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HUMAN CLINICAL ARTICLE

Ex vivo-expanded autologous adipose tissue-derived stromal cells ensure enhanced fat graft retention in breast augmentation: A randomized controlled clinical trial

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Abstract

Autologous fat grafting and implant surgery are used for volume restoration in plastic surgery. With the aim of producing a treatment superior to current solutions, we report a randomized, controlled, data assessor-blinded clinical trial comparing fat grafts enriched with ex vivo-expanded autologous adipose-derived stromal cells (ASCs) to nonenriched fat grafts in breast augmentation. The intervention group received ASC-enriched fat grafts (≥20 × 10^6 viable ex vivo-expanded ASCs per milliliter fat), and the control group received conventional nonenriched fat grafts. Volume retention was measured by magnetic resonance imaging, and clinical photographs were taken simultaneously for outcome evaluation. ASC-enriched fat grafts had significantly higher retention rates (mean = 80.2%) compared with conventional fat grafts (mean = 45.1%). Clinical photos showed statistically significant superior results in the intervention group, assessed by independent clinical experts. These results improve the prospects for using culture-expanded ASCs in both reconstructive and cosmetic volume restoration and make the procedure an attractive alternative to conventional fat grafting and implants. This study is registered at www.ClinicalTrials.gov, number H-16046960.

KEYWORDS

adipose-derived stromal cells, breast augmentation, cell-enriched fat grafting, ex vivo-expanded, fat grafting, plastic surgery

1 | INTRODUCTION

The repair of disfiguring volume defects of the female breast following cancer resection and congenital anomalies or secondary to the aging process and breastfeeding represents a significant surgical challenge.[1] Silicone implants are the gold standard for correcting breasts aesthetically, and breast augmentation continues to be the number one plastic surgical procedure throughout the world.[2] Breast implants are associated with significant complications, and trust is currently at risk because of breast implant-associated anaplastic large cell lymphoma[3]; however, the long-term implications are not yet clear. With the goal of maximizing appearance as well as patient safety and satisfaction, an alternative strategy for breast augmentation and correction is autologous fat grafting.[4] Harvesting fat through liposuction is possible with

minimal trauma, and the indications for the transfer of fat from one part of the body to another have expanded beyond treatment of small contour irregularities to larger volume deficits.[5] Autologous fat is gaining acceptance as an ideal soft tissue filler because it is biocompatible, versatile, nonimmunogenic, and readily available.[4] However, clinical outcomes have varied extensively, and reported retention rates for transferred fat range from 25% to 80%, regularly necessitating repeated procedures.[5,6] Methods to increase graft retention are required to make the procedure a reliable and attractive alternative to implants, especially in slim patients with limited fat resources.

In this respect, cell enrichment has been shown to be a promising approach. Two different cell populations have been evaluated, either the mixed, heterogeneous, and nonexpanded population extracted from the stromal vascular fraction (SVF) or the more homogenous population of ex vivo-expanded adipose-derived stromal cells (ASCs). Regarding SVF cells, one clinical study demonstrated a beneficial effect,[7] but most studies failed to demonstrate a substantial benefit, especially with larger graft volumes.[8,9] Notably, SVF contains only a minor fraction of ASCs.[10] With respect to using ASCs, animal and human studies have demonstrated that enrichment with ex vivo-expanded ASCs markedly improved the residual volume and histological appearance of fat grafts,[11,12] and the use of ASCs has in some studies been reported to be advantageous when compared with the use of unpurified SVF cells.[13] Previously, we reported a profound effect in a randomized, placebo-controlled, triple-blind trial of ASC-enriched fat grafting with excellent feasibility and safety.[12]

We report the results of the first randomized, controlled, blinded clinical trial comparing breast augmentation in patients treated with fat grafts enriched with ex vivo-expanded autologous ASCs with those treated with nonenriched grafts. The purpose was to examine whether this procedure will improve the results of conventional lipofilling, thereby offering a safer, more natural, and long-lasting alternative to current artificial solutions.

Lessons learned

- Expanded adipose-derived stromal cells (ASC) ensured enhanced fat graft retention.
- ASC-enriched fat grafting resulted in a superior clinical outcome in breast augmentation.
- With a high survival of the injected volume, no second procedure was needed.
- No adverse side effects of high concentration ASC injections were found.
- ASC-enriched fat grafting was found to be safe, improving the prospects of using expanded ASCs in reconstructive and cosmetic breast surgery.

Significance statement

Currently, applications of adipose-derived stromal cells are registered for investigation in hundreds of clinical trials for a wide range of applications. This study demonstrated that adipose-derived stromal cells significantly improve the ability of fat grafts to retain their volume compared with conventional fat grafting, and the clinical outcomes were assessed as noticeably improved by independent plastic surgeons. This study demonstrated a safe profile for the use of adipose-derived stromal cells. The results are likely transferable to most soft tissue augmentations and may, therefore, be beneficial to a broad spectrum of patients.

2 | MATERIALS AND METHODS

2.1 | Study design

The study protocol, a blinded, controlled, randomized, prospective clinical study on healthy subjects, was approved by the local Danish Committee on Biomedical Research Ethics (H-16046960) for Copenhagen, the Danish Data Protection Agency, and the Danish Patient Safety Authority. This study is registered at www.ClinicalTrials.gov, number H-16046960.

Sixteen healthy participants were enrolled in the study (inclusion and exclusion criteria are summarized in Table 1). A minimum sample size of six subjects per group was determined via a power calculation. The study participants were divided into two groups by randomization. The allocation sequence was generated 1:1 using www.randomization.com and concealed by a medical doctor unrelated to the trial management group. Both the data generator (radiologist analyzing the magnetic resonance imaging [MRI]) and the outcome data assessor (statistician and surgeons evaluating the clinical outcomes) were blinded, and blinding was maintained until the data were analyzed. The patients and surgeons were not blinded, as this would have necessitated an additional minor liposuction for the patients in the nonintervention group. Participants were recruited through Aleris Hamlet hospitals in Denmark. The principal investigator and sponsor were responsible for coordinating inquiries of possible candidates. No compensation for study participation was offered. Before a subject's participation in the trial, the principal investigator obtained written informed consent from the participant after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific screening procedures or any investigational products were administered. All candidates were evaluated for suitability to be included in the study by physical examination and by the criteria stated in Table 1.

2.2 | Interventions and outcomes

The procedures were performed in patients with small breasts who had a desire for breast augmentation. All study participants received breast augmentation using autologous fat grafting according to the

TABLE 1 Inclusion/exclusion criteria for cosmetic breast augmentation

Inclusion criteria
1. Age 18-50 years
2. Healthy females
3. BMI 18-30 kg/m^2
4. 1000 mL of fat (lipoaspirate) available for liposuction in the abdomen and/or thighs
5. Desire for breast augmentation
6. Speaks and reads Danish or English
7. Signed informed consent
Exclusion criteria
1. Smoking
2. Previous breast surgery
3. Previous cancer or predisposition to breast cancer
4. BMI >2 points during the study
5. Pregnancy or planned pregnancy within 1 year after the procedure
6. Breastfeeding <6 months prior to inclusion
7. Known chronic disease associated with metabolic malfunction or poor healing
8. Pacemaker or other implanted foreign objects
9. Allergy to necessary anesthesia
10. Intention of weight loss or weight gain within the trial period

Abbreviation: BMI, body mass index.

randomization sequence. The intervention group received ASC-enriched fat grafts, whereas the control group received conventional nonenriched fat grafts. The enriched fat grafts were supplemented with ≥20 × 10^6 viable ex vivo-expanded ASCs per milliliter of fat, and the dose depended on the available number of ASCs after culturing. This concentration was chosen based on the results of a previous clinical study by the first author.[12] One month prior to the augmentation procedure, an MRI scan for volume determination of the breasts was conducted, and blood samples were obtained according to the Danish Tissue Act. Patients in the intervention group underwent minor liposuction 2 weeks prior to the augmentation procedure for the isolation and subsequent expansion of ASCs. Each participant was then subjected to standard liposuction and breast augmentation by fat transplantation with or without ASC enrichment of the fat graft under general anesthesia. Patients were followed for a minimum of 18 months and will be given a 5-year follow-up safety evaluation. Total breast volume was determined by MRI preoperatively and again after 4 months. In addition, clinical photos were taken preoperatively at 4- and 18-months follow-up. A flowchart overview of the study is shown in Figure 1.

The primary endpoint was volume retention of the breast, measured by MRI after 4 months, as transplanted fat resorption stops after approximately 3 months.[14] Secondary endpoints were the assessment of group variance and the determination of safety, as evaluated by possible adverse events not normally occurring in relation to fat grafting to the breast and not described in the risk assessment of the procedure. Finally, the feasibility and clinical outcomes of conventional and ASC-enriched fat injection in the breast were investigated.

2.3 | Liposuction, graft preparation, and lipofilling

Eight subjects underwent outpatient minor liposuction under local or general anesthesia to harvest 100 mL of fat for the isolation and ex vivo expansion of ASCs. The lipoaspirate was harvested by sterile liposuction techniques. Through incisions, a wetting Klein's solution (lidocaine, epinephrine, and Ringer's Lactate) was infiltrated into the subcutaneous fat. The lipoaspirate was procured with a standard liposuction device and sealed in a sterile single-use container (GID SVF-2; GID Europe, East Sussex, United Kingdom).

For the breast augmentation procedure, the sites (abdomen/thighs/hips) to be used for tissue harvesting were marked before the major liposuction in all patients while the participant was in an upright position. The procedure was conducted using sterile technique and under general anesthesia. A wetting Klein's solution was infiltrated into the donor site. Liposuction was performed to obtain lipoaspirate for the transplantation while simultaneously contouring the body shape at the donor site. Liposuction was performed with the use of standard equipment for fat transplantation. The obtained lipoaspirate was kept in the liposuction container (GID700; GID Europe), and the adipose tissue was washed three times with warm (37°C) Ringer's Lactate to remove residual Klein's solution and blood, followed by spinning for 2 minutes using the built-in mixing blades. Oil content and fluids were continuously drained during the process using the attached vacuum pressure. Next, the fat tissue, ready for transplantation, was extracted using a 100-mL catheter tip syringe.

In the group that received nonenriched transplants, the lipoaspirate was transferred to 10-mL luer-lock syringes (Becton, Dickinson and Company, Franklin Lakes, New Jersey).

In the group that received ASC-enriched lipoaspirates, the fat was mixed with autologous ex vivo-expanded ASCs in the syringes, corresponding to ≥20 × 10^6 ASCs per milliliter of fat transplanted. To ensure a homogenous solution of adipocytes and ASCs, the transplant product was mixed by rotation and gentle stirring until uniform in color and consistency. The obtained ASC-enriched lipoaspirate was transferred to 10-mL luer-lock syringes (Becton, Dickinson and Company).

A blunt 14-gauge cannula was used for injection of grafts in both groups. Four 14-gauge incisions were made in each breast. Fat grafts were distributed in as many channels and planes in the subcutaneous, subglandular, intramuscular, and submuscular levels as possible to ensure proper placement of small aliquots. The injected volume was dependent on the patient fat reserves available and the size of the skin envelope of the breast. The injection and donor sites were closed with an interrupted suture, which was removed after 7 days. Postoperative compression garments were applied to the donor sites, and a supportive bra was applied to the breasts.

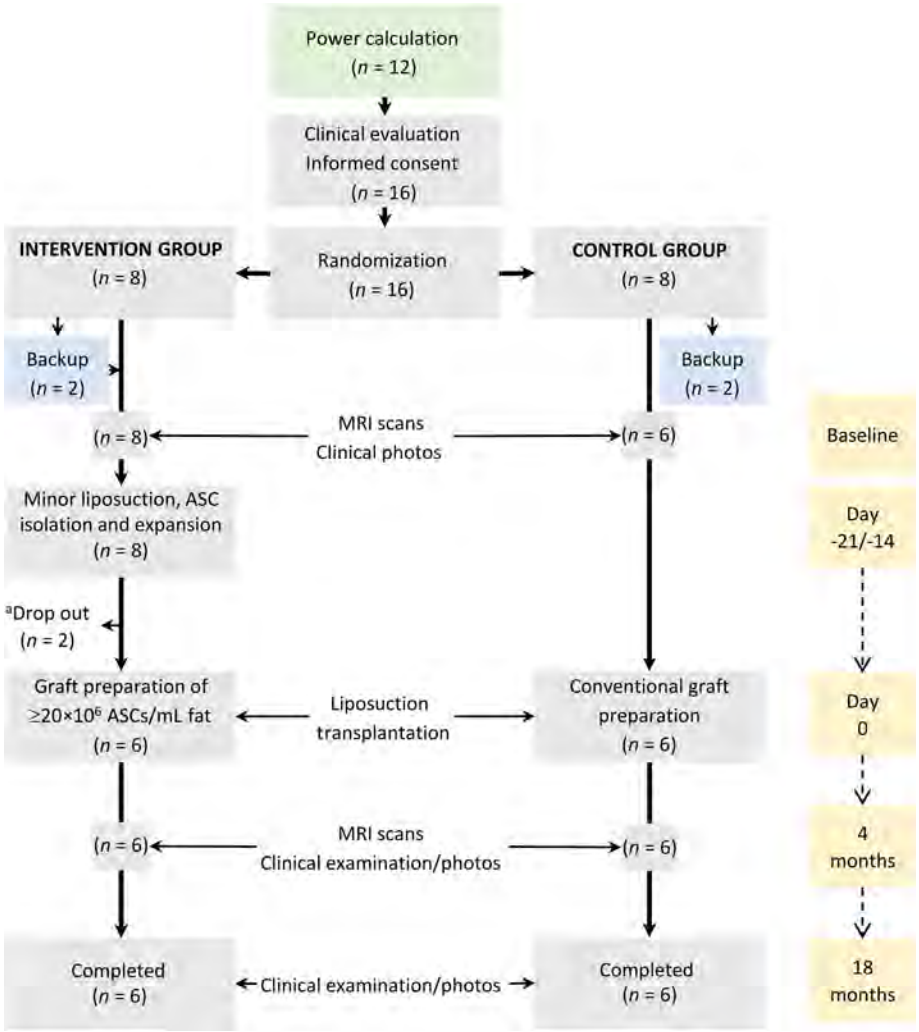

FIGURE 1 Overview of the study. [a]Drop out 1: Due to overconfluency of the adipose-derived stromal cells, resulting in loss of cells during the harvesting procedure, leaving too few cells to reach the desired concentration. Drop out 2: A malignant melanoma was diagnosed prior to the breast augmentation procedure. ASCs, adipose-derived stromal cells; MRI, magnetic resonance imaging

2.4 | Isolation and culturing of ASCs

All handling of the ASCs, including donation, procurement, processing, and testing, was performed in accordance with the approved protocol and following good manufacturing practice standards in a laboratory regulated by the tissue establishment Stemform and approved by The Danish Patient Safety Authority.

At the site of the cell manufacturing facility, the washed fat tissue contained in the GID SVF-2 (GID Europe) was further processed to liberate the ASCs according to the manufacturer's instructions. Briefly, the GID SVF-2 collagenase was added, followed by incubation at 37°C for 75 minutes with end-over-end mixing. The released SVF cells were pelleted by centrifugation for 10 minutes at 600*g*, and the pellet was resuspended in complete medium (Dulbecco's modified Eagle's medium, 2 IU/mL preservative-free heparin, 1% penicillin-streptomycin, and 10%-15% pooled human platelet lysate [pHPL; PL Bioscience, Aachen, Germany]). We extracted an average of 100 million mononuclear cells (MNCs) from 100 mL of lipoaspirate. The SVF cells were seeded at a cell density of 2500-5000 SVF cells per cm^2, depending on SVF yield, in cell factories (NUNC; Thermo Fisher, Roskilde, Denmark) and placed in a cell culture chamber (85% N_2, 10% O_2, and 5% CO_2 at 37°C) in an isolator (BioSpherix Xvivo System X2; BioSpherix, Parish, New York). Cell culture medium was replenished once a week. After 14-21 days in culture, the cells were harvested at near-full/full cell confluency using a standard trypsinization protocol (TrypLE; Gibco, Thermo Fisher Scientific).

The MNCs were seeded at a density of 10 000-12 000 MNCs per cm^2 and harvested in the primary passage at near confluency. The culturing process resulted in 10-12 population doublings.

Following harvest, the ASCs were washed twice in isotonic NaCl with 1% human albumin (CSL Behring, Kgs. Lyngby, Denmark). The suspension was transferred to a sterile container labeled according to current regulations, which was transferred to the operating room in a sealed sterile bag. Prior to release for clinical use, the ASCs were evaluated for (a) the absence of pathogen contamination, as assessed by visual inspection and sterility testing of the medium during culture; (b) ASC viability above 90%; (c) characteristic ASC morphology, as assessed using an inverted microscope; and (d) the absence of visible clumps in the cell suspension. Cryopreserved reference samples will be stored for 2 years after the study has ended, after which they will

be discarded as biological waste material. The samples are stored in the Cell Facility of Stemform, Denmark.

2.5 | Differentiation potential and cell surface markers

The culturing procedure was validated both before and during the study by measuring cell surface markers and differentiation capacity according to the International Society for Cellular Therapy criteria.[15] Samples from ex vivo-expanded cells were successfully tri-lineage differentiated into adipocytes, chondrocytes, and osteoblasts. Cell surface markers characteristic for ASCs ($CD90^+$, $CD73^+$, $CD105^+$, $CD34^-$, $CD45^-$, $CD14^-$, $CD19^-$, and anti-human leukocyte antigen (HLA)-DR^-) were confirmed by flow cytometry using an mesenchymal stem cells (MSC) Phenotyping Kit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). No differences between the patients were observed.

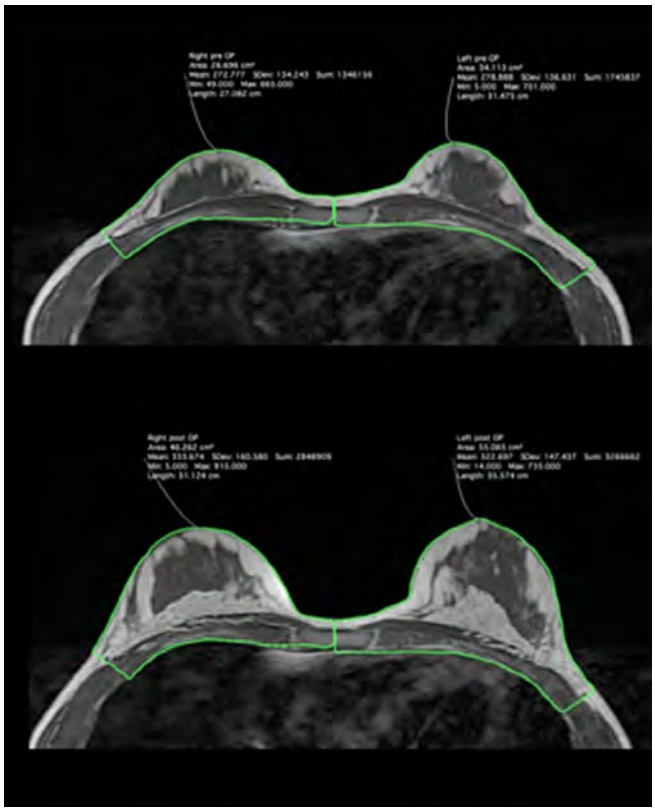

FIGURE 2 Magnetic resonance images and delineation of the implanted grafts. Methods: Pre- and postoperational scans were linked according to the manubriosternal joint. A region of interest (ROI) was drawn on each axial slide, making sure to include the whole breast. Landmarks for the ROI: 1. Medial: a perpendicular line through the center of the sternum/midsagittal plane 2. Posterior: The transition from lung to costa. 3. Lateral: A straight line perpendicular to the skin surface going through an anatomical landmark, for example, the thoracoepigastric vein. 4. Anterior: The transition from skin to air. After ensuring that the entire transplant was included in the ROIs, the volumes were calculated automatically

2.6 | Magnetic resonance imaging

MRI of the fat-grafted breasts was performed using a 1.5-Tesla GE Signa HD (2008) with a dedicated mamma coil. Imaging was performed with an axial and coronal T1-weighted Fast Spin Echo, with a thickness of 5 mm and a 512 × 512 matrix size. The scanning included the entire anterior chest wall and was performed 1 month prior to and 4 months after breast augmentation (Figure 2). The estimated fat graft volume was calculated based on changes in total breast volume over time assessed by MRI.

Method used for volume assessment: Using dedicated imaging analysis software (Osirix version 8.5 for mac; Bernex, Switzerland), pre- and postoperation images (axial T1-weighted) were linked according to the manubriosternal joint. Regions of interest (ROIs) were drawn on all axial slides, making sure to include the whole breast. Landmarks for the ROI: 1. Medial: a perpendicular line through the center of sternum/midsagittal plane. 2. Posterior: The transition from lung to the costa. 3. Lateral: A straight line perpendicular to the skin surface going through an anatomical landmark, for example, the thoracoepigastric vein. 4. Anterior: The transition from skin to air (Figure 2).

MRI scans (including T2-weighted short-tau inversion recovery and volume imaging for breast assessment sequences) were also accessed to detect any newly formed radiological changes at the recipient site.

2.7 | Assessment of clinical results

Clinical results were evaluated by 10 independent board-certified plastic surgeons with years of clinical experience. Photos of the patients before the operation, 4 months after the operation, and 18 months after the operation were presented blinded and at an equal profile to each surgeon and assessed by the question: "If you performed one fat transplantation for breast augmentation, how satisfied would you be with the retention/survival and the cosmetic result?"

1. Below average
2. Average
3. Above average
4. Good
5. Excellent

2.8 | Statistical analysis

The sample size was calculated to be significant, with 12 participants allocated equally (1:1) to show a significant difference in residual graft volume of 20%, with an expected SD of 10% using a study power of 90% and a significance level of 5%. The comparative analysis (Mann-Whitney test) was performed using SPSS (IBM, Chicago, Illinois), and graphs were generated using Prism 8 (GraphPad Software, La Jolla, California). A two-sided P value <.05 was considered statistically significant. All values are presented as the median with upper and lower quartile, Q_3-Q_1 (interquartile range), unless otherwise explicitly stated.

2.9 | Role of the funding source

The funding source had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. The authors are responsible for the design and conduct of this study, all study analyses, and the drafting and editing of the paper and its final contents. All authors had full access to all the data after the allocation sequence code was broken. The corresponding author, Dr Kølle, confirms having final responsibility for the decision to submit for publication.

3 | RESULTS

3.1 | Patient demographics

Sixteen healthy female participants were enrolled and randomized, four of whom served as backup, leaving two backup patients in each group. Two participants were excluded; one was excluded owing to an unforeseen loss of cells during the cell harvesting procedure, and one was diagnosed with a malignant melanoma prior to breast augmentation. The additional two participants, randomized to the control group, served as standby patients and were never treated. Twelve participants completed the study according to the randomized sequence, intervention group (n = 6) and control group (n = 6; Figure 1). Participants who completed the study were included in the data analysis (n = 12). The patients were recruited from 1 December 2016, to 1 April 2017, and followed from 1 April 2017, to 1 June 2019.

3.2 | Graft implantation

Comparable volumes of fat were injected into each group. A median total volume of 222.5 mL (270-182.5 mL) was injected into each breast in the intervention group, and 260 mL (310.6-240 mL) was injected into each breast in the control group, with no statistically significant difference between them (P = .087; Figure 3A). Subtracting the volume of cells added to the enriched grafts, a median of 197.5 mL (238.8-164.4 mL) of fat was injected. Considering the volume of saline in the grafts, a median of 175.25 mL (211.8-146.1 mL) of pure dry fat was injected in the intervention group, whereas each breast in the control group received a median of 234 mL (279.6-216 mL).

3.3 | ASC-enriched grafts maintain greater residual volumes than control grafts

The ASC-enriched fat grafts (n = 6) showed significantly higher residual volumes than the control grafts (n = 6), with a median volume retention of 80.2% (124.2%-66.1%) vs 45.1% (50.7%-36.5%; P = .0022) in the control group, as measured by MRI 4 months postoperatively (Figure 3B). Subtracting cell volume and saline from the graft, the corresponding values were 100.2% (164.1%-84.4%) vs 50.1% (56.3%-40.5%; P = .0022) in the controls (Figure 3C). The intervention group had an enlargement median of 2.6 (1.94-3.37) vs 1.57 (1.69-1.43) in the control group (P = .0043; Figure 3D). Collectively, 10 plastic surgeons assessed the patients who had ASC-enriched fat grafts as having significantly better augmentation and cosmetic results compared with the control patients (Figure 4). The intervention group had a median score of 4.50 (4.67-4.33). The control group had a median score of 2.67 (3.33-2.25; P < .00001).

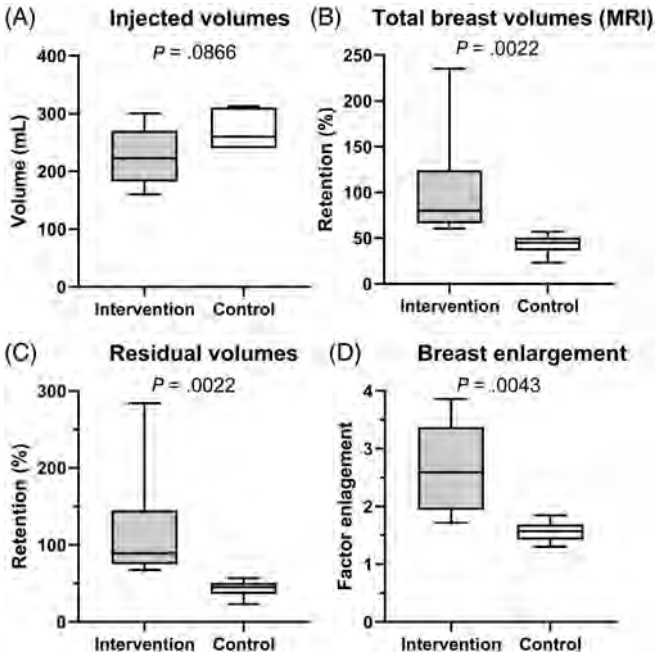

FIGURE 3 Statistical outcomes. A, Injected volumes: Injected graft received by the intervention group compared with the control group. B, Total breast volumes (magnetic resonance imaging [MRI]) at 4 months: Transplant retention volumes in adipose-derived stromal cell (ASC)-enriched grafts and control grafts on day 120. C, Residual volumes at 4 months: The residual volumes of ASC-enriched fat grafts and regular fat grafts were calculated 4 months after the procedure after subtracting the cell volume and saline from the graft. D, Breast enlargement: Enlargement of the breasts, as measured by MRI 4 months after the operation. All boxplots show the median with Q_1-Q_3 and full range

3.4 | Safety and feasibility

No serious adverse events were noted in any of the study groups. Common complications such as swelling and bruising occurred in both groups but subsided as expected. No oil cysts were found on MRI 4 months postoperatively in any subjects. The time spent on the surgical procedures was no longer than that spent on regular fat transplantation.

4 | DISCUSSION

In this study, we demonstrated that enrichment of fat grafts with ex vivo-expanded autologous ASCs significantly improved breast volume

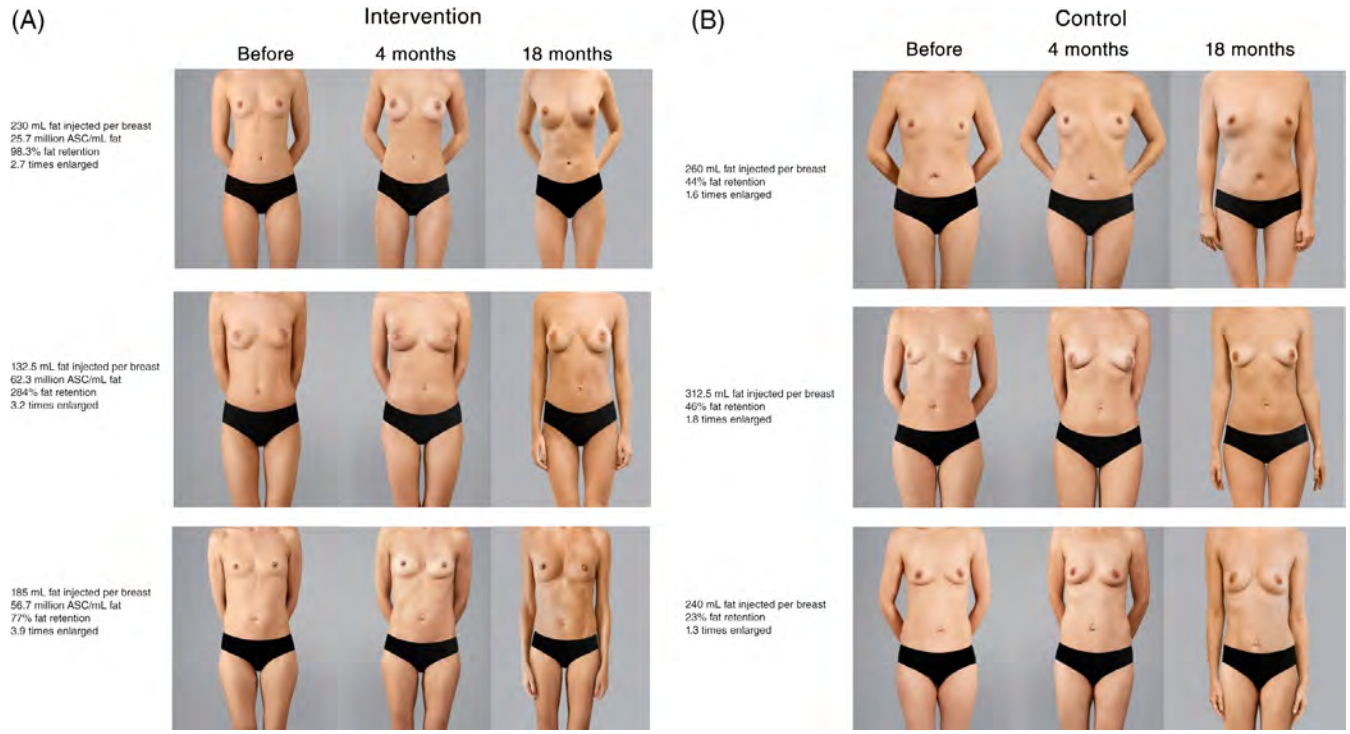

FIGURE 4 Clinical outcomes. A, Clinical outcomes intervention group: Front photographs of three patients who received adipose-derived stromal cell-enriched fat grafts. B, Clinical outcomes control group: Front photographs of three patients who received regular fat grafts. ASC, adipose-derived stromal cell

retention, as assessed by MRI after 4 months. Additionally, photo evaluation after 18 months by blinded surgeons revealed significantly better clinical results in the ASC-treated group than in the controls receiving nonenriched fat grafts. These findings are in accordance with our previous human study in which we, in an experimental setting with bolus injections on the backs of the upper arms, demonstrated significantly increased fat graft survival and quality of ASC-enriched fat grafts compared with controls.[12] Thus, in the two studies, we found equivalent retention rates of the ASC-enriched grafts but differences in the retention rates of the control grafts. Several factors may have contributed to this difference, including different injection techniques and recipient beds.

The present study is the first to investigate the effect of expanded ASCs on large-volume fat grafting in a clinically relevant setting. In comparison, several studies have investigated possible effects of SVF enrichment, although only a small subset of the reports include both volumetric measurement and proper control groups. The advantages of SVF are its easy accessibility and in-operation-room extraction, which limits treatment to a single operation, whereas ASC-enriched fat grafting requires a small liposuction procedure prior to the primary operation. ASC expansion also requires cultivation procedures at cell facilities that are currently costly and time consuming. Therefore, the use of ASCs rather than conventional fat grafting and enrichment with the mixed population of SVF cells needs to be justified by superior results.

We demonstrated a median of 80.2% residual fat graft volume in the ASC-enriched group compared with 45.1% in the control group. In a review, Laloze et al[9] reported retention results of 64% for cell-assisted lipotransfer (SVF and expanded ASCs, but mainly SVF) and 44% for nonenriched fat grafts; however, significant differences involved only volumes <100 mL. The control group results were similar to our findings.[9]

Additional important measures include the need for repeated procedures and the persistent fold expansion in breast enlargement. We did not evaluate the first factor, but for the latter factor, we demonstrated a significant 2.60-fold increase in breast volume in the ASC group compared with a 1.57-fold increase in the controls. Notably, the participants were all very slim, making this increase even more remarkable.

Conventional fat grafting is known to deviate in retention from 25% to 80%,[12,14,16-18] likely caused by differences in fat grafting techniques, as proposed by Gir et al.[19] This includes fat harvesting, processing, storage, and reinjection. Additionally, the retention rate has been proposed to correlate with the number of viable fat cells.[20] However, the survival of these cells is multifactorial. The oxygen supply in grafts is initially limited, as vessels first need to form in the grafted lipoaspirate. Mathematical models predict central necrosis of fat injections larger than 0.16 cm in radius and a rise of 9 mmHg or higher to critically decrease capillary perfusion, thus limiting the survival of fat grafts. Survival can be optimized by creating as many channels as possible to ensure better diffusion.[21]

The limitations of the present study include the fact that the study was not paired, which would have diminished the impact of interindividual variation. We chose this approach acknowledging the weaker scientific strength because of concerns about possible asymmetry after the procedure, which could be difficult to correct properly, both because of the patient's insufficient fat deposit for a second procedure and because of the slightly different structure of the ASC-enriched fat grafts in our previous study.[12]

Additionally, the study was not blinded to the participants or the surgeons. The first choice was made to avoid additional liposuction procedures for the control patients, and the latter choice was made because the infrastructure of the organization hampered this approach. However, the possible bias due to this setup, the fat grafting results in the control group, was fully in line with our own previous experiences and other reports, as described above. Moreover, and very importantly, the data assessors were all blinded.

The randomization of subjects generated minor differences in basic characteristics between the study groups (summarized in Table 2). A trend was found in a comparison of median initial breast volumes: 155 mL in the intervention group and 195 mL per breast in the controls. However, this difference did not reach statistical significance. To achieve the best possible surgical results and to optimize the conditions for fat graft survival, we did not use a predefined standard volume but rather determined the optimal volume for each individual patient. However, a proportionally larger amount in relation to initial breast volume was injected in the intervention group (2.72 [3.49-1.95]) than in the control group (2.33 [2.62-1.96]). This difference could cause reduced survival relative to the control group because of greater pressure on the adipocytes.

One of the patients treated with ASCs had an increase in injected volume at 4 months postoperatively of 235%. Very importantly, the clinical result was assessed as "good" by the independent surgeons after 18 months. This residual volume above the injection volume could be a result of a considerable weight gain subsequent to the breast augmentation procedure but slimming prior to the control MRI scan 4 months after the procedure, thereby avoiding exclusion. It should be considered that the initial weight gain resulted in better graft survival by ensuring sufficient nourishment and by stimulating the ASCs toward adipose tissue. An additional reason for the high survival may be due to injection of the highest concentration of ASCs (Table 3), although we found no statistically significant differences in ASC concentration and graft retention between 20×10^6 and 62×10^6 ASCs per milliliter fat. Different ASC concentrations were not found to conclusively correlate with graft retention; by excluding one patient who deviated substantially, a statistically significant correlation was found. Additionally, it is reasonable to believe that there is a lower limit beyond which there is no effect of ASC enrichment. A larger patient population must be enrolled to assess this approach further.

Two control patients gained weight greater than one body mass index point from the first to second MRI scan, promoting an increased breast volume compared with weight-stable patients.

TABLE 2 Baseline characteristics

Characteristics	Intervention group (n = 6)	Control group (n = 6)
Sex	Female	Female
Age (years)	29 (21-42)	32.5 (24-39)
BMI	19.2 (18-22.5)	20.8 (19.7-23.8)
IBV (mL)	155 (50-200)	195 (170-275)

Note: Data are the median with the full range. Data include all participants who completed the study.
Abbreviations: BMI, body mass index; IBV, initial breast volume.

TABLE 3 Overview of the number and concentration of injected adipose-derived stromal cells (ASCs)

Intervention patients (n = 6)	Injected ASCs per milliliter of fat graft	Total number of ASCs injected
1	25.7×10^6	11.8×10^9
2	20.8×10^6	11.0×10^9
3	62.3×10^6	16.5×10^9
4	20.0×10^6	7.0×10^9
5	56.8×10^6	21.0^9
6	26.9×10^6	11.3×10^9
Median	26.3×10^6	11.6×10^9

Whether ASC-enriched fat grafting is effective enough to be worth the trouble is still a matter of debate, and surely, the expansion process is challenging to obtain large numbers of autologous ASCs. We used Cell Factories, which involved labor-intensive manual procedures, especially to harvest the cells. Moreover, in a pilot study (data not shown), we found a rapid drop in ASC viability within hours of harvest. Additionally, an even larger drop was observed for vitality, as assessed by apoptotic markers, which is not routinely used as a release criterion. We found a cell viability of ~90%, with 1%-3% of these cells already induced to undergo apoptosis just minutes after harvest, depending on the methods. In our study, ASCs were transported to the operating room within a few minutes of harvesting, thus minimizing this critical stage. Delivering ASC-enriched treatments as a substitute for current breast augmentation will demand improved survival over longer transportation times.

Whereas SVF cell preparations are a heterogeneous cell population composed of perivascular cells, inflammatory cells (ie, leukocytes), endothelial cells, erythrocytes, and other stromal cells, ASCs are notably more homogenous owing to the enrichment of stromal cells capable of adherence to culture plastic, which is associated with the loss of cells such as leukocytes and endothelial cells.

Apart from their paracrine effect, in the specific setting of fat grafting, ASCs are more resistant to hypoxia than mature adipocytes and thus better survive the grafting procedure.[22,23]

The high yield of perivascular cells from adipose tissue, the easy and repeatable access to subcutaneous adipose tissue via liposuction, and the uncomplicated enzyme-based isolation procedures and in

vitro expansion of ASCs make this tissue attractive to investigators for overcoming a wide array of clinical challenges.

Allogeneic cells as an off-the-shelf product have been used for a variety of conditions and have the benefit of avoiding the initial minor liposuction. However, apart from the possible transmission of viral or bacterial agents, a potential immune rejection and, consequently, a significant reduction in the clinical effect should be considered in this specific setting. In contrast to most other studies with ASCs in which the effect is most likely caused by a paracrine effect,[24] a possible mechanism in cell-enriched fat grafting is that the ASCs better resist the hypoxic period and actually differentiate into mature adipocytes expressing normal levels of class 1 HLA molecules. To date, to our knowledge, no clinical studies of cell-enriched fat grafting have used allogeneic cells, so the effect and safety should be verified first in preclinical and later in clinical studies.

In addition to concerns about conventional fat grafting,[1] there is an ongoing debate regarding the possible oncological risks after autologous fat grafting with cell enrichment. In this respect, clinical studies investigating SVF-enriched fat tissue in breast augmentation and in patients with volume defects after breast cancer surgery have shown no increased incidence of cancer recurrence.[14] With respect to the use of ex vivo-expanded ASCs, despite extensive research, there are no data indicating malignant transformation of expanded human MSCs/ASCs.[25] Additionally, in a previous in vitro study, we found no signs of chromosome abnormalities, as indicated by array comparative genomic hybridization analyses in ASCs cultured under conditions similar to those used in the present study.[26] In addition, the fat grafts transplanted in our previous clinical trial, which were excised 121 days postoperatively, showed no signs of malignancy in the histological examination of the specimens, which all revealed normal fat tissue quality.[12]

Apart from theoretical de novo MSC/ASC-derived tumorigenesis, several studies have investigated the possible effect of MSCs on tumor growth. A range of in vitro and in vivo animal studies have been conducted with different types of cancer cells, cocultured or cotransplanted with MSCs. No conclusions have been made so far, because MSCs have shown both promoting and inhibitory effects on tumor cell growth.[25,27] Of note, most of these experiments have been performed in immunodeficient animals, which differ greatly from immunocompetent animals, not to mention human subjects. Consensus to withhold transplantation of MSCs to patients with known active cancer disease has been made because of a lack of definitive evidence of whether MSCs may be promoting and/or inhibitory to active cancer cells. Thousands of patients in more than 500 clinical trials have received ex vivo-expanded MSCs/ASCs, without reports of serious adverse events.[28] Additionally, a very high dose of ASCs (240×10^6 ASCs per kilogram) was tested in a murine study and showed no signs of cancer, organ toxicity, or changes in body weight after 12 months.[29]

5 | CONCLUSION

Our data show that fat transplantation has the potential to optimize the procedures used for breast augmentation and correction. We demonstrate that ASC graft enrichment may improve conventional lipofilling procedures because the resorption rate is significantly reduced, and no adverse effects were observed. With a median survival of 80.2% of the total injected volume and a median enlargement of 2.6 times the initial breast volume, no second augmentation procedure was needed; the procedure may therefore be an alternative to breast augmentation or breast repair with artificial materials. These results will likely transfer to other soft tissue augmentations and reconstructions, thereby holding potential for a large number of patients.

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CONFLICT OF INTEREST

S.-F.T.K. is chief executive officer and on the board of directors at Stemform, Inc., and reports grants from Stemform and Aleris Hamlet Fund as well as public funding. A.K. is founder, board director, and chief medical officer of The GID Group, Inc., the company that designs and manufactures the SVF-1 device. He is also a consultant for Allergan, Inc., the company that sells the Revolve fat harvest/graft device. D.D. and M.T. are consultants to Stemform. A.F.-N., L.M.-F., B.J., and P.S. declared stock ownership in and hold patents at Stemform. J.S. received a fee from Stemform during the development of this article. The other authors indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

S.-F.T.K.: conception and design, administrative support, manuscript writing, grant application, final approval; D.D.: draft of manuscript, review and revision; M.T.: data collection, analysis, and interpretation, manuscript writing, review and revision; A.F.-N. and A.J.K.: conception and design, review and revision; J.D.S.: culturing of adipose stromal cells; L.M.-F.: culturing of adipose stromal cells, review and revision; B.J.: provision of patients; P.B.S.: administrative support, provision of patients, collection of data; F.P.M.: manuscript writing, data interpretation, review and revision.

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