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Research

Isolation and Differentiation Potential of Human Mesenchymal Stem Cells From Adipose Tissue Harvested by Water Jet-Assisted Liposuction

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Abstract

Background: In recent years the therapeutic application of extracted adipose tissue for autologous fat grafting and the application of adipose tissue-derived mesenchymal stem cells (adMSC) isolated thereof has progressed. Water-jet assisted liposuction (WAL) is 1 procedure for harvesting adipose tissue and provides a favorable aesthetic outcome combined with high tissue protection. Tissue aspirated by WAL has been successfully applied in grafting procedures.

Objectives: The aims of this study were to confirm the tissue viability and to understand the abundance and mesenchymal differentiation capacity of stem cells within the tissue.

Methods: We analyzed tissue integrity of WAL tissue particles via fluorescence microscopy. The adMSC content was determined by isolating the cells from the tissue. The mesenchymal differentiation capacity was confirmed with cytochemical staining methods.

Results: The stromal vascular fraction of WAL tissue showed high viability and contained an average of 2.6 × 105 CD34-positive cells per milliliter of tissue. Thus WAL tissue contains a high number of stem cells. Furthermore adMSC isolated from WAL tissue showed typical mesenchymal differentiation potential.

Conclusions: WAL of adipose tissue is well suited for autologous fat grafting because it retains tissue viability. Furthermore it is a valid source for the subsequent isolation of adMSC with multipotent differentiation potential.

Level of Evidence: 3

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Through the therapeutic application of extracted adipose tissue for autologous fat grafting, liposuction has become an important issue.[1,2] In this context the properties of the aspirated tissue, like tissue particle size, viability, adipocytes number, and other adipose-resident cell types or functional structures (eg, blood vessels) need to be investigated in closer detail.

Adipose tissue is composed of various cell types (adipocytes, endothelial cells, fibroblasts, blood and blood-derived cells, and adipose tissue-derived mesenchymal stem cells [adMSC]).[3-6] Enzymatic digestion of the adipose tissue and depletion of adipocytes yields the so-called stromal vascular fraction (SVF), which already has successfully been used in

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different clinical approaches such as supplementation of fat grafts and treatment of urinary incontinence and chronic wounds.[7-10]

Zuk et al were the first to describe adMSC.[6] By their intrinsic capacity for self-renewal and multipotent mesenchymal differentiation, adMSC are important in tissue homeostasis and regeneration.[11] Human adMSC were shown to directly differentiate into osteoblasts and endothelial cells in a nonunion fracture model.[12] In case studies, human adMSC were already successfully used to treat critical-size calvaria[13] and maxilla defects.[14] Furthermore, adMSC were demonstrated to engraft at long-term at the transplantation site.[15,16]

Compared with bone marrow, adipose tissue can be harvested in higher amounts, with a higher abundance of stem cells and less donor-site morbidity.[17] adMSC are present at a concentration of roughly 50,000 cells per mL adipose tissue (approximately 7% of the cells found in enzyme-dissolved adipose tissue),[18,19] which is 100-fold higher than found for mesenchymal stem cells (MSC) isolated from bone marrow.[17] Also, the use of adult stem cells does not entail the ethical concerns that the use of embryonic stem cells does. Finally adMSC fulfill all MSC characteristics defined by the International Society for Cellular Therapy, the International Federation of Adipose Therapeutics and Sciences, and others.[20,21]

Adipose tissue can be harvested by various methods, such as resection, conventional liposuction, syringe-aspiration, and others.[2] The specific liposuction methodology applied may impact the viability of cells present in the harvested tissue.[22,23] Water jet-assisted liposuction (WAL) is a liposuction procedure that applies the tumescent solution as a thin, fan-shaped, targeted, pulsating jet.[24,25] The cannula used during the operation comprises 2 channels that make it possible to infiltrate the tumescent solution and aspirate the emerging water and fat suspension at the same time. Thus, the amount of fluid infiltrated into the patient is much lower than during conventional liposuction procedures, which lowers the risk of cardiovascular side effects. This aims at ensuring the desired aesthetic outcome and safety of the patient as well as protecting the harvested adipose tissue. Our examinations assure quality of autologous fat grafts obtained by WAL and show differentiation reliability of mesenchymal stem cells isolated from WAL tissue. To this end, we examined the viability, number and adipogenic or osteogenic differentiation potential of adMSC isolated from WAL-harvested adipose tissue.

METHODS

If not stated otherwise, all plastic-ware was from Greiner Bio-One (Frickenhausen, Germany) and all reagents were from Sigma-Aldrich (Steinheim, Germany).

Donors

This study examined 13 consecutive patients (1 man and 12 women) over a 27-month period from August 2012 to October 2014. The patients gave written informed consent that they were willing to take part in this study. The study was approved by the ethics committee of Rostock University Medical Center under the registration number A2013-0112, and it complies with the ethical standards defined by the World Medical Association Declaration of Helsinki.

Aspiration of Human Adipose Tissue

The liposuction procedure was carried out according to the BEAULI protocol described by Ueberreiter et al.[26] Briefly, a pulsating water jet was applied for infiltration with simultaneous suction. For infiltration the ranges 1 to 3 are available, ranging from 90 mL/minutes ± 15% to 130 mL/minutes ± 15%. The infiltration solution used was classical Klein's tumescence solution that was prewarmed to 37 to 38°C. The cannulas for harvesting have an outer diameter of 38 mm and bear sharp tips. After the first infiltration, there was no required waiting period before the suction could be commenced. The negative pressure for the suction was adjusted to 500 mbar.

Viability Assessment of the WAL-Isolated Tissue

In order to evaluate the viability of the aspirated tissue, a live/dead staining was performed as described previously.[27] Therefore, the aspirated tissue was incubated for 10 minutes with cell culture medium (Dulbecco's Modified Eagle Medium (DMEM), Life Technologies, Darmstadt, Germany) supplemented with 3 µM Hoechst 33342 (Bis-benzimide H33342 trihydrochloride), 500 nM propidium iodide, and 1 µM calcein acetoxymethyl ester (both Life Technologies). The tissue was then analyzed by fluorescence microscopy (Carl Zeiss Microscopy GmbH, Göttingen, Germany) using the blue, green, and red emission filters. Emission maxima of the dyes are 460 nm (Hoechst 33342), 516 nm (calcein), and 617 nm (propidium iodide). Excitation maxima are 360 nm (Hoechst 33342), 496 nm (calcein), and 535 nm (propidium iodide).

Isolation and Culture of adMSC

As described previously,[28] adipose tissue was digested using 1.6 mg/mL Collagenase NB4 (SERVA, Heidelberg, Germany), a mixture of a neutral protease and the collagenases I and II in phosphate-buffered saline (PBS) with calcium and magnesium (PAA Laboratories, Coelbe, Germany). Through a series of steps of washing in PBS with 10% fetal calf serum (FCS; PAN Biotech, Aidenbach,

Germany) and centrifugation, the SVF was separated from the adipose tissue remains. At this point the SVF cell number and content of CD34-positive cells were determined with flow cytometry (see Flow Cytometric Analysis section). The cells liberated from the digested tissue were incubated for 24 hours under standard cell culture conditions (5% CO_2 and 37°C in a humidified atmosphere). CD34-positive cells were isolated from total plastic-adherent cells using a magnetic bead depletion system (Life Technologies). During this selection procedure, the number of cells that were plastic adherent after 24 hours and the number of cells that were plastic adherent and CD34 positive were determined using the Scepter cell counter (Merck KGaA, Darmstadt, Germany). Since the bead depletion is a standard procedure to attain the cells used for the experiments, we desisted from the reapplication of flow cytometry. After 3 passages, adMSC were seeded into experimentation at 20,000 cells per cm^2. Absence of *Mycoplasma* species was confirmed microscopically after DNA staining.

AdMSC were cultured in standard culture medium, adipogenic differentiation stimulating medium, and osteogenic differentiation-stimulating medium. Standard culture medium was DMEM, high glucose, GlutaMAX-I, supplemented with 1% penicillin/streptomycin (100 U/mL and 100 µg/mL; both Life Technologies), and 10% FCS. For adipogenic stimulation, 500 µM 3-isobutyl-1-methylxanthin (SERVA), 10 µM insulin, 1 µM dexamethasone, and 200 µM indomethacin (Fluka, Seelze & Buchs, Germany) were added to standard culture medium. For osteogenic stimulation, standard culture medium was supplemented with 0.25 g/L ascorbic acid, 1 µM dexamethasone, and 10 µM β-glycerophosphate (Fluka). At confluence, cultivation in standard and differentiation-inducing media began, termed day 0, and took up to 35 days. Change of medium was done every 2 to 3 days.

Flow Cytometric Analysis

In order to quantify the CD34 positive cell population of the SVF, isolated cell-suspension specimens from 7 patients were stained using a CD34 single platform staining kit (Stem-Kit, Beckman-Coulter, Krefeld, Germany). The Stem-Kit Reagents consist of a 2-color fluorescent (CD45-FITC/CD34-PE) murine monoclonal antibody reagent, a 2-color murine fluorescent (CD45-FITC/isoclonic control-PE) reagent to check the non-specific binding of the CD34-PE monoclonal antibody, a nucleic acid viability dye (7-amino-actinomycin-D/7-AAD), a reagent (ammonium chloride) to lyse erythrocytes, and Stem-Count fluorospheres for absolute count determination of CD34-positive cells. Therefore, tubes were prefilled with 20 µL of a mixture of CD45-FITC (clone J33) and CD34-PE (clone 581) and 20 µL of 7-AAD-solution. 100 µL of cell suspension was added and incubated for 15 minutes at room temperature (20°C) in the dark. A second incubation for 10 minutes was done after addition of 2 mL lysis reagent. 100 µL Stem-Count fluorospheres were added to the cell suspension directly before measurement. The flow cytometer FC500 Flow Cytometer (Beckman-Coulter) was equipped with a 20 mW argon-ion-laser providing excitation at a wavelength of 488 nm.

Analysis was done following a modified ISHAGE protocol[29] for single platform analysis.[30] Cells were gated for CD45 and 7-AAD-negative cells to exclude leucocytes and dead cells. Viable CD34-positive cells and fluorospheres were counted using the CXP analysis software (Beckman-Coulter). The stem cell concentration was then calculated on the basis of the fluorosphere concentration provided by the test kit.

Quantification of Cell Number

The cell number in tissue from 8 individuals was determined by crystal violet staining as described previously.[28] Crystal violet stains the negatively charged cellular DNA via ionic attraction[31] in a linear fashion.[32]

Analysis of Mesenchymal Differentiation

Analysis of Adipogenic Differentiation

Intracellular accumulation of lipids in the isolated adMSC of 5 individuals was detected by fluorescence staining of lipid vacuoles after 14 days of culture as described previously.[33] To this end the cells were washed with PBS and fixed in 4% paraformaldehyde (PFA). After washing with PBS, the cells were incubated in the Bodipy staining solution (1 µg/mL 150 mM NaCl, Life Technologies) in the dark. The cells were then washed with PBS and H_2O. The fluorescence intensity was measured in a fluorescence microplate reader (TECAN, Crailsheim, Germany) using an excitation wavelength of 480 nm and an emission wavelength of 515 nm. Fluorescence microscopy was done to document the formation of lipid vacuoles within the cells using an Axiocam MRc fitted to an Axio Scope.A1 (Carl Zeiss Microscopy GmbH).

Analysis of Osteogenic Differentiation

Quantification of alkaline phosphatase (ALP) activity in the isolated adMSC of 6 patients and the extracellular matrix (ECM) calcium content in the isolated adMSC of 4 patients were used to detect osteogenic differentiation of the cells as previously described.[28] For photo documentation of ALP (liver/bone/kidney isoenzyme) activity, cells were stained with a solution of 67 mM 2-Amino-2-methyl-1.3-propanediol (AMPED), 2.7 mM Naphtol AS-MX phosphate, and 2.7 mM Fast Red Violet LB Salt in H_2O. Quantification of ALP activity was done after 21 days of culture by washing cells with Tris-buffered saline and permeabilizing lysis buffer (1% Tween 20 and 100 mM phenylmethanesulfonyl fluoride in H_2O) and incubating with ALP substrate solution (10 mM para-nitrophenylphosphate (pNPP), 100 mM AMPED, and 5 mM $MgCl_2$ in H_2O for 1 hour under standard culture

conditions. To stop ALP activity, 2 M NaOH was added. The absorbance of the supernatant was quantified in a microplate reader (anthos Mikrosysteme) at 405 nm.

For the visual documentation of the calcium deposition in the ECM, the cells were stained with 30 mM Alizarin Red S dissolved in H_2O. Pictures were taken with the camera AxioCam ICc1 fitted to an Axiovert 25 (Carl Zeiss Microscopy GmbH). Quantification of ECM calcium content based on Ca^{2+} complexation by *ortho*-cresolphthalein was done after 35 days. Briefly, after washing cells with PBS and fixation with 4% PFA pre-warmed to 37°C, cells were washed with H_2O, and cresolphthalein buffer (0.1 mg/mL *ortho*-cresolphthalein complexon, 1 mg/mL 8-hydroxy quinoline, and 6% of 37% HCl in H_2O) was added. After incubation for 5 minutes at room temperature (19°C to 21°C), AMP buffer (15% 2-Amino-2-methyl-1-propanol [AMP] in H_2O, pH 10.7) was added and incubated for 15 minutes at room temperature (20°C). The optical density of the supernatant was quantified in a microplate reader (anthos Mikrosysteme) at 580 nm.

Data Illustration and Statistics

All measurements were performed in triplicate. Data are illustrated as bar and line charts as well as box plots and were created using the R software environment for statistical computing and graphics.[34] The data displayed are the medians with positive and negative error bars representing the third and first quartile, respectively. Since the data obtained were in most cases not normally distributed, testing for significance in the difference between 2 datasets was done using the nonparametric Mann-Whitney *U* test. The level of significance was set to a *P*-value of lower or equal to .05 ($P \leq .05$) and calculated using R.

RESULTS

The average patient age was 42 years (range, 24-59 years). All patients were healthy adults seeking contouring of one or more of the following areas: arms, hips, buttocks, upper and lower abdomen, upper and lower back, flanks, inner thighs, and outer thighs. The patients had an average body mass index of 25.8 (range, 21-35). The time from tissue harvest to cell isolation was less than 20 hours.

Viability of the Aspirated Tissue

The combined live/dead staining of WAL tissue resulted in a comprehensive green fluorescence emission of the tissue and thus indicates its viability, while the nuclei of all cells are stained in blue (Figure 1A). A thin green circumferential fluorescent line reflects the spherical shape of the mature adipocytes. The biggest part of the adipocytes is filled by

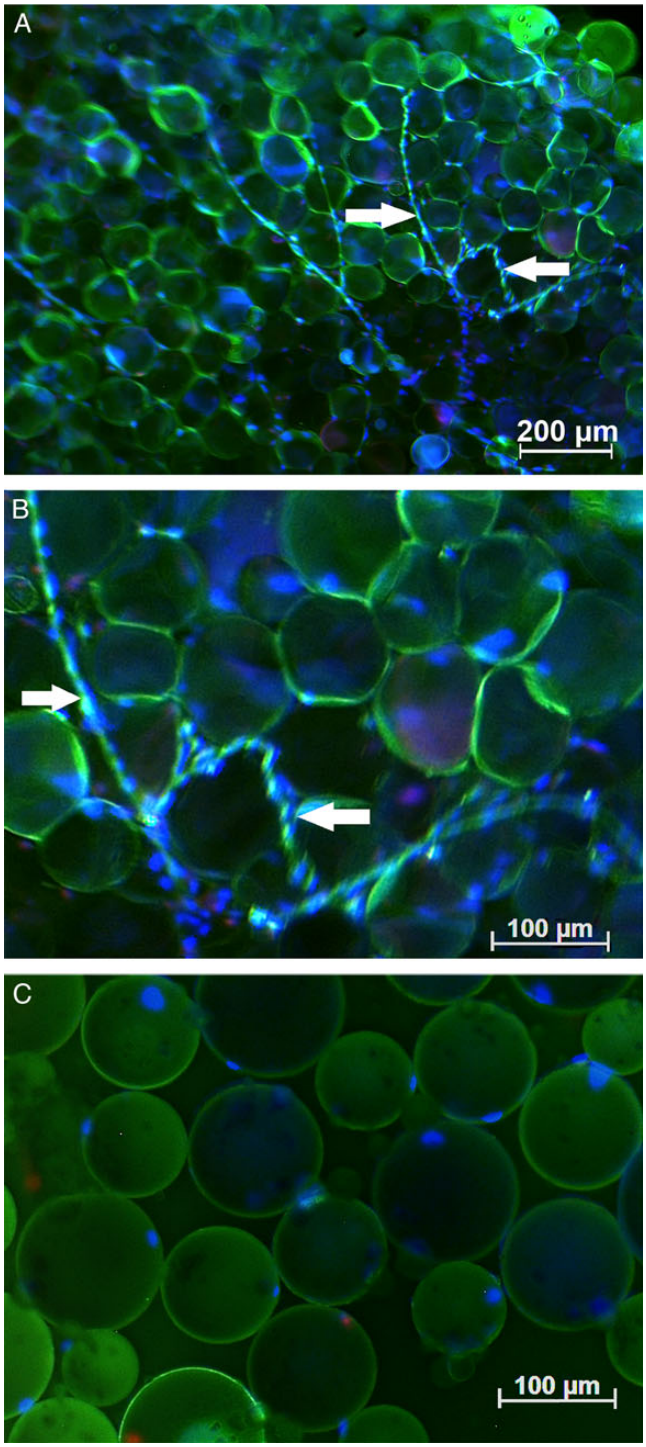

Figure 1. Live/dead-stained adipose tissue directly after the isolation by WAL (A; green: viable cells, red: dead cells, blue: nuclei); detail from isolated tissue showing vasculature-like structures (B); single cell suspension resulting from enzymatic digestion of the tissue (C). Most cells showed an intensive green staining and were thus identified as viable. A number of blood vessel-like structures were detectable (arrows). Only a few dead cells (red fluorescence) were found.

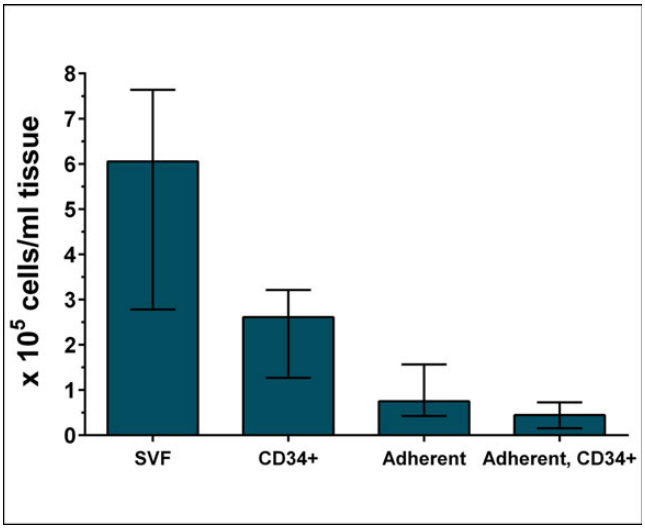

Figure 2. Quantification of cell amounts after different steps of isolation (cell concentration in the SVF; concentration of CD34-positive cells within the SVF; concentration of plastic-adherent cells after 24 hours of culture; concentration of plastic-adherent and CD34-positive cells after 24 hours of culture; n = 7). The SVF on average contains 6.1 × 10^5 cells per mL harvested adipose tissue and an average of 2.6 × 10^5 cells per mL tissue was positive for CD34. After 24 hours of cultivation, an average of 0.8 × 10^5 cells per mL tissue was plastic adherent, and thereof 0.45 × 10^5 cells per mL tissue, on average, were positive for CD34.

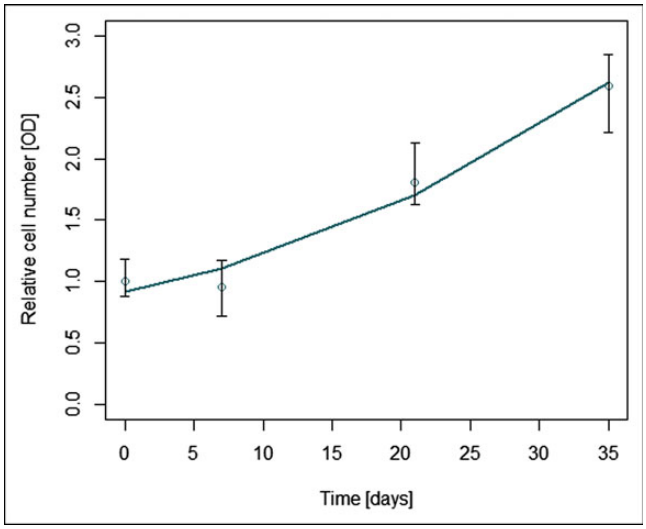

Figure 3. Proliferation of adMSC under steady state cultivation conditions; adMSC were cultured for 35 days. Cell numbers were determined at 0, 7, 21, and 35 days. All values were normalized to the 0 day value. Cells show continuous proliferation, ie, 2.5-fold increase of cell number over 35 days of culture (n = 8).

a unilocular lipid vacuole that is unstained, due to an organelle-inherent lack of esterase activity required for fluorophore activation. Only a few dead cells were detected (Figure 1B, red fluoresecence). Furthermore, blood vessel-like structures were detectable throughout the WAL-derived tissue (white arrows, Figure 1A and B). Collagenase treatment of WAL-isolated tissue led to a single cell suspension of mostly viable cells (Figure 1C).

Number of Cells in Aspirated Tissue

After preparation of a single cell suspension, the SVF was obtained by removal of adipocytes. The SVF on average contained 6.1 × 10^5 cells per mL aspirated tissue. Almost half of that fraction was identified as CD34 positive (averaging 43%, about 2.6 × 10^5 cells per mL aspirated tissue). Moreover, an average of 18% of the SVF (0.8 × 10^5 cells per mL aspirated tissue) was adherent after 24 hours of standard cell culture, roughly half of this portion was adherent and CD34 positive (0.45 × 10^5 cells per mL aspirated tissue from the SVF) (Figure 2).

Proliferation of adMSC In Vitro

To evaluate the proliferation behavior of WAL-isolated adMSC during cultivation and under differentiation conditions, cells were seeded into steady state conditions and the relative cell numbers were determined after 0, 7, 21, and 35 days of cultivation. We found a continuous increase in cell number and thus continuous proliferation of adMSC (Figure 3).

Mesenchymal Differentiation Potential

Adipogenic Differentiation

After 14 days of adipogenic stimulation, the formation of lipid-filled vacuoles in adMSC was detectable, whereas no lipid vacuoles were found in adMSC under control conditions (Figure 4A,B). Quantification of the amount of accumulated intracellular lipid revealed a significant 3-fold increase under adipogenic differentiation conditions (Figure 4C).

Osteogenic Differentiation

To evaluate the degree of osteogenic differentiation, the activity of the enzyme ALP and the amount of calcium phosphate deposited to the ECM were determined. In contrast to the control cultures showing a mild red staining (Figure 5A), osteogenically stimulated adMSC showed a distinct red staining for ALP after 21 days of cultivation (Figure 5B). After 35 days of osteogenic stimulation, calcium phosphate deposits were found, which was not true for the unstimulated control cultures (Figure 5D,E). Quantification of these differentiation markers confirmed distinct changes in the cellular phenotypes: after 21 days in osteogenic differentiation medium, cells displayed a more than 4-fold higher activity of ALP than cells that had been cultured under standard conditions

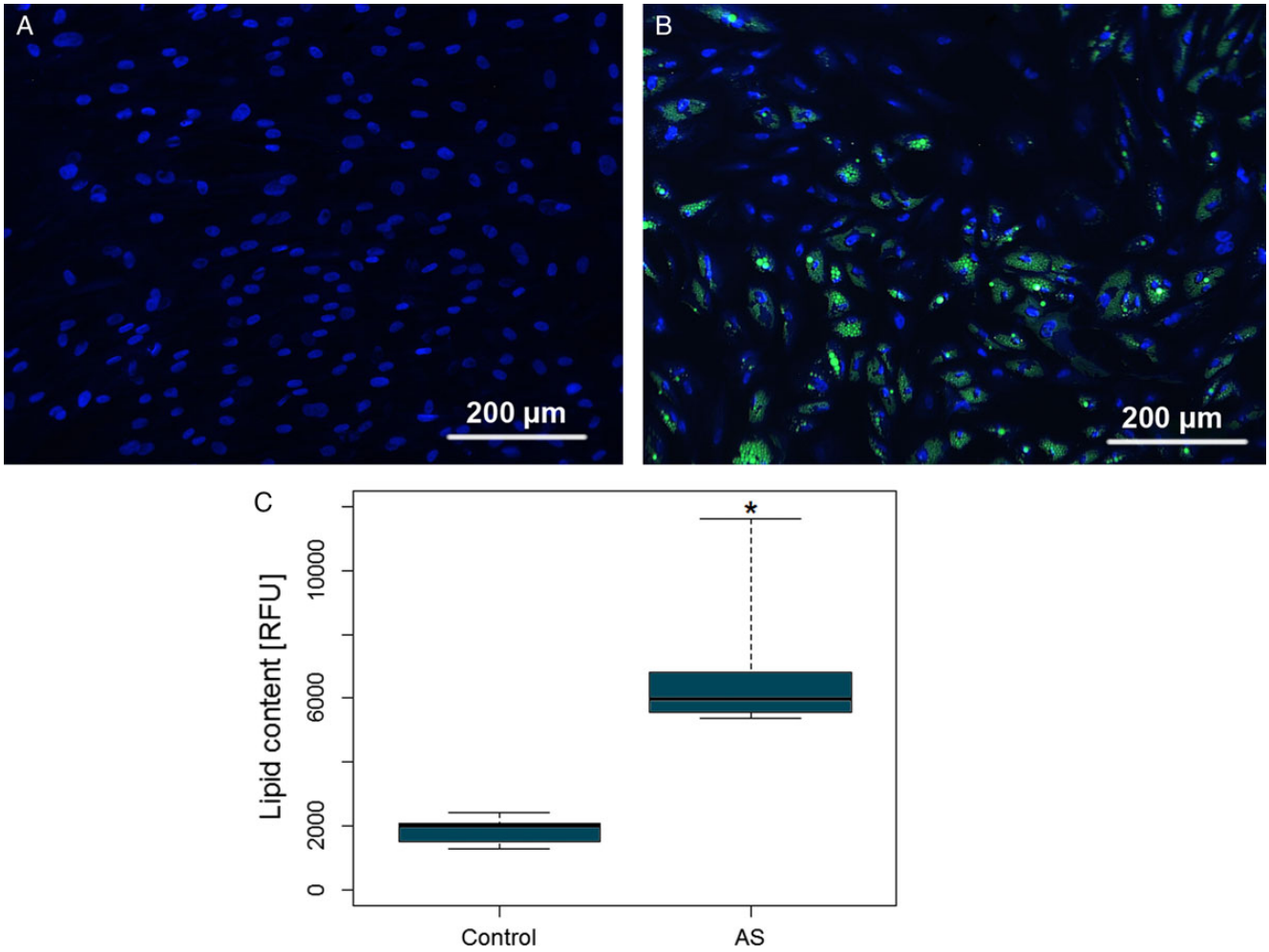

Figure 4. Adipogenic differentiation degree of adMSC. Staining of lipid vacuoles (green) and nuclei (blue) in unstimulated control cultures (A) and adipogenically stimulated adMSC (AS) after 14 days (B). Quantification of lipid content (C, quantification of fluorescence intensity; n = 5; Mann-Whitney *U* test). Only adipogenically stimulated adMSC showed lipid accumulation.

(Figure 5C). Calcium phosphate deposition by adMSC stimulated osteogenically for 35 days was almost 100-times higher than found for cells in the control cultures, which showed almost no calcium phosphate deposition (Figure 5F).

DISCUSSION

WAL is a liposuction procedure that provides a favorable aesthetic outcome combined with high tissue protection and lower risk of cardiovascular side effects due to lowered amount of infiltrated fluid compared with conventional liposuction procedures.[24,25,35,36] Moreover tissue aspirated by WAL has already been successfully applied in grafting procedures.[26,37] Peltoniemi et al proposed that WAL is a preferable technique for autologous fat grafting because it yields a transplant rich in stem cells and enables easy injection without pressure, thus preventing damage to cells.[38]

To confirm the high viability and abundance of stem cells in adipose tissue aspirated by WAL, we analyzed WAL tissue particles regarding integrity, adMSC yield, and mesenchymal differentiation capacity. The scope of this study did not include the examination of other liposuction techniques, such as a direct comparison of tissue harvested from the same patient in the same operation but with 2 different techniques, which would have been a more direct approach for the evaluation of the WAL technique. Nonetheless using data from current publications, the WAL procedure can be put into context with other liposuction techniques. Through visual evaluation, we can show that WAL aspiration results in viable tissue with intact vasculature-like structures. This is consistent with previous findings revealing that WAL tissue consists of intact cell aggregates and small blood vessels.[35] The assessment of the overall cell content and consecutive quantification of dead and viable cells could be a subject of future studies. We

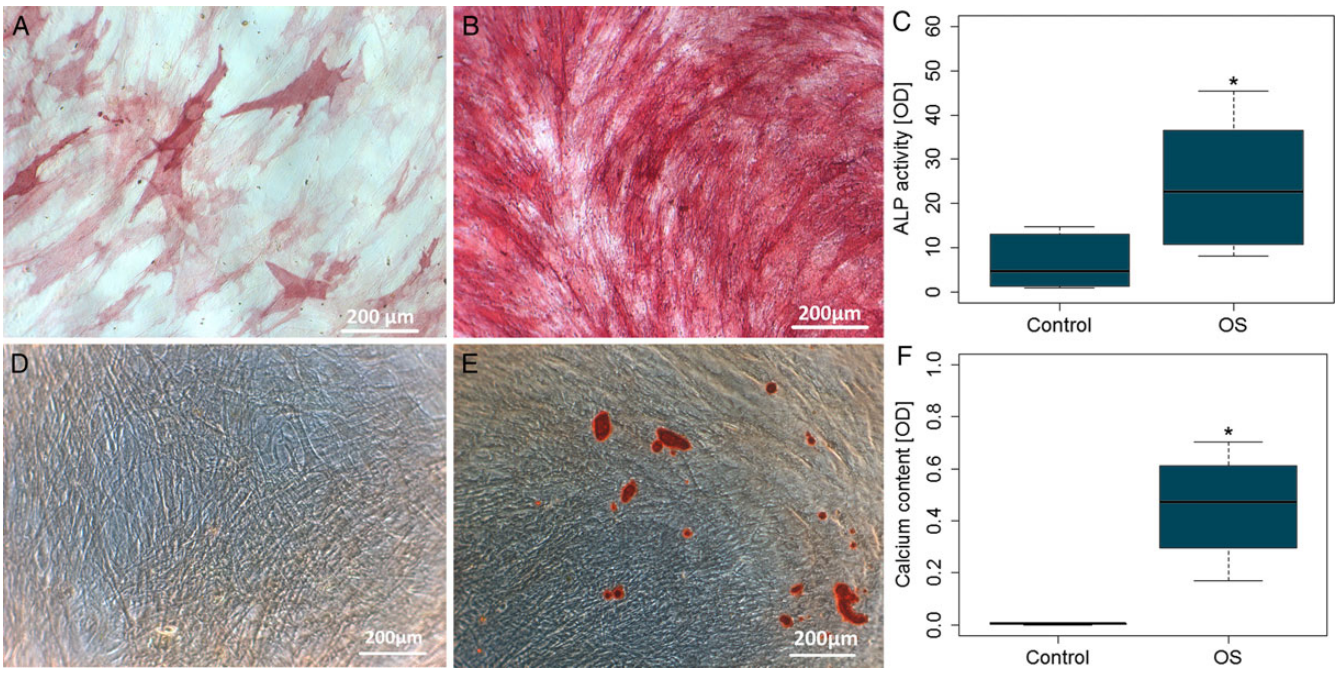

Figure 5. Osteogenic differentiation degree of adMSC. ALP-staining (red) in control cultures (A) and in osteogenically stimulated cultures (OS) after cultivation for 21 days (B). Quantification of ALP activity (C; n = 6; Mann-Whitney *U* test). Staining of ECM calcium deposition in control cultures (D) and osteogenically induced cultures after cultivation for 35 days (E). Quantification of ECM calcium content (F, cresolphthalein staining; n = 4; Mann-Whitney *U* test). Compared with non-stimulated control cultures, osteogenically stimulated cultures showed a 4-fold increase in ALP activity after 21 days and a 100-fold higher calcium deposition in the ECM after 35 days.

found that the enzymatic digestion procedure yields a single cell suspension consisting of adipocytes and an SVF with an average of 6.1×10^5 cells per mL WAL tissue. Differences in cell number of the SVF have been found for different tissue harvesting methods. For example an average of 3×10^5 SVF cells per mL tissue was found after liposuction not performed by WAL,[39] 6×10^5 after ultrasound-assisted liposuction and 7×10^5 after adipose tissue resection.[40] Since the SVF is a heterogeneous mixture of cells and thus the SVF cell content is not a direct measure of stem cell content, we have analyzed the presence of CD34-positive cells within the SVF. CD34 was first identified in stromal precursors in normal human adult bone marrow,[41] and the Stro-1 antibody frequently used for prospective isolation of MSC was consequently generated using CD34-positive MSC.[42] Subsequently, CD34 was found to be expressed also by hematopoietic stem cells in activated state and thus cycling in the blood stream, while hematopoietic stem cells in quiescent state and residing to the bone marrow are CD34 negative.[43] Subsequently, the panel of CD34-expressing cells was even more extended to muscle satellite cells, interstitial cells, epithelial, and endothelial progenitors[44] and is currently thought to be expressed by all perivascular cells in situ,[45] though not always at constant level, as reported for MSC whose CD34-expression level declines upon passaging in vitro.[46] The SVF from WAL tissue contained an average of 2.6×10^5 CD34-positive cells per mL tissue (an average of 43% of SVF). Results from other groups are an average of 22.5%[17] and 15% to 40% cells per mL tissue.[5] Following the statements made above, 1 portion of the CD34-positive cells is of hematopoietic origin while the other is adMSC. This was confirmed by Bourin et al by detecting a CD34-positive, but CD45-negative population of cells within the SVF of adipose tissue because hematopoietic precursors would be positive for both surface markers.[20] The 2 cell populations could be separated by selecting them for adherence to standard tissue culture polystyrene because adMSC are plastic adherent[47] whereas hematopoietic stem cells are not.[48,49] Thus, the CD34-positive and plastic-adherent population of 0.45×10^5 cells per mL tissue (8% of the SVF and 18.9% of the CD34-positive cells) that we isolated from the SVF 24 hours after tissue digestion represents a homogenous population of adMSC. Other groups have found 6.3% ± 1.8% to be functional adMSC residing in the SVF of resected adipose tissue, 1.9% ± 1.3% functional adMSC in the SVF of tissue obtained by tumescent liposuction, and 0.4% ± 0.1% functional adMSC in the SVF of tissue obtained by ultrasound-assisted liposuction.[40] A wide range of adMSC

content in the SVF is discernible comparing a single patient's material. While the average is 8%, as mentioned above, the range varied from 2.3% to 17.9%. Philips et al, in experiments where tissue was harvested with the Coleman technique, found this range to vary from 4% to 37%.[50] The differences between the study outcomes result from the fact that the methods used for the analysis of adMSC content in adipose tissue differ between almost every single research group. In this case not only the aspiration method was different but also the adMSC content was determined by flow cytometric analysis of the SVF, while in our study the absolute adMSC content was determined after 24 hours of plastic adherence.

Supportingly adipogenic stimulation of the isolated adMSC led to intracellular lipid accumulation, which proves the adipogenic differentiation potential of the adMSC obtained from WAL tissue. Also, the osteogenic differentiation potential could be shown after specific stimulation by means of differentiation markers ALP activity and ECM calcification. Thus, adMSC from WAL tissue show mesenchymal differentiation potential. It has been shown that the stem cell content in harvested tissue may be a positive measure for the long-term survival and retention of fat grafts.[50] Thus, not only does WAL represent a very gentle operation technique concerning the patient comfort and the viability of the harvested tissue,[24,38] but the possibility of enriching fat grafts with autologous stem cells isolated from the same tissue represents a promising clinical application. Another important point will be the future application of stem cells in the treatment of, eg, degenerative arthritis, and a possible wide variety of other clinical indications. The comparative examination of tissues from the same patient harvested by WAL and other liposuction techniques would be an interesting prospect for future studies.

CONCLUSIONS

WAL is a liposuction procedure that provides favorable results concerning patient comfort and aesthetic outcome. We show that WAL tissue is a valid source of adMSC, yielding numbers of cells that lie within the range of cell numbers isolatable from adipose tissue harvested with other techniques. The adMSC isolated from WAL tissue are viable and possess mesenchymal differentiation potential. In this respect WAL tissue may be used for autologous fat grafting and is an appropriate source for adMSC isolation.

Disclosures

Dr Kleine is on the Executive Board of Seracell Stammzelltechnologie GmbH (Rostock, Germany) Dr Matthiesen is an employee of human med AG (Schwerin, Germany). The other authors have no financial disclosures to report.

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REFERENCES

1. Hsu VM, Stransky CA, Bucky LP, Percec I. Fat grafting's past, present, and future: why adipose tissue is emerging as a critical link to the advancement of regenerative medicine. *Aesthet Surg J*. 2012;32(7):892-899.
2. Kakagia D, Pallua N. Autologous Fat Grafting: In Search of the Optimal Technique. *Surg Innov*. 2014;21(3):327-336.
3. Astori G, Vignati F, Bardelli S, et al. "In vitro" and multicolor phenotypic characterization of cell subpopulations identified in fresh human adipose tissue stromal vascular fraction and in the derived mesenchymal stem cells. *J Transl Med*. 2007;5:55.
4. Gimble JM, Guilak F, Bunnell BA. Clinical and preclinical translation of cell-based therapies using adipose tissue-derived cells. *Stem Cell Res Ther*. 2010;1(2):19.
5. Yoshimura K, Shigeura T, Matsumoto D, et al. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. *J Cell Physiol*. 2006;208(1):64-76.
6. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001;7(2):211-228.
7. Sterodimas A, de Faria J, Nicaretta B, Boriani F. Autologous fat transplantation versus adipose-derived stem cell-enriched lipografts: a study. *Aesthet Surg J*. 2011;31(6):682-693.
8. Yamamoto T, Gotoh M, Kato M, et al. Periurethral injection of autologous adipose-derived regenerative cells for the treatment of male stress urinary incontinence: Report of three initial cases. *Int J Urol*. 2012;19(7):652-659.
9. Akita S, Yoshimoto H, Akino K, et al. Early experiences with stem cells in treating chronic wounds. *Clin Plast Surg*. 2012;39(3):281-292.
10. Zhu M, Zhou Z, Chen Y, et al. Supplementation of fat grafts with adipose-derived regenerative cells improves long-term graft retention. *Ann Plast Surg*. 2010;64(2):222-228.
11. Majka SM, Barak Y, Klemm DJ. Concise review: adipocyte origins: weighing the possibilities. *Stem Cells*. 2011;29(7):1034-1040.
12. Shoji T, Ii M, Mifune Y, et al. Local transplantation of human multipotent adipose-derived stem cells accelerates fracture healing via enhanced osteogenesis and angiogenesis. *Lab Invest*. 2010;90(4):637-649.
13. Lendeckel S, Jodicke A, Christophis P, et al. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. *J Craniomaxillofac Surg*. 2004;32(6):370-373.
14. Mesimaki K, Lindroos B, Tornwall J, et al. Novel maxillary reconstruction with ectopic bone formation by GMP

adipose stem cells. *Int J Oral Maxillofac Surg*. 2009;38 (3):201-209.
15. Toupet K, Maumus M, Peyrafitte JA, et al. Long-term detection of human adipose-derived mesenchymal stem cells after intraarticular injection in SCID mice. *Arthritis Rheum*. 2013;65(7):1786-1794.
16. Nowacki M, Pietkun K, Pokrywczynska M, et al. Filling Effects, Persistence, and Safety of Dermal Fillers Formulated With Stem Cells in an Animal Model. *Aesthet Surg J*. 2014;34(8):1261-1269.
17. James AW, Zara JN, Zhang X, et al. Perivascular stem cells: a prospectively purified mesenchymal stem cell population for bone tissue engineering. *Stem Cells Transl Med*. 2012;1(6):510-519.
18. Levi B, Longaker MT. Concise review: adipose-derived stromal cells for skeletal regenerative medicine. *Stem Cells*. 2011;29(4):576-582.
19. Shi Y, Niedzinski JR, Samaniego A, Bogdansky S, Atkinson BL. Adipose-derived stem cells combined with a demineralized cancellous bone substrate for bone regeneration. *Tissue Eng Part A*. 2012;18(13-14):1313-1321.
20. Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy*. 2013;15 (6):641-648.
21. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8(4):315-317.
22. Lalikos JF, Li YQ, Roth TP, Doyle JW, Matory WE, Lawrence WT. Biochemical assessment of cellular damage after adipocyte harvest. *J Surg Res*. 1997;70 (1):95-100.
23. Schreml S, Babilas P, Fruth S, et al. Harvesting human adipose tissue-derived adult stem cells: resection versus liposuction. *Cytotherapy*. 2009;11(7):947-957.
24. Man D, Meyer H. Water jet-assisted lipoplasty. *Aesthet Surg J*. 2007;27(3):342-346.
25. Steinert M. [Physical lipolysis]. *Hautarzt*. 2010;61 (10):856-863.
26. Ueberreiter K, von Finckenstein JG, Cromme F, Herold C, Tanzella U, Vogt PM. [BEAULI--a new and easy method for large-volume fat grafts]. *Handchir Mikrochir Plast Chir*. 2010;42(6):379-385.
27. Salamon A, van Vlierberghe S, van Nieuwenhove I, et al. Gelatin-Based Hydrogels Promote Chondrogenic Differentiation of Human Adipose Tissue-Derived Mesenchymal Stem Cells In Vitro. *Materials*. 2014;7(2): 1342-1359.
28. Salamon A, Jonitz-Heincke A, Adam S, et al. Articular cartilage-derived cells hold a strong osteogenic differentiation potential in comparison to mesenchymal stem cells in vitro. *Exp Cell Res*. 2013;319(18):2856-2865.
29. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. *J Hematother*. 1996;5(3):213-226.
30. Keeney M, Chin-Yee I, Weir K, Popma J, Nayar R, Sutherland DR. Single platform flow cytometric absolute CD34+ cell counts based on the ISHAGE guidelines. International Society of Hematotherapy and Graft Engineering. *Cytometry*. 1998;34(2):61-70.
31. Noeske K. [The binding of crystal violet on deoxyribonucleic acid. Cytophotometric studies on normal and tumor cell nuclei]. *Histochemie*. 1966;7(3):273-287.
32. Gillies RJ, Didier N, Denton M. Determination of cell number in monolayer cultures. *Anal Biochem*. 1986;159 (1):109-113.
33. Fiedler T, Salamon A, Adam S, Herzmann N, Taubenheim J, Peters K. Impact of bacteria and bacterial components on osteogenic and adipogenic differentiation of adipose-derived mesenchymal stem cells. *Exp Cell Res*. 2013;319(18):2883-2892.
34. R_Core_Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R_Core_Team, 2013.
35. Stutz JJ, Krahl D. Water jet-assisted liposuction for patients with lipoedema: histologic and immunohistologic analysis of the aspirates of 30 lipoedema patients. *Aesthetic Plast Surg*. 2009;33(2):153-162.
36. Stabile M, Ueberreiter K, Schaller HE, Hoppe DL. Jet-assisted fat transfer to the female breast: preliminary experiences. *Eur J Plast Surg*. 2014;37(5):267-272.
37. Sasaki GH. Water-assisted liposuction for body contouring and lipoharvesting: safety and efficacy in 41 consecutive patients. *Aesthet Surg J*. 2011;31(1):76-88.
38. Peltoniemi HH, Salmi A, Miettinen S, et al. Stem cell enrichment does not warrant a higher graft survival in lipofilling of the breast: a prospective comparative study. *J Plast Reconstr Aesthet Surg*. 2013;66(11):1494-1503.
39. Mitchell JB, McIntosh K, Zvonic S, et al. Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. *Stem Cells*. 2006;24(2):376-385.
40. Oedayrajsingh-Varma MJ, van Ham SM, Knippenberg M, et al. Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure. *Cytotherapy*. 2006;8(2):166-177.
41. Simmons PJ, Torok-Storb B. CD34 expression by stromal precursors in normal human adult bone marrow. *Blood*. 1991;78(11):2848-2853.
42. Simmons PJ, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. *Blood*. 1991;78(1):55-62.
43. Lin CS, Ning H, Lin G, Lue TF. Is CD34 truly a negative marker for mesenchymal stromal cells? *Cytotherapy*. 2012;14(10):1159-1163.
44. Sidney LE, Branch MJ, Dunphy SE, Dua HS, Hopkinson A. Concise Review: Evidence for CD34 as a Common Marker for Diverse Progenitors. *Stem Cells*. 2014;32(6):1380-1389.
45. Crisan M, Corselli M, Chen WC, Peault B. Perivascular cells for regenerative medicine. *J Cell Mol Med*. 2012;16 (12):2851-2860.

46. Kaiser S, Hackanson B, Follo M, et al. BM cells giving rise to MSC in culture have a heterogeneous CD34 and CD45 phenotype. *Cytotherapy*. 2007;9(5):439-450.
47. Rada T, Reis RL, Gomes ME. Distinct stem cells subpopulations isolated from human adipose tissue exhibit different chondrogenic and osteogenic differentiation potential. *Stem Cell Rev*. 2011;7(1):64-76.
48. Glimm H, Eaves CJ. Direct evidence for multiple self-renewal divisions of human in vivo repopulating hematopoietic cells in short-term culture. *Blood*. 1999;94(7):2161-2168.
49. Handgretinger R, Gordon PR, Leimig T, et al. Biology and plasticity of CD133 + hematopoietic stem cells. *Ann N Y Acad Sci*. 2003;996:141-151.
50. Philips BJ, Grahovac TL, Valentin JE, et al. Prevalence of endogenous CD34 + adipose stem cells predicts human fat graft retention in a xenograft model. *Plast Reconstr Surg*. 2013;132(4):845-858.