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Cell-assisted Lipotransfer for the Correction of Facial Aging and Facial Contour Deformities

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ABSTRACT

Introduction: With increasing use of autologous fat grafting in the clinical setting, it is important that techniques be refined in order to improve cosmetic outcomes, improve volumetric retention, and reduce the need for repeat procedures. Here we investigate the safety and efficacy of cell-assisted lipotransfer (CAL) using uncultured stromal vascular fraction cells as a method to replace traditional autologous fat grafting for facial applications.

Methods: 32 patients received cell-assisted lipotransfer for a variety of indications including facial aging, facial contour defects, and facial augmentation. Subjects were assessed in terms of safety and tolerability as well as skin quality, patient satisfaction, and volume restoration.

Results: The mean volume of cell-enhanced fat grafted was 23.5mL (range: 5-70 mL). The mean volume of tissue submitted for point of care SVF cell isolation was 228.0 mL (range: 50-700 mL). The average nucleated cell yield per gram of tissue processed was $3.38x10^5$ nucleated cells per gram of lipoaspirate ($1.10x10^5$ - $7.0x10^5$ nucleated cells/g) with an average viability of 86.4% (67.2-97.8%). Overall, CAL to the face was well tolerated by all the subjects. There were no instances of infection and no serious adverse events reported. Only 1 patient out of 32 elected to undergo an additional round of CAL.

Discussion: The results reported here demonstrate that CAL for facial indications can be done safely at the point of care without introducing any additional risk to the subject and can potentially reduce the need for additional procedures to achieve the desired cosmetic result.

INTRODUCTION

In the past 20 years, significant advances in the basic science of engraftment and surgical technique have improved fat grafting outcomes and led to widespread acceptance of autologous fat grafting as an option for a variety indications requiring soft tissue augmentation and/or reconstruction. Adipose tissue is an attractive tissue graft because it is easily acquired in large amounts with liposuction techniques with little donor site morbidity. Autologous fat grafting (AFG) of the face is now used for a wide variety of both reconstructive and cosmetic indications including repair of congenital and post traumatic defects, facial rejuvenation/augmentation and facial atrophy [1-3].

Facial fat grafting, as with other anatomic sites, is still characterized by unpredictable rates of final engraftment volume frequently requiring multiple graft procedures to achieve the desired clinical outcome. One approach commonly used to combat this is over correction by injection of excess fat graft, but over correction is typically unfeasible in the face since the face can only accommodate small graft volumes. Another approach is supplementation fat graft with stromal vascular fraction (SVF) cells, a method termed cell-assisted lipotransfer (CAL) [4]. CAL is currently the most promising strategy proposed to improve fat graft retention. Preclinical studies show that CAL decreases the

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resorption rate of grafted adipose tissue and results in improved final volume retention [4-6]. Unambiguous efficacy compared to unenhanced fat grafting and dose-response curves have proven more difficult to establish in the clinical setting but the supportive evidence is clearly accumulating.

The SVF from adipose is a heterogeneous mixture of various nucleated blood cells, preadipocytes, fibroblasts, smooth muscle cells, and both vascular endothelial progenitors and adipose-derived stem cells [7-9]. These cells can be quickly and safely isolated from excess lipoaspirate at the point of care [10]. The adipose-derived stem cells (ASCs) contained within the stromal vascular fraction have been shown to improve the permanent graft volume through adipogenic and endothelial differentiation as well as through the production of anti-inflammatory, antiapoptotic and proangiogenic cytokines which act in a paracrine manner to maintain the viability of nearby adipocytes [11-14].

Because of volume limitations, fat grafting in the face is an excellent opportunity to apply the promising strategy of CAL. In this paper we report on the clinical results and safety in a retrospective, single center case series review of 32 patients who underwent CAL to the face for a variety of indications.

METHODS

A total of 32 subjects (3 male, 29 female) at Tower Outpatient Surgery Center received cell-assisted lipotransfer to the face between September 2010 and September 2015. All patients provided written informed consent prior to treatment under an IRB approved protocol. Subjects were treated for a variety of indications including facial aging, facial contour defects, and facial augmentation. Under IV sedation, subjects underwent infiltration with a standard tumescent solution (lidocaine 1% with 1/100,000 epinephrine and Marcaine 0.5% with 1/200,000 epinephrine) followed by suction-assisted lipoplasty using a 3.0 mm cannula in order to harvest lipoaspirate. A portion of lipoaspirate was set aside to decant for use as graft material and the remaining volume was submitted to a trained technician in the operating room for stromal vascular fraction cell separation.

In order to isolate SVF cells (**Figure 1**), freshly harvested lipoaspirate is washed 3 times using an equivalent volume of Lactated Ringer's Solution (**Figure 2a**). Washed lipoaspirate is aseptically aliquotted into sterile 50 mL conical tube with collagenase solution in a 1:1 ratio. Final collagenase concentration during digestion is 0.08 Wünsch units per mL (4 Wünsch units per 25 mL lipoaspirate). Lipoaspirate is incubated with collagenase in a heated shaking unit at 200 rpm for 20 minutes, inverting to mix every 5 minutes. Digested lipoaspirate is then centrifuged for 10 minutes at 700xg. The fluid and lipid layers are discarded (**Figure 2b**). Cell pellets are concentrated and washed 3 times with Lactated Ringer's Solution. Washed cells are then strained through a 100um cell strainer in order to remove detritus and other tissue fragments which may remain after washing. Freshly isolated SVF cells (**Figure 2c**) and graft material were homogenized and injected in a retrograde fashion using a 19-gauge injection cannula into a grid covering the desired treatment area. A portion of the isolated SVF cells were analyzed for nucleated cell counting and viability, infection control (gram stain and aerobic culture), and bacterial endotoxin testing.

RESULTS

The mean age of subjects was 56.3 years old (range: 28-75 years old) with an average BMI of 22.2 kg/m^2 (range: 17.1-27.1 kg/m^2). Subjects we followed for at least 6 months and as needed after that. Average follow-up time was 8.6 months (range: 6-14 months). **Table 1.** summarizes the indications treated. The mean volume of cell-enhanced fat grafted was 23.5mL (range: 5-70 mL). The mean volume of tissue submitted for point of care SVF cell isolation was 228.0 mL (range: 50-700 mL). The mean total nucleated cell yield was $7.54x10^7$ cells (range: $6.6x10^6$ - $2.5x10^8$ nucleated cells). The average nucleated cell yield per gram of tissue processed was $3.38x10^5$ nucleated cells per gram of lipoaspirate ($1.10x10^5$ - $7.0x10^5$ nucleated cells/g) with an average viability of 86.4% (67.2-97.8%). No bacteria were seen on any gram strains. Bacterial endotoxin levels were below the acceptable endotoxin limit in all cases. The mean time to isolate cells was 65 minutes (range: 60-90 min). Overall, CAL to the face was well tolerated by all the subjects. There were no instances of infection and no serious adverse events reported. Only 1 patient out of 32 elected to undergo an additional round of CAL.

Table 1. Indications Treated

Indication	# of subjects
Facial Aging	20
Cosmetic Augmentation	3
Facial Scarring	3
Facial contour deformity	3
Facial atrophy	3

This case series also highlights the versatility of facial fat grafting for a variety of indications. The subject pictured in **Figure 3a, 3b, 3c** is a 72 year old male who is HIV+. He presented with an HIV associated contour deformity on the left cheek. **Figure 3d, 3e, 3f** show the results at 6 months. He did not require additional fat grafting and facial symmetry was restored.

The subject shown in Figure 4 is a healthy, 75 year old female who presented with typical aging of the face (**Figure 4a, 4b, 4c**). The subject received injections into the

nasolabial fold and surrounding areas. Again, the procedure was well tolerated and the subject did not elect to receive any additional fat grafting. At 6 months (**Figure 4d, 4e, 4f**), we see an overall improvement in the skin tone and quality as well as significant softening of the age lines around the mouth and chin.

The subject shown in Figure 5 is a 57 year old male who presented with acne scarring (**Figure 5 a, 5b, 5c**). The subject received cell-enhanced fat injections throughout the affected areas. The subject did not require additional treatment and as a result of injections scarring was noticeably reduced and overall skin quality was improved (**Figure 5d, 5e, 5f).**

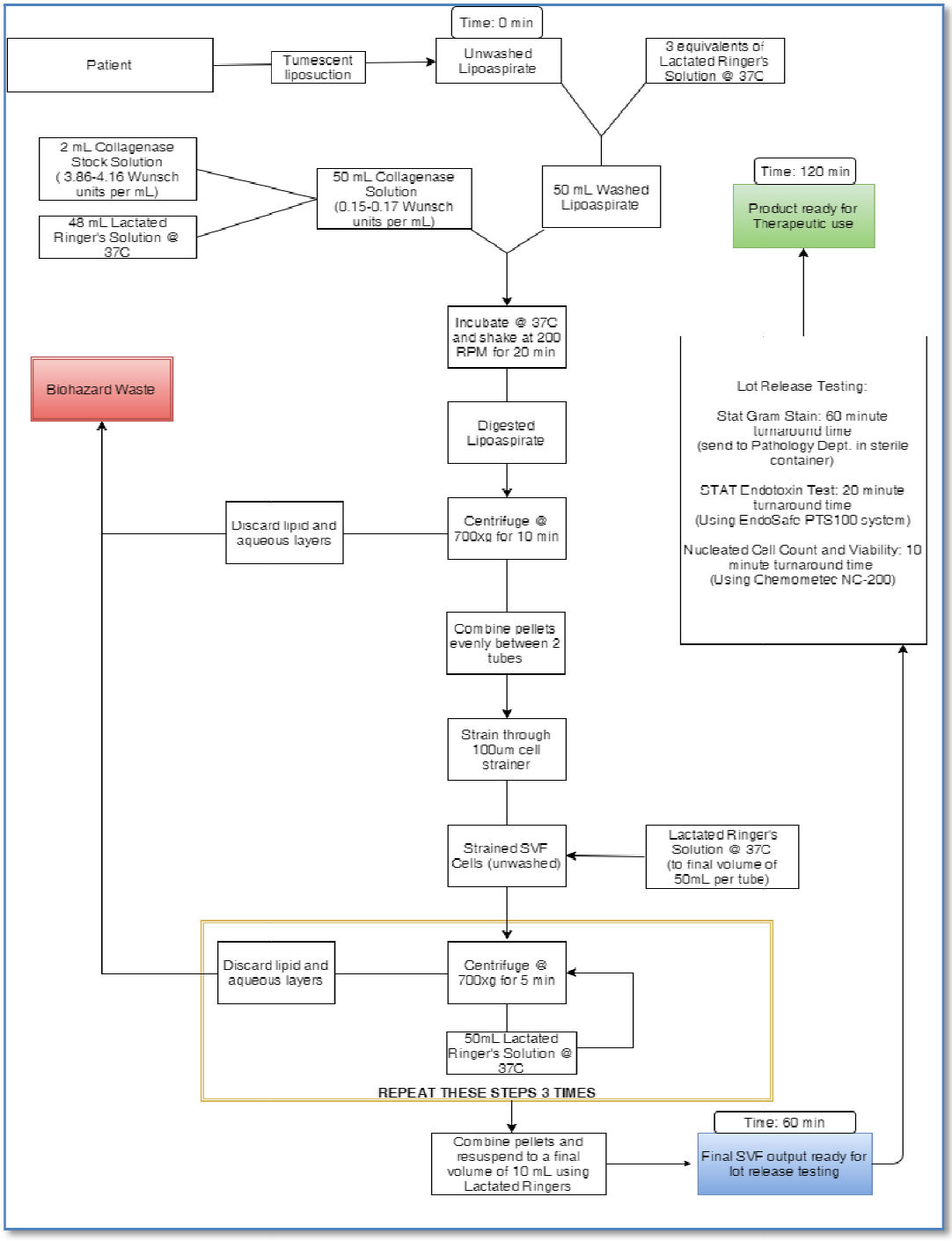

Figure 1. Summary of SVF cell isolation process.

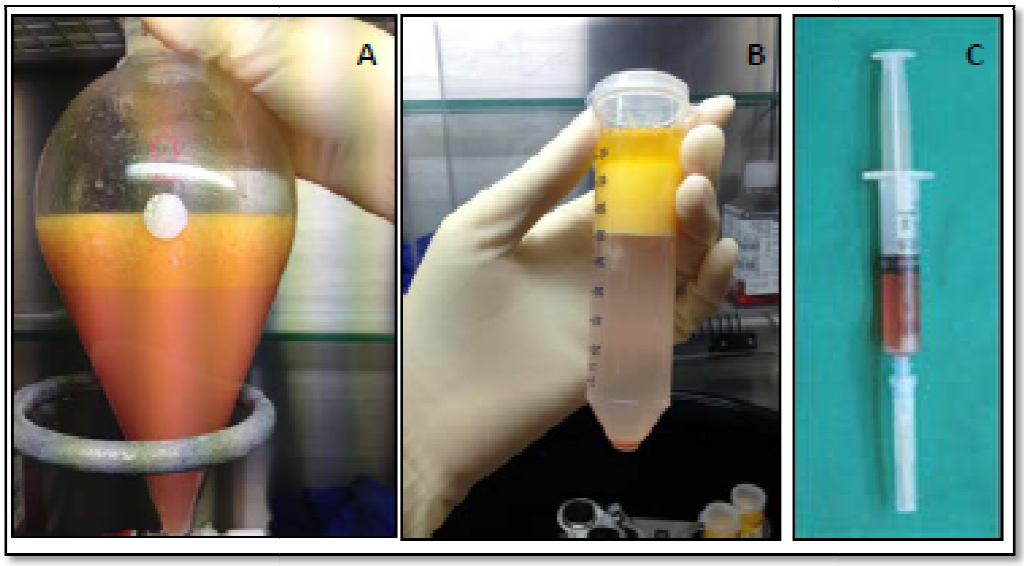

Figure 2 (a). Lipoaspirate mixed with Lactated Ringer's Solution decanting to remove tumescent solution, **(b).** 25 mL of digested lipoaspirate after centrifugation. Pellets are collected, **(c).** Concentrated SVF cells.

The subject shown in Figure 6 is a 74 year old female who presented with a facial contour defect resulting from the excision of a facial malignancy 2 years prior (**Figure 6a**). Using CAL, the defect was able to be filled and eliminated in a single procedure (**Figure 6b**). There was no recurrence of malignancy in the treated area.

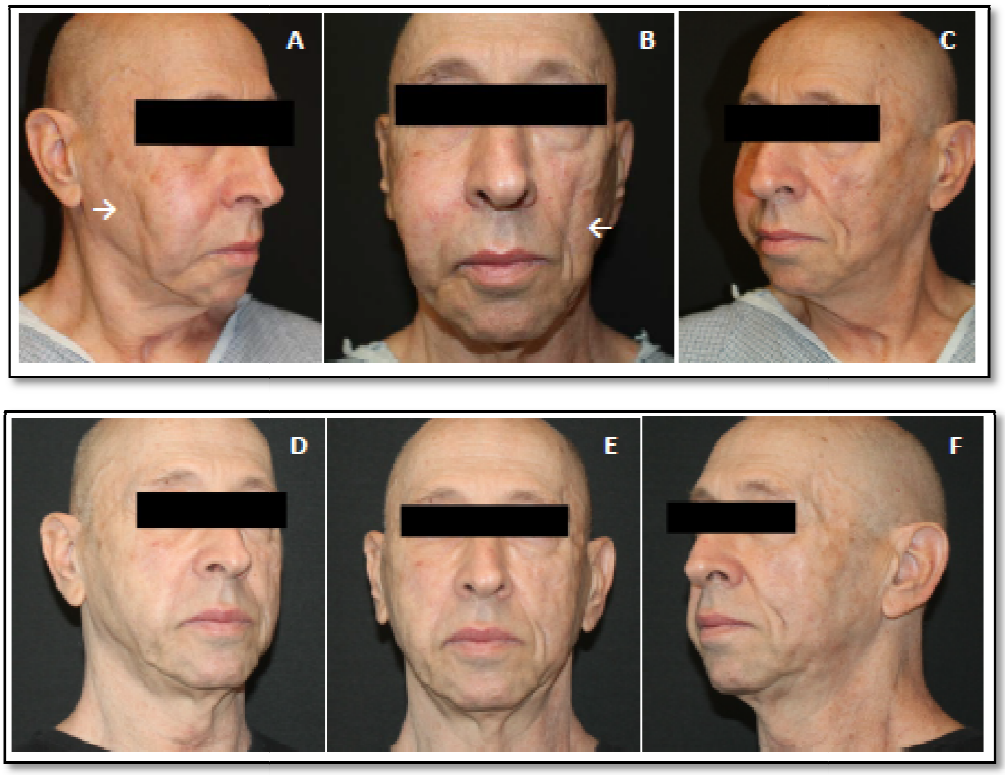

Figure 3. (a, b, c) Pre-operative: an HIV-associated contour defect of the left cheek, **(d, e, f)**. 6 months post-operative: photos

DISCUSSION

With autologous fat grafting in the last 20 years, there still remains a large amount of variability in the clinical outcomes due to a wide range of factors including graft harvest and preparation techniques, volume of fat transferred, injection technique, and patient to patient variability. With such a large amount of variability, specifically in techniques used, there is no clear consensus as to the best methods which should be employed. The major deterrent of autologous fat grafting is that the final graft volume retention is unpredictable and varies significantly with reports ranging from 10-90% retention [15]. This unpredictability often leads to multiple treatment sessions being required in order to achieve a satisfactory result [16]. This can make the treatment very expensive if multiple sessions are required. Improving the single session outcomes is beneficial because it can reduce the overall cost to achieve the required results and ultimately make the procedure available to a wider range of potential subjects. While more research is required in order to find the optimal technique for fat grafting, supplementing the graft material with autologous stromal vascular fraction cells (cell-assisted lipotransfer) has been shown to increase the overall volume of tissue which becomes permanently engrafted [3].

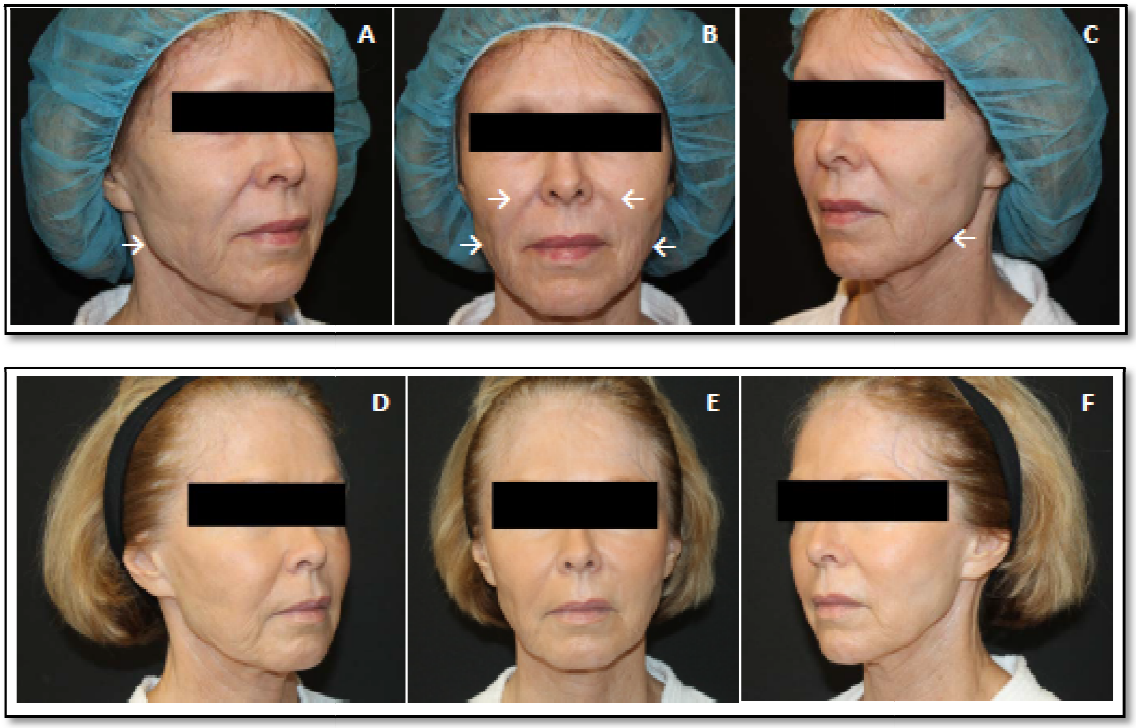

Figure 4. (a, b, c). Pre-operative: a 75-year old female presenting with facial aging, **(d, e, f)**. 6 month post-operative: photos demonstrate facial rejuvenation, improved skin quality and reduction in wrinkles.

Several studies have been published demonstrating outcomes of cell-assisted lipotransfer to the face. Schendel et al. (2015) examined the 3D volumetric retention of cell-assisted lipotransfer to the face [3]. 12 subjects received CAL to the face and were followed with facial scans to track 3D volumetric retention over the course of a year (average follow-up 12.6 months). Schendel et al. reported 68% mean volume retention at 12 months. Schendel et al. drew comparison to another study by Gerth et al. (2014) which reported results from 26 patients (mean follow-up 17 months) with 41.2% mean volume retention in a study using similar methods, but using autologous fat grafting instead of cell-assisted lipotransfer [17]. The comparison of the two supports the idea that SVF-cell supplementation does improve the long-term volume retention.

Sterodimas et al. [16] compared results of AFG and CAL in a group of 20 subjects with congenital or acquired facial tissue defects. Of the 10 subjects who received AFG alone, 7 required additional procedures in order to achieve satisfactory results, whereas all 10 subjects in the CAL groups were satisfied after only 1 procedure. Another study by Yoshimura et al. [2] compared clinical outcomes of AFG and CAL in 6 subjects with facial lipoatrophy due to lupus profundus or Parry-Romberg syndrome [18]. All subjects obtained improved facial contour, but subjects in the CAL group (n=3) had better clinical improvement reported overall. Overall, the general trend observed in clinical publications of CAL to the face is that the use of CAL is superior to AFG alone in terms of clinical outcome and can reduce the need for additional procedures.

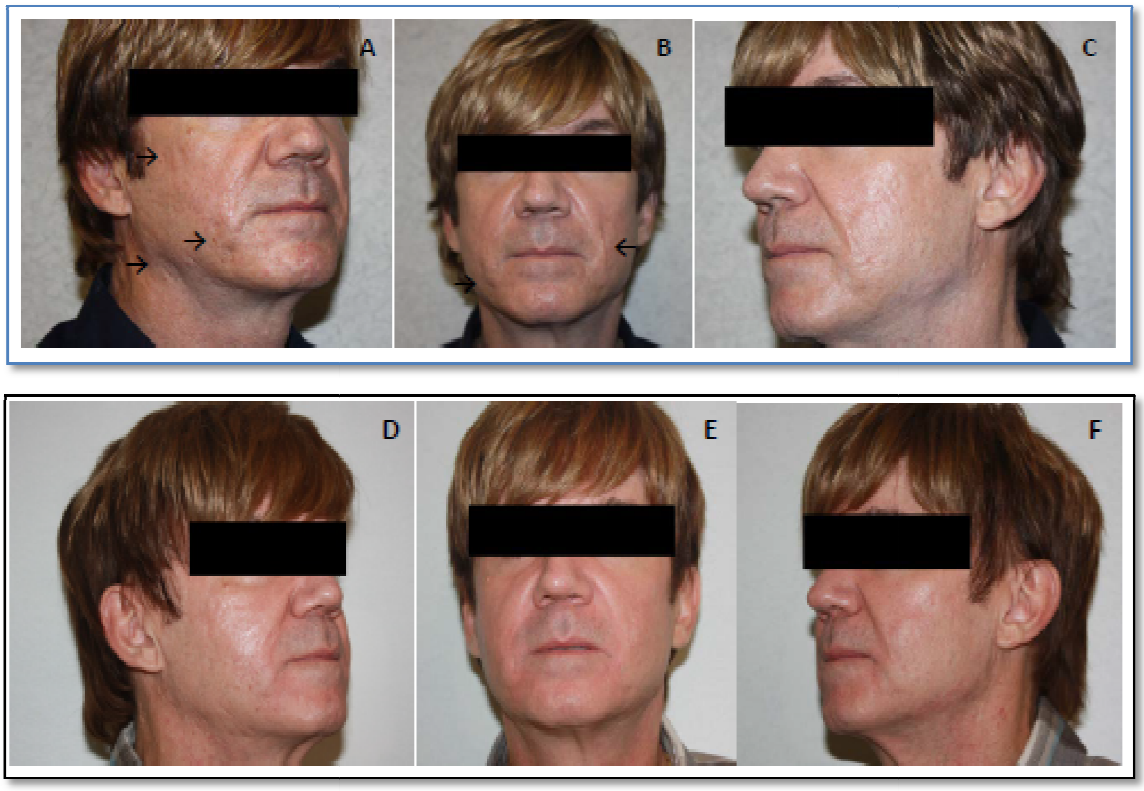

Figure 5. (a, b, c). Pre-operative: a 57 year old male who presented with acne scarring, **(d, e, f)**. 12-months post-operative: photos depict softening of scars and overall improvement in skin tone/quality.

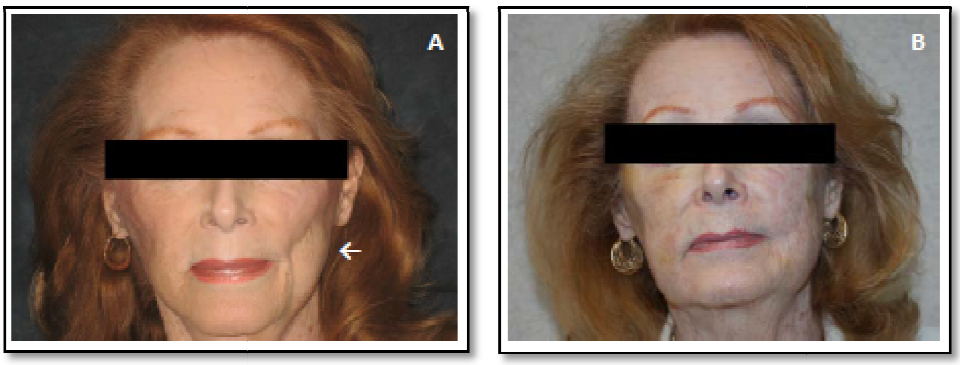

Figure 6. (a). Pre-operative: photo showing acquired facial contour defect of the left cheek, **(b).** 11 months post-op: Restoration of symmetry and volume.

CONCLUSION

The results reported here further demonstrate the applicability of CAL as a suitable substitute to conventional AFG in the clinical setting for facial applications. Only 1 patient desired additional treatment after receiving the initial treatment. Another important aspect of this case series is that the procedure was well tolerated and there were no adverse events reported in any of the 32 subjects treated. As SVF cell-based therapies begin to translate into the clinical setting, it is important to highlight the safety and tolerability of the procedures. The results reported here demonstrate that CAL for facial indications can be done safely at the point of care without introducing any additional risk to the subject compared to standard autologous fat grafting while negligibly increasing the operation time if concurrent procedures are being conducted.

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