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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

vWF, all primary antibodies were monoclonal antibodies from mice. vWF is a polyclonal antibody from rabbits. Secondary antibodies were Alexa Fluor 488 goat anti-mouse (dilution 1:100; Invitrogen, USA) and in case of vWF Alexa Fluor 488 anti-rabbit (dilution 1:100; Invitrogen, USA). Staining protocols performed according to the manufacturer's protocols. Stained cells examined with the fluorescence microscope Axioplan 2 (Carl Zeiss, Germany). Negative as well as positive controls were implemented according to manufacturer's protocols.

2.1.7. Alizarin Red staining

For detection of calcification during osteogenic differentiation, the induced cells were rinsed twice with PBS and fixed with methanol for 20 min. The fixed cells were stained with 1 mL of Alizarin Red solution (Chroma-Gesellschaft mBH & Co., Germany/Carl Roth GmbH & Co. KG, Germany) and incubated for 2 min at room temperature. Cells culture in HGEM was used as negative control.

3. Results

3.1. Cell morphology

Human adipose-derived stromal cells show a spindle-cell-like bipolar morphology. By comparison, there are morphological differences in cell structure among cells isolated from subcutaneous (Fig. 1), visceral (Fig. 2), and omental (Fig. 3) adipose tissue. Cells of subcutaneous preparations have a mean diameter of 133.5 mm (adherent) in passage 1 (Table 1). The diameter increases in further passages, depending on primary cell density. Although cell density declines in further passages, the complete population upholds its homogeneity.

Preparations of human visceral adipose tissue show a more heterogeneous cellular architecture. It also has a bipolar morphology, but in comparison with subcutaneous preparations, cells appear more voluminous and tend to build ramified cell junctions. The diameter varies considerably between lower (P1: 49.6 mm) and higher (P3: 338.9 mm) passages (Table 1). Visceral adipose tissue preparations can be processed analogously; the cultures are marked by contaminating cells, especially spindle-shaped fibroblasts or cobblestone-like mesothelial cells. Mesothelial cells have a polygonal shape with deep blue cytoplasm and clearly visible nuclei, appearing as part of small insular clusters, especially in lower passages. However, their survival seems to be transient: within a few days these cells tend to hypertrophy, including embedding of vesicles and finally decay (Fig. 2).

Different cell types are found in human omental adipose tissue preparations, mainly mesothelial cells and only a few visceral-type ADSC (Fig. 3). Due to the short survival of these cells, the predominating cell type switches during passages 4 and 5 to visceral-type ADSC, which show similar characteristics compared to subcutaneously or viscerally derived preparations. This majority switch is also visible in the sudden increase of cell diameter between these passages (Table 1).

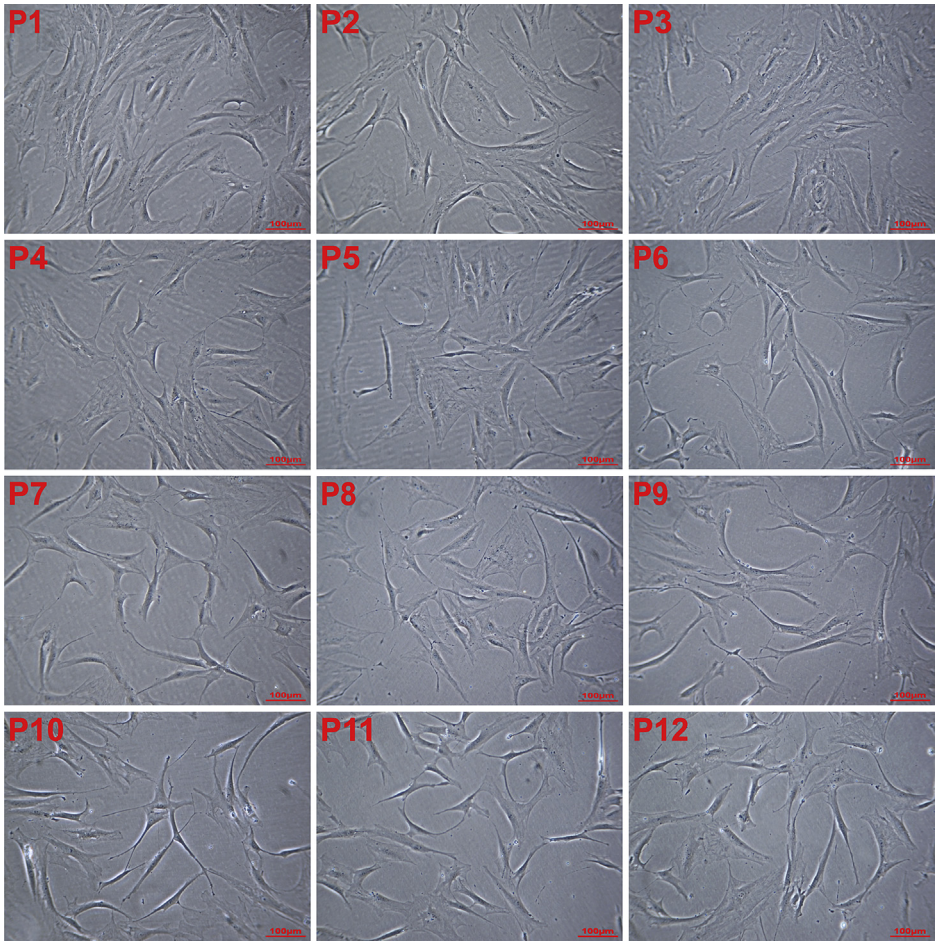

Fig. 1. Human subcutaneous fat tissue-derived cells in culture, passages P1–P12, ×10 magnification.

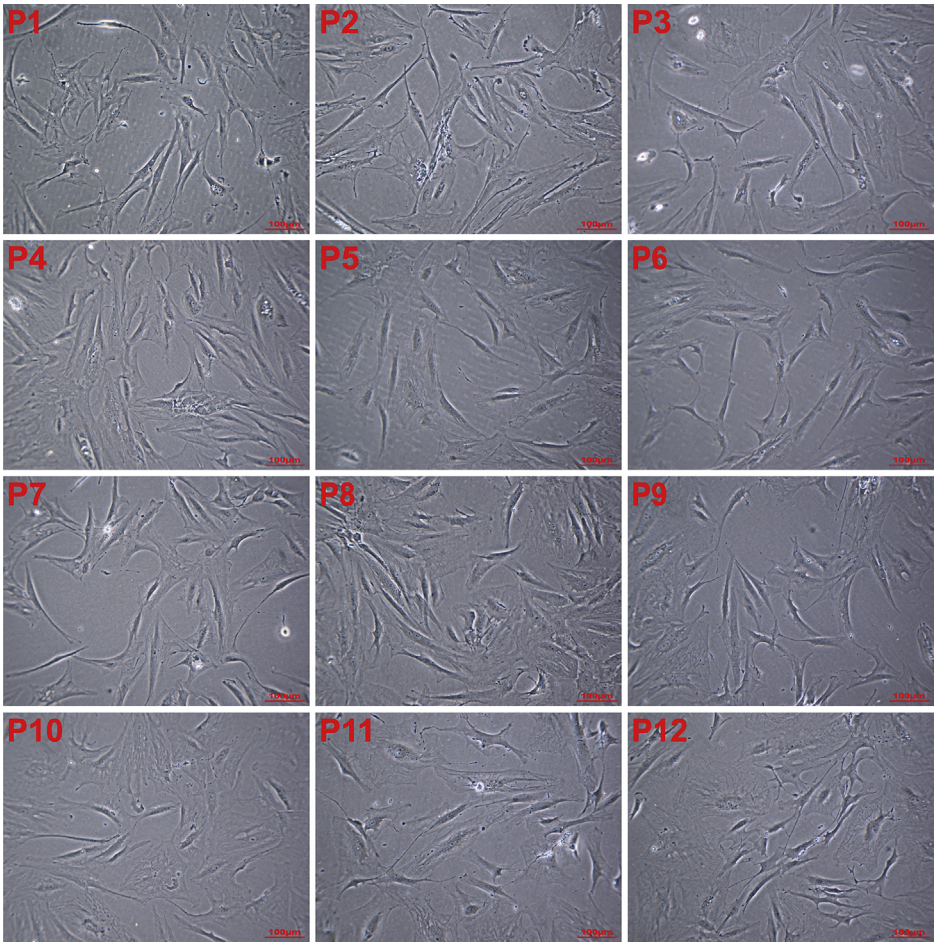

Fig. 2. Human visceral fat tissue-derived cells in culture, passages P1–P12, ×10 magnification.

3.2. Immunophenotype

Spindle-shaped cells from subcutaneous, visceral, and omental preparations express CD13, CD44, and CD90 in all cultivated passages with similar intensity; the expression of CD44 is more intense. The endothelial antigen vWF can also be detected from passage 1 to 12, whereas the expression of CD31 is absent. α-SMA is also expressed by the cells from passages 1 to 12 (Fig. 4).

Mesothelial cells, derived from omental adipose tissue, also express CD13, CD44, and CD90, comparable to multipotent cells. These cells also express vWF with high intensity (Fig. 4).

3.3. Osteogenic differentiation

Fig. 5 shows the immunohistochemical analysis according to osteogenic differentiation. The staining of the adipose-derived cells is correlated with human osteoblasts as a control group. Within 10 days in HGEM, cells derived from subcutaneous, visceral, and omental tissue change their morphology towards a more osteoblast-like shape: The width doubles, length decreases, cell nucleus becomes larger, and the culture condenses (Fig. 5). The mean cell diameter is 19.7 mm. Subcutaneous cells differentiates faster than visceral or omental cells. Visceral and omental adipose tissue cell cultures need more time for the differentiating process because of contaminating mesothelial cells, which cause the cultures to remain inhomogeneous for several weeks. Omental tissue-derived cell cultures tend to decease after a few weeks.

HGEM supplemented with α-glycerophosphate and ascorbic acid accelerates the differentiation process in all cell lineages, also in visceral and omental fat tissue cultures. The extrinsic cells such as fibroblasts or mesothelial cells decay within 10–30 days.

The immunophenotype of differentiated adipose-derived cells correlates with human osteoblasts: The proteins osteocalcin, osteonectin, and collagen I are expressed (Fig. 5). Subcutaneous tissue-derived cells, as compared with visceral- or even omental tissue-derived cells, show a high intensity of protein expression, depending on the amount of differentiated cells in culture (Fig. 5). In visceral- and omental tissue-derived cell cultures, cobblestone-like mesothelial cells express the antigens as well, which suggests an osteogenic differentiating potential. A culturing time of about approximately 40 days in HGEM including supplement leads to mineralisation of all cell lineages indicated by positive Alizarin red staining (Fig. 5).

3.4. Endothelial differentiation

After 2 days of cultivation, the cells start to proliferate quickly. Cell growth increases while using EGM2. Subcutaneous tissue-derived cell cultures become confluent within 3–5 days as opposed to 7–10 days using EGM. Comparable to osteogenic differentiation, visceral- and omental tissue-derived cells needed more time to express endothelial characteristics. Differentiated cells are small and spindle-shaped, containing a large cell nucleus; the mean diameter of detached cells is 16.5 mm.

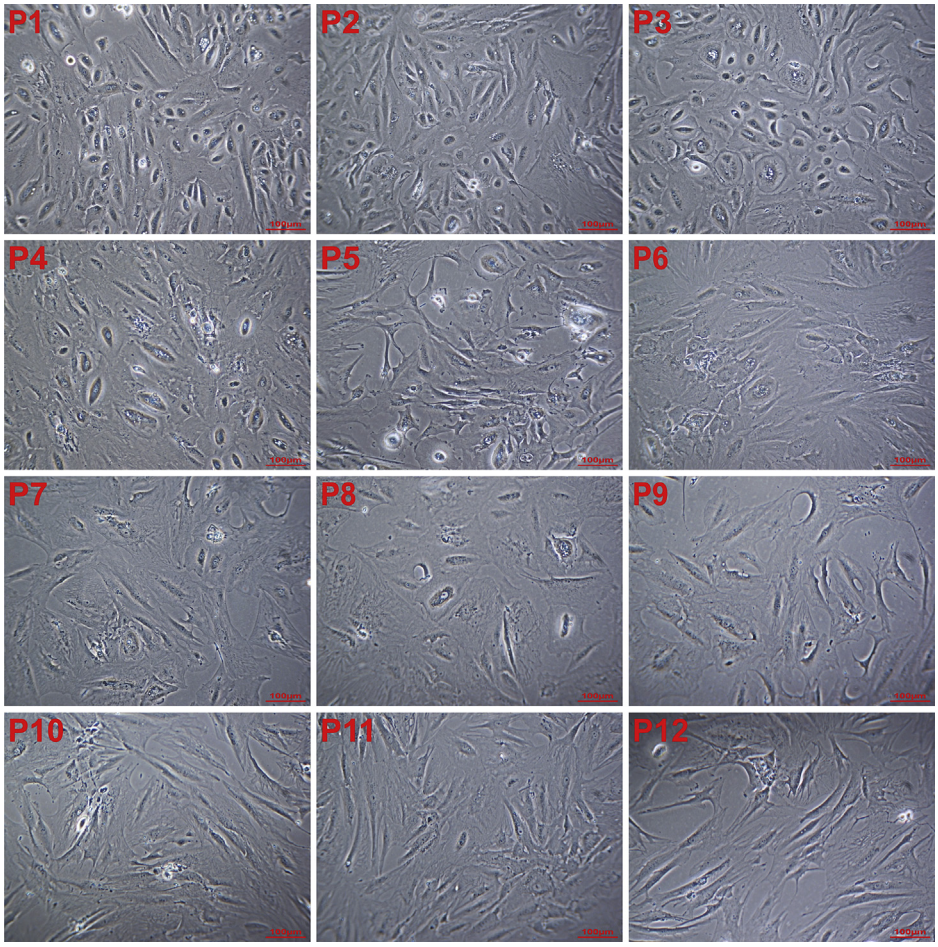

Fig. 3. Human omental fat tissue-derived cells in culture, passages P1–P12, ×10 magnification.

Fig. 6 shows the immunohistochemical analysis according to endothelial differentiation. The staining of the adipose tissue-derived cells is correlated with human endothelial cells as a control group. In the immunohistochemical analysis, the differentiated cells of all tissues express vWF, which is less marked while using EGM instead of EGM2. The expression of CD31 is detectable only on differentiated cells from subcutaneous- and visceral tissue-derived tissues using EGM2. Nevertheless, the antigen is expressed on fewer cells and shows a more spindle-shaped structure, compared to the reticular structure of the marked cell junctions of human endothelial cells (Fig. 6).

Table 1
Mean diameter of adherent cells derived from subcutaneous, visceral, and omental fat tissue per passage growth in α-MEM.

Passage	Subcutaneous		Visceral		Omental	
	Mean diameter (μm)	SD (μm)	Mean diameter (μm)	SD (μm)	Mean diameter (μm)	SD (μm)
P0	132.180	24.168	129.920	33.383	41.190	12.914
P1	131.620	27.001	151.420	45.551	45.530	28.934
P2	139.220	35.536	168.400	38.068	74.260	29.417
P3	133.415	37.420	171.230	54.398	70.610	20.023
P4	134.820	32.293	172.840	52.389	74.000	21.558
P5	129.415	34.585	174.610	36.402	110.255	27.762
P6	128.705	33.843	159.980	48.834	121.550	38.791
P7	130.830	35.773	172.450	41.295	133.510	36.228
P8	126.310	39.990	150.870	39.344	148.360	37.603
P9	128.250	42.745	161.410	35.814	139.930	30.520
P10	149.030	40.855	152.240	40.652	145.340	39.158
P11	133.970	40.338	166.290	48.117	162.250	38.000
P12	137.930	41.028	171.250	35.820	177.800	38.632

4. Discussion

In the present study, the isolation, cultivation, and induction towards osteogenic and angiogenic differentiation from cells derived by miscellaneous adipose tissue is discussed. One purpose of this work is to determine whether all types of adipose tissue can be used from donor sites for the isolation of mesenchymal stromal cells, and also whether the characteristic surface markers are expressed equally.

Adipose tissues are a rich source of multipotent adult cells. Not only the cells harvested as by-products after liposuction, but also cells dissected and harvested during standard surgical procedures from visceral or omental adipose tissue might provide multipotent cells for autologous regenerative purposes.

Lipoaspirate samples provide the advantage of gaining a large amount of cells during the standard surgical procedure. It is not possible, however, to purposely omit areas containing vascular structures or fibroblast-rich connective tissue to provide a more homogeneous cell culture without contamination. By leading the needle subcutaneously, optical control is not feasible. By selectively dissecting the fat tissue, a contamination at least of macroscopically recognisable different cell types is possible and may lead to a more

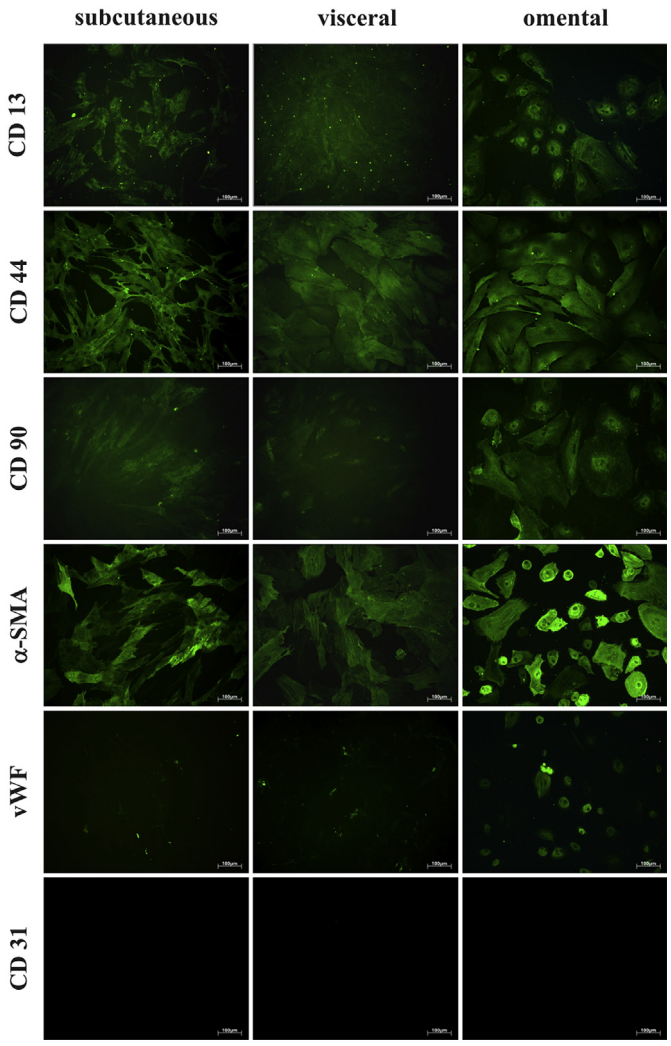

Fig. 4. Expression of ADSC marker CD13, CD44, CD90, α-SMA, and endothelial markers vWF and CD31 on human subcutaneous-, visceral-, and omental fat tissue-derived cells.

homogenous cell culture. The presence and interference of mesothelial cells in the omental and visceral cell cultures that have been dissected under visual control contradicts this hypothesis, however. The observed decay of the additional cells without weakening the culture results leads to the assumption that a contamination with mesothelial cells has no adverse effect on cell proliferation and differentiation.

Toyoda et al. compared the characteristics of omental and subcutaneous fat and concluded that there are no differences concerning either the amount of cells or their proliferation (Toyoda et al., 2009). Due to the high density of endothelial progenitor cells in the subcutaneous tissue, the angiogenic differentiation is more successful as compared to omental tissue. These findings are underlined by the study at hand (Toyoda et al., 2009). The capability of osteogenic differentiation seems to be more pronounced in multipotent cells from abdominal adipose tissue; this observation might be related to the respective culture conditions. In the direct comparison of identical cultural conditions of this investigation, no advantage of the omental cells was observed.

Morphologically, the cells from different origins are comparable. Zuk et al. describe the morphological appearance of these isolated cells as being similar to fibroblasts and resembling bone marrow-derived mesenchymal stem cells (Zuk et al., 2001). Multipotent cells from donor sites other than the bone marrow emerge as fibroblast-like spindle-shaped cells (Ashjian et al., 2003). The subcutaneous cells in this study, harvested by tissue resection during general surgery, match these morphological characteristics. Alternative donor regions as omental or visceral fat tissue are less prominent and not as well investigated. Sachs et al. studied adipose-derived multipotent cells from visceral fat and described comparable morphological, immunohistochemical, and multipotent features as in subcutaneous fat cells (Sachs et al., 2012). When dealing with these cells, the crucial first task is to clearly define the investigated cells at hand clearly as multipotent mesenchymal cells and to differentiate them from nearly ubiquitous fibroblasts or leukocytes. The mesenchymal stromal cells are characterised immunohistochemically by different surface markers; the most prominent are CD13, alanyl aminopeptidase N, expressed especially in endothelial cells of the intestines, CD44, a surface marker and collagen receptor, and CD90, an essential glycoprotein with substantial importance in cell–cell adhesion and neuronal growth (Jones et al., 2002; Dimitroff et al., 2001; Saalbach et al., 2000; Mitchell et al., 2006).

In the characterisation of adipose tissue–derived multipotent cells, the overexpression of CD13, CD44, and CD90 among others proved to be the most pronounced and therefore characteristic of the studied cells (Zuk et al., 2002). Another relevant marker is α–smooth muscle actin (α-SMA), an intracellular contractile structural filament of the cytoskeleton. It is expressed in smooth muscle cells or myofibroblasts. Its presence represents a negative control for endothelial cells. α-SMA is transitorily expressed by a variety of mesodermally derived cells during their proliferation and development or in processes of tissue repair (Liu et al., 2013). The presence of α-SMA as hADSC marker has already been described in the literature (Cheng et al., 2011). In addition to morphological examination, characterisation by defined surface marker stands for the unambiguous identification of the studied cells and traces their development. The selected surface markers in the present investigation were CD13, CD44, and CD90 and α-SMA. The absence of CD31 ruled out the presence of a mature endothelial culture.

In the current literature, a plethora of relevant surface markers to confirm the identity of the studied, allegedly multipotent cells are discussed: the experimental proof of CD105, CD73, or CD90 combined with the lack of CD14, CD45, or c-kit substantiate the identity of the MSCs (Kuhbier et al., 2010). CD44 and CD90 have been described as surface markers of adipose derived cells from subcutaneous and omental origin. CD90 seems to be expressed more strongly than on cells from visceral origin (Baglioni et al., 2009; Klopp et al., 2012). Festy et al. observed CD13 as a strong indicator for subcutaneous as well as visceral derived multipotent cells; CD13 therefore was chosen for the study at hand (Festy et al., 2005). The aim of this characterisation is to be sure to avoid fibroblasts, endothelial cells, and leukocytes. In the present study, the combination of cell harvesting and preparation, the confirmation of selected surface markers, and the morphologic analysis accounts for the identity of adipose tissue-derived multipotent cells. Considering the strong similarity to fibroblasts, the ability to differentiate finally reveals the multipotent cells (Alipour et al., 2010; Yu et al., 2010). One crucial finding of this investigation was the reproducible osteogenic differentiation in all studied cell lineages. This result might be of some relevance in the development of autologous regenerative therapy concepts of bony critical-sized defects or non-unions following trauma or tumour resections. Schubert at al. introduced an autologous graft consisting of a 3D scaffold of demineralised bone matrix and autologous subcutaneous fat cells in a minipig model, and were able to show increased bone formation in a spine fusion model as well as a critical-sized

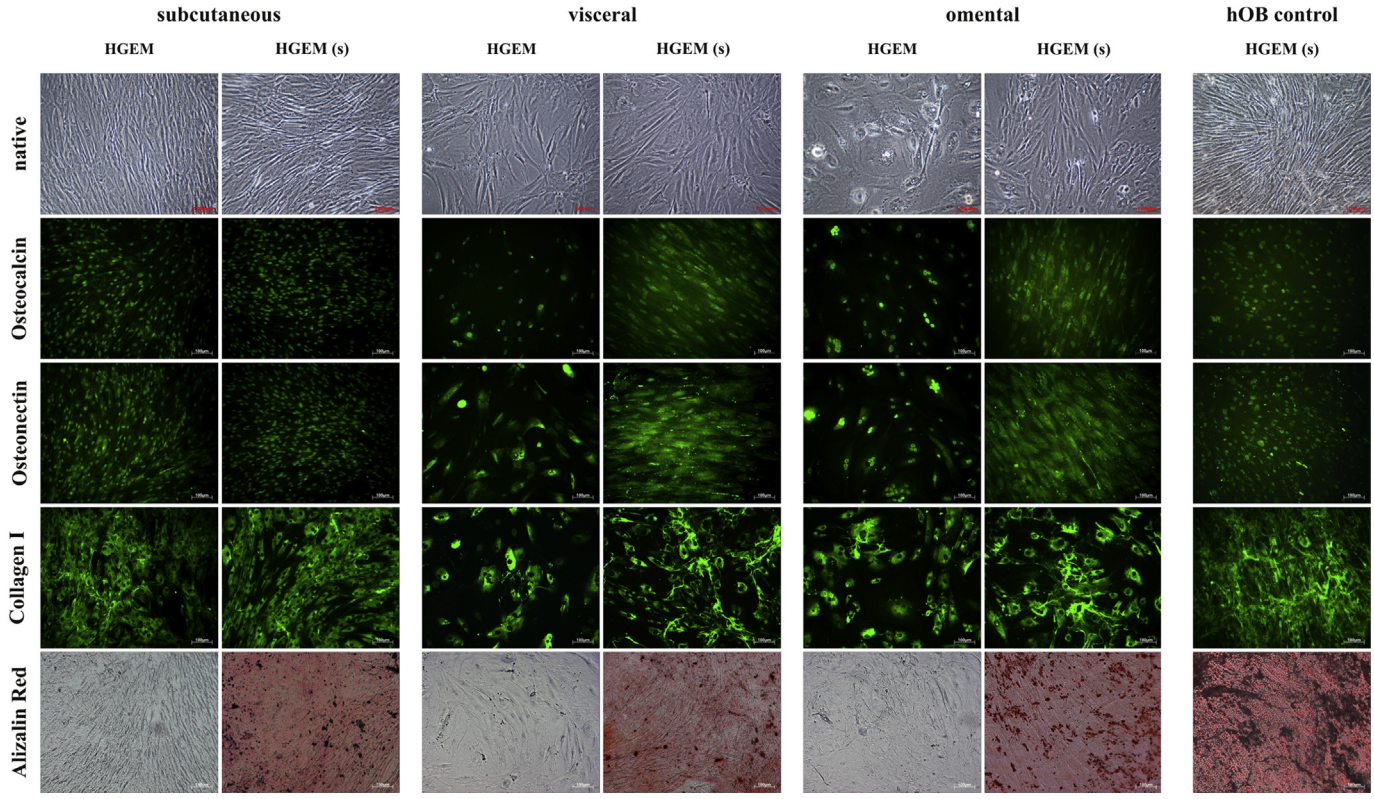

Fig. 5. Expression of osteoblastic markers osteocalcin, osteonectin, collagen I on subcutaneous-, visceral-, and omental fat tissue-derived cells in HGEM and supplemented HGEM undergoing osteogenesis compared to human osteoblasts. The differentiation process of subcutaneous preparations shows the best result in cell density, homogeneity, and protein expression. A positive mineralisation is visible in all lineages after 40 days of culture with $HGEM_{(s)}$, supplemented with α-glycerophosphate 10 mM (Sigma, Germany) and L-ascorbic acid 25 μg/mL (Sigma, Germany).

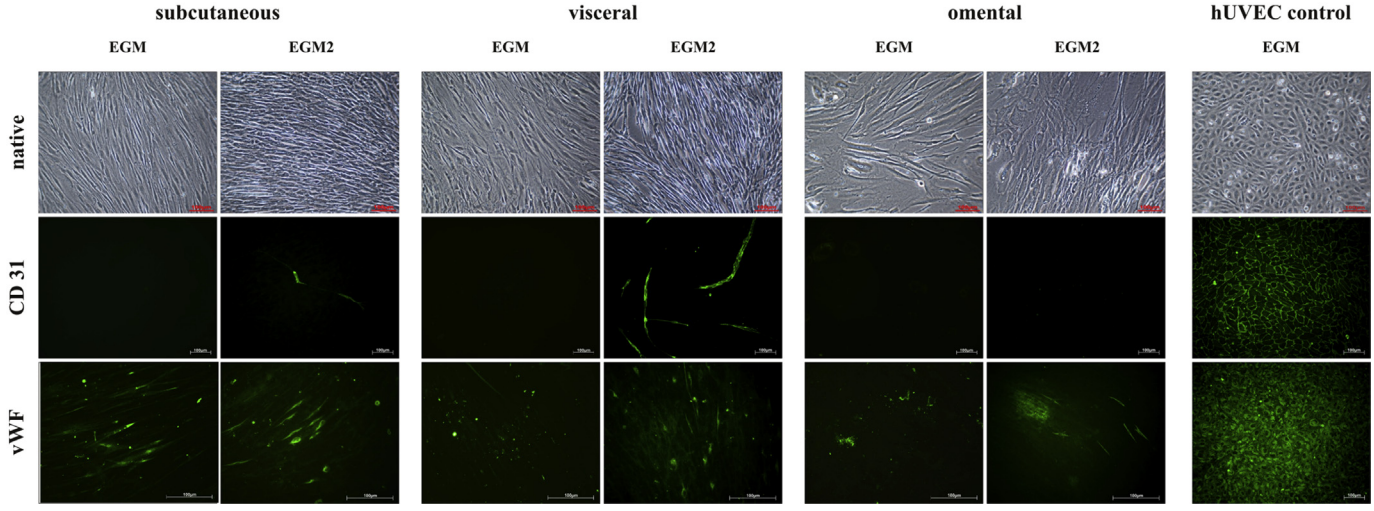

Fig. 6. Expression of endothelial markers CD31 and vWF on subcutaneous-, visceral-, and omental fat tissue-derived cells in EGM and EGM2 undergoing angiogenesis compared to HUVECs. In general there is a difference in cell morphology and protein expression on fat tissue cells compared to endothelial cells. The differentiation process of subcutaneous preparations shows the best result in cell density and homogeneity. The expression of vWF is detectable on cells of all cultures. CD31 is visible on differentiated subcutaneous- and visceral fat tissue-derived cells using EGM2.

femoral defect (Schubert et al., 2013). On the basis of the current findings, visceral and omental multipotent cells can be considered as a potential cell base as well. The regulation of the differentiation seems to stand under the regulation of miRNA: Li et al. observed adipogenic and osteogenic differentiation of MSCs from lipoaspirates with respect to the aspect of miRNA-dependent regulation: Generally the harvested cells hold the potential for adipogenic and osteogenic differentiation. The regulation of which cell type emerges is governed by miRNA expression: Whereas an overexpression of certain miRNAs induces adipogenic differentiation, their inhibition supports osteogenic differentiation (Li et al., 2013). The angiogenic differentiation in this setting has not been

as successful as described in comparable concepts: in animal models, adipose-derived cells in EGM2 medium show the typical endothelial morphology and express the characteristic surface markers CD31 and vWF after two weeks (Ning et al., 2009; Konno et al., 2010). During the investigation at hand, using human cells, the immunohistochemical markers were not as strongly expressed. Especially, stromal cells from the omental tissue are less able to perform endothelial differentiation and do not express CD31 in this study concept.

Several key factors for endothelial differentiation have been discussed. The presence of CD34 and the application of VEGF or culture condition using shear stress are studied and seem to favour endothelial differentiation, but not all critical factors are yet understood (Marino et al., 2012; Fischer et al., 2009; Colazzo et al., 2011).

Regarding a developing vasculature, the optimal adipose-derived cellular source for regenerative purposes are subcutaneous cells.

5. Conclusion

This study setup aims to systematically analyse different types of adipose tissue with special regard to their proliferation and differentiation potential concerning the angiogenic and osteogenic lineage. The cultivation of subcutaneous, visceral, and omental fat cells under osteogenic conditions led to a morphological and immunohistochemical differentiation; mineralization could be detected, most reliably 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, even if the immunohistochemical analysis provided less pronounced results of endothelial differentiation compared to osteogenic differentiation.

The findings of this investigation provide the basis for more specific analysis of the different multipotent adipose tissue-derived cells, and might improve existing concepts of hADSs-related individualized regenerative therapies.

Conflict of interest

The authors declare no conflict of interest.

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