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Comparative Analysis of a New Automatic System and Four Existing Techniques for Autologous Fat Grafting

Xavier Nelissen, MD*
Séverine Licciardi, MS†
Christophe Nizet, MD*
Emmanuel Delay, MD‡
Régis Roche, PhD†

Background: Autologous fat grafting is increasingly used worldwide and is a very attractive technique in many ways. However, treatment duration and postinjection tissue resorption remain problematic elements, which are largely related to the preparation method used. Moreover, few scientific studies objectively compare different fat preparation methods. This study analyzes the efficiency and quality of lipoaspirates prepared with a new filtration/centrifugation system (Adipure) in comparison with several existing techniques.

Methods: Patient lipoaspirates were processed by five different techniques: decantation, centrifugation, Macrofill, Puregraft, and Adipure. Adipose tissue was evaluated in vitro for tissue resorption and oil formation, as well as in vivo after subcutaneous injections in immunodeficient mice. Adipose grafts were collected after 1 month, weighed, and analyzed by histology with a detailed scoring method.

Results: Decanting gives inferior results to all other techniques, in terms of amount of tissue and oil in vitro, or graft weight and histological analysis in vivo. Methods using classical Coleman centrifugation (1200*g*), or a modified one (400*g*) associated with washes (Macrofill) produce very similar results, both in vitro and in vivo. Techniques using filtration systems (Puregraft and Adipure) produce less oil overall and have a higher grafting efficiency. The best results regarding grafting efficiency and oil quantity are found with the Adipure device.

Conclusions: A combination of filtration and very low-speed centrifugation potentiates the advantages of these techniques, in terms of graft efficiency. The adipose tissue purification being done in a few minutes, in an automatic way, undoubtedly provides a strong advantage for the use of this new system. *(Plast Reconstr Surg Glob Open 2023; 11:e5349; doi: 10.1097/GOX.0000000000005349; Published online 16 October 2023.)*

INTRODUCTION

Whether for reconstructive or cosmetic surgery, autologous fat grafting (AFG) is increasingly used throughout the world. This technique uses the patient's fat tissue mainly for its volumizing effect, but also for its healing and regenerative properties.[1–5]

Autologous fat grafts are in many ways ideal fillers with several attractive features,[6] and the technique has experienced tremendous development in the last 10 years for breast reconstruction, due to its extremely natural results and its very low complications.[7–10]

However, one of the major problems concerns the resorption of adipose tissue after reinjection, which will lead to a significant decrease in injected volume in the months following injection.[6] Indeed, conditions of harvesting, preparation, and reinjection of the adipose tissue will considerably influence its survival during and after surgery. Depending on the techniques and volumes used, tissue survival after injection can vary from 30% to 70%,[11–13] which significantly impacts the outcome and number of surgical procedures to achieve the expected result. However, very few scientific studies compare objectively the different fat preparation methods.

Fat processing can be broadly divided into four steps: infiltration, aspiration, purification, and reinjection. Each of these steps is essential for the survival of both fat cells and vascular stromal cells and will each influence, to a greater or lesser extent, postinjection results.[6,11,14,15] Historically, the majority of surgeons harvested fat with a syringe coupled with manual aspiration,[16] then performed

*From the *Department of Plastic, Reconstructive, Maxillofacial Surgery and Burn Center, University Hospital of Liege, Liege, Belgium; †Neosyad Company, Aix en Provence, France; and ‡Department of Plastic and Reconstructive Surgery, Centre Léon Bérard, Lyon, France.*

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centrifugation. However, since high-speed centrifugation is harmful to adipose tissue cells,[17–19] other techniques have been developed using decantation, filtration, or gentle centrifugations.[15,20–23] In addition, washing the tissue to remove potentially pro-inflammatory substances such as blood, oil, and cellular debris has been shown to improve outcomes.[14,24–28] However, since this washout requires more preparation time, it is still only rarely used by practitioners.

In view of all studies on the subject and various devices already developed, some consensus seems to be emerging regarding the most effective preparation technique for adipose tissue. Indeed, washing coupled with passive filtration is less traumatic than centrifugation, whereas the latter is the most effective in removing liquid from the tissue. However, studies comparing these two techniques have found similar results.[20] Furthermore, washing coupled with active filtration is faster to perform than passive filtration[23,29] and seems to yield better results regarding the number of viable adipocytes,[30] but the purification duration is still significant for large volumes of tissue.

Ideally, the fat should be washed and filtered, together with soft centrifugation, to eliminate the maximum amount of liquid in a very short time. Based on these elements, we developed a new device dedicated to the lipofilling technique, to improve results and make them more reproducible. (Fig. 1) The device is composed of an automaton (AdiMate) associated with a single-use medical device (Adipure), allowing it to perform washes, together with active filtration by centrifugation at very low speed (26*g*), in an automatic way.

In this study, we compare this new device to classical techniques (decantation and Coleman) and two existing devices on the market (Puregraft and Macrofill) (Figs. 1 and 2). We analyzed the different quantities of tissue, liquid, and oil in vitro after adipose tissue purification, as well as the efficiency of the graft in vivo, in immunodeficient mice. The effectiveness of the graft was evaluated through a detailed analysis via assessment of several parameters, including weight, size of the grafts, oil amount, and presence of fibrosis.

Takeaways

Question: How to improve the preparation of fat tissue for autologous fat grafting?

Findings: Our team has developed a new technique, combining filtration and centrifugation at very low speed, to purify adipose tissue fully and automatically in less than 10 minutes. The quality of the treated fat was tested in vitro and in vivo on immunodeficient mice and compared with four other techniques.

Meaning: Development of a new lipofilling machine allows for automatic fat purification.

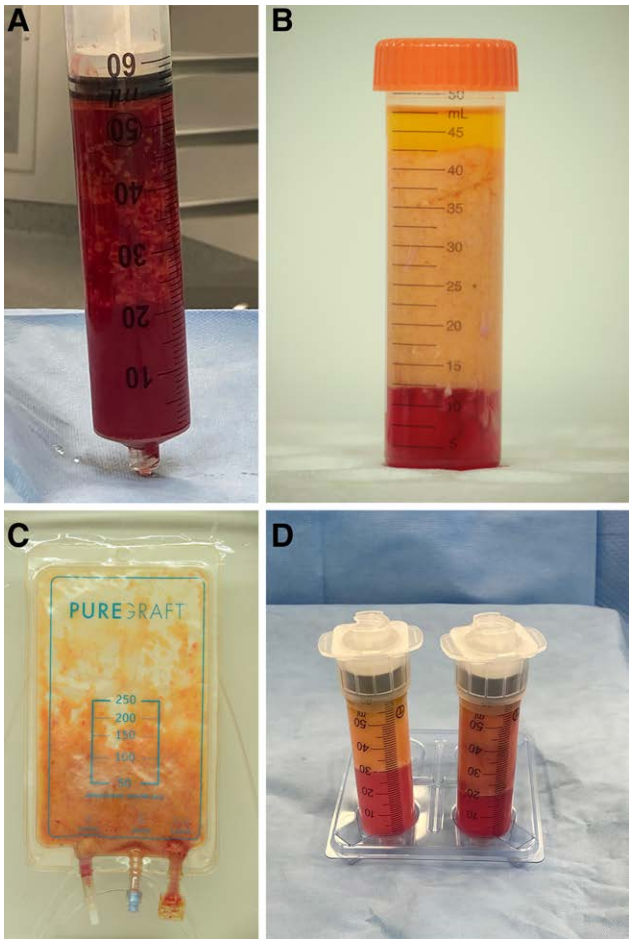

Fig. 1. Comparative and representative photographs of adipose tissue preparations with the different devices and techniques used. A, Decantation; B, Coleman, C, Puregraft, D, Macrofill.

MATERIAL AND METHODS

Patients

Lipoaspirates were obtained from five patients undergoing abdominal dermolipectomy associated with liposuction. Patients were infiltrated with a tumescent solution (0.9% NaCl saline solution, 2% adrenaline) before aspiration. Approximately 500 mL was collected manually per patient, with classical 50 mL syringes or 60 mL specific syringes (Macrofill kit), respecting a vacuum of less than 0.5 atm (plunger pulled 10 mL per 10 mL) using a 3.5-mm cannula with 20 holes of 2.5-mm diameter.

Adipose Tissue Processing

Between 50 and 150 mL of lipoaspirate was used for each condition. The 50 mL syringes were left to settle for 10 minutes to remove the infiltration mixture. Decantation protocol: 1×50 mL syringe was left to stand for a further 10 minutes before removing the liquid phase. Coleman method: 80 mL of fat tissue was transferred into 2×50 mL tubes and centrifuged for 3 minutes at 1200*g*. The oil supernatant and the lower liquid phase were discarded. Macrofill (2×60 mL manufacturer's specific syringes): after decantation for 5 minutes and removal of the lower liquid phase, fat tissue was washed by the addition of 15 mL of Ringer lactate for 35 mL of tissue and centrifuged quickly at 100*g* for 1 second (centrifugation was stopped as soon as 100*g* was

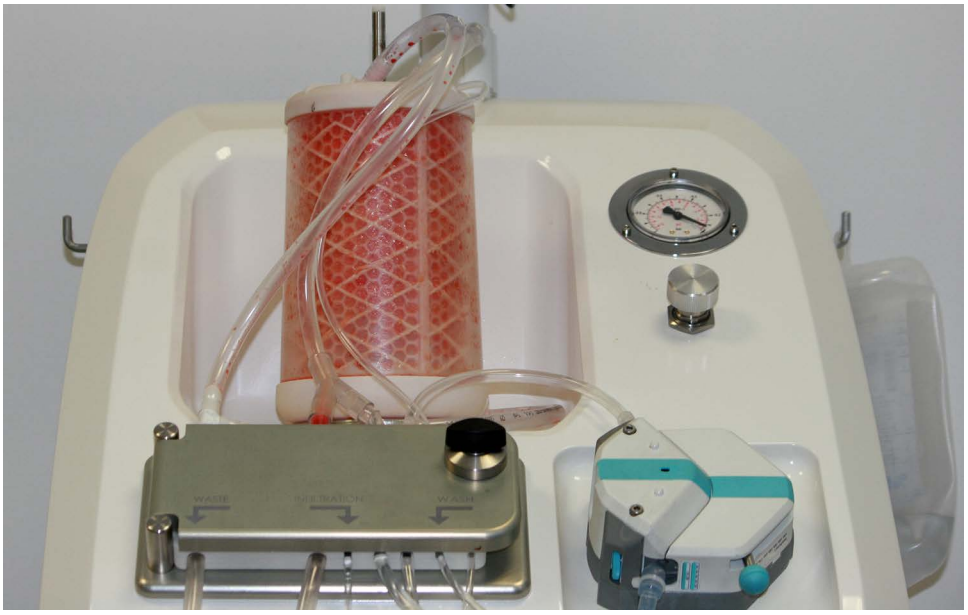

Fig. 2. AdiMate machine and Adipure single-use kit.

reached for gentle phase separation, according to manufacturer's purification protocol). This procedure was repeated twice. The final washing step was followed by centrifugation at 400*g* for 1 minute. At each step, the oil supernatant and the lower liquid phase were discarded. Puregraft: 150 mL of tissue was processed according to the manufacturer's protocol. Fat tissue was washed twice with 150 mL NaCl and allowed to settle for 5 minutes between the two washes and liquid removal. Adipure (automatic protocol): 150mL of adipose tissue were aspirated into the Adipure device, first centrifuged at 26*g* for 30 seconds (liquid removal), then mixed with 300 mL of NaCl under intermittent rotation for 1 minute 30 seconds (washing), followed by centrifugation at 26*g* for 30 seconds (liquid removal). This washing cycle was repeated twice with the last centrifugation at 26*g* for 1 minute 15 seconds (last step of liquid removal).

After processing, 50 mL of fat from each condition was transferred to 10 mL syringes, with 40 mL dedicated to in vitro assays, and 10 mL for mice injection.

In Vitro Experiments

Tissue Culture

Processed fat tissue from each protocol was cultured in 24-well microplates, and 0.3 mL of fat was distributed into each well and combined with 0.6 mL of DMEM (supplemented with 1% sodium pyruvate, 10% FBS, 1% Ultraglutamine and 1% penicillin/streptomycin). The plates were then incubated at 37°C, 5% CO_2 for 48h. After two days, 24 wells were harvested, pooled, and centrifuged at 400*g* for 2 minutes. The oil and tissue layers were weighed to assess their quantities.

In Vivo Experiments

Animals

The study was performed on 75 adult (7 weeks old) CB17 severe combined immunodeficient (SCID) female mice (Envigo, France). Animal care was provided by the CER Animal Facility (Marche-en-Famenne, Belgium). This study protocol was approved by the local ethical commission (PS-2022-NEO-001). Animals were acclimatized to the animal facility conditions for 1 week, maintained at 20–24°C and given a 12 hour/12 hour light/dark cycle. The experiments were realized with the adipose tissue from five patients, with 15 mice per patient (n = 6 injections per protocol per patient). Mice were kept under isoflurane during the entire surgical procedure. In each flank, a volume of 1 mL of human fat was injected using a 1.25-mm cannula connected to a preloaded 1-mL luer-lock syringe. The weight of each graft was accurately determined by weighing syringes before and after injection. After 4 weeks, each mouse was euthanized, and human fat grafts were separated from the skin, weighted with a precision scale, and fixed in 4% formaldehyde (weight/volume) for 48 hours for further histological analysis.

Histological Analysis

Fixed samples were dehydrated in alcohol baths and embedded in paraffin. Paraffin blocks were then sectioned with a microtome in 4-µm tissue sections (two levels, three slides per level) and stained with hematoxylin-eosin (HE) or with Masson trichrome. HE slides were used to evaluate oil vacuole presence, signs of inflammation, and homogeneity of adipocyte size and shape, whereas Masson trichrome was used for the evaluation of the fibrosis score in fat graft samples. Slides were scanned (S360 Hamamatsu, Japan), and area measurements and histological scoring were performed using the NDPview software (Hamamatsu, Japan). Histological scoring was adapted from previous studies.[22,27]

Statistical Analysis

All statistical analyses were conducted using the Prism (GraphPad, La Jolla-USA) software. Significance was

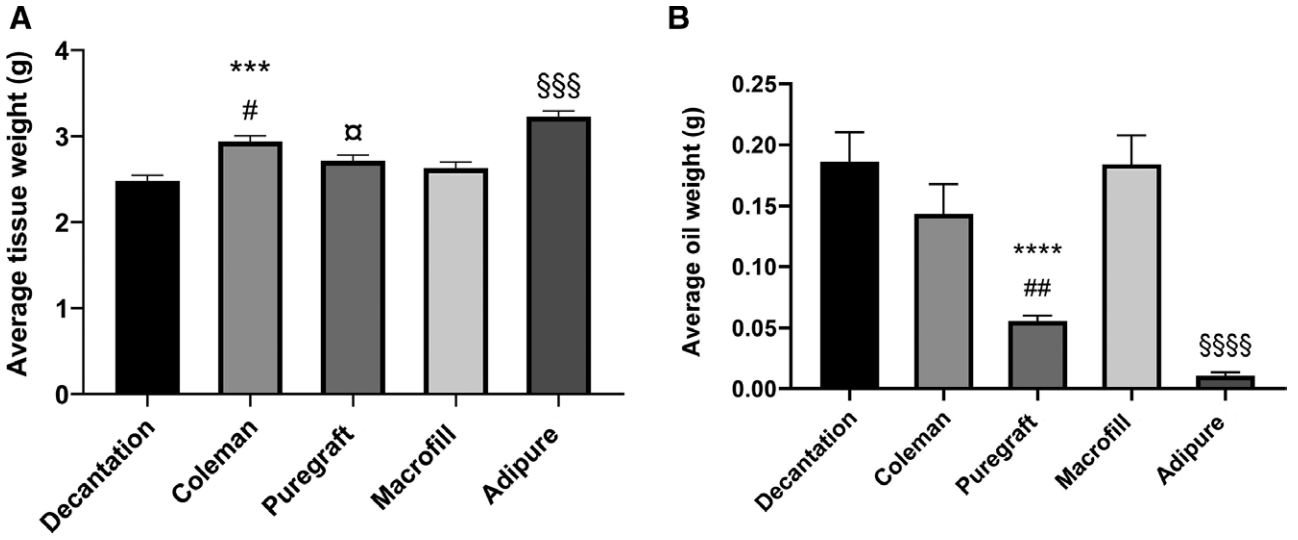

Fig. 3. In vitro evaluation of adipose tissue (A) and oil quantity (B) after purification with five different techniques (n = 5 patients). Graphs represent the mean of 25 plates of 24 wells (five plates per patient) per technique ± SEM. Purified samples were incubated in the presence of a culture medium for 48 hours. A, The adipose tissue phase was measured. §§§P < 0.005 compared with all conditions. ***P < 0.005 compared with decantation and Macrofill conditions. # P < 0.05 compared with Puregraft condition. ¤P < 0.05 compared with decantation. B, The oil supernatant was measured. §§§§P < 0.0001 compared with all conditions. ****P < 0.0001 compared with decantation and Macrofill conditions. ##P < 0.01 compared with Coleman's condition.

determined by an unpaired parametric t test for each condition, compared with each of the other conditions. A P value of less than or equal to 0.1 was considered significant.

RESULTS

In Vitro Analysis

Tissue Resorption in Culture

Lipoaspirates obtained from five donors and purified with the five different protocols were seeded in culture plates with a culture medium. After 2 days of culture, the remaining tissue phase was significantly higher with the Adipure and Coleman techniques (3.23 ± 0.19 g for Adipure and 2.93 ± 0.21 g for Coleman method) compared with decantation (2.48 ± 0.17 g), Puregraft (2.71 ± 0.2 g) or Macrofill (2.62 ± 0.21 g) (Fig. 3A). In addition, the amount of remaining tissue is significantly higher with Adipure compared with all other techniques, including the Coleman method.

Oil Formation in Culture

In addition to the adipose tissue phase, oil formation was also measured (Fig. 3B). In protocols using filtration, only a limited amount of oil was detected (0.055 ± 0.013 g for Puregraft and 0.01 ± 0.007 g for Adipure, corresponding respectively to 2% and 0.3% of tissue quantity), whereas higher quantities are measured with the other conditions (0.185 ± 0.077 g for decantation, 0.143 ± 0.078 g for Coleman method, and 0.185 ± 0.067 g for Macrofill, corresponding respectively to 7.45%, 4.9%, and 7% of tissue quantity). The amount of oil is significantly lower (close to zero) with Adipure compared with all other techniques.

In Vivo Analysis

Tissue Survival after 1 Month

After 1 month, grafts were harvested, and the remaining tissue weight was assessed (Fig. 4). Protocols using a filtration method, either passive (Puregraft) or active (Adipure with low-speed centrifugation), give the best results (0.87 ± 0.0155 g for Puregraft and 1.02 ± 0.058 g for Adipure) compared with other techniques (0.63 ± 0.064 g for decantation, 0.77 ± 0.065 g for Coleman method and 0.75 ± 0.048 g for Macrofill). The Adipure technique resulted in statistically higher remaining tissue size and weight (1.02 ± 0.058 g) than all other techniques.

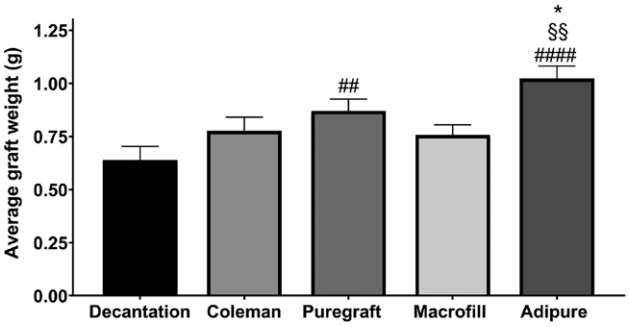

Fig. 4. In vivo evaluation of fat grafts 1 month after subcutaneous implantation. Fat grafts were performed in mice after purification with five different techniques (n = 5 patients, n = 6 injections per technique per patient). The entire graft was removed after 1 month and weighed. Results are expressed as mean ± SEM. *P < 0.1 compared with Puregraft condition. ##P < 0.01 and ####P < 0.0001 compared with decantation condition. §§P < 0.01 compared with Coleman and Macrofill conditions.

Table 1. Detailed Histological Scoring Grid of Adipose Tissue Grafts

Scoring Criteria	Oil Vacuoles		Signs of Fibrosis		Signs of Inflammation		Adipocyte Size and Shape		Maximal Score
Evaluation Score	<2%	5	Absent	3	Absent	3	Homogeneous	1	12
	2%–5%	4	Minimal	2	Minimal	2	Heterogeneous	0	
	5%–10%	3	Moderate	1	Moderate	1			
	10%–15%	2	Extensive	0	Extensive	0			
	15%–20%	1							
	>20%	0							

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

Histological Scoring at 1 Month

Harvested grafts were histologically analyzed for the presence of oil vacuoles, fibrous tissue, signs of inflammation, and adipocyte size and shape (Table 1). The lower the scores, the worse the condition of the adipose tissue (more oil cysts, fibrous tissue and signs of inflammation).

The results of global histological scoring are presented in Figure 5A. Protocols using a filtration method, either passive (Puregraft) or active (Adipure with low-speed centrifugation), give the highest scores (5.8 ± 0.41 and 6.14 ± 0.45 respectively), whereas decantation shows the lowest score (4.09 ± 0.5). Techniques using conventional centrifugation (strong, 1200*g* for the Coleman method or soft, 400*g* for Macrofill) give intermediate results (5 ± 0.38 and 5.17 ± 0.47 respectively).

Figure 5B shows the results of the specific histological score of oil vacuoles (maximum score 5), which is the main marker of adipose tissue integrity. In the same way as for the global score, but to a greater extent, protocols using a filtration method give the highest scores (3.09 ± 0.19 for Puregraft and 3.45 ± 0.11 for Adipure), whereas decantation still shows the lowest score (1.68 ± 0.3). Techniques using conventional centrifugation give intermediate results (2.47 ± 0.22 for Coleman and 2.42 ± 0.26 for Macrofill). The oil vacuole score is significantly higher with Puregraft and Adipure compared with all other techniques. Representative histological results for each condition are illustrated in Figure 6.

DISCUSSION

AFG has seen a strong revival of interest over the past decade, thanks to the multiple indications of this technique for breast reconstruction and augmentation. Unlike facial lipofilling, breast AFG requires the preparation of

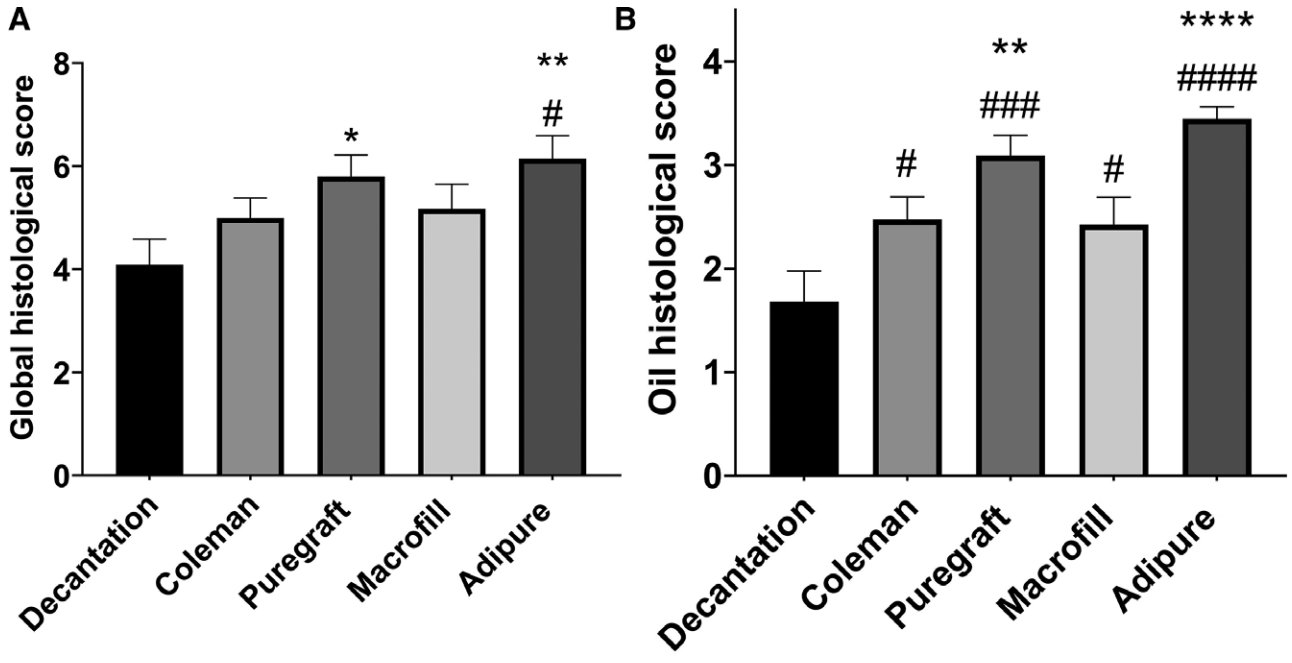

Fig. 5. In vivo evaluation of fat grafts 1 month after subcutaneous implantation. Fat grafts were performed in mice following purification with five different techniques (n = 5 patients, n = 6 injections per technique per patient). The entire graft was removed after 1 month. Histological scoring was performed on HE with Masson's trichrome-stained sections. Longitudinal sections were prepared from two different graft depths. A, Global scoring includes oil vacuole, inflammation, fibrosis and adipocyte size and shape. *$P < 0.05$ and **$P < 0.01$ compared with decantation condition. #$P < 0.1$ compared with Coleman condition. Results are expressed as mean ± SEM. B, Oil vacuoles specific scoring. **$P < 0.05$ and ****$P < 0.001$ compared with Macrofill and Coleman conditions. #$P < 0.1$; ###$P < 0.001$ and ####$P < 0.0001$ compared with decantation condition.

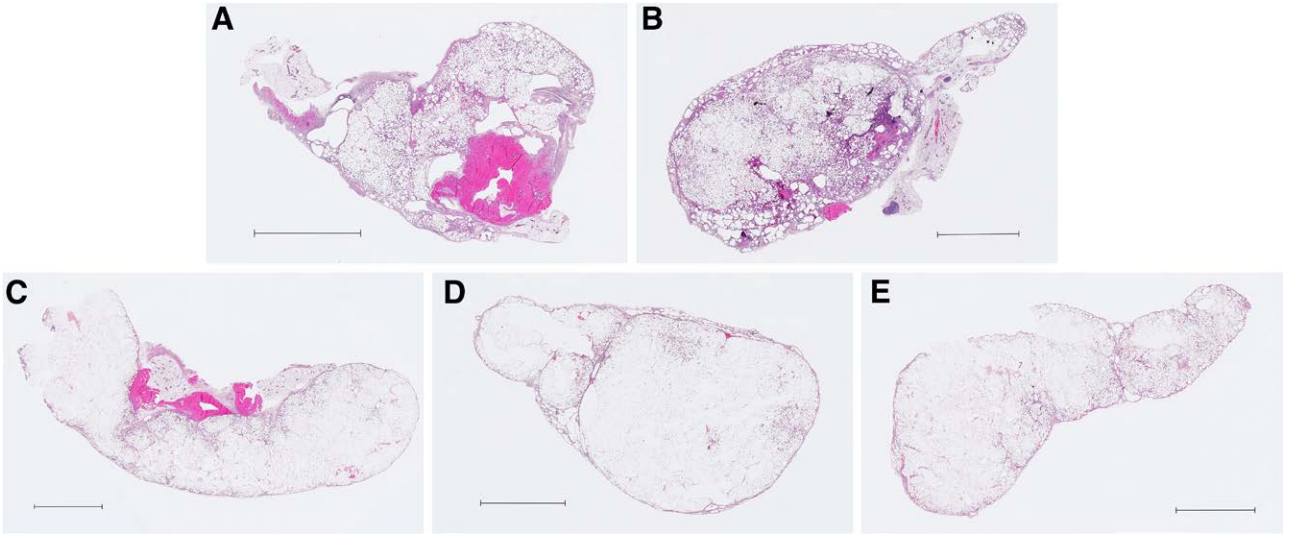

Fig. 6. Histological results of grafts recovered 1 month after injection according to the different techniques. Representative histological images of HE-stained sections from (A) decantation technique, (B) Coleman technique, (C) Puregraft protocol, (D) Macrofill protocol, and (E) Adipure protocol. The scale bar represents 5mm.

much larger quantities of adipose tissue, thus becoming a real challenge for the operating team if considering time and sterility constraints. In fact, several medical devices have appeared on the market in recent years; however, even if the protocols are evolving favorably by adding washes that have shown their effectiveness,[15,26,27] AFG for large volumes still comes up against two major problems: for patients, tissue resorption after injection and, for the operating team, time and aseptic conditions for fat preparation. Indeed, the handling of adipose tissue remains mainly manual, while in vitro/in vivo studies struggle to show a clear superiority of one device or technique over the other, with some showing an advantage to centrifugation and washing,[22,27] some showing better results with filtration and washing,[30] and others showing no significant differences in vivo[26] or at the clinical level,[20,30,31] between centrifugation and active or passive filtration.

Based on this statement, we wished to develop a new device dedicated to the AFG technique, which would optimize both graft efficiency and preparation time in the operating room. This device is composed of an automaton that allows the surgeon to perform liposuction and infiltration, as well as a single-use kit allowing adipose tissue treatment to be performed in an automated way. The tissue preparation technique consists of filtration combined with 26*g* centrifugation and integrates two automatically performed washes.

In this study, we compared the quality of the fat obtained with this new device to that obtained with other devices on the market, using centrifugation or filtration, as well as the classical and widespread techniques (Coleman and decantation). We have chosen an in vivo approach to obtain indications of the potential clinical effectiveness. So, even if it obviously cannot compete with a clinical study in humans, the xenograft model in immunodeficient mice is an excellent model because it allows for precise histological quantification of the success and quality of the graft.[22,26,27]

In addition, adipose tissue was harvested in an identical way between all the techniques (manually with a syringe, with the same cannula), while respecting a low depression (lower than 0.5 atm), without using local anesthetics in the infiltration solution (general anesthesia) to limit cell death during harvesting and later.[32,33]

Firstly, the results we obtained allow us to confirm results from several previous studies, namely that simple decantation of the tissue leads to inferior results to all other techniques tested.[24,27,30,34,35] Indeed, in this technique, the liquid represents up to 30% of the volume,[17] which may explain the poor grafting efficiency of about 60% (Fig. 4). In addition, we also note overall poor histological results (4 out 12, Fig. 5A) as well as an important number of oily vacuoles within the tissue (1.68 out 4, Figs. 5B and 6). In fact, even though it is fast and inexpensive, decantation should probably be considered the least effective technique for purifying adipose tissue and should be avoided.

Secondly, our results also confirm that the centrifugation technique, at high speed without washing (1200*g*, Coleman) or medium speed with washing (400*g*, Macrofill), gives globally similar results in vivo, whether for grafting efficiency (respectively, 0.77 g and 0.75 g of graft weight, Fig. 4), global histological score (respectively, 5 ± 0. 38 and 5.17 ± 0.47 out 12, Fig. 5A), or specific score concerning oil quantity (respectively, 2.47 ± 0.22 and 2.42 ± 0.26 out 4, Fig. 5B). Results of Macrofill in mice (75% graft efficiency) are close to those obtained clinically for breast reconstructions in a study dedicated to this device[36] where authors obtained a graft survival rate of 70%.

Surprisingly, in vitro, results seem to favor the Coleman method over the Macrofill device, whereas the Macrofill purification conditions are rather in favor of adipose tissue preservation (lower speed + washes). However, the difference is only significant for the tissue quantity, and with a rather small difference between the two techniques

(2.93 ± 0.21 for the Coleman method compared with 2.62 ± 0.21 g for Macrofill). In any case, this confirms that a reliable comparative study cannot be limited to in vitro experiments and that a living model provides a lot of important data that can be transposed to the clinic more reliably.

Finally, our study corroborates other works showing the interest of preserving integrity and cell viability of adipose tissue, by using a filtration technique rather than a classical centrifugation.[15,30] Indeed, and even if protocols are based on very different technologies, results observed with Puregraft and Adipure, which both use filtration with two washes, are superior to the other techniques. This is globally reflected both in vitro (oil quantity) and in vivo on graft efficiency (weight) and quality (histology). The fact that Puregraft seems to give inferior results to the Coleman technique in vitro (Fig. 3A) can probably be explained by the amount of liquid still present in the tissue after the Puregraft protocol, measured at an average range of 20% to 25% (data not shown), because of the passive filtration. On the other hand, it is possible that in vivo, Puregraft has an advantage over the Coleman technique because the tissue is less damaged by filtration than centrifugation (Fig. 4).

However, between the two filtration techniques, Adipure gives significantly better results than Puregraft, both in vitro and in vivo on graft efficiency (graft weight, Fig. 4), with a remarkable efficiency of 100% after one month. This superiority can again be explained by the amount of liquid present in the tissue after purification, measured at an average range of 5%–10% after the Adipure protocol (data not shown), ie, approximately 15% less than with Puregraft, and this finally has a beneficial impact on graft efficiency (Fig. 4). The histological studies confirm this hypothesis because no significant difference is observed in the sections between the two methods, which confirms the interest of filtration for the preservation of fat cell integrity (Fig. 5 and Fig. 6C and 6E).

CONCLUSIONS

Overall, this study confirms both in vitro and in a proven animal model that AFG efficacy is highly dependent on used protocols, as well as the superiority of adipose tissue preparation techniques involving filtration and washing rather than centrifugation or decantation. The Adipure device, which uses active filtration by rotation, provides results superior to those of conventional methods and the Puregraft and Macrofill devices. If these encouraging graft efficacy results are confirmed by medium- and long-term clinical studies, a preparation time of less than 10 minutes combined with a fully automated protocol will make this system highly attractive for the operating room.

Régis Roche, PhD
260 Rue René Descartes, 13290
Aix en Provence, France
E-mail: r.roche@neosyad.com

DISCLOSURE

Régis Roche and Séverine Licciardi are employees of Neosyad. Régis Roche holds stocks in Neosyad. Xavier Nelissen holds stocks in Neosyad. Drs. Nizet and Delay have no financial interest to declare in relation to the content of this article. This study was funded by the Neosyad Company.

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