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Conventional vs. micro-fat harvesting: How fat harvesting technique affects tissue-engineering approaches using adipose tissue-derived stem/stromal cells

Ziyad Alharbi [a,b,*,d], Christian Opländer [a,d], Sultan Almakadi [a], Andrea Fritz [a], Michael Vogt [c], Norbert Pallua [a]

[a] *Department of Plastic, Reconstructive and Hand Surgery — Burn Center, Medical Faculty, RWTH Aachen University, Pauwelsstr. 30, D-52074 Aachen, Germany*
[b] *Division of Plastic and Reconstructive Surgery, Department of Surgery, King Abdullah Medical City, Kingdom of Saudi Arabia*
[c] *Two-Photon Imaging Core Facility, Interdisciplinary Center for Clinical Research (IZKF), Medical Faculty, RWTH Aachen University, Pauwelsstr. 30, D-52074 Aachen, Germany*

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Summary *Background:* Biocompatible scaffolds as dermal substitutes are used commonly in soft tissue reconstruction and tissue-engineering approaches. The combination of these scaffolds with mesenchymal stem and stromal cells would have additional benefits in multilayer soft tissue reconstruction. In addition, the use of lipoaspirate may be beneficial for this purpose containing high levels of regenerative cells and relevant growth factors. However there are many factors, which may impact the lipoaspirate content of isolated cells, cell behaviour and growth factors. There is a lack of data as to whether fat-harvesting procedures using different cannulas of small diameter will impact these parameters, which are relevant not only for tissue engineering but also for clinical outcome.
Methods: Abdominal liposuctions were performed on 10 patients using the conventional fat harvesting by the Coleman cannula (3 mm, one-hole blunt tip) and the micro-fat-harvesting technique by the st'RIM cannula (2 mm, multi-perforated hole blunt tip) on contralateral area. Lipoaspirate contents of insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) were measured by enzyme-linked immunosorbent assay (ELISA). The *in vitro* viability of

* Corresponding author. Department of Plastic, Reconstructive and Hand Surgery — Burns Unit University Hospital of the RWTH Aachen University, Pauwelsstr. 30, 52057 Aachen, Germany. Tel.: +49 241 80 0; fax: +49 241 80 82634.
E-mail address: zalharbi@ukaaachen.de (Z. Alharbi).
[d] Both authors contributed equally to this work.

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lipoaspirates was tested by the alamarBlue™ assay. Adipose-derived stem/stromal cells (ASCs) were isolated and the yields determined. Furthermore, ACSs were seeded on collagen elastin matrices (Matriderm™) and cell migration/adhesion rate was examined by the alamarBlue™ assay and visualised by two-photon microscopy.
Results: Conventionally obtained lipoaspirates were found to contain significantly higher concentrations of IGF and VEGF, but not PDGF or bFGF. No significant effects on the yields of ASCs or the *in vitro* viability of lipoaspirates obtained from different cannula sizes were observable. However, the viability and migration of isolated ASCs obtained from micro-harvested lipoaspirates were significantly higher. Moreover, a significant high adherence rate of isolated ASCs from the micro-fat-harvesting technique onto matrices was observed.
Conclusion: The different sizes and surface/volume ratios of pieces of fatty tissue obtained by using different cannula sizes may be responsible for the observed differences and effects. Thus, micro-fat harvesting may be more suitable for tissue-engineering and -regenerative approaches using ASCs and collagen elastin matrices.

Soft tissue augmentation by lipofilling with adipose tissue derived from liposuction procedures has shown promising results with less invasive techniques, but variation in long-term outcome in terms of loss of transplanted volume has been reported.[1–3] One reason for a decreasing volume is insufficient blood supply after transplantation. Therefore, angiogenesis process is crucial for the survival of adipocytes, which are subject to apoptosis and necrosis if not supported by a network of capillary vessels by the fourth day after implantation.[4] Studies have shown that the plasmatic nutritional supply of adipocytes can only go as far as 1.5 mm into tissues[5] and that only 40% of the fat at a distance of 1.5 mm from the boundary of the tissue block survives.[6] Growth factors, in particular, vascular endothelial growth factor (VEGF), are essential for angiogenesis, and after a short latency period the sprouting vessels organise into a capillary blood supply network, enabling adipocytes to survive.[7]

Lipoaspirates, obtained from liposuction, contain VEGF and many other growth factors, including basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF) and platelet-derived growth factor (PDGF).[8] In animal studies, these growth factors have been shown to improve transplantation results.[9–12] In the adipocyte-free fraction of lipoaspirate, the stromal vascular fraction (SVF) contains a variety of cells such as pre-adipocytes, endothelial cells, smooth muscle cells, pericytes, fibroblasts, macrophages and adult stem cells.[13] Under certain conditions, these cells, called adipose-derived stem/stromal cells (ASCs), can be induced to differentiate into not only adipocytes but also bone, neuronal or endothelial cells. Therefore, ASCs, and especially the fraction of stem and progenitor cells, present an innovative option for regenerative cell therapy.[14,15] For example, ASCs have been shown to play a major role in promoting wound healing[16,17] and secrete almost all growth factors involved in normal wound healing.[18] Furthermore, it has been reported that ASCs promote vessel density, the granulation process and collagen thickness[16] and thus may improve the cosmetic aspect of the resultant scar.[18] Since fatty tissue is a major source of stem cells[19] and growth factors, the use of fatty tissue may provide more clinical benefits than augmentation alone. However, studies have shown that apart from sex and depot-dependent differences,[20–22] the yield of stem cells isolated from lipoaspirates can be affected by the fat-harvesting procedure and/or lipofilling techniques.[23]

Furthermore, the combination of isolated adipose tissue derived cells with collagen-based scaffolds would provide another dimension in soft tissue reconstruction through tissue-engineering approaches. Clinically, collagen elastin matrices (Matriderm®) have been used in multistage operations or even in one-stage operations as they have been cooperated with autologous split thickness skin transfer for soft tissue reconstruction.

Variables such as the ideal cannula and technique for harvesting, the best way of processing fat to ensure maximum uptake and viability of the graft and ACS on collagen elastin matrices, are all factors which require clarification.[2] For using fresh-isolated ACSs for tissue-engineering or -regenerative approaches the harvesting technique may be crucial, because cells have to adhere fast onto scaffolds to avoid 'cell slipping' away from scaffold during transplantation or from the original site of recipient area after transplantation. Also factors, such as cell yield, viability and growth factors, influenced by the harvesting technique may affect the performance of ACS-based tissue engineering for regenerative medicine purposes. Therefore, the aim of this study is to compare the influence of the fat-harvesting procedures (conventional vs. micro-fat-harvesting technique) on the viability and content of growth factors of the grafts obtained. Furthermore, we have investigated the effects of both methods on the yield, migration and adhesion rate of the ASCs which have been isolated directly from the corresponding lipoaspirates.

Methods and materials

Patients

With the patients' consents, lipoaspirates were obtained from 10 healthy patients (five males and five females; age range 27—59) having undergone an elective liposuction procedure