Title:

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

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Abstract

Lipoinjection is a promising treatment, but is currently limited by unpredictable outcomes and a low rate of graft survival due to partial necrosis. To address these problems we developed a novel strategy called Cell-Assisted Lipotransfer (CAL) in which autologous adipose-derived stem (stromal) cell (ASC) supplementation is used in combination with lipoinjection. A stromal vascular fraction (SVF) containing ASCs is isolated from half of an aspirated fat sample and is recombined with the remaining half of the aspirated fat sample. This process converts relatively progenitor-poor aspirated fat to progenitor-rich fat. Our experience with the CAL technique showed that by transplanting the ASC-enriched fat tissue post-operative atrophy of transplanted fat grafts was minimal and satisfactory clinical results were generally achieved without any major complications, suggesting that ASC supplementation is both effective and safe. Further studies with longer follow up are necessary to establish the value of this technique. Continued improvements in the technique could make autologous tissue transfer the first choice for breast augmentation in the future.

I. 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 due to the lack of consensus on whether it is safe and appropriate because of 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 [1-5]. This re-evaluation may reflect recent advances in autologous fat transfer and the radiological detection of breast cancer.

In this chapter, we introduce a novel approach to autologous fat grafting called cell-assisted lipotransfer (CAL). CAL is the concurrent transplantation of aspirated fat tissue and adipose progenitor cells called adipose-derived stem/stromal cells (ASCs), which is the grafting of progenitor-enriched fat tissue (Fig. 1). 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 [6].

II. Adipose tissue-specific progenitors with multipotency called adipose-derived stem/stromal cells

It has been shown that fibroblast-like stromal cells obtained from liposuction aspirates can differentiate into various cell lineages [7,8] such as adipogenic, osteogenic, chondrogenic,

myogenic, cardiomyogenic, and neurogenic. Thus, the adipose tissue-specific progenitor cells are now called “adipose-derived stem/stromal cells (ASCs)” and are expected to become valuable tools in a wide range of cell-based therapies [9] (Fig. 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 [6,11,12]. 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 [8].

ASCs are currently being used in clinical trials of treatments for bone defect (autologous fresh ASCs) [13], rectovaginal fistula (autologous cultured ASCs) [14], graft-versus-host disease (non-autologous ASCs) [15], and soft tissue augmentation by CAL (autologous fresh ASCs) [5]. 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.

III. 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. 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 about 50-60% of the total cell number[16] (Fig. 3). ASCs are adipose tissue-specific progenitor cells that

contribute to adipose tissue turnover (adipose tissue is considered to turnover every 2-3 years [17]) 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 [12].

2) Aspirated fat versus intact fat 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 revealed that aspirated fat contains only half the number of ASCs as intact whole fat [6] (Fig. 4). The two main reasons for this relative deficiency of ASCs are 1) a major portion of ASCs are located around large vessels (within tunica adventitia) and are left at the donor site, and 2) part of ASCs are released into the fluid portion of liposuction aspirates [8]. Our histological studies indicated that ASCs are located not only between adipocytes but also around vessels. Large-sized 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.

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. 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 [16] 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, the freshly isolated autologous SVF is used to supplement fat graft tissue without any manipulations such as cell sorting or culture.

4) Progenitor:adipocyte ratio In general, tissue grafting is performed using graft tissue with an intact organ-specific ratio of progenitor cells: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 has. The ratio of basal keratinocyte number to other differentiated cell number is the progenitor:mature-cell ratio for the epidermis.

In adipose tissue, aspirated fat has a significantly lower progenitor:mature-cell ratio, as mentioned above, and this low ASC:adipocyte ratio might be the main reason for long-term atrophy of transplanted adipose tissue. There are at least three experimental studies, including ours [6,18,19], demonstrating that supplementing adipose progenitor cells enhances the volume or weight of survived adipose tissue.

We 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 [20] (Fig. 5). This leads to condensation of cell numbers per volume of adipocytes and ASCs by 25% and 43%,

respectively, and improved the ASC/adipocyte ratio by 14%. Thus, even centrifugation alone is likely to lead to better aspirated fat engraftment.

5) Concept of Cell-Assisted Lipotransfer: Enrichment of adipose progenitor cells by supplementation with the stromal vascular fraction Supplementation with SVF improves the progenitor/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. 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 paragraph.

6) Possible roles of adipose-derived stem/stromal cells in Cell-Assisted Lipotransfer There are four possible roles for ASCs in CAL, which have partly confirmed in pre-clinical studies [6,18,19]. First, ASCs differentiate into adipocytes and contribute to the regeneration of adipose tissue. Second, ASCs differentiate into endothelial cells and possibly vascular mural cells [6,11,12], 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 [21, our unpublished data], and these factors influence surrounding host tissue. Finally, and possibly most importantly, some ASCs survive as original ASCs [6]. In the adipose, ASCs reside between adipocytes or in the extracellular matrix, especially around vessels, and contribute to the turnover of adipose tissue, which is known to be very slow (2 years or more) [17]. However, surviving adipose grafts probably turn over during the first 2 to 3 months after transplantation because they experience temporary ischemia followed by reperfusion injury. This turnover, 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 post-operative atrophy of grafted fat, which commonly occurs during the first 6 months following lipoinjection.

IV. Technique

1) **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 the 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 [8], 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) that is placed in an ice water bath.

For the injection syringe, we use a 10 cc LeVeen™ inflator (Boston Scientific Corp., MA) or our 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. 6). We use two syringes 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 Figure 7A. 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. 7B), 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 three months.

For patients with artificial breast implants, CAL can be performed immediately following implant removal. 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 it on 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 inflammammary 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.

2) **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 minutes. 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.

V. Results of clinical trials

1) **Patients** From 2003 to 2008, we performed CAL in 188 patients at various anatomical sites, including 164 breast procedures (20 patients had breast reconstruction after mastectomy), 37 facial procedures, two procedures in the hand, and three in the hip (CAL was performed at two different sites in 17 patients). In 164 breast cases, 26 patients underwent CAL immediately after removal of breast implants. All of the patients were females 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 mean volume of injected fat was 268.6 ± 48.2 ml on the left side and 273.1 ± 40.4 ml on the right.

2) **Pre- and post-operative evaluations** In order to evaluate outcomes, we took physical measurements (maximum and bottom breast circumferences, etc.), and performed mammography, magnetic resonance imaging (MRI), echography, photography, and videography. We have also adopted a three-dimensional (3D) measurement system that 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. 8). The digital images are then analyzed using customized software. We calculate the volume and projection of each breast above a standard plane designated by three fixed points (the shoulder, suprasternal notch, and xiphoid process) that do not usually shift after breast augmentation.

3) **Outcomes** The procedure takes about 3.5-4 hours 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 one to two 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 two months. The 3D measurements taken at six months follow up showed that the surviving fat volume was 100-250 ml, meaning that the graft take ranged from 40-90% (Fig. 9). Compared to breasts augmented with implants of the same size, breasts augmented with CAL were lower but

had a more natural contour and softness without any palpable nodules at six 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 six 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 [22].

4) **Representative cases** Two representative cases of breast augmentation by CAL and two cases of breast augmentation by CAL immediately after implant removal are illustrated in Figures 10-13.

VI. Discussion

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 [23]. Because it requires a substantial length of time to perform ideal, diffuse distribution of suctioned fat into the breast [1], we use a disposable syringe with a threaded plunger and connections and a very long needle (150 mm). These devices are critical for performing large-volume lipoinjection safely and precisely in the shortest length of time possible. We use a relatively large-sized 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 versus non-centrifuged fat is used, although we have yet to perform a quantitative and statistical analysis of this observation. The reason that centrifuged fat produces better outcomes could be that the ASC:adipocyte ratio is improved following centrifugation [20]. In addition, 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 interacts 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.

2) **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 cc of fat from these patients. Some lean young patients with no history of pregnancy have flat chests and high skin tension, therefore they cannot accept a large volume fat graft due to skin shortage. Some patients have oily aspirates and others have fibrous aspirates. Mastectomy patients 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 our 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. Similarly, the breast skin of women with a history of pregnancy and breast feeding has expanded due to enlargement of the mammary glands and their breasts can more easily accept a larger injection volume than those with no history of pregnancy.

3) **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) only detected 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 which 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 three months; CT scan detected unexpected fibrosis in the subcutaneous fat layers of the breast mounds and fibrosis on the sternum [24]. Therefore, ASCs should be adhered to cells, tissue, extracellular matrix, or some type of biological scaffold prior to administration in order to avoid their unexpected differentiation, migration, or other behavior.

VII. Conclusions

Transplanting ASC-enriched fat tissue 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.

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Legends

Fig. 1. Scheme of Cell-Assisted Lipotransfer (CAL). Relatively adipose-derived stromal/stem cells (ASC)-poor aspirated fat is converted to ASC-rich fat by supplementation with ASCs isolated from half of the aspirated fat sample. The ASCs are attached to the aspirated fat, which is used as a scaffold in this strategy (cited from ref #22). SVF, stromal vascular fraction.

Fig. 2. The stromal vascular fraction (SVF) can be obtained from adipose and fluid portions of liposuction aspirates through collagenase digestion. SVF contains 10-35% adipose-derived stromal cells (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. 3. Scheme of adipose tissue components. Adipocytes constitute more than 90% of tissue volume but only 50-60% of the total cell number. Adipose-derived stromal/stem cells (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.

Fig. 4. Comparison of human aspirated fat and excised whole fat obtained from a single site of a single patient. (top) Histology of aspirated fat and excised fat. (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 stromal vascular fractions, which were then cultured for one 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 (cited and revised from ref. #6).

Fig. 5. Effects of centrifugation on aspirated adipose tissue. The adipose portion was concentrated to 71.0% of the original volume after centrifugation for three minutes at 1200×g. The volume of the adipose portion was significantly reduced and the volume of the fluid and oil portions was significantly increased. However, the number of adipose-derived stromal cells (ASCs) contained in the adipose portion was not significantly changed 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:adipocyte ratio by 14% (cited and revised from #19).

Fig. 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. 7. Schematic instruction of the 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 (cited and revised from ref. #5).

Fig. 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. 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 six months, corresponding to 40-90% survival of transplanted adipose tissue.

Fig. 10. Case 1 (breast augmentation): Preoperative views (top) and postoperative (bottom) views at 24 months. A 30 year-old woman underwent 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 (cited and revised from ref. #5).

Fig. 11. Case 2 (breast augmentation): Preoperative view (top) and postoperative (bottom) view at 12 months. A 36 year-old woman whose body mass index was 17.3 underwent breast augmentation with CAL (245 ml in each breast). The breast mounds were soft with no subcutaneous indurations or visible scars at 12 months.

Fig. 12. Case 3 (breast augmentation immediately after implant removal): A 33 year-old woman who had 210 ml saline implants underwent implant removal and simultaneous CAL (260 ml in each breast). The preoperative view showed capsular contractures and upward displacement of the left implant (top). At 12 months the breasts were symmetric and had a natural appearance (bottom). MRI at 12 months 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 at 12 months. Augmented breast mounds maintained a sufficient breast volume even after implant removal and were naturally soft without any subcutaneous indurations.

Fig. 13 Case 4 (breast augmentation immediately after implant removal): A 25 year-old woman who had 165 ml hydrogel implants (ruptured) underwent implant removal and simultaneous CAL (260 ml in each breast). The preoperative view showed capsular contractures and displacement of the right implant (top). At 12 months the breasts were symmetric and had a natural appearance (bottom), and mammography revealed no

abnormalities. Augmented breast mounds were soft and natural appearing without injection scars or subcutaneous indurations.

Photos

Figure 1.

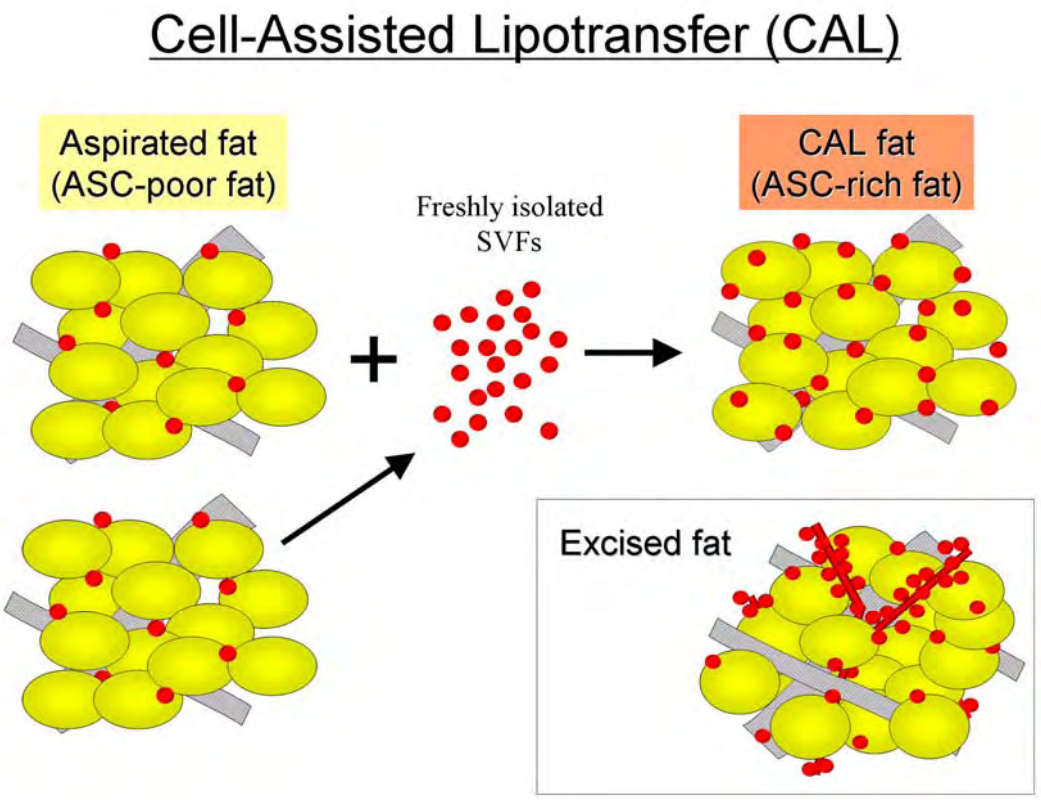


Figure 2

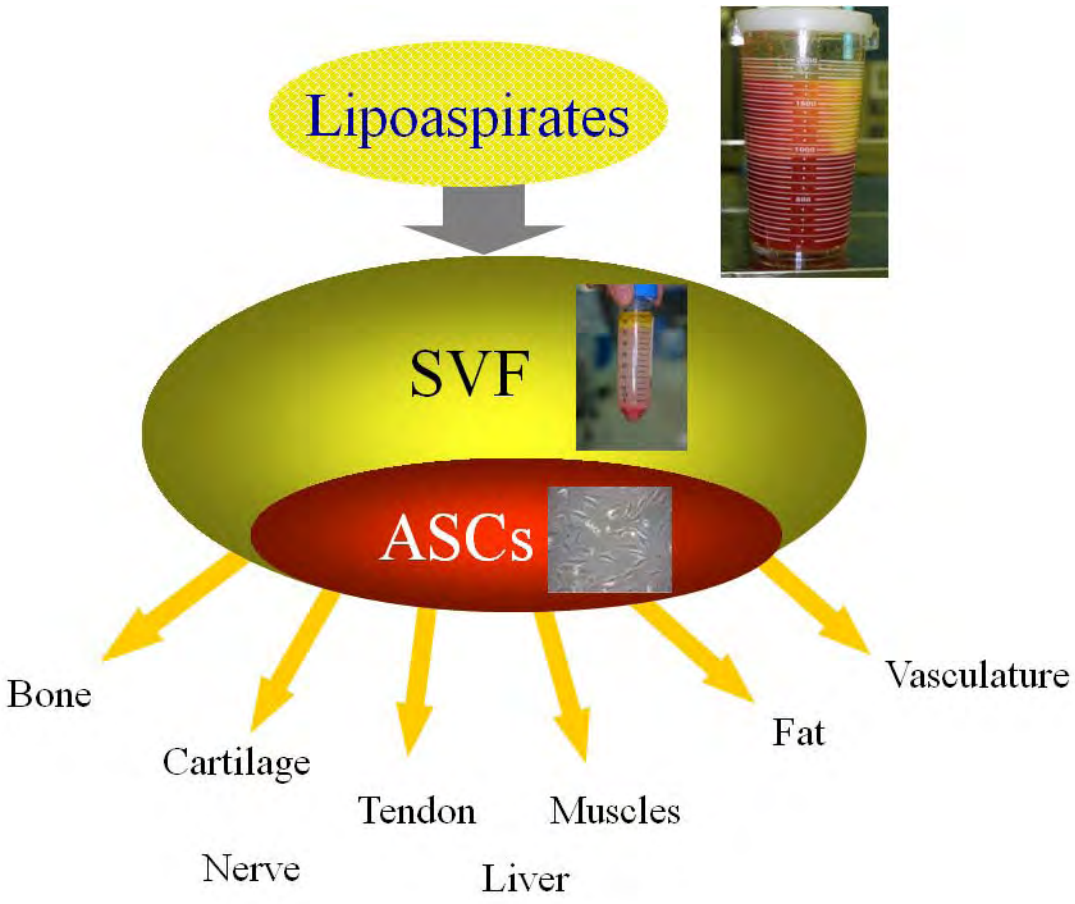

Lipoaspirates
SVF
ASCs
Bone
Cartilage
Nerve
Tendon
Liver
Muscles
Fat
Vasculature

Figure 3

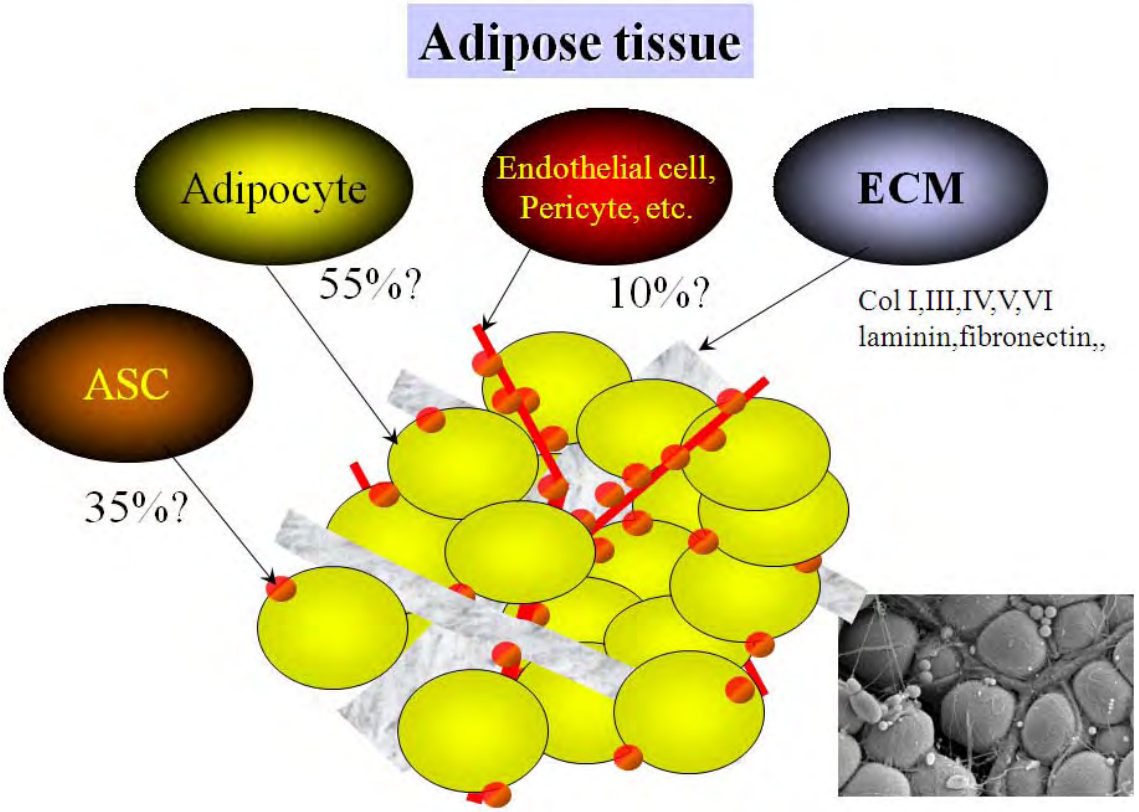

Adipose tissue
Adipocyte
Endothelial cell,
Pericyte, etc.
ECM
55%?
10%?
Col I,III,IV,V,VI
laminin,fibronectin,,
ASC
35%?

Figure 4

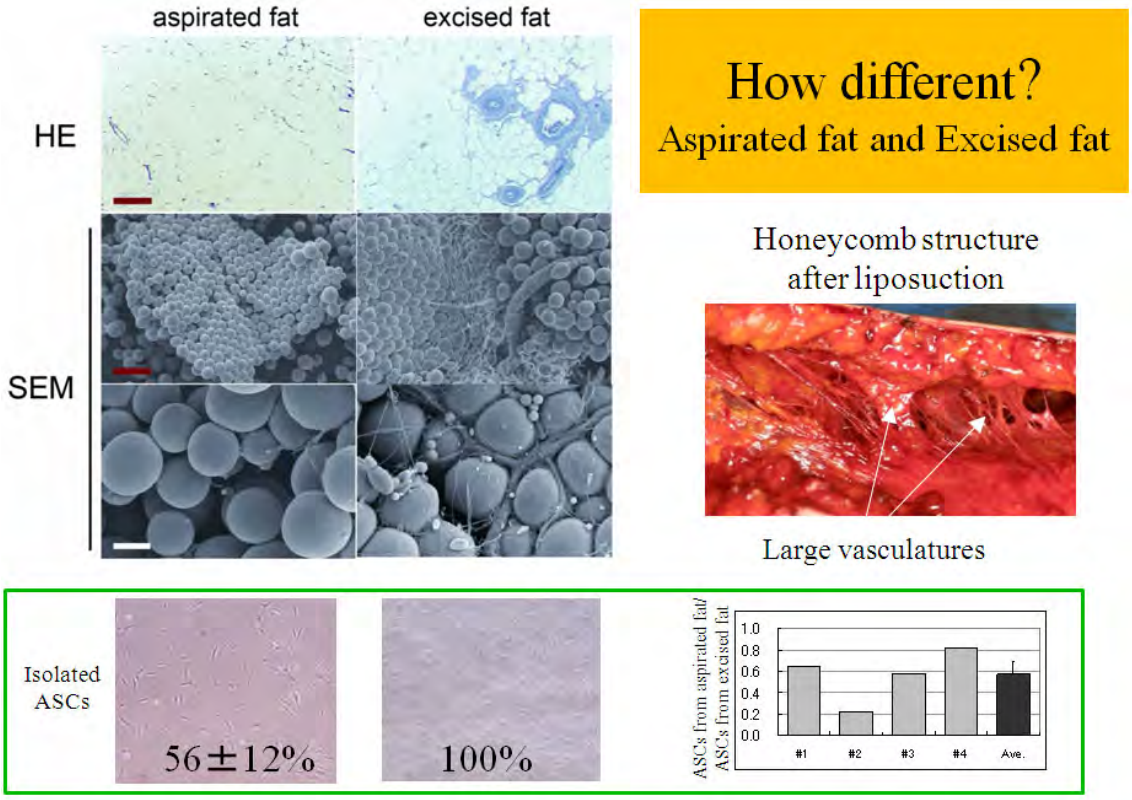

aspirated fat
excised fat
HE
SEM
How different?
Aspirated fat and Excised fat
Honeycomb structure
after liposuction
Large vasculatures
Isolated
ASCs
56±12%
100%
ASCs from aspirated fat/
ASCs from excised fat
1.0
0.8
0.6
0.4
0.2
0.0
#1
#2
#3
#4
Ave.

Figure 5

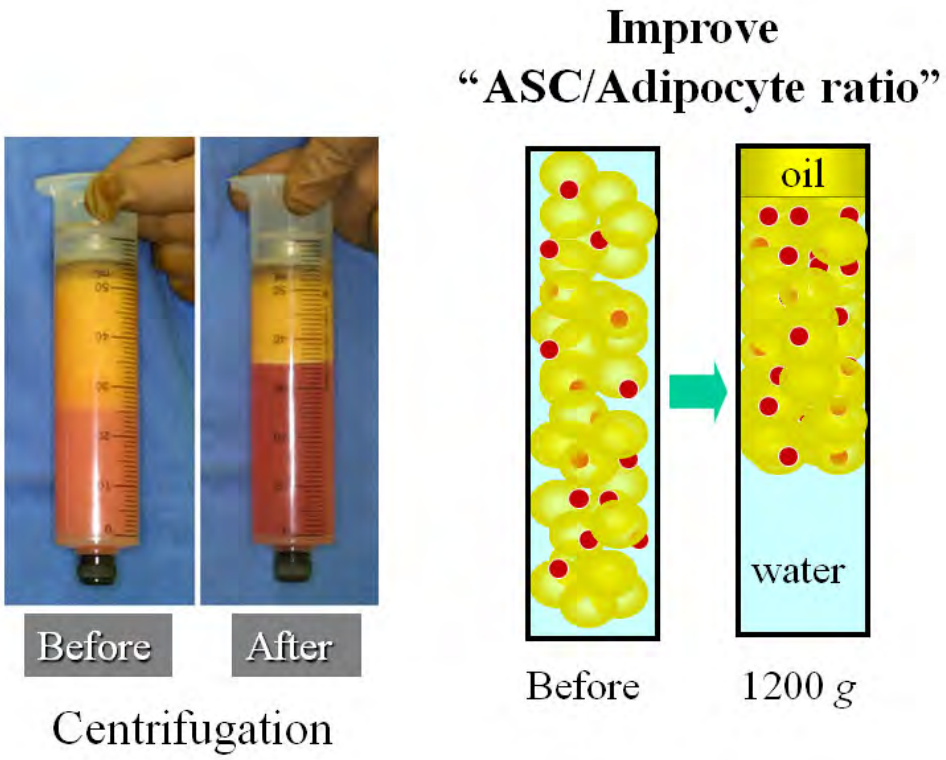

Improve
"ASC/Adipocyte ratio"
Before
After
Centrifugation
oil
water
Before
1200 g

Figure 6

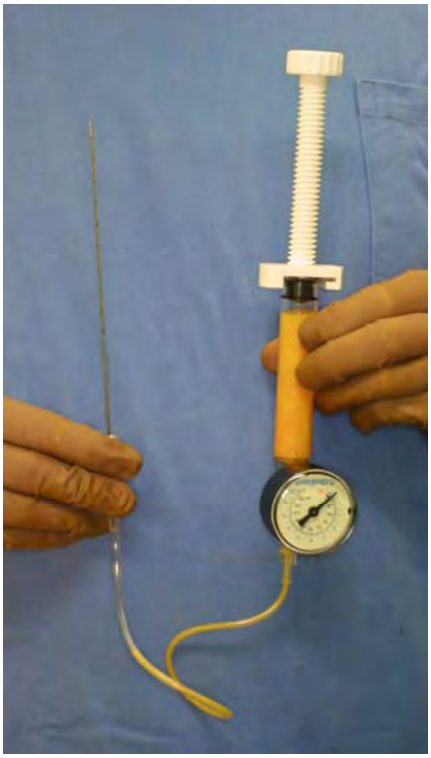


Figure 7

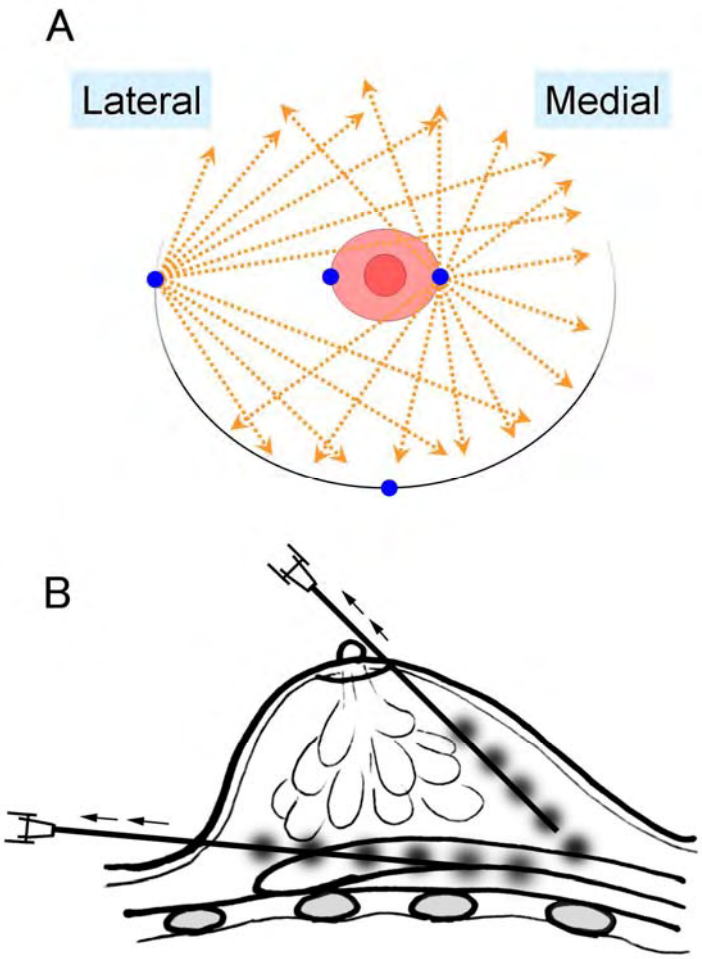
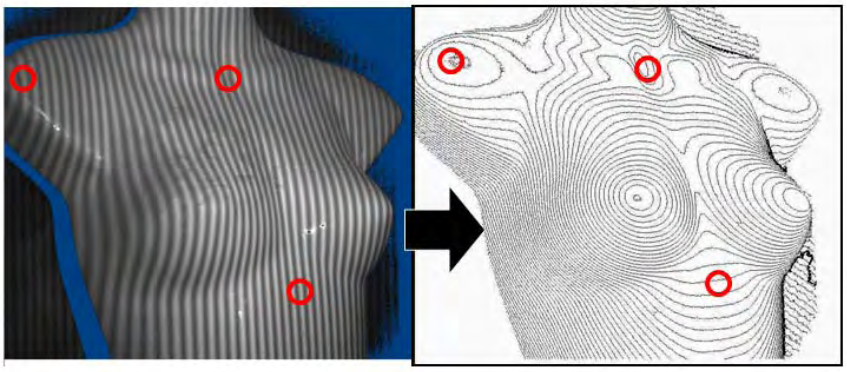


Figure 8

Quantitative analysis of volume change



A standard plane is defined by plotting 3 points.

The volume of breast that extends beyond the plane is calculated.

Figure 9

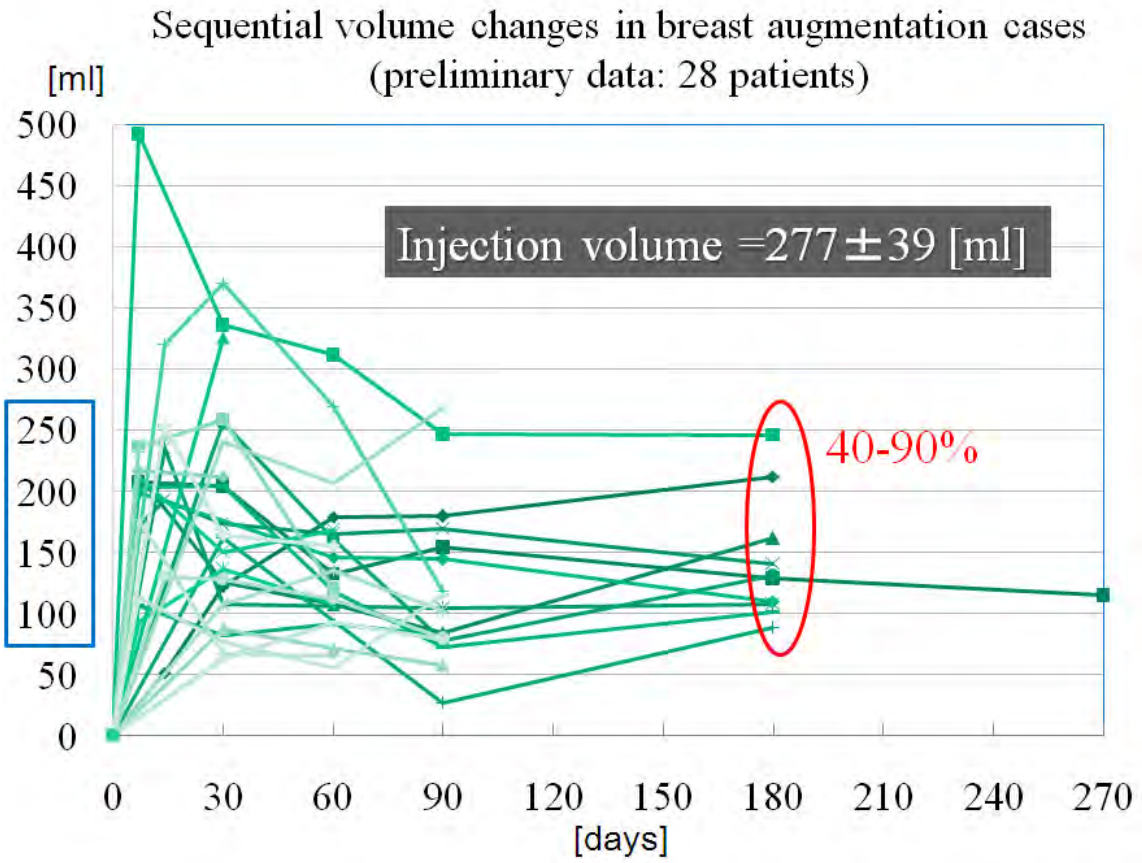

Sequential volume changes in breast augmentation cases
(preliminary data: 28 patients)
[ml]
500
450
400
350
300
250
200
150
100
50
0
Injection volume =277±39 [ml]
40-90%
0
30
60
90
120
150
180
210
240
270
[days]

Figure 10

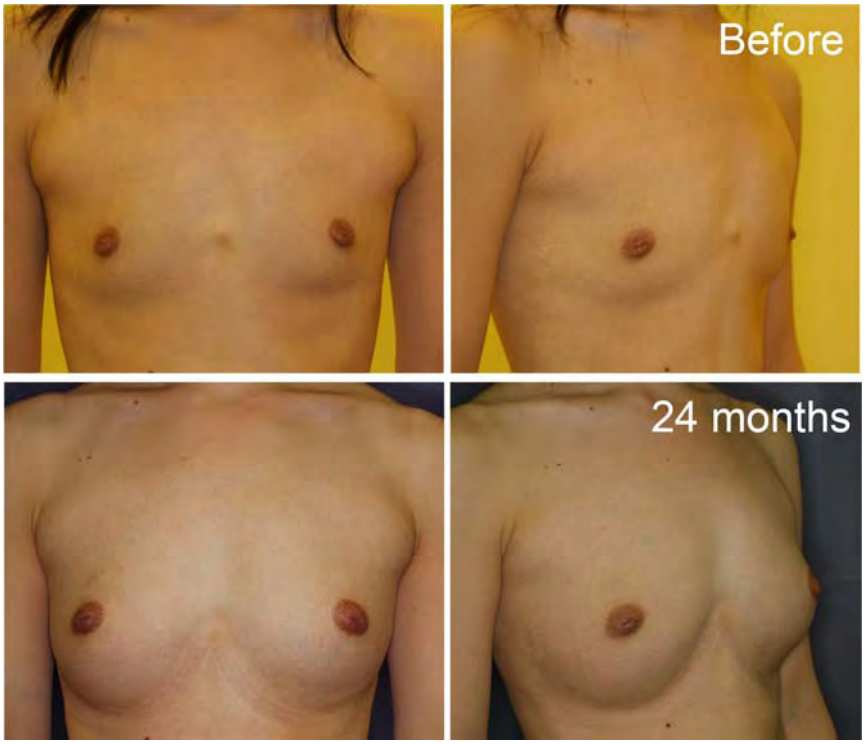


Figure 11

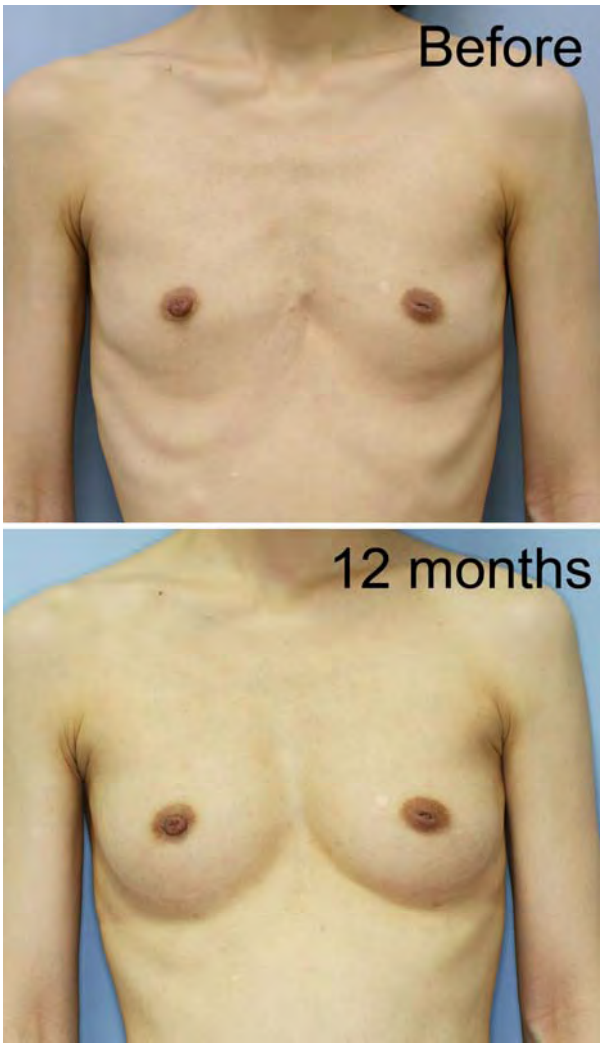


Figure 12

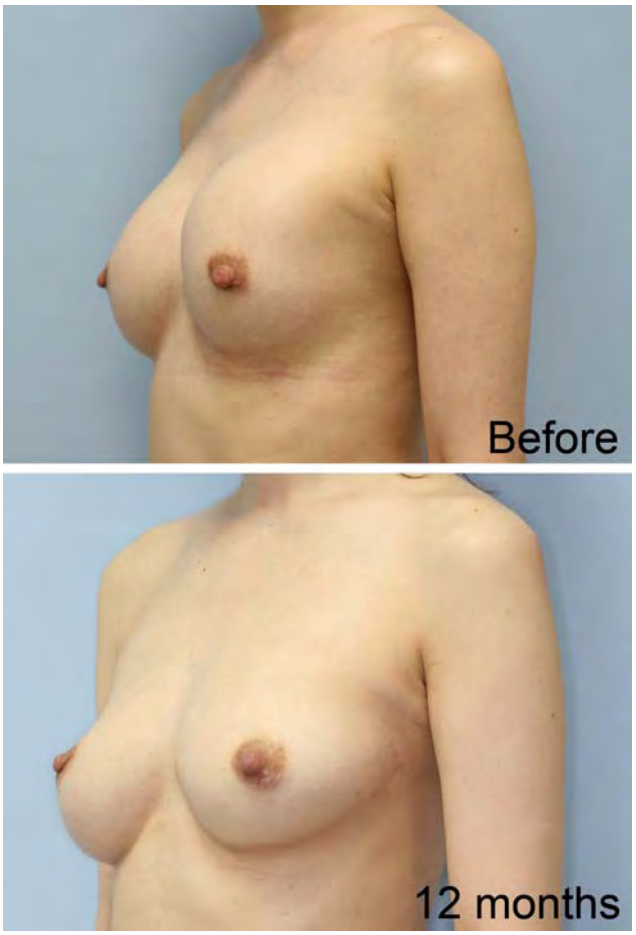


Figure 13