, up to 55 cells per passage were measured by means of measurement bars on microscopic photos with Adobe Photoshop CS2 Version 9.0 (Adobe Systems Inc., USA).

2.1.2. Osteogenic differentiation of hADSC

Cells of second passage were transferred to osteogenic medium: α-MEM was replaced by High Growth Enhancement Medium (HGEM) (ICN Biomedicals GmbH, Germany) without α-glycerophosphate and ascorbic acid. Culture techniques were adopted of Zuk et al. (2001). The medium was changed every 4–5 days. To initiate mineralisation, HGEM was supplemented ($HGEM_{(s)}$) with 10 mM α-glycerophosphate and 25 μg/mL ascorbic acid (Sigma, Germany). Cells were cultivated at 37 °C with 5% CO_2. Mineralisation was visualized by Alizarin red staining.

2.1.3. Endothelial differentiation of hADSC

The cells of the second passage were transferred to an endothelial medium. To stimulate endothelial differentiation, α-MEM was replaced by endothelial cell growth medium (EGM) (Promocell, Heidelberg, Germany) and EGM 2 (Promocell, Heidelberg, Germany). Cells were cultivated at 37 °C with 5% CO_2.

2.1.4. Human endothelial cells

Human umbilical cords were obtained by the Department of Neonatology, University Hospital, Münster (Germany). After washing umbilical cords in 70% ethanol, the umbilical vein was rinsed with PBS (Sigma–Aldrich Chemie GmbH, Germany). While being occluded with arterial clips on both sides, the vein was filled with PBS containing 0.05% collagenase (Biochrom AG Seromed, Germany), and incubated for 10 min at 37 °C. Cell suspension was collected in a Falcon tube and centrifuged for 7 min at 1200 *g*. The pellet was resuspended in EGM and plated in gelatine-coated 56.7-cm^2 culture dishes. Coating was prepared beforehand by incubating dishes with 0.2% gelatin (Sigma–Aldrich Chemie GmbH, Germany) for at least 30 min at 37 °C, followed by washing with PBS. Cells were cultivated at 37 °C with 5% CO_2 while being fed every 3–4 days and passaged every 7–10 days after reaching nearly total confluence.

2.1.5. Human osteoblasts

Human spongiosa was obtained from Department of Craniofacial Surgery, University Hospital, Münster (Germany). The tissue was collected anonymously from leftover tissue after informed consent of the patients. Pieces were washed several times with HGEM and placed in a 90-mm culture dish in HGEM without α-glycerophosphate and ascorbic acid at 37 °C with 5% CO_2. After 7 days bone pieces were removed. Medium was replaced every week, containing 0.04% Fortecortin (Merck Pharma GmbH, Germany) for osteogenic differentiation until growing cells reached 90% of confluence. Cells were passaged as described above.

2.1.6. Immunohistochemistry

Primary antibodies applied in this work: α-SMA (clone 1A4; dilution 1:100; MP Biomedicals, USA), CD13 (clone WM 15, dilution 1:25, Thermo Fischer Scientific, USA), CD31 (clone JC70A; dilution 1:100; Dako, Germany), CD44 (clone A3D8, dilution 1:50; Sigma Aldrich, Germany), CD90 (clone AF-9; dilution 1:50; Thermo Fischer Scientific, USA), collagen 1 (#ab6308, dilution 1:100; abcam, England), osteocalcin (clone OC4-30; dilution 1:50; TaKaRa, Japan), osteonectin (OSN4-2; dilution 1:50, TaKaRa, Japan), and vWF (#F3520, dilution 1:100, Sigma Aldrich, Germany). Other than