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A Single-Center Trial to Evaluate the Efficacy and Tolerability of Four Microneedling Treatments on Fine Lines and Wrinkles of Facial and Neck Skin in Subjects With Fitzpatrick Skin Types I-IV: An Objective Assessment Using Noninvasive Devices and 0.33-mm Microbiopsies

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Abstract

Background: Although ablative techniques are the standard of care for the treatment of fine lines and wrinkles, microneedling offers a minimally invasive alternative.

Objectives: The purpose of this study was to assess the efficacy of microneedling on facial and neck fine lines and wrinkles.

Methods: Thirty-five subjects between 44 and 65 years old with Fitzpatrick skin types I to IV received 4 monthly microneedling treatments over the face and neck. Subjects returned 1 and 3 months posttreatment. At every visit, high-resolution ultrasonography and optical coherence tomography were performed, and transepidermal water loss and skin elasticity were measured. Microbiopsies (0.33 mm) were collected pretreatment, before the fourth treatment and 3 months posttreatment.

Results: Thirty-two subjects (30 females, 2 males) completed all 7 visits. Facial dermal and epidermal density increased 101.86% and 19.28%, respectively, from baseline at 3 months posttreatment. Facial elasticity increased 28.2% from baseline at 3 months posttreatment. Facial attenuation coefficient increased 15.65% and 17.33% at 1 and 3 months posttreatment, respectively. At study completion, blood flow at a depth of 300 µm decreased 25.8% in the face and 42.3% in the neck. Relative collagen type III and elastin gene expression was statistically higher 3 months posttreatment. However, total elastin protein levels were unchanged from baseline values. Dermal muscle formation was observed in 58% of biopsies extracted 3 months posttreatment compared with 15.3% at baseline.

Conclusions: The results illustrate the effects of microneedling treatments. Noninvasive measurements and biopsy data showed changes in skin architecture and collagen/elastin gene expression, suggesting skin rejuvenation, with new extracellular matrix production and muscle formation.

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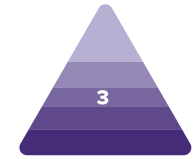
Level of Evidence: 3



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Ablative techniques, such as laser resurfacing, chemical peels, and dermabrasions, have long been the standard of care for the treatment of facial fine lines and wrinkles.[1,2] These treatments are based on the premise that removal of the epidermis and a variable portion of the dermis may trigger the growth of new skin, replacing damaged or aged tissue.[1,3] Some of these procedures may be associated with prolonged healing time, fibrosis of the papillary dermis,[4] and the risk of infection, scarring, and changes in pigmentation.[4-6] Concerns over these complications and an extended recovery have led to the increasing popularity of nonablative therapies and less invasive treatments that trigger dermal neocollagenesis while preserving the barrier function of the epidermis.[1]

Microneedling is a minimally invasive technique that has been used to treat a variety of cosmetic and dermatologic conditions. In 1995, Orentreich et al first discussed the use of a tri-beveled hypodermic needle for the treatment of depressed scars and wrinkles. The authors proposed that this method may correct depressed skin by 2 mechanisms: the surgical act of cutting under the skin releases the tethered site and the controlled dermal damage leads to new connective tissue formation.[7] Two years later, Camirand and Doucet introduced the concept of needle dermabrasion.[8] In their study, 2 patients with facial postsurgical hypochromic scars were treated with a tattooing apparatus that did not contain ink, leading to softening of the scars. The authors theorized that puncturing the skin broke down and realigned collagen bundles, resulting in this softening. In 1998, Henry et al first coined the term microneedling.[9] Because the stratum corneum, the superficial 10 to 15 µm of epidermis, forms the primary barrier to drug transport, they proposed that transepidermal drug delivery via microneedles approximately 150 µm in length could penetrate the epidermis while avoiding pain caused by stimulating nerves located in deeper dermal tissue. Several microneedling devices are currently available on the market, including manual rollers, which can be rolled back and forth over the skin to induce thousands of microwounds via uniformly separated needles, and electric-powered pens, which deliver a pulsating, stamp-like motion of needles over the skin surface.[1,10] These motor-operated devices allow for adjustable settings, controlling both the speed and depth of needle penetration.[1,11]

Although studies have been published investigating the application of microneedling to treat a variety of conditions, particularly acne scars, most evaluations of its efficacy are subjective in nature.[11-14] This includes blinded and unblinded clinician assessments according to standardized grading scales or the Clinician Global Aesthetic Improvement Scale, as well as patient self-assessments. Tissue biopsies have successfully been extracted in a number of acne scar studies in order to perform histologic analyses of skin treated with microneedling.[12,15-17] This analysis provides a more objective evaluation of skin structure and function beyond the top layers of the epidermis (M. Kislevitz et al, unpublished work). High-resolution photography with filters and polarized light can be used to evaluate wrinkles, pigmentation, texture, pore size, and the presence of bacteria.[18] Additionally, there are numerous noninvasive methods capable of objectively characterizing the dermis and epidermis. Beneath the surface, advanced sound- and light-based scanners, such as ultrasound and optical coherence tomography (OCT), can measure not only dermal and epidermal thickness, but also density, texture, and blood flow.[18-20] Finally, there are devices that quantify the viscoelastic properties of the skin, such as elasticity, laxity, and stiffness, by applying a high-resolution infrared laser coupled with a negative pressure transducer.[18,21,22] These noninvasive devices can provide objective measurements of skin characteristics in order to assess the efficacy of facial rejuvenation treatments (M. Kislevitz et al, unpublished work).[18]

The purpose of this single-center, prospective clinical trial was to objectively assess the efficacy and tolerability of a motor-operated microneedling device on the appearance of fine lines and wrinkles of the face and neck.

METHODS

Study Design

The single-center, prospective, open-label study protocol was approved by the University of Texas Southwestern Medical

Table 1. Inclusion and Exclusion Criteria

Inclusion criteria
1. Male and female volunteers aged between 35 and 65 years with general good health.
2. Individuals who desire correction of their fine lines and wrinkles.
3. Individuals willing to withhold aesthetic therapies to the face and neck during the course of the study.
4. Women of childbearing potential agree to take a urine pregnancy test at the baseline visit and 3 months posttreatment. a. This test had to be negative at the baseline visit in order to be eligible for enrollment.
5. Individuals of childbearing potential willing and able to use an acceptable method of birth control throughout the study, including any of the following: hormonal methods of contraception, barrier methods of contraception with spermicide, intrauterine device or system, surgical sterilization, or abstinence from heterosexual intercourse.
6. Individuals willing to provide written informed consent and able to read, speak, write, and understand English.
7. Individuals willing to sign a photography release.
8. Individuals willing to comply with the study requirements.
Exclusion criteria
1. Individuals with allergies to facial or general skin care products.
2. Individuals with sensitivity to topical lidocaine.
3. Individuals with a history of poor wound healing or hypertrophic or keloid scar formation.
4. Individuals with severe solar elastosis.
5. History of trauma to the treatment area in the last 6 months.
6. Any significant scarring other than acne scars in the treatment areas.
7. Individuals with severe or cystic active acne in the treatment areas.
8. History of inflammatory skin disease or cancerous/precancerous skin lesions.
9. History of smoking within the past 5 years.
10. History of chronic drug or alcohol abuse.
11. Use of oral retinoids within the past 6 months and topical retinoids in the past 2 weeks.
12. Use of any antiwrinkle or skin-lightening device or medication within 2 weeks.
13. Use of anticoagulants.
14. Use of any psychiatric drugs that in the investigator's opinion would impair the subject from understanding study requirements or providing informed consent.
15. Pregnancy or planned pregnancy during the course of the study.
16. History of immunosuppression or use of immunosuppressive medications.
17. History of any uncontrolled disease.
18. Initiation of hormone replacement therapies or hormonal birth control within the past 3 months.
19. Any dermatologic disease or condition that could interfere with the outcome of the study.
20. Individuals with a history of the following cosmetic treatments: a. skin-tightening procedures within the past year b. hyaluronic acid or calcium hydroxyapatite fillers within the past year c. poly-L-lactic acid fillers within the past 2 years d. any permanent fillers e. neurotoxins within the past 3 months f. ablative resurfacing laser treatment g. nonablative laser or light treatment within the past 6 months h. microdermabrasion, chemical peel, or glycolic acid treatment in the past month
21. Individuals participating in any other clinical trial within 4 weeks prior to inclusion in the study.

Center IRB and was conducted between January 2019 and January 2020. Table 1 highlights the inclusion and exclusion criteria. All inclusion criteria and no exclusion criteria had to be met for the subject to be eligible for enrollment.

Skin Treatments

All subjects underwent the same treatment protocol for this study. Figure 1 outlines the study timeline. A screening visit was first conducted to determine eligibility, to perform a urine pregnancy test for women of child-bearing potential, and to examine the treatment area. All study procedures were reviewed and informed consent was obtained. Participants were asked to refrain from applying topical medications and topical retinoids 2 weeks prior to their first treatment and for the duration of their study participation.

Each participant received 4 treatments with the investigational device (The SkinPen Precision System, Bellus Medical, Dallas, TX), once every month for 4 months, over the entire face and neck. Three clinicians were trained and performed the microneedling treatments for this study: Clinician 1 (D.B., 6 treatments performed), Clinician 2 (M.K., 33 treatments performed), Clinician 3 (C.E.W., 89 treatments performed). After the removal of any make-up and jewelry from the treatment areas and a 15-minute acclimation period, a topical numbing cream (20% benzocaine/8% lidocaine/4% tetracaine; Carie Boyd's Prescription Shop, Irving, TX) was applied to the treatment areas for 30 minutes and the skin was washed with saline followed by isopropyl alcohol. Treatment was performed according to the manufacturer's instructions with microneedle settings between 1.0 and 2.5 mm, depending on patient tolerability and the ease of achieving the clinical endpoint, a confluent zone of pinpoint bleeding, over various regions of the face and neck. Figure 2 illustrates the average needle length (in mm) at which each region of the face and neck was treated. See Supplemental Table 1 for the average needle length in numerical form, as well as the minimum and maximum lengths with which all subjects were treated. All noninvasive measurements were taken prior to application of numbing cream and microneedling therapy. Additionally, microbiopsy punches were performed at 3 visits, as depicted in Figure 1, after application of topical numbing cream.

Noninvasive Devices for Skin Function and Ultrastructure

AquaFlux AF200-01 (BioX Systems Ltd, London, UK)

To evaluate skin barrier function, transepidermal water loss was measured prior to the first treatment and at

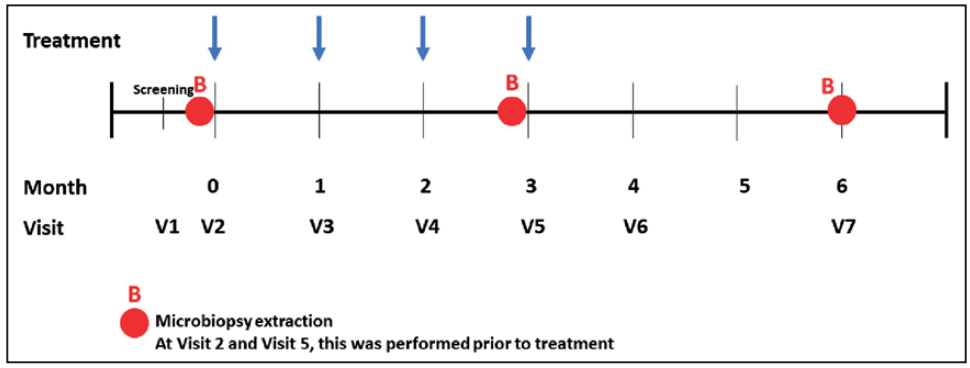

Figure 1. Study timeline.

each subsequent visit through Visit 7. Measurements were obtained approximately 1 cm lateral to the left nasolabial fold.

DUB SkinScanner75 (taberna pro medicum GmbH, Lüneberg, Germany)

High-resolution ultrasonography images of the skin were taken by placing the probe 90° to the skin surface, 1 cm lateral to the left nasolabial fold. The measurements were started at 250% amplification and 4-mm penetration depth with a 75-MHz applicator. Measurements were also taken at 5, 6.4, 8, 10, 13, and 16 mm. Images were obtained prior to the first treatment and at each subsequent visit through Visit 7.

BTC-2000 (SRLI, Nashville, TN)

This suction device utilizes an infrared laser to measure skin changes within a vacuum-sealed environment. The vacuum aperture is placed 90° to the skin surface, 1 cm lateral to the left nasolabial fold, and attached to the skin with a double-sided adhesive disc. The pressure within the chamber is then raised to 150 mmHg, maintained for 3 seconds, and then released for a 3-second relaxation period. Elastic deformation, viscoelastic deformation, ultimate deformation, energy absorption, elasticity, and stiffness are recorded. Elastic deformation (mm) is the amount of deformation occurring at the point of maximum pressure. Viscoelastic deformation (mm) is defined as the time-dependent, viscous deformation of tissue caused by a constant application of pressure. Ultimate deformation (mm) is the sum of elastic and viscoelastic deformation. Energy absorption (mmHg × mm), is a measure of overall tissue compliance or softness, and higher energy reflects a softer, more compliant material. Elasticity (mm) is the amount of elastic recovery, or reverse deformation that occurs upon the release of pressure. Stiffness (mmHg/mm) is the mechanical behavior of the structure, and several factors account for this property, including material shape, size, and content.[23] Measurements were obtained at Visits 2 through 7. The settings included: maximum pressure = 150 mmHg, time period for relaxation = 3 seconds, number of cycles = 1, data sample rate = 10 Hz, linear negative pressure rate = 10 mmHg/second, test chamber size = 10 mm.

VivoSight (Michelson Diagnostics, Maidstone, UK)

OCT technology was used to acquire facial skin images at 1305-nm laser center wavelength, creating a 6-mm × 6-mm 250-frame real-time image 1 to 2 mm below the skin surface. OCT measures vasculature density, surface roughness parameters, and the optical attenuation coefficient (OAC).[24] The arithmetic mean roughness, Ra, is the average measurement of skin curvatures and takes into account all variances in the skin roughness profile from the center line.[25] Average depth of roughness, Rz, is the difference between the highest and lowest data points from the mean surface measurement. The OAC is a measure of light absorption and scattering; substances that are strongly absorbing or scattering, such as those containing dense collagen bundles, have a higher OAC (M. Kislevitz et al, unpublished work). The probe was placed 1 cm lateral to the left nasolabial fold and images were obtained at Visits 2 through 7.

Microbiopsy

Core skin biopsies were extracted with a 0.33-mm-diameter microbiopsy punch (catalog number YA0016; World Precision Instruments, Sarasota, FL), as previously described, prior to the first microneedling treatment at Visit 2, immediately prior to the final microneedling treatment at Visit 5, and 3 months after the last microneedling treatment at Visit 7. The samples were taken 1 cm lateral to the left alar base of the facial skin for both histologic and gene expression assessments.

Immunostaining

For histologic analyses, 16 microbiopsy samples were obtained at baseline, 13 were obtained at Visit 5,

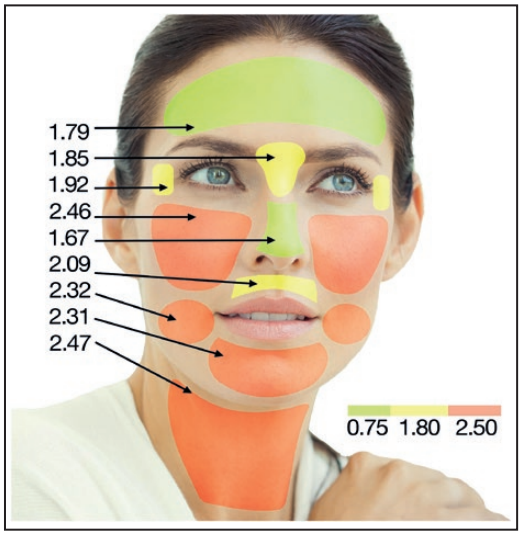

Figure 2. Average needle lengths over each region of the face. The settings recorded for each visit for each subject were averaged. Regions are color coded based on microneedle length, ranging from lower (green) to higher settings (red).

and 7 were obtained at Visit 7. Immediately after extraction, microbiopsy samples were fixed in a 4% paraformaldehyde solution (Sigma-Aldrich, St Louis, MO) for 4 hours, then kept in 50% ethanol until paraffin embedding. Immunostainings were done as described previously.[26] Briefly, 5-µm-thick sections were deparaffinized and blocked with 10% normal goat serum (Life Technologies, Carlsbad, CA) for 20 minutes at room temperature. Tissue sections were then incubated with primary antibody, biotinylated hyaluronic acid binding protein (HKD-BC41; Cosmo Bio, Carlsbad, CA), collagen I (ab6308; Abcam, Cambridge, MA), collagen III (ab7778; Abcam), elastin (ab2160; Abcam), or Ki67 (catalog number VP-K452; Vector Labs, Burlingame, CA) for approximately 16 hours at 4°C. The slides were washed 3 times with phosphate-buffered saline, then incubated with a solution containing fluorescein-tagged streptavidin (1:500 dilution), Alexa Fluor 488– or 555–conjugated secondary antibodies (Ig, heavy and light chains; 1:1000 dilution; Life Technologies) in 10% normal goat serum for 1 hour at 25°C. The slides were washed again 3 times in phosphate-buffered saline, and coverslips were mounted to the slides with Prolong Gold containing 4′,6-diamidino-2-phenylindole (Life Technologies). Collagen and elastin sections were subject to antigen retrieval with boiling in sodium citrate buffer (10 mM, pH 6) for 20 minutes prior to blocking. TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) staining for apoptotic cells was done with 5-µm-thick paraffin sections according to the protocol supplied with the Promega DeadEnd Fluorometric TUNEL System (Promega, Madison, WI). Apoptotic cells were labeled with fluorescein, and sections were counterstained with propidium iodide. Images were obtained with a Zeiss laser scanning confocal microscope. ImageJ software was used to generate individual images. Paraffin processing, embedding, sectioning, and histologic staining with hematoxylin-eosin and Masson's trichrome were all performed as described previously.[26,27]

RNA isolation, amplification, and quantitative real-time polymerase chain reaction

Total RNA was extracted with a microRNeasy kit (QIAGEN, Hilden, Germany) and treated with DNase-I (DNA-Free; Ambion, Austin, TX). cDNA synthesis was performed with 1 µL of total RNA volume iScript Reverse Transcription Supermix, followed by SSO Advanced PreAmp supermix according to the protocol supplied by the manufacturer (Bio-Rad, Hercules, CA). Quantitative real-time polymerase chain reaction was performed with Taqman primers and a PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Aliquots of amplified cDNA were used for each reaction and were run in triplicate. Each gene was normalized to the expression of the housekeeping gene, human ActinB. Gene expression was calculated according to the $2^{-\Delta\Delta Ct}$ method, as described by the manufacturer (user bulletin number 2; Applied Biosystems) (M. Kislevitz et al, unpublished work).

Statistical Analysis

Statistical analysis was performed by the Bioinformatics Core Facility at UT Southwestern. The per-protocol population included all subjects who received 4 treatments and attended the 2 follow-up visits in order to complete the study in accordance with the protocol. The mean percentage change from baseline (day 0) was calculated for each time point (Days 30, 60, 90, 120, and 180), and a paired *t* test was performed to evaluate the significant change from baseline for data acquired from noninvasive skin assessments. One-sample *t* tests were used to evaluate biopsy results.

RESULTS

Subject Demographics

A total of 35 subjects were enrolled in this study, of whom 32 successfully completed all 7 visits. One

subject was withdrawn due to noncompliance and 2 subjects were lost to follow-up; these 2 subjects failed to appear for a scheduled clinic visit and the research team was unable to contact them despite multiple attempts. The loss of subjects at follow-up was unrelated to study outcomes. Of those who completed all visits, 30 were females (93.75%) and 2 (6.25%) were males. At the time of enrollment, the mean [standard deviation] age of subjects was 56.3 [4.96] years (range, 44-65 years). Subject Fitzpatrick scores ranged from II to IV, with the distribution depicted in Figure 3: 75% of patients had a Fitzpatrick score of II, whereas the remaining 25% of patients were distributed evenly between Fitzpatrick scores of III and IV. Figure 4 illustrates pre- and posttreatment photographs taken for 2 subjects, 55 years old (Figure 4A,B) and 49 years old (Figure 4C,D), at baseline and 3 months following the treatment series.

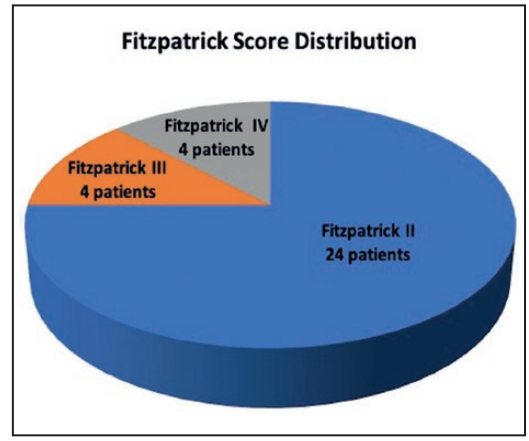

Figure 3. Fitzpatrick skin type distribution for the subjects.

Safety Evaluations

The clinician performing microneedling visually examined each subject immediately after treatment. All subjects had erythema and a confluent zone of pinpoint bleeding immediately posttreatment: this was the clinical endpoint the clinicians intended to achieve. It was ensured that any pinpoint bleeding subsided before the subjects were allowed to leave clinic following treatment, and all subjects reported that the erythema resolved within 72 hours posttreatment. In 1 subject, the clinicians noted mild bruising immediately after the second treatment. Another subject reported that mild bruising developed the day following the first treatment. However, in both subjects, all bruising resolved within 48 hours of treatment. No unanticipated adverse events were reported throughout the course of the study.

Noninvasive Skin Assessments

AquaFlux

Skin barrier function, as measured via transepidermal water loss, decreased in the face by 9.08% ($P = 0.0329$) and in the neck by 12.8% ($P = 0.049$) at Day 30, immediately prior to the second treatment. It also decreased at Day 60 in the neck by 13.85% ($P = 0.0027$). Additionally, 1 month posttreatment, there was a 13.22% decrease from baseline in the neck ($P = 0.0302$) (Figure 5). Epidermal barrier function values of the face and neck were not significantly different from baseline values by study completion, 3 months posttreatment.

DUB SkinScanner75

High-resolution ultrasound imaging assessed with a DUB SkinScanner75 system showed significant changes from baseline in the dermal and epidermal thickness and density, as illustrated in Figure 6. The most dramatic changes measured via ultrasound were noted in facial dermal and epidermal density. At Visits 4 to 7, facial dermal density was increased 39.35% ($P = 0.0013$), 65.44% ($P = 0.023$), 46.58% ($P = 0.0152$), and 101.86% $P< 0.001$) from baseline, respectively (Figure 6D). Additionally, at Visits 5 to 7, facial epidermal density had increased 10.73% ($P = 0.0376$), 9.97% ($P = 0.0305$), 19.28% ($P = 0.0078$) from baseline, respectively (Figure 6C).

At 1 month posttreatment, dermal thickness increased by 8.79% from baseline in the neck ($P = 0.0348$) (Figure 6B). No significant changes were noted in epidermal thickness of the face or neck at any visit (Figure 6A).

BTC-2000

Assessment of skin elastic properties with the BTC-2000 showed significant changes in several parameters at both the face and neck throughout the study. At Day 30, immediately prior to the second treatment, elasticity in the face had increased 7.9% from baseline ($P = 0.0465$). Facial elasticity was also increased 28.22% from baseline at Visit 7, 3 months posttreatment ($P = 0.0039$). Although elasticity of the neck had increased 189.69% in the neck at 3 months posttreatment, this value was not statistically significant ($P = 0.187$, Figure 7A). Stiffness was increased in both the face (9.52%, $P = 0.007$) and neck (49.40%, $P = 0.049$) at Day 60 and in the face at Day 90 (12.17%, $P = 0.0031$). However, there were no significant changes from baseline in the stiffness of the face or neck measured at the 2 follow-up visits (Figure 7B). At Day 120, 1 month posttreatment, only a 55.25% increase in viscoelastic deformation of the neck was statistically significant ($P = 0.0322$, Figure 7C). At Day 180, facial elastic deformation increased 15.61% from baseline ($P = 0.0003$, Figure 7D) and facial ultimate deformation had increased 14.55% from baseline ($P = 0.0004$,

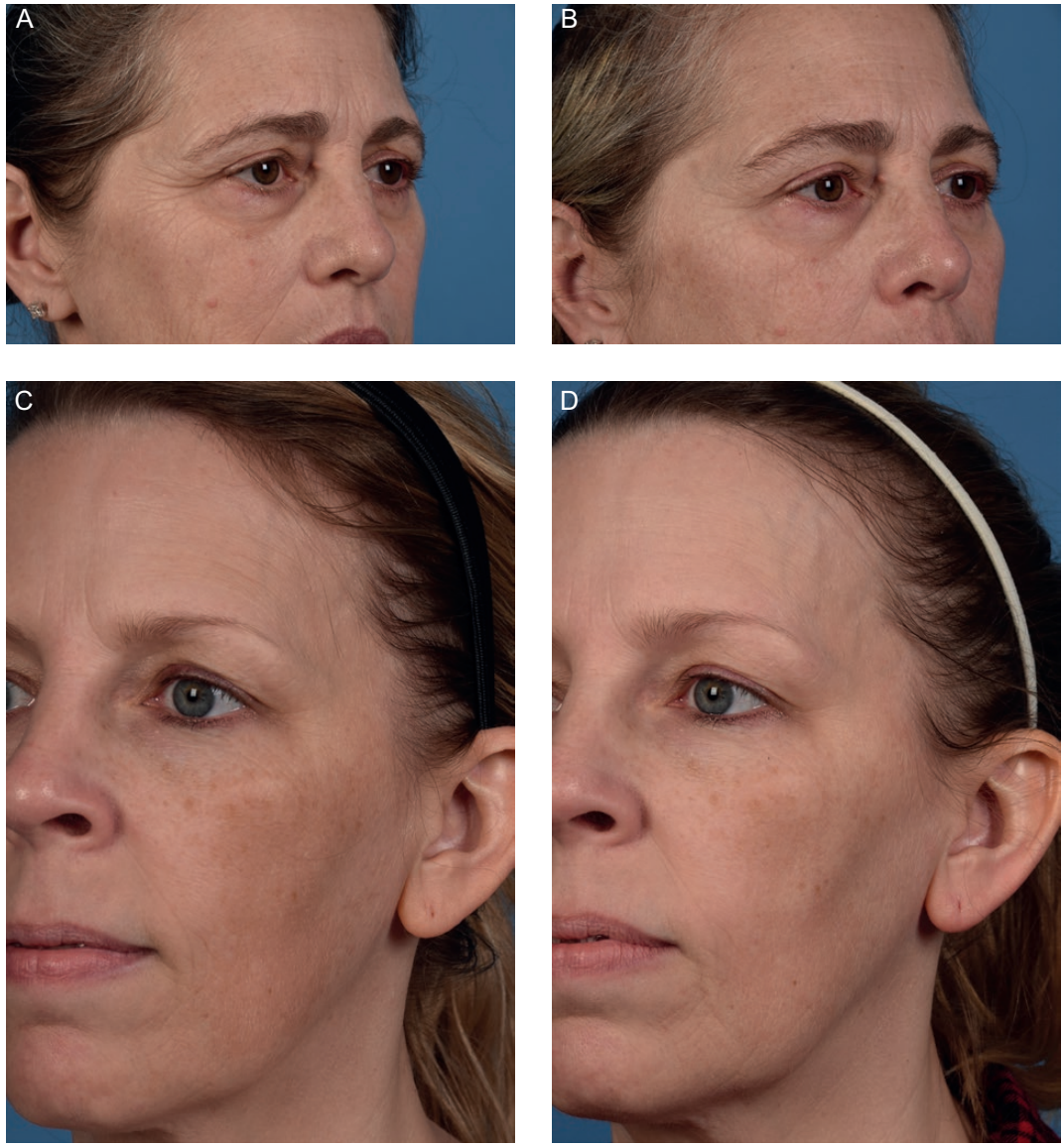

Figure 4. Pre- and posttreatment clinical examples of 2 subjects: a 55-year-old female and a 49-year-old female. Pretreatment photos (A, C) were taken immediately prior to the first microneedling treatment. Posttreatment photos (B, D) were taken 3 months posttreatment.

Figure 7E). No statistically significant changes in laxity were measured at any visit (Figure 7F).

VivoSight

Skin roughness measurements, as assessed by OCT, were not significantly changed from baseline at any visit. Skin density in the face, as reflected by the OAC, was increased from baseline at several time points throughout the course of the study. At Day 60, the OAC had increased 8.58% from baseline in the face ($P = 0.0056$) and 8.53% in the neck ($P = 0.0312$). At Day 90, the OAC had increased 14.02% from baseline in the face ($P = 0.0003$). One month posttreatment, the OAC was increased 15.65% from baseline in the face ($P < 0.0001$) and 7.99% in the neck ($P = 0.014$). Three months posttreatment, it was further increased in the face, 17.33% from baseline ($P = 0.0012$, Figure 8).

Blood flow also significantly changed from baseline at the follow-up visits, particularly between 300 and 500 µm below the skin surface as shown in Figure 9. Beginning at Day 60, mean blood flow was statistically lower than at baseline between these depths in both the face and neck and continued to decrease through the end of the study (Figure 9A,B). In the face, average blood flow at 300 µm decreased from baseline, by 17.9% at Day 60 ($P = 0.0074$), 20.1% at Day 90

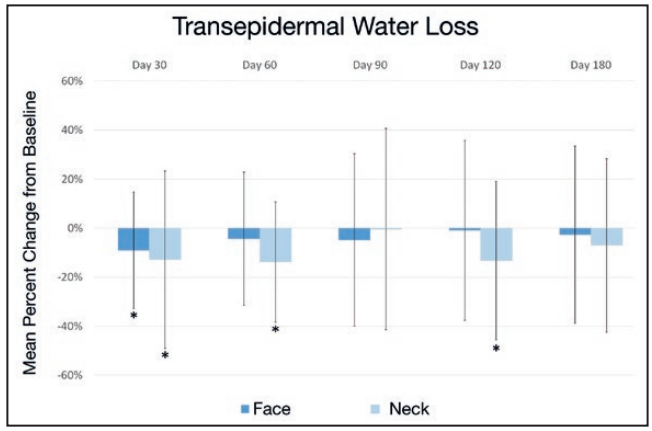

Figure 5. Mean percentage change from baseline for transepidermal water loss measurements. An asterisk indicates a statistically significant change ($P < 0.05$). Day 120 corresponds to 1 month posttreatment and Day 180 corresponds to 3 months posttreatment. Error bars indicate percentage standard deviation.

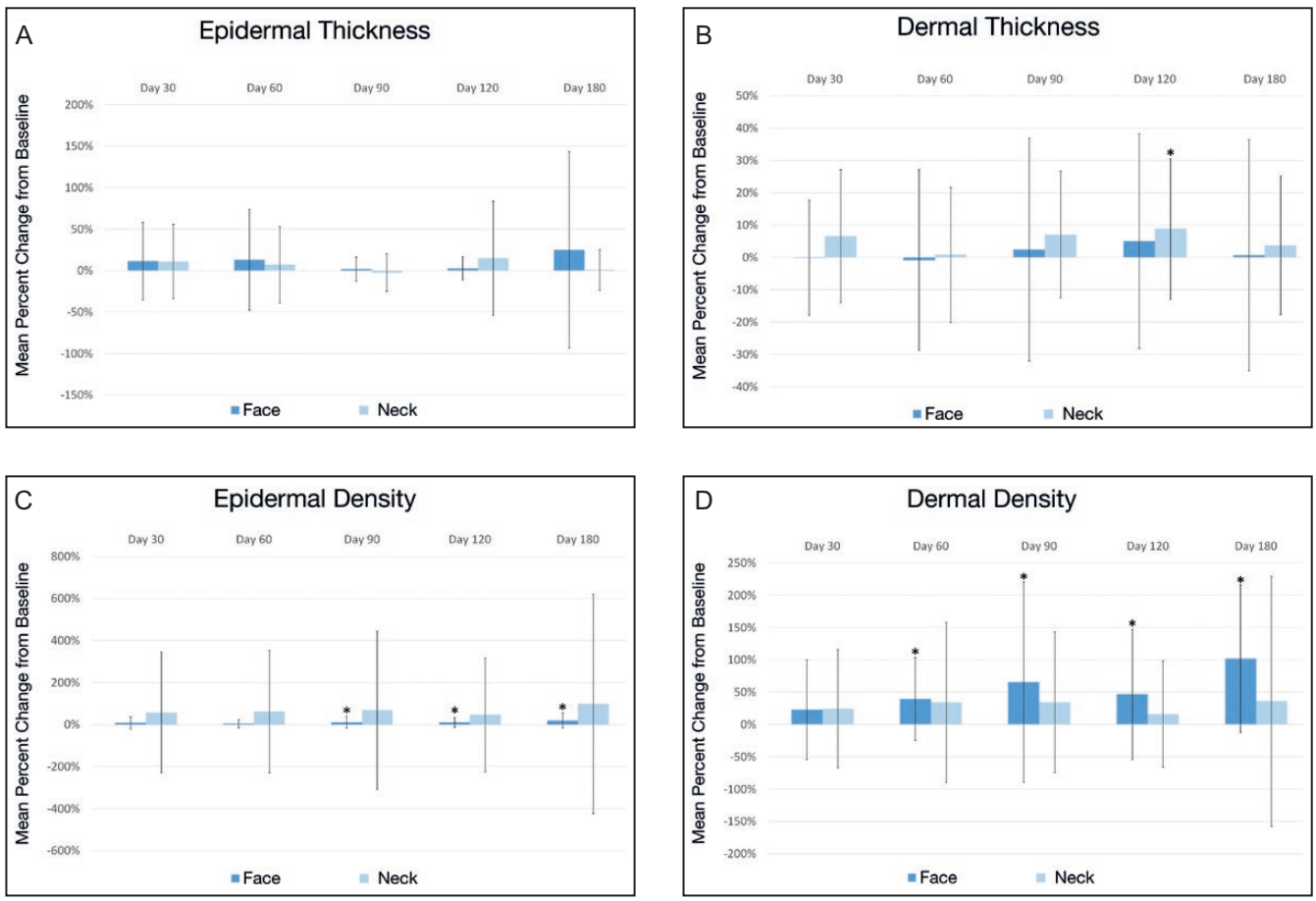

Figure 6. Mean percentage change from baseline for epidermal thickness (A), dermal thickness (B), epidermal density (C), and dermal density (D) as assessed by high-frequency ultrasound imaging. An asterisk indicates a statistically significant change ($P < 0.05$). Day 120 corresponds to 1 month posttreatment and Day 180 corresponds to 3 months posttreatment. Error bars indicate percentage standard deviation.

($P = 0.0061$), 27.5% at Day 120 ($P = 0.0010$), and 25.8% at Day 180 ($P = 0.0084$). In the neck, average blood flow at 300µm decreased from baseline by 28.0% at Day 60 ($P = 0.0035$), 26.9% at Day 90 ($P = 0.0131$), 37.1% at Day 120 ($P = 0.0007$), and 42.3% at Day 180 ($P = 0.0008$).

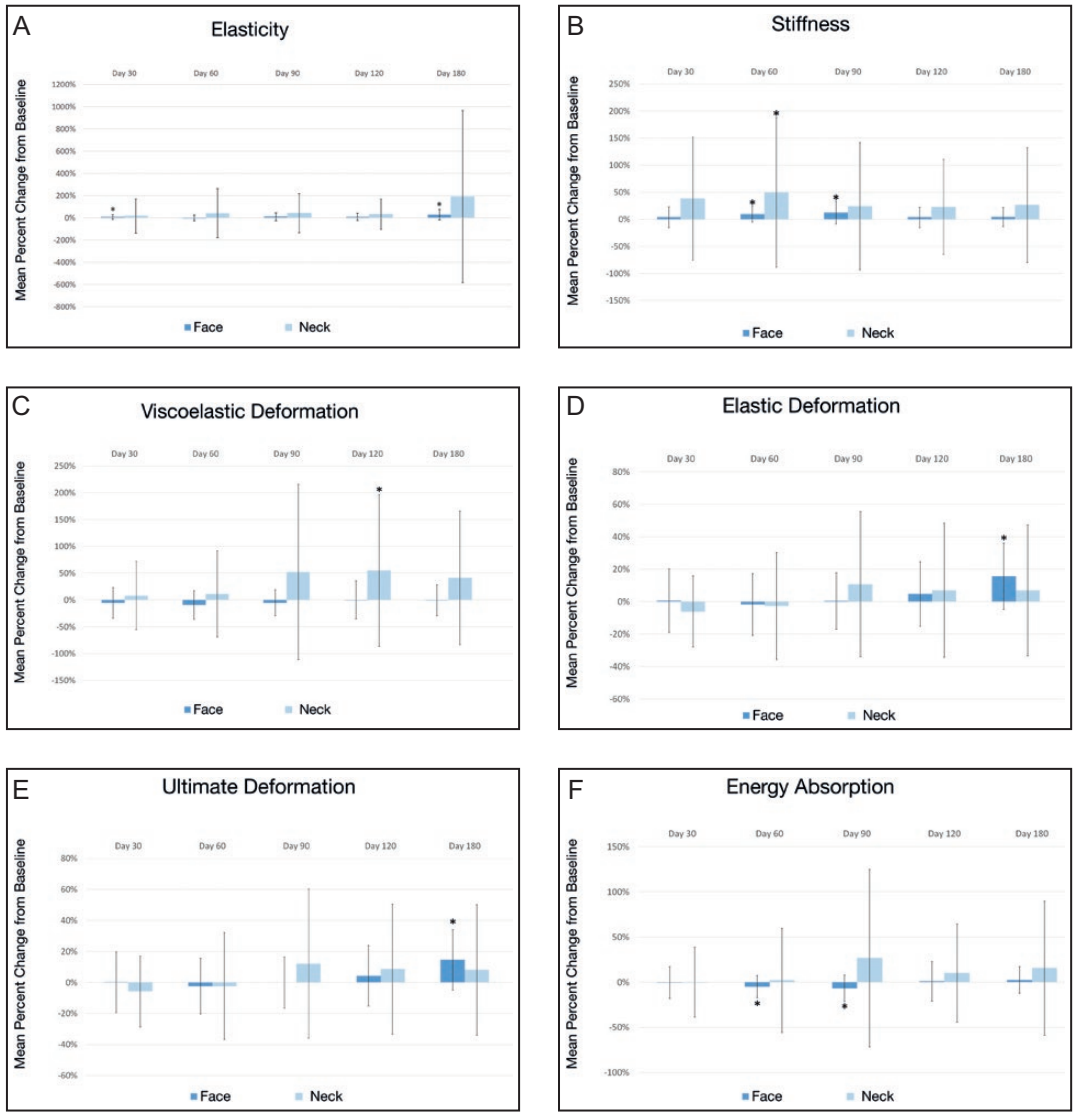

Figure 7. BTC-2000 measurements: mean percentage change from baseline for elasticity (A), stiffness (B), viscoelastic deformation (C), elastic deformation (D), ultimate deformation (E), and energy absorption (F). An asterisk indicates a statistically significant change ($P < 0.05$). Day 120 corresponds to 1 month posttreatment and Day 180 corresponds to 3 months posttreatment. Error bars indicate percentage standard deviation.

Histology and Gene Expression Analysis of Microbiopsy Samples

Although there was a gradual increase of collagen type I protein and gene abundance in the subepidermis following treatment at Visit 7 (3 months posttreatment) compared with baseline (Visit 2), it was not statistically significant (Figures 10 and 11A). The collagen type III expression in the subepidermis was statistically higher 3 months posttreatment ($P = 0.02$). The gene expression of collagen-degrading matrix metalloproteases (MMP-2 and MMP-9) trended lower following treatment along with the collagen and the elastin crosslinking molecule lysyloxidase (Figure 11B). Interestingly, elastin protein abundance in the subepidermis was not changed following microneedling (Figure 10F), although elastin gene expression was upregulated at 3 months posttreatment (Figure 11A). There was no statistical difference in collagen-degrading MMP-2 and MMP-9 genes partly due to large variations between samples of each group (Figure 11B).

Inflammation was recorded higher at Visit 5 compared with baseline with no statistical significance and large variations between samples. On average, the interleukin 1β (IL-1β) gene expression was 30-fold higher,

whereas the IL-6 gene was upregulated 6-fold (Figure 12A). Supporting the inflammatory gene expression, there was around a 16% increase in hyaluronic acid abundance at Visit 5 compared with baseline in the skin (Figure 12B, green staining).

There was no change in cell proliferation and growth factor gene expressions following treatment (Supplemental Figure 1).

It was recorded that only 2 of the 13 samples (15.3%) extracted before treatment exhibited evidence of muscle formation (Figure 13A), whereas 2 of the 7 samples (28.6%) at Visit 5 (Figure 13B) and 7 of the 12 samples (58.3%) at Visit 7 (Figure 13C) exhibited muscle formation.

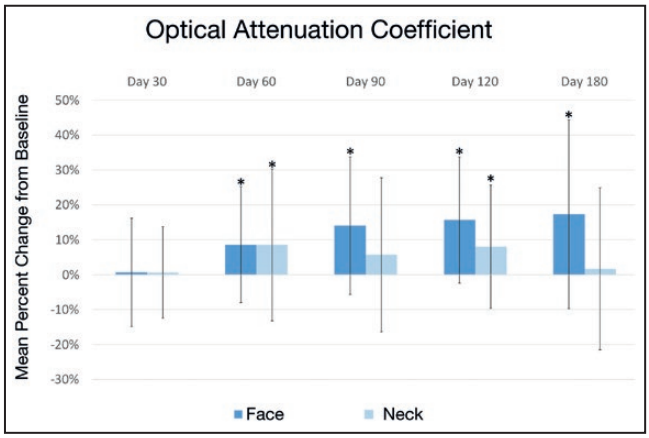

Figure 8. Percentage change from baseline of the optical attenuation coefficient. An asterisk indicates a statistically significant change ($P < 0.05$). Error bars indicate percentage standard deviation.

DISCUSSION

Although ablative technologies, such as laser resurfacing, are regarded as the gold standard for facial skin rejuvenation, there has been a recent drive to develop less invasive therapies, which offer a shorter recovery time and milder side effect profile. One minimally invasive option that has emerged over the past decades is microneedling, or percutaneous collagen induction therapy. It is theorized that controlled microinjury can stimulate the wound-healing process, leading to the production of elastin and collagen, while preserving the structural integrity of the epidermis.[5,7,11] Our study showed that a series of 4 microneedling treatments resulted in a 28.22% increase in facial elasticity 3 months posttreatment. Additionally, facial dermal and epidermal density increased 101.86% and 19.28% from baseline at 3 months posttreatment, respectively. This coincides with the increase in the OAC in the face and neck noted throughout the study. The relative expression of collagen type III in the subepidermis was statistically higher 3 months posttreatment. Finally, muscle formation increased from 15.3% for samples at baseline to 58.3% for samples 3 months posttreatment.

Microneedling has most extensively been studied as a treatment for acne scarring.[10,11] Histologic analyses have objectively substantiated the benefits of these treatments, showing increased epidermal thickness with well-developed rete ridges,[28-30] increased expression of collagen types I to IV and VII[12,28] and fibronectin,[30] and increased thickness of elastin fibers in the upper dermis.[30] In addition to atrophic acne scars, the applications for microneedling have expanded over the past decades[31] to include the treatment of hypertrophic and keloid scars[32] and traumatic scars.[11,29,33] In particular,

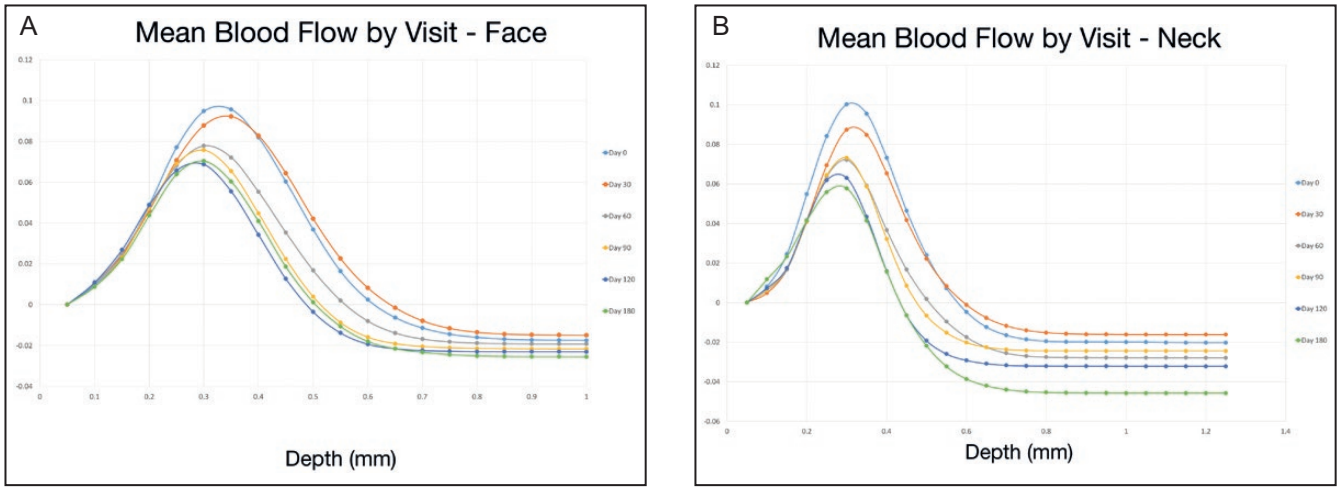

Figure 9. Mean blood flow of the face (A) and neck (B) at each visit as assessed by optical coherence tomography.

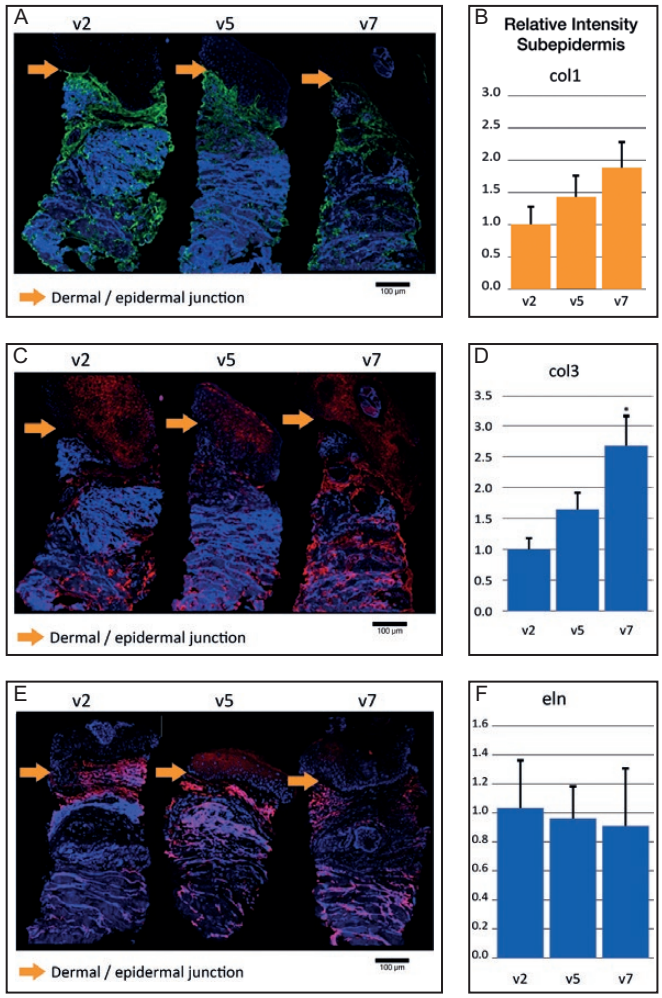

Figure 10. Immunostaining of collagen type I (A), collagen type III (C), and elastin (E) and relative subdermal intensity of collagen type I (B), collagen type III (D), and elastin (F) at Visits 2, 5, and 7. The orange arrows delineates the dermal-epidermal junction. N = 16 (Visit 2), 13 (Visit 5), and 7 (Visit 7).

microneedling has been found to normalize the extracellular collagen matrix in the reticular dermis, providing a possible treatment to improve skin structure and promote contracture relaxation at burn sites.[29,33,34] It has also been investigated as a treatment for melasma,[35,36] striae distensae, or stretch marks,[37-39] and androgenic alopecia.[40,41]

Relatively little research, however, has been conducted regarding microneedling's effects on fine lines and wrinkles. Fernandes first discussed the concept of percutaneous collagen induction therapy as a minimally invasive treatment facial rejuvenation. In 2002, he presented 2 cases showing long-term visual improvement of wrinkles on the abdomen and upper lip.[2] Aust et al retrospectively analyzed 480 patients who were treated with a manual roller for fine wrinkles, laxity, scarring, or stretch marks.[42] Six months after needling, Van Gieson staining showed a considerable increase in collagen and elastin deposition, with collagen being laid in a normal lattice pattern, as compared to parallel bundles seen in scar tissue. Furthermore, among 35 patients with wrinkles, patient satisfaction was high, as assessed by a visual analog scale (0 = absolutely dissatisfied, 10 = completely satisfied). Fabbrocini et al performed 2 sessions on 8 patients with a manual roller for the treatment of the aging neck.[43] Skin ultrasonography showed a statistically significant increase in dermal thickness by an average of 0.45 mm. Skin cast images showed a reduction in the degree of irregularity of skin texture by 29%, on average, whereas average skin roughness and maximum depth of wrinkles decreased by 24% and 31%, respectively. Finally, wrinkle depth severity was reduced in most patients, as assessed with the Wrinkle Severity Rating Scale. A parallel study was performed by Fabbrocini et al for treatment of upper lip lines of 10 subjects, and similar

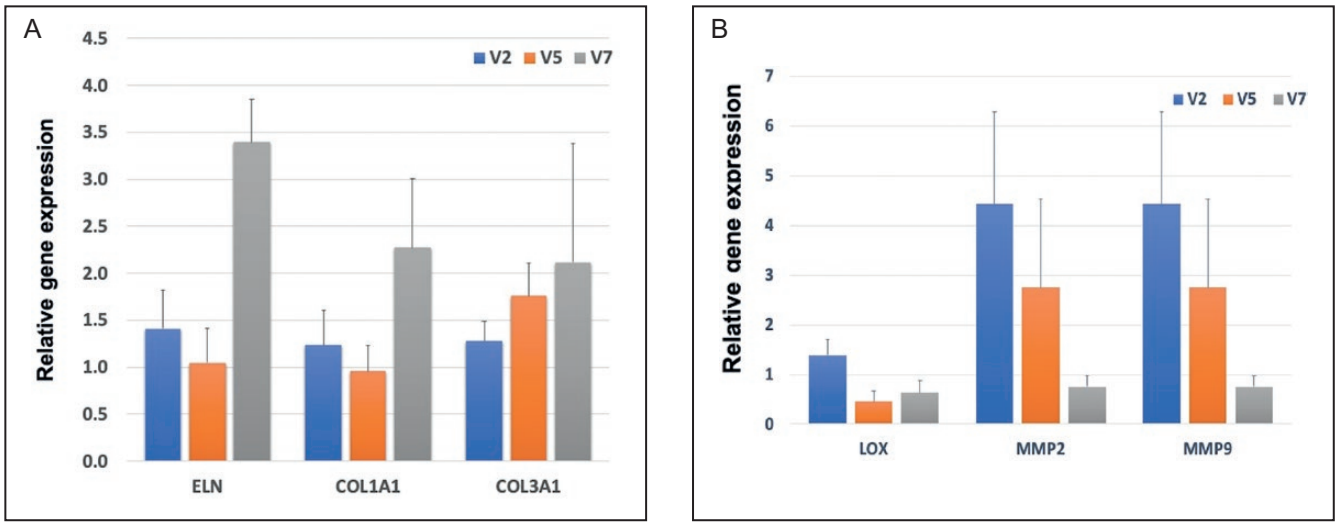

Figure 11. Relative gene expression of elastin, collagen type I, collagen type III (A) and lysyl oxidase, matrix metalloproteinase 2 (MMP-2), and MMP-9 (B) at Visits 2, 5, and 7.

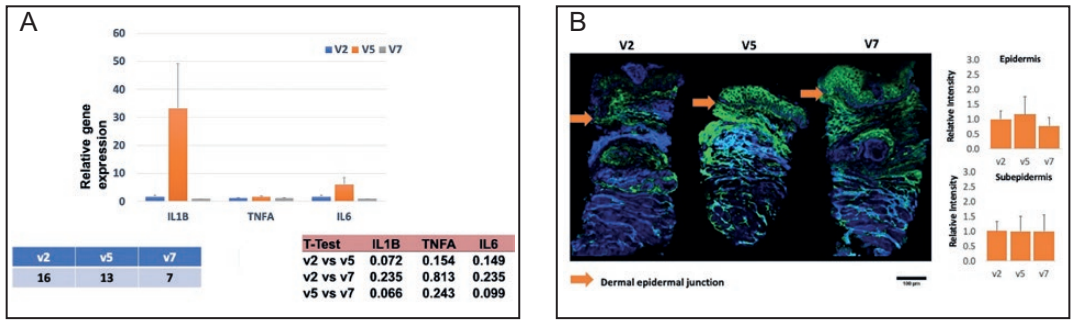

Figure 12. Relative gene expression of inflammatory genes interleukin (IL) 1β, tumor necrosis factor α, and IL-6 (A), and immunostaining and relative intensity of hyaluronic acid (B). The orange arrows delineate the dermal-epidermal junction. N = 16 (Visit 2), 13 (Visit 5), and 7 (Visit 7).

results were reported; the degree of irregularity of skin texture and skin roughness were found to be significantly reduced posttreatment, as assessed from silicon-printed upper lip impression casts, and the wrinkles' severity grade was reduced in most patients.[44] El-Domyati et al used histologic and immunochemical staining to demonstrate statistically significant increases in epidermal thickness and tropoelastin content, as well as dermal collagen types I, III, and VII levels after 6 microneedling sessions.[45] Finally, 1 study was found that assessed the safety and efficacy specifically of an automated microneedling device for the treatment of photoaging. Among the 48 subjects, improvements were observed for line roughness and surface roughness in the periorbital and mesolabial regions 3 and 6 months posttreatment, as measured by digital fringe projection technology. Significant improvements were also noted in wrinkle severity, as assessed by the Lemperle Grading Scale, as well as in skin laxity and texture, as assessed by a modified Alexiades-Armenakas Grading Scale.[46,47]

The results of our study provide further objective evidence of the safety and efficacy of microneedling for the treatment of fine lines and wrinkles of the face and neck. Noninvasive skin measurements illustrated several structural and functional changes resulting from microneedling treatment. Particularly noteworthy is the 28.22% increase in facial elasticity 3 months posttreatment, as measured with a BTC-2000 (Figure 7A). High-resolution ultrasonography showed statistically significant increases in facial dermal density at Days 60, 90, 120, and 180 (Figure 6D), whereas facial epidermal density was also increased compared to

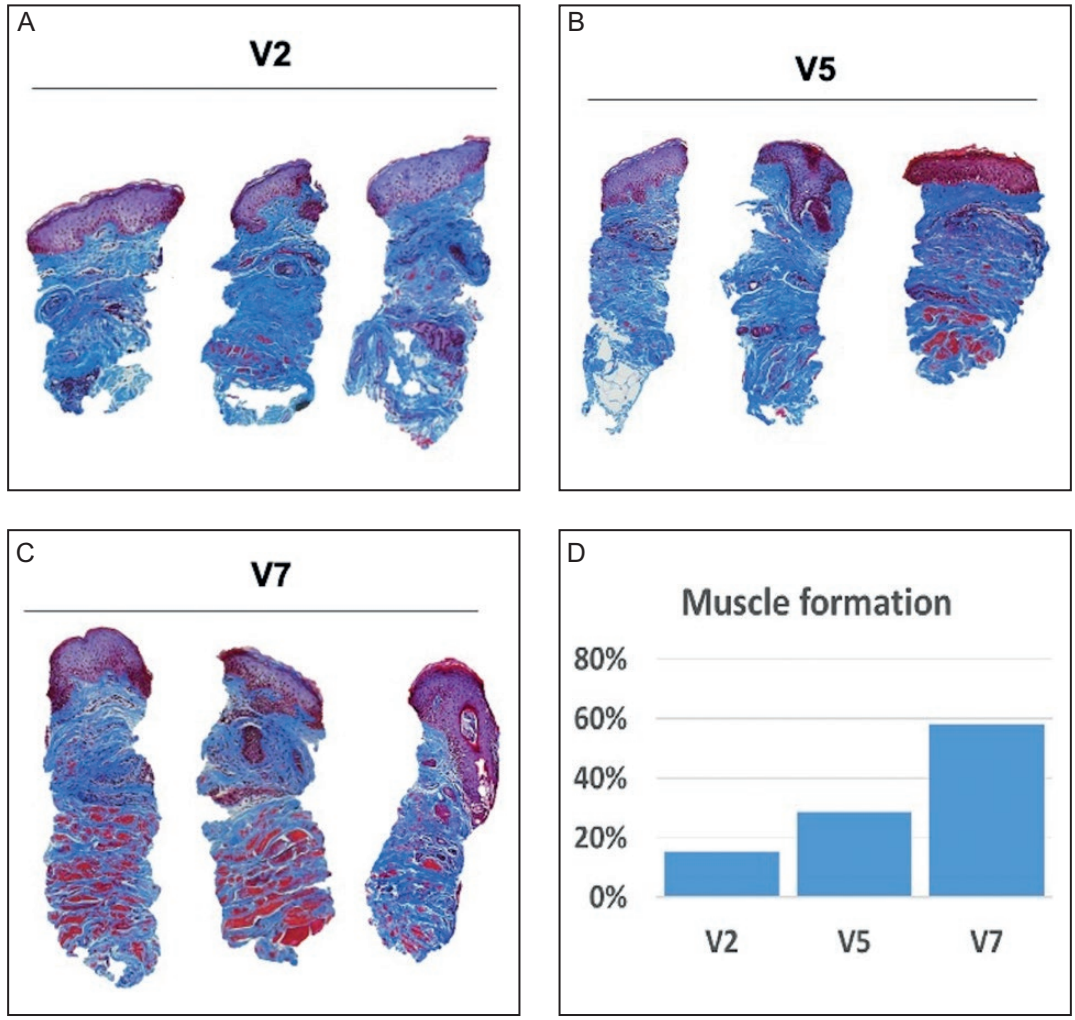

Figure 13. Representative trichrome stain images at Visits 2 (A), 5 (B), and 7 (C), and percentage muscle formation (D). The blue regions of the sample indicate collagen; the red is cell cytoplasm, composed of the epidermis and muscle. N = 13 (Visit 2), 7 (Visit 5), and 12 (Visit 7).

baseline at Days 90, 120, and 180 (Figure 6C). These results complement those obtained via OCT.

A slight, yet statistically significant increase in the attenuation coefficient compared with baseline was observed in the face at several time points (Figure 8). The OAC measures the amount of light reflection (scattering) and absorption in a tissue.[48] Denser tissues, or those that contain a large number of structures within the dermis and epidermis, scatter light to a greater degree and thus exhibit a higher OAC.[49] On the other hand, water in the skin could cause a greater absorption of light, resulting in a darker appearance and lower OAC (M. Kislevitz et al, unpublished work). An increase in attenuation coefficient, therefore, may be seen with denser distribution of extracellular matrix structures, due to decreased water content or increased collagen bundle thickness.[50] Indeed, collagen I and III gene expression was higher following treatment compared with baseline values. Elastin gene expression was upregulated over 3-fold, suggesting new elastin production in the facial skin following microneedling. However, the increase in elastin gene expression did not translate into an increase in elastin protein level, suggesting an insignificant addition of new elastin to previously made elastin (Figures 10 and 13). Therefore, it could be theorized that the microneedling treatments performed in this study resulted in increased collagen production and density, which is responsible for the increased OAC values as well as dermal and epidermal densities measured in subjects as the study progressed.

Facial skin inflammation prior to the last treatment (Visit 5) was higher but returned to baseline, untreated skin levels at Visit 7, as assessed by inflammatory genes IL-1β, tumor necrosis factor α, and IL-6 (Figure

12B). The change in inflammation was supported by an increase in hyaluronic acid abundance, mostly in the epidermis, at Visit 5 compared with baseline (Figure 12A). Hyaluronic acid increases in the tissue following injury or infection as high-molecular-weight hyaluronic acid acts as protection against inflammation.[26,51] However, these changes in inflammation were not statistically significant due to large variations between skin samples.

Finally, we looked at the changes in vasculature and blood flow induced by the treatment to demonstrate whether microneedling results in significant tissue regeneration with vascularization. Surprisingly, from Day 60 through study completion, mean blood flow was decreased from baseline in the face and neck between 300 and 500μm below the skin surface compared with untreated skin (Figure 9). This finding was in agreement with limited studies showing a decrease in vasculature during the remodeling phase of the skin regeneration/wound healing following acne scarring.[52] Our finding differs from a full-thickness wound healing, where neovascularization increases following skin injury during the proliferative phase of normal wound healing[53] and gradually decreases to uninjured skin vasculature levels during the remodeling phase[54] This decrease in vasculature/blood flow may also be a result of the noted increase in muscle formation recorded at 6 months following the first miconeedling session, which may fill the space occupied by blood vessels and prevent new vessel formation. We speculate that the microneedling may increase tissue oxygen levels, leading to stimulation of fibroblast differentiation into myofibroblasts and inhibition of neovascularization. Studies with human primary dermal fibroblast and pulmonary adventitial fibroblasts have shown that hypoxia inhibits fibroblast differentiation to myofibroblasts.[55] Although myofibroblast increase is associated with fibrosis and scarring, vascularization increases in scars, unlike our current study.[56]

This paper focuses on objective analyses of the microneedling treatment series. We believe that combining both objective and subjective data, including blinded photographic evaluations and patient satisfaction surveys, into one article would be too lengthy. Therefore, a subsequent manuscript is being prepared that will solely discuss the subjective data acquired in this study.

Further limitations of this study included a relatively small study population lacking racial diversity. Although we initially attempted to recruit all Fitzpatrick skin types, there was difficulty enrolling subjects with a skin type V or VI due to minimal fine lines and wrinkles in interested candidates or candidates having exclusionary criteria. In order to proceed with the study in a timely manner, it was ultimately decided to enroll any subject in the order in which they qualified. As a result, the study was limited to Fitzpatrick skin types I through IV. Further studies may evaluate this treatment in skin types V and VI. Treatments are also technique dependent and can vary between treating providers, although every attempt was made to maintain consistency. This included holding the device in the same manner, performing the same strokes of the device over the subjects' skin, and treating each region at similar microneedle settings across subjects until pinpoint bleeding was achieved. Finally, this study focused specifically on the effects of microneedling; we did not investigate whether greater improvements were achieved in subjects who followed a regular skincare routine, including products such as moisturizer and sunscreen. Further studies may investigate the use of microneedling on other regions of the body or the application of topical treatment, cosmeceuticals, and/or growth factors to further stimulate collagen production.

CONCLUSIONS

Microneedling can provide a minimally invasive alternative for the treatment of fine lines and wrinkles of the face and neck. Production of a wound via microneedling is minimally invasive, yet stimulates normal collagen production while preserving the normal epidermal structure. This study illustrated the safety and efficacy of an automated microneedling device to treat signs of aging based on objective evaluations. Overall, the current study suggests that 4 microneedling treatments of the face and neck can lead to skin improvements in terms of increased skin density and elasticity lasting up to 3 months posttherapy.

Supplemental Material

This article contains supplemental material located online at www.aestheticsurgeryjournal.com.

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