published in peer-reviewed scientific journals years ago versus the current situation of a technology that has not been characterized. There seems to be a big rush to sell laser boxes and to promote laser-assisted lipoplasty, but the data that show science, safety, improved outcomes over existing lipoplasty technologies, and patient satisfaction are missing.

Disclosures

Mark Jewell, MD, is a paid consultant for Allergan, Medicis, and COAPT. He is a clinical investigator for Mentor, Allergan, Medicis, Excaliard, and Kythera. He is an unpaid consultant for Keller Medical, Sound Surgical, *New Beauty Magazine*, and AorTech.

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INTERNATIONAL CONTRIBUTION BRAZIL

Research

Effects of Centrifugation on Cell Composition and Viability of Aspirated Adipose Tissue Processed for Transplantation

Aesthetic Surgery Journal
30(2) 249–255

DOI: 10.1177/1090820X10369512
www.aestheticsurgeryjournal.com
SAGE

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Abstract

Background: Centrifugation is one of the preferred methods of fat processing. Although it has been promoted for nearly three decades to separate adipose tissue components before grafting, there remain many controversies regarding the results obtained with centrifuged adipose tissue.
Objectives: The authors demonstrate the effects of centrifugation on the cellular components of aspirated fat.
Methods: Fat harvested from the lower abdomen of 10 female patients undergoing liposuction was divided in two equal parts, then processed by decantation or centrifugation and sent to the laboratory. Each processed lipoaspirate was analyzed histologically after hematoxylin and periodic acid-Schiff staining for the presence of intact adipocytes. It was then cultured and analyzed by multicolor flow cytometry for identification of adipose-derived mesenchymal stem cells.
Results: The middle layer of the centrifuged lipoaspirate, which is used by many surgeons, showed a great majority of altered adipocytes and very few mesenchymal stem cells in comparison with the decanted sample, which maintained the integrity of the adipocytes and showed a greater number of mesenchymal stem cells. The pellet observed as a fourth layer at the bottom of the centrifuged lipoaspirate showed the greatest concentration of endothelial cells and mesenchymal stem cells, which play a crucial role in the angiogenic and adipogenic effect of the grafted tissue.
Conclusions: If centrifuged lipoaspirate is used, the pellet (rich in adipose-derived mesenchymal stem cells) and the middle layer should be employed to increase fat graft survival.

Keywords

adipose tissue, centrifugation, pellet, stem cells

Accepted for publication August 28, 2009.

Successful treatment of soft tissue asymmetry and depressions remains elusive. Autologous adipose tissue is very attractive for this purpose because it is readily available and easily harvested. Unfortunately, fat transplantation often has unreliable results caused by tissue resorption and volume loss.[1-5] Furthermore, the optimal technique for processing the fat after harvesting by liposuction remains to be determined.

The transplanted tissue essentially contains two components: acellular material and cells.[6] Introduction of acellular material into the receptor site elicits activation of proteolytic and lipolytic enzymes as well as cells (mainly macrophages, which are normally involved in tissue cleaning). Also, the associated inflammatory reaction accelerates clearance, decreasing the gained volume. The implanted cellular components at the recipient site contain cells harvested from the donor site and cells mobilized from adjacent tissues, as well as circulating cells that are involved in the formation of local inflammatory infiltrate

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and tissue repair.[7] They can also elicit a fibrotic scarring reaction, which can stabilize the volume gain and delay resorption of the implanted material at the receptor site.

Two main categories of cells are present in fat tissue: mature adipocytes and cells from blood vessel walls.[8] Because adipose tissue contains a rich blood vessel network, the quantity of vessel-associated cells is considerable. Preadipocytes (which differentiate into mature adipocytes) are perivascular cells. They are derived from perivascular pericytes or mesenchymal stem cells (MSC), which have a broad capacity for differentiation, including formation of new blood vessels, connective tissue cells, and components of the musculoskeletal system.[9] They can participate at different levels in the formation of a new tissue at the receptor site, making the cellular component relevant for the duration and stabilization of the tissue volume gain for the determined therapy.[10] Consequently, the preservation of cell integrity and viability is one of the major concerns in processing the tissue to be transplanted.

Centrifugation of the adipose tissue harvested by liposuction has been used for nearly three decades. It is one of the preferred methods of fat processing designed to concentrate the adipocytes and separate them from debris and other substances that can damage them. Several studies have reported satisfactory results from grafting the middle layer of the centrifuged lipoaspirate,[11] whereas others insist on the importance of grafting a less-manipulated lipoaspirate and obtaining more viable adipocytes to increase the likelihood of graft survival.[12]

The investigation of methods to isolate cells from adipose tissue began in the mid-1960s[13] with minced fat pads. They were washed extensively to remove contaminating blood, incubated with collagenase, and centrifuged to separate the floating population of mature adipocytes from the pelleted stromal cells. With the advent of liposuction, which generates finely minced fat tissue,[14] fat samples were directly incubated with collagenase to isolate MSC. This enzymatic digestion, which is the current protocol employed by many laboratories to isolate MSC,[15,16] limits the volume of lipoaspirate to be processed for two main reasons: difficulties with the method for large volumes and the high cost of collagenase itself. For this reason, we have chosen mechanical dissociation to achieve red blood cell lysis without tissue digestion. The cells isolated in this study have shown mesenchymal stem cell properties similar to the lipoaspirate cells isolated with enzymatic procedures by other researchers.[17,18] The reproducibility of the mechanical process makes it a preferred method for larger volumes of samples, such as those needed in tissue engineering,[19] at a reduced cost and in less time.

METHODS

The present study focused on the effects of centrifugation on adipocytes and MSC in harvested adipose tissue to be used in autologous grafting. With approval from the Ivo Pitanguy Institutional Review Board and the Institute's Ethics Committee, we conducted a prospective study with discarded adipose tissue obtained from 10 healthy women, ages 35 to 58 years, who underwent aesthetic liposuction in October 2008. All 10 patients who participated in this study received detailed information and breakdown of the procedure. They signed a form acknowledging their consent for both the procedure and for the use of a small amount of their aspirated adipose tissue for analysis rather than it being discarded. Mean body weight was 67 kg; mean body mass index was 23.5. Exclusion criteria included prior abdominal surgeries, the presence of hematologic and other systemic metabolic disorders, previous obesity, and pathologic lipodystrophies.

With the patient under locoregional anesthesia associated with light sedation, infiltration of the abdomen with a solution containing normal saline with 1:500,000 of epinephrine was performed in a 1:1 ratio (1 mL of solution per 1 mL of aspirated tissue). Then, 80 mL of adipose tissue was aspirated from the lower abdomen with a 3-mm diameter blunt cannula attached to a 10-mL Luer-Lok syringe (Figure 1A). The aspirated fat was divided in two equal parts. Half (40 mL) was left to decant slowly in the syringe for 30 minutes under the action of gravity. The other half was processed by centrifugation in the IEC Medlite microcentrifuge (Thermoelectron Corporation, Byron BodyAesthetics, Mentor, Inc, Scottsdale, Arizona) at 3000 rpm over three minutes. Separation into three distinct layers was observed in both decanted (Figure 1B) and centrifuged (Figure 1C) lipoaspirates: the top, least dense layer (composed of oil from ruptured parcels of fat); the middle layer (primarily consisting of compact adipose tissue and cells); and the bottom layer (composed mostly of blood and substances used for infiltration). In the centrifuged sample, another denser layer was observed at the bottom of the syringe, identified as the pellet (Figure 2).

Each set of processed lipoaspirates was sent to the laboratory and analyzed within 24 hours. After discarding the superior and inferior layers, the middle layer of each sample and the pellet of the centrifuged samples were isolated and analyzed as follows:

1. Lipoaspirate fragments of 3 cm^3 of each sample were placed on paper filters, fixed in 10% buffered formalin, and embedded in paraffin. Five-micron sections cut at 200-µm intervals on an American optical microtome were stained by hematoxylin and periodic acid-Schiff (PAS) and examined histologically. Two sections of each set of processed samples were examined for architectural disruption (ie, degeneration of the adipocytes) with an image acquisition program (Q capture, Quantitative Image Corporation, Surrey, British Columbia, Canada). The cell count was measured only for intact adipocytes per field on 15 high-powered (× 200) images of randomly selected areas of each paraffin section with Image Pro-Plus software (Media Cybernetics, Bethesda, Maryland). Parametric one-way analysis of variance (ANOVA) tests were performed and results were expressed as mean ± standard deviation (SD).

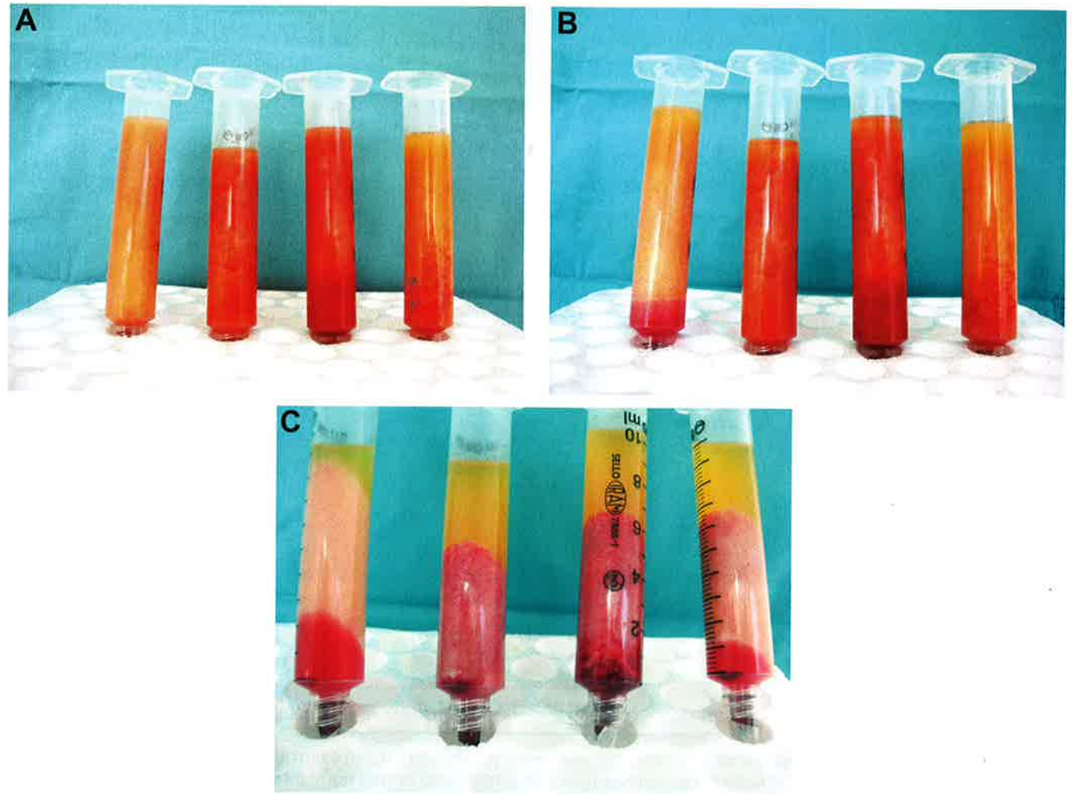

Figure 1. Photographs of the lipoaspirates before and after decantation and centrifugation. (A) Fresh adipose tissue aspirated with 10-mL Luer-Lok syringes. (B) Decanted lipoaspirates in 10-mL syringes, showing the three distinct layers. (C) Centrifuged lipoaspirates in 10-mL syringes, showing more distinctly the three layers of separation plus the pellet identified at the bottom.

Figure 2. Close-up photograph of the four distinct layers issued from the centrifuged lipoaspirate: the superior, middle, and inferior layers and pellet.

2. Isolation of MSC from each sample was performed by mechanical dissociation with simultaneous red blood cell lysis buffer treatment (ACK buffer solution at 1/1, v/v) as described in a recent study,[20] obtaining mechanically processed lipoaspirated (MPLA) cells without tissue digestion (by collagenase). The obtained suspension of cells and pellets derived from centrifuged samples was monitored for surface marker expression with monoclonal antibodies (CD45, CD105, CD34, CD31) conjugated with fluorescent dyes (Becton & Dickinson Biosciences, Franklin Lakes, New Jersey). Multicolor flow cytometry analyses were performed with a FACScanto (BD Biosciences) equipped with the software FACS Diva 4.0, as described by Baptista et al.[20] Alternatively, cells were plated in tissue culture flasks with Dulbecco's modified Eagle's medium (DMEM-LGC; Cotia, SP, Brazil) containing

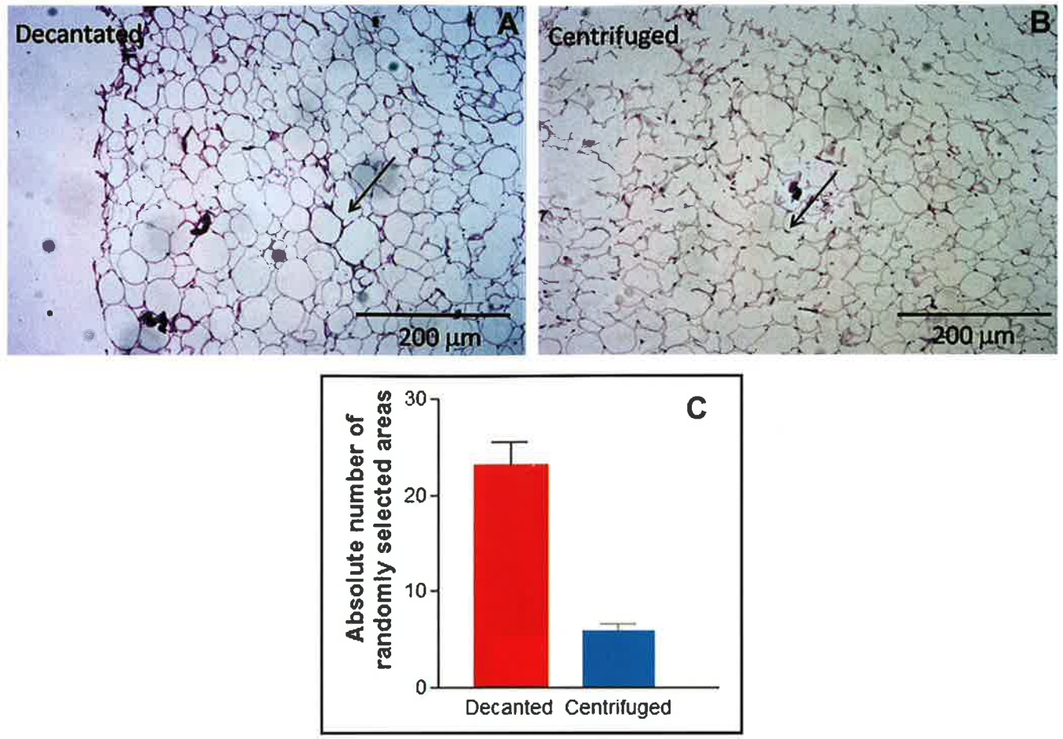

Figure 3. Histologic sections of aspirated adipose tissue processed by decantation and centrifugation. (A) The decanted lipoaspirate sample shows relatively intact, nucleated adipocytes (*arrow*) and overall normal morphology. (B) The centrifuged lipoaspirate sample has a small number of intact adipocytes (*arrow*) and more extensive trauma. The adipocyte count was performed based on morphology observation and represents only intact cells. (C) A graph depicting the mean ± standard derivation of four patients, where $P < .05$ among all samples. Bar size: 200 µm.

10% fetal bovine serum (FBS; Cultilab, Campinas, SP, Brazil), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were also stained with propidium iodide (Sigma-Aldrich, St. Louis, Missouri) to assess their viability.

RESULTS

A total of 30 samples—10 samples of the middle layer of decanted lipoaspirates, 10 samples of the middle layer of centrifuged lipoaspirates, and 10 pellet samples issued from centrifuged lipoaspirates—were analyzed by the same group of the laboratory staff. Macroscopically, we observed a greater distinction between the different layers after centrifugation, which separated the middle layer from most of the other substances that are of no use in fat grafting (such as infiltration substances, oil, and nonviable blood components). On histologic examination, we observed that the middle layer of the centrifuged lipoaspirates contained a great majority of adipocytes with disrupted membranes, whereas the adipocytes' integrity was maintained in the decanted samples (Figure 3A,B).

In the cell population obtained by mechanical dissociation with simultaneous lysis buffer treatment, we identified two distinct cell populations: cells positive for CD45 (corresponding to peripheral blood-derived cells) and cells negative for CD45 (consisting of adipose-derived mesenchymal stem cells expressing CD34 and CD105 as well as blood vessel endothelial cells CD31; see Table 1).

The middle layer of the decanted lipoaspirates contained more blood-derived CD45-positive cells than centrifuged lipoaspirates. In the centrifuged samples, we observed cells and different debris materials at the bottom of the syringe, in the pellet. This fourth layer showed a significantly higher quantity of MSC. We found that 4.3% ± 2.3% were CD45–CD34+ cells and 4.7% ± 1.6% were CD45–CD105+ cells. This was a significantly higher quantity of MSC compared to the middle layer of both decanted and centrifuged samples—2.8% ± 2.7% CD45–CD34+ cells and 2.9% ± 2.4% CD45–CD105+ cells in the former one, as well as 2.3% ± 1.7% CD45–CD34+ and 2.1% ± 1.5% CD45–CD105+ cells in the latter one.

This reduction of cells possibly caused the negative results observed in the cultures derived from centrifuged samples, in which the mesenchymal stromal cells did not reach confluence (Figure 4).

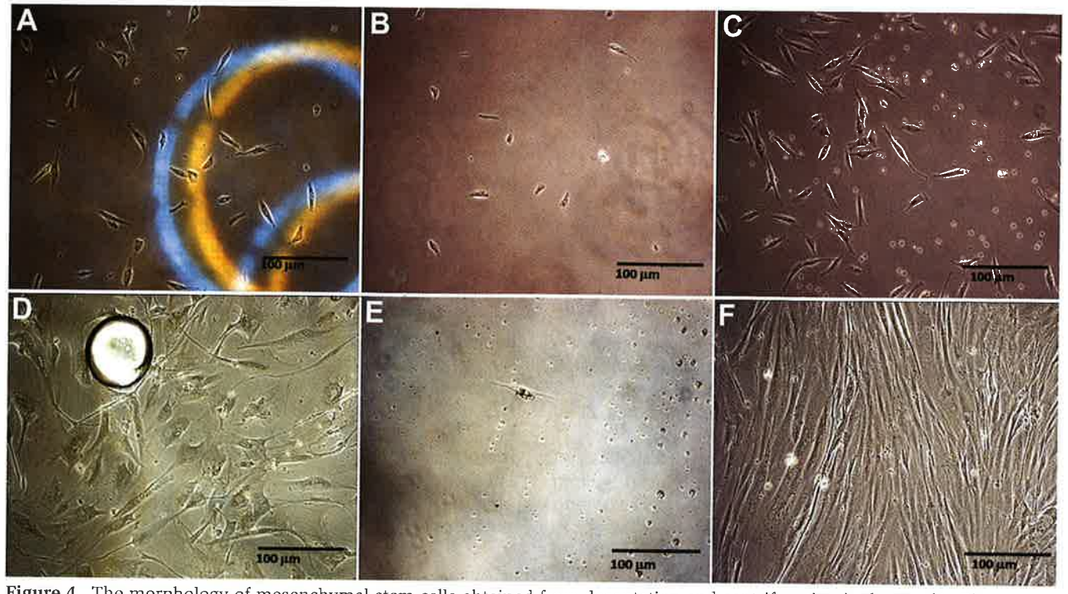

Figure 4. The morphology of mesenchymal stem cells obtained from decantation and centrifugation is shown. (A, D) Decanted, (B, E) centrifuged, and (C, F) pellet from centrifuged lipoaspirate samples. Cultures were maintained for (A-C) three days and (D-F) 15 days. Bar size: 100 µm.

Table 1. Quantitative Representation of Hematopoietic (CD45+) and Nonhematopoietic (CD45–) Cell Fractions in Decanted and Centrifuged Lipoaspirates

	Decanted	Centrifuged	Pellet
CD45+	6.2 ± 2.9	2.7 ± 1.6	8.8 ± 1.4
CD45–CD31+	3.8 ± 3.1	2.0 ± 0.7	7.1 ± 3.6
CD45–CD34+	2.8 ± 2.7	2.3 ± 1.7	4.3 ± 2.3
CD45–CD105+	2.9 ± 2.4	2.1 ± 1.5	4.7 ± 1.6

The table represents percentage of cells (mean ± standard deviation of 10 patients).

DISCUSSION

When fat tissue harvesting and subsequent fat tissue grafting are performed in the same surgical intervention, the time for processing the fat may be relevant and centrifugation may be preferable to decantation because it rapidly separates the different components. When the search for improved final results is also taken into account, the quality and the viability of implanted cells have to be considered.

Centrifugation concentrates adipocytes and separates them from blood cells, lipids, proteases, and other components that can degrade them,[21] yielding a more compact, heavier tissue in the middle layer, which gives a better idea of the amount of tissue, which will be grafted[22] and even reduces the volume of fat to be transplanted.[23]

However, considering that viable adipocytes may be relevant for graft survival, as stated by Peer,[24] decantation preserves the adipocytes' integrity, although it is limited to separating the adipocytes from other contaminating blood cells. By contrast, centrifugation destroys a great majority of adipocytes, yielding a mass of disrupted cells that may result in poor integration of the grafted tissue with its surroundings. We have found that the middle layer of the centrifuged lipoaspirates used by many surgeons (after discarding the oily top and serosanguinous bottom layers) contains mostly altered adipocytes. We believe that the satisfactory results shown by those who employ this method[25] are probably due to (1) good standardization of the technique, (2) the injection method of maximizing the contact area between the donor and receptor area,[26] (3) overinjection of fat to compensate for the percentage of fat lost to possible absorption, or (4) the presence of an inflammatory reaction and subsequent fibrosis, which help to at least partially maintain the volume of the transplanted tissue.

It is also questionable whether the adipocytes of the middle layer that have not preserved their overall structure after centrifugation, as observed by histology, are still able to reconstitute adequate cellular tissue in the site that receives the graft. Mature adipocytes with a large single fat droplet (which are the majority of cells in adult fat tissue) have a limited capacity to proliferate.[27] The slow natural turnover of perivascular cells in the adipose tissue is equivalent to MSC. These cells have a high capacity to proliferate and differentiate into separate connective tissue cells and blood vessel components. Vascularization of the new tissue is fundamental because it enables nutrient and oxygen supplies. Hypoxia, expected to occur within the transplanted tissue, stimulates the proliferation and differentiation of endothelial cells,[8] the progenitors of which are also present in the lipoaspirate and can also be derived from MSC.[28] Formation of functional, stable blood vessels is thus also dependent on mesenchymal cell mobilization,[10] which is another potential benefit of introducing them into the grafted tissue.

Centrifugation is able to concentrate a great majority of processed lipoaspirated cells in the pellet, which is rich in MSC (Figure 4C,F). They can be sedimented alone or with other components of blood vessels. Because fat is poor in connective tissue, the majority of larger debris found in the pellet are vascular and perivascular cells. These cells are more resistant to trauma than adult adipocytes because they are smaller and devoid of structural discontinuities, such as fat droplets. Because of this good resistance to the trauma generated during the harvesting, processing, and injecting of adipose tissue, adipose-derived mesenchymal stem cells are considered by some authors to be the most important elements for adipose tissue graft take.[29] Aspirated fat has demonstrated an increased survival rate when transplanted with adipose-derived stromal cells; this cell-assisted lipotransfer has proven to be effective, safe, and superior to conventional lipoinjection in procedures such as breast reconstruction, cosmetic breast augmentation, and facial lipoatrophy.[30,31]

Preadipocytes and adipose-derived stem cells may be the only cells that survive adipose tissue transplant, and the variable quantity of cells between individuals may be responsible for the end result in adipose tissue grafting. Therefore, the paucity of these cells shown both in the middle layer of decanted and, to a greater extent, in centrifuged lipoaspirates can be responsible for the decreased survival rate and eventual absorption of the grafted fat[32] because both MSC and endothelial cells play a crucial role in the angiogenesis of grafted tissue by secreting angiogenic factors and increasing capillary density and blood circulation.[33]

It appears that there is no single ideal fat processing method that can increase graft take. A combination of methods can yield a rich and better quality product. Recently, the Cytori Therapeutics group has shown promising results with the use of the Celution technology, in which stem and regenerative cells from adipose matrix are separated, concentrated, and mixed with adipose tissue in a single procedure with a single device, resulting in a variety of mechanisms promoting tissue survival.[34] However, the cost of this device is a limiting factor for many plastic surgery centers or small private practices. Therefore, we suggest mixing the pellet with the middle layer whenever centrifugation is chosen as a processing method, rather than using only the middle layer. Another alternative would be to mix the pellet obtained from centrifuged samples with the middle layer of decanted lipoaspirates to take advantage of the preserved structure of the adipocytes found in the latter (although the high quantity of blood contaminants is a limiting factor).

Based on these theories and our findings, centrifugation can be considered a key component in converting naturally low concentrated stem cell adipose tissue into a relatively rich stem cell preparation (because of its high content in the pellet, specifically), increasing the chance of graft survival and preventing secondary atrophy.[35]

These observations are the preliminary results of a long-term project involving a group of studies needed to optimize actual adipose tissue graft survival and, more importantly, take advantage of the rich source of mesenchymal stem cells and endothelial cells found in adipose tissue for application in all fields of regenerative medicine.

CONCLUSIONS

Centrifugation plays a very important role in separating adipose tissue from blood remnants and other substances that are aspirated, permitting a better evaluation of the amount of tissue that will be transplanted. However, centrifugation seems to have controversial effects on the cell population of aspirated adipose tissue: a negative effect on adipocytes (altering their morphology and thus compromising their viability) and a positive effect on MSC (concentrating them in the pellet at the bottom layer of the centrifuged lipoaspirate). According to our results, if centrifuged adipose tissue is used, the association of the pellet, which is rich in adipose-derived MSC, with the middle layer, which is poor in MSC (representing a fat tissue scaffold that would provide mechanical support to cells), should be recommended to increase graft survival.

Disclosures

The author(s) declared no conflicts of interests with respect to the authorship and/or publication of this article.

Funding

The author(s) received no financial support for the research and/or authorship of this article.

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