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Power assisted liposuction to obtain adipose-derived stem cells: Impact on viability and differentiation to adipocytes in comparison to manual aspiration

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KEYWORDS

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Summary *Background:* Adipose-derived stem cells (ASCs) play a key role in tissue engineering approaches and are probably of major importance in the context of autologous fat transfer. A number of different tools for harvesting ASCs-containing fat tissue have been established. Such devices should be easy to handle, time saving, low priced, safe and provide a high amount of viable ASCs in the aspirate. Power-assisted liposuction (PAL) has not yet been described in the literature as a tool for fat harvesting for lipotransfer. Aim of this study was to investigate ASCs' viability in fat tissue harvested using PAL versus manual aspiration (MA).

Methods: Fat tissue was obtained from 9 donors undergoing abdominoplasty. Samples were divided into two sections. Out of each section fat was harvested using either PAL or MA. Number of isolated ASCs was defined, proliferation rate was determined and cell viability was assessed by flow cytometry. The ability of isolated ASCs to differentiate into mature adipocytes was analyzed by gene marker expression.

Results: The number of viable ASCs and the proliferation rates did not significantly differ between PAL and MA but cells harvested using PAL showed significantly higher expression levels of differentiation markers adiponectin, GLUT4 and PPAR γ .

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Conclusion: Our results show that PAL is a feasible method for harvesting fat tissue containing viable ASCs. Quantity and quality of PAL-harvested ASC is similar or even better, respectively, compared to ASCs harvested by MA.

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Introduction

Adipose-derived stem cells (ASCs) play a key role in tissue engineering approaches and are probably of major importance in the context of autologous fat transfer.¹ ASCs have the ability to proliferate and differentiate into different cell types such as adipocytes, chondrocytes and osteoblasts.^{2–4} Although ASCs have a higher survival rate under ischemic conditions than mature adipocytes, explantation of adipose tissue as performed during the procedure of autologous fat transfer confers stress to the cells due to mechanical trauma and disruption of blood supply, which may result in hypoxia and apoptosis of ASCs.^{5–8} Therefore, current research activities focus on identifying factors that positively influence cell viability during the process of autologous fat transfer. A number of different tools for harvesting fat tissue have been established. The device of choice should be easy to handle, time saving, low priced, safe and provide a high amount of viable cells in the aspirate. In 1977, Illouz demonstrated the possibility to extract fat by suction rather than excision.⁵ Another significant development of fat transplantation was Klein's concept of the tumescent technique⁹ reducing the risks of liposuction. In the 1990s, Sydney Coleman brought up the concept of lipostucture.¹⁰ His technique involved infiltration of the donor site with tumescent solution, fat harvest using specific cannulas and a 10 ml LuerLock syringe, centrifugation as well as injecting small portions of fat into the recipient site to enhance fat survival. Coleman emphasized that the nonviable elements of fat aspirate like oil, blood, serum, and tumescent solution should be removed by centrifugation.⁶ However, whether or not centrifugation has an adverse impact on fat graft viability remains discussed. Some authors have shown a negative impact,^{7,8} some found a positive effect¹¹ while others could not find any difference between centrifugation and non-centrifugation.^{1,12}

Power assisted liposuction is an automatic vacuum liposuction with a vibrating tip of the cannula. Fat can be harvested into a sterile container for reinjection. To date, it is used as a harvesting tool for autologous fat transfer and adipose derived stem cells but it has not been described in the literature, though many surgeons already use PAL in clinical practice with promising results.

Therefore, aim of this study was to clarify whether PAL is a feasible method for harvesting ASCs by comparing it to manual aspiration (MA), a standard harvesting technique. Furthermore the influence of centrifugation in combination with both techniques on ASC viability was evaluated.

Materials and methods

Patients and tissue harvesting

This study was approved by the ethics committee of the Medical University of Vienna and the General Hospital

Vienna (EK no. 560/2010). All subjects gave written informed consent before taking part in the study.

Fat tissue was obtained from 9 donors undergoing abdominoplasty. Samples were divided into two sections in a randomized manner. Out of each section fat was harvested using either PAL (PAL-200E MicoAire power-assisted lipoplasty device, MicroAire Surgical Instruments LLC, Charlottesville, VA, USA) or MA. No tumescent solution was used.

PAL involved a 3.0 mm-diameter and 30-cm-long cannula with a blunt tip and several side holes with a negative pressure of 0.5 Bar. A negative pressure of 0.5 bar was used as this is state of the art in our clinic and also recommended by other authors.¹³ A sterile container was interconnected to collect the harvested fat. MA used a blunt-tipped cannula with several side holes measuring 3 mm in diameter and 12 cm in length (Byron Medical) connected to a 10-cc syringe adapted with 2 cc of negative pressure applied.

10 ml of liposuction material of each technique was immediately sent to the laboratory for cell evaluation. A second portion of 10 ml was centrifuged for 5 min at 380 G and only the adipose tissue phase was used for further investigations. In the literature a variety of different adjustments are described.^{6,11,14–16} We have chosen 380 g for 5 min as this is the standard setting in our clinic.

Ratio of fat, oil and aqueous material in lipoaspirates

Samples (20 ml) of two donors were centrifuged for 5 min at 380 G and relative volumes of the resulting layers were evaluated. In addition samples were digested with 2 mg/ml Collagenase Type IV (Sigma–Aldrich, St. Louis, MO, USA) in Hanks' buffered salt solution (HBSS, PAA Laboratories GmbH) for 1 h at 37 °C with constant shaking. Centrifugation for 5 min at 380 G was performed and afterwards the ration of fat, oil and aqueous material was determined.

Isolation of ASCs

Tissue was washed in PBS (phosphate buffered saline, PAA Laboratories GmbH, Pasching, Austria) and digested with 2 mg/ml Collagenase Type IV (Sigma–Aldrich, St. Louis, MO, USA) in Hanks' buffered salt solution (HBSS, PAA Laboratories GmbH) for 1 h at 37 °C with constant shaking. Cells were filtered through cotton gauze and centrifuged for 5 min at 380 G (1500 RPM). Red blood cells in the stromal vascular fraction were lysed in 2 ml Red Blood Cell Lysing Buffer (Sigma–Aldrich) and incubated on ice for 8 min. Cold medium was added and suspension was filtered through a 70 µm cell filter. Cells were centrifuged for 5 min at 380 G and cell pellet was re-suspended in proliferation medium DMEM (PAA Laboratories GmbH) supplemented with 10% fetal calf serum (Hyclone, Fisher Scientific GmbH,

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