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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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Centrifugation

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 lipotranfer. 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 PPARg.

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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.

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, chrondrocytes 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 technique[9] reducing the risks of liposuction. In the 1990s, Sydney Coleman brought up the concept of lipostructure.[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 effect[11] 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,

Schwerte, Germany), 100 units/ml penicillin and 100 μg/ml streptomycin (Life Technologies Ltd, Paisley, UK).

Isolated cells were counted in a Bürker-Türk counting chamber (Hecht Assistant, Sondheim, Germany) with trypan blue stain (0.4%, Gibco, Life Technologies Ltd., Paisley UK) ASCs were cultured as a monolayer at 37 °C in supplemented proliferation medium in a humidified atmosphere with 5% CO_2.

Cell viability and proliferation

Immediately after isolation, part of the ASCs were used to quantify cell viability by staining against annexin-V and propidium iodide (PI, BD Biosciences, San Jose, CA) using a Beckman Coulter FC500 (Beckman Coulter, Indianapolis, IN) to examine cell viability. For flow cytometric analysis, cells (5×10^5) were incubated with FITC-labelled annexin V and/or PI in 1× binding buffer for 15 min at room temperature in the dark following the manufacturer's protocol. As viability control we used ASCs isolated from excised fat tissue of the same donor.

Proliferation of cells was measured using a CellTiter96® non-radioactive proliferation Assay (Promega Corporation, Madison, WI). Therefore, cells were seeded in 96-well plates (8×10^4 cells). After 48 h cell number was evaluated according to manufacturer's protocol: 15 μl Dye Solution was added to100 μl medium. After two hours of incubation 100 μl stop solution was added and absorbance was measured after one hour on a Wallac 1420 VICTOR2 plate reader (PerkinElmer, Waltham, MA, USA) after gentle shaking.

Adipocyte differentiation

To assess adipocyte maturation, ASCs were plated at equal viable cell densities (0.5×10^6) for 4 days. For morphology analysis viable ASCs were washed with PBS, fixed with 4% formalin and permeabilized with 0.9% Triton X-100 (Bio-Rad Laboratories, Hercules, CA, USA). Cells were stained for 15 min with FITC-phalloidin (Invitrogen, Carlsbad, CA, USA). Nuclei were stained 1 min with DAPI (Serva Electrophoresis, Heidelberg, Germany).

To induce adipocyte differentiation, cells were incubated for 2 days in Preadipocyte Differentiation Medium (PromoCell GmbH, Heidelberg, Germany). Afterwards cells were incubated in Adipocyte Nutrition Medium (PromoCell GmbH, Heidelberg, Germany). Differentiation was evaluated by fluorenscent AdipoRed staining and by q-RT PCR analysis on day 14 after induction of differentiation.

For lipid staining, viable differentiated cells were washed once with PBS and incubated with AdipoRed Assay Reagent (Lonza, Walkersville, MD, USA) according to manufacturer's protocol. After 15 min, cells were rinsed with PBS, fixed with 4% formalin, permeabilized with 0.9% Triton X-100 and stained for 15 min with TRITC-phalloidin (Sigma—Aldrich). Nuclei were stained 1 min with DAPI. Cells were analyzed using an AxioImager microscope (Zeiss, Jena, Germany).

A specific feature of mature adipocytes is expression of adiponectin (gene: *ADIPOQ*), PPARg (gene: *PPARG*) and *GLUT4*.[2] Total RNA was prepared from 4 samples by homogenizing cells in Trizol reagent (Invitrogen) followed by RNA extraction according to a standard protocol. RNA was transcribed into cDNA by Superscript II using random hexamer priming (all Invitrogen). Quantitative real-time PCR was performed using an adiponectin, GLUT4-, and PPARγ -specific commercial Assay-on-Demand (Applied Biosystems) normalized to ubiquitin c expression as endogenous control (Applied Biosystems). Expression of specific mRNA in each sample was quantitated in duplicates on an ABI PRISM 7000 Cycler (Applied Biosystems) using the DDCt method.

Statistics

Data are expressed as means ± SD of at least 3 independent experiments. Statistical analysis was performed with software SPSS statistics 19. Statistical comparisons for all experimental settings were based on two sample *T*-test and ANOVA with $p < 0.05$ considered as significant.

Results

Comparable amounts of viable ASCs in aspirates after PAL and MAL

Total numbers of viable ASCs per ml of aspirated fat were between 0.15×10^6 cells and 0.8×10^6. There were no significant differences in the number of isolated ASCs using PAL compared to MA (Figure 1). Cell density was higher in both groups when the lipoaspirates were subjected to centrifugation.

Cell viability was assessed by annexin/PI flow-cytometric analysis. No significant differences could be seen between the two harvesting methods. ASCs from both harvesting methods were similarly viable independently of centrifugation and comparable of ASCs isolated from excised fat tissue of the same donor (Figure 2).

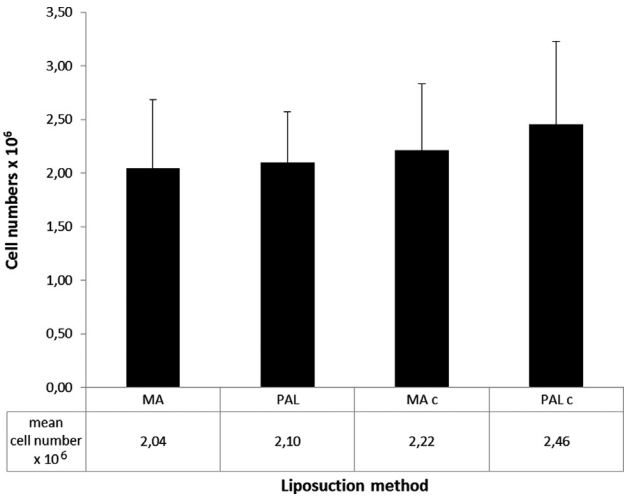

Figure 1 Number of ASCs in 1 ml lipoaspirate comparing PAL to MA with and without centrifugation. Cells were isolated, stained and trypan blue-excluding cells counted in a Bürker-Türk counting chamber. Centrifuged samples are marked as PAL c and MA c. Data are expressed as means ± SD of 9 independent experiments.

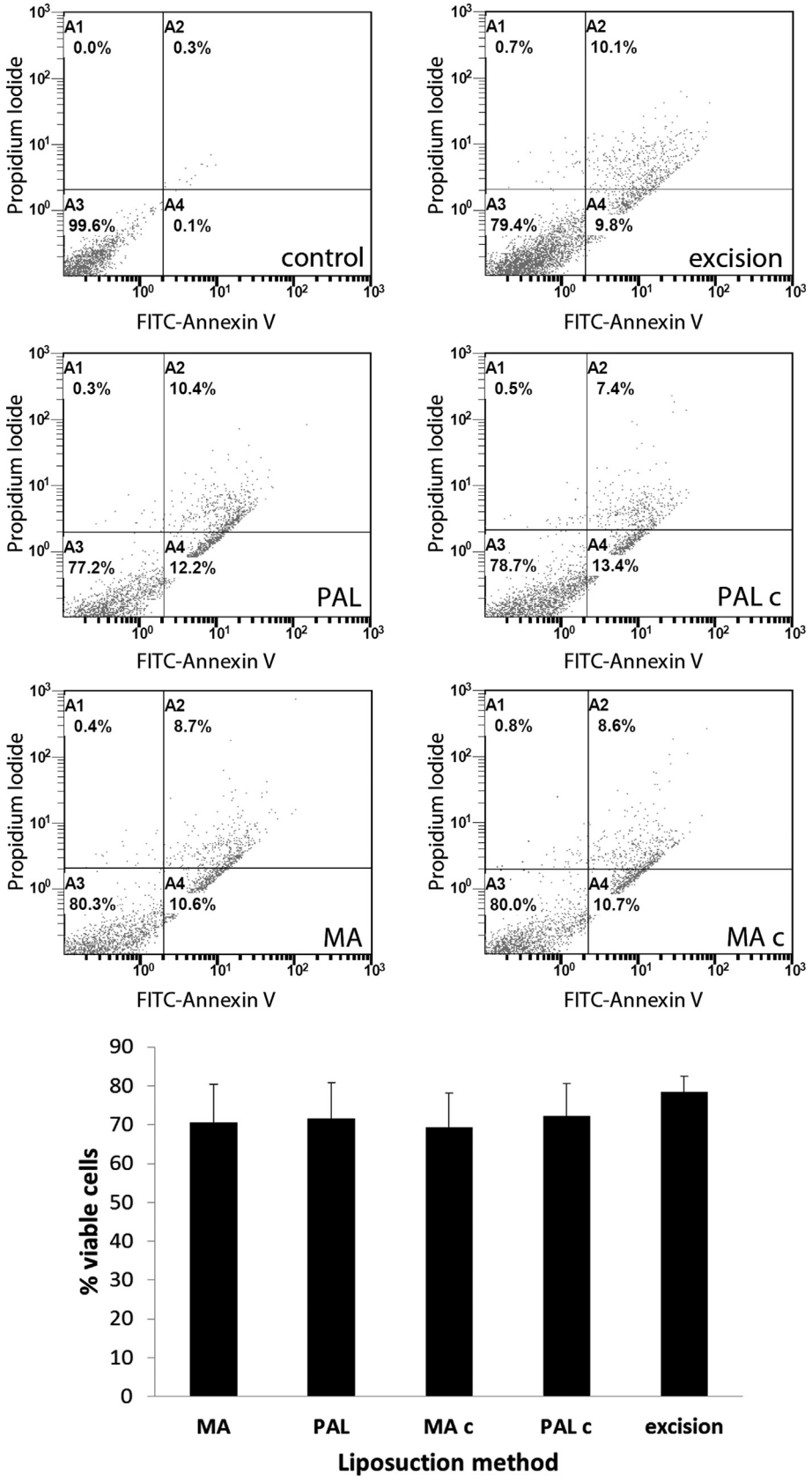

Figure 2 Cell viability was assessed by Annexin/PI FACS analysis. Centrifuged samples are marked as PAL c and MA c. (A) 5×10^5 cells were incubated with FITC Annexin V and propidium iodide (PI). Histogram set of one of four experiments with similar outcome is displayed. Unstained cells are shown as control. As viability control, cells isolated from excised fat tissue of the same donor were stained. (B) Statistical evaluation of four independent donors; viable cells are shown in percentage relative to total cell number. Data are expressed as means $\pm$ SD.

Ratio of fat, oil and aqueous material in lipoaspirates

The ratio of resulting layers of lipoaspirates after centrifugation as well as after collagenase digestion and centrifugation as shown in Figure 3 were comparable, although in both donors MA released slightly more oil than PAL (5 ml vs. 6 ml after centrifugation and 2 ml vs. 3 ml after collagenase digestions and centrifugation). As a consequence the fat layer was slightly higher using PAL.

No difference in proliferation rates between PAL and MA

Proliferation rates of ASCs harvested using PAL or MA showed no significant differences after 48 h as shown in Figure 4. After centrifugation, PAL-derived cultures only by trend slightly more proliferated than cells harvested using MA.

Significant differences in expression of differentiation markers

In addition to these quantitative analyses, we investigated the effects of the two harvesting methods on the quality of ASCs as assessed by their ability to differentiate into adipocytes. Irrespective of the harvesting method, cells started to form lipid droplets on day eight after induction of differentiation. Also on day 14 after induction of differentiation, no differences in cell size and lipid droplet formation were detectable comparing the cells harvested with different liposuction methods. Typical example photographs of cells taken after isolation (Figure 5A) and 14 days after induction of differentiation (Figure 5B) are shown.

In contrast to this normal phenotypical appearance of cells, harvesting methods affected adipocyte differentiation as determined by their expression of the differentiation markers adiponectin, PPARγ and GLUT4. Expression levels of adiponectin, GLUT4 and PPARγ were markedly (up to 5-fold) and statistically significantly higher in PAL than in MA (Figure 6). After centrifugation, these differences were detectable only in trend.

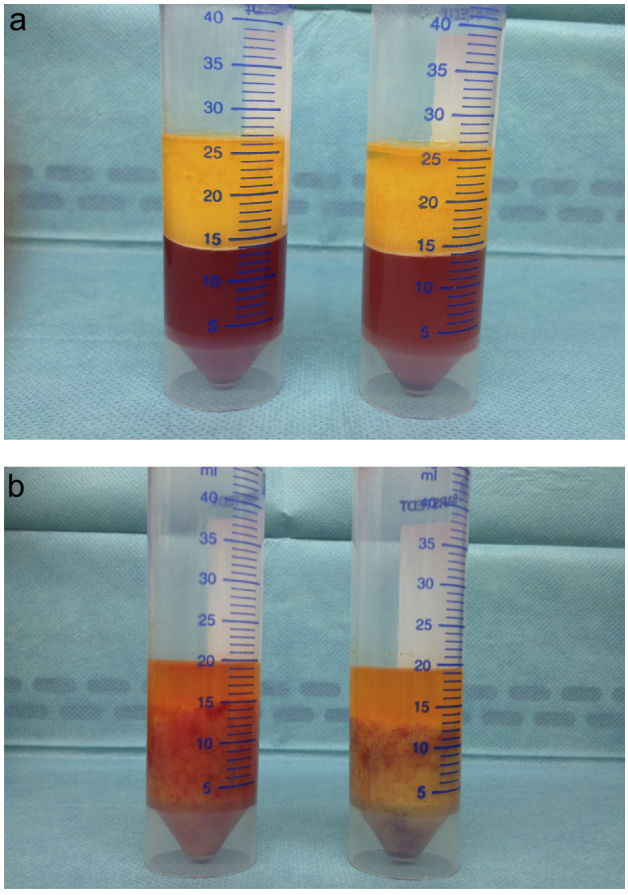

Figure 3 a) Lipoaspirates using PAL (left) and MA (right) after centrifugation with 380 G for 5 min b) Lipoaspirates using PAL (left) and MA (right) after collagenase digestion and centrifugation with 380 G for 3 min. MA released slightly more oil than PAL. The aqueous phase is comparable using both methods.

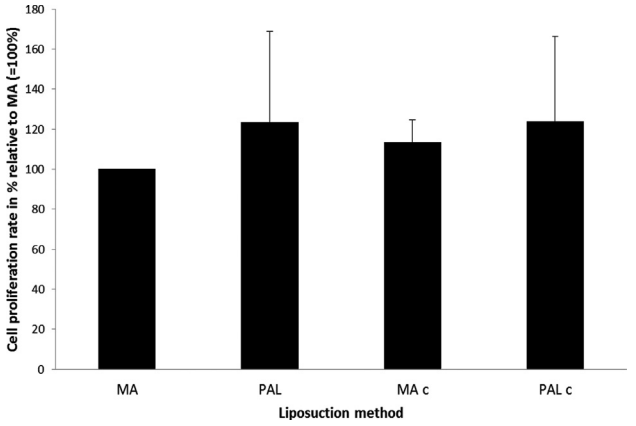

Figure 4 Proliferation rates were assessed by CellTiter96® Non-Radioactive Proliferation Assay. 8×10^4 cells were seeded in 96-well plates and proliferation rates were determined after 48 h. Cell proliferation rates are shown in percentage relative to MA (=100%). Data are expressed as means ± SD of three independent experiments. Centrifuged samples are marked as PAL c and MA c.

Discussion

Among a variety of stromal-vascular cells, ASCs are located between mature adipocytes in fat tissue. Different methods of harvesting have been described.[3,4,17] To date, no study has evaluated the use of PAL for fat harvesting in context of lipotransfer or ASC retrieval. Not only the quantity of ASCs surviving the autotransplantation, but also their ability to differentiate into adipocytes is of great importance when evaluating the harvesting procedure.[9,10] In the present study we evaluate the PAL technique and compare this technique to MA — the generally accepted gold standard for reference.

We measured the amount and viability of ASCs in fat tissue harvested using PAL versus MA. Furthermore, we investigated the ability of ASCs to proliferate and to differentiate into mature adipocytes as determined by their ability to express adiponectin, GLUT4 and PPARγ, differentiation markers with multiple beneficial local and systemic functions.[18]

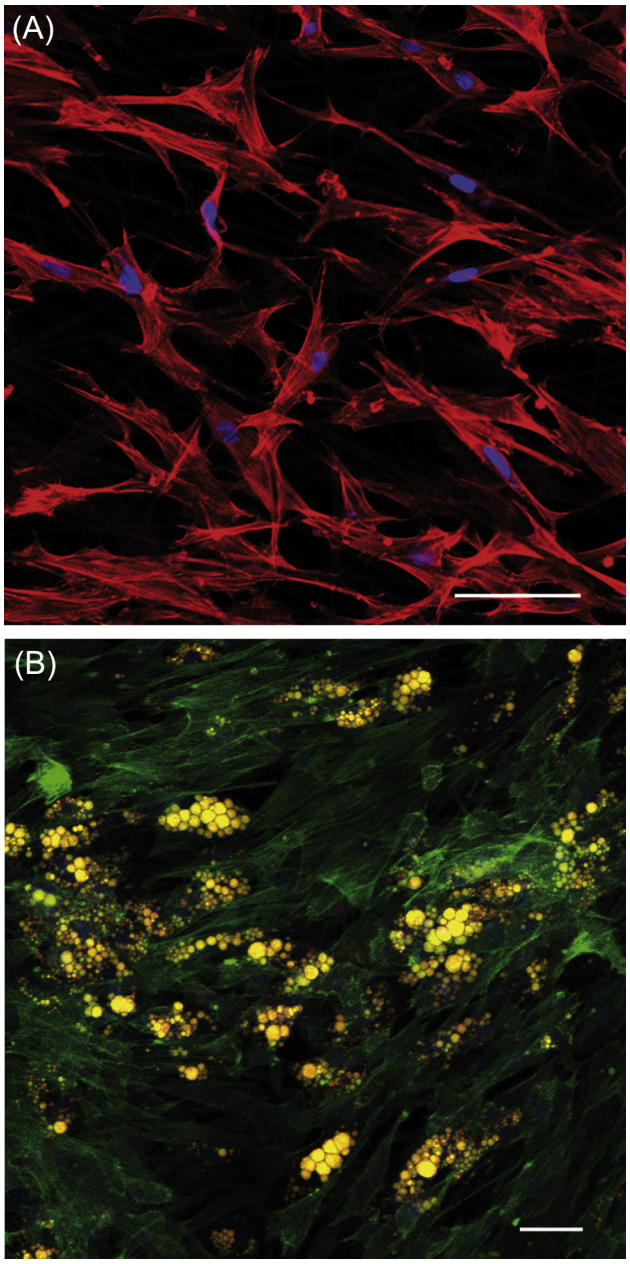

Figure 5 Typical example photographs of cultivated ASCs taken 4 days after isolation are shown (size bar indicates 50 μm) (A). For differentiation experiments ASCs were seeded at same viable cell densities, cultivated, and induced to differentiate to adipocytes as described in Materials and Methods. Typical example photographs of differentiated cells taken on day 14 after induction of differentiation are shown (size bar indicates 50 μm) (B).

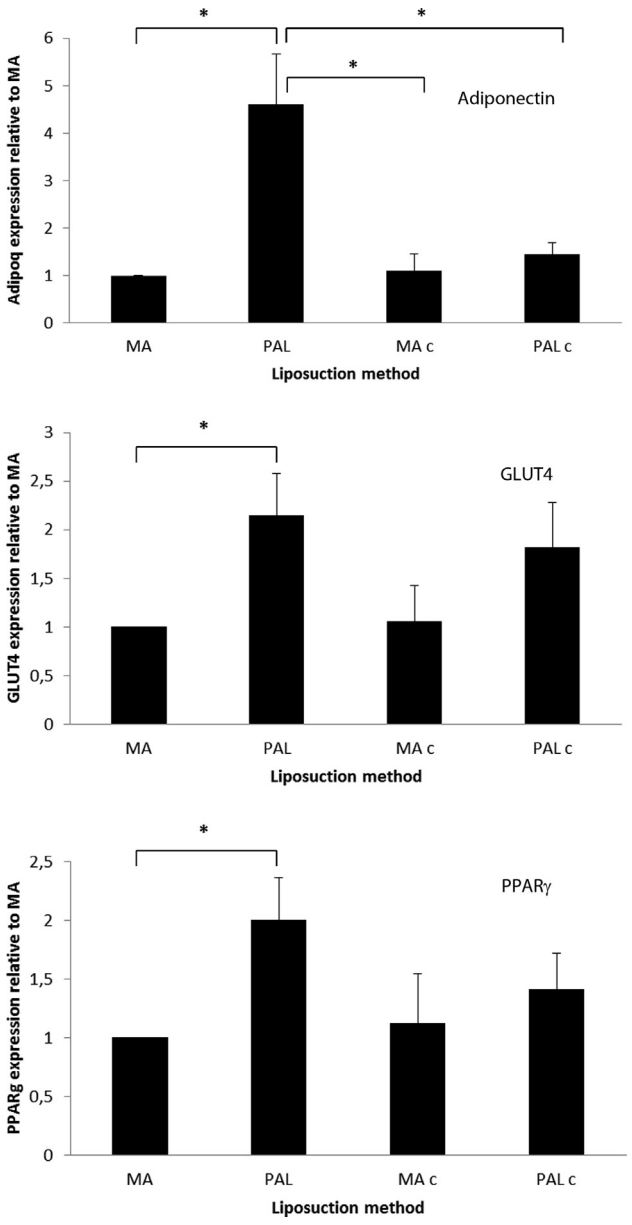

Figure 6 Expression levels of adiponectin, GLUT4 and PPARγ are significantly higher in PAL than in MA. Quantitative real-time PCR was performed using an adiponectin, GLUT4-, and PPARγ-specific commercial Assay-on-Demand normalized to ubiquitin c expression as endogenous control. Expression of specific mRNA in each sample was quantitated in duplicates. Expression levels are shown in percentage relative to MA (=100%). Data are expressed as means ± SD of four independent experiments. Significant differences are indicated by asterisks: *, $p < 0.05$. Centrifuged samples are marked as PAL c and MA c.

We also considered the fact that centrifugation might have an influence on cell viability and therefore evaluated the harvested tissue with and without centrifugation for 5 min at 380 G. Manual aspiration can be performed with centrifugation as described by Coleman or without centrifugation.[3] PAL has not been described for its use in lipotransfer, hence no recommendations concerning centrifugation have been made in the literature.

Both methods are using continuous suction and in both methods air can enter the suction system if the surgeon pulls out the cannula, which leads to a strong change in pressure. Unfortunately, it is not possible to completely avoid this phenomenon and we still don't know exactly what mean pressure actually acts on the cells in both groups.

We decided not to use tumescent solution in this study in order to avoid it as another factor to influence cell viability, as we were able to describe in our previous work.[19] The results of our investigations show that fat tissue harvested using PAL contains a comparable amount of viable ASCs as when using MA. Furthermore, proliferation rates as evaluated using proliferation assays did not show any significant differences between both methods. However, although the phenotypical appearance of cells 14 days after induction did not show any striking differences, adipocytes differentiated from ASCs harvested by PAL showed higher expression of differentiation markers than those obtained by MA, indicating a qualitative difference of the ASC derived by these methods with respect to fully their differentiation capacity.

It was shown that reduced levels of adiponectin alone could have serious consequences on the function of reconstituted adipose tissue.[20] However, whether and how reduced expression of adiponectin, GLUT4 and PPARγ in normally appearing adipocytes affects autologous fat transfer or tissue engineering approaches, which aim rather at the structural than the metabolic functions of adipose tissue, remains to be elucidated in conjunction with functional defects of adipocytes derived from harvested ASCs. Nevertheless, any impairment of ASC differentiation by the harvesting method may promote a negative outcome and should be kept minimal.

The effect of the centrifugation on cell viability is discussed controversially in the literature.[1,6,7,11,12] In our study centrifugation did not have any impact on ASCs viability or proliferation potential. Cell density was higher in both groups when the lipoaspirates were subjected to centrifugation. Since cell count was performed out of one ml of aspirated tissue, these results can be explained by the fact that after centrifugation fluid, oil and cell debris were removed.

Interestingly, expression of the differentiation marker adiponectin was significantly higher in ASCs isolated from PAL-gained fat tissue not only compared to the manual aspiration but also to PAL after centrifugation. This effect could not be observed between MA and MA c. Adiponectin is — in contrast to the other observed markers - a protein which is typical for late adipogenesis as it is produced exclusively by the adipocyte.[21] This could be an indication for differentiation rate and that not centrifuged stem cells harvested by PAL show a faster development towards mature adipocytes.

Main goal of this study was to evaluate ASCs viability, proliferation and differentiation which can only be investigated in an in vitro setting. The next step would be to evaluate the influence of cell viability, proliferation and differentiation properties on the clinical outcome.

In our study we focused on the quantity and quality of ASCs rather than adipocytes and were able to demonstrate that PAL is an adequate technique for fat harvesting to obtain viable ASCs. But in context of autologous fat transfer adipocytes and multiple other factors may influence the outcome as well; the number of ASCs and their ability to proliferate and differentiate is only one aspect. Further investigations are needed to evaluate the actual clinical relevance of our findings.

Additionally, the surgeon's choice for a particular harvesting system is much more influenced by factors such as pain, complications, ease of handling, time, price and the availability. MA is low priced and available in most of the clinics, whereas PAL is faster and easier to use than MA and therefore might be favorable when larger volumes are required.

Conclusion

In this study we were able to show that PAL is a feasible technique to harvest viable ASCs showing proliferation rates comparable to cells harvested by MA. ASCs harvested using PAL show the same phenotype as manually aspirated ASCs while the expression of adiponectin, GLUT-4 and PPARγ was even higher.

Financial disclosures

None.

Conflict of interest

None.

Acknowledgements

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