

Preliminary Report

Enriched Autologous Facial Fat Grafts in Aesthetic Surgery: 3D Volumetric Results

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Abstract

Background: Improved results with aesthetic fat augmentation of the face have been recently described by the concomitant use of autologous stem cells from the stromal vascular fraction (SVF).

Objective: There are no studies in the literature regarding facial fat augmentation results with the use of SVF using 3D computer volumetric analyses. This prospective study was thus undertaken to answer this question.

Methods: Fat was harvested by a standard liposuction technique for reinjection. A 50 cc aliquot of fat was also processed to obtain the SVF using a standard collagenase technique. A cell count was done using a cytometer, and the amount of injected fat and cells were recorded. The Vultus 3D photogrammetric scanning system was used to scan the face pre- and posttreatment and long-term, and volume changes were then calculated at the different time intervals. The data was then correlated to the variables.

Results: Ten subjects were included in the study, with an average follow-up of 12.6 months. The average amount of fat injected was 18.4 cc, of which 68% was retained. The average cell count of the SVF was 4.8×10^5 . The amount of retained fat by volume was found to be positively correlated to the number of cells in the SVF. There was no correlation between the age and number of cells in the SVF.

Conclusions: There is a correlation between the number of cells in the SVF and the amount of fat retained.

Level of Evidence: 3



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The interest in and utilization of autologous fat grafts is increasing in both reconstructive and aesthetic surgery.^{1,2} Clinical outcomes, though, show variable and unpredictable resorption of the graft that remains a concern, with widely variable rates reported in the literature based on a number of factors. Fat graft survival is firstly influenced by the harvest method, handling, and implantation technique.³⁻⁵ Secondly, enriched autologous fat grafts have demonstrated better results with a higher retention percentage in some areas of the body, such as the breast.^{6,7} Recently, adipose stem cell (ASC)-enriched autologous fat grafts have also shown a significantly higher residual volume when placed in the arm.⁸ Other studies have shown that adipose-derived stromal vascular fraction cells survive implantation and benefit fat graft retention.⁹ Tanikawa et al¹⁰ have looked at a protocol for faster isolation of adipose-derived stem cells and their

combination with fat in the treatment of craniofacial microsomia; however, results were not actually reported for fat retention in the face after injection in these cases. Kato et al¹¹ clarified the dynamic remodeling that occurs after fat grafting and clarified the fate of adipocytes, which depends on the microenvironment. Much remains to be learned about autologous fat grafting and to date little information is available regarding enriched fat grafting to the face and

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subsequent results. This study was undertaken to better understand the results of fat grafting to the face enriched by stromal vascular fraction (SVF) using a sophisticated three-dimensional (3D) photogrammetric facial scanning system.

METHODS

Twelve consecutive female patients were enrolled in the study, ten of which were included in the final study. One patient was dropped because of insufficient posttreatment scans and one because of large weight fluctuations and additional facial surgery during the study interval. The study was approved by the Institutional Review Board of the Stanford University and Laser Institute (Palo Alto, CA), protocol #22451, on October 11, 2011, and ran until October 2013. All subjects signed an informed consent form. The study was set up to compare autologous fat with and without the addition of the stromal vascular fraction (SVF). All candidates were presented both options. Unfortunately, no one took the simple fat grafting option when presented with the SVF option. Thus the comparison between methods was based on the results from this study and the literature. All patients underwent autologous fat grafting to the face with lipoaspirate by a single surgeon. The amount of fat injected and the injection location location were charted for each patient intra-operatively. In addition, the stromal vascular fraction was separated from 50 cc of autologous lipoaspirated fat using the technique of Yoshimura et al¹² and re-injected with the fat using fine Coleman cannulae. The cells in the SVF were counted using the nucleocounter from New Brunswick Scientific (Enfield, CT) and recorded. Each patient underwent a 3D facial photogrammetric scan prior to surgery and between 1 to 3 months posttreatment, then again at intervals of one year for the longest follow-up. The 3dMD photogrammetric system (Atlanta, GA) was used for all scans and has proven reliable and accurate.¹³ The same scanning machine was used for all scans, which were all performed by the same operator. The patients could have no other surgical procedures on the face during the study interval and their weight was monitored.

Facial Scan

The facial scans were taken using the 3dMD system, which records a photogrammetric volume scan in color which can then be analyzed volumetrically or manipulated. The Vultus software from 3dMD was used to analyze the volume of the face and posttreatment changes. The facial scans were registered for each patient at the different posttreatment time intervals, and the volume change calculated to compare the volume injected to the volumes measured from the scans at the various posttreatment time intervals. The registration technique has been shown to be reliable with a value of <0.3 mm difference.¹⁴ The volume change could

then be measured and also recorded by means of a color histogram demonstrating the changes as negative, neutral, or positive. The injected fat volume was recorded during surgery by a technician and each cc was marked on a generic face drawing as to where it was injected and the amount in each area. The total volume was then calculated for each patient. The data was then statistically analyzed and correlated using the variables of age, SVF cell count, amount of fat injected, and amount of fat retained by volume.

Fat Harvest and SVF Processing Technique

The extracted fat was processed via the technique developed by Yoshimura et al¹² at the University of Tokyo, Japan, and modified for reduced time of processing. Physiologic buffered saline (PBS) was used to neutralize the collagenase. The fat was harvested by using the Lipivage Closed System (Genesis Biosystems, Lewisville, TX) using a 3 mm, two hole under low pressure and then put aside. 50 cc of the fat was then placed into a sterile plastic tube and centrifuged for 5 minutes, to remove the infranatant blood/tumescent fluid and the supernatant oil/disrupted fat. The centrifuged fat was washed with PBS and centrifuged at 700G for 5 minutes. The infranatant solution was pipetted out and discarded. The washed fat was then preheated in an incubator, allowing the fat to reach 37°C prior to the next step.

Collagenase GMP grade, non-animal source collagenase from VitaCyte (Indianapolis, IN) was used. The product is a Blend 1 formulation, Cat. No.005-1010. This is a mixture of collagenase I and collagenase II, derived from bacterial production (*Clostridium histolyticum*) and a neutral protease (derived from *B. polymyxa*). One vial of Blend 1 formulation was used per 100 gm of centrifuged fat processed. The enzyme activity of each vial is 25 Wunsch units for the collagenases and 200,000 neutral protease units for the *B. polymyxa* neutral protease. Hank's balanced salt solution (HBSS) was pre-heated in an incubator at 37°C and 5 cc of this was then added to 1 vial of collagenase (100 mg of enzyme) and left in the incubator at 37°C until the fat was ready. This solution was then added to 95 cc of balanced saline, giving a total volume of 100 cc of collagenase solution ready for mixture with fat (1:1 mixing ratio).

Equal volumes of the washed fat and collagenase were now mixed and incubated in a shaker set at 200 rpm for 30 minutes at 37°C. The collagenase/fat solution was then centrifuged at 800G for 5 minutes at room temperature. The fat was removed from the supernatant layer of the centrifuge tube and discarded. The lower layer was then re-suspended by removing this with a pipette and placing this in a new 50 cc centrifuge tube with PBS. Three cycles of centrifugation at 800G were done for a total of 5 minutes at room temperature. The pellet was removed and resuspended and the cells counted using a nucleocounter and the total cell number

recorded. The residual cell mixture with buffered saline was a total volume of 10 cc. The surgeon then mixed the SVF suspension with the fat to be injected by transfer to opposing 60 cc syringes until the fat and PBS were well mixed. This mixture was then transferred into 1 cc syringes using a female-to-female transfer hub. The SVF-enriched fat grafts were then injected into the face using Coleman cannulae based on the discretion of the surgeon, the underlying deformity, and the patient's desired correction.

Fat Injection Technique

The fat/SVF mixture was injected into the face using Coleman cannulae following the standard technique. The amount of fat injected was based on the individual variations of each patient as far as age and aging of the face. The areas and amounts of fat injected for each patient were recorded on a generic face. The areas usually injected, based on volume, in order, were the temples, malar areas, forehead and glabella, eyelid area, lips, and chin.

RESULTS

The average patient age was 51.6 years (standard deviation [SD] 9.57) with an age range of 36 to 71 years. The average amount of fat injected was 18.4 cc (SD 15.34 cc). The average SVF cell count was 4.97×10^4 per cc. In all patients, 60 cc of fat was processed, so the relative numbers of stem cells correlated. The volume of facial augmentation was compared pretreatment and twice posttreatment (mean, 4 and 12.6 months). Follow-up ranged from 6 to 17 months. The total amount of fat injected increased with the age of the patient (Figure 1). Figure 2 shows the relationship between the final volume of retained fat in relation to the number of cells in the SVF for each patient. The final volume retention of the augmentation was 68%. It was found that the greatest change occurred in the first 3 months after the surgery, and in 8 of the 10 cases there was a loss of volume. Following that time period, six of the ten cases had an increase in volume. A clinical example is shown in Figures 3-6. Figure 4 shows the pretreatment, early posttreatment (4 months), and long-term (12 months) follow-up 3D scans. Figure 5 demonstrates the facial scans registered as histograms, with changes in facial volume color coordinated. The warmer the color the more volume increase was seen, and the cooler the color the more there was a loss of volume. In the illustration showing the placement of the fat and SVF grafts for this patient, it can be seen that fat was injected around the eyes and cheeks and in the chin (Figure 3). The histogram shows good retention in the peri-orbital area by the pink colors and in the chin (Figure 5). Conversely, the upper lip lost volume, as did the nasolabial grooves. This patient's clinical pretreatment and posttreatment results are shown in Figure 6. A statistical analysis and correlation matrix was performed in

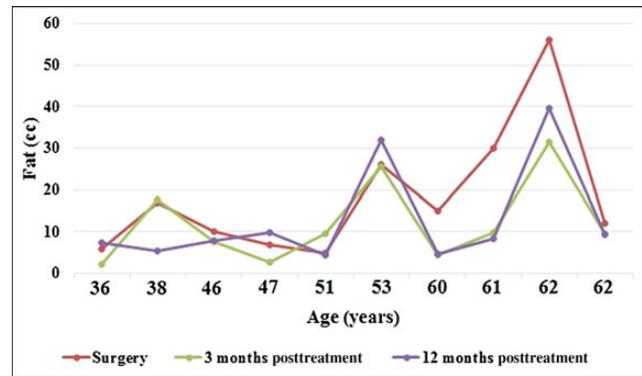


Figure 1. The amount of fat grafted in each case, plotted by age. The initial amount is in brown, the first posttreatment period in red, and the final in blue. It can be seen that more fat was grafted in the older age group. Also, final fat volume retention was higher than the initial amount in the older age groups (after age 47), while fat was lost only in the younger group.

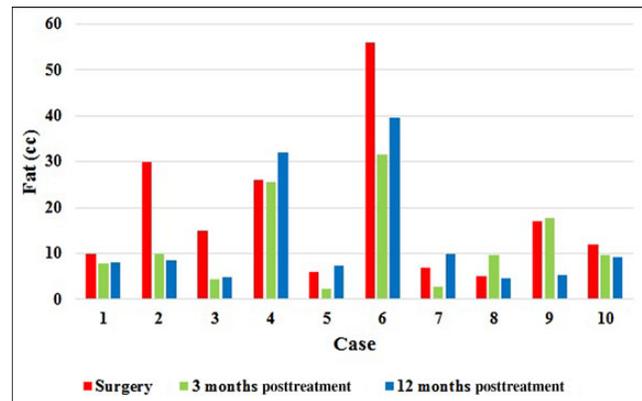


Figure 2. Graph showing the amount of fat grafted, the amount of fat remaining at 3 months, and the final amount of fat. It can be seen that after 12 months, volume was lost in 7 of the 10 cases compared to the injected volume. In six cases there was a volume gain, which was sometimes significant between 3 months and 12 months posttreatment, demonstrating an increase in volume after the initial loss of volume between surgery and 3 months posttreatment.

addition to a Wilcoxon signed rank test, and Bootstrap at 1000 times was also run to see if any further significance could be found. The analyses showed that patient age did not correlate to the number of stem cells, but the volume retention of fat did correlate to the number of stem cells per each individual, at 0.61 ($p = .0588$; Figure 7). The age of the patient did not correlate to the percentage retention of fat volume. There were no complications.

DISCUSSION

The variability of fat harvesting and processing techniques can influence the end result of fat grafting and thus the

ultimate success. A recent study by Gerth et al¹⁵ looked at long-term volumetric retention of autologous fat processed by a closed system. There were 26 patients in the study with

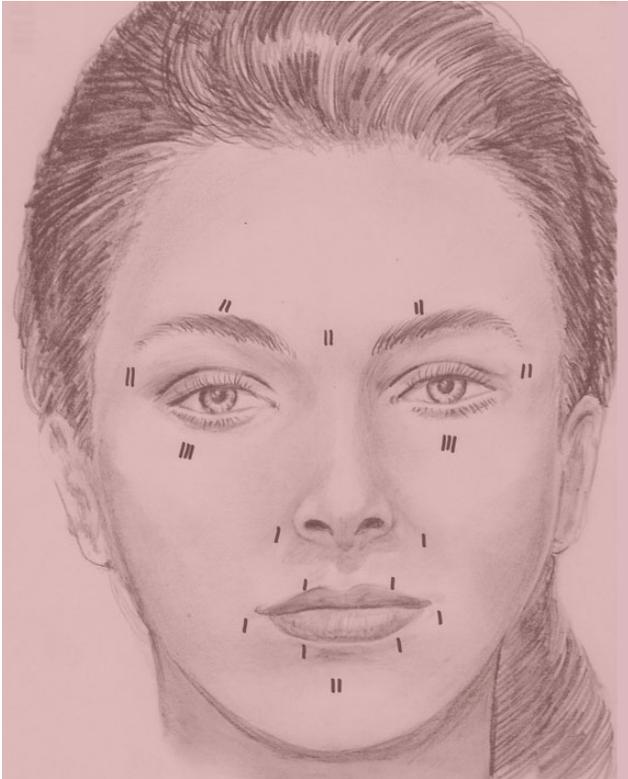


Figure 3. Facial illustration showing the placement of the fat and stromal vascular fraction (SVF) grafts in the patient seen in Figures 4-6. The fat was injected around the eyes and mouth for a total of 27 cc combined fat and SVF.

a follow-up period of 10 months. The 3D results demonstrated long-term fat retention by volume of 41.2% using closed filtration, which was superior to the 31.8% long-term retention found with centrifuged fat. Also in this study, fat retention was better in patients under age 55, which is not the case in the present study. When the 68% volume retention of fat seen in this study is compared to the study by Gerth et al¹⁵ of 41.2% with similar methodology, we could make the assumption that addition of the SVF did improve the overall volume retention of fat. This impression is also confirmed by the positive correlation between the volume of fat retained and the number of stem cells injected with the fat.

Fisher et al⁴ have compared the properties of fat grafts prepared by different methods by assessing the retained volume in a nude mouse model. The suction-assisted lipos aspirate was processed by centrifugation, cotton gauze rolling, or filtration. Cotton gauze rolling resulted in the highest stromal vascular fraction cell count per gram but was time consuming. Both centrifugation and filtration resulted in comparable graft retention rates, which were lower than the cotton gauze rolling rate. Cotton gauze rolling resulted in a 70% retention, while centrifugation resulted in 47% retention and filtration in 58% fat graft volume retention at 6 weeks. In this present study, the fat was harvested in a commercially available closed syringe system which also condenses the fat and removes any fluid or oils by filtration (Lipivage, Genesis Biosystems, Lewisville, TX).

Yoshimura and colleagues¹² have shown improved fat grafting in the breast when stromal vascular fraction cells are combined with the fat. The graft retention was improved with no evidence of fibrosis. Fu et al⁹ have also shown in an animal model that cell-assisted lipotransfer

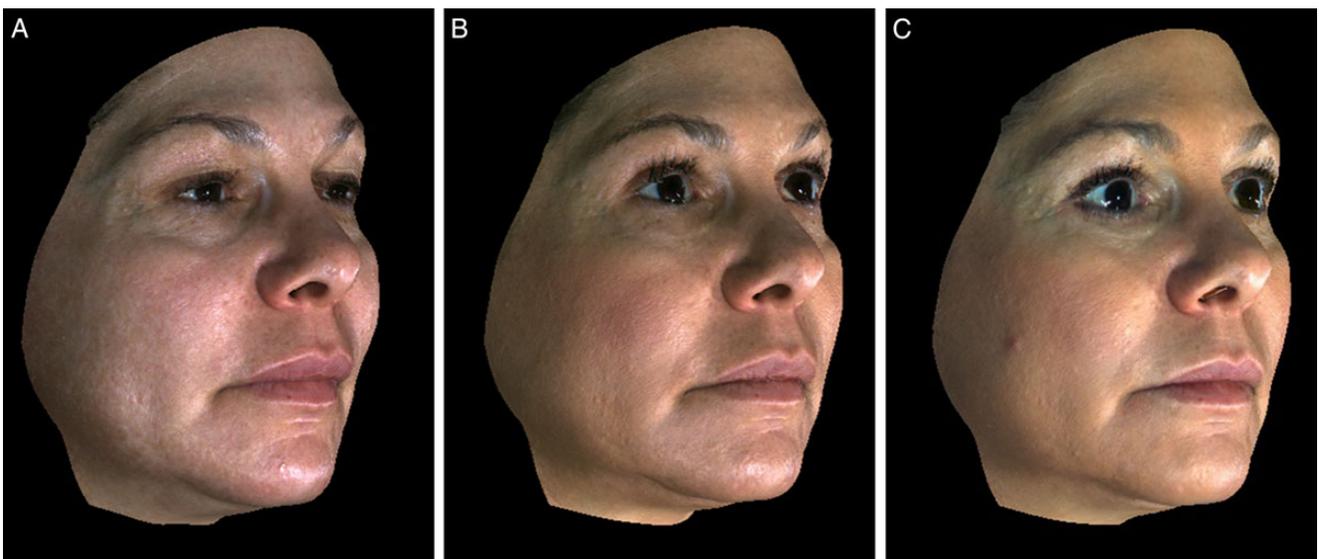


Figure 4. A series of 3D facial scans of a 49-year-old woman (A) pretreatment, (B) 4 months posttreatment, and (C) 12 months posttreatment. The increase in volume can best be seen in the malar areas.



Figure 5. Superimposed pretreatment and 12-month histograms of a 49-year-old woman (the same patient shown in Figure 4). The pink areas around the mouth show the retained volume increase in these areas.

results in subpopulations of stromal vascular fraction cells that can survive long-term after co-implantation with fat. In addition, there was spontaneous adipogenic differentiation of the implanted stromal vascular fraction cells over time.

Kølle et al⁸ reported a triple-blind study which was a placebo-controlled trial to compare fat grafts enriched with adipose-derived stem cells and non-enriched fat grafts. ASC isolation and expansion was performed first, and 14 days later they were injected along with fat at 20×10^6 cells per ml of fat in the upper arm. After 121 days, the enriched fat graft retention rate was 80.9%, whereas the non-enriched fat grafts had a retention rate of 16.3%. Tanikawa et al¹⁰ fat grafted hemifacial microsomia patients with ASC enriched fat. In the blinded and randomized study, the surviving fat volume after 6 months was 88% in the enriched group and 54% in the regular group. Mosely et al¹⁶ have shown a better result when a higher volume of fat is injected, as was also demonstrated in this study, where the two patients with smaller volume fat grafts had poorer volume retention in the table; however, this was not statistically significant in our study. The improved retention of fat volume seen in this study was also related to the presence of stromal vascular fraction cells, although the retention volume rate was 68% in this study, which is lower than the previous studies.

The comparison cannot be directly made, as this was a volume retention study using a different method of volume measuring.

The biological properties of ASCs were looked at by Philips et al.¹⁷ Lipoaspirate from 8 subjects was processed by a standardized technique and injected into nude mice. The average retention volume at 8 weeks was 52%. They found inherent differences in the concentration of CD34+ progenitor cells between patients, which may be one of the factors used to predict human fat graft retention. In the present study, the number of cells in the SVF did not correlate with age. The average SVF cell count was 4.97×10^4 per cc, which is lower than other authors. This could be a result of the study population or harvest technique. Also in this study, the cell scanner only counted live cells whereas some counters will count all cells, which can give a higher number. The volume retention after fat grafting, however, did correlate to the initial number of cells in the SVF. This could be an effect from the injection of stem cells on the grafted fat. On the other hand, individuals with an initial higher number of cells in the SVF perhaps were better candidates for fat grafting in the beginning.¹⁸ Three months after treatment, more fat volume was lost in four cases while in six cases the volume actually increased. This could be secondary to the continued growth and maturation of peridipocytes that were transplanted, and the initial loss could be a result of cell death and/or cell fat volume decreases. The results of this study are limited by the small number of cases and the variable amounts of fat injected, and a larger study population may demonstrate improved correlations. In addition, there are limitations with the 3D measuring technology, which is accurate in this scenario to 0.3 mm.¹⁴ The time difference in follow-up periods is also a limitation, as is the lack of a cohort group receiving facial fat augmentation without SVF. The overall volume retained per patient has been shown here: there was no attempt to break this down by specific regions of the face, as the volume differences and variation of the regions injected and amounts injected for each patient, together with the small number of patients in the study, made objective measurement of this unreliable in this study and is an obvious topic for further investigation, especially when related to the mobility of each region. Lastly, this study measured volume retention, which is subject to possible weight changes and changes in status of the adipocytes.

This study provides evidence that addition of the SVF fraction when fat grafting the face results in an improved retention of fat volume in comparison to historical controls. In addition there is evidence that the fat volume retention is improved with increase numbers of cells in the SVF. Much more needs to be examined in this regard to technique of harvesting, processing and injection of the fat. This study does show that the simultaneous harvest of fat and processing of the SVF and reinjection can be done without complication in the outpatient setting.



Figure 6. (A, C, E) Pretreatment and (B, D, F) 12-month posttreatment photographs of a 49-year-old woman (the same patient shown in Figures 4 and 5).

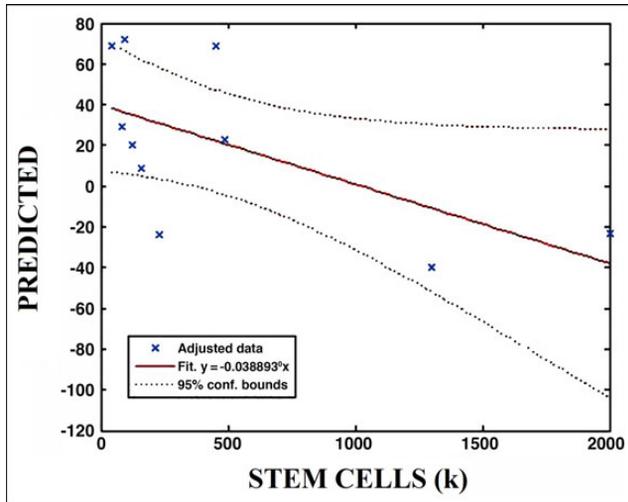


Figure 7. The relationship between the volume of fat retention and the number of stem cells for each patient. Note that the stem cell numbers are on the horizontal axis and they increase as one goes to the right. The percentage fat volume lost is on the left vertical axis and the lower the number, the more volume retained.

CONCLUSION

Fat grafting to the face for soft tissue augmentation has a high acceptance among patients. The volume retention after grafting with SVF cell supplementation is around 68%. The only correlation in this small sample size study was a higher end volume retention of fat with an initial higher number of cells in the SVF. Further work in this area is necessary. Since there was not a control group without SVF in this study, we cannot say if the result was improved by use of SVF with the fat using this technique. However, when compared to an equivalent article in the literature it appears that there is a beneficial result with the addition of the SVF in the graft volume retention.

Disclosures

Dr Schendel is a consultant for 3dMD (Atlanta, GA) and the medical director for Genesis Biosystems (Lewisville, TX).

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