

Unfiltered Nanofat Injections Rejuvenate Postburn Scars of Face

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Abstract: The aim of this study was to compare the quality of postburn facial scars before and after injection of unfiltered nanofat. The study was performed in the Plastic Surgery Department of Mayo Hospital, Lahore, Pakistan, from January 2015 to December 2016. Forty-eight patients with postburn facial scars were included; age range was 4 to 32 years with Fitzpatrick skin types between 3 and 4. Patients with hypertrophic scars, contractures, or keloids were excluded. Scars were assessed by a senior plastic surgeon and the patient on the POSAS (Patient Observer Scar Assessment Scale). Fat was harvested from the abdomen and/or thighs with a 3-mm multiport liposuction cannula (containing several sharp side holes of 1 mm) using Coleman technique. The harvested fat was emulsified and transferred into 1-mL Luer-Lock syringes for injection into the subdermal or intradermal plane. Final follow-up was scheduled at 6 months, and scar was rated by the patient and the same surgeon on the POSAS. Preoperative and postoperative scar scores were compared, and *P* values were calculated. Results indicated that after nanofat grafting, there was a statistically significant improvement in scar quality. The most significant improvements on the observer scale were seen in pigmentation and pliability (*P* < 0.0001). Thickness and relief were the least improved variables (*P* = 0.785 and 0.99, respectively). ImageJ scanning also showed pigmentation change (*P* = 0.076). A statistically significant improvement was seen in all parameters of the patient section of the POSAS (*P* < 0.0001). In conclusion, unfiltered nanofat grafting seems to be a promising and effective therapeutic approach in postburn facial scars, showing significant improvement in scar quality. The trial was registered on www.clinicaltrials.gov with following ID NCT03352297.

Key Words: liposuction, nanofat, rejuvenation, postburn scars

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Fat grafting in its various forms could be considered as a hallmark in scar rejuvenation therapy in the 21st century. With aesthetic appearances increasingly proving to be a major factor for social survival, it is only logical that reconstructive surgery has become inextricable from its cosmetic counterpart. Techniques described to improve the cosmetic appearance of scars include scar revisions, lasers, steroid injections, pressure garments, skin grafting, tissue expansion, chemical peels, dermabrasion, topical creams, and ointments.

Fat grafting was conceptualized by Neuber¹ in 1893. It further gained impetus after Illouz² introduced liposuction for harvesting fat in the 1980s. A refinement of his technique, proposed by Coleman,^{3,4} has become the standard today. A breakthrough in fat grafting occurred when multipotent stem cells were discovered in the stromal vascular

fraction (SVF) of adipose tissue in 2001.⁵ The term “nanofat grafting” was conceived by Tonnard et al⁶ in 2013. The “element of interest” in nanofat is the tissue stromal vascular fraction (t-SVF), which is essentially a bioactive extracellular matrix adherent to a mixture of cells.⁷ Among these, the adipose-derived stem cells (ASCs) have a unique, uncanny proclivity for differentiating into tissues in which they are injected. Inevitably, therefore, nanofat is of indispensable value in regenerative and rejuvenative therapies^{8–10} including scar rejuvenation. In addition, the SVF of adipose tissue contains growth factors such as basic fibroblast growth factor, insulinlike growth factor 1, vascular endothelial factor, and platelet-derived growth factor.¹¹ As such, ASCs could also be potential key players in scar rejuvenation.¹² Further processing of the t-SVF by enzymatic degradation of the matrix yields the cellular SVF, which is a mixture of pluripotent cells and growth factors without the connective tissue matrix.

A large number of facial postburn scars present in our outpatient department every year. Thus, despite the fact that the face possesses an excellent healing propensity, a significant proportion of patients present with postburn facial stigmata. Exacerbating this predicament are the darker skin types prevalent in our race with a penchant for hyperpigmentation that further aggravates the appearance of these scars. Face being the most evident part of the body, any imperfection has adverse and indelible psychosocial implications.¹³ With studies expounding the beneficial effects of ASCs,¹³ nanofat grafting for scar modulation seemed an exciting and encouraging proposition.¹³ Given the significant number of postburn facial scars in our setup, our study aimed to investigate the efficacy of nanofat grafting for improvement of facial postburn scars using a standard scar scoring scale.

MATERIALS AND METHODS

This prospective study was held at the Department of Plastic and Reconstructive Surgery, Mayo Hospital Lahore, from January 2015 to December 2016. Forty-eight patients were included in the study. Facial scars of more than 1-year duration with Fitzpatrick skin types 3 or 4 were selected for rejuvenation with nanofat therapy. Patients who had any previous treatment for their scars were excluded. Patients with hypertrophic scars/keloids (defined for the purpose of this study as scars raised >5 mm above the skin when measured with a caliper), contractures, and comorbid conditions known to affect wound healing, for example, diabetes, liver or connective tissue disease, or a body mass index of 14 kg/m² or less (expected to yield insufficient fat), were also excluded. Patients taking blood thinners were advised to stop their medication 2 weeks before surgery to minimize bruising and hematoma formation. After informed consent, photographs of the scars were taken with a 10-megapixel camera. Standard lighting and views were ensured. Scars were assigned an initial score based on the Patient Observer Scar Assessment Scale (POSAS).¹⁴ Considering that only mature scars were included in the study, the vascularity of the scar on the observer scale was not relevant and therefore not scored. Similarly, no change in surface area of the scar was expected; thus, this parameter was also deleted from the scale. Apart from a subjective analysis of pigmentation on the POSAS, quantitative analysis of pigmentation levels before therapy was assessed by ImageJ version 4.1 software (<https://imagej.nih.gov/ij/>).

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Fat was mainly harvested from the abdomen, the lateral thigh, and/or the gluteal region. Tumescence solution comprising of 0.9% saline 1000 mL, lidocaine 30 mL, and 1 mL of 1:1000 epinephrine was infiltrated into the donor sites. After making a stab incision at the site of harvest, fat was harvested using a 3-mm cannula with multiple sharp side holes of 1 mm attached to a 20-mL syringe. Negative pressure was created in the syringe by pulling the plunger back by 2 mL. The fat was transferred to a commercially available sterilized sieve. It was rinsed well with 0.9% saline to wash off all the blood and tumescent fluid suctioned along with the fat. This fat was then transferred to a 10-mL syringe connected to another similar syringe with a female Luer-Lock. Emulsification of this fat was achieved manually by 30 passes of this fat between two 10-mL syringes. No local anesthesia was used at the recipient site. Pretunneling (subcision) was done in the intradermal or subdermal layer depending on the thickness of the dermis. Fat was injected in a fanwise pattern with an 18-gauge needle connected to a 1-mL syringe until the skin blanched or displayed a yellowish discoloration.⁶ Adhesive surgical strips (Steri-Strip) were applied over and around the recipient area for 5 days to minimize edema and fat shift. Stab incisions in the donor area were sutured or dressed with Steri-Strip, and an elastic bandage was applied to contain edema and bruising. The patients were discharged the next day in the absence of any complications. Sun protection was advised to all patients. They were also asked to take a soft diet and immobilize the area as much as possible for the first 14 days to minimize shift and edema. Gram-positive coverage with cephradine was provided until 2 days postoperatively. First follow-up was at 10 days. The grafted area was examined for any infection or edema. The patients were asked to revisit after 6 months in case of no complaints in the interim period.

After 6 months, photographs of the scar were taken again using the same views and lighting as used preoperatively and reanalyzed with ImageJ version 4.1 software.^{15,16} A final POSAS score was ascribed to the scar. Data were analyzed using SPSS version 22 and tabulated for interpretation. In order to apply paired-sample *t* test, we checked the normality of data using 1-sample Kolmogorov-Smirnov test. If the normality assumptions were not fulfilled, the Wilcoxon test was applied.

RESULTS

The mean age of patients was 22.25 ± 5.79 years with an age range between 4 and 32 years. There were 20 (41.7%) male and 28 (58.3%) female cases. The mean scar size was 24.20 ± 16.39 cm². The mean volume of fat injected was 12.34 ± 10.07 mL. The observer (Tables 1 and 2) and patient (Tables 3 and 4) results were tabulated. The scores were also presented on a bar graph for a quick overview of the overall improvement after nanofat therapy (Tables 1 and 3, lower panels). The most significant improvements were found in pliability and pigmentation on the observer scale (Table 1 and Fig. 1). Improvement in thickness and relief (the extent to which surface irregularities are present, preferably compared with adjacent normal skin) were, however, unremarkable. Decrease in scar pigmentation “post therapy” was also recorded on ImageJ scanning (Fig. 2) but was not statistically significant with a $P = 0.076$. Statistically significant improvement ($P < 0.0001$) was observed on all parameters of the patient section of the POSAS (Table 3 and Fig. 2). Figure 1 shows a visible improvement in the relief and overall appearance of the scar postoperatively. There was mild edema in 25 patients (62.5%) that settled per se within 2 weeks in all cases. No bruising was noted in any patient. No fat cysts or granulomas were observed in any patient.

DISCUSSION

Nanofat in our study proved to be an effective and viable entrant within the wide domain of scar therapies. Scar appearance was statistically better on almost all parameters following a single session of nanofat

TABLE 1. Pretherapy and Posttherapy Observer Values on the POSAS

Variable	Mean	SD	Median	IQR	P
Preprocedure pigmentation	7.58	1.23	8.00	1.00	<0.0001*
Postprocedure pigmentation	6.42	1.51	6.00	2.00	
Pre procedure thickness	4.38	1.95	3.00	3.75	0.785†
Postprocedure thickness	4.25	1.78	3.00	3.00	
Preprocedure relief	4.25	2.07	3.00	3.00	0.099†
Postprocedure relief	3.58	1.65	3.00	3.00	
Preprocedure pliability	6.75	1.76	7.00	2.50	<0.0001*
Postprocedure pliability	3.42	0.77	3.00	1.00	
Preprocedure overall scar score	7.50	0.77	8.00	1.00	<0.0001*
Postprocedure overall scar score	4.33	0.48	4.0	1.0	

Note that maximum benefit obtained was for improvement in pigmentation and pliability.

*Wilcoxon test was applied (comparison was made on median $\pm$ interquartile range [IQR]).

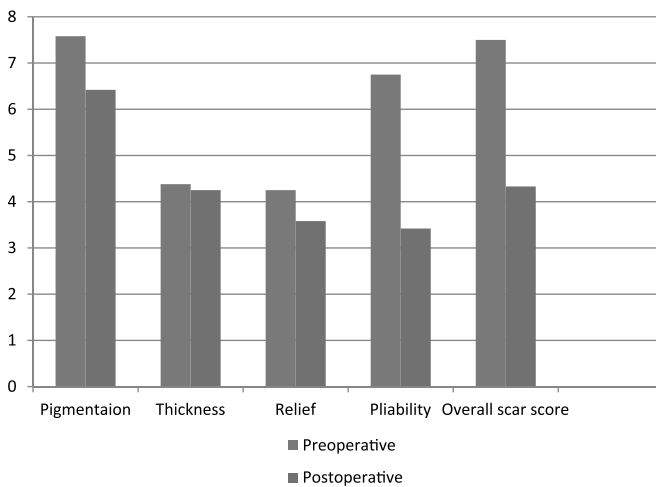
†Paired-sample *t* test was applied (comparison was made on mean $\pm$ SD).

injection using a simple technique requiring no special equipment. We excluded hypertrophic scars and keloids from our study as the pathology in these scars requires their regression rather than rejuvenation. Accordingly, the study recorded least improvement in scar thickness and relief. Scars of less than 1-year duration were also excluded as they are amenable to improvement with standard postburn scar therapy at this stage. However, we regard not recording the age of individual scars a limitation of our study.

Microfat grafting has been used successfully in treating radiotherapy ulcers and scars,¹⁷ filling of rhytides,¹⁸ facial scar improvement,¹⁹ and breast augmentation,^{20,21} among others. However, its progeny, the nanofat, is still a relatively new concept; as such, literature pertaining to it is scanty, limiting our comparisons with other studies. Microfat grafting on the contrary has been around for a longer period and is therefore more researched, established, and prevalent.

Tonnard et al,⁶ in his seminal article, described emulsifying and filtering microfat through a nylon gauze with 0.5-mm perforations to produce nanofat. The resultant effluent was fluid enough to be injected easily through a 27-gauge needle. Ten milliliters of lipoaspirate typically yields about 1 mL of nanofat with this method.⁶ In our study, nanofat was created by simple emulsification of microfat without further filtration. Emulsification of fat harvested with multiperforated cannulas is sufficient to generate tiny nanofat particles per se while filtration would facilitate injection with small 27-gauge needles. Significant improvement in scar appearance occurred with emulsified fat alone. A predecessor of Tonnard and colleagues⁶ filtered nanofat, it would logically contain all elements as its filtered offspring. Moreover, the deceased adipocytes retained in the unfiltered specimen are indefatigable even in their death, serving to release cytokines and attract growth factor–releasing macrophages to the injected areas.²² These factors further incite stem cell proliferation and tissue regeneration.^{22,23}

Lo Furno et al²² compared the number of ASCs in samples of microfat, nanofat, and unfiltered-emulsified fat that was given the appellation “nanofat 2.0.” A greater number of ASCs were recorded in the nanofat 2.0 as compared with the conventional nanofat sample, filtration being culpable for the destruction of the stem cell population. In contrast to Tonnard and colleagues⁶ findings, the number of CD34⁺ hemopoietic stem cells was low by the 20th day in all 3 samples, whereas the population of CD90, CD44, and CD105 positive mesenchymal stem cells (MSCs) increased. Lipoaspirate derived CD34 stem cells area transient species compared with their more tenacious peers in hemopoietic

TABLE 2. Mean Preoperative and Postoperative Observer POSAS Shown

tissue. Tonnard and colleagues⁶ CD34⁺ cell count was performed at 10 days, whereas Lo Furno et al²² extended the count time to 20 days.

Unfiltered emulsified nanofat was also used by Goisis et al²⁴ to treat tear trough deformities. Filtration was omitted because medical-grade nylon gauze was not available. Instead 1.5-mm cannulas were used to suction smaller fat particles that would not plug fine-bore needles. In our study, the thicker nanofat had to be injected with large-gauge needles. Fortunately, no conspicuous scars remained at the puncture sites.

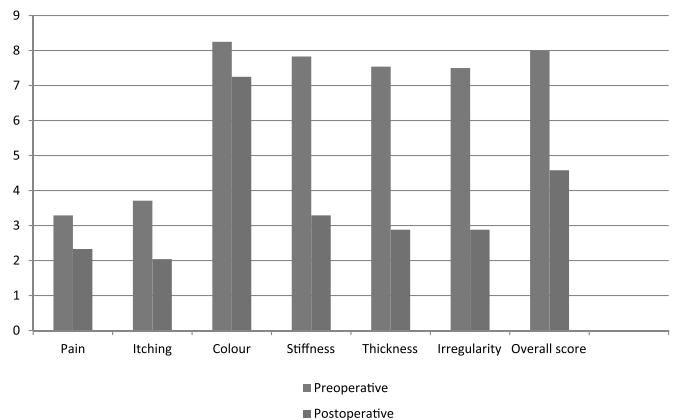
One of the earliest manifestations of nanofat therapy is improved pliability. Although long-term improvement in scar pliability is a function of stem cell–induced collagen and elastin synthesis and remodeling, early improvement in scar pliability stems from the separation of the scarred skin from underlying tissues and the placement of soft, fluid fat in between, whereas assessment of pliability in our study was limited

TABLE 3. Pre- and Post-6 Months of Therapy Patient Scoring on the POSAS

Variable	Mean	SD	Median	IQR	P
Preprocedure pain	3.29	2.19	2.00	2.75	<0.0001*
Postprocedure pain	2.33	0.63	2.00	1.00	
Preprocedure itching	3.71	2.70	2.00	5.00	<0.0001*
Postprocedure itching	2.04	0.46	2.00	0.00	
Preprocedure color	8.25	0.67	8.00	1.00	<0.0001*
Postprocedure color	7.25	1.18	8.00	1.00	
Preprocedure stiffness	7.83	1.29	8.00	0.75	<0.0001*
Postprocedure stiffness	3.29	0.62	3.00	1.00	
Preprocedure thickness	7.54	1.62	8.00	1.75	<0.0001*
Postprocedure thickness	2.88	0.73	3.00	1.00	
Preprocedure irregularity	7.50	1.70	8.00	0.75	<0.0001*
Postprocedure irregularity	2.88	0.79	3.00	1.75	
Preprocedure overall score	8.00	1.05	8.00	1.00	<0.0001*

Patients recorded significantly better scores on all parameters after nanofat grafting.

*Wilcoxon test was applied (comparison was made on median $\pm$ interquartile range [IQR]).

TABLE 4. Mean Preoperative and Postoperative Patient POSAS Shown

to a mere subjective assessment by a feel of the skin on pinch due to nonavailability of objective tools such as a cutometer or a durometer. Jaspers et al²⁵ objectively measured and recorded improvement in pliability of scars after microfat grafting using a cutometer. Jaspers et al²⁵ recorded statistically significant improvements in pliability and an overall POSAS score over a 3-month follow-up period. We reckon the short follow-up period is too early to preempt the very veritable effect of persisting erythema on scar pigmentation. However, in our study too, pigmentation changes on POSAS over a 1-year follow-up were insignificant.

Tonnard et al⁶ postulate the use of nanofat to treat dark circles under the eyes. Similarly, in another study, nanofat was successfully used for treatment of dark circles under the eyes.²⁶ Nanofat forms a layer between the thin almost translucent eyelid skin and the underlying vasculature that imparts the dark shadows. Although the skin of postburn scars selected for our study group was mostly thin similar to eyelid skin, hyperpigmentation was largely the result of persistent postinflammatory melanocytic hyperactivity in our darker skin types, rather than a show of the underlying vasculature. Even so, we surmise that in our study the interposition of pearly white nanofat between the thinned-out dermal layers and the deeper tissues contributed to the improvement in pigmentation. Moreover, enhanced contours may be misperceived as paler areas due to increased reflection of light from them. Thus, the significantly improved pigmentation recorded on the subjective POSAS could partly be the result of a visual perception contradicted by the more objective ImageJ scan. However, we would place greater emphasis on subjective scar scores because in such assessments patient satisfaction carries more weight than nonchalant mathematical values.

Mailey et al²⁷ claim to be the first to report improvement in skin pigmentation after autologous fat grafting. This was attributed to the antioxidant and wound healing properties of the constituents of the SVF.^{27,28} In vitro, SVF is reported to cause whitening by suppressing melanin synthesis and tyrosinase activity.²⁸ Only Fitzpatrick types 3 and 4 were selected for this study to minimize bias, as different Fitzpatrick skin types respond differently to the various stimuli that effect pigmentation. ImageJ software can be used to detect the density of color. A lower density corresponds to a darker color and vice versa, with white possessing the highest density. Using the ImageJ to identify a numerical shift in color density was a distinctive feature of our study.²⁹ Gu et al³⁰ also used ImageJ to highlight improvement in pigmentation improvement after condensed (unfiltered, twice centrifuged nanofat to remove the oleaginous components) nanofat therapy. As described previously, nanofat in this study refers to the milky fluid produced after emulsification of microfat. Very few adipocytes responsible for approximately 70% of the filling effect survive the



FIGURE 1. Visible improvement in scar appearance 6 months after a single session of nanofat grafting. Representative photographs of 3 patients are shown.

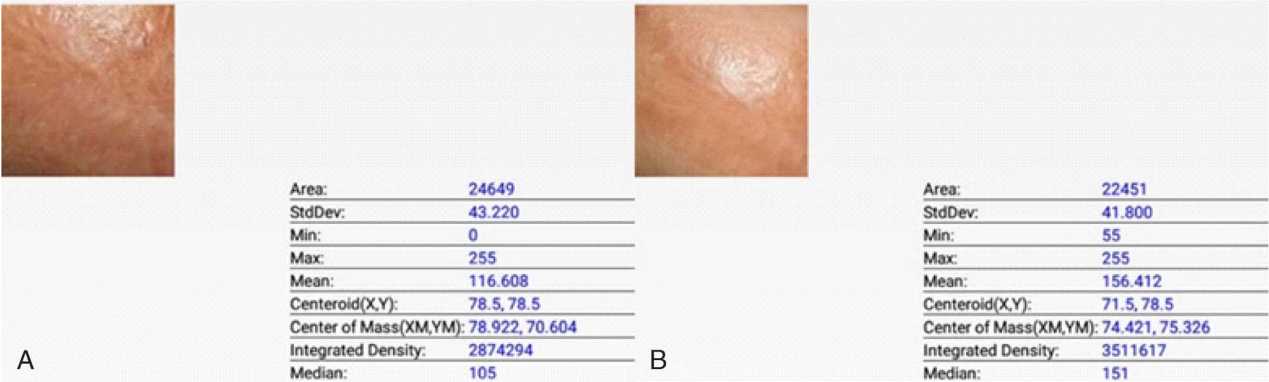


FIGURE 2. ImageJ scanning showing increase in optical density corresponding to a decrease in pigmentation after nanofat grafting. ImageJ values are shown before (A) and after (B) nanofat injections.

mechanical stress induced by emulsification of fat.^{6,31} Hence, unlike microfat grafting, filling is not a primary component of nanofat grafting. Any augmentation in volume is only a consequent of proliferation incited by growth factors provided by the nanofat and therefore not evident before 3 weeks.⁶ Microfat or macrofat is conclusively the material of choice when provision of volume is the prime reason for fat grafting. Conversely, nanofat is more suited for aesthetic enhancement. The small size of the nanofat particles facilitates injection in and under scars; the stem cells contained in the nanofat make it ideal for skin rejuvenation. Unlike macro fat and microfat, nanofat is not known to be associated with infection, granulomas, or fat cysts.^{32,33}

As cited previously, the most relevant component of nanofat is the adipose-derived tissue stromal vascular fraction that survives the emulsification process and is responsible for the rejuvenative, proliferative, and subsequent filling effects of nanofat. The heterogeneous multipotent stem cell population in the adipose-derived t-SVF has the ability to transform into the host cell lineage such as adipogenic, chondrogenic, cardiogenic, or even neurogenic.^{34–36} Apart from these stem cells, the SVF contains other cell populations such as preadipocytes, endothelial cells, and cells of hemopoietic lineage, as well as fibroblasts. Growth factors are secreted in a paracrine fashion by the SVF, triggered by inflammation during pretunneling and hypoxia during suctioning.^{10,37,38} In addition, t-SVF acts as a bioactive matrix to provide a scaffold for the attachment of proliferating stem cells. Stem cells can also be harvested from the bone marrow, but the procedure is more invasive and painful. Enzymatic digestion of the stromal element in this bioactive t-SVF results in the creation of adipose tissue–derived cellular stromal vascular fraction that is an even more refined source of stem cells. It is clear that the nanofat functions at a cellular level. Keeping this in view, Tonnard et al⁶ are skeptical if it should in fact be classified as a fat graft or an in vivo tissue engineering process.⁶ The highest concentration of MSCs is clustered around the pericytes of the adipose tissue vasculature. Based on this knowledge, Lipogem technology^{39–40} (Lipogems; Lipogems International SpA, Milan, Italy) was developed in 2013 that enabled isolation of nanofat with an intact perivascular structure. Lipogems are being used in many fields of medicines today for their regenerative properties.^{41–44} Relying on a closed, low-pressure system to inflict minimal trauma to the stromal vasculature and hence to the MSCs, it is very similar to the emulsification sans filtration method practiced in this study.

Similar to Lo Furno,²² skipping the filtration step in our study was driven by necessity rather than a moment of eureka as we too lacked the relevant filtration capabilities. As noted previously, the survival of ASCs depends on the treatment meted to them.²² Filtration has been cited to damage the ASCs.^{22,39} Its only benefit seems to be easy infiltration that can be overruled by other methods or even skipped altogether as observed in our study without impacting on the results. Given our own success with unfiltered nanofat and that of other studies cited, it is food for thought that it might in fact be beneficial to preclude the step of filtration from nanofat processing. Having procured filtration equipment, studies are now underway to compare the results of filtered with unfiltered nanofat in scar rejuvenation.

CONCLUSIONS

Injection of nanofat sourced from unfiltered, emulsified fat in nonhypertrophic, postburn scars caused statistically significant improvements in scar appearance.

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