Journal of Plastic, Reconstructive & Aesthetic Surgery (2016) xx, 1–9

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

Autologous fat grafting: A comparative study of four current commercial protocols

Vincent Hivernaud [a,b,c,*], Bruno Lefourn [e], Myriam Robard [f], Jérôme Guicheux [a,b,c,d], Pierre Weiss [a,b,c,d]

[a] *INSERM, LIOAD, UMR-S 791, Nantes, France*
[b] *Université de Nantes, UMR-S 791, LIOAD, UFR Odontologie, Nantes, France*
[c] *ONIRIS, UMR-S 791, LIOAD, Nantes, France*
[d] *CHU Nantes, PHU 4 OTONN, Nantes, France*
[e] *Clinique Bretéché, Département de Chirurgie Plastique et Reconstructive, Nantes, France*
[f] *Cellular and Tissular Imaging Core Facility of Nantes University (MicroPICell), Structure Fédérative de Recherche François Bonamy, Université de Nantes, Nantes, France*

Received 9 March 2016; accepted 30 November 2016

KEYWORDS
Autologous fat grafting;
Comparison study;
Mice model;
Fat processing;
Adipose tissue;
Lipotransfer

Summary *Background:* Autologous fat grafting is a widely used technique that gives natural results when treating soft tissue deficiencies. However, there is no consensus on which is the best procedure to use, leading to unpredictable results because of fat graft resorption.
Objectives: This study compared four commercial lipotransfer devices by analyzing the behavior of the processed adipose tissue and outcome of the adipose graft in an *in vivo* model.
Methods: Four different protocols that used manual, power-assisted or water-assisted lipoaspiration and then decantation, centrifugation, or filtration were used on each of eight patients to process lipoaspirate. Harvested adipose tissue was assessed *in vitro* for tissue resorption, oil formation, and cytokine secretion. Graft resorption rate was calculated and histological analyses were performed after subcutaneously injecting the harvested adipose tissue in a murine model.
Results: All protocols resulted in very low oil formation and histologically healthy grafts. The tissue volume was significantly greater after 2 days in culture when using manual lipoaspiration and soft centrifugations/washing steps (Microfill®/Macrofill®) compared to Water-Assisted Lipoaspiration/Decantation (BodyJet®) and Power-Assisted Lipoaspiration/Filtration (PAL® + PureGraft®). These results were confirmed in mice 1 month after subcutaneous injection, with greater efficiency obtained with protocols that used (A) manual aspiration, (B) soft centrifugations, and (C) washing steps.

* Corresponding author. INSERM, LIOAD, UMR-S 791, Nantes, France.
E-mail address: vincent.hivernaud@hotmail.fr (V. Hivernaud).

http://dx.doi.org/10.1016/j.bjps.2016.11.022
1748-6815/© 2016 British Association of Plastic, Reconstructive and Aesthetic Surgeons. Published by Elsevier Ltd. All rights reserved.

Please cite this article in press as: Hivernaud V, et al., Autologous fat grafting: A comparative study of four current commercial protocols, Journal of Plastic, Reconstructive & Aesthetic Surgery (2016), http://dx.doi.org/10.1016/j.bjps.2016.11.022

Conclusions: We confirmed that the choice of technique used to process adipose tissue during lipotransfer surgery can highly influence fat grafting efficacy. In our study, the use of manual aspiration combined with soft centrifugations led to the best results in the selected models.
© 2016 British Association of Plastic, Reconstructive and Aesthetic Surgeons. Published by Elsevier Ltd. All rights reserved.

Introduction

Interest in autologous fat grafting (AFG) has increased since the advent of lipoaspiration in the 1970's. It is used both for esthetic purposes (volume augmentation and wrinkles) and plastic reconstruction to treat adipose tissue affections (lipodystrophy) or injuries (burn marks, mastectomy, scars, etc.).[1] The principle of this procedure is to provide soft tissue filling of a chosen area of the body through subcutaneous injection of adipose tissue that has been harvested from a donor site (mostly abdomen according to the American Society of Plastic Surgeons in 2013).

However, high levels of graft resorption several months after injection (from 30% to 80% depending on site, volumes, and protocols) remains a major clinical limitation to AFG.[2,3]

The AFG technique can be divided into four steps: infiltration, aspiration, purification, and reinjection. Each of these steps is critical for the survival of adipose and stromal vascular cells and are influential in postinjection outcome.[2,4,5] The majority of surgeons harvest fat with a syringe and manual suction,[6] but new alternative devices are also available. Centrifugation is commonly used during purification, but high-speed centrifugation has been shown to be harmful to adipose tissue cells.[7,8] For this reason, other techniques have been developed that utilize decantation, filtration systems, or soft centrifugations.[5,9,10] Finally, washing the tissue to remove blood and infiltration solutions has been shown to improve results,[7] but this technique is presently used only in a handful of protocols.

Currently, surgeons have numerous devices at their disposal that use different processes to harvest and purify tissue. Despite growing interest in this field, there is still no consensus regarding the manner in which fat should be processed from harvesting through fat transfer, leading to highly variable results.

In this study, the adipose tissue graft was harvested and processed by four "new generation" lipotransfer protocols that represent three different concepts: Body-Jet® [water-assisted lipoaspiration (WAL) and decantation], PAL® + PureGraft® [power-assisted lipoaspiration (PAL) and filtration], and Microfill® and Macrofill® (manual aspiration; soft centrifugation and washing). The harvested tissue was compared *in vitro* and *in vivo* to determine which protocol gives the best results in terms of quality (oil formation and histological aspect) and quantity (resorption rate).

Material and methods

Patients

All protocols were approved by the French National Ethics Committee (code DC-2011-1399). Lipoaspirates were obtained (January 2014 to May 2015) from eight female patients undergoing abdominal lipoplasty or dermolipectomy [mean age: 41.5 years old (range 29—55) and mean body mass index: 26.2 kg/m^2 (range 23.5—29.5)]. All patients provided informed consent. Exclusion criteria were BMI $>$ 30 and lipodystrophy syndrome. For each of the eight patients, the abdomen was divided into four sites (left upper quadrant, left lower quadrant, right upper quadrant, and right lower quadrant), avoiding the sub-umbilical region. The study protocols were randomly attributed to each site (harvesting was performed with each technique at least once from each quadrant). For each protocol, the same amount of lipoaspirate (75 cc) was harvested, with a total of 300 cc per patient.

For each protocol, except the WAL, a tumescent solution (0.9% NaCl saline solution, 2% adrenaline) was infiltrated prior to aspiration with 1 cc infiltrate for 1 cc of harvested tissue. With the WAL protocol, the tumescent solution was infiltrated before and during the harvesting step in accordance with the manufacturer's instructions (Bodyjet® — Human med, Schwerin-Germany).

Harvesting and processing methods

Harvesting products and consumables were kindly provided by Adip'sculpt and Human-med companies.

WAL protocol

Infiltration and tissue aspiration were performed using the Bodyjet® device. Infiltration was set at 2 atm and aspiration at 0.5 atm (approximately 380 mmHg). The aspiration device was connected to a fat trap (Lipocollector 3® — Human-med, Schwerin-Germany) in which the tissue was allowed to decant for at least 15 min. The lower liquid phase was then discarded, and the adipose tissue phase was used for our experiments.

PAL and filtration

Following infiltration, the fat tissue was harvested using a 3-mm multi-hole cannula connected to a PAL device (PAL® — Microaire, Charlottesville-USA) with vibration set at 5/10 and a 0.5-atm aspiration. The harvested adipose tissue was

Please cite this article in press as: Hivernaud V, et al., Autologous fat grafting: A comparative study of four current commercial protocols, Journal of Plastic, Reconstructive & Aesthetic Surgery (2016), http://dx.doi.org/10.1016/j.bjps.2016.11.022

then washed through a filtration device (Puregraft® – Cytori Therapeutics, San Diego-USA) according to the manufacturer's instructions. Briefly, fat was inserted into the Puregraft® bag and then washed twice with the same amount of washing solution (Ringer Lactate – Bbraun, Melsungen-Germany). After each washing step, the liquid and oil fraction were allowed to evacuate into the appropriate bag. The remaining tissue was used for our experiments.

Multiple washing and centrifugation protocol
The adipose tissue was harvested and processed by two slightly different protocols according to the manufacturer's instructions (Microfill® and Macrofill® – Adip'sculpt, Sainte Clotilde-France). With Microfill®, the tissue was harvested with a 2-mm multi-hole cannula connected to a 10-cc syringe and a vacuum of −0.5 atm (2 cc aspiration). With Macrofill®, a 3.5-mm multi-hole aspiration cannula connected to a 50-cc vacuum-controlled syringe (−0.5 atm) was used. Once harvested with either of the two protocols, fat tissue was washed by the addition of 15 cc of Ringer lactate for 35 cc of tissue and centrifuged at 100 × *g* for 1 s. This procedure was repeated twice. The final washing step was followed by centrifugation at 400 × *g* for 1 min. At each step, the oil supernatant and the lower liquid phase were discarded. The remaining tissue was used for our experiments.

In vitro experiments

Tissue culture
With the tissue harvested from five patients (with all the four protocols performed on every patient), processed fat tissue from each protocol was cultured in 24-well microplates (Corning, Corning-USA). One cubic centimeter of fat was distributed into each well using a 5-cc syringe and was combined with 2 cc of standard medium: Dulbecco's modified Eagle's medium—GlutaMax (Thermo Fischer Scientific, Waltham-USA), 10% fetal calf serum (PAN-Biotech, Aidenbach-Germany), penicillin 10 000 U/mL, and streptomycin 10,000 μg/mL (Thermo Fischer Scientific). Plates were incubated at 37 °C with 21% oxygen and 5% carbon dioxide. After 2 days of culture, tissue and medium were collected and gently centrifuged at 400 × *g* for 1 min to determine the volume of oil (upper phase) and the remaining volume of tissue (middle phase). Each phase volume (oil and tissue) was calculated using a calibrated pipette, and measurements were confirmed with an additional weighing.

Tissue secretion of inflammatory cytokines
After 2 days of adipose tissue culture and centrifugation at 400 × *g* for 1 min, the lower liquid phase was collected and monocyte chemotactic protein 1 (MCP1) and interleukin 6 (IL6) secretion were quantified by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (eBioscience, San Diego-USA). Briefly, plates coated with an anti-MCP1 or anti-IL6 antibody were incubated with the samples. A biotinylated antibody (anti-MCP1 or anti-IL6) coupled with an enzyme was added.

Stromal vascular fraction cell isolation
For four patients, part of tissue samples obtained by liposuction were digested under agitation for 1 h at 37 °C in Ringer lactate buffer containing 1.5% collagenase (NB4, SERVA, Heidelberg-Germany, PZ activity 0.175 U/mg) followed by centrifugation at 900 × *g* for 3 min. The cell pellet (stromal vascular fraction; SVF) was then resuspended in Ringer lactate and washed with centrifugation at 900 × *g* for 3 min. This step was repeated twice. After the third wash, the pellet was resuspended in Ringer lactate and filtered through Steriflip 100 μm (Millipore, Molsheim-France). After centrifugation at 900 × *g* for 3 min, cells were resuspended in standard medium, and then cell number and viability were assessed microscopically by trypan blue dye exclusion in triplicate at a 1/1000 dilution on a cell counter (Malassez-VWR international, Fontenay sous bois, France).

In vivo experiments

Animals
The study was performed on 36 adult (2 months old) CB17 severe combined immunodeficient (SCID) female mice provided by a certified breeding center (Charles River, l'Arbresle, France). Animal care was provided by the Department of Experimental Therapeutics Unit in Nantes, France. The Ethics Committee of the "Region Pays de la Loire" reviewed and approved the study design (CEEA 2012-249). The animals were acclimatized to the conditions of the local vivarium for 1 week, which was maintained at 24 °C and given a 12 h/12 h light/dark cycle. Two subcutaneous tunnels were created in the mice flanks to administer two injections per mouse. The experiments were realized with the adipose tissue from three patients, with 12 mice per patient (n = 6 injections per protocol per patient). One cubic centimeter of fat was injected with a retrograde movement using a 2-mm cannula connected to a 1-cc syringe. After 4 weeks, mice were sacrificed using carbon dioxide and fat grafts were retrieved. Samples were fixed in 4% formaldehyde for 48 h. This experiment was repeated thrice with the tissues obtained from the three last patients.

Implant characterization
Grafts were retrieved and fixed 1 month after implantation. Fixed samples were quickly dried and weighed using a precision scale.

Histological analyses
Fixed samples were dehydrated in a series of alcohol baths and embedded in paraffin. Five-micrometer tissue sections were obtained on a microtome and stained with hematoxylin/phloxin/saffron (HPS) staining. Slides were scanned (Hamamatsu nanozoomer), and area measurements and histological scoring were performed using the NDPview software (Hamamatsu, Hamamatsu-Japan). Histological scoring was adapted from a previous study.[11] The inflammation state of the tissue was added to the previous scoring system with a four-degree scale. An example of inflammation infiltrate is shown in Figure 5C.

Statistical analysis
All statistical analyses were conducted using the Prism (GraphPad, La Jolla-USA) software. Significance was determined by Kruskal–Wallis analysis combined with a Dunn's multiple comparison test.

Please cite this article in press as: Hivernaud V, et al., Autologous fat grafting: A comparative study of four current commercial protocols, Journal of Plastic, Reconstructive & Aesthetic Surgery (2016), http://dx.doi.org/10.1016/j.bjps.2016.11.022

Results

In vitro analysis of harvested lipoaspirates

Cellularity and stem cell contents

SVF cells from the lipoaspirates of the first four patients were isolated with no significant cellular differences being observed ($7.68.10^6 \pm 5.46$ cells/mL for BodyJet®, $10.24.10^6 \pm 4.22$ cells/mL for Macrofill®, $9.05.10^6 \pm 2.40$ cells/mL for Microfill®, and $11.86.10^6 \pm 4.16$ cells/mL for PAL® + PureGraft®) (Figure 1A). Moreover, adipose stromal cells (ASC) were isolated by plastic adhesion over a period of 24 h, and again no significant differences were found in the number of cells/mL of lipoaspirate (Figure 1B).

Tissue resorption in culture

The lipoaspirates obtained from five donors and harvested/purified by four protocols were seeded in culture plates with culture medium. After 2 days of culturing, the remaining tissue phase was significantly higher with the two manual aspiration/centrifugation techniques (77.7 ± 5.46% and 79.5 ± 5.12%) compared to the WAL/decantation (48.9 ± 5.06%) (Figure 2A). The proportion of residual tissue seemed intermediate with the PAL/filtration (62.2 ± 4.67%) protocol, but no statistical differences were noted.

Oil formation in culture

In addition to the adipose tissue phase, oil formation was also measured (Figure 2B). In all protocols, only a limited amount of oil was detected (<2% of initial volume: 0.9, 1.5, 0.9, and 0.8% respectively).

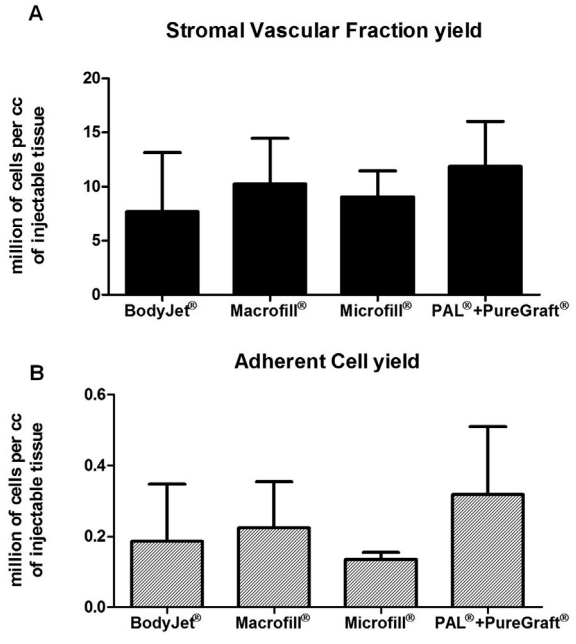

Figure 1 *In vitro* evaluation of adipose tissue cellular content after harvesting and processing by the four different protocols (n = 4 patients). Processed lipoaspirates were digested with collagenase and cells from SVF were counted (A). SVF cells were cultured for 24 h on culture plates. After 24 h, nonadherent cells were washed and adherent cells were counted (B). Results are expressed as mean ± SEM.

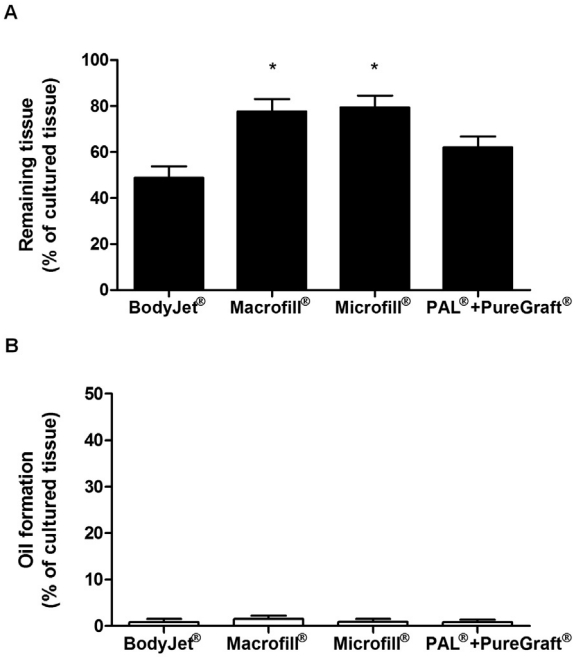

Figure 2 *In vitro* evaluation of adipose tissue and oil quantity after harvesting and processing by the four different protocols (n = 5 patients). Processed lipoaspirates were incubated in the presence of culture medium for 48 h. (A) The adipose phase was assessed, and the results are expressed as a percentage of initial volume. *p < 0.01 compared to the BodyJet® condition. (B) The oil supernatant was measured and the results are expressed as a percentage of initial volume as mean ± SEM.

Cytokine secretion in culture

After 2 days of culture, the tissue medium (lower phase) was harvested and filtered, and cytokine secretion was evaluated by ELISA. Extensive patient-dependent cytokine secretion was observed. When comparing the techniques, no significant differences were found for MCP1 or IL6 secretion (Figure 3A and B). In all cases, cytokine secretion was lower than 100 ng/mL.

In vivo analyses of implanted lipoaspirates

Histological scoring at 1 month

Harvested grafts were histologically analyzed for the presence of oil cysts, fibrous tissue, and inflammatory cell infiltration (Table 1). The lower the histological score was, the more oil cysts, fibrous tissue, and cell infiltration were present in the samples (examples are listed in Figure 5).

The results of histological scoring are presented in Figure 4A and illustrated in Figure 6. No significant histological differences were observed when the grafts were compared (with scores of 4.72 for WAL/decantation, 5.75 and 5.25 for manual aspiration/centrifugation, and 5.76 for PAL/filtration).

Tissue resorption after 1 month

One cubic centimeter of tissue obtained by each protocol was implanted in the subcutaneous tissue of

Please cite this article in press as: Hivernaud V, et al., Autologous fat grafting: A comparative study of four current commercial protocols, Journal of Plastic, Reconstructive & Aesthetic Surgery (2016), http://dx.doi.org/10.1016/j.bjps.2016.11.022

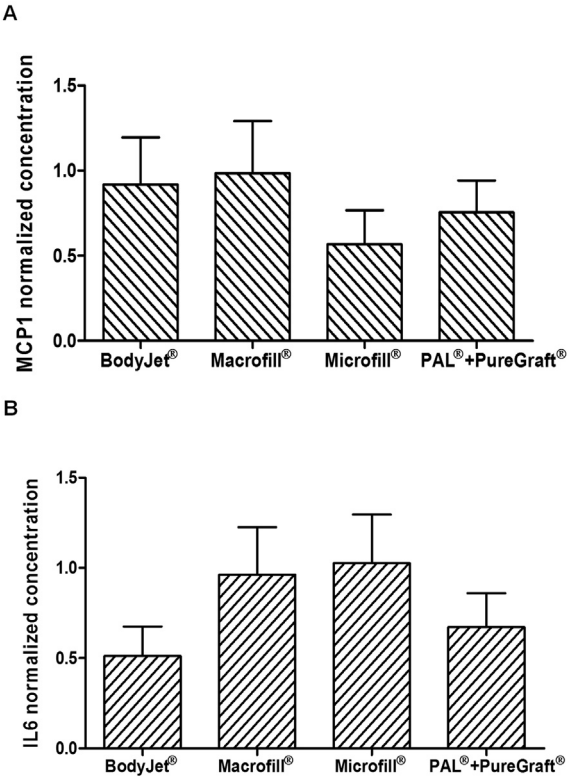

Figure 3 Adipose tissue cytokine secretion after 48 h of culture. Processed lipoaspirates were incubated in the presence of culture medium for 48 h. Then medium subnatant was retrieved to assess MCP1 (A) or IL6 (B) concentration by ELISA (n = 5 patients). Results are expressed as mean ± SEM.

immunodeficient mice (n = 18 injections per protocol). After 1 month, grafts were harvested and the remaining tissue weight was assessed (Figure 4B). Once again, manual aspiration/centrifugation techniques resulted in higher

Table 1 Detailed histological scoring grid of adipose tissue grafts.

Scoring criteria	Evaluation	Score
Oil vacuoles	<2%	5
	2–5%	4
	5–10%	3
	10–15%	2
	15–20%	1
	>20%	0
Signs of fibrosis	Absent	3
	Minimal	2
	Moderate	1
	Extensive	0
Signs of inflammation	Absent	3
	Minimal	2
	Moderate	1
	Extensive	0
Adipocyte size and shape	Homogeneous	1
	Heterogeneous	0
Maximal score		**12**

The higher scores are attributed to healthy criteria, whereas the lower scores are assigned to injured grafts.

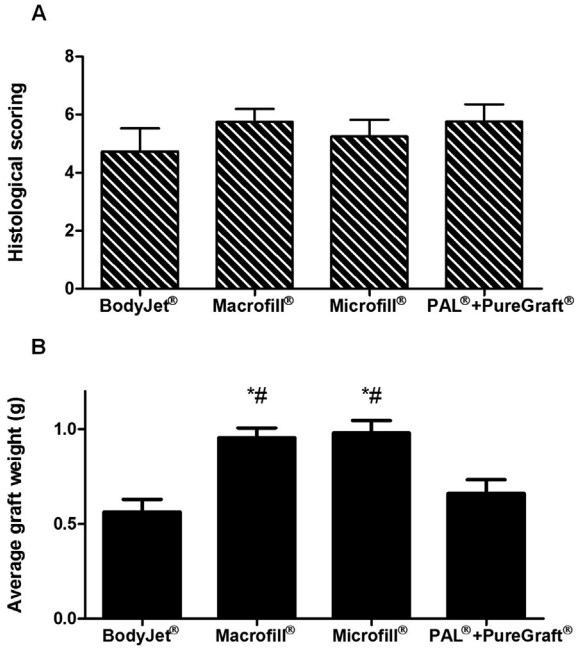

Figure 4 *In vivo* evaluation of fat grafts 1 month after subcutaneous implantation. Fat grafts were performed in mice following liposuction and lipoaspirate purification with four different protocols (n = 3 patients, n = 18 injections per protocols). The entire graft was removed after 1 month. (A) Histological scoring was performed on HPS-stained sections. Longitudinal sections were prepared from three different graft depths. (B) Harvested grafts were weighed. *p < 0.05 compared to the BodyJet® condition. #p < 0.05 compared to the PAL® + PureGraft® condition. Results are expressed as mean ± SEM.

remaining tissue size and weight (means of 0.95 ± 0.05 and 0.97 ± 0.07 g, respectively) than the other two techniques (a mean of 0.56 ± 0.07 g for WAL/decantation and a mean of 0.66 ± 0.07 g for PAL/filtration).

Discussion

The actual context of AFG

From simple decantation to the use of Coleman's centrifugation protocol, lipotransfer protocols are increasingly being used worldwide. Presently, most of the techniques in use are based on decantation in a fat trap or centrifugation by Coleman's protocol, but other methods using filtration, washing steps, or centrifugation with a range of settings are also used.[6]

Faced with reaching the limits of graft resorption during AFG, several teams have set out to define the critical points that can improve the technique to develop a new and more efficient approach[7,10,12–14] that could lead to the commercialization of new lipotransfer devices.

In recent years, there have been great advances in lipotransfer tissue processing. Use of a controlled vacuum during aspiration is now a commonly accepted critical step.[15,16] Similarly, reducing the use of local anesthetic,[11,17] removing any potential blood waste, or slowly

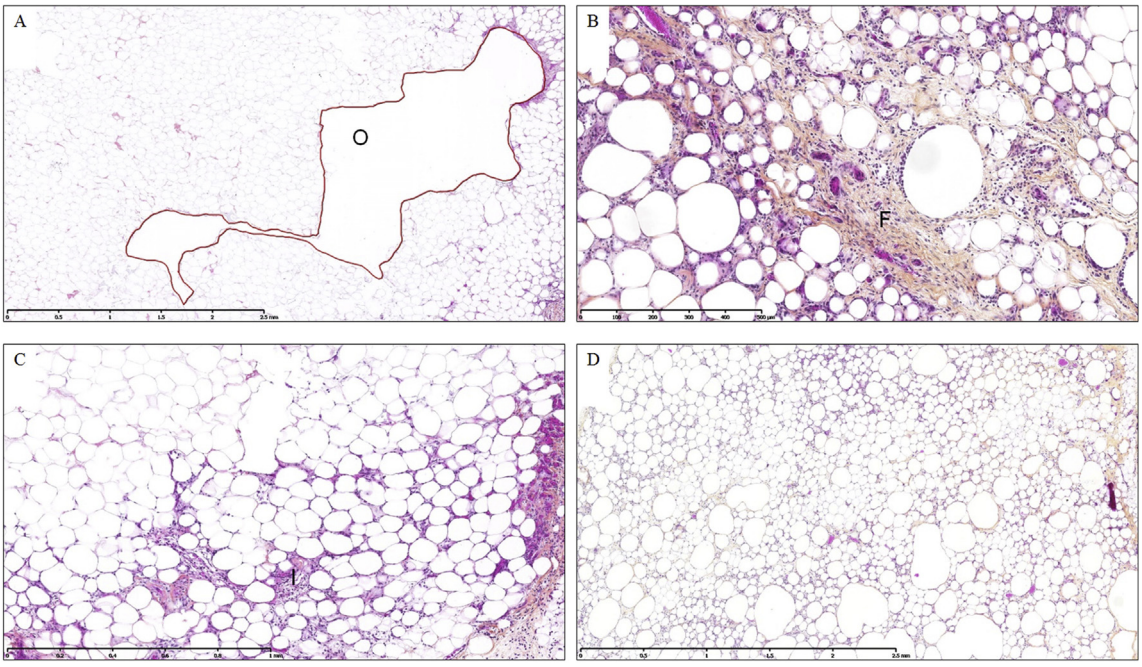

Figure 5 Examples of histological area that represent the items used for the scoring used in this study. (A) Example of the presence of oil lacuna, marked "O." (B) Example of the presence of fibrous tissue, marked "F." (C) Example of the presence of inflammatory infiltration, marked "I." (D) Example of heterogeneity of adipocyte size and shape.

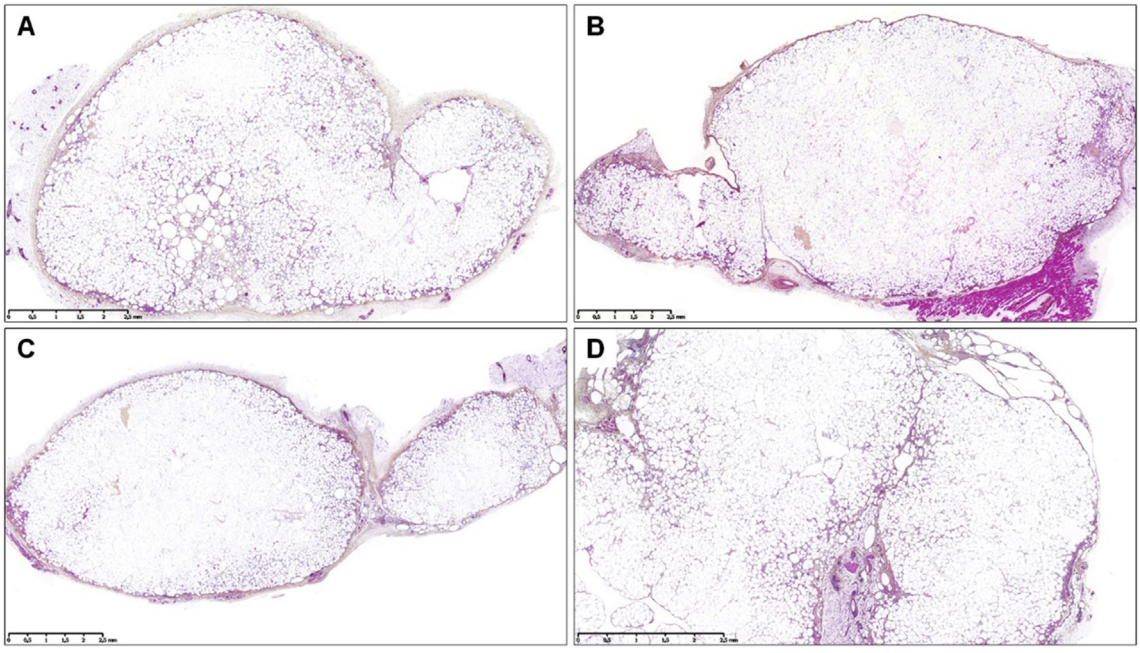

Figure 6 Histological results of grafts recovered 1 month after injection according to the compared protocols. Representative histological images of HPS-stained sections of (A) graft processed by the BodyJet® protocol, (B) graft processed by the Macrofill® protocol, (C) graft processed by the Microfill® protocol, and (D) graft processed by the PAL® + PureGraft® protocol.

reinjecting small aliquots to improve neovascularization and reduce shear stress[18] are all equally important.

However, the appearance of several new devices for aspiration and purification has not led to the identification of a protocol that provides the best results in terms of patient volume maintenance, decreasing oil cysts, and avoidance of over-correction or multiple AFG procedures.

Our study aimed to compare four "new generation" AFG clinical products with respect to their distinct harvesting and purification methods. The potential for patient-

Please cite this article in press as: Hivernaud V, et al., Autologous fat grafting: A comparative study of four current commercial protocols, Journal of Plastic, Reconstructive & Aesthetic Surgery (2016), http://dx.doi.org/10.1016/j.bjps.2016.11.022

dependent results was limited by comparing all four protocols in the same patient.[19] Each of the 4 protocols had its own specific harvesting cannula, suction, and purification methods. However, in our study we did not have a "control group." The reason for this is that there is currently no fat grafting clinical standard. Moreover, all the existing protocols are hampered by their high levels of graft resorption. Faced with numerous differences between the protocols and the variations in the quantity of tissue harvested, we therefore decided to focus on the comparison between the different protocols.

Good handling of tissue viability

All protocols used a controlled harvesting vacuum to improve the viability of the cells and limit oil formation. In fact, as described previously, no or very little oil formation was detected in the *in vitro* 48-h graft culture model for all conditions.[5,7,20] Filtration, decantation, and soft centrifugations did not produce more oil after culture or implantation.

It is now commonly accepted that the survival of SVF cells is critical for graft vascularization and survival. In our study, there were no significant differences in SVF cell yield proportions between techniques, possibly because of the great variation between patients. We obtained more than 90% viability in all cases. Moreover, when isolating the ASC from the SVF, no difference was found in number, viability, or proliferation rate after 1 week. These results are concordant with previously described studies.[20–22]

The lipotransfer protocol, particularly the harvesting of cells from their environment and the concentration of the cellular phase prior to reinjection, can be stressful for the cells, leading to the production of pro-inflammatory cytokines. IL6 and MCP1 are two cytokines secreted by the adipose tissue during inflammation.[7] This inflammatory environment can lower the viability of cells. In our experiments, no difference in IL6 and MCP1 secretion was detected in whole adipose tissue (Figure 3) or SVF cells (data not shown), with cytokine secretion remaining low with all protocols.

Histological analysis showed that all the techniques resulted in relatively healthy tissue after injection (low rate of oil formation and limited signs of fibrosis or inflammation). These results can be explained by the use of our *in vivo* model (relatively small amounts of fat were injected for a limited period of time). Moreover, in our study, the tested protocols maintained adipose graft viability with low vacuum aspiration (<700 mmHg), without the use of local anesthetic, and with fat processed in a closed circuit (no contact with the air).

However, the main limitation of AFG is not the shape of the graft but the resorption rate. To compare the different clinical processing techniques, we calculated the remaining tissue weight in our *in vitro* and *in vivo* models (the density of the graft was constant for all tested conditions).

Presence of liquid

In this study, we chose to compare volumes of processed lipoaspirates. Thus, the proportion of the adipose tissue and the liquid phase may vary between processing protocols. However, the comparison was made with "injectable" tissue because we believe that it is necessary to think in terms of injected volume. Moreover, the maximum volume required at the recipient site and the desired outcome determines the injected volume. Over-correction can alter the viability of injected tissue and increase the necessity for several lipofilling sessions.[23] In this context, we believe it is necessary to compare the injected volume of each operation with the protocols used.

Graft volume maintenance

Major differences between the tested conditions were, in general, observed when we investigated the remaining tissue after processing in our two models: 48 h *in vitro* and 1 month *in vivo*. The two manual-aspiration/centrifugations/washing protocols that were tested in these two models resulted in higher volumes of fat tissue than the other protocols.

The BodyJet® protocol uses the WAL technique with continuous infiltration during aspiration. This process allows the harvesting of small aliquots of fat, with lobules separated with a jet of water. An advantage of infiltration is that there is a low bleeding rate that results in "blood-free" lipoaspirate. Although the lipoaspirate is much more diluted by the presence of infiltration liquid, the decantation process with the Lipocollector® device enables the liquid phase to collect in the bottom of the fat trap through the action of gravity, thus eliminating it almost completely. However, it is extremely likely that a large part of the liquid phase will still be trapped in the adipose phase.[24,25] This liquid will be the first to be reabsorbed after injection, which explains the lower remaining quantity of tissue obtained with this technique in our study. It would be very interesting to assess the combination of WAL fat harvesting with centrifugation to eliminate most of the liquid component prior to reinjection.[26]

Similar to the WAL process, PAL® enables the harvesting of small fat lobules by dissecting the tissue with cannula vibration. However, care should be taken to not use vibration at full capacity as this can lead to tissue damage. The PAL® system is only a fat-harvesting aspiration device and does not purify the fat. Therefore, like in a number of other clinical protocols, we combined the PAL® aspiration device with the PureGraft® filtration bag. The PureGraft® system enables the removal of the liquid and oil phases while washing the tissue, without the use of gravity (decantation or centrifugation). Our study did not enable us to assess which of the points is critical in improving graft efficacy in this combined system. Nevertheless, to our knowledge, this is the first time that such a combination has been tested in these experimental models.

Good results have previously been obtained with each device.[5,20] However, previous studies have focused on cell viability and differentiation, blood cell content, or growth factors. In our study, we focused on short-term evaluation with an *in vitro* model and long-term resorption of fat grafts in addition to the histological appearance of the grafts 1 month post injection. We highlighted significant differences in terms of graft resorption with the PAL/

Please cite this article in press as: Hivernaud V, et al., Autologous fat grafting: A comparative study of four current commercial protocols, Journal of Plastic, Reconstructive & Aesthetic Surgery (2016), http://dx.doi.org/10.1016/j.bjps.2016.11.022

filtration combination, whereas the amount of stem cells per volume of adipose tissue remained equivalent to other techniques.

In our study, the Macrofill® and Microfill® kits, using manual aspiration, washing steps, and soft centrifugations, gave significantly better results than the other techniques as far as remaining tissue was concerned.

The results that we obtained are consistent with previous studies that reported the advantages of gentle centrifugation(s)[4,7,13] and tissue washes.[11,27]

Firstly, controlled aspiration with a syringe or smooth aspiration with WAL or PAL are crucial for adipocyte survival. However, this alone is insufficient for good resorption results. To achieve this, the removal of the majority of the liquid phase of the lipoaspirate is critical, especially if WAL is used.

Centrifugation can be harmful to the tissue when the duration and speed are excessive. The use of several short and soft centrifugations overcomes these constraints while at the same time concentrating the fat tissue. The other advantage of using multiple centrifugations is that it allows washing steps to be included to eliminate possible dead cells, residual local anesthetics, and cell debris.[11,28] Therefore, a filtration method, for example, with a filtration bag, is of interest when washing the lipoaspirate. Furthermore, the addition of soft centrifugations to WAL or PAL harvesting could improve liquid removal and post-reinjection tissue maintenance.[26] In this study, the *in vitro* results indicate that there is higher graft resorption with decantation/filtration-purified tissue than tissue purified with centrifugation alone. Our *in vitro* results confirm that more liquid is present in injected tissue that has not undergone centrifugation. This liquid part is likely to be resorbed after injection as is probably the case in our model. The use of centrifugation to remove more liquid from the graft results in decreased resorption.

This study compares four fat preparation protocols, with manual aspiration, WAL, PAL, decantation, centrifugation, and filtration and represents the majority of the techniques in current use. Although this study has made use of *in vitro* and animal models to try and elucidate what is the best way of handling fat grafts to decrease resorption, it would now be highly interesting to take the next step forward and conduct a comparative study in a human clinical trial.

Conclusion

In this study, although the histological parameters of the implanted fat tissue remain in the same range for all the compared protocols, the adipose tissue processing significantly influenced the resorption rate. Comparison of the *in vitro* and *in vivo* results obtained by the four protocols suggests that using manual aspiration combined with soft centrifugations is advantageous. This method for handling the adipose graft has already produced good results in a recent clinical evaluation.[29] In our models, concentration of the adipose graft by the removal of the lipoaspirate liquid component seems to be critical to obtain the best graft maintenance.

Include answer in your manuscript text as requested above.

Conflict of interest

VH received a scholarship grant from the Association Nationale de la Recherche et de la Technologie (France) and from the Stemcis company.

Funding

This work was financially supported by the Ministère de l'Enseignement Supérieur et de la Recherche. VH received a scholarship grant from the Association Nationale de la Recherche et de la Technologie (France) (2012-1508) and from the Stemcis company. Stemcis company paid for laboratory costs.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bjps.2016.11.022.

References

1. Gutowski K, Force AFGT. Current applications and safety of autologous fat grafts: a report of the ASPS fat graft task force. *Plast Reconstr Surg* 2009;**124**(1):272—80.
2. Hivernaud V, Lefourn B, Guicheux J, et al. Autologous fat grafting in the breast: critical points and technique improvements. *Aesthetic Plast Surg* 2015;**39**(4):547—61.
3. Leopardi D, Thavaneswaran P, Mutimer K, Olbourne N, Maddern G. Autologous fat transfer for breast augmentation: a systematic review. *ANZ J Surg* 2014;**84**(4):225—30.
4. Condé-Green A, de Amorim N, Pitanguy I. Influence of decantation, washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: a comparative study. *J Plast Reconstr Aesthetic Surg — JPRAS* 2010;**63**(8):1375—81.
5. Zhu M, Cohen S, Hicok K, et al. Comparison of three different fat graft preparation methods: gravity separation, centrifugation, and simultaneous washing with filtration in a closed system. *Plast Reconstr Surg* 2013;**131**(4):873—80.
6. Kling R, Mehrara B, Pusic A, et al. Trends in autologous fat grafting to the breast: a national survey of the american society of plastic surgeons. *Plast Reconstr Surg* 2013;**132**(1):35—46.
7. Hoareau L, Bencharif K, Girard A, et al. Effect of centrifugation and washing on adipose graft viability: a new method to improve graft efficiency. *J Plast Reconstr Aesthetic Surg — JPRAS* 2013;**66**(5):712—9.
8. Kurita M, Matsumoto D, Shigeura T, et al. Influences of centrifugation on cells and tissues in liposuction aspirates: optimized centrifugation for lipotransfer and cell isolation. *Plast Reconstr Surg* 2008;**121**(3):1033.
9. Fodor P, Vogt P. Power-assisted lipoplasty (PAL): a clinical pilot study comparing PAL to traditional lipoplasty (TL). *Aesthetic Plast Surg* 1999;**23**(6):379—85.
10. Hoppe D, Ueberreiter K, Surlemont Y, Peltoniemi H, Stabile M, Kauhanen S. Breast reconstruction de novo by water-jet assisted autologous fat grafting—a retrospective study. *GMS Ger Med Sci* 2013;**11**.
11. Girard A, Mirbeau S, Gence L, et al. Effect of washes and centrifugation on the efficacy of lipofilling with or without local anesthetic. *Surg—Global Open* 2015;**3**(8):e496.
12. Ansorge H, Garza J, McCormack M, et al. Autologous fat processing via the revolve system: quality and quantity of fat

retention evaluated in an animal model. *Aesthetic Surg J* 2014;**34**(3):438—47.
13. Ferraro G, De Francesco F, Tirino V, et al. Effects of a new centrifugation method on adipose cell viability for autologous fat grafting. *Aesthetic Plast Surg* 2011;**35**(3):341—8.
14. Minn K, Min K, Chang H, Kim S, Heo E. Effects of fat preparation methods on the viabilities of autologous fat grafts. *Aesthetic Plast Surg* 2010;**34**(5):626—31.
15. Cheriyan T, Kao H, Qiao X, Guo L. Low harvest pressure enhances autologous fat graft viability. *Plast Reconstr Surg* 2014;**133**(6):13651368.
16. Shiffman MA, Mirrafati S. Fat transfer techniques: the effect of harvest and transfer methods on adipocyte viability and review of the literature. *Dermatol Surg Off Publ Am Soc Dermatol Surg* 2001;**27**(9):819—26.
17. Wang W, Fang X, Williams S, et al. Lidocaine-induced ASC apoptosis (tumescent vs. local anesthesia). *Aesthetic Plast Surg* 2014;**38**(5):1017—23.
18. Lee J, Kirkham J, McCormack M, Nicholls A, Randolph M, Austen W. The effect of pressure and shear on autologous fat grafting. *Plast Reconstr Surg* 2013;**131**(5):1125—36.
19. Li K, Gao J, Zhang Z, et al. Selection of donor site for fat grafting and cell isolation. *Aesthetic Plast Surg* 2013;**37**(1):153—8.
20. Keck M, Kober J, Riedl O, et al. Power assisted liposuction to obtain adipose-derived stem cells: impact on viability and differentiation to adipocytes in comparison to manual aspiration. *J Plast Reconstr Aesthet Surg — JPRAS* 2014;**67**(1):8.
21. Fisher C, Grahovac T, Schafer M, Shippert R, Marra K, Rubin J. Comparison of harvest and processing techniques for fat grafting and adipose stem cell isolation. *Plast Reconstr Surg* 2013;**132**(2):351—61.
22. Salinas H, Broelsch G, Fernandes J, et al. Comparative analysis of processing methods in fat grafting. *Plast Reconstr Surg* 2014;**1**.
23. Khouri RK, Rigotti G, Cardoso E, Khouri RK, Biggs TM. Megavolume autologous fat transfer: Part I. Theory and principles. *Plast Reconstr Surg* 2014;**133**(3):550.
24. Peltoniemi H, 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 — JPRAS* 2013;**66**(11):1494—503.
25. Ueberreiter K, von Finckenstein JG, Cromme F, Herold C, Tanzella U, Vogt P. BEAULI™—a new and easy method for large-volume fat grafts. *Handchir Mikrochir Plast Chir: Organ der Deutschsprachigen Arbeitsgemeinschaft für Handchirurgie: Organ der Deutschsprachigen Arbeitsgemeinschaft für Mikrochirurgie der Peripheren Nerven und Gefässe: Organ der Vereinigung der Deutschen Plastischen Chirurgen* 2010;**42**(6):379—85.
26. Yin S, Luan J, Fu S, Zhuang Q. Is centrifugation necessary for processing lipoaspirate harvested via water-jet force assisted technique before grafting? Evidence of lipoaspirate concentration with enhanced fat graft survival. *Ann Plast Surg* 2016;**77**(4):477—84.
27. Dos-Anjos Vilaboa S, Llull R, Mendel T. Returning fat grafts to physiologic conditions using washing. *Plast Reconstr Surg* 2013;**132**(2):323—6.
28. Moore J, Kolaczynski J, Morales L, et al. Viability of fat obtained by syringe suction lipectomy: effects of local anesthesia with lidocaine. *Aesthetic Plast Surg* 1995;**19**(4):335—9.
29. Nelissen X, Lhoest F, Preud'Homme L. Refined method of lipofilling following DIEP breast reconstruction: 3D analysis of graft survival. *Plast Reconstr Surg — Glob Open* 2015;**3**(9).

Please cite this article in press as: Hivernaud V, et al., Autologous fat grafting: A comparative study of four current commercial protocols, Journal of Plastic, Reconstructive & Aesthetic Surgery (2016), http://dx.doi.org/10.1016/j.bjps.2016.11.022