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REVIEW

Towards more rationalized approach to autologous fat grafting[☆]

Lee L.Q. Pu*

Division of Plastic Surgery, University of California at Davis, 2221 Stockton Boulevard, Suite 2123, Sacramento, CA 95817, USA

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Summary *Background:* Even though fat grafting has become widely used by plastic surgeons, most surgeons choose their method of fat grafting based almost entirely on anecdotal evidence. As more and more scientific studies appear in the literature, we now may have more objective, scientific evidence to support the use of specific techniques.

Methods: The author critically reviewed all studies related to fat grafting research in the last 20 years in the English literature. To better summarize the findings of the studies, the fat grafting procedure was arbitrarily divided into 4 essential parts to determine the best donor sites and how the fat grafts should be harvested, processed, and placed.

Results: While most studies show no difference in the viability of fat grafts harvested from different donor sites, one study shows that more adipose-derived stem cells (ADSCs) are found within the fat grafts harvested from the lower abdomen or inner thigh. Fat grafts harvested with syringe aspiration and processed with centrifugation at 1200 g for 3 min clearly have better viability. In addition, viable adipocytes and ADSCs as well as growth factors can be concentrated within the fat grafts after proper centrifugation. The studies also reinforce the principle that fat grafts, once placed, should have a maximal amount of contact with the vascularized tissue in the recipient site for better survival.

Conclusions: Most scientific studies in fat grafting research support a more rationalized technique that should be selected by surgeons for fat grafting. The technique includes proper selection of donor sites, proper harvest and process of fat grafts, and proper placement of fat grafts.

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* Tel.: +1 916 734 2130; fax: +1 916 734 7104.

E-mail address: lee.pu@ucdmc.ucdavis.edu.

Introduction

Autologous fat grafting has long been considered as a valid option for soft-tissue augmentation in plastic surgery.^{1,2} However, whether fat grafting can be a valid option has always been somewhat controversial among plastic surgeons and its long-term result is still considered poor or unpredictable because the final outcome can largely be the surgeon-dependant.^{3,4} Variable outcomes after fat grafting may probably be due to lack of a "standardized" surgical technique that was not sufficiently studied in the past.⁵

Despite a growing interest and enthusiasm about autologous fat grafting in plastic surgery community worldwide, there is no consensus and agreement on what is the best technique to perform fat grafting.^{6,7} Many techniques of fat grafting have been described in the literature.^{8–15} Unfortunately, many of those techniques are not supported by well-conducted scientific studies. In addition, the assessment of the final outcome after fat grafting has been criticized by lack of quantitative evidence of fat graft survivability and predictability of volume restoration.⁷

More recently, one particular technique of fat grafting has been popularized and known to many surgeons. This technique emphasizes on atraumatic method of fat harvesting, proper centrifugation, and injection aimed at maximizing nutrition and structural integrity at the recipient site.⁸ However, whether recent scientific studies have supported the use of this technique over any other technique has not been clearly presented. In order to help surgeons to gain more insight to fat grafting technique and understand why fat grafting should be performed in a certain way, the author conducted this review of the current objective literature on fat grafting research. To better summarize the findings of the studies, the basic fat grafting procedure was arbitrarily divided into 4 parts: donor site selection, harvesting, processing and placement. We hope that the surgeon will use the information presented in this review article to choose a scientifically sound approach to fat grafting based on objective findings rather than anecdotal reports.

Basic fat grafting technique

Donor site selection

It has been a common practice that the donor site of fat grafting is usually selected by the surgeon based on his or her preference or the "desired" areas chosen by the patient. The question of which is the best donor site to harvest fat grafts remains unclear as most of the previous studies were not conducted scientifically to adequately address this question.³ However, recent studies may help us find a more optimal donor site.

Rohrich et al examined adipocyte viability from 4 commonly used donor sites (Abdomen, thigh, flank, and knee) in 5 patients. They found no statistical differences in adipocyte viability of fat grafts among these donor sites based on an *in vitro* colorimetric assay of cell proliferation.¹⁶ In an *in vivo* study, Ullmann et al examined 3 donor areas (Breast, abdomen, and thigh) of a female patient. After

centrifugation, 1 cc of fat grafts was injected subcutaneously into the scalp of the nude mouse. They did not find any significant differences among 3 investigated groups in terms of weight, volume, and histology of "survived" fat grafts at the end of the study.¹⁷ Therefore, there is no evidence of a favorable donor site for harvest of fat grafts. According to these two studies, the viability of lipocytes within the fat grafts from different donor sites may be considered equal.

Adipose tissue has recently been identified as a source of processed lipoaspirate cells or adipose-derived stem cells (ADSCs).¹⁸ Padoin et al evaluated the cell concentration of processed lipoaspirate cells in 6 commonly used donor sites for fat grafting (Upper abdomen, lower abdomen, trochanteric region, inner thigh, knee, and flank) in 25 women. Based on this well-conducted study, they conclude that both lower abdomen and inner thigh have higher concentrations of processed lipoaspirate cells and these areas may be the better donor sites of adult adipose-derived stem cells.¹⁹

Adipose tissue may contribute in many ways to the optimal outcome of soft-tissue replacement. For example, it not only serves as filler but also improves the quality of aged and scarred skins. In addition, it may have the potential to heal radiation induced chronic ulcers.^{20,21} An amazing experimental study in animals demonstrates that freshly isolated ADSCs when mixed with donor fat grafts can improve the longevity and the volume of these fat grafts.²² This work, although still in a preliminary stage, suggests one potential mechanism by which ADSCs may be able to improve the blood supply at the fat graft recipient sites. Another clinical study has also demonstrated that an amazing result after fat grafting to the breasts together with injection of freshly isolated ADSCs.¹² Although above studies still need to be validated by further studies, it has become clear that ADSCs may play a significant role in autologous fat grafting.

With what we know about the potential role of ADSCs in autologous fat grafting, the lower abdomen and inner thighs should, therefore, be chosen as the better donor sites for fat transplantation. These donor sites are not only easily accessible by the surgeons with a patient in the supine position, but also scientifically sound because they have higher concentration of stem cells than other donor sites as long as patients have adequate amount of adipose tissue in those areas.²³

Method of harvest

The optimal method for harvest of fat grafts remains controversial. Some surgeons still routinely harvest fat grafts with conventional liposuction. Many studies assess the viability of fat grafts harvested with different techniques. Unfortunately, several frequently referenced studies have made their conclusions based on a single measurement selected by the investigators. For example, Smith et al evaluated fat grafts harvested with either standard liposuction or syringe aspiration. They conclude that there is no significant difference in adipocyte viability of fat grafts harvested with standard liposuction compared with syringe aspiration using only weights and the colorimetric assay of cell proliferation.²⁴ Shiffman and Mirrafati studied histology of adipocytes in the fat grafts harvested

from different settings of suction force. Based on only histologic examination, they conclude that adipocytes become damaged and disrupted only when as high as -700 mmHg vacuum is used for collection of lipoaspirates.²⁵ However, Crawford et al reported that less traumatic harvest of fat grafts with a syringe yields significantly higher viable adipocyte counts compared with the grafts harvested with standard power-assisted liposuction based only on Trypan blue staining.²⁶

However, several more comprehensive studies consistently support the superiority of atraumatic technique for harvest of fat grafts than conventional liposuction. Pu et al examined the potential role of adipose aspirates collected from conventional liposuction as a source for autologous fat grafting. In this study, the viability of adipose aspirates was evaluated by viable cell count, glycerol-3-phosphate dehydrogenase assay, and histology. They conclude that although adipose aspirates collected from conventional liposuction maintain normal structure with near the same number of viable adipocytes compared with fresh fatty tissues, but they have a less-than-optimal level of cellular function and therefore may not survive well after they are transplanted.²⁷

Another comprehensive study was conducted by Pu et al to determine the viability of fat grafts harvested and refined with the Coleman technique compared with a common technique (conventional liposuction and low force centrifugation) proposed by Boschert et al.²⁸ In this study, the viability of fat grafts was evaluated again by viable cell count, glycerol-3-phosphate dehydrogenase assay, and histology. They conclude that although fat grafts harvested and refined by both techniques maintain normal histological structure, the Coleman technique yields a great number of viable adipocytes and sustain a more optimal level of cellular function within fat grafts and should be considered superior to the conventional liposuction as a preferred method of choice for fat graft harvesting.²⁹

What is the proper size of cannula and syringe used for syringe aspiration to harvest fat grafts also remains controversial. Erdim et al conclude that the use of larger cannulas for syringe aspiration appears to provide more viable adipocytes of the fat grafts based on the viable cell counts.³⁰ However, a comprehensive study (Viable cell count, a cell proliferation assay, an enzyme assay, and Oil Red O stain) conducted by Gonzalez et al conclude that the viability of fat grafts is significantly better when fat graft is harvested by 2 mm diameter cannula with a blunt tip and several side holes connected to a 10cc syringe as compared to a 3 mm diameter blunt tipped cannula connected to a 60cc syringe. They concluded that the larger size of cannula for syringe aspiration may not have very much advantage and the use of smaller syringes (10cc) is advisable to maintain a minimal negative pressure during harvesting.³¹

Overall, the syringe aspiration as a relatively less traumatic method to harvest fat grafts is supported by the recent more comprehensive studies and should be considered as a standardized technique of choice for harvest of fat grafts. (Figure 1) However, this technique can be time-consuming even for experienced surgeons and the large quantity of fat grafts may not easily be obtained with this technique. Several manufacturers have attempted to develop an "ideal" device that combines fat harvest, process, and transfer. Such a device

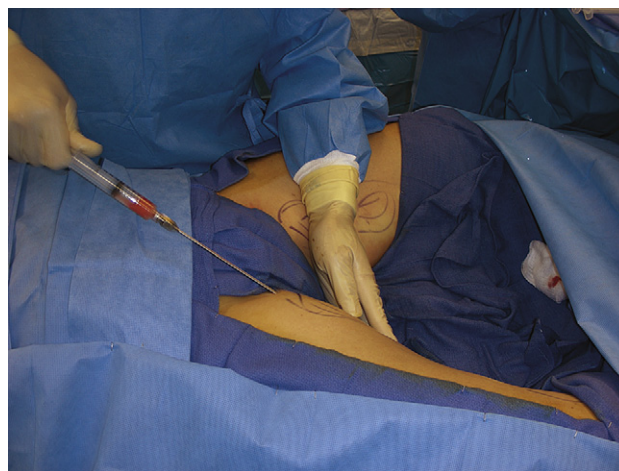


Figure 1 Syringe aspiration, as a relatively atraumatic technique, is a better technique and should be used for harvest of fat grafts.

may potentially become a "preferred" method of choice by some surgeons for more extensive fat grafting procedures that require a large-quantity of fat grafts or for less-experienced surgeons who desire to harvest fat grafts with more predictable viability. Unfortunately, only a few such devices have been studied comprehensively for their reliability.³²

Method of process

Most surgeons believe that fat grafts harvested with syringe aspiration or conventional liposuction need to be processed in some way in order to limit the blood or oil within the lipoaspirates so that only pure fat as a soft-tissue filler will be used for injection. However, this has become a highly controversial issue and currently there is no agreement among surgeons in terms of which is the best method for processing fat grafts. Three primary methods (Sedimentation by gravity, filtering technique, and centrifugation) have been used clinically to process fat grafts. Many experimental studies designed to compare these 3 refinement techniques were evaluated only by a single measurement selected by the investigators and thus which method is better still remains debatable.

Boschert et al evaluated the viability of adipocytes after liposuction when fat grafts were processed with centrifugation. The total number of viable adipocytes was counted under microscopy after trypan blue staining. They found that centrifugation at 50 g for 2 min separates fat, lipids, and blood cells well with more viable adipocytes being found at the deepest layer of the fat portion, which is the middle portion after centrifugation while oil is in the upper portion and blood cells in the lower portion.²⁸ The effect of centrifugation with different forces (1000–4000 rpm) on viability of fat grafts was evaluated further by Xie et al with the glucose transport test, a colorimetric assay of cell proliferation, and histology. They found that there was a linear reduction of viability of fat grafts with the increase in centrifugal force. Histologically, significantly distorted and fractured adipocytes were seen when the centrifugal speed reached 4000 rpm.³³ The optimal speed and duration of centrifugation for processing fat grafts was determined by

Kim et al. They found that adipocyte survival rates, evaluated by trypan blue staining, were significantly lower when fat grafts were centrifuged at 1500 and 3000 rpm for more than 5 min or centrifuged at 5000 rpm for more than 1 min. In addition, the ruptured cell membranes, fusion of cells, and irregular cell shape were identified when fat grafts were centrifuged at 5000 rpm for 5 min. Therefore, they conclude that centrifugation with 3000 rpm for 3 min is optimal and should be recommended for processing fat grafts.³⁴

Rammon et al studied an open method using cotton towel as a platform for concentrating the lipoaspirates and separating them from fluids, oil, and debris. They compared this method with centrifugation in an animal study. No significant differences were found regarding fat graft weight and volume between the two methods. However, the histologic study revealed significantly less fibrosis in the animals when the fat grafts were processed by their cotton towel method.¹¹ Minn et al compared viability of fat grafts via a cell proliferation assay and rate of fat graft survival in animals when fat grafts were processed with centrifugation or cotton gauze. They found there are no significant differences of the viability of fat grafts or the rate of fat graft survival if fat grafts were processed with either technique.³⁵ However, what these authors did is not necessarily clinically applicable since fat grafts are administered in a bolus but not in small amounts with multiple passes in their studies. Conde-Green et al compared the content of adipocytes and mesenchymal stem cells of fat grafts processed by decantation or centrifugation. Although there are significantly more viable adipocytes in the decanted group, the fat grafts processed with sedimentation still contain a great quantity of contaminating blood cells and fewer stem cells. Centrifugation, although may be more aggressive on adipocytes, clears the fat from most blood remnants and is able to possibly maintain the highest concentration of stem cells within the processed lipoaspirates.³⁶

A more comprehensive study was conducted by Yoshimura's group in Japan and reported in the journal recently. In this study, liposuction aspirates were either not centrifuged or centrifuged at 400, 700, 1200, 3000, or 4200 g for 3 min. They found that centrifugation concentrates adipose tissues and ADSCs in the adipose portion. Centrifugation enhances fat graft take per 1 cc centrifuged adipose tissue. However, centrifugation at more than 3000 g significantly damages ADSCs. They conclude that excess centrifugation can destroy adipocytes and ADSCs, but appropriate centrifugation concentrates these cells, resulting in enhanced fat graft take. They recommend 1200 g as an optimal centrifugal force for processing fat grafts.³⁷ Interestingly, such a centrifugal force is close to the 1286 g generated by the centrifuge from the Coleman's instrument set if one would use this centrifuge to process fat graft.

The benefits of centrifugation for processing fat grafts were also studied by others. Pallua et al studied the content of growth factors in the presence of the various fractions of liposuction aspirates after centrifugation. They found significant quantities of angiogenic growth factors, such as basic fibroblast growth factor, vascular endothelial growth factor, are left in the middle portion of the tube where the adipose tissues are concentrated since centrifugation according to Coleman's protocol effectively separates those

growth factors from oil or blood.³⁸ In addition, centrifugation creates a unique fraction of adipose tissue and "high" density of fat grafts may survive better than "low" density ones based on a recent study.³⁹

Overall, fat grafts should be processed by either centrifugation or cotton towel technique. However, proper centrifugation would concentrate not only adipocytes and ADSCs but also several angiogenic growth factors within the processed fat grafts. Since stem cell or angiogenic growth factor may play a role in fat graft survival, centrifugation at 3000 rpm (about 1289 g) for 3 min appears to offer more benefits and should be a better method of choice for processing fat grafts (Figure 2).

Method of placement

How fat grafts are placed into the recipient site can be one of the most important steps in fat grafting.^{8,15} However, there is no standardized technique on how fat grafts should be placed for soft-tissue augmentation although most surgeons believe a single bolus injection with large amount of fat would lead poor outcome or even significant complications such as fat necrosis, blindness or strokes.^{40,41} An early study by Carpaneda and Ribeiro demonstrate that the ability of fat grafts to obtain nutrition through plasmatic imbibition occurs approximately 1.5 ± 0.5 mm from the edge of the vascularized tissue and only 40% of fat grafts is viable from the graft edges at 60 days.⁴² The authors further demonstrate, in a subsequent study, that the percentage of fat graft viability depends on the thickness and geometric shape of the graft in the recipient bed. The percentage of graft survival gradually decreases as the injected volume surpasses the total diameter of the graft.⁴³ Another study by Nishimura et al shows fat grafts become vascularized around day 7 after transplantation. The study also confirms that angiogenic factors, such as vascular endothelial growth factor, are responsible for revascularizing fat grafts in the recipient site.⁴⁴

The placement of fat grafts in different tissue planes was also studied by Karacaoglu et al in a rabbit face model.

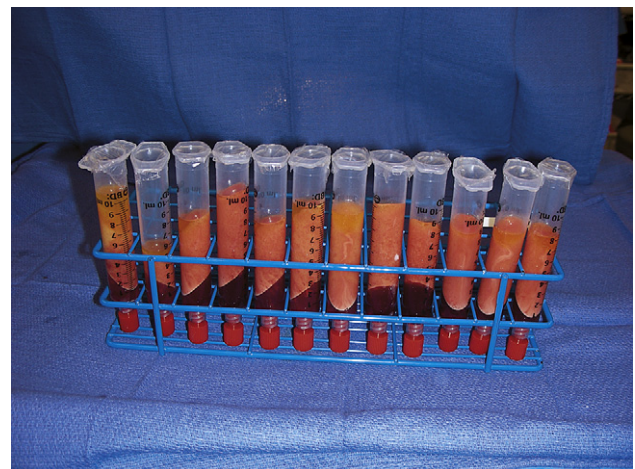


Figure 2 Fat grafts are processed after centrifugation at about 1200 g for 3 min. The upper (oil) portion and lower (red blood cells) portion should all be discarded. Only the middle portion with more condensed adipose tissues should be used.

By measuring transplanted fat grafts morphometrically and histologically, the results reveal the survival of fat grafts is significantly higher if they are placed in supramuscular layer than in subcutaneous or submuscular layer. The findings of the study support the placement of fat grafts in different tissue planes to achieve better result clinically.⁴⁵

In a well-conducted study, one fat grafting technique was evaluated in a murine model that was specifically designed to study the fate of injected lipoaspirates. Fat grafts were infiltrated using a blunt-tip cannula through a 2 mm incision over the dorsum of the animal. The infiltration cannula was advanced and withdrawn in a fan-like pattern and the fat grafts were placed in an even layer over the entire dorsal surface. The grafts were injected only as the cannula was withdrawn, in small volumes of approximately 1/30 ml per withdrawal. In this study, Thanik et al, for the first time, demonstrate fat grafts could persistently survive well in a high level (82%) with minimal inflammatory reaction and also confirm the efficacy of one established technique for placement of fat grafts that has been used clinically by many surgeons. The "survived" fat grafts indeed appear to be viable and well-vascularized.⁴⁶

Overall, the above well-conducted experimental study has shown convincing evidence that fat grafts are taken in a high percentage when they are placed with above described technique. This technique emphasizes to place fat grafts in a small amount with each pass as the cannula is withdrawn but to place them with multiple passes in multiple tunnels and at multiple tissue levels so that fat grafts have a maximal amount of contact with the vascularized tissue in the grafted area for better survival. (Figure 3).

Other considerations

Influence of local anesthetic on adipose tissue

There have been concerns about the effect of local anesthetics such as lidocaine on the viability of fat grafts

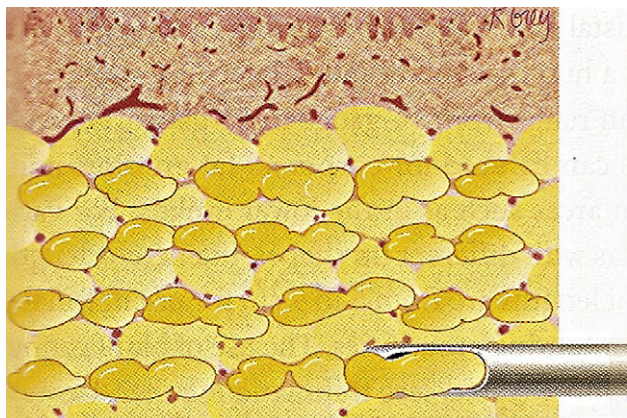


Figure 3 Schematic diagram shows a proper technique of fat injection. Placement of minuscule amounts of fat grafts with each pass as the cannula is withdrawn and in multiple tissue planes and tunnels after multiple passes are critical to successful fat grafting (Reprinted with permission from Coleman SR and Mazzola RF. Fat Grafting: From Filling to Regeneration. St. Louis: Quality Medical Publishing, 2009).

harvested by any kind of liposuction since such an agent is routinely used for analgesia of the donor site. Such a concern is based on an *in vitro* study reported by Moore et al that lidocaine may inhibit a variety of adipocyte functions in tissue culture. However, the effect is found to be totally reversible once the agent has been washed out.⁴⁷ The viability and differentiation of preadipocytes are also found to be impaired but only after being isolated from fat tissue and then exposed directly to a higher concentration of lidocaine (2%) *in vitro* for 30 min. The effect is thought to be independent of lipophilic properties and the resulted *in vivo* concentration of lidocaine may be different due to dilution effects.⁴⁸ However, Shoshani et al demonstrate, in an animal study, that local anesthetic solution, consisted of lidocaine (0.06%) and epinephrine (1:1000000) does not alter the take of fat grafts based on an *in vivo* volume and histological study.⁴⁹ Therefore, a commonly used tumescent solution with low concentration of lidocaine (0.05% or less) and only a short exposure to adipose tissue (less than 20 min) can be used for analgesia of the fat graft donor site without very much harmful effects to adipocytes or preadipocytes.

Necessity for overcorrection

Whether overcorrection would be necessary for fat grafting remain unclear. Since the viable fat grafts are only observed in the peripheral zone approximately 1.5 mm from the edge of the grafts and the percentage of graft viability depends on its thickness and geometrical shape,^{42,43} overcorrection for "better" graft survival in the recipient site appears to be lack of scientific support. In addition, significant overcorrection may increase the incidence of fat necrosis and subsequent calcification or even severe infection.^{13,40} Therefore, significant overcorrection should be avoided at the present time until its necessity and safety can be confirmed by future studies.

Timing for subsequent injection

Since overall take rate of fat grafting by even more experienced surgeons ranges from about 50 to 90 percent,⁸⁻¹⁵ additional procedures are always necessary to achieve an optimal result. However, there is no scientific study which has addressed the timing of subsequent fat grafting. So far, only "expert" opinion has been mentioned in the literature regarding this specific issue. It has been described as "the timing of additional fat grafting sessions should be deferred until 6 months postoperatively to diminish the inflammatory response".¹³

Conclusion

Much of the current scientific studies support one rationalized approach to autologous fat grafting. Beside the proper selection of donor sites (i.e. the lower abdomen or inner thigh), fat grafts should be harvested with a less traumatic method such as syringe aspiration and then processed with proper centrifugation (at about 1200 g for 3 min). Fat grafts should be placed in a small amount each pass as the cannula is withdrawn but with multiple passes in multiple tunnels and

multiple tissue levels. In addition, solutions with low lidocaine concentration should be used for infiltration of the donor site and significant overcorrection should be avoided to minimize complications such as fat necrosis. However, the optimal timing for subsequent injection and necessity and safety of overcorrection in the receipt site still need to be determined by future studies. Other factors such as pre-operative expansion or post-operative care may also affect the clinical outcome after fat grafting. As evidence-based medicine evolving in our specialty, a future prospective randomized controlled clinical trial with objective measurements should be conducted to confirm the efficacy of fat grafting as an effective means for soft-tissue augmentation in plastic surgery.

Ethical approval

Not required.

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Conflicts of interest

None declared.

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