Fat Graft Enhanced With Adipose-Derived Stem Cells in Aesthetic Breast Augmentation: Clinical, Histological, and Instrumental Evaluation

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Abstract

Background: Fat graft enhanced with adipose-derived stem cells (FG-e-ASCs) has been utilized in outcomes of radiotherapy after mastectomy, breast soft tissue defects, ulcers, and loss of substance. The authors present their experience utilizing FG-e-ASCs in breast augmentation.

Objectives: The aim of this study was to evaluate the safety and efficacy of a study group (SG) regarding utilization of FG-e-ASCs in breast augmentation for aesthetic improvement, comparing the results with a control group (CG).

Methods: A total of 46 patients affected by breast hypoplasia were treated with FG-e-ASCs, comparing results with those of a CG (n = 30) treated with fat graft not enhanced with adipose-derived stem cells (FG-ne-ASCs). The preoperative evaluation included a complete clinical evaluation, a photographic assessment, magnetic resonance imaging of the soft tissue, ultrasound, and mammography. Postoperative follow-up took place at 1, 3, 7, 12, 24, and 48 weeks and then annually.

Results: The patients treated with FG-e-ASCs showed 58% maintenance of the contour restoring and of 3-dimensional (3D) volume after 3 years compared with the patients of the CG treated with FG-ne-ASCs, who showed 29% maintenance. In 67.4% (n = 31) of breast augmentations treated with FG-e-ASCs, we observed a restoration of the breast contour and an increase of 10.3 mm in the 3D volume after 36 months, which was observed in only 20.0% (n = 6) of patients in the CG treated with FG-ne-ASCs. Volumetric persistence in the SG was higher than that in the CG (P < 0.0001 SG vs CG).

Conclusions: Utilization of FG-e-ASCs was safe and effective in this series of cases performed.

Level of Evidence: 4

4

Therapeutic
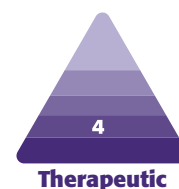
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Adipose-derived stem cells (ASCs) are identified in the stromal vascular fraction (SVF) portion of subcutaneous fat tissue,[1] which has a heterogeneous mesenchymal cell set.[2,3] These cells can be further isolated by utilizing an enzymatic digestion to deplete most of the hematopoietic cell population from the SVF cells or by utilizing a combination of the filtration and centrifugation steps as mechanical digestion.

In the last 10 years, an increasing number of papers have reported on the utilization of fat graft enhanced with ASCs, including radiotherapy-based tissue damage after

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mastectomy,[4,5] breast augmentation,[6] ulcers,[7] and calvarial defects.[8] ASCs can be identified in the mixed cell population referred to as "SVF cells."[9] SVFs might improve fat graft maintenance by increasing vascularity and through the secretion of growth factors that improve fat survival. Some authors have published studies employing fat grafts enhanced with SVFs and ASCs, called in some cases "cell-assisted lipotransfer,"[6,10] yielding some favorable and some unfavorable results employing different methods of cell isolation.[11-13] Cell-assisted lipotransfer was utilized in primary breast augmentation[6,14] and for correcting the outcomes of conservative breast cancer surgeries[9] and congenital deformities,[15] but none of these studies reported on the long-term follow-up in fat graft maintenance.

Many studies[16,17] have been published on the utilization of centrifuged fat graft with the Coleman procedure[18,19] enriched with platelet-rich plasma in plastic surgery in ulcers and loss of substance.[20] Now, we feel the necessity to report the long-term follow-up (36 months) of fat graft maintenance for aesthetic purposes in patients treated with fat graft enhanced with adipose-derived stem cells (FG-e-ASCs).

A multimodal imaging approach performed with magnetic resonance imaging (MRI), ultrasound (US), and mammography (MG) was necessary for studying breast tissue modification following fat graft injection.[21] In addition, we will describe how actual instrumental techniques of imaging, which is now available, can show the physiological modifications of the breast tissue, estimate the injected volume fat reabsorption, and evaluate fat replication throughout neoangiogenesis in addition to tissue characterization. The results obtained suggest the efficacy of FG-e-ASCs and the satisfaction of the patients treated with this method.

METHODS

The retrospective observational case-series study reported was conducted following the principles outlined in the Declaration of Helsinki and internationally consented ethics in clinical research.[22] A quality assessment was carried out based on the Strengthening the Reporting of Observational studies in Epidemiology checklist.[23] All patients received detailed oral and written information about the study, including the risks, benefits, and alternative therapies. All patients signed an informed consent form before any study procedures were undertaken. The study protocol was performed according to the European rules (1394/2007 EC) and EMA/CAT recommendations (20 June 2014 EMA/CAT/600280/2010 Rev 1).

Patients

Between January 2008 and December 2016, 46 patients (study group [SG]) diagnosed with breast hypoplasia (19

patients with a moderate grade [Figure 1A-C] of bilateral hypoplasia, 9 patients affected by a high grade of bilateral hypoplasia [Figure 2A,D], 3 patients with prostheses removal, 12 patients with a low grade of bilateral hypoplasia [Figure 3A,D], and 3 patients with unilateral breast hypoplasia) were treated with FG-e-ASCs for breast augmentation. The SG was composed of 46 females aged 22 to 53 years (average age, 36.52 years). There were 33 premenopausal patients (71.8%). To establish the long-term follow-up of fat graft maintenance, we compared the results obtained with a control group (CG) made up of 30 patients treated with fat graft not enhanced with adipose-derived stem cells (FG-ne-ASCs) according to the Coleman technique[18,19] (centrifuged fat graft alone). The CG comprised 30 females aged 21 to 56 years (average age, 38.5 years), all of whom were affected by breast hypoplasia (2 patients affected by unilateral breast hypoplasia, 2 patients after prosthesis removal, 7 patients affected by a high grade of bilateral hypoplasia, 12 patients with a moderate grade of bilateral hypoplasia, and 7 patients with a low grade of bilateral hypoplasia). There were 21 premenopausal patients (70%). All enrolled patients (the SG and CG were composed exclusively of females) underwent a full preoperative screening, including a complete clinical evaluation and photographic and instrumental assessment performed with MRI, US, and MG. Postoperative follow-up took place at 1, 3, 7, 12, 24, and 48 weeks and then annually for 5 years.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: age 18 to 75 years and a history of breast hypoplasia or prostheses removal. Additional inclusion criteria in both groups were patients with BMI between 20 and 35 kg/m^2 and sufficient fat in the abdomen, thigh, flank, and inner knee regions (sites of fat harvesting). The exclusion criteria were divided into 2 types: local and systemic. The systemic criteria include anti-aggregating therapy, bone marrow aplasia, uncompensated diabetes, sepsis, and cancer. The local criteria include cancer loss of substance, outcomes of breast cancer, and uncontrolled comorbidities. Tobacco use or genetic disorders were not considered as exclusion criteria.

Clinical Data Assessment

The following characteristics were prospectively recorded in the dataset: demographic data, age, BMI, surgical management, and surgical complications (Table 1). All of the therapeutic options were discussed and decided by a multidisciplinary team, including a breast surgeon, a plastic surgeon, and a radiologist. During the first 5 years, patients followed up every 6 months by clinical examination and every 12 months by surveillance with MG, US, and MRI.
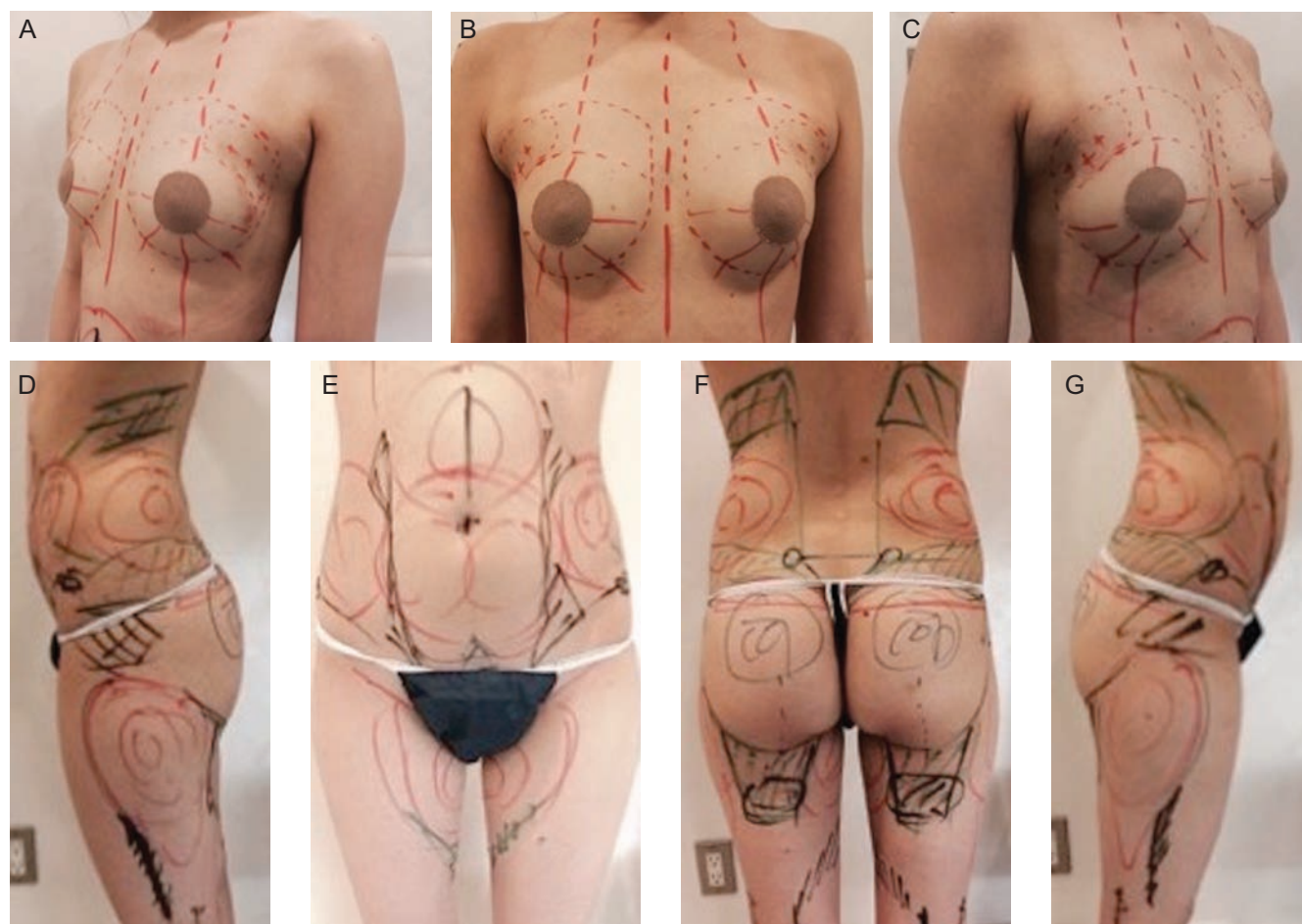
Figure 1. Technical analysis of this 29-year-old female patient's study design affected by breast hypoplasia. (A) Oblique left preoperative view with details of the site injections in the left breast (superior and inferior lateral quadrant and periareolar regions). (B) Preoperative design in frontal view with complete details of the site injections in both breasts (superior and inferior lateral and internal quadrants, peri-areolar region, inferior and superior poles). (C) Oblique right preoperative view with details of the site injections in the right breast (superior and inferior lateral quadrants and periareolar region). (D) Lateral left preoperative view with details of the anatomical areas in which the fat harvesting was performed (abdomen, outer thigh, flank). (E) Frontal (abdomen, inner thigh, flank) and (F) back preoperative views with details of the anatomical areas in which fat harvesting was performed. (G) Lateral right preoperative view with details of the anatomical areas in which fat harvesting was performed (abdomen, outer thigh, flank).

Abnormal clinical findings were further investigated as appropriate. Breast soft tissue modifications, such as cysts, macrocalcifications, and microcalcifications, were documented by clinical examination, radiological tests, and/or pathological assessment.

Fat Graft Enhanced With ASCs

The cell extraction and fat tissue purification procedure mainly entails 2 steps. Step 1 starts with liposuction (715.4 mL average in all patients; range, 250-1080 mL) in the abdominal region and/or flank and thighs (Figure 1D-G) using 3-mm cannulae. Maintaining an aseptic technique, we removed the plunger of the 60-mL Luer-Lock syringes and closed the tip with a cap. One-half of the fat tissue harvested (234.46 mL average) was placed into the tissue collection container of the Celution 800/CRS System (Cytori Therapeutics Inc., San Diego, CA, http://www.cytoritx.com).

Through a wash cycle, blood and free lipid were removed from the tissue, and the Celase 835/CRS Reagent was added to enzymatically digest the tissue, which released SVFs containing ASCs. After additional wash and centrifugation cycles, 4 to 5 mL of the SVF-ASC suspension was extracted from the system. In the second step, the remaining part of the fat tissue harvested was added to the tissue collection container, and a washing step was automatically carried out. Once completed, the 4 to 5 mL of the SVF-ASC suspension was added and mixed with
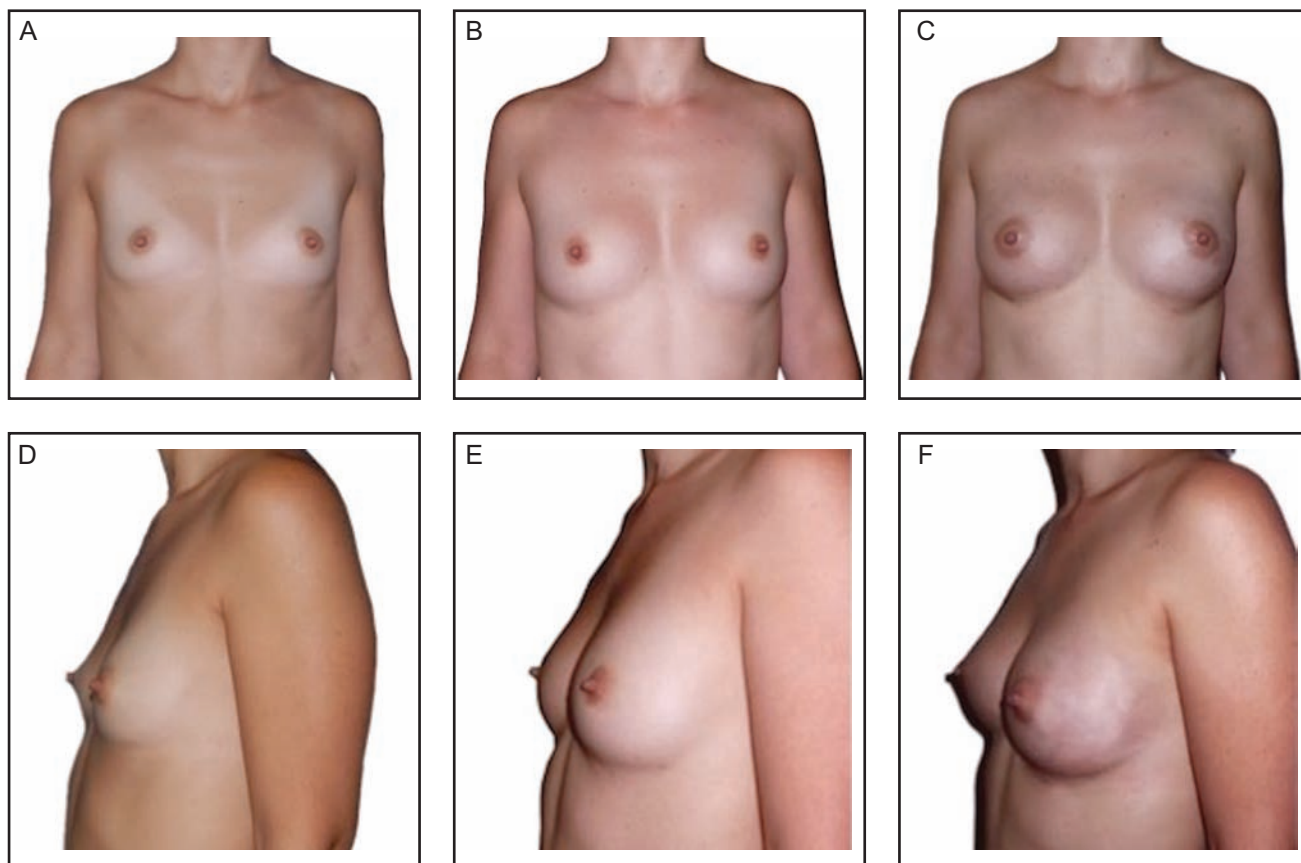
Figure 2. This 29-year-old female (the same patient shown in Figure 1) with breast hypoplasia was treated with 2 fat graft enhanced with adipose-derived stem cells injections. (A, D) Preoperative views at baseline with bilateral hypoplasia. (B, E) Postoperative views after 6 months and 1 treatment, with breast volume improving in both breasts. (C, F) Postoperative view 12 months after the second treatment with improving breast volume that is evenly distributed in the superior and inferior lateral and internal quadrants, periareolar region, and inferior and superior poles. Details of the improving soft tissue volume are shown in the superior and inferior lateral quadrants and periareolar region.

the washed fat graft, resulting in approximately 429.61 mL (range, 60-620 mL) of ASC-enhanced fat tissue for grafting that we call FG-e-ASCs (Table 1). This newly processed cell-enhanced fat graft typically consists of 25% to 30% of water, which will be reabsorbed by the body in the postoperative period. This overall process is controlled through automated sensors and processing algorithms that ensure standard handling of the tissue and cells, and the process is completed within 160 minutes. The FG-e-ASC was transferred into 10-mL Luer-Lock syringes and aseptically re-injected into the patient employing specific 1.5-mm-diameter microcannulae for implantation.

Fat Donor Site and Anesthesia

The donor site region (abdomen and/or flanks and/or thighs and/or inner knees) was infiltrated with a cold solution containing 1 mL of adrenaline per 500 mL of saline solution to reduce the bleeding during the treatment. An inverse

relationship has been observed between the amount of blood in the harvested fat and the viable number of adipocytes.[24] Local anesthesia was not utilized. The procedure was performed utilizing sedation and general anesthesia. Fat tissue was harvested after 6 minutes employing a 3-mm-diameter cannula and a 60 mL Toomey syringe.

Fat Graft Not Enhanced With ASCs

FG-ne-ASCs was performed according to the Coleman technique. Adipose tissue was collected from the abdomen and/or flanks and/or thighs and/or inner knees (Figure 1D-G) with the same cannula utilized in FG-e-ASCs. Maintaining asepsis, we removed the plungers from the syringes; after closing them with a cap, we positioned them flat in the sterile centrifuge. The syringes containing fat tissue were processed for 3 minutes at 3000 revolutions per minute. This procedure obtained purified adipose tissue, preserving the integrity of the adipocytes but
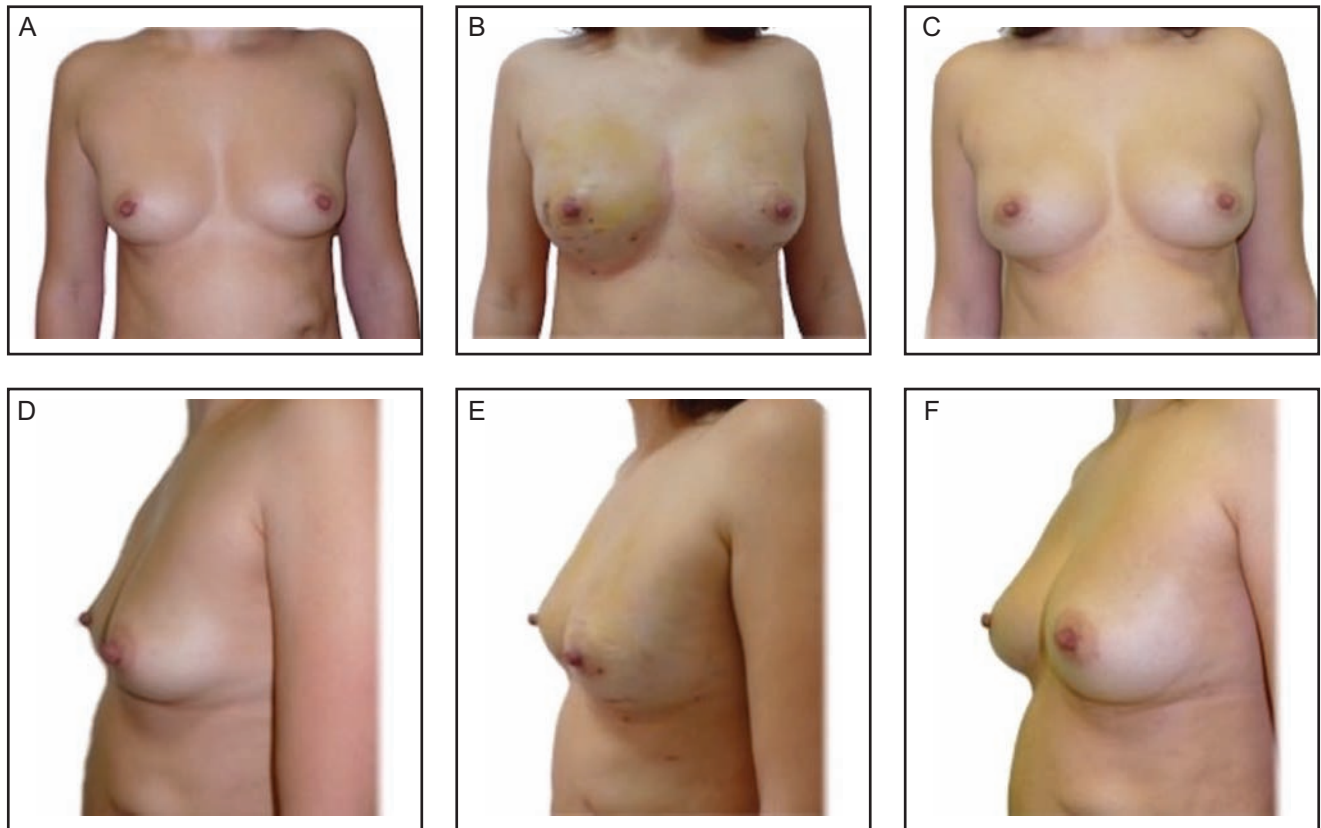
Figure 3. This 43-year-old female with breast hypoplasia was treated with 1 fat graft enhanced with adipose-derived stem cells injection. (A, D) Frontal preoperative view at baseline with bilateral hypoplasia. (B, E) Postoperative view after 3 weeks and 1 treatment show volume improving in both breasts. (C, F) Postoperative view 12 months later shows good fat graft maintenance that is evenly distributed in the superior and inferior lateral and internal quadrants, periareolar region, and inferior and superior poles. The soft tissue volume has improved particularly in the superior and inferior lateral quadrants and the periareolar region.

separating the fluid fat portion from the serous bloody part. The purified and centrifuged fat was placed in 1-mL Luer Lock syringes and aseptically re-injected utilizing 1.5-mm-diameter microcannulae for implanting. No additional SVF or ASC was performed.

Fat Injection Technique

The processed fat tissue was injected for breast augmentation into 7 regions: the superior and inferior lateral and internal quadrants, the periareolar region, and the inferior and superior poles (Figure 1A-C). The area destined to receive the fat injection was decided, in all patients, based on the necessary corrections analyzed through MRI scans and clinical assessment. The processed fat tissue injection was performed utilizing the "gentle technique"[24] based on a slow and gentle injection implanting linear deposits of fat graft in the suprafascial, retroglandular, and intraglandular spaces.[24,25] For this reason, the FG-e-A and FG-ne-A were implanted only into the subcutaneous space (not into the pectoralis major muscle and parenchyma) in multiple tunnels with slow and controlled movements through different entrances (inframammary fold, higher and lower external quadrants, higher and lower internal quadrants, and periareolar) to underscore the importance of a nontraumatic procedure to maximize the integrity of the grafted tissue and to maximize the contact surface between the fat processed and the host's capillaries.[24,26] The diffusion of nutrients from the neighboring capillaries is essential for adipocyte survival and favors their integration with the surrounding tissue.[24,26] According to the patient's needs, 80 to 280 mL (average, 180 mL) of fat grafting was injected into each breast for a total of 360 mL (range, 160-560 mL) per patient (Table 1). The incisions were closed with 5-0 nylon sutures.

Evaluation of Fat Amount to Inject Into Each Breast

A careful anamnesis (patient's needs), a clinical examination (measurement of breast volume), and photographs

Table 1. Patient Data and Fat Graft Assessment

	Study group	Control group
No. of patients	46	30
Mean age at surgery, y	36.2 (range, 22-53)	38.5 (range, 21-56)
Mean BMI at surgery, kg/m^2	27 (21-33.16)	27 (21-33.16)
Bilateral hypoplasia	19 (moderate), 9 (high), 12 (low)	12 (moderate), 7 (high), 7 (low)
Unilateral hypoplasia	3	2
Outcomes of prostheses removal	3	2
Premenopausal	33 (71.8%)	21 (70%)
Fat maintenance percentage (1 year later)	70.8% ± 5% (all patients)	41.4% ± 5% (all patients)
Fat maintenance percentage (3 y later)	58% ± 8% (all patients)	29% ± 8% (all patients)
Fat maintenance volume (3 y later)	10.3 mm ≥ (31 patients)	10.3 mm ≥ (6 patients)
Fat maintenance volume analysis during follow up (first year)	•43.3 mm ≥ 3 weeks later (35 patients) •29.5 mm ≥ 6 months later (35 patients) •25.7 mm ≥ 12 months later (35 patients)	•43.3 mm ≥ 3 weeks later (35 patients) •29.5 mm ≥ 6 months later (18 patients) •25.7 mm ≥ 12 months later (7 patients)
Cyst formation and calcification	11	9
Fat necrosis	0	0
Second-fat injection (re-treatment)	7 (range, 60-190 mL for each breast)	9 (range, 60-190 mL for each breast)
Mean fat graft injected volume for each breast	180 mL (range, 80-280 mL)	180 mL (range, 80-280 mL)
Mean fat graft injected for each patient	360 mL (range, 160-560 mL)	360 mL (range, 160-560 mL)
Mean fat graft harvested volume	715.4 mL (range, 250-1080 mL)	715.4 mL (range, 250-1080 mL)
Mean fat graft obtained volume	715.4 mL (range, 250-1080 mL)	715.4 mL (range, 250-1080 mL)

(defects evaluation) were performed to evaluate the optimal volume of fat to graft. In particular, each breast has been considered to be like a "cone" geometric figure. For this reason, the geometric formula *Volume = π × r^2 × h / 3* (base area × height, divided by 3) to evaluate the initial volume of each breast was applied. In addition, the MRI scansion was performed in all patients before the FG-e-ASCs and FG-ne-ASCs injections, with the aim to complete the breast volume evaluation with 3-mm-thick slices. The patient's needs were matched with the initial breast volume calculated and with the fat obtained. The optimal volume of fat to inject into each breast was established by applying the geometric formula $V = π × r^2 × h / 3$ and injecting the same amount of fat in milliliters corresponding to the initial volume expressed in cm^3 (eg, breast with radius (*r*) of 6 cm and height (*h*) 5 cm; cone volume = 3.14 × 36 × 5 = 565.2 / 3 = 188.4 cm^3. The amount of fat graft injected will be 188.4 mL).

Breast Volume Evaluation

The MRI scansion was performed in all patients before the first treatment, again at 6 and 12 months after the FG-e-ASCs and FG-ne-ASCs injections, and then annually. In fact, in the postoperative follow-up, the US, MG, and MRI scansions were performed annually after the first year, with the aim of determining the breast volume, macrocalcifications, and microcalcifications. A 1.5-Tesla scanner (Hitachi, MS, Echelon Oval, Tokyo, Japan) was employed with 3-mm-thick slices. OsiriX software, 32 bits, free version (Pixmeo, CA) was utilized to calculate breast volume. Two calculations were conducted per examination, and the determined average was taken as the final breast volume. Based on the acquired MRI scans, volumetric fat site assessments into the breasts were also calculated, utilizing as edges the anterior axillary line, anterior margin of the pectoral muscle, medio-sternal line, skin, and nipple. They were assessed utilizing a 3D reconstruction on a separate workstation (ADW 4.0; GE Medical Systems, Milwaukee, WI).

Clinical Evaluation Assessment

Clinical outcomes were evaluated with Equipe-evaluation and patient self-evaluation. The Equipe-evaluation was based on clinical observation employing a scale of 6 values

(excellent, good, discreet, enough, poor, inadequate). The patient-based self-evaluation utilized these same 6 values. The factors and variables considered were pigmentation, vascularization, pliability, thickness, itching, and pain.

Histological Fat Tissue Characterization

As a secondary endpoint of the paper, a histological evaluation was conducted to compare on a translational basis the 2 different fat grafting procedures. The postoperative biopsy was not considered necessary. The radiological findings have not showed suspected lesions, confirming the safety of the procedures.

Characterization, Isolation, and Expansion of ASCs and SVFs Derived From Fat-Harvested Tissue

The harvested fat tissue was washed 3 times with phosphate-buffered saline (PBS) and suspended in an equal volume of PBS and 0.1% collagenase type I (C130; Sigma- Aldrich, Milan, Italy) prewarmed to 37°C. Fat graft was positioned in a shaking water bath at 37°C with agitation for 60 minutes and centrifuged for 10 minutes at 600 g. The supernatant with mature adipocytes was collected. The ASCs-SVFs pellet was resuspended in erythrocyte lysis buffer (155 mm NH_4Cl, 10 mm $KHCO_3$, and 0.1 mm Ethylenediamine tetra-acetic acid) and incubated for 5 minutes at room temperature. After centrifugation for 5 minutes, the pellet was resuspended in a few microliters of growth medium and passed through a 100-mm Falcon strainer (Becton and Dickinson, Sunnyvale, CA), and the cellular population was counted utilizing a hemocytometer. In 17 selected patients by simple randomization, we calculated nucleated SVFs that were 448.403 ± 35.645 cells/mL of fat processed.

The allocation sequence was generated utilizing an online randomization generator (https://www.randomizer.org) and was concealed by a person unrelated to the trial management group. The participants, study personnel, and outcome assessors were all blinded to treatment allocation, and blinding was maintained until all data were analyzed. Then digestion was plated in Dulbecco's Modified Eagle Medium (DMEM) (Euroclone, Pavia, Italy) added with 10% (v/v) fetal bovine serum (FBS; Euroclone), 2 mm L-glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin, and 0.25 mg/mL Fungizone (Invitrogen, Milan, Italy) at a density of 2500/5000 cells/cm^2 of surface area. This initial passage of primary cell culture was referred to as passage 0. After 48 hours of incubation at 37°C at 5% CO_2, the cultures were washed with PBS and maintained in stromal medium until they achieved 75% to 90% confluence. ASCs were passaged by trypsin (0.05%) digestion and plated at

a density of 5000 cells/cm^2 (passage 1). The medium was changed every 3 days, as previous reported.[16]

Statistical Analysis

Comparison between the SG and CG was conducted with a *t* test or Mann-Whitney U test for breast volumetry, fat graft volume, cell counting, answers to questions on the self-assessment questionnaire, and surface markers expression. The data are expressed by mean (range and standard deviation), median (range), and percentages. For the assessment of histological parameters, data are expressed as mean values ± standard error of the mean. Statistical significance was defined as *P* < 0.05 (2-tailed).

RESULTS

Clinical Assessment

The injections of FG-e-ASCs and FG-ne-ASCs were successfully performed in all patients (SG and CG). The follow-up was performed after baseline at 1 week (T1), 3 weeks (T2), 7 weeks, 3 months, 6 months, 12 months, and then annually. The follow-up was performed in all patients (SG and CG) until the third year after the last fat graft injection. Many patients in the CG were not available to come back 2 years later. In fact, after the third year, 21 patients (46%) of the SG and 13 patients (43%) of the CG were controlled at the fourth year, whereas 11 patients (24%) of the SG and only 2 patients (7%) of the CG were controlled in the fifth year. Mean follow-up was 36 months (range, 12-60 months). The average age of the patients was 36.24 years (range, 22-53 years).

In 76.1% (n = 35) of the breast augmentations treated with FG-e-ASCs (SG), we observed a restoration of the breast contour and an increase of 43.3 mm in the 3D volume after 3 weeks (T2) (Figure 3B,E), 29.5 mm after 6 months (T5) (Figure 2B,E) and 25.7 mm after 12 months (T6) (Figures 2C,F and 3C,F), which was observed in only 23.3% (n = 7) of patients in the CG treated with FG-ne-ASCs.

The patients treated with FG-e-ASCs showed 58% maintenance of the contour restoration and 3D volume after 3 years compared with the patients of the CG treated with FG-ne-ASCs, who showed 29% maintenance. In 67.4% (n = 31) of the breast augmentations treated with FG-e-ASCs, we observed a restoration of the breast contour and an increase of 10.3 mm in the 3D volume after 36 months, which was observed in only 20.0% (n = 6) of the patients in the CG treated with FG-ne-ASCs.

All patients in both the SG and CG who underwent the treatments were satisfied with the resulting texture, softness, and volume contours. In both groups (SG and CG), the majority were satisfied with the results of the fat grafting

Table 2. Patient Satisfaction Data

	Study group	Control group
Number of patients	46	30
Self-evaluation of cosmetic results (score range, 1 ([excellent] - 6 [very poor])	Excellent (1): 13 Very good (2): 10 Good (3): 12 Sufficient (4): 11	Good (3): 7 Sufficient (4): 12 Poor (5): 8 Very poor (6): 3
Satisfaction with final result (self-evaluated as sufficient or better)	46 (100%)	19 (63%)
Available to next fat grafting	35	7
Would recommend the fat injection to a friend	46 (100%)	19 (63%)
Available to breast augmentation with fat injections	46 (100%)	30 (100%)
Sufficiently informed about risks and complications	46 (100%)	30 (100%)

(P = 0.401), were available to undergo the fat grafting procedure again (P > 0.789), and would recommend the fat injection treatment to a friend (P = 0.373). Regarding the self-evaluation of cosmetic results, scores ranged from 3 to 6 in the CG and from 1 to 4 in the SG (P = 0.033). The results showed a hard trend for patients in the SG to be more satisfied than patients in the CG (Table 2). Satisfaction grade assessment questionnaire analysis showed that all people in both groups (SG and CG) would choose to undergo breast augmentation with fat injections and that they were sufficiently informed about the risks and complications of this treatment, including the risk of reabsorption of the fat graft and the strong possibility of needing to repeat the treatment (Appendix A, available as Supplementary Material online at www.aestheticsurgeryjournal.com).

When the satisfaction grade was evaluated through a visual analog scale, individuals in both the SG and CG were similarly satisfied (P = 0.23). Figures 2 and 3 show females who were categorized as showing "improvement" by all peers. When computing the new scores, patients in the SG and CG respectively scored (average) 4.8 and 2.7 (P = 0.34) and, therefore, were regarded as presenting better improvement in the SG.

Imaging Assessment: Breast Surveillance

During breast surveillance, any tissue modification needs to be well known to avoid a misleading interpretation between benign and malignant growth. To better identify the glandular and adipose tissues, a multimodal imaging approach seems to provide the right answer for studying breast tissue modification following fat grafting. Figure 4 shows the physiological modifications of the breast tissue and evaluates fat replication throughout neoangiogenesis. In particular, Figure 4 shows the increase in the vascularity of the left breast compared with the contralateral right breast after fat grafting. In light of this concept, it is also possible to identify the new fat (placed and maintained) in MRI scans during postoperative evaluation as the adipose tissue with new
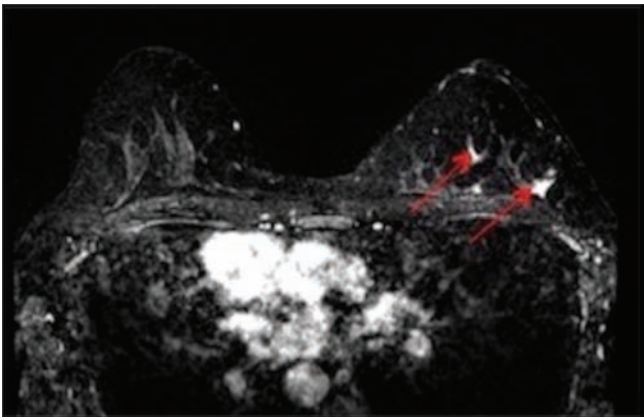

Figure 4. Bilateral axial T2-weighted short tau inversion recovery MRI scans of the breasts obtained 12 months after the last fat graft session (in the same patient reported in Figure 3) in which contrast uptake is evident in the glandular tissue and the fat graft boundary. In the left breast, very small vessels are depicted in the glandular area (red arrows).

small vessels prevalently distributed in the subcutaneous space and retroglandular area where it was initially injected.

Fat Reabsorption

Injected fat tissue reabsorption was evaluated with instrumental MRI and US. The volumetric persistence of fat graft in the SG was higher (70.8%) than that in the CG (41.4%) (P < 0.0001 vs CG) 1 year after the last fat grafting. Compared with breast augmentation utilizing FG-ne-ASCs, augmentation with FG-e-ASCs showed a lower rate of fat reabsorption. Cyst formation, microcalcifications, macrocalcifications, and cytosteatonecrotic areas were detected by MRI. Cyst formation and calcifications were identified in 11 patients in the SG and in 9 patients in the CG (P = 0.089). Fat necrosis was not identified. Seven patients underwent a second treatment. In the long-term follow-up, adverse events such as infections and skin necrosis were not observed in either group.
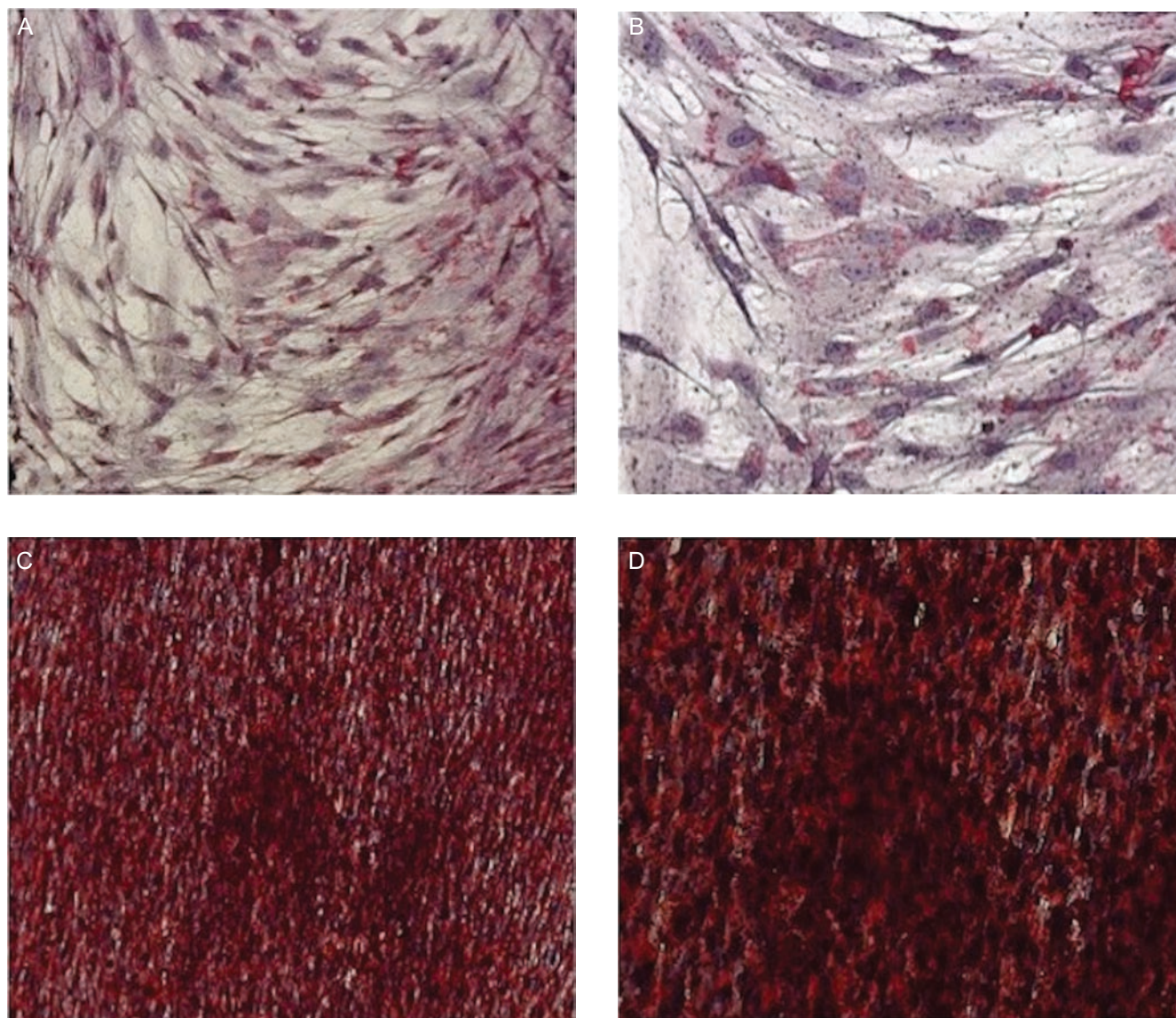
Figure 5. Adipogenic differentiation of adipose-derived stem cells. (A, B) Oil Red O staining of control adipose-derived stem cells (Dulbecco's Modified Eagle Medium and 10% fetal bovine serum) at (A) 10× magnification and (B) 20× magnification, and adipose-derived stem cells after induction of adipocyte differentiation at (C) 10× magnification, and (D) 20× magnification.

Histological Assessment

Differentiation of cultured ASCs (Figures 5A,B and 6A,B) in both an osteogenic sense (Figures 6C,D and 7A,B) and an adipogenic sense (Figures 5C,D and 8A,B) was verified in third-passage confluent cells. Briefly, for adipogenesis, ASCs were cultured in DMEM supplemented with 10% FBS, 100 mm L-ascorbic acid, 1 mm dexamethasone, 0.5 mm 1-methyl-3-isobutylxanthine, 100 mm indomethacin, and 10 mg/mL human recombinant insulin (Sigma-Aldrich S.r.l, Milan, Italy). Control was cultured in DMEM plus 10% FBS. The medium was changed every 3 days for 3 weeks, and adipogenesis was assessed by Oil Red O staining (Figure 5A-D). Osteogenic differentiation was induced in DMEM supplemented with 10% FBS, 200 mm L-ascorbic acid, 0.1 mm dexamethasone, and 10 mMb glycerol phosphate (Sigma-Aldrich S.r.l). Control was cultured in DMEM plus 10% FBS. The medium was changed every 3 days for 21 days. To assess mineralization corresponding to osteogenic differentiation, intracellular calcium deposits were stained with Alizarin red (Figure 6C-D). Images were obtained at 20× magnification through a digital camera (Dxm1200F; Nikon, NY, NJ) connected to a computer utilizing Nikon ACT-1 software.

Histological Analysis of Fat Graft (±ASCs-SVF) Before Transplantation

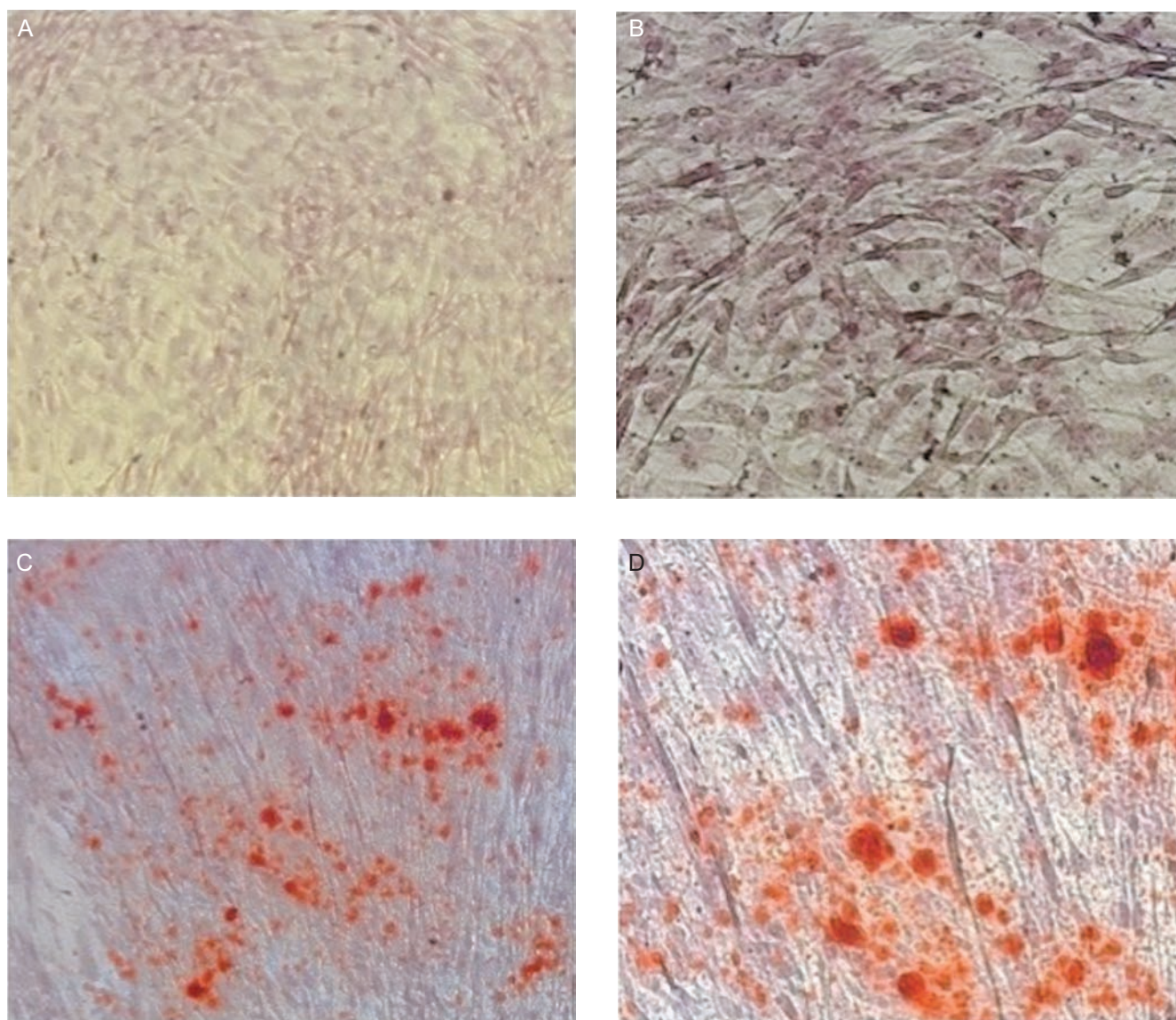Histological analysis reporting the different architectural distribution of adipocytes was performed for FG-ne-ASC

Figure 6. Osteogenic differentiation of adipose-derived stem cells. Oil Red O staining of control adipose-derived stem cells (Dulbecco's Modified Eagle Medium 10% fetal bovine serum) at (A) 10× magnification and (B) 20× magnification. (C, D) Alizarin red-stained adipose-derived stem cells after induction of osteogenic differentiation at 10× magnification (C) and 20× magnification (D).

and FG-e-ASC samples (n = 17). Figure 9 shows representative images of FG-ne-ASCs and FG-e-ASCs. The FG-ne-ASCs (Figure 9A,C) represents the part of fat tissue composed by normal-shaped adipocytes vs the FG-e-ASCs (Figure 9B,D) composed of stromal scaffolding of adipose tissue, with cell clusters that identify a small group (>15 cells) of round-shaped cells within the fat context.

DISCUSSION

Fat grafting is an important clinical application in the treatment of breast hypoplasia. The simplicity of the procedures and the absence of prostheses and of subsequent

visible scarring prompted increased interest in this technique. The breast fat grafting technique has been utilized for many years by surgeons and scientists with documented experience in the manipulation of fat, as reported by Gentile et al.[24,26] Therefore, we feel the necessity to better explain the correct approach to achieve more natural results with less fat reabsorption, represented by breast volume evaluation, fat injection technique, and methods of fat enrichment.

Regarding the fat injection technique, it is fundamental to choose a correct procedure. We have utilized the Coleman technique for many years for both preparation (through a 3000-rpm centrifugation per 3 minutes)
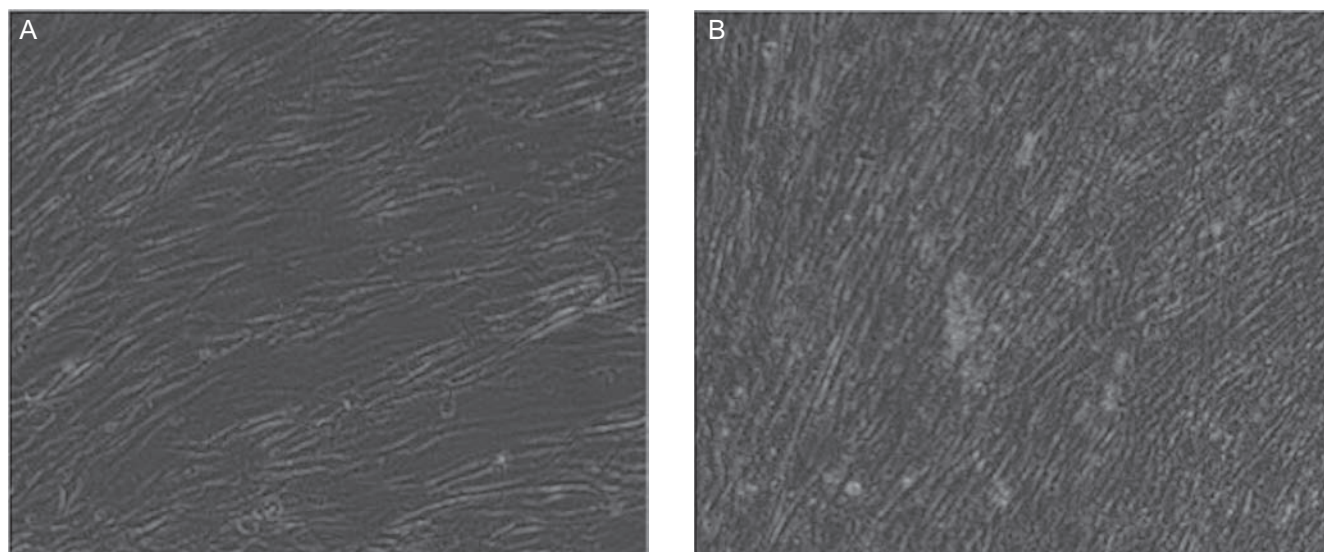
Figure 7. Cellular morphology for adipose-derived stem cells under phase contrast microscopy. (A, B) Contrast phase micrographs of adipose-derived stem cells (A) showing the osteogenic differentiations at 10× magnification (B).
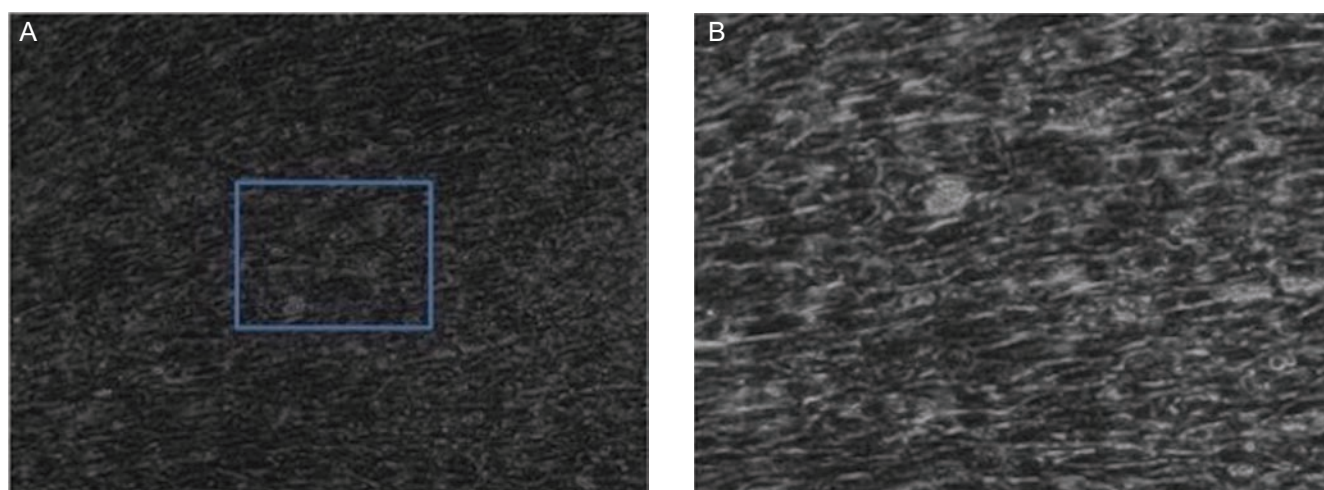

Figure 8. Cellular morphology for adipose-derived stem cells under phase contrast microscopy. (A, B) Contrast-phase micrographs showing the adipogenic differentiations of adipose-derived stem cells at 10× magnification (A) and 20× magnification (B).

and for the fast and dynamic way of infiltration. For several years, Gentile et al[24,26] have compared the different methods of centrifugation, filtration, and fat enrichment for various uses in plastic surgery, verifying that a more delicate and 3D infiltration distributed in the different compartments of the breast will reduce the percentage of fat reabsorption over time. For this reason, in the present study, the processed fat tissue was injected into 7 regions for breast augmentation: superior and inferior lateral and internal quadrants, periareolar region, and inferior and superior poles. The area destined to receive the fat injection was decided based on the necessary corrections.

The processed fat tissue injection was performed with the "gentle technique"[24] based on a slow and gentle injection implanting linear deposits of fat graft in the supra-fascial, retro-glandular and intra-glandular space.[24,25]

With respect to breast composition, this technique is mainly based on a mixture of 3 tissue types: glandular, fat, and fibrous tissue. For this reason, the FG-e-ASCs and FG-ne-ASCs were implanted in multiple tunnels with slow and controlled movements through different entrances (inframammary fold, higher and lower external quadrants, higher and lower internal quadrants, periareolar) to emphasize the importance of a nontraumatic procedure to
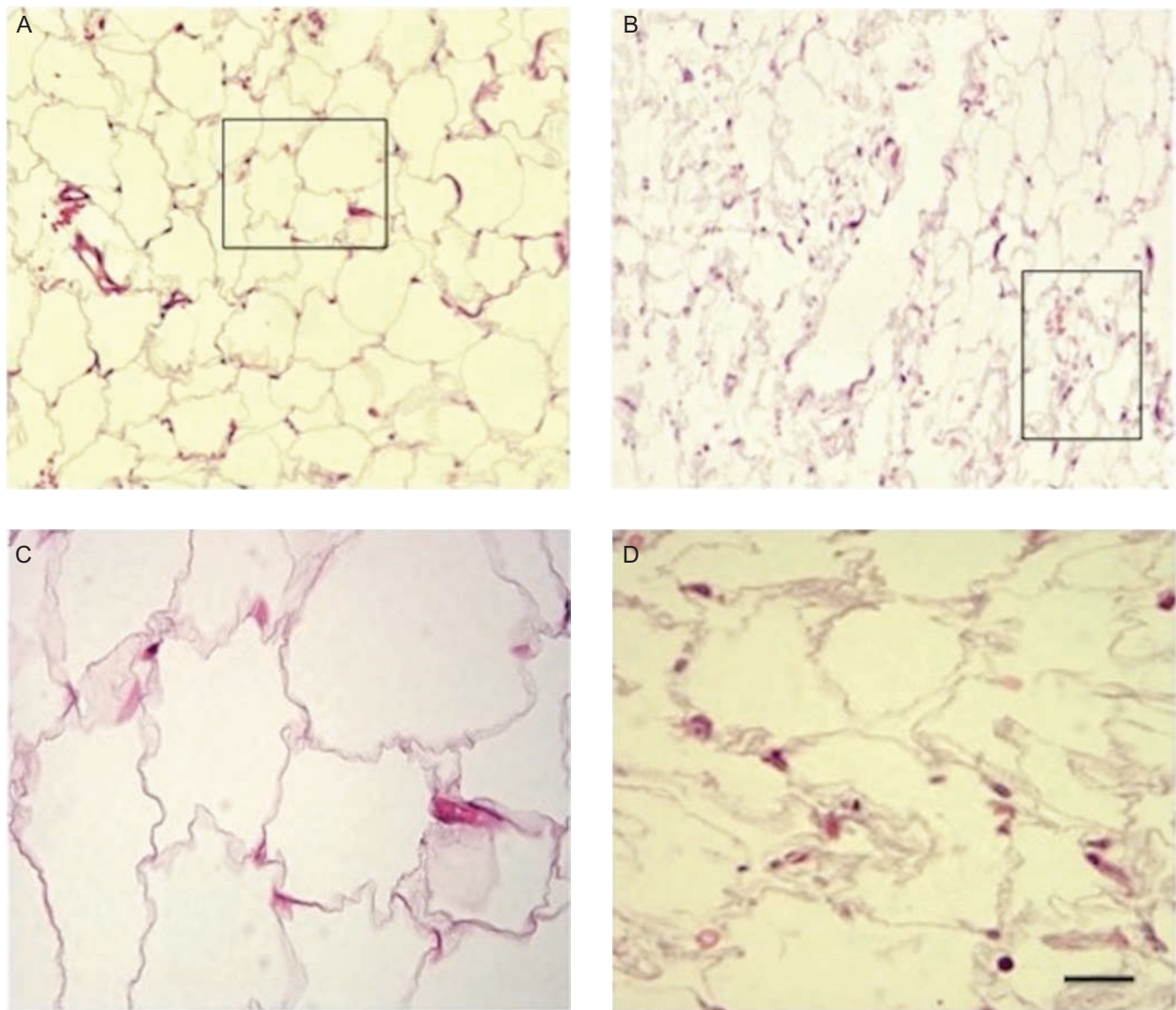
Figure 9. Histological analysis of fat graft (± stromal vascular fraction) before transplantation performed by hematoxylin and eosin staining. The fat graft not enhanced with adipose-derived stem cells (normal-shaped adipocytes) at (A) 10× magnification and (C) 40× magnification. The fat graft enhanced with adipose-derived stem cells with (stromal scaffolding of adipose tissue), with cell clusters (small group >15 cells of round-shaped cells within the fat context) at (B) 10× magnification and (D) 40× magnification (inset 40×).

maximize the integrity of the grafted tissue and maximize the contact surface between the processed fat and the host capillaries.[24,26] Employing this technique, we favor the diffusion of nutrients from neighboring capillaries, which are essential for adipocyte survival by improving their integration with the surrounding tissue.[24,26]

Regarding the methods of fat enrichment in this study, supplementation of autologous fat injection utilizing FG-e-ASCs improves the soft tissue volume of the breast compared with utilizing FG-ne-ASCs. FG-e-ASCs presented less reabsorption compared with FG-ne-ASCs. The reabsorption rate reported over the first year is highly variable,

ranging from 37% with FG-e-ASCs to 61% for FG-ne-ASCs.[26] To prevent reabsorption, it is fundamental to conduct each step of the procedure carefully and meticulously. In this study, breast augmentation with FG-e-ASCs showed a lower height but a more natural contour and softness of the breasts compared with prostheses.[27]

The positive outcomes derived from adding ASCs and SVFs could be explained by the cells' capacity to secrete multiple growth factors, such as vascular endothelial growth factor, which is a potent pro-angiogenic factor that improves neoangiogenesis and fat vascularization and provides physical extracellular matrix guidance cues

promoting endothelial sprouting.[28-30] This SVF-ASC addition can increase fat survival through improved vascularization, leading to reduced reabsorption of the graft, as observed in this study. This concept, which is related to the activity of ASCs-SVFs that improve fat graft survival and maintenance, is supported by observations from other surgical treatments such as that for a calvarial defect and breast reconstruction after partial mastectomy with radiotherapy damage, as previously reported.

In fact, injected adipose tissue must survive through a diffusion mechanism until an active blood supply is reestablished. Thus, survival of the graft must be balanced between this mechanism and hypoxia-induced cell death. Growth factors released by ASCs-SVFs may therefore improve the fat injected through increased blood vessel density within the same graft with a significant improvement in graft retention, as also demonstrated in an animal study employing gene therapy to deliver vascular endothelial growth factor to the graft.[31] In fact, in this animal study, a significant improvement in graft retention at 15 weeks was reported. Also, the early establishment of new microcapillary networks, which deliver the proper nutrients and oxygen to the implant, might contribute to the improved outcomes observed.[31]

In addition, ASCs-SVFs can improve fibrogenic activity of fibroblasts that favor vascularization, such as fat tissue survival and 3D organization. Thus, fat graft survival is more probable when the addition of ASCs-SVFs is performed and fat necrosis is reduced potentially due to improved vascular development in the treated area. This research suggests that an in vivo tissue-engineering approach is based on an optimized microenvironment, supporting the correct architectural adipocyte distribution, and on better cell-to-cell interaction that favor adipose tissue survival; this approach could offer early protection from surrounding inflammatory events.

Regarding breast volume evaluation, it is fundamental, in the preoperative phase, to identify the regions needing correction and to perform breast volume analysis, shape, and symmetry evaluation. Clinical evaluation is very fast and simple and is mainly based on experience and preferences. On the other hand, the systematic application of different methods and techniques are being tested to standardize the measurement obtaining numbers for breast volume. Kayar et al[32] compared 5 methods for breast volume estimation—mammographic, anthropometric, the Grossman-Roudner device, the Archimedes procedure, and the casting technique—demonstrating that mammographic evaluation seems to be much more accurate.[32] Moreover, 3D evaluation with MRI proved to be an alternative method that is accurate and effective for volume estimation depicting in vivo breast shape and symmetry.

During MRI examination, both breasts are located in prone position in the coil area without compression. Volume can be obtained with automatic or manual contouring on week 1-weighted images. With the patient in the uncompressed prone position, both shape and symmetry in vivo can be depicted with volume-rendering 3D images.

Utilizing MRI, any deformity, asymmetry, and postoperative changes, including loss of fat volume, can be correctly located, estimated, and evaluated. Breast volume modification and shape changes can be compared during the follow-up as a result of the reproducibility of the assessment.[32,33]

The intrinsic differences between water and fat protons in the MRI environment allow selective interrogation of their contribution to the magnetic resonance (MR) signal. Fat-suppression techniques and chemical shift imaging are routinely utilized to expand the dynamic range of the MR images for better depiction of physiologic and pathologic processes.[32,33] Short time inversion recovery and T2-weighted sequence are usually applied in the MRI study of the breast to show the perifocal edema. Moreover, utilizing the new sequence as iterative decomposition of water and fat with echo asymmetry with the least squares estimation (IDEAL) sequence is a novel imaging technique for separating fat and water.[32,33]

IDEAL is a single acquisition technique that generates 4 series of images: water only, fat only, in-phase images, and out-of-phase images. All images are inherently registered, leading to faster interpretation and higher diagnostic confidence. IDEAL consistently separates fat from water in challenging anatomical areas, resulting in excellent image quality even in those areas in which fat tissue of the breast and fat grafting chemical shift could be slightly different.[32,33]

MRI angiography is routinely utilized both clinically and experimentally for the identification of tumor-feeding and -draining vessels, and treatment planning can be also utilized with specific contrast agents to show the morphological structure of angiogenesis in relation to vessel permeability. Noninvasive quantification of angiogenesis may also be possible with MRI. Moreover, it may include the so-called 4-dimensional MR angiography, in which high-resolution 3D MRI angiography is combined with dynamic contrast-enhanced MRI.[32,33] Nowadays, an MRI of the breast, thanks to very high sensitivity and specificity, is considered a problem-solving technique that is able to depict the large majority of physiological and pathological processes in which morphology and vascularity changes are involved and well depicted.[32-34]

Despite the appeal of the fat grafting technique and the advantages reported, some problems still remain concerning the final breast volume, the application of a standardized method for injection technique aimed to improve

the fat survival reduction and the cyst formation, the controversial role of ASCs, and the necessity to repeat the treatment in some cases.

In fact, maximum breast augmentation utilizing the technique described in the present work varied among the patients and appeared to be 80 to 280 mL. Although these volumes may be smaller than those achieved with large artificial implants, a definite advantage is that patients need not be concerned about postoperative complications induced by artificial implants, such as rupture, infection, capsular contracture, unnatural contour, hardness, neurologic symptoms, and immune response. However, postoperative sequelae of fat grafting may occur such as cyst formation, microcalcifications, macrocalcifications, and cytosteatonecrotic areas, as detected by MRI scans in our present work. In fact, cyst formation and calcifications were identified in 11 patients in the SG and in 9 patients in the CG ($P = 0.089$). Fat necrosis, on the other hand, was not reported. Seven patients underwent a second treatment. In the long-term follow-up, adverse events such as infections and skin necrosis were not observed in either group. In light of this concept, the use of a "gentle technique," the injection of fat only in the subcutaneous space, and the identification of an optimal volume of fat to inject into each breast reduced but did not prevent calcification and cyst formation.

As reported in previous studies,[35,36] the enrichment of ASCs and SVFs to the fat graft does not seem to improve the carcinogenesis. On the other hand, the secretions of the adipose tissue, that is, adipokines that are modulated during obesity, could have "remote" effects on mammary carcinogenesis.[35-38] Breast cancer cells are surrounded and locally influenced by an adipocyte microenvironment, which is probably more extensive in obese people.[35-38] In fact, the percentage of recurrences, as reported in our previous work[35] related to the use of enhanced fat graft with ASCs in outcomes of breast cancer, was significant in a group of patients (called control group 2) not treated with fat graft injection, in which all of the patients were affected by obesity, suggesting the crucial role of obesity in breast cancer. In fact, in a group of 7 patients (control group 2), 3 recurrences (2 systemic and 1 local) were recorded compared with 4 recurrences (3 systemic and 1 local) in the SG that was composed of 121 patients and had 5 recurrences (2 systemic and 3 local) in control group 1 composed of 50 patients. In a study by Delort et al[38], leptin appears to be strongly involved in mammary carcinogenesis and may contribute to the local proinflammatory mechanisms, especially in obese patients, who have increased metastatic potential and a greater risk of mortality.

More additional randomized controlled trials are necessary to further evaluate the efficacy of this method.

CONCLUSIONS

We demonstrated that the gentle injection of FG-e-ASCs results in increased fat graft survival in patients affected by breast hypoplasia. We concluded that the approach based on a correct breast evaluation performed by MRI, SVF-enhanced fat graft as FG-e-ASCs (based on automated fat washing in a closed system [Cytory] and the addition of ASCs), and a gentle injection is a reliable alternative to breast implant. In addition, the results obtained suggested that the utilization of FG-e-ASCs is effective and safe and that SVFs and ASCs favor adipose tissue survival. Also, the FG-ne-ASCs performed utilizing the Coleman technique were obtained through the purification procedure of adipose tissue, preserving the integrity of the adipocytes but separating the fluid fat portion from the serous bloody part.

In both cases, both the SG and CG patients were treated with procedures based on the purification of fat tissue (washing/centrifugation/filtration); therefore, in this work, we confirmed, through the results reported, the necessity of utilizing fat tissue that underwent the purification procedure. Recent papers (since March 2019) published by Gentile et al[35-37] suggest that ASCs may have potential usefulness in different conditions such as neurodegenerative disease[36] and wound repair,[37] as well breast cancer[35-38] and osteochondral defects.[39] These data can be helpful in developing new therapeutic approaches in personalized cell therapy that are aimed to develop the autologous and allogeneic uses of ASCs in aesthetic, regenerative, and functional ways in different pathologies.

Supplementary Material

This article contains supplementary material located online at www.aestheticsurgeryjournal.com.

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