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Report

Multiple microneedling sessions for minimally invasive facial rejuvenation: an objective assessment

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Abstract

Background Microneedling or percutaneous collagen induction is a new modality used for skin rejuvenation, tightening, and scar remodeling. It offers a simple and effective treatment for photoaged skin with minimal disruption of the epidermis, thus limiting adverse effects and minimizing downtime.

Objectives To evaluate the efficacy, coupled with quantitative assessment, of the histological changes in response to multiple sessions of skin microneedling in the treatment of aging skin.

Patients and methods Ten patients with Fitzpatrick skin type III and IV and Glogau class II to III wrinkles were subjected to six skin microneedling sessions at 2-week intervals. Standard photographs and skin biopsy specimens were obtained at baseline and at one and three months after the start of treatment. Histometry for epidermal thickness and quantitative evaluation of collagen types I, III, and VII, newly synthesized collagen, total elastin, and tropoelastin were performed for all skin biopsies.

Results Skin microneedling produced noticeable clinical improvement of photoaged skin, with corresponding histological enhancement. Compared to the baseline, collagen types I, III, and VII, as well as newly synthesized collagen, together with tropoelastin showed a statistically significant increase ($P < 0.05$) in response to treatment, while the mean level of total elastin was significantly decreased ($P < 0.05$) after treatment.

Conclusions Skin microneedling is a promising minimally invasive treatment option with the advantage of increased collagen production. However, multiple sessions are usually needed to maintain the improvement achieved.

Introduction

Extrinsic skin aging (photoaged skin) was first described in 1986 by Kligman and Kligman,[1] as the consequences of repeated exposure to environmental elements, primarily ultraviolet radiation on the skin, which enhances the breakdown of collagen with decrease in its production[2–6] and thus eventually will be manifested by wrinkles and other signs of photoaged skin.[7]

The desire of people to appear young relates to various modalities on offer, including medical preparations, dermabrasion, chemical peeling, and lasers. Many of these procedures have been well studied, whereas others have not.[8,9] Recently, minimally invasive rejuvenation was reported to be an effective aesthetic treatment that reverses the signs of photo-damaged skin.[10–12] Skin microneedling therapy, also known as collagen induction therapy or percutaneous collagen induction, is a recent addition to the treatment armamentarium for managing skin aging. The treatment is performed as an office procedure after application of a local anesthetic cream, by means of an instrument known as a skin needling roller.[12]

A skin needling roller is a simple, drum-shaped, handheld device consisting of a handle with a roll having fine stainless steel needles 0.25–3 mm in length. This needle-studded drum is rolled on the skin in different directions to achieve a therapeutic benefit.[13,14]

Needling of the skin aims to stimulate the fibroblast with deposition and reorientation of collagen bundles by producing microwounds and initiating the normal postinflammatory chemical cascade leading to induction of percutaneous collagen. Subsequent remodeling and reorientation of collagen bundles and the formation of new collagen is achieved over months of treatment.[15]

Clinical studies that have documented clinical and histopathological improvement of skin aging after microneedling treatment are few.[16] In the present study, to

investigate the effect of collagen induction therapy by skin microneedling for facial rejuvenation, we aimed to evaluate the clinical effect and objectively quantify the histological changes in response to multiple microneedling sessions in the treatment of aging skin.

Patients and methods

Study population

Ten volunteers (three men and seven women) with Fitzpatrick skin type III and IV and Glogau class II–III wrinkles[17] that desired an improvement in the appearance of signs of photoaging, were enrolled in the present study. The individuals, ranging in age from 38 to 60 years with an average of 49.2 ± 6.7, were recruited from the dermatology outpatient clinic of Al-Minya University Hospital, Al-Minya, Egypt. Treatment and study protocol were fully explained to subjects, and all gave informed consent. The study was approved by the postgraduate and research committee of Al-Minya University.

Procedure, treatment regimen, and follow-up

After cleaning the face with saline and povidone iodine, we marked the periorbital area on both sides and forehead (treatment areas). Topical anesthetic cream (lidocaine 2.5% + prilocaine 2.5%) was applied as a thick coating and left for 60 minutes under occlusion. The cream was then gently removed, and the subject was positioned for the session. The treatment was then carried out by rolling a dermaroller with a needle length of 1 mm (Directive MDD 93/42 EEU. DERMAROLLER Deutschland S.a.r.l. Lindener Strasse15, D38300 Wolfenbüttle, Germany; Model MF8, 192 needles in eight rows, needle diameter at penetration point of 0.25 mm, width and diameter of the roller head, 20 mm) on the treatment area, six passes were used per area treated, and each pass was carried out in eight directions (vertical, up and down, horizontal, to the right and left, and in both diagonal directions). By rolling in various directions with minimal pressure, an even distribution of the holes could be achieved. The needles penetrate the epidermis and because it is only punctured, healing of the epidermis would be achieved rapidly. Cold saline soaks were then performed to wash out any oozing or pinpoint bleeding. Potential side effects or complications, including erythema, edema, crusting, hypo- or hyperpigmentation, and ecchymosis, were monitored.

Over a 3-month treatment period, volunteers were subjected to six sessions at 2-week intervals. They were instructed to use topical antibiotic "fusidic acid" for 24 hours, to guard against secondary infection, after each session. In addition, sun exposure was avoided by using sunscreens with sun protection factor value of 30 or more during the daytime. Photographs were taken before each session and at three months post-treatment. Punch biopsies (3 mm) were obtained from facial skin at baseline, two weeks after two sessions (1 month), and three months post-treatment (2 weeks after six sessions).

Histological staining

Skin specimens were stained for standard hematoxylin and eosin (H&E), Verhoeff-van Gieson (elastic fibers) (HT25A; Sigma, St. Louis, MO, USA), and picrosirius red staining (Direct Red 80; Sigma) for newly synthesized collagen. When tissues are stained with picrosirius red and viewed under polarized microscope, large collagen fibers stain red while the thinner ones, which represent the newly synthesized fibers, are stained yellow to orange.[18,19]

Immunohistochemical staining

The immunoperoxidase technique was performed to investigate total elastin and collagen types I and III.[9,20,21] Following deparaffinization in xylene and rehydration in ascending grades of alcohol, endogenous peroxidase activity was quenched by incubation of tissue sections in 3% H_2O_2 for 10 minutes at room temperature. Antigen retrieval was performed by the microwave method in 0.1 M sodium citrate (pH 6.0) for five minutes. Nonspecific sites were blocked, and the tissues were incubated with antibodies to total elastin (1 : 300; E4013; Sigma), type I collagen (1 : 400; sc-59772; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and type III collagen (1 : 600; ab6310; Abcam, Cambridge, MA, USA). Tissues were washed and incubated with biotinylated secondary antibody (1 : 200; PK-6102; Vector Labs, Burlingame, CA, USA), ABC reagent (Vectastain Elite ABC Peroxidase Kits Mouse; PK-6102; Vector Labs), and signal developed with DAB Chromagen Substrate Kit (K3468; Dako, Carpinteria, CA, USA). All tissue sections were stained under similar conditions to ensure equal staining intensity.

Tropoelastin and collagen type VII were detected by using indirect immunofluorescence staining technique. Tissues were incubated with antibodies to tropoelastin (1 : 400; Elastin Products, Owensville, MO, USA) and to type VII collagen (1 : 600; sc-33710; Santa Cruz Biotechnology), secondary antibody, and 4′,6-diamidino-2-phenylindole dihydrochloride (1 : 1000; D8417; Sigma) for nuclear staining.

Histometry and quantitative evaluation of stained tissues

Using a computer-based software (analysis®Five by Olympus Soft Imaging Solutions GmbH, Münster, Germany), epidermal thickness was determined using H&E-stained sections. Five measurements for each section were calculated between the top of the granular cell layer to the dermoepidermal junction.

Quantitative evaluation of immune-stained tissues was carried out using computer-based software to detect the positively stained dermis and color density for staining; a representative square area of 1 × 1 cm was used to measure luminosity for fluorescent-stained sections, while another 2.5 × 2.5 cm square was used to measure the color density for immunoperoxidase staining. All values were normalized to the baseline.

Statistical analysis

Data were tabulated and analyzed using the Software Package for Statistical Science (Version 16; SPSS, Chicago, IL, USA). Statistical analysis was performed using Wilcoxon-matched pairs signed ranks, one-way ANOVA test, and chi-squared tests. Data were expressed as mean ± SD. Statistical significance was defined as $P \leq 0.05$.

Results

Clinical evaluation

All study participants completed the microneedling treatment and follow-up period and showed both subjective as well as objective improvement in the clinical appearance of wrinkles and skin texture (Fig. 1). At each endpoint (before, after 1 month, and 3 months after starting treatment), the volunteers, two dermatologists, and two independent observers were asked to evaluate the following criteria: wrinkle improvement, skin texture, and overall satisfaction, based on a five-point scale (none = 0%; mild = 1–25%; moderate = 26–50%; good = 51–75%; and very good = 76–100%). The results were tabulated and compared to baseline for statistical significance with the Pearson chi-squared test (Table 1). The independent observers' and dermatologists' evaluation were comparable to the volunteers' assessment rates.

In addition, potential side effects were evaluated at each visit. All patients reported slight pain, erythema, and facial edema after the procedure, which resolved 24 hours later. All patients were able to return to normal daily activity two days after the session (downtime). With the 1 mm dermaroller and the minimally invasive technique employed with minimal pressure, only slight oozing and pinpoint bleeding were observed at the end of the microneedling session. This was washed out by cold saline soaks, leaving no crusting. During the follow-up period (3 months), patients were monitored for pigmentary changes, and no postinflammatory pigment alteration was recorded.

Epidermal changes

The epidermis showed acanthosis with development of rete ridges in response to skin microneedling treatment. This increase was reflected by a significant increase in the mean thickness of the epidermis from 58 ± 4.1 μm before treatment to 63 ± 3.8 μm at one month of treatment ($P = 0.03$), which continued to increase to 72.9 ± 5.4 μm

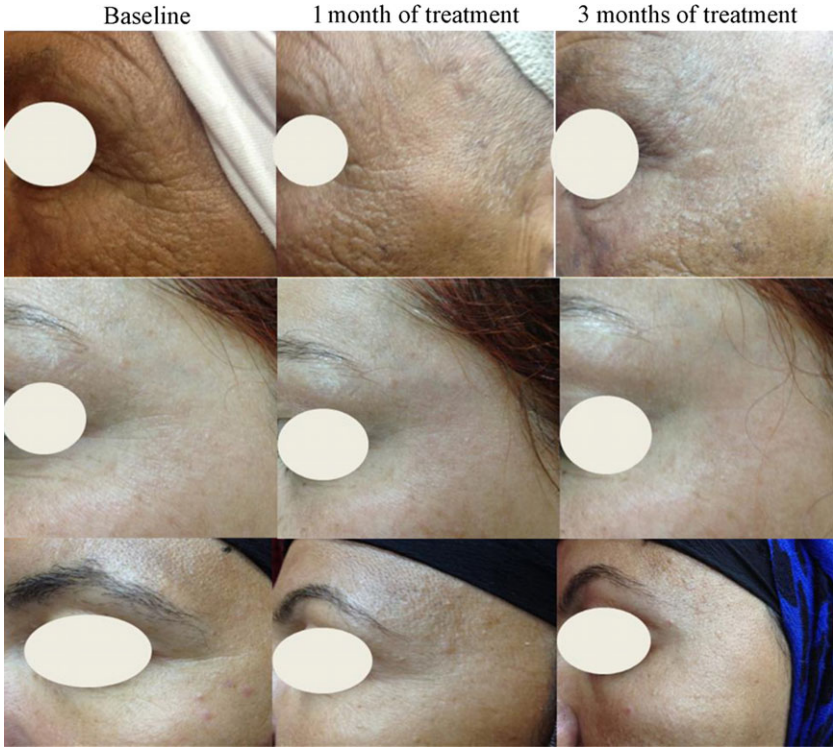

Figure 1 Representative examples of photographs of patients with pre-orbital wrinkles showing clinically favorable improvement in response to microneedling therapy after 1 month of treatment (two sessions), which continued to improve 3 months post-treatment (after six sessions) when compared to baseline

Table 1 Clinical improvement relative to baseline in response to microneedling treatment for skin aging

% Improvement (n = 10)	Wrinkles	Skin texture	Overall satisfaction
After 1 month of treatment (after 2 sessions)	20–25 Mild	20–25 Mild	35–45* Moderate
After 3 months post-treatment (after 6 sessions)	55–65* Good	55–60* Good	80–90* Very good

*$P < 0.05$.

three months post-treatment ($P = 0.007$), when compared to baseline (Table 2).

Evaluation of changes in dermal elastic structures

Through different decades of life, both quantitative and qualitative changes in the elastic and collagen fibers are observed. The histological changes associated with aged skin include the accumulation of elastotic material in the papillary and mid dermis, a process known as solar elastosis.[12,22] We investigated the effects of skin microneedling treatment for aging skin on total dermal elastin by measuring the percentage of dermis occupied by immunohistochemically stained elastin. The elastin level showed a slight but statistically insignificant ($P = 0.511$) decrease in level from 56.9 ± 5.3% at baseline to 55.2 ± 4.1% after one month of treatment; this was followed by a statistically significant ($P = 0.02$) decrease in the elastin level to 48.1 ± 4.9%, at three months post-treatment when compared to baseline (Table 2 and Fig. 2). The decline in elastin content was associated with partial restoration of normal-appearing elastic fibers within the papillary and upper reticular dermis after displacement of the solar elastotic material away from the epidermis.

Elastic fibers are composed mainly of elastin, a connective tissue protein, which is initially synthesized as tropoelastin.[20,23] The rate of elastin biosynthesis was then assessed by quantifying for newly synthesized tropoelastin. Compared to baseline, we observed a slight but statistically non-significant increase in the mean of tropoelastin level from 15.1 ± 4.2% to 16.6 ± 3.3% ($P = 0.478$) after one month of microneedling therapy. Meanwhile, the content of tropoelastin showed a statistically significant increase in level to 21.9 ± 2.9% at three months post-treatment when compared to baseline ($P = 0.02$) (Table 2 and Fig. 2).

Quantitation of dermal collagen content

When tissues are stained with picrosirius red and viewed under polarized light, mature collagen fibers have special physical characters, as they show change in color (birefringence) with a subsequent decrease in light penetration. In the present study, we noticed a slight statistically non-significant ($P = 0.165$) increase in newly synthesized collagen, as reflected by the presence of yellow–orange birefringence, from 14.1 ± 4.9% before treatment to 16.9 ± 4.2% after one month of microneedling treatment. Meanwhile, this was followed by a statistically significant ($P = 0.01$) increase to 21.8 ± 3.8% three months post-treatment when compared to baseline (Table 2 and Fig. 3).

The effect of microneedling treatment on collagen types I and III was quantitatively evaluated, and the values were compared to baseline for statistical significance. Quantitative evaluation of the percentage of dermis occupied by

Table 2 Quantitative analysis of epidermal thickness and extracellular matrix proteins before and after microneedling treatment for skin aging

	(Mean ± SD)			*P* value		
(n = 10)	Baseline	After 1 month of treatment (after 2 sessions)	After 3 months post-treatment (after 6 sessions)	Baseline vs. 1 month of treatment	One month of treatment vs. 3 months after starting treatment	Baseline vs. 3 months after starting treatment
Epidermal thickness (μm)	58 ± 4.1	63 ± 3.8	72.9 ± 5.4	0.03*	0.01*	0.007*
Total elastin (%)	56.9 ± 5.3	55.2 ± 4.1	48.1 ± 4.9	0.511	0.03*	0.02*
Tropoelastin (%)	15.1 ± 4.2	16.6 ± 3.3	21.9 ± 2.9	0.478	0.03*	0.02*
Newly synthesized collagen (%)	14.1 ± 4.9	16.9 ± 4.2	21.8 ± 3.8	0.165	0.02*	0.01*
Collagen I (%)	58.1 ± 5.1	61.2 ± 4.9	69.9 ± 5.4	0.369	0.02*	0.01*
Collagen III (%)	55.6 ± 4.7	58.5 ± 5.6	67.8 ± 6.1	0.462	0.02*	0.01*
Collagen VII (%)	13.5 ± 2.7	14.6 ± 2.1	19.1 ± 1.9	0.621	0.02*	0.02*

*$P \leq 0.05$.

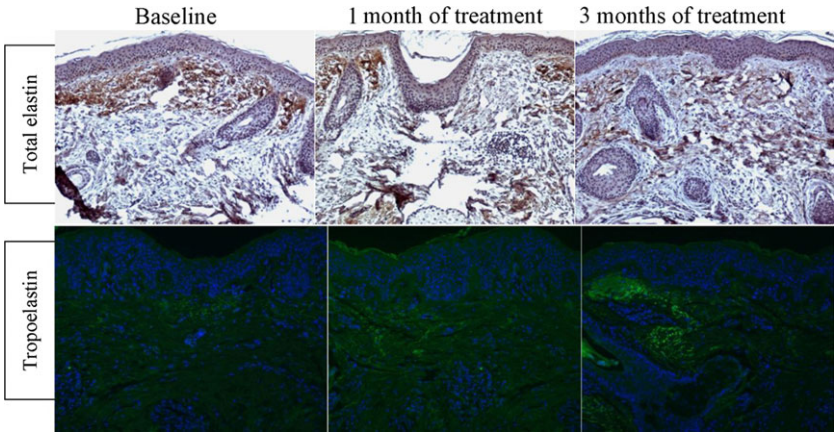

Figure 2 Immunoperoxidase (IP) staining for total elastin (top row) showing a decrease in total elastin level after 1 month and 3 months post-treatment when compared to baseline. Immunofluorescence (IF) staining of tropoelastin (second row) shows increased deposition of newly synthesized tropoelastin in dermis in response to microneedling therapy (IP, first row and IF, second row staining; × 200)

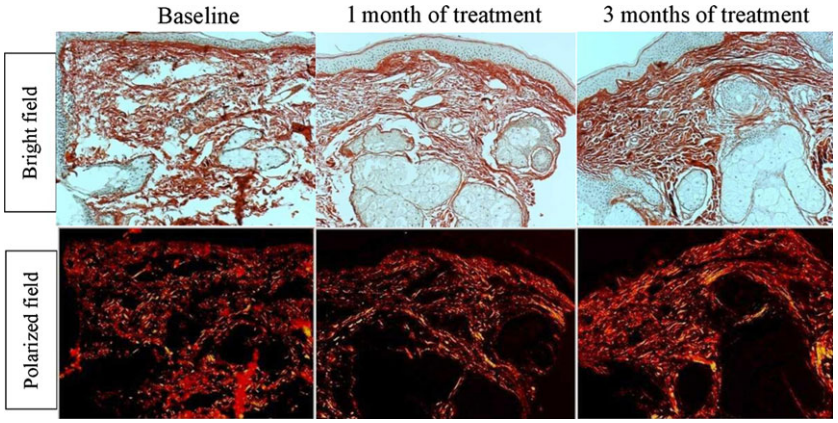

Figure 3 Skin biopsies stained with picrosirius red for newly synthesized collagen viewed under bright field microscope (top panels) and polarized field (bottom panels) showing increase in newly formed collagen level in response to microneedling treatment. Polarized light shows an increase in yellow to orange birefringence for newly synthesized collagen (picrosirius red, × 200)

collagen showed a statistically insignificant ($P = 0.369$) increase in level from 58.1 ± 5.1% before treatment to 61.2 ± 4.9% after one month of skin microneedling treatment, which was followed by a statistically significant ($P = 0.01$) increase to 69.9 ± 5.4% three months post-treatment when compared to baseline (Table 2 and Fig. 4).

In addition, assessment of collagen type III revealed a slight but insignificant increase ($P = 0.462$) from 55.6 ± 4.7% at baseline to 58.5 ± 5.6% after one month of treatment, but the level of collagen type III expression increased significantly ($P = 0.01$) three months post-treatment (67.8 ± 6.1%) when compared to baseline (Table 2 and Fig. 4).

Type VII collagen is the main component of the anchoring fibrils mediating dermal–epidermal adherence in human skin.[24] Different studies had reported the effect of the aging process on collagen VII biosynthesis and degradation,[6,25,26] so we quantified the level of collagen VII expression in response to microneedling therapy. Our data showed a statistically non-significant ($P = 0.621$) increase from 13.5 ± 2.7% before treatment to 14.6 ± 2.1% after one month of microneedling treatment. On the other hand, it was statistically significantly increased ($P = 0.02$) to 19.1 ± 1.9% at three months after starting treatment when compared to baseline (Table 2 and Fig. 4).

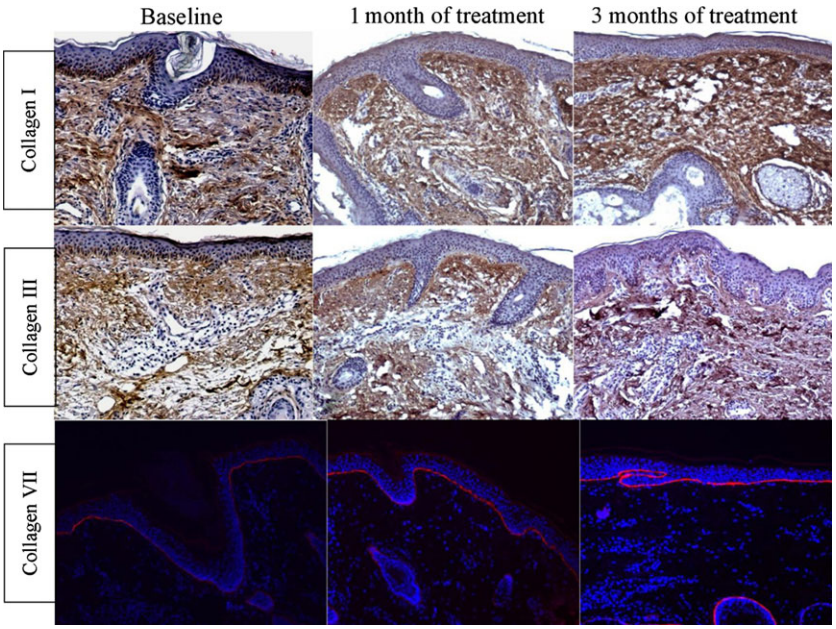

Figure 4 Immunohistochemical staining of collagen. Immunoperoxidase staining for collagen type I (first row) and collagen type III (second row) and immunofluorescence staining for collagen type VII expression (third row) of skin tissues showing an increase in the level of collagen in response to skin microneedling therapy for skin aging 1 month after treatment and 3 months post-microneedling treatment compared to baseline (IP, first and second rows, and IF, third row staining; ×200)

Discussion

In the last few years, patients have been seeking information on how to improve the signs of skin aging; it is the responsibility of physicians to select the most suitable procedure(s) based on the patient's age, physical needs, concerns, and cosmetic goals.[20,27] Different therapeutic maneuvers were used throughout the years to give the face a youthful appearance. However, because each person is unique, there is no one modality that is best for everyone.[12,28–30]

Interest in ablative rejuvenation has waned significantly while non-ablative modalities have become attractive alternative procedures, as they are used to rejuvenate skin with minimal downtime and complications.[11,21] Many different terms have been used to describe these procedures, including subsurface resurfacing and minimally invasive skin rejuvenation. These modalities are designed to produce many cosmetic benefits, including improvement of wrinkles, skin laxity, and texture with minimal downtime.[31]

The goal of most minimally invasive treatments is to induce selective dermal injury, which results in wound repair response, while the epidermis remains intact.[31] The wound healing response with microneedling occurs in three successive phases. The first phase (inflammation phase) begins shortly after injury with the release of chemotactic factors from platelets; resulting in invasion of other platelets, neutrophils, and fibroblasts. In the second phase, proliferation phase, monocytes replace neutrophils and change into macrophages with subsequent release of numerous growth factors, including platelet-derived growth factor, fibroblast growth factor, and transforming growth factors α and β, thus stimulating the migration and proliferation of fibroblasts. Keratinocytes then start to re-establish the basement membrane by enhancing the production of laminin and collagen types IV and VII. The last phase, the remodeling phase (phase 3), which is mainly achieved by the fibroblasts, continues for months after the injury, and collagen is formed in the upper dermis over a period of a year or longer.[32,33]

Collagen III is the main type of collagen formed in the early wound healing phase. It is gradually replaced by collagen I over a period of a year or more resulting in continued tissue remodeling for months after the injury. Collagenases and matrix proteinases are involved in the gradual conversion of collagen III into collagen I, which remains in the area for 5–7 years.[16]

Orentreich and Orentreich[34] described subcision or dermal needling by pricking the skin with a needle to free the dermis and build up connective tissue under scars and wrinkles. This technique, however, could not be used on large body surface areas. Camirand and Doucet[35] used a tattoo pistol to treat scars with needle abrasion. Although

this technique can be used on larger areas, it is slow and laborious. The fundamental similarity of these different techniques is that the needles break old collagen structures that connect the scar with the upper dermis.

Skin microneedling treatment is a new modality to the minimally invasive treatment armamentarium for skin rejuvenation. It is becoming popular all over the world, not only in the management of scars but also as an anti-aging therapy.[36] Many studies concerning the efficacy of minimally invasive modalities have been published, yet most of them were based on subjective evaluation of the results.[37–39] We have used a simple skin needling device to objectively evaluate the effectiveness of the microneedling technique in skin rejuvenation, both on clinical and histological levels.

In the present study, evaluation of patients' clinical results showed noticeable improvement in response to microneedling treatment. Improvements in wrinkle appearance and skin texture increased from 20–25% (mild improvement) at one month of treatment (after two sessions) to good improvement (55–65% and 55–60%; respectively) at three months post-treatment. Meanwhile, patient satisfaction was reported as moderate (35–45%) at one month of treatment and then increased to 80–90% (very good) at three months post-treatment (Table 1). After skin microneedling, our patients experienced transient erythema and edema, which resolved two days later. The result of the present work is consistent with previously published studies using the skin microneedling technique for treatment of skin aging, which evaluated the efficacy of skin microneedling in the management of signs of skin aging and showed improvement in all patients without any side effects except for redness and swelling, which disappeared within 2–3 days.[13,15,16,40]

The present study also showed that multiple sessions (six) of minimally invasive microneedling initialize collagen synthesis and do not interfere with normal lifestyle of the patient. However, other investigators[32] used a 3 mm roller to produce better results but with a longer downtime.

It was shown in a retrospective analysis of 480 patients that percutaneous collagen induction therapy is a safe and successful method for skin rejuvenation, and none of the patients developed postoperative pigmentary disorder. Histologic examination was carried out on only 20 patients, using H&E and Van Gieson staining, and showed a considerable increase in collagen and elastic fiber deposition at six months postoperatively.[15] The collagen also appears to have been laid down in a normal lattice pattern rather than in parallel bundles, as seen in scar tissue.[15,40,41]

To the best of our knowledge, no previous work concerning quantitative changes of elastin, tropoelastin, and collagen (newly formed, types I, III, and VII) in response to skin microneedling therapy for the treatment of skin aging were reported. The results of the present study aim to improve the subjective evaluation in the context of objective means by quantitatively evaluating the role of skin microneedling on extracellular matrix remodeling. Quantitative evaluation of histological changes showed a statistically significant ($P < 0.05$) increase in epidermal thickness with enhanced formation of dermal papillae. Furthermore, evaluation of extracellular matrix proteins showed statistically significant ($P < 0.05$) improvement of elastin and collagen deposition and formation in response to microneedling therapy. Although there was a slight but statistically insignificant ($P > 0.05$) decrease in total elastin level, which was accompanied by an increase in tropoelastin, collagen (types I, III, VII), and newly synthesized collagen at one month of treatment, these changes continued to improve to show statistically significant ($P < 0.05$) enhancement three months after starting treatment.

Our results were consistent with Aust *et al.*[15] and Fernandes and Signorini,[16] who used H&E staining to evaluate the effect of microneedling on skin rejuvenation. They reported a noticeable increase in epidermal thickening and rete ridges at one month postoperatively, which was accompanied by a subjective increase in collagen deposition in response to treatment.

Conclusions

Microneedling is an effective and valuable procedure that can be used to tighten and rejuvenate aged skin. This modality reverses the clinical as well as histopathological signs of aging, with the advantage of being an inexpensive office maneuver and relatively risk-free procedure, avoiding significant downtime. The results of the present study recommend that continued skin microneedling treatment is required to maintain the clinical and histological improvement. Meanwhile, further in-depth, long-term studies are required to assess the ability to maintain the clinical and histological improvement and the maximum duration of treatment required to achieve clinical or histological beneficial effects.

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