34 Cell-Assisted Lipotransfer for Breast Augmentation: Grafting of Progenitor-Enriched Fat Tissue

Kotaro Yoshimura, Katsujiro Sato, and Daisuke Matsumoto

34.1 Introduction

Autologous fat transplantation is a promising cosmetic treatment for facial rejuvenation and soft-tissue augmentation because of the lack of an incision scar and complications associated with foreign materials. However, certain problems remain, including unpredictable outcomes and a low rate of graft survival due to partial necrosis. Autologous fat transplantation has been used for breast augmentation by only a limited number of plastic surgeons (1). This procedure is controversial because of the lack of consensus on whether it is safe and appropriate due to associated microcalcifications that might cause confusion during the evaluation of mammograms. Recently, autologous fat injection has been re-evaluated as a potential alternative to artificial implants for breast augmentation (1–7). This re-evaluation may reflect recent advances in autologous fat transfer and the radiological detection of breast cancer.

A novel approach to autologous fat grafting called cell-assisted lipotransfer (CAL) is the current preferred method by the authors for transplantation of aspirated fat tissue and adipose progenitor cells or adipose-derived stem/stromal cells (ASCs), which consists of the grafting of progenitor-enriched fat tissue (Fig. 34.1) (8). The therapeutic strategy is based on the observation that aspirated fat is vessel-poor and adipose progenitor cell-poor as compared to intact whole fat (9).

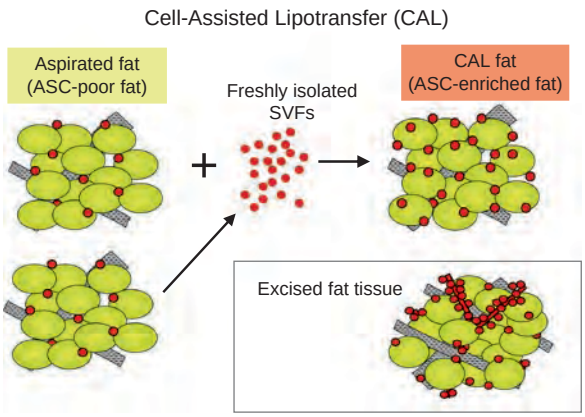

Fig. 34.1 Scheme of cell-assisted lipotransfer (CAL). Relatively adipose-derived stromal/stem cells (ASC)-poor aspirated fat tissue is converted to ASC-rich fat tissue by supplementation with ASCs isolated from the other half of the aspirated fat sample. The ASCs are attached to the aspirated fat, which is used as a scaffold in this strategy (8, 19)

34.2 Adipose Tissue-Specific Progenitors with Multipotency

It has been shown that fibroblast-like stromal cells obtained from liposuction aspirates can differentiate into various cell lineages (10, 11) such as adipogenic, osteogenic, chondrogenic, myogenic, cardiomyogenic, and neurogenic. Thus, the adipose tissue-specific progenitor cells are now called "Adipose-derived stem/pogenitor cells (ASCs)" and are expected to become valuable tools in a wide range of cell-based therapies (12) (Fig. 34.2). Adipose tissue is known to be rich in microvasculature (10), and ASCs were shown to have angiogenic characteristics and to experimentally differentiate into vascular endothelial cells (9, 14, 15).

K. Yoshimura (✉)
Department of Plastic Surgery, University of Tokyo School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: yoshimura-pla@h.u-tokyo.ac.jp

M. A. Shiffman (Ed.), *Autologous Fat Transfer*
DOI: 10.1007/978-3-642-00473-5_34, © Springer-Verlag Berlin Heidelberg 2010

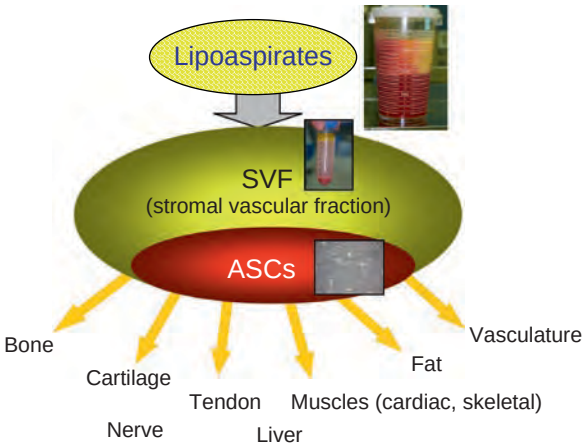

Fig. 34.2 The stromal vascular fraction (SVF) can be obtained from adipose and fluid portions of liposuction aspirates through collagenase digestion. SVF contains 10–35% ASCs, some of which are multipotent and have been shown to differentiate into several lineages in vitro. SVF also contains blood-derived cells such as leukocytes

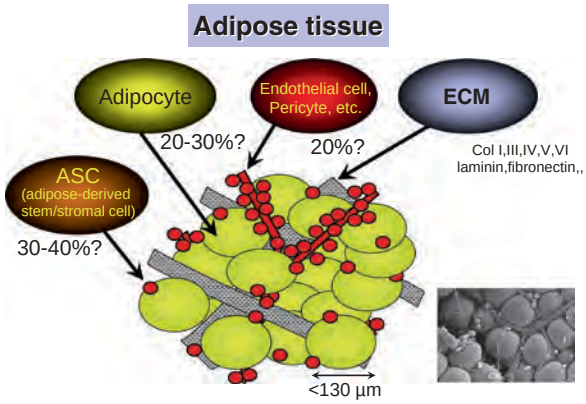

Fig. 34.3 Scheme of adipose tissue components. Adipocytes constitute more than 90% of tissue volume but only 50–60% of the total cell number. ASCs, endothelial cells, fibroblasts, and other cells constitute the remainder. Extracellular matrix (ECM) of the adipose tissue contains various collagens, laminin, fibrinogen, and other ECM substances

Human ASCs are distinct from other mesenchymal progenitors in their surface marker expression profile; notably, only ASCs express stem cell-associated marker CD34 in higher percentages than do bone marrow-derived mesenchymal stem cells and dermal fibroblasts (11).

ASCs are currently used in clinical trials of treatments for bone defect (autologous fresh ASCs) (16), rectovaginal fistula (autologous cultured ASCs) (17), graft vs. host disease (nonautologous ASCs) (18), and soft-tissue augmentation by CAL (autologous fresh ASCs) (5–7, 19). If ASCs are harvested from a large volume (e.g., 500 mL) of liposuction aspirates, ASCs can be used without cell expansion because a sufficient number can be obtained from such a volume. Furthermore, the use of minimally manipulated fresh cells might lead to higher safety and efficacy in actual treatments.

34.3 Biological and Therapeutic Concepts of Cell-Assisted Lipotransfer

1. Cell components of adipose tissue

Adipose tissue consists predominantly of adipocytes, ASCs, endothelial cells, pericytes, fibroblasts, and extracellular matrix (ECM). Adipocytes constitute more than 90% of tissue volume but they are much larger in size than the other cells, and the number of adipocytes is estimated to be only less than 50% about of the total cell number (20, 21) (Fig. 34.3). ASCs are adipose tissue-specific progenitor cells that contribute to adipose tissue turnover (adipose tissue is considered to turnover every several to 10 years (22, 23)) and provide cells for the next generation. Based on recent studies, ASCs are considered to be bipotent progenitors, being sources of both adipogenic and angiogenic lineages (15).

2. Aspirated fat tissue vs. intact fat tissue

In general, we can use only aspirated fat tissue as lipoinjection material. Aspirated fat is a fragile part of the adipose tissue taken under negative pressure. Indeed, a fibrous honeycomb structure is left in the donor tissue after liposuction. Our research has revealed that aspirated fat contains only half the number of ASCs as intact whole fat (9, 21) (Fig. 34.4). The two main reasons for this relative deficiency of ASCs are the following. (a) A major portion of ASCs is located around large vessels (within tunica adventitia) and is left at the donor site. (b) A part of ASCs is released into the fluid portion of liposuction aspirates (11). Our histological studies have indicated that ASCs are located not only between adipocytes but also around vessels. Large vessels are located in the fibrous part of the tissue contained by intact whole fat but much less by aspirated fat. Thus, aspirated fat tissue is regarded as progenitor-poor fat tissue as compared to intact fat tissue.

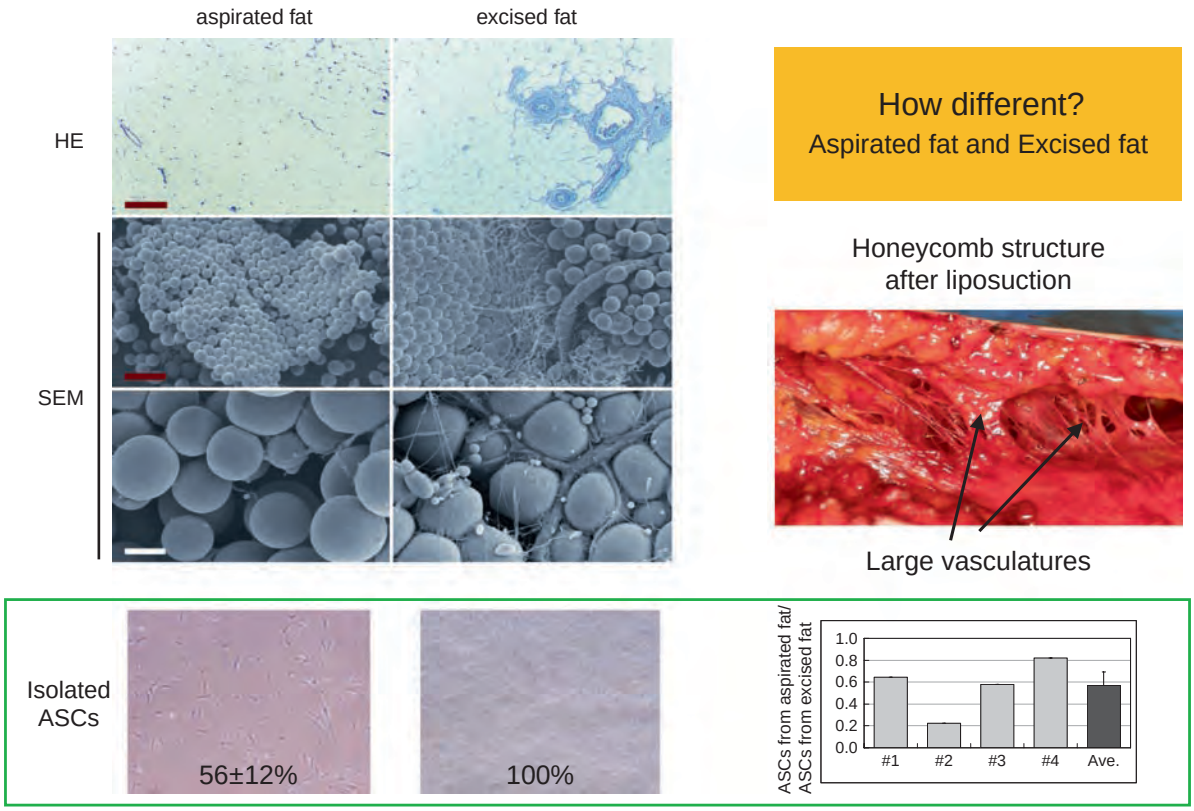

Fig. 34.4 Comparison of human aspirated fat and excised whole fat obtained from a single site of a single patient. Histology of aspirated fat and excised fat (*top*) (hematoxylin and eosin-stained microphotographs and scanning electron micrographs; red scale bar = 200 μm, white scale bar = 40 μm). The basic structure of adipose tissue was preserved in the aspirated fat, while significantly fewer vascular vessels, especially those of large size, were detected in aspirated fat than in excised fat. It is well known that the honeycomb structures of vascular and neural perforator networks are left intact in aspirated sites following liposuction procedures (*bottom*). Adipose-derived stromal/stem cell (ASC) yield from aspirated fat and excised fat. Both tissues were processed for isolation of SVFs, which were then cultured for 1 week. Ratios of ASC yields from aspirated fat to ASC yields from excised fat of the same volume were calculated; data from three patients (#1–4) and their average value are shown. The ASC yield from aspirated fat was significantly less (56 ± 12%) than the yield from excised fat (9, 21)

3. Stromal vascular fraction

Through collagenase digestion, a heterogeneous cell mixture can be extracted from adipose tissue as a cell pellet. This cell fraction is called the "stromal vascular fraction" (SVF) (Fig. 34.2) because it contains mostly stromal cells, vascular endothelial cells, and mural cells, but not adipocytes. In the clinical setting, the SVF contains a substantial amount of blood-derived cells such as leucocytes and erythrocytes as well as adipose-derived cells such as ASCs and vascular endothelial cells. Our pilot study (20) revealed that nucleated cells contained in SVF are composed of 37% white blood cells, 35% ASCs, and 15% endothelial cells and other cells, though the percentage of blood-derived cells strongly depends on individual hemorrhage volumes. In CAL, freshly isolated autologous SVF is used to supplement fat graft tissue without any manipulations such as cell sorting or culture.

4. Progenitor to adipocyte ratio

In general, tissue grafting is performed using graft tissue with an intact organ-specific ratio of progenitor cells to differentiated adult cells. For example, in split- or full-thickness skin grafting, the graft skin has the same number of basal keratinocytes and other keratinocytes as intact skin. The ratio of basal keratinocyte number to other differentiated cell number is the progenitor to mature cell ratio for the epidermis.

In adipose tissue, aspirated fat has a significantly lower progenitor to mature cell ratio and this low ASC to adipocyte ratio might be the main reason for long-term

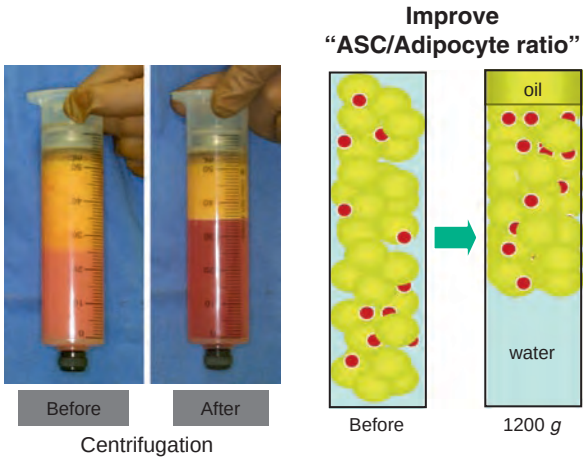

Fig. 34.5 Effects of centrifugation on aspirated adipose tissue. The adipose portion was concentrated to 71.0% of the original volume after centrifugation for 3 min at 1,200 × *g*. The volume of the adipose portion significantly reduced and the volume of the fluid and oil portions significantly increased. However, the number of ASCs contained in the adipose portion did not significantly change by the centrifugation. Thus, centrifugation at 1,200 × *g* led to condensation of cell numbers per volume of adipocytes and ASCs by 25 and 43%, respectively, and improved the ASC to adipocyte ratio by 14% (26)

atrophy of transplanted adipose tissue. There are at least three experimental studies (9, 24, 25) demonstrating that supplementing adipose progenitor cells enhances the volume or weight of survived adipose tissue.

The authors have found that centrifugation of the aspirated fat influences engraftment efficiency substantially, because centrifugation at 1,200 × *g* decreases the fat volume by 30%, damaging 12% of the adipocytes and 0% of the ASCs (26) (Fig. 34.5). This leads to condensation of cell numbers per volume of adipocytes and ASCs by 25 and 43%, respectively, and improves the ASC to adipocyte ratio by 14%. Thus, even centrifugation alone is likely to lead to better aspirated fat engraftment.

34.4 Concept of Cell-Assisted Lipotransfer

Enrichment of adipose progenitor cells can be supplemented with the SVF. Supplementation with SVF improves the progenitor to adipocyte ratio – progenitor-poor aspirated fat tissue is converted to progenitor-rich fat tissue. It was hypothesized that this progenitor-enriched fat tissue would not only survive better but would also preserve its volume with minimal atrophy. In CAL, freshly isolated SVF, which contains ASCs, is added to progenitor-poor aspirated fat tissue; the cells are attached to the aspirated fat before transplantation, with the fat acting as a living bioscaffold (Fig. 34.1). After transplantation, the ASC-supplemented adipose tissue encounters ischemia and subsequent reperfusion, the high pressure of edema, and inflammatory changes in the host tissue. The microenvironments, injury-associated growth factors, and inflammation-associated cytokines and chemokines influence ASC behavior during the acute phase of tissue repair, as discussed in the next section.

34.5 Possible Roles of Adipose-Derived Stem/Stromal Cells in Cell-Assisted Lipotransfer

There are four possible roles for ASCs in CAL, which have partly been confirmed in preclinical studies (9, 24, 25). First, ASCs differentiate into adipocytes and contribute to the regeneration of adipose tissue. Second, ASCs differentiate into endothelial cells and possibly vascular mural cells (9, 14, 15), thereby promoting angiogenesis and graft survival. Third, ASCs release angiogenic growth factors such as hepatocyte growth factor in response to injury, hypoxia, and other conditions (27, 28), and these factors influence the surrounding host tissue. Finally, and possibly most importantly, some ASCs survive as original ASCs (9). In the adipose, ASCs reside between adipocytes or in the ECM, especially around vessels, and contribute to the turnover of adipose tissue, which is known to be very slow (2 years or more) (22, 23). However, surviving adipose grafts probably turn over during the first 2–3 months after transplantation because they experience temporary ischemia followed by reperfusion injury (8). This turnover, i.e., the replacement process of the adipose tissue, is conducted by tissue-specific progenitor cells, which are ASCs. The relative deficiency of ASCs in aspirated fat could affect the replacement process and lead to postoperative atrophy of grafted fat, which commonly occurs during the first 6 months following lipoinjection.

34.6 Technique

Surgical procedures

Donor sites are determined according to patient's preference and body mass index (BMI). If the patient's BMI is greater than 25, then 1,500 mL of aspirated fat can usually be harvested from either the abdomen and flanks, or posterior, medial, and lateral thighs. If BMI is less than 20, fat should be harvested from both abdomen and thighs. After the liposuction site is infiltrated with saline solution containing diluted epinephrine (0.001%) under general anesthesia, the adipose tissue is suctioned using a cannula with 2.5 mm inner diameter and a conventional liposuction machine. About a half of the collected liposuction aspirate (500–800 mL of aspirated fat) is used to harvest the SVF. The SVF is isolated from both the adipose portion and the fluid portion of liposuction aspirates, as described below (11), and the cell processing procedure takes about 80 min. During the processing period, the remaining half of lipoaspirate is harvested and prepared as a graft material. The aspirate is centrifuged at 700 × *g* for 3 min, and the floating adipose portion is placed in a metal jar (1,000 mL) placed in an ice water bath.

For the injection syringe, the authors use a 10-mL LeVeen inflator (Boston Scientific Corp., MA) or the authors' original syringe (20 mL) because they are both screw-type syringes (with a threaded plunger) with threaded connections that fit both the connecting tube and the needle, providing precise control during injection (Fig. 34.6). Two syringes are used in order to reduce the time of the procedure. While one syringe is being used for an injection the other is filled with the graft material in preparation for the next injection. A 16- or 18-gauge needle (150 mm long) is used for lipoinjection and inserted subcutaneously at one of the four points indicated in Fig. 34.7. Care is taken to insert the needle horizontally (parallel to the body) in order to avoid damaging the pleura and causing a pneumothorax. The needle is inserted in several layers and directions and is continuously and gradually retracted as the plunger is advanced (Fig. 34.7), thereby ensuring diffuse distribution of the graft material. The grafts are placed into the fatty layers on, around, and under the mammary glands (but not intentionally into the mammary glands), as well as into the pectoralis muscles. After training, the operator can easily recognize the difference between mammary gland or pectoralis fascia, which are harder tissues, and the fat or muscle tissue. After the surgery, the breasts are maintained in the proper position using a brassier; massage of the breasts is prohibited during the first 3 months.

For patients with artificial breast implants, CAL can be performed immediately following implant removal (6, 7). During the cell isolation period, the breast implants are removed through a periareolar incision made at the caudal third of the areola margin. Lipoinjection is initiated at the deepest layer under the implant capsule and completed with injection into the most superficial subcutaneous layer. Again, in the deepest layer, it is important to insert and place the needle horizontally (parallel to the body) in order to avoid damaging the pleura. The operator can insert a finger into the implant capsule and place

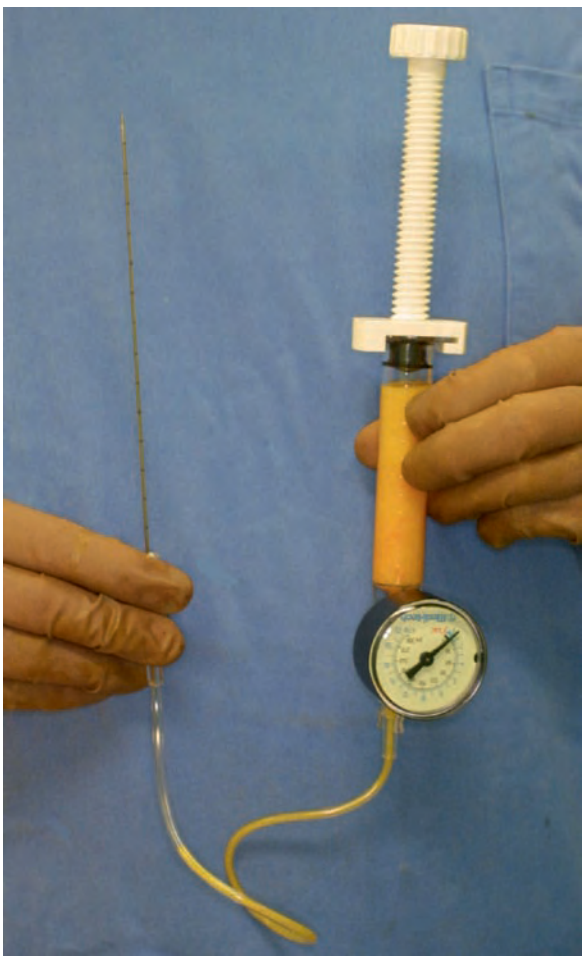

Fig. 34.6 Injection devices. A high-pressure injection can be performed with a disposable syringe with a threaded plunger. A 150-mm long 16- or 18-gauge needle is connected to the syringe with a connecting tube threaded at both ends. The injection needle is rigidly manipulated by an operator, while an assistant rotates the plunger according to the operator's instruction

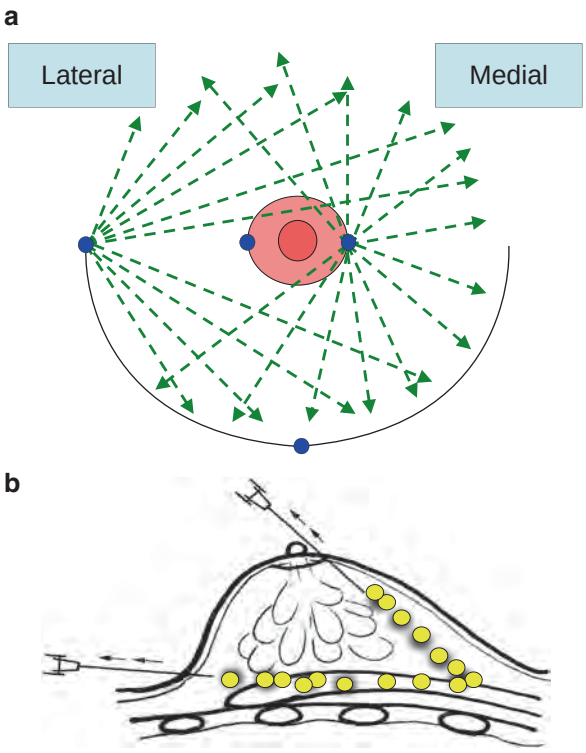

Fig. 34.7 Injection method. (**a**) The needle is inserted from either one of two points on the areola margin or one of two points at the infra-mammary fold in various directions and planes to achieve a diffuse distribution. (**b**) A small amount of fat tissue is injected in small aliquots or a thin string with a long needle on a syringe with a threaded plunger while the needle is continuously withdrawn (5)

it at the bottom of the capsule to recognize the location of the injection needle. The needle is inserted from the lateral margin of the breast and from a point on the inflamammary fold. Lipoinjection between the capsule and the skin is done from the same two points and from the periareolar incision. This technique helps to ensure a diffuse distribution of the graft material; no injections are made into the mammary glands or into the capsular cavity. Finally, the capsular cavity is washed with saline and the periareolar incision is closed.

34.7 Cell Processing (Stromal Vascular Fraction Isolation Procedure)

Processed lipoaspirate cells (PLA) cells and liposuction aspirate fluid (LAF) cells are separated from the fatty and fluid portions of liposuction aspirates, respectively. For PLA cells, the suctioned fat is digested with 0.075% collagenase in phosphate buffered saline for 30 min on a shaker at 37°C after centrifugation. Mature adipocytes and connective tissues are separated from cell pellets by centrifugation (800 × *g*, 10 min); the pellets are then resuspended in erythrocyte lysis buffer (155 mM NH_4Cl, 10 mM, $KHCO_3$, 0.1 mM EDTA) and incubated for 5 min at room temperature. The pellets are resuspended and passed through a 100-mm mesh filter (Millipore, Billerica, MA). To eliminate any remaining collagenase, the cells pellets are washed at least three times in Dulbecco's Modified Eagle's Medium (DMEM) by repeated suspension and centrifugation. For LAF cells, the suctioned fluid is centrifuged (400 × *g*, 10 min) and the pellets resuspended in erythrocyte lysis buffer. After 5 min at room temperature, lysates are passed through a 100-mm mesh filter. Again, the cell pellets are washed at least three times in DMEM and passed through a 100-mm mesh filter.

The entire procedure should be performed by well-trained physicians or technicians in an aseptic room (preferably at a level of good manufacturing practice) according to a designated standard operating procedure. Isolated cells should be strictly evaluated regarding quantity and quality. Cell counts for erythrocytes and nucleated cells are performed using a cell counter used for standard blood testing. The whole process of cell isolation takes about 70–80 min. We also recommend that a fraction of the isolated SVF be seeded and cultured to verify cell viability, and another fraction be frozen and stored in a deep freezer or liquid nitrogen for future cell tracing.

34.8 Results of Clinical Trials (2003–2009)

CAL was performed on 307 patients at various anatomical sites, including 269 breast procedures; 40 patients had breast reconstruction after mastectomy, 48 facial procedures, three procedures in the hand, and three in the hip. CAL was performed at two different sites in 17 patients. In 269 breast cases, 52 patients underwent CAL immediately after removal of breast implants. All patients were women with a BMI of 19.6 ± 2.1 (mean ± standard deviation) and the patient's ages varied from 13 to 73 years (34.9 ± 11.2). The

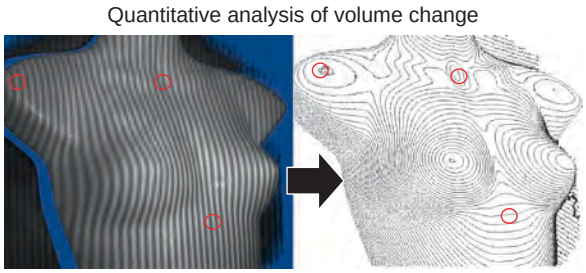

Fig. 34.8 Three-dimensional system for measuring breast volume. Using this system, breast volume can be measured while the patient is in a sitting position

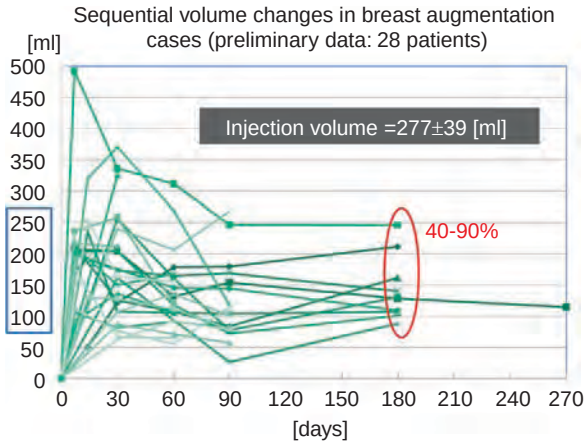

Fig. 34.9 Sequential volume changes after cell-assisted lipotransfer measured using the three-dimensional system (preliminary results for 28 patients). Augmented volume among patients varied between 100 and 250 mL at 6 months, corresponding to 40–90% survival of transplanted adipose tissue

mean volume of injected fat was 268.6 ± 48.2 mL on the left side and 273.1 ± 40.4 mL on the right.

34.9 Preoperative and Postoperative Evaluations

In order to evaluate outcomes, physical measurements (maximum and bottom breast circumferences, etc.) were taken, and mammography, magnetic resonance imaging (MRI), echography, photography, and videography performed. A three-dimensional (3D) measurement system was adopted, which enables volumetric evaluation of the breast mound in a standing position. Perpendicular striped lights are projected onto the breasts and photographed using a stereo-type digital camera (Fig. 34.8). The digital images are then analyzed using customized software. The volume and projection of each breast are calculated above a standard plane designated by three fixed points (the shoulder, suprasternal notch, and xiphoid process), which do not usually shift after breast augmentation.

34.10 Outcomes

The procedure takes about 3.5–4 h including SVF isolation. The injection process requires 35–60 min for both breasts. Subcutaneous bleeding and edema are typical on some parts of the breasts, but this usually resolves within 1–2 weeks.

Transplanted adipose tissue was gradually absorbed during the first two postoperative months, particularly during the first month, and the breast volume changed minimally thereafter, although skin tension sometimes decreased after 2 months. The 3D measurements taken at 6 months follow-up showed that the surviving fat volume was 100–250 mL, meaning that the graft take ranged from 40 to 90% (Fig. 34.9). Compared to breasts augmented with implants of the same size, those augmented with CAL were lower but had a more natural contour and softness without any palpable nodules at 6 months follow-up. Patients were satisfied with the outcome despite the limited size increase possible with autologous tissue transfer. Computed tomography (CT) scans and MRI showed that transplanted fat tissue survived and formed a significant thickness of the fatty layer not only subcutaneously on and around the mammary glands but also between the mammary glands and the pectoralis muscles.

Regarding CAL-mediated breast augmentation immediately after implant removal, most cases showed natural softness of the breasts without any palpable nodules at 6 months follow-up, and the patients were satisfied with the texture, softness, contour, symmetry, and the absence of foreign material.

These satisfactory outcomes are similar to those observed in other soft-tissue augmentation cases, such as in patients with hemifacial lipoatrophy (19) (Figs. 34.10–34.13).

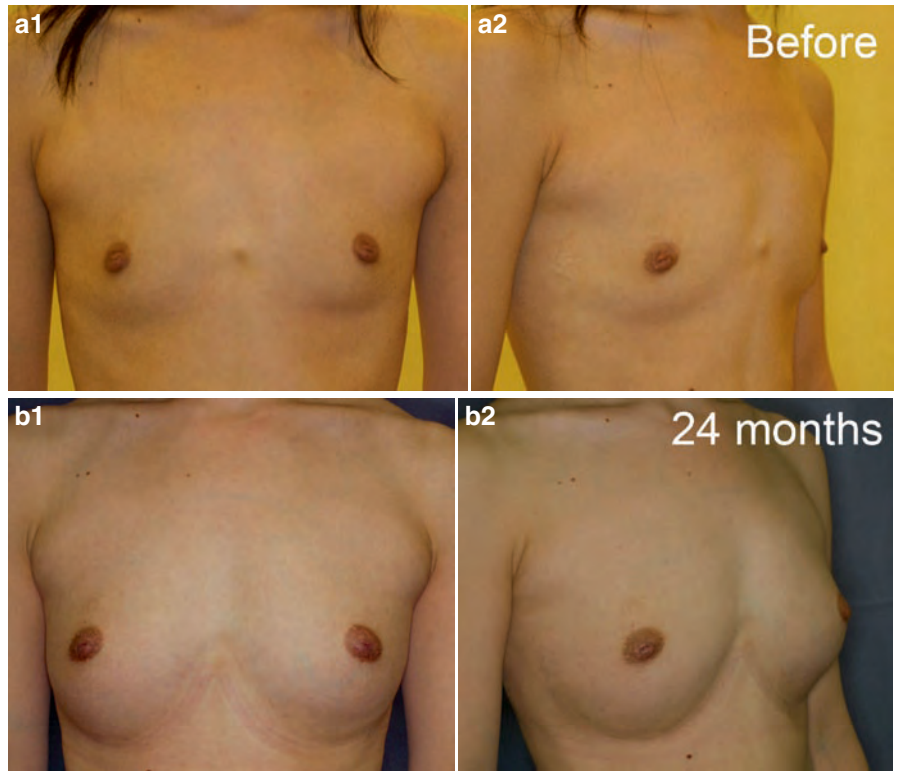

Fig. 34.10 Breast augmentation. (**a1**–**a2**) Preoperative 30-year-old woman. (**b1**–**b2**) Twenty-four months after breast augmentation with CAL (310 mL in each breast). Her breasts were augmented dramatically with an 8.0 cm increase in breast circumference at 24 months. The breast mounds were soft with no subcutaneous indurations. An original infra-mammary fold on the left breast is slightly visible, but injection scars are not visible (5)

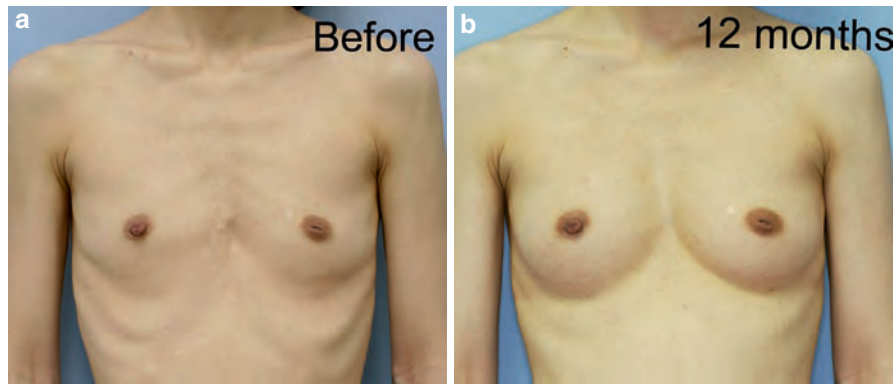

Fig. 34.11 Breast augmentation. (**a**) Preoperative 36-year-old woman whose body mass index was 17.3. (**b**) Twelve-months postoperative after breast augmentation with CAL (245 mL in each breast). The breast mounds were soft with no subcutaneous indurations or visible scars at 12 months

34.11 Discussion

34.11.1 Refinement of Autologous Fat Graft Techniques

It is well accepted that adipose tissue should be grafted in small aliquots, preferably within an area 3 mm in diameter (29). Because it requires a substantial length of time to perform ideal diffuse distribution of suctioned fat into the breast (1), a disposable syringe with a threaded plunger and connections and a very long needle (150 mm) are used. These devices are critical for performing large-volume lipoinjection safely and precisely in the shortest time possible. We use a relatively large suction cannula (2.5–3.5 mm inner diameter), centrifuge the aspirated fat, and keep it cooled until transplantation. In our experience, outcomes (increase in breast size) are superior when centrifuged vs. noncentrifuged fat is used, although we are yet to perform a quantitative and statistical analysis of this observation. The reason that centrifuged fat produces better outcomes could be that the ASC to adipocyte ratio is improved following centrifugation (26). In addition,

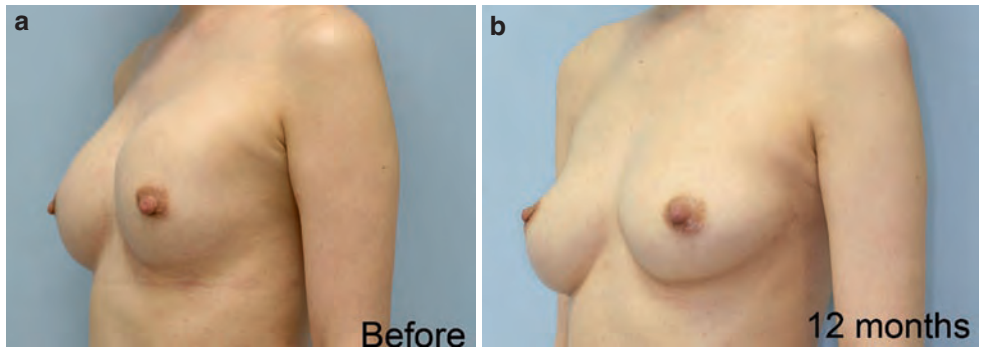

Fig. 34.12 Breast augmentation immediately after implant removal. (**a**) Preoperative 33-year-old woman who had 210 mL saline implants and capsular contractures with upward displacement of the left implant. (**b**) Twelve-months postoperative after implant removal and simultaneous CAL (260 mL in each breast). The breasts were symmetric and had a natural appearance (*bottom*). MRI revealed that transplanted adipose tissues had survived and formed thick layers around and under the mammary gland. Mammograms showed no calcifications or other abnormal signs in either breast. Augmented breast mounds maintained sufficient breast volume even after implant removal and were naturally soft without any subcutaneous indurations

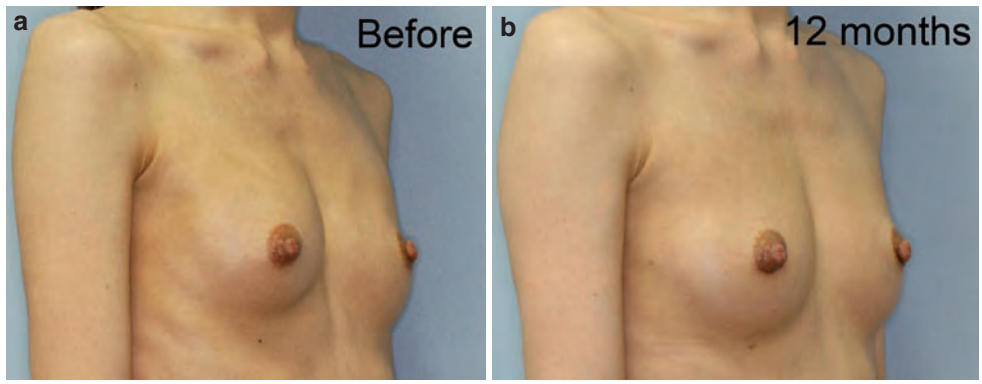

Fig. 34.13 Breast augmentation immediately after implant removal. (**a**) Preoperative 25-year-old woman who had 165 mL hydrogel implants (ruptured) with capsular contractures and displacement of the right implant. (**b**) Twelve-months postoperatively following implant removal and simultaneous CAL (260 mL in each breast). The breasts were symmetrical, had a natural appearance, and mammography revealed no abnormalities. Augmented breast mounds were soft without injection scars or subcutaneous indurations

centrifugation may be of particular benefit in this procedure because centrifugation decreases the water content in the graft material. Higher water content could disturb the ASCs to adhere to the adipose tissue, leading to unexpected behaviors of ASCs, as discussed below.

After transplantation, ASCs probably interact with other cells contained in SVF such as vascular endothelial cells. Therefore, in this treatment, supplementation with the SVF might be superior to supplementation with ASCs alone. Further studies are needed to elucidate the synergistic effects of ASCs with other cells contained in the graft.

34.12 Indications

There are several patient factors that may affect the clinical outcome of CAL, such as skin redundancy of the breasts, age, BMI, personal quality or character of fat, scars and adhesions, breast implant and its capsule, systemic disease such as autoimmune disease, and oral corticosteroid use. Lean patients have a disadvantage because it is not easy to obtain 1,500 mL of fat from these patients. Some lean young patients with no history of pregnancy have flat chests and high skin tension, and therefore they cannot accept a large-volume fat graft due to skin shortage. Some patients have oily aspirates and others have

fibrous aspirates. Patients who have undergone mastectomy have scarring and adhesions to the underlying fascia and some have a history of radiation therapy.

Good candidates for CAL are those who have sufficient fat at the donor sites and sufficient skin redundancy on breasts with healthy skin vasculatity and no scars. In the authors' experience, age does not appear to affect the clinical result.

Patients with breast implants, who are already familiar with drawbacks of implants and have sufficient breast skin expanded by implants, are considered good candidates for CAL even though they have implant capsules in place (7). Similarly, the breast skin of women with a history of pregnancy and breast-feeding would have expanded owing to enlargement of the mammary glands, and their breasts can more easily accept a larger injection volume than those with no history of pregnancy.

34.13 Complications

Cyst formation (5–15 mm diameter) was detected by MRI in two patients and by echogram in six patients. Tiny cyst formation (smaller than 5 mm), detected only by echogram, might happen more frequently, but no treatment is needed as long as the cyst diameter is less than 10 mm. Small calcifications were detected by mammogram in two patients at 24 months follow-up, but the calcifications were easily distinguished from those associated with breast cancer. Postoperative donor site problems, such as irregularity or seroma, could be more commonly associated with CAL than with conventional treatment because of the large volumes removed during liposuction.

In two patients in whom an SVF cell suspension was injected into each breast mound (30 mL per side) immediately after conventional lipoinjection, the breast mounds were somewhat hard to the touch at 3 months; CT scan detected unexpected fibrosis in the subcutaneous fat layers of the breast mounds and on the sternum (30). Therefore, ASCs should be adhered to cells, tissue, ECM, or some type of biological scaffold prior to administration in order to avoid their unexpected differentiation, migration, or other behavior.

34.14 Conclusions

Transplanting ASC-enriched fat tissue has provided satisfactory outcomes without any major complications. Our experiences with the CAL technique suggest that ASC supplementation is a safe and effective means of breast augmentation. Controlled studies with longer follow-up are necessary to establish the value of this technique. Continued improvements to this technique could make autologous tissue transfer the first choice for breast augmentation in the future.

References

1. Coleman SR, Saboeiro AP. Fat grafting to the breast revisited: Safety and efficacy. Plast Reconstr Surg 2007;119(3):775–785.
2. Spear SL, Wilson HB, Lockwood MD. Fat injection to correct contour deformities in the reconstructed breast. Plast Reconstr Surg 2005;116(5):1300–1305.
3. Yoshimura K, Matsumoto D, Gonda K. A clinical trial of soft tissue augmentation by lipoinjection with adipose-derived stromal cells (ASCs). Proceedings of the 3rd annual meeting of International Fat Applied Technology Society (IFATS), Charlotteville, Virginia 2005, pp. 9–10.
4. Spear SL, Newman MK. Discussion: Fat grafting to the breast revisited: Safety and efficacy. Plast Reconstr Surg 2007;119(3):786–787.
5. Yoshimura K, Sato K, Aoi N, Kurita M, Hirohi T, Harii K. Cell-assisted lipotransfer (CAL) for cosmetic breast augmentation -supportive use of adipose-derived stem/stromal cells. Aesthetic Plast Surg 2008;32(1):48–55.
6. Yoshimura K, Asano Y, Aoi N, Kurita M, Oshima Y, Sato K, Inoue K, Suga H, Eto H, Kato H, Hirohi T, Harii K. Progenitor-enriched adipose tissue transplantation as rescue for breast implant complications, Breast J, in press.
7. Yoshimura K, Asano Y. Fat injection to the breasts: cosmetic augmentation, implant replacement, inborn deformity, and reconstruction after mastectomy. In Aesthetic and Reconstructive Surgery of the Breast (Eds) Hall-Findlay EJ, Evans GRD., Elsevier Ltd, London, in press.
8. Yoshimura K, Suga H, Eto H. Adipose-derived stem/progenitor cells: roles in adipose tissue remodeling and potential use for soft tissue augmentation. Regen Med 2009; 4:265–273.
9. Matsumoto D, Sato K, Gonda K, Takaki Y, Shigeura T, Sato T, Aiba-Kojima E, Iizuka F, Inoue K, Suga H, Yoshimura K. Cell-assisted lipotransfer: Supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. Tissue Eng 2006;12(12):3375–3382.
10. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13(12):4279–4295.

11. Yoshimura K, Shigeura T, Matsumoto D, Sato T, Takaki Y, Aiba-Kojima E, Sato K, Inoue K, Nagase T, Koshima I, Gonda K. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. J Cell Physiol 2006;208(1):64–76.
12. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Circ Res 2007;100(9):1249–1260.
13. van Harmelen V, Skurk T, Hauner H. Primary culture and differentiation of human adipocyte precursor cells. Methods Mol Med 2005;107:125–135.
14. Miranville A, Heeschen C, Sengenes C, Curat CA, Busse R, Bouloumie A. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. Circulation 2004;110(3):349–355.
15. Planat-Benard V, Silvestre JS, Cousin B, Andre M, Nibbelink M, Tamarat R, Clergue M, Manneville C, Saillan-Barreau C, Duriez M, Tedgui A, Levy B, Penicaud L, Casteilla L. Plasticity of human adipose lineage cells toward endothelial cells: Physiological and therapeutic perspectives. Circulation 2004;109(5):656–663.
16. Lendeckel S, Jodicke A, Christophis P, Heidinger K, Wolff J, Fraser JK, Hedrick MH, Berthold L, Howaldt HP. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: Case report. J Craniomaxillofac Surg 2004;32(6):370–373.
17. Garcia-Olmo D, Garcia-Arranz M, Herreros D, Pascual I, Peiro C, Rodriguez-Montes JA. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. Dis Colon Rectum 2005; 48(7):1416–1423.
18. Fang B, Song Y, Lin Q, Zhang Y, Cao Y, Zhao RC, Ma Y. Human adipose tissue-derived mesenchymal stromal cells as salvage therapy for treatment of severe refractory acute graft-vs.-host disease in two children. Pediatr Transplant 2007;11(7):814–817.
19. Yoshimura K, Sato K, Aoi N, Kurita M, Inoue K, Suga H, Eto H, Kato H, Hirohi T, Harii K. Cell-assisted lipotransfer for facial lipoatrophy: Efficacy of clinical use of adipose-derived stem cells. Dermatol Surg 2008;34(5):1178–1185.
20. Suga H, Matsumoto D, Inoue K, Shigeura T, Eto H, Aoi N, Kato H, Abe H, Yoshimura K. Numerical measurement of viable and nonviable adipocytes and other cellular components in aspirated fat tissue. Plast Reconstr Surg 2008;122(1):103–114.
21. Eto H, Suga H, Matsumoto D, Shigeura T, Inoue K, Aoi N, Kato H, Araki J, Yoshimura K. Characterization of adipose tissue structure and cellular components: Differences between aspirated adipose tissue and excised adipose tissue. Plast Reconstr Surg, in press.
22. Strawford A, Antelo F, Christiansen M, Hellerstein MK. Adipose tissue triglyceride turnover, de novo lipogenesis, and cell proliferation in humans measured with 2H2O. Am J Physiol Endocrinol Metab 2004;286(4):E577–E588.
23. Spalding KL, Arner E, Westermark PO, et al.: Dynamics of fat cell turnover in humans. Nature 2008;453(7196):783–787.
24. Masuda T, Furue M, Matsuda T. Novel strategy for soft tissue augmentation based on transplantation of fragmented omentum and preadipocytes. Tissue Eng 2004;10(11–12): 1672–1683.
25. Moseley TA, Zhu M, Hedrick MH. Adipose-derived stem and progenitor cells as fillers in plastic and reconstructive surgery. Plast Reconstr Surg 2006;118(3 Suppl): 121S–128S.
26. Kurita M, Matsumoto D, Shigeura T, Sato K, Gonda K, Harii K, Yoshimura K. Influences of centrifugation on cells and tissues in liposuction aspirates: Optimized centrifugation for lipotransfer and cell isolation. Plast Reconstr Surg 2008;121(3):1033–1041.
27. Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, Pell CL, Johnstone BH, Considine RV, March KL. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation 2004;109(10):1292–1298.
28. Suga H, Eto H, Shigeura T, Inoue K, Aoi N, Kato H, Nishimura S, Manabe I, Gonda K, Yoshimura K. IFATS collection: FGF-2-induced HGF secretion by adipose-derived stromal cells inhibits post-injury fibrogenesis through a JNK-dependent mechanism. Stem Cells 2009;27:238-249.
29. Carpaneda CA, Ribeiro MT. Percentage of graft viability versus injected volume in adipose autotransplants. Aesthetic Plast Surg 1994;18(1):17–19.
30. Yoshimura K, Sato K, Aoi N, Kurita M, Suga H, Inoue K, Eto H, Hirohi T, Harii K. Ectopic fibrogenesis induced by transplantation of adipose-derived progenitor cell suspension immediately after lipoinjection. Transplantation 2008; 85(12):1868–1869.