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ORIGINAL ARTICLE

Autologous Cell-Enriched Fat Grafting for Breast Augmentation

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Abstract Autologous fat grafting for breast augmentation has faced some historical hurdles. However, in recent years it has been gaining acceptance from the medical community. This prospective, nonrandomized open-label study of 20 Japanese women supports the use of autologous fat grafting in breast augmentation and explores enhancement of fat graft tissue with autologous adipose-derived regenerative cells (ADRCs). After adipose harvesting using syringe liposuction, the tissue is processed in the Celution 800 System®, which washes the graft and isolates ADRCs. The average cells per gram of harvested adipose tissue was 3.42×10^5, and the mean cell viability measured using an automated cell counting system before graft delivery was 85.3%. All patients demonstrated improvement in circumferential breast measurement (BRM) from their baseline state, and breast measurements were stable by 3 months after surgery. The mean BRM 9 months after surgery had increased 3.3 cm from preoperative measurements. Through 9 months, overall physician satisfaction was 69% and patient satisfaction was 75%. No serious or unexpected adverse events were reported, and the procedure was safe and well tolerated in all patients. Postoperative cyst formation was seen in two patients. These prospective results demonstrate that ADRC-enriched fat grafts processed with a closed automated system maintain high cell viability and that the procedure is safe and effective, with all patients showing improvement after a single treatment.

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In the current environment, most breast augmentation procedures use synthetic prosthetic implants, available in a variety of sizes and shapes with a well-described safety and efficacy profile. An alternative to prosthetic breast implants is breast augmentation using autologous tissue.

Autologous fat transfer, described initially more than 100 years ago [1], currently is a well-recognized alternative to prosthetic breast implants [2–5]. However, unpredictable long-term volume retention has led to variable aesthetic outcomes [6, 7].

In recent years, research has indicated that subcutaneous fat contains many stem and regenerative cells replete with cells important in tissue survival and vascularization [8, 9]. Until recently, stem cells were most commonly harvested from adult bone marrow or blood, with cell culturing required due to the low frequency of stem cells from these sources. By comparison, adipose-derived regenerative cells (ADRC) are plentiful and have the ability to differentiate into various cells types. Furthermore, it is possible to use ADRCs therapeutically at the time of their harvest without the need for culture [10–14].

As the nonbuoyant cellular fraction derived from the enzymatic digestion of adipose tissue, ADRCs contain several types of stem and regenerative cells including adipose-derived stem cells (ADSCs), vessel-forming cells such as endothelial and smooth muscle cells and their progenitors, and preadipocytes. Studies show that ADRCs can secrete growth factors that have pro-angiogenic, anti-apoptotic, and pro-adipogenic affects [15–17]. Furthermore, the abundance of vessel-forming cells in ADRCs suggests that these cells

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may have the potential to increase neovascularization. In summary, it is believed that ADRCs may increase graft survival by increasing neoangiogenesis, preventing apoptosis, and encouraging adipocyte differentiation.

With the growing adoption of autologous fat grafting, published preclinical and clinical data describing cell-enriched adipose tissue transplants in breast defect repairs [18] and breast augmentation are increasing [19]. Various methods for preparing adipose tissue grafts have been used including gravitational separation, centrifuge separation, and pressure separation. This pilot study aimed to ensure the safety and efficacy of the Celution 800 System® (Cytori Therapeutics, San Diego, CA, USA), which is the most technologically sophisticated and clinically proven system on the market for ADRC-enriched fat grafting in breast augmentation.

Materials and Methods

Patient Selection

Between November 2007 and April 2008, 20 Japanese women with a mean age of 35.6 years (range, 21–52 years) desiring natural breast augmentation were considered for entry into the study. This prospective physician-initiated clinical study was approved by the institution's ethics committee, and all the patients provided written consent before participation. The study excluded patients with comorbidities including malignant carcinoma within the preceding 5 years, uncontrolled diabetes or cardiovascular disease, autoimmune disorder (i.e., systemic lupus erythematosus), history of keloid formation, thin or flaccid skin, infectious disease, anticoagulation therapy, contraindications to magnetic resonance imaging (MRI) or mammogram, and pregnancy.

Preoperative assessments included medical history, physical examination, height and weight including body mass index (BMI), breast photographs (anteroposterior, lateral, obliques), laboratory blood tests, and breast MRI. Mammograms were obtained for all patients to rule out malignancy within 30 days of the procedure, and pregnancy tests were administered before surgery. To measure change in breast size (BRM), circumferential breast (B) and circumferential chest (C) measurements were obtained preoperatively and at each follow-up visit. In this patient series, improvement in breast size was measured using the formula

$$B - C = BRM. \quad (1)$$

Follow-up evaluations were conducted at 2 weeks, then at 1, 3, 6, and 9 months. Breast measurements and photographs were obtained. At 9 months, MRI, mammography, ultrasound, and patient and physician satisfaction evaluations were collected. Physician satisfaction with procedure results was based on the magnitude of change in breast size (B – C = BRM) from pretreatment to 9 months after the procedure.

Surgical Technique

In this study, patients underwent a single bilateral breast augmentation treatment in an outpatient surgical setting. With the patients under conscious sedation, liposuction and breast augmentation were performed. After the required amount of adipose tissue graft for processing and augmentation was determined preoperatively, tissue was harvested via syringe liposuction followed by processing and delivery of the ADRC-enriched adipose tissue graft. The general procedure flow of adipose tissue harvesting, tissue processing, and delivery of the autologous cell-enriched adipose tissue graft is shown in Fig. 1. All liposuction and breast augmentation procedures in this study were performed by the authors (T.K. and K.I.).

Adipose Tissue Harvesting

Before liposuction, target areas were infiltrated with standard tumescent solution (lactated Ringers, 1% lidocaine and epinephrine). Through 5- to 6-mm skin incisions, adipose tissue was collected from the thighs, hips, buttocks, or abdominal area using a 3-mm Mercedes tip three-hole blunt cannula (Byron Medical, Tucson, AZ, USA) under low negative pressure. Adipose tissue then was divided into two fractions: one for the extraction of regenerative cells and one for use as the fat graft.

Preparation of the ADRC-Enriched Fat Graft

Adipose tissue used for ADRC isolation was injected into the first-generation Celution 800 System®, which with the addition of a proteolytic enzyme reagent, washed and digested the tissue to concentrate the regenerative cells. The Celution 800 System® is a CE Marked device currently under review by the Food and Drug Administration (FDA). The final suspension of ADRCs (~5 ml) was retrieved from the Celution processing chamber using a 10-ml syringe with an 18-gauge spinal needle. The second fraction of adipose tissue then was added into the Celution 800 System®, which washed and gravity filtered it to create an aqueous fat graft. The concentrated ADRCs were added to the fat graft tissue in the Celution 800 System® and mixed to create the ADRC-enriched fat graft. After the completion of tissue processing, the ADRC-enriched fat graft was aseptically transferred to the sterile field using 60-ml Toomey syringes.

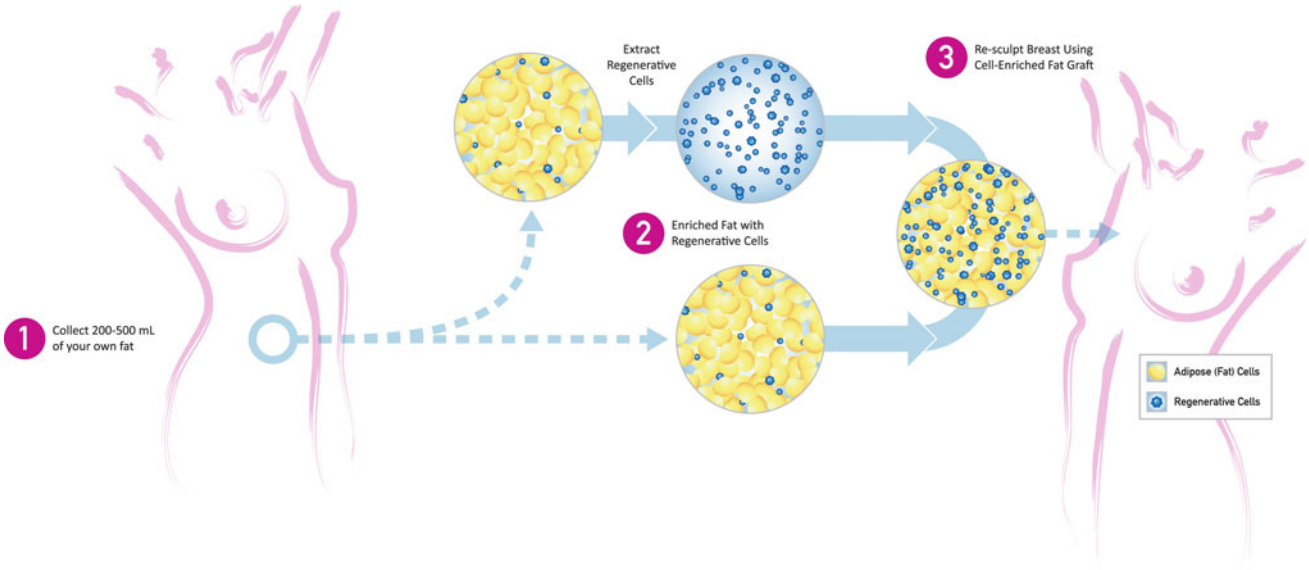

Fig. 1 Surgery flow of harvesting, processing, and delivery of adipose-derived regenerative cell (ADRC)-enriched fat grafts

Delivery of the ADRC-Enriched Fat Graft

Stab incisions were made in the axillary regions of the breasts. Axillary rather than submammary incisions were used because Japanese women tend to heal with discolored or hypertrophic scars, and their small breasts would not adequately hide a submammary scar. Approximately half of the tissue was injected into the subglandular space. Subsequently, two-thirds of the remaining tissue was injected in the subcutaneous space, and one-third was injected into and immediately below the pectoralis major muscle, which provided a good vascular supply to the graft. Multilayer injections of ADRC-enriched fat were made in a fanning manner until the desired results were achieved using the Celbrush® (Cytori Therapeutics, San Diego, CA, USA). The Celbrush® (Fig. 2) is a stainless steel thumb-brush syringe adaptor designed to provide microdroplet dispersion of tissue in 0.50-ml increments. The threaded thumb-brush plunger design minimizes pressure buildup and provides tactile feedback during tissue dispersion.

Injections were performed with the patients in a supine position so that the breast tissue shifted superiorly a few centimeters relative to that of the patient in a sitting position. Generally, about two-thirds of the total graft volume was injected in the lower half and one-third in the upper half. After approximately two-thirds of the total volume was injected, the patient was moved to a sitting position momentarily for assessment of the injection progress, then returned to the supine position for completion of the injections until the desired results were achieved. After routine wound closure, dressings with microfoam tape were applied to support the breasts.

All the patients were discharged from the surgery center after recovery from anesthesia. Postoperative care included antibiotic therapy, analgesics as needed, limitation of activity for 3–7 days, and cold compresses as needed to reduce secondary injury from edema and inflammation.

Fig. 2 Celbrush® (*left*) provides uniform microdroplet graft delivery compared with a conventional syringe (*right*)

Results

The mean total volume of fat harvested via liposuction for cell processing and graft preparation was 1,026.5 ml (range, 660–1,125 ml), which was obtained from the thighs, waist, abdomen, and upper arms. The mean volumes of aqueous fat graft injected were 244.9 ml (range, 166–330 ml) into the right breast and 235.1 ml (range, 166–290 ml) into the left breast. The mean surgery time was 3.5 h (range, 3.0–4.75 h), as seen in Table 1.

Table 1 Liposuction volume, donor site, and graft volume

Patient	Age (years)	Liposuction volume (g)	Liposuction donor site	Amount grafted (ml)	
				Right	Left
1	49	945	T, W, A	200	170
2	33	1,080	T, W, A	250	240
3	21	1,120	T, W, A	250	250
4	33	1,020	T, W, A	215	215
5	36	1,045	T, U	230	230
6	47	1,075	T	240	240
7	42	1,040	T	208	208
8	40	660	T, W, A	166	166
9	52	1,035	T, A	235	249
10	26	1,125	T, A	330	220
11	27	1,100	T, A	270	270
12	23	1,080	T, A	274	274
13	31	905	T, A	226.5	226.5
14	28	1,040	T, A	240	240
15	34	930	T	248.5	248.5
16	42	1,115	T	255	260
17	43	1,005	T	219	192
18	27	1,100	T	290	290
19	37	1,040	T	265.5	227.5
20	41	1,070	T	285	285

Liposuction donor site: thighs (*T*), waist (*W*), abdomen (*A*), upper arms (*U*)

Both viable cell count and cell viability were measured for the ADRCs recovered from adipose tissue before augmentation. After isolation of ADRCs, cell count and cell viability were measured using the NucleoCounter Automated Cell Counting System (New Brunswick Scientific, Edison, NJ, USA). The average number of recovered viable cells per gram of tissue was $3.42 \times 10^5 \pm 1.39 \times 10^5$, and the average cell viability was $85.3 \pm 6.2\%$.

Breast Measurements

The mean preoperative BRM was 8.3 cm (range, 5.0–11.5 cm), whereas 36 weeks after surgery it was 11.6 cm, an increase of 3.3 cm. A minor decrease in BRM (solid line) was noted 2–12 weeks after surgery, with it subsequently remaining stable (Fig. 3). Of the 16 cases, 4 (25%) showed BRM increases of 5 cm or more through 36 weeks. For reference, the dashed line in Fig. 3 represents the chest circumference over the course of the study, and the solid line is the breast volume change over time.

Physician Satisfaction

Physician satisfaction with the treatment result was deemed "excellent" with a BRM increase of 4 cm or more, "good" if BRM increased 2.5–3.9 cm, and "fair" if the BRM increase was less than 2.5 cm. Pre- and postoperative breast measurements were available for 16 of the 20 patients through 9 months. Physician satisfaction was excellent ($n = 6$, 38%), good ($n = 5$, 31%), and fair ($n = 5$, 31%), demonstrating an overall excellent or good physician satisfaction rate of 69% (Fig. 4).

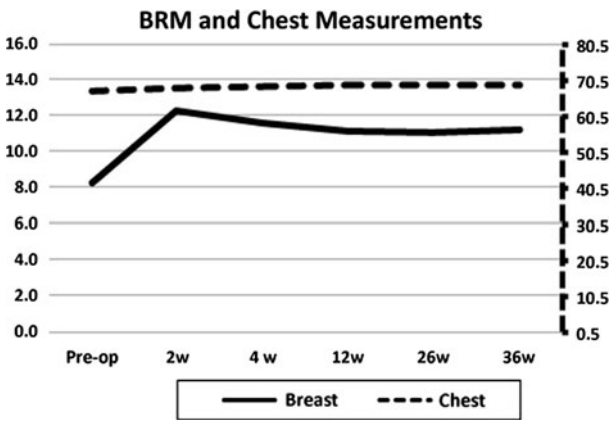

Fig. 3 Change in circumferential breast measurement (BRM) through 36 weeks

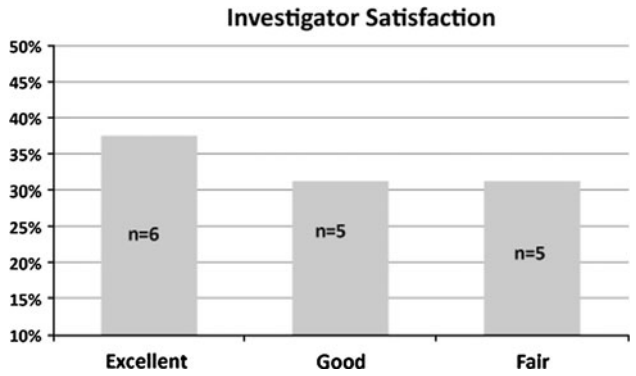

Fig. 4 Physician satisfaction with treatment results at the 9-month follow-up evaluation

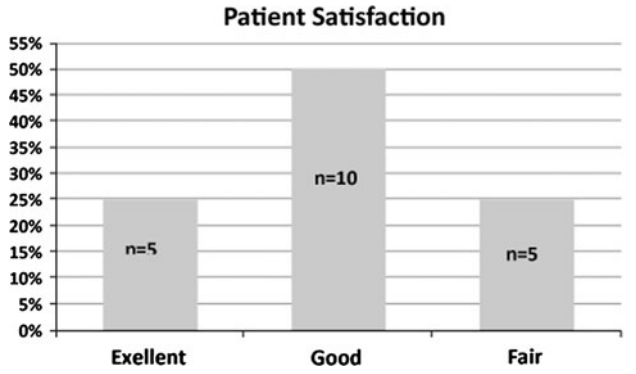

Fig. 5 Patient-reported satisfaction with treatment results at 9 months

Patient Satisfaction

Patient satisfaction with the overall treatment results were collected at 9 months. Patients were asked their overall satisfaction with treatment results in terms of three possible responses: excellent, good, or fair. Satisfaction was reported as excellent ($n = 5$, 25%), good ($n = 10$, 50%), and fair ($n = 5$, 25%), demonstrating "good" or "excellent" overall satisfaction with the treatment results by 75% of the patients (Fig. 5).

Case Photographs

Case 1

A 27-year-old woman had 1,100 g of tissue harvested via liposuction from the thighs and abdomen. Each breast was injected with 270 ml of ADRC-enriched fat graft (540 ml total) during a procedure time of 3:55. From baseline to 9 months after surgery, BRM increased 6 cm (from 7 to 13 cm). Patient and physician satisfaction with the treatment results at 9 months was reported to be "excellent" (Fig. 6).

Case 2

A 21-year-old woman had 1,120 g of tissue harvested via liposuction from the thighs, waist, and abdomen. Each breast was injected with 250 ml of ADRC-enriched fat graft (500 ml total) during a procedure time of 4:20. From baseline to 9 months after surgery, BRM increased 5 cm (from 6.5 to 11.5 cm). Patient and physician satisfaction

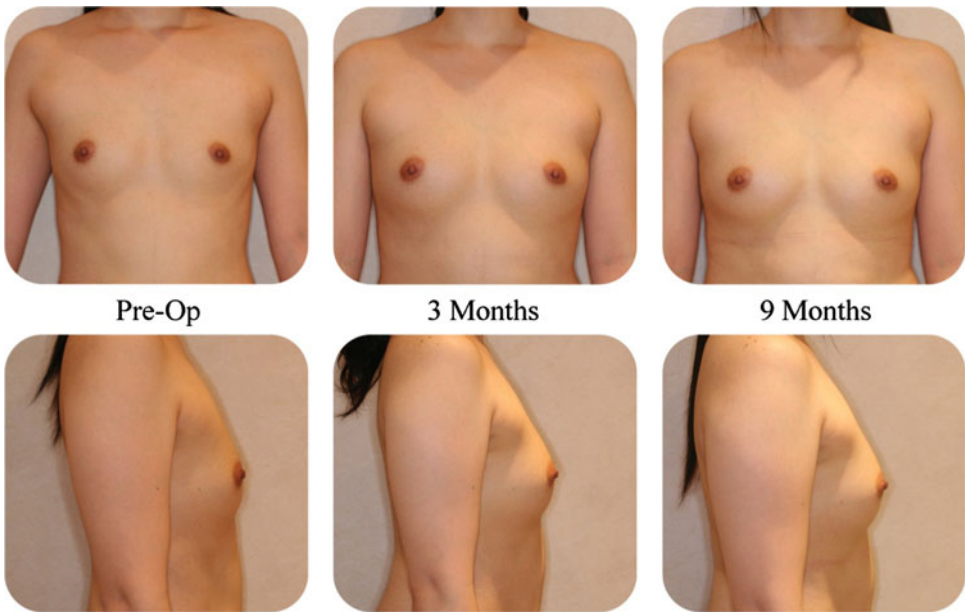

Fig. 6 Case 1 patient photographs pre-procedure, 3 months post-procedure and 9 months post-procedure

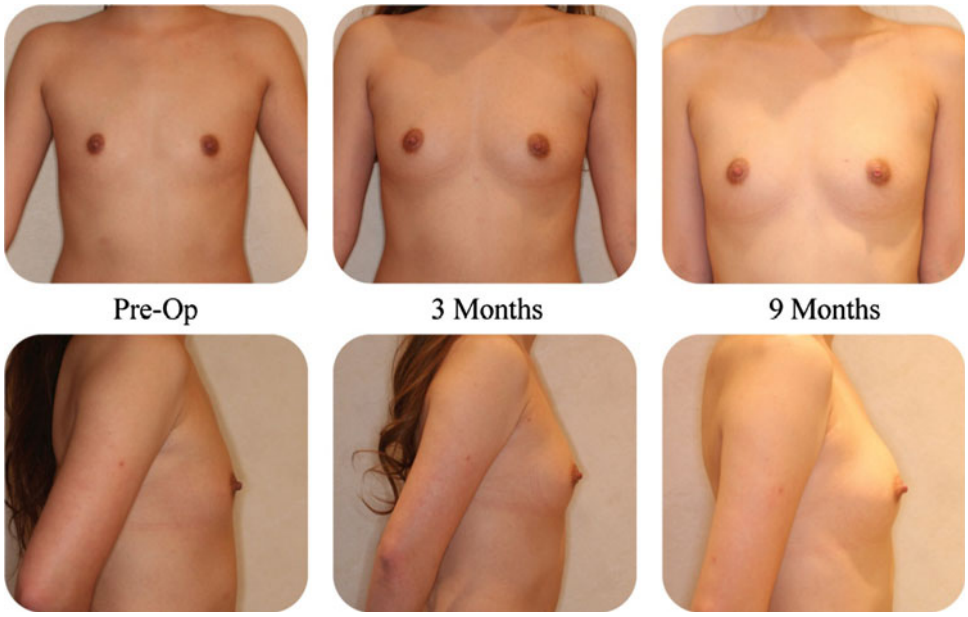

Fig. 7 Case 2 patient photographs pre-procedure, 3 months post-procedure and 9 months post-procedure

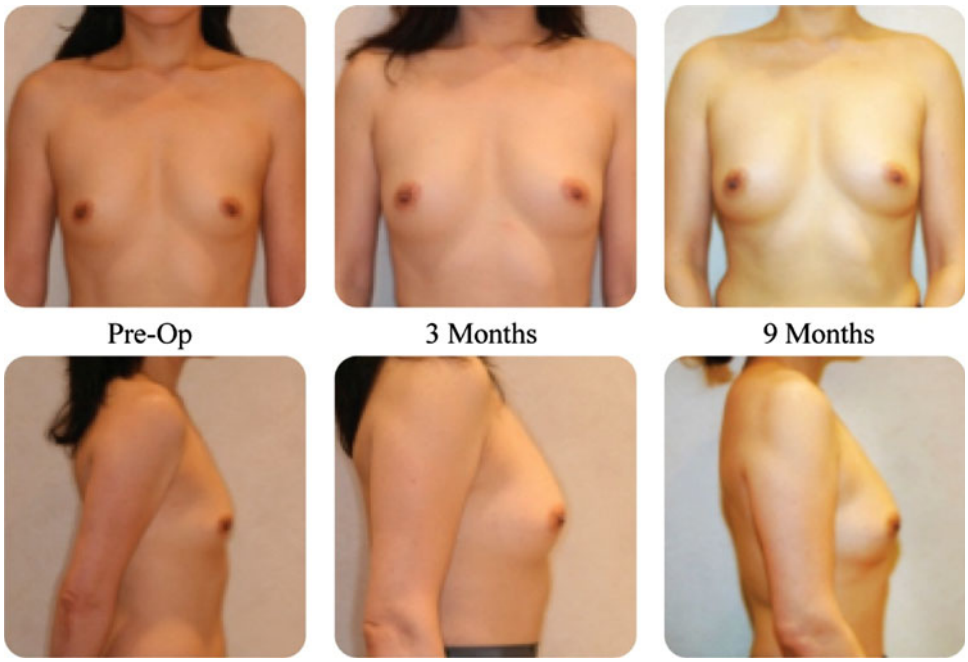

Fig. 8 Case 3 patient photographs pre-procedure, 3 months post-procedure and 9 months post-procedure

with the treatment results at 9 months was reported to be "excellent" (Fig. 7).

Case 3

A 41-year-old woman had 1,070 g of tissue harvested via liposuction from the thighs, waist, and abdomen. Each breast was injected with 285 ml of ADRC-enriched fat graft (570 ml total) during a procedure time of 4:08. From baseline to 9 months after treatment, BRM increased 3 cm (from 10 to 13 cm). Patient satisfaction with treatment results at 9 months was "excellent." However, physician satisfaction with the treatment results was reported to be "good" based on a BRM improvement of 3 cm (Fig. 8).

Frequency of Complications

No serious or unexpected adverse events were noted or reported through the 9-month follow-up period. As with all invasive breast procedures, possible complications after fat injections to the breast include fat necrosis, oil cysts, and calcifications [20–31], some with rates up to 57% [23]. In

this series, 19 of the 20 patients had 9-month mammograms (1 excluded due to pregnancy), and liponecrotic cyst formation with subsequent calcification was seen in a relatively low number of patients ($n = 2$, 11%). The cysts were approximately 1–2 cm in diameter, with calcification noted along the capsule. One cyst improved after needle aspiration, and biopsy was not required of either cyst because there was no indication of a possible malignancy. No other injection-related adverse events were reported.

Discussion

Several factors have an effect on autologous graft survival rates including the method of fat harvesting, the volume of graft injected into a single area, the quality of the harvested fat, and the selection of cannulas for aspiration and injection. During this study, it was noted that results improved as physicians gained experience with the technique. As with any other technical skill, proficiency at producing excellent results is conditional on practice and perfection of the technique.

In this case series, patient selection was not based on the patient's native breast characteristics. However, we have since developed a better understanding of the patients most likely to have the best outcomes. Although we have not yet completed a formal comparative analysis, we believe that better outcomes are achieved when the patient's skin over the breast is thinner and easier to stretch. We expect that more careful patient selection will lead to improved outcomes.

Pre- and postoperative planning also was based on the aqueous nature of the fat graft created by the Celution 800 System®. Because the ADRC-enriched fat graft was not concentrated by a technique such as centrifugation, injected ADRC-enriched grafts contained approximately 35% fluid. This fluid in combination with postoperative edema creates a temporary over-enhanced appearance to the breasts that normalizes approximately 4 weeks after surgery. A new product that we have incorporated into our practice, PureGraft® (Cytori Therapeutics, San Diego, CA, USA), enables removal of almost all the free lipid and fluid before grafting, which has led to a decreased need for overcorrection to account for the fluid.

When the results from autologous fat grafting are evaluated, the following four phases need to be accounted for: the injection of fat + ADRCs + aqueous suspension, the postoperative edema phase, the aqueous resorbtion phase, and the volume retention phase. Figure 9 is an idealized graph demonstrating volume changes and graft retention through the course of treatment with ADRC-enriched fat grafts.

Finally, routine breast imaging is an important part of health maintenance for women. Breast imaging should be obtained before and after any breast surgery to establish a baseline for comparison with future screening mammograms. Working with an experienced radiologist is essential in early detection of breast health issues.

The expectations of both patients and physicians must be managed. A surgeon learning curve must be expected, and regular breast health monitoring should occur. Patient age, tissue thickness, extensibility of the breast skin, and breast mound size before treatment must be taken into consideration because proper patient selection is vital to the success of the procedure.

Study Limitations

The limitations of this clinical study include a prospective cohort study design and a relatively small sample size ($n = 20$). To our knowledge, a randomized, controlled study has not been performed to compare the outcomes of cell-enriched fat transfer with those of traditional fat transfer. Furthermore, graft take is difficult to measure accurately. In this series, BRM was used to measure graft

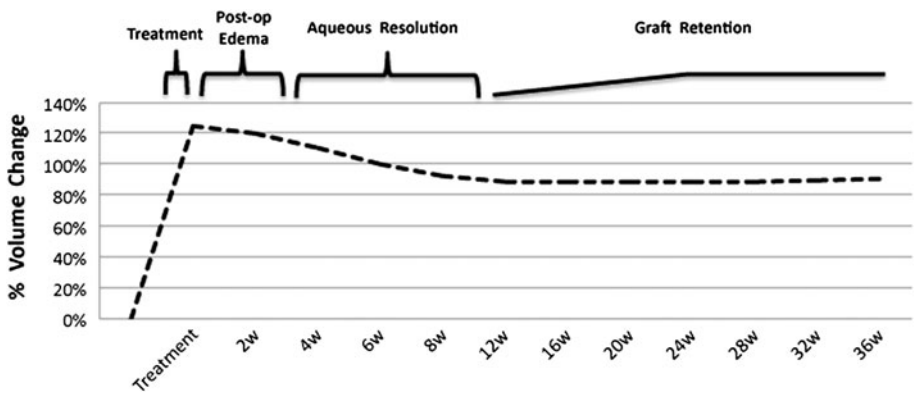

Fig. 9 Phases of volume change after adipose-derived regenerative cell (ADRC)-enriched fat graft augmentation

take, but future studies could consider the use of three-dimensional imaging methods for more accurate documentation of outcomes.

This clinical study was conducted using a homogeneous population of Japanese women with a relatively short follow-up period of 9 months. Our clinical experience suggests that graft take does not change after 9 months, but future studies could consider longer follow-up assessment to document this observation. Larger randomized, controlled clinical trials are warranted for further substantiation of these findings.

Conclusion

With all the patients showing a mean BRM improvement of 3.3 cm from baseline, the results of this study conclusively show that single-treatment breast augmentation using ADRC-enriched fat grafts is safe, effective, and predictable, demonstrating BRM stability over time. Of the 16 cases, 4 (25%) obtained a BRM increase of 5 cm or more through 9 months. Compared with breast sizes attainable using implants, sizes attainable with a single ADRC-enriched fat grafting are much smaller.

This series had a physician satisfaction of 69% and a patient satisfaction of 75% through 9 months, substantiating that ADRC-enriched fat grafting is useful for patients who want slightly larger breasts that have a more natural shape and a soft feel but are not looking for an increase of more than one or two breast cup sizes.

The safety profile of breast augmentation with autologous adipose tissue is consistent with that of other breast procedures, and in our series, we saw a low rate of postoperative benign cystic findings on mammograms (11%). It is our expectation that the data from this study will help to build on the existing data to increase confidence that this option is safe, results in minimal complications, and does not increase mammography abnormalities more than any other breast procedure.

The Celution 800 System® offers physicians an automated closed-circuit system that allows for isolation of regenerative cells and preparation of an ADRC-enriched fat graft. Although ongoing research will continue to improve on our current technique, this study demonstrates that we can confidently provide this treatment to patients and that it is safe, demonstrates minimal complications, and produces long-term results that are predictable.

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Conflict of interest The authors have no other conflicts of interest and did not receive any other support, financial or otherwise, from Cytori Therapeutics.

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