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Preliminary Report

Topical Nanofat Biocrème Improves Aesthetic Outcomes of Nonablative Fractionated Laser Treatment: A Preliminary Report

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Abstract

Background: Improvements in skin erythema and elasticity have been observed with topical application of platelet-rich plasma after fractional laser (FXD) treatment. Injections of nanofat via small needles into the dermis improves tissue thickness, discoloration and wrinkle depth.

Objectives: The aim of this study was to evaluate improvements in skin following a nonablative FXD treatment combined with the application of a novel topical nanofat biocrème, called neo-U.

Methods: Fifty patients were treated with a nonablative FXD followed by application of a topical nanofat biocrème. Harvested fat was processed into nanofat, which was compounded with a transdermal liposomal delivery vector to produce a topical biocrème. In 2 patients, postauricular skin punch biopsies were performed before and after treatment and examined for histologic changes. Photographs of a historical group treated with only the FXD were compared with photographs of patients treated with a combination of topical nanofat biocrème and FXD. Skin types were evaluated for improvements in nasolabial folds, wrinkles, and skin texture.

Results: Findings from postauricular skin biopsies show the skin exposed to FXD with nanofat biocrème had more elastin fibers and a slight increase in the thickness of the epidermis. Patients treated with FXD plus nanofat biocrème had a statistically significant improvement in the degree of wrinkles, nasolabial fold depth, and texture compared with historical controls.

Conclusions: Transdermal delivery of nanofat topical biocrème applied after FXD treatment can serve as a delivery system to improve fine lines, nasolabial fold depth, and overall texture of the tissue to a greater degree than laser resurfacing alone.

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Topical tissue regeneration in which nanofat serves as an adjunct to laser resurfacing has not been explored and is a new concept in cosmetic surgery. Nanofat as a biological crème compounded with a liposomal delivery vehicle was used in combination with a fractionated 1550-nm erbium/1927-nm thulium fiber laser (FXD) to deliver adipose stromal vascular fraction (SVF) to improve healing and rejuvenation of the treated skin. Conventional fat grafting and treatment with platelet-rich plasma (PRP), nanofat, and adipose-derived SVF, used alone and in combination

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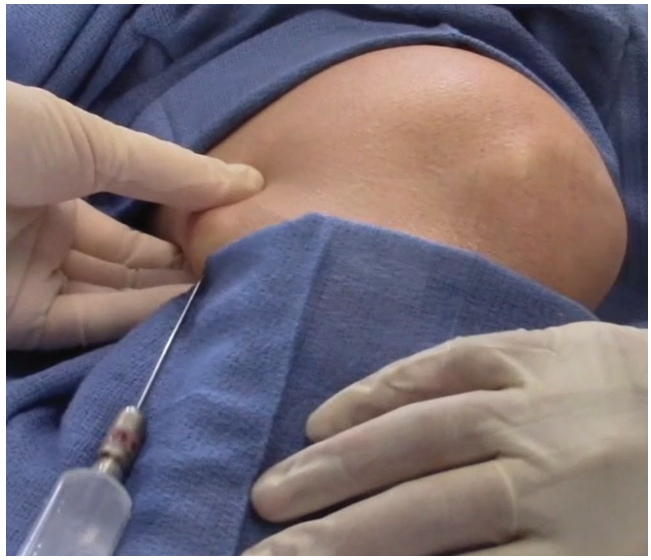
with resurfacing lasers and microneedling devices, have been performed in our practice since 2004.[1-3] Patients treated with PRP, nanofat, and SVF by needle infiltration and microneedling have shown improvements in the appearance of atrophic scars and reductions in the degree of hyperpigmentation of the lower eyelid, tear troughs, fine lines of the face and neck, fine rhytids around the mouth as well as wounds, incisions, scars, and alopecia.[2,4-6] Improvements in skin erythema and elasticity have been seen with PRP topical application after FXD treatment.[7] To our knowledge this is the first study to evaluate improvements in skin following an FXD treatment combined with the application of a novel topical biocrème, called neo-U (FacesPlus, San Diego, CA).

METHODS

The medical records of 50 patients treated between January 2017 and December 2018 were reviewed. A total of 50 patients (94% females, 6% males), median age 58 years, gave consent, and were treated with FXD and topical nanofat biocrème. Laser settings varied based on the patient's condition, severity, and skin type, and the variation in treatment parameters between the 2 groups (patients who received topical nanofat biocreme in addition to laser vs patients who only received laser) was similar. Photographs were taken of all patients both before and after treatment. Seventeen participants were eliminated due to incomplete data. Patients treated with additional modalities were excluded from the present study in order to focus on the impact of nanofat biocrème when combined with a specific laser regimen. The remaining 33 patients, with Fitzpatrick skin types I-IV (18.2%, type I; 45.5%, type II; 21.2%, type III; 18.2%, type IV), referred to as the topical nanofat biocrème group, constitute the basis of the study and were compared with 84 patients receiving the same laser treatment, but without topical nanofat.[1]

Treatment Protocol

A topical lidocaine 23%/tetracaine 7% numbing ointment was applied to the face for 1 hour, during which time fat was harvested via 14-gauge needle puncture from the patient's medial thighs. All procedures were performed under local anesthesia with tumescent solution. A total of 20 mL of fat per patient was harvested by syringe-assisted liposuction with a 14-gauge 10 cm Carraway harvest cannula. Harvested fat was emulsified by first passing it through a 2.4-mm diameter connector, a 1.2-mm connector, and a final double filter of 400 and 600 μm to produce the nanofat.[8] This yields a more liquid-like product of matrix, stromal vascular cells, and free fatty acids. The nanofat was centrifuged (1200 rpm for approximately 2 minutes)



Video 1. Watch now at http://academic.oup.com/asj/article-lookup/doi/10.1093/asj/sjz240

in order to separate the free fatty acids, which were aspirated and discarded. The remaining nanofat was compounded with a liposomal delivery vehicle to create the topical nanofat biocrème. The liposomal delivery vehicle is a transdermal preparation base containing a liposomal component that increases the permeation of a variety of actives.[9-11] The nanofat biocrème was applied to the skin after each quadrant of the face was treated with the FXD (Video 1). Finally, the nanofat biocrème was sent home with the patients, who were instructed to keep it at 4°C, and recommended to apply it twice per day for the next 72 hours (Supplementary Figure 1). All patients were also instructed to apply SPF 55 and a Neo-Cleanse Biocream for 2 weeks before and 10 days after treatment (Neocutis, Pully, Switzerland).

Data

Two of the 33 patients consented to biopsies before and 3 months following treatment. The FXD treatment was administered to the face as well as behind both ears, whereas the nanofat biocreme was applied to the face and postauricular region on only one side as specified by the practitioner (Figure 1). The histologic examination involved a Verhoeff-Van Gieson stain followed by a trichome stain to evaluate differences in elastin fibers.

Photographs taken before and after treatment were subjected to a blind review performed by a research intern who evaluated improvements in wrinkles, nasolabial folds, and pigmentation on a scale of 0 (no improvement) to 5 (near full improvement) (Figures 2, 3). Eighty-four patients who had undergone FXD treatment alone between 2009 and 2017 served as our historical control group.[1]

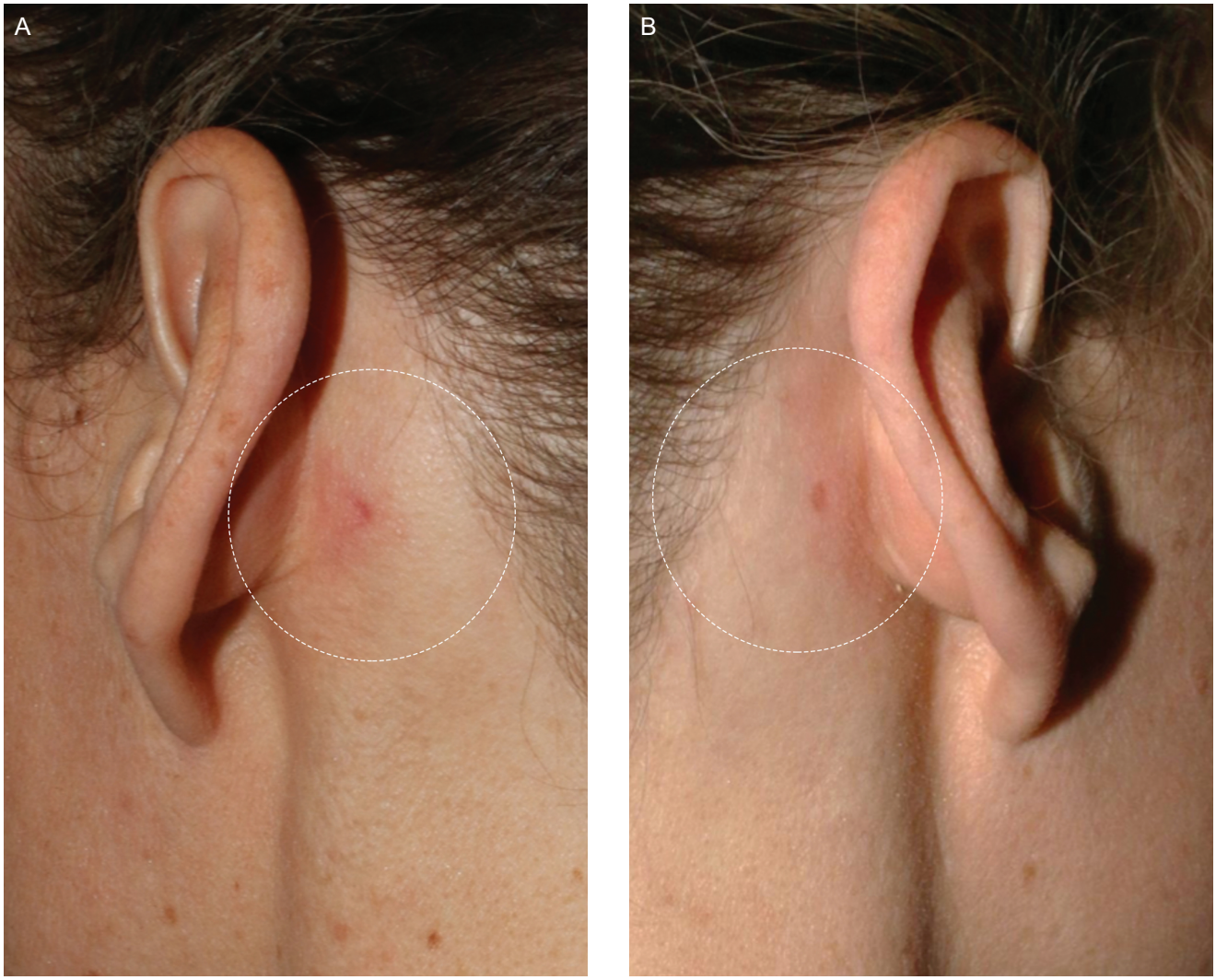


Figure 1. The postauricular regions where skin punch biopsies were performed 3 months earlier on a 38-year-old female patient. (A) On the left side, where only FXD laser treatment was performed, there is a triangle of redness around the punch biopsies. (B) On the right side, where the topical nanofat biocrème and FXD laser treatments were performed, very little surrounding redness other than the punch biopsy is observed. FXD, fractional laser.

Improvement ratings of wrinkles, nasolabial folds, and skin texture in our topical nanofat plus FXD group were then compared with the historical control group.

It was not necessary to obtain approval from an institutional review board as fat-grafting is a long-established procedure and the microinjection device used in this study has received ISO 13485 certification and CE marking. All devices used for fat harvesting and processing were manufactured in the United States, and were registered and listed with the US Food and Drug Administration (FDA). Patients gave written informed consent prior to all surgical procedures, anesthesia, intraoperative video recording, and photography in accordance to both the Helsinki Declaration and Good Clinical Practice (GCP).

RESULTS

Follow-up photos were taken 1 to 18 months after treatment, with a mean follow-up time of 3 months. The topical nanofat plus FXD group consisted of 33 patients (32 females and 1 male), ranging in age from 35 to 74 years (median, 58 years), with Fitzpatrick skin types I-IV, who underwent FXD treatment between 2017 and 2018 with topical application of the nanofat biocrème. Two patients from the nanofat plus FXD group underwent biopsies prior to and 3 months after treatment. A histologic blind examination performed by a dermatopathologist reported a greater amount of new elastin fibers as well as a slight increase in the thickness of the epidermis in the skin of

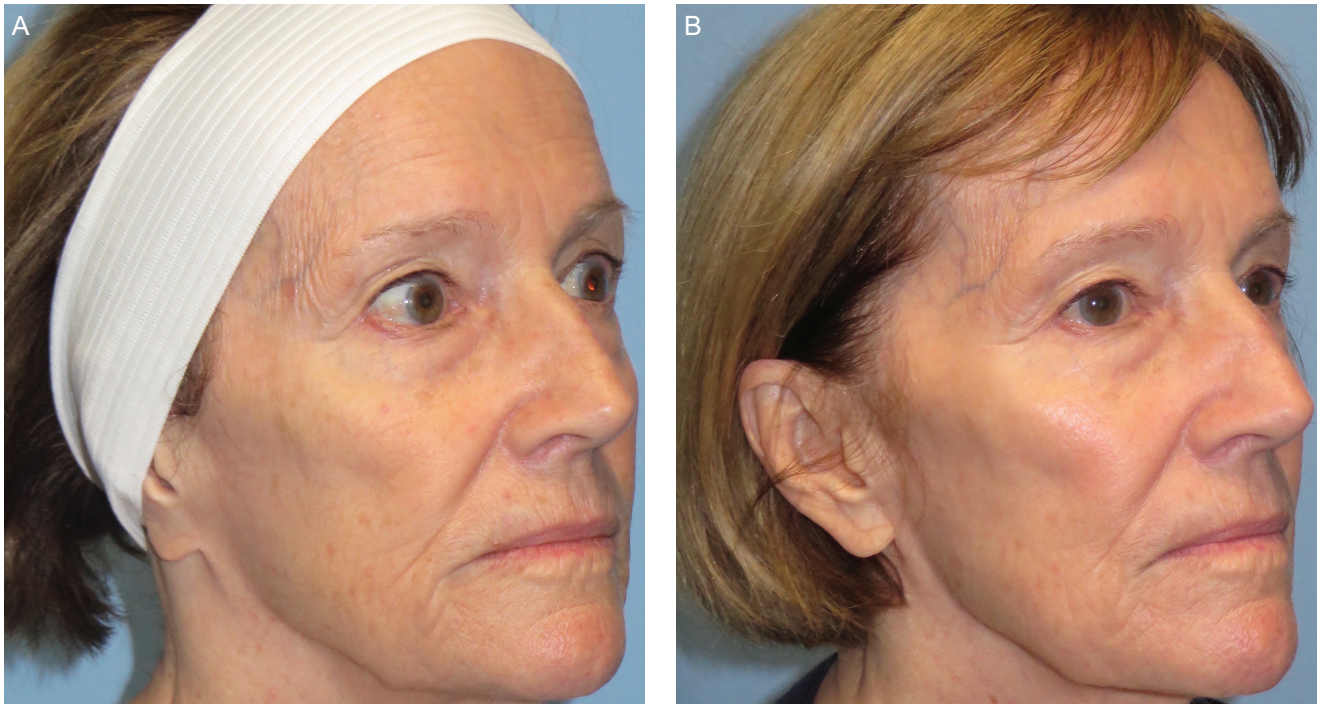


Figure 2. (A) A 72-year-old female patient with a Fitzpatrick type II skin type before fractional laser and topical nanofat biocrème treatment. (B) The patient 3 months after treatment, showing a 1-point wrinkle improvement, a 1-point improvement in nasolabial folds, and a 5-point improvement in overall skin texture.

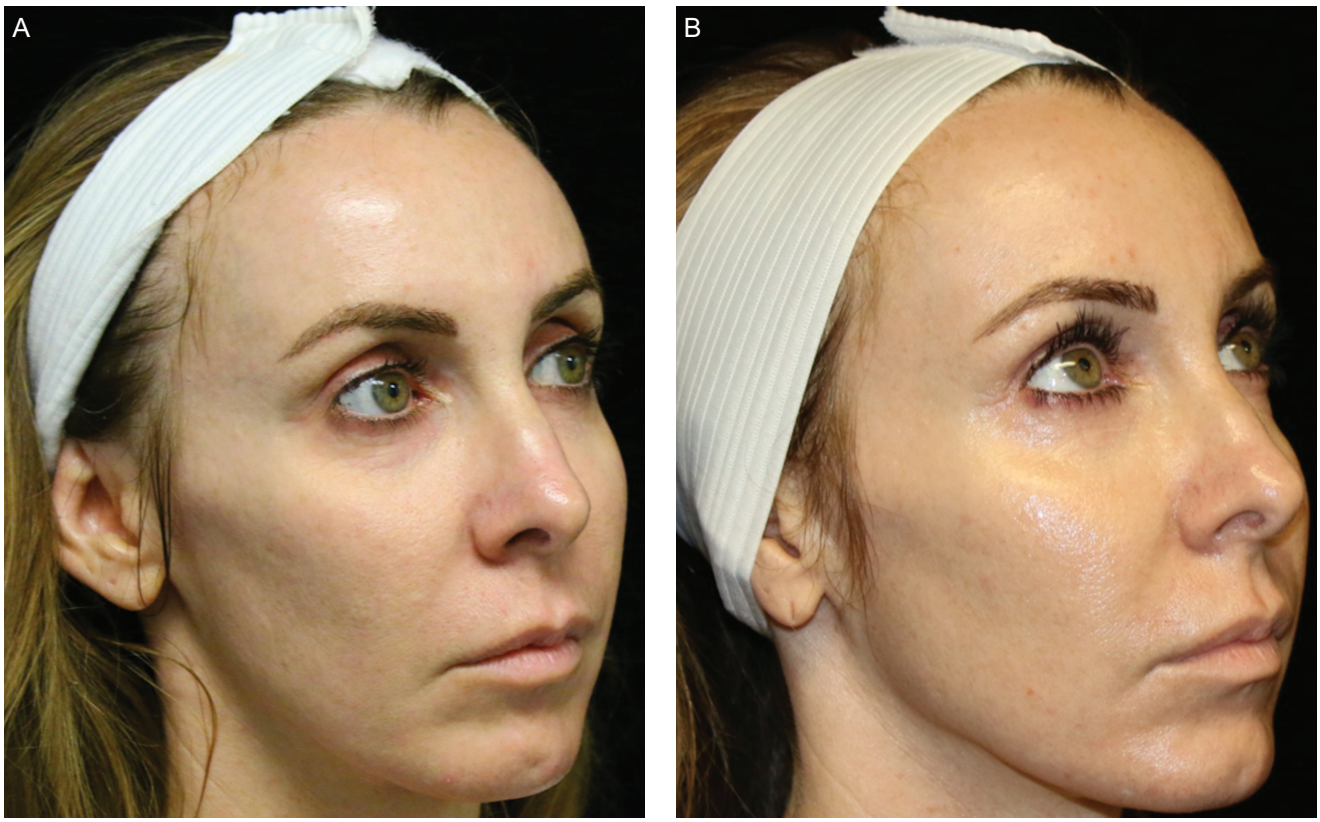


Figure 3. (A) A 39-year-old female patient with a Fitzpatrick type II skin type before fractional laser and topical nanofat biocrème treatment. (B) The patient 3 months after treatment, showing a 1-point wrinkle improvement, a 1-point improvement in nasolabial folds, and a 3-point improvement in overall skin texture.

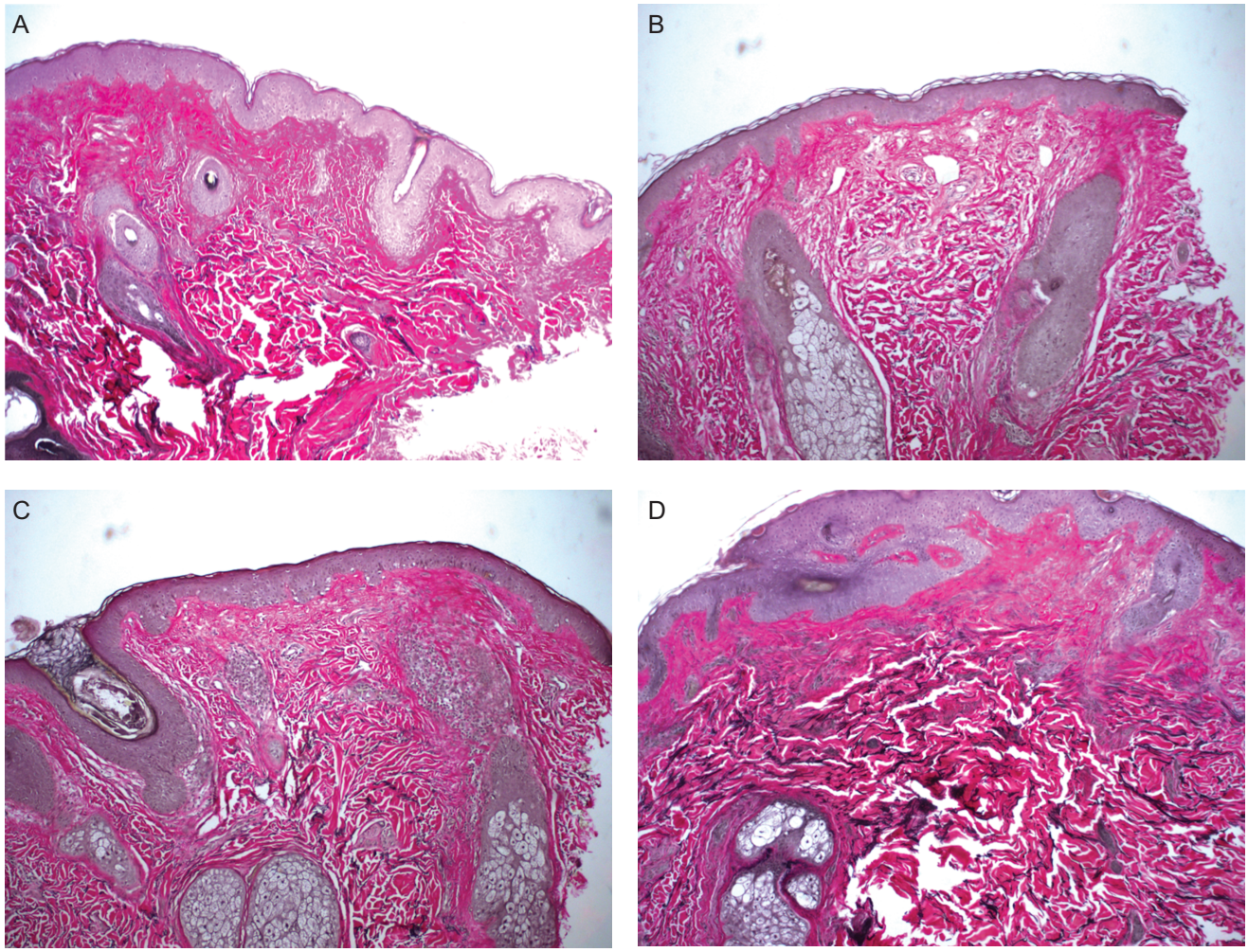


Figure 4. Pretreatment punch skin biopsy of 38-year-old female patient's (A) left postauricular and (B) right postauricular regions. Histologic examination revealed that pretreatment punch biopsies of left and right postauricular skin showed similar features. Punch biopsy taken 3 months after treatment of the patient's (C) left postauricular skin, which received only FXD treatment, and (D) right postauricular skin, which received FXD treatment plus topical nanofat biocrème. Blind histologic examination identified greater numbers of new elastin fibers as well as a slight increase in the thickness of the epidermis on the skin in the area treated with topical nanofat biocrème and FXD (D). FXD, fractional laser.

Table 1. Fitzpatrick Skin Type and Point Improvement in the Historical Control Group

	Historical control group		
Fitzpatrick skin type	Wrinkles (average point improvement)	Nasolabial fold (average point improvement)	Texture (average point improvement)
I (n = 2, 2.4%)	1.5	1	2
II (n = 33, 39.3%)	1.03	0.79	2.79
III (n = 35, 41.7%)	0.97	0.83	2.63
IV (n = 14, 16.7%)	0.71	0.57	3.07

FXD, fractional laser.

Table 2. Fitzpatrick Skin Type and Point Improvement in the FXD Treatment Plus Topical Nanofat Group

	Topical nanofat group plus FXD		
Fitzpatrick skin type	Wrinkles (average point improvement)	Nasolabial fold (average point improvement)	Texture (average point improvement)
I (n = 6, 18.2%)	1.17	2	3
II (n = 15, 45.5%)	2.85	2	3.46
III (n = 7, 21.2%)	1.43	3.5	4.14
IV (n = 6, 18.2%)	2.67	2.5	3.4

FXD, fractional laser.

Table 3. Comparison of Average Point Improvement Between the Historical Group and the FXD Treatment Plus Topical Nanofat Group

	Comparison between historical and topical nanofat plus FXD groups		
Fitzpatrick skin type	Wrinkles (average point improvement)	Nasolabial fold (average point improvement)	Texture (average point improvement)
I	0.33	1	1
II	1.82	1.21	0.67
III	0.46	2.67	1.51
IV	1.96	1.93	0.33

FXD, fractional laser.

the nanofat plus FXD group when compared with skin exposed to FXD alone (Figure 4).

The breakdown of the Fitzpatrick skin types in the historical controls as well as the average point improvement in each category, which included wrinkles, nasolabial fold depth, and texture, are shown in Table 1. The breakdown of the Fitzpatrick skin types in the nanofat biocrème plus FXD group as well as the average point improvement in each category, which included wrinkles, nasolabial fold depth, and texture, are displayed and compared to the historical control group (Table 2).

The topical nanofat plus FXD group showed a 1-point improvement in both nasolabial folds and texture compared with the historical controls; however, no wrinkle improvement in Fitzpatrick skin type I patients was seen. This was the only category not to show any improvement. Fitzpatrick skin type II saw a 1.82-, 1.21-, and 0.67-point improvement in wrinkles, depth nasolabial folds, and overall skin texture, respectively, when compared with the control group. In Fitzpatrick type III patients, 0.46-, 2.67-, and 1.51-point improvements were seen in wrinkles, nasolabial folds, and texture, respectively. Finally, when compared with the control group, Fitzpatrick Type IV patients showed 1.96-, 1.93-, and 0.33-point average improvements in wrinkles, nasolabial fold depth, and overall skin texture, respectively (Table 3). A 2-tailed t test comparing the Likert-scale results showed that the topical nanofat plus FXD group achieved significant improvements in nasolabial fold depths ($P < 0.05$), wrinkles ($P < 0.05$), and overall skin texture ($P < 0.05$) when compared with the historical control group.

DISCUSSION

Topical nanofat biocrème, a new concept in aesthetics, may have applications in many different areas of regenerative medicine. Depending on the depth of penetration and the type of transdermal transport vehicle, delivery of various substances into the deep dermis and into the subcutaneous fat is possible. Dermal fibroblasts, which can differentiate into adipose tissue, have been shown to be integrally involved in the aging processes of skin. A study by Zhang et al [12] identified transforming growth factor β (TGF-β) as a key regulator of age-related losses of dermal fat, decreased adipogenesis, and decreased production of cathlidicin antimicrobial–producing peptide in response to a *Staphylococcus aureus* skin infection. Mice that were treated with TGF-β receptor inhibitors displayed restoration of both antimicrobial function of dermal fibroblasts and adipogenesis and an increased resistance to *S. aureus* skin infection.[12] Breakdown of the skin barrier has implications beyond the aesthetic aspects of aging, including permitting entry of bacteria and other pathogens—events that provide insight into the dermal changes that occur as a result of aging. Nanofat microneedling, laser-assisted delivery, and pharmaceutical-assisted delivery of biologically active tissues such as SVF and nanofat may upregulate fibroblasts and reduce levels of TGF-β, maintaining youthful skin and its underlying adipose cells.[13-18]

The transdermal delivery product used in this study has 3 drawbacks: substances intended for percutaneous permeation must have low molecular weights (<5500 Da), high lipophilicity (oil solubility), and a dose requirement not exceeding 40% of the biocrème mixture (PCCA, Houston, TX). Nanofat itself is too large to penetrate the skin transcutaneously in this compound, and even individual cells and adipocyte-released growth factors are too large. However, certain peptides, amino acids, various DNA fragments, and other substances under 5500 Da are likely to be the biologically active products transported through the skin. More basic scientific research on the specific action of nanofat, SVF, and other components of fat is needed. Nevertheless, the clinical results of this study indicate that nanofat compounded with a unique transdermal transport vehicle penetrates the skin, leading to improvements in wrinkling, nasolabial fold depth, and skin texture.

Nanofat when injected via small needles into the dermis has shown improvements in tissue thickness, discoloration, and wrinkle depth.[19-21] We speculated that SVF and nanofat could be microneedled or delivered by FXD into the skin to produce a beneficial effect.[8] In search of a topical route of nanofat delivery, we combined nanofat with a liposomal transport agent in the belief that increased penetration of the skin with biologically active components of nanofat would further improve clinical results. Furthermore, the findings of Duncan et al[16] showed that holes in the skin created by FXD treatment stay open longer and thus enhance drug or biological uptake due to the dermal sponge injury pattern that is created.

This small retrospective study appears to confirm our notion that a nanofat biocrème may be complimentary to laser treatment. In clinical practice, both our nanofat microneedling technique as well as the nanofat biocrème are used together. In patients who decline treatment due to cost constraints and/or downtime required for nanofat collection, PRP can be combined with the liposomal delivery vehicle in place of the nanofat.

Study Limitations

Although this study yielded statistically significant findings there are a number of limitations in comparing topical nanofat-treated patients with historical controls. Ideally, a prospective study that validates these findings would provide much more substantial evidence than a retrospective comparison. In addition, we only had biopsies from 2 patients, which provided some information, but the results would require corroboration from a larger prospective study. Our assessment of the increased amount of elastin fibers was based on a simple visual estimation. It would have been preferable to have used a morphologic approach to count actual fibers. In future studies, objective histometric studies would be preferable. This study was based on observation of photographs, which even under the best circumstances are not uniform. A better study would be to prospectively evaluate skin changes with more objective photomorphologic evaluations. Nevertheless, the strongly significant changes in this small study, which in and of themselves were a surprise, point toward a treatment impact. It is our hope that this small preliminary study stimulates other researchers to corroborate these findings.

CONCLUSION

This study shows that the transdermal delivery of nanofat topical biocrème applied following FXD treatment can serve as a particulate delivery system to improve fine lines, nasolabial fold depth, tissue texture, pigmentation and healing. Further studies should be done in order to determine the effects and longevity of nanofat biocrème on wound healing and aging skin in combination with lasers and alone, and to compare these treatments with other regenerative modalities. At present, patients undergoing laser resurfacing are evaluated for possible nanofat biocrème treatment. When significant facial volume loss is present, fat grafting via a technique called Injectable Tissue Replacement and Regeneration (ITR^2) is offered. In patients being treated by laser resurfacing alone, topical nanofat biocrème may be used to accelerate healing and improve long-term results.

Disclosures

Dr Cohen is a shareholder in and receives royalties from Lipocube, Inc, has options in and receives royalties from Millennium Medical Inc, and receives royalties from Tulip, Inc. Dr Cohen holds a trademark on ITR^2 and has a patent pending on the biological crème and ITR^2 discussed in this paper. Dr Tiryaki is a shareholder in the Mage Group, the owner of Lipocube. The other authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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