Original Contribution

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Multiple fractional erbium: yttrium–aluminum–garnet laser sessions for upper facial rejuvenation: clinical and histological implications and expectations

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Summary

Background Fractional photothermolysis is a modern resurfacing technique, in which microscopic zones of thermal injury are created, stimulating turnover of both epidermis and dermis. Fractional laser rejuvenation has been developed to overcome the drawbacks of traditional ablative laser.

Objectives To objectively evaluate the effectiveness of multiple sessions of fractional Er: YAG laser rejuvenation for aging upper face clinically, histologically and immunohistochemically.

Patients/Methods Ten volunteers asking for facial rejuvenation were subjected to multiple sessions (3–5) of fractional Er:YAG laser. Clinical evaluation with both histopathological and immunohistochemical assessment for skin biopsies was carried out before, after 1 month and 6 months of laser resurfacing. Histometry for epidermal thickness and quantitative assessment for neocollagen formation, collagen I, III, and VII, elastin and tropoelastin were carried out for all skin biopsies.

Results Comparing before, after 1 month and 6 months of fractional Er:YAG laser resurfacing resulted in improved clinical appearance with increased epidermal thickness ($P < 0.001$). Dermal collagen showed increased neocollagen formation ($P = 0.006$), with increased concentration of collagen types I ($P < 0.001$), III ($P < 0.001$), and VII ($P = 0.001$). Dermal elastic tissue studies revealed decreased elastin, while tropoelastin concentration increased after laser resurfacing ($P < 0.001$). An increase in collagen (I and III) and tropoelastin level and decreased elastin content was encountered with increasing the number of sessions, yet it was not significant.

Conclusions Multiple sessions are effective in rejuvenation of the aging face with high safety, short downtime, and no adverse effects. They stimulated formation of new collagen (type I, III, and VII) up to 6 months after treatment with better improvement in skin texture and fine wrinkles. The variable number of fractional Er: YAG laser sessions (3–5) showed no significant difference as regards efficacy.

Keywords: fractional, Er: YAG, rejuvenation, elastin, collagen, skin aging

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Introduction

Fractional resurfacing is a novel variation on the theory of selective photothermolysis, wherein microscopic treatment zones (MTZs) of controlled width, depth, and densities are created.[1,2] These controlled zones of thermal heating and tissue damage are surrounded by spared areas of viable epidermis and dermis that allow for rapid repair of the MTZs.[3]

Fractional laser resurfacing first became commercially available in 2003, when *Fraxel* SR device was introduced to fill the gap between ablative and nonablative devices.[1] It targets water as a chromophore but, in contrast to traditional ablative devices, uses an objective lens and adjustable beam to focus the laser beam within the dermis, thereby targeting specific depths in the skin, a phenomenon termed fractional photothermolysis. When operated using a scanning motion, it creates an array of MTZs measuring 50–150 microns in diameter. Each MTZ forms a column of thermally denatured collagen spanning from the epidermis to mid-dermis.[1,4]

Fractional delivery systems for both carbon dioxide and erbium:yttrium–aluminum–garnet (Er:YAG) lasers were developed in an attempt to achieve the clinical results observed with traditional ablative lasers with minimal downtime and side effects. These devices cause true ablation of the epidermis in addition to variable depths of ablative damage to the dermis.[3]

A fractional system based on the Er:YAG laser has become commercially available. When the Er:YAG laser is used for resurfacing in the fractional mode, recovery time is considerably shortened, and traditional postresurfacing sequelae are minimal or absent. Consequently, this allows patients a rapid return to their social or work environments. Fractional Er:YAG laser is not only to improve the appearance of wrinkles, but comprehensively to remove other symptoms of photoaging, meeting all the criteria of skin rejuvenation.[5]

Fractional resurfacing with fractional microscopic delivery of high energies to targeted depths in the dermis has made possible significant clinical improvements often approaching that of ablative lasers without any reports of permanent hypopigmentation, hyperpigmentation, or scarring.[1,2,6] The combination of epidermal and dermal ablation appears to lead to a more robust wound healing response and accompanying dermal fibrosis, which may explain the rapid and significant clinical effects that can be achieved with ablative *vs.* nonablative devices.[3]

Recent studies showed that multiple sessions of fractional lasers could give better results.[7,8] Moreover, multiple fractional Er:YAG laser sessions induced clinically and histologically correlated improvement in collagen when compared to single ablative Er:YAG laser resurfacing session.[9]

This study aims to objectively assess the result of multiple sessions (3–5) of fractional Er:YAG laser rejuvenation for aging upper face as regards clinical improvement and both histological and immunohistochemical evaluation of skin biopsies before, after 1 month and 6 months of resurfacing.

Materials and methods

The present study was conducted on 10 volunteers attending the dermatology outpatient clinic of Al-Minya University Hospital, Al-Minya, Egypt, seeking for improvement of aging face. The subjects were 35–60 years old, 4 males, and 6 females, with Fitzpatrick skin types III–V, and class II–IV wrinkles based on Glogau's scale.[10]

The patients were not using any anti-aging topical medications and did not perform any other cosmetic procedure. The study details were fully explained to the volunteers, and they gave an informed consent for fractional laser facial rejuvenation, photography, and skin biopsies. The study was approved by the Committee for Postgraduate Studies and Research of Al-Minya University.

The fractional laser device was Fotona ablative fractional laser system Er:YAG 2940 nm model Dualis XS, "Ljabljana Slovania". The laser system has 5 levels (with MTZ [pixel] number ranging from 4–256 and MTZ size 20–300 micrometer according to the selected level).

Volunteers were subjected to a total of 3–5 sessions at 2-week interval (2 subjects, 3 sessions; 6 subjects, 4 sessions, and 2 subjects, 5 sessions) according to clinical response and volunteer's opinion. Four complete laser passes per session were performed over the forehead and crow's feet areas.

Acyclovir 400 mg tablets/8 h was given 1 day before the fractional laser sessions and for 3 days after resurfacing. No anesthesia was needed; 70% ethyl alcohol was used to clean the skin before and after it had been sterilized by povidone iodine. Patient eye protection goggles were applied. The fractional Er:YAG 2940 nm laser was used at a fluency of 1200 mJ/cm^2, with pixel number: 30/cm^2 and pixel size: 270 micrometer via the short-pulse mode (300 μs) and 10 mm spot size.

After treatment, a thin layer of antibiotic ointment "fusidic acid" was applied and the patient was

instructed to use it 2–3 times each day for 4 days. Following re-epithelialization of the skin, all volunteers were instructed to apply sunscreens, with sun protection factor (SPF) of 35 or more, every 2 h during daytime.

Follow-up and assessment

Patients were seen daily for 1 week after the procedure, then weekly during first month after resurfacing and monthly for 6 months. Skin biopsies (3 mm) were obtained from the crow's feet area, before and after 1 month and 6 months. Any possible complaint or complication was evaluated at every visit. At each endpoint (before, 1 month and 6 months after treatment), the volunteers, doctors, and two independent observers were asked to evaluate the following criteria: improvement in rhytides, skin tightening, and texture, and overall volunteer satisfaction. Their evaluations and improvement were assessed on a four point scale structured questionnaire (poor = 1%–25%, fair = 26%–50%, good = 51%–75%, and very good = 76%–100%). Skin biopsies were processed and sectioned for histometry, histopathological, and immunohistochemical study.

Histologic staining and histometry

Skin biopsy specimens were stained with hematoxylin–eosin (H&E), orcein for elastic fibers, and both Masson trichrome and picrosirius red (Direct Red 80, Sigma, St Louis, MO, USA) for collagen. Epidermal thickness was estimated histometrically (mean distance between the outermost surface of the epidermis, excluding the stratum corneum, and the dermo-epidermal junction). Five measurements for each skin biopsy specimen were taken by a computer-assisted program (analySIS Five, Olympus Soft Imaging Solutions GmbH, Münster, Germany). Picrosirius red was evaluated using a microscope (Nikon, Melville, NY, USA) equipped with filters to provide circularly polarized illumination. Immunohistochemical and picrosirius red staining were quantified using software (IMAGE-PRO PLUS, Media Cybernetics Inc, Silver Spring, MD, USA). Epidermal thickness and immunohistochemical evaluation were carried out by 2 independent blinded dermatopathologists. A single staining technique was used for each stain and each marker.[9]

Immunohistochemical study

Immunoperoxidase technique was used for detection of total elastin (1:300; E4013, Sigma), Collagen type I (1:400; sc-59772, Santa Cruz, CA, USA), and type III (1:600; ab6310, Abcam, MA, USA).[11,12] Indirect immunofluorescence was used to evaluate type VII collagen (1:600; sc-33710, Santa Cruz Biotechnology) and tropoelastin (tropoelastin GA317, 1:400; Elastin Products, Owensville, MO, USA).[12,13]

Statistical analysis

The collected data were analyzed and figured using a computer-based program, SPSS software package for statistical analysis (SPSS for Windows, Version 16.0, copyright © SPSS Inc., USA). The data were summarized in the form of mean ± standard deviation (SD). The significance was assessed using dependent (paired) *t*-test to compare 2 variables (before *vs.* 1 month, before *vs.* 6 months and 1 month *vs.* 6 months), while ANOVA test was used to compare all the 3 variables (before, after 1 month, and after 6 months). Statistical significance for all results was defined as "$P \leq 0.05$".

Results

Clinical results

All 10 volunteers completed the study to achieve acceptable clinical response of their photoaged skin. The clinical response after these multiple sessions of fractional Er:YAG laser resurfacing, especially of the crow's feet area, showed improvement in the skin texture rather than static wrinkles (Fig. 1). Fine wrinkles showed better response than coarse wrinkles. The overall response were rated as: very good (40%), good (30%), and fair (30%), and it was constant during the 6 months after treatment. The number of sessions for each volunteer had no significant effect on overall clinical response in the studied volunteers.

Complete healing occurred within 2–4 days. By the 2nd week (at the time of the next session), there was no erythema in all cases. No pigmentary changes were observed in-between sessions.

One week after the last session, the facial skin of all patients has healed with a smooth and soft texture with no erythema or hyperpigmentation and no scar or keloid formation. Also, after 6 months, no complications were observed.

Histological and Histometric evaluation of epidermal changes

The epidermis had improved after fractional Er:YAG laser resurfacing. The keratinocytes became more organized, and the cell layers increased in number.

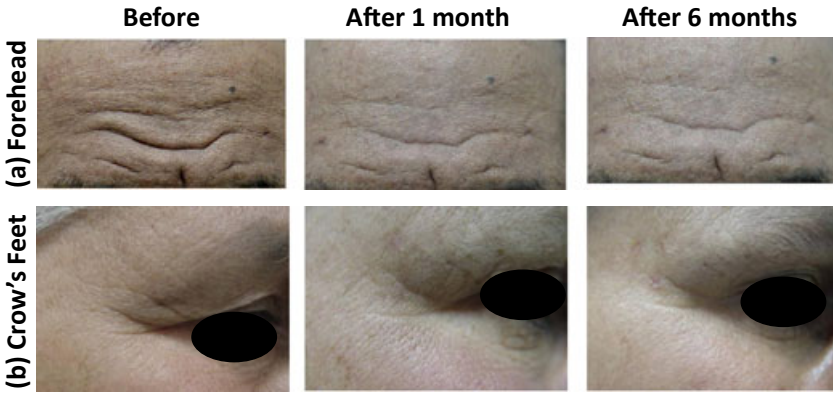

Figure 1 Improvement in forehead wrinkles (a) and skin texture rather than static wrinkles in crow's feet (b) after Er:YAG fractional laser resurfacing with no adverse effects.

The epidermal thickness changed significantly ($P < 0.001$) after fractional Er:YAG laser resurfacing. It increased significantly from a mean ± SD of 43 ± 4.6 μm before treatment to 51.8 ± 6.9 μm after 1 month ($P < 0.001$). It then decreased significantly after 6 months ($P = 0.001$) to 46 ± 5.3 μm, but still more than the pretreatment level ($P = 0.013$) (Fig. 2, Table 1).

Evaluation of dermal collagen

Collagen type I concentration increased significantly ($P < 0.001$) after fractional laser sessions. It increased significantly from 60.2 ± 1.8 before treatment to 63 ± 2.4 after 1 month ($P = 0.001$) with more increase after 6 months to 68.4 ± 5.8 ($P < 0.001$) when compared with the pretreatment level (Fig. 3 (A), Table 1).

After fractional laser resurfacing collagen type III concentration had also increased significantly ($P < 0.001$). The pretreatment level was 55.9 ± 3.5 and increased after 1 month to 58.2 ± 3.9 ($P = 0.001$) and after 6 months to 63.1 ± 35.9 ($P = 0.001$) as compared with pretreatment level (Fig. 3 (B), Table 1).

When tissues are stained with picrosirius red and examined under a polarized microscope, collagen fibers display characteristic optical properties: large fibers stain red, while the thinner ones, which represent the newly synthesized collagen, are stained yellow to orange.[14,15]

Our data showed significant increase in the newly synthesized collagen fibers in response to fractional Er:YAG laser resurfacing ($P = 0.006$), as reflected by the increase in yellow–orange birefringence. It increased significantly from 18.3 ± 1.1 before treatment to 21.8 ± 2.9 after 1 month ($P = 0.001$) with further increase after 6 months to 25.5 ± 2.3 ($P < 0.001$) when compared with the pretreatment level (Fig. 3 (C), Table 1).

Collagen VII increased significantly after fractional Er: YAG facial resurfacing ($P = 0.001$). Pretreatment level (11 ± 1.1) showed significant increase after 1 month (12.9 ± 1.3) and 6 months (16 ± 2.5) ($P = 0.001$, $P < 0.001$, respectively) (Fig. 3 (D), Table 1).

Evaluation of dermal elastic tissue

The elastotic material was displaced downwards and decreased in density after fractional laser resurfacing. Elastin concentration decreased gradually after frac-

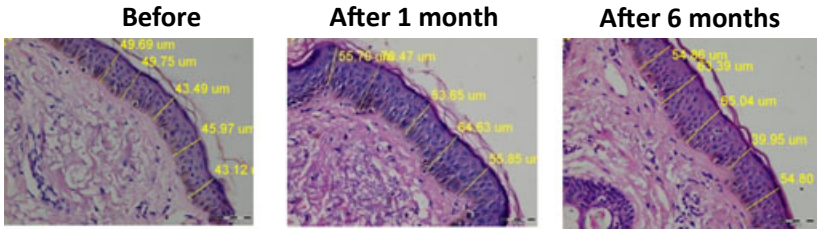

Figure 2 Histometrical measurement of epidermal thickness increased after 1 month with gradual decrease, after 6 month, but still more than before resurfacing (H&E stain; original magnifications ×200).

Table 1 Mean results of fractional Er:YAG laser resurfacing.

	Fractional Er:YAG laser resurfacing			Paired samples *t*-test (*P* value) (Compare 2 end points)			
	Before	After 1 month	After 6 months	Before *vs.* 1 month after treatment	Before *vs.* 6 months after treatment	1 month *vs.* 6 months after treatment	ANOVA test (Compare 3 end points)
Epidermal thickness (μm)	43 ± 4.6	51.8 ± 6.9	46 ± 5.3	<0.001*	0.013*	0.001*	<0.001*
Neocollagen (%)	18.3 ± 1.1	21.8 ± 2.9	25.5 ± 2.3	0.001*	<0.001*	0.001*	0.006*
Collagen I (%)	60.2 ± 1.8	63 ± 2.4	68.4 ± 5.8	0.001*	<0.001*	0.001*	<0.001*
Collagen III (%)	55.9 ± 3.5	58.2 ± 3.9	63.1 ± 3.9	0.001*	0.001*	0.001*	<0.001*
Collagen VII (%)	11 ± 1.1	12.9 ± 1.3	16 ± 2.5	0.001*	<0.001*	<0.001*	0.001*
Elastin (%)	52.8 ± 2.1	48.9 ± 4	44.3 ± 5.3	0.001*	<0.001*	0.008*	<0.001*
Tropoelastin (%)	14 ± 1.4	16.8 ± 2.4	19.3 ± 3	0.001*	<0.001*	<0.001*	<0.001*

*Significant: $P \leq 0.05$.

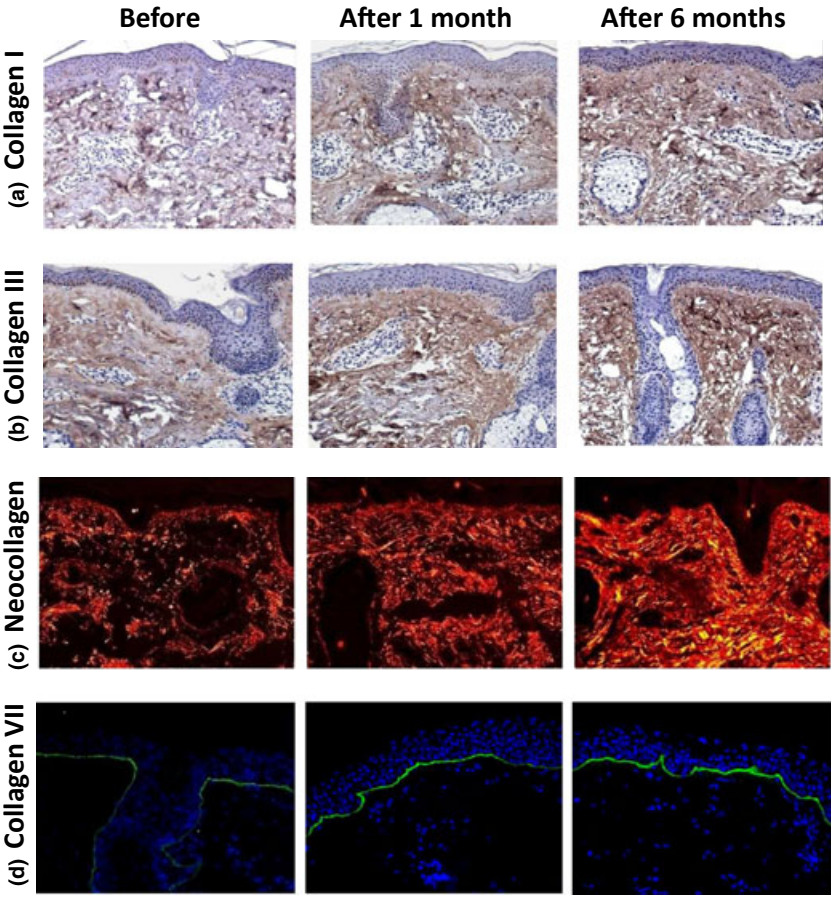

Figure 3 After laser resurfacing: Increase in collagen I (a) (Immunohistochemistry) and collagen III (b) (Immunohistochemistry); neocollagen formation (c) (Picrosirius red stain under polarized light microscopy) and collagen VII (d) fluorescence at dermo-epidermal junction (immunofluorescence) (a–d, original magnifications ×200).

tional laser resurfacing ($P < 0.001$). The pretreatment concentration was the highest (52.8 ± 2.1), then it decreased to 48.9 ± 4 after 1 month ($P = 0.001$) with further reduction to 44.3 ± 5.3 after 6 months ($P < 0.001$) when compared with pretreatment level (Fig. 4 (A), Table 1).

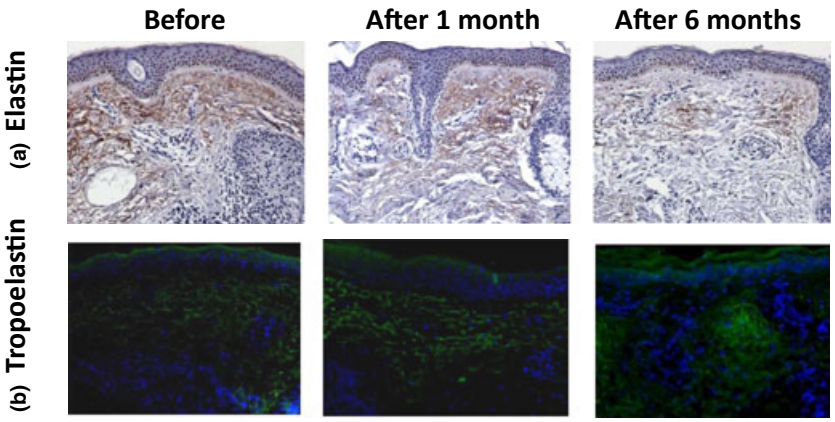

Figure 4 After fractional laser: elastin decreased (a) after laser resurfacing (Immunohistochemistry) and tropoelastin fluorescence increased (b) (Immunofluorescence with DAPI) (a and b, original magnifications ×200).

Fractional Er:YAG laser induced significant increase in the concentration of tropoelastin ($P < 0.001$). After 1 month, the tropoelastin concentration increased from 14 ± 1.4 (before treatment) to 16.8 ± 2.4 ($P < 0.001$), then to 19.3 ± 3 after 6 months ($P < 0.001$) (Fig. 4 (B), Table 1).

Discussion

Laser ablative resurfacing remains the "gold standard" for rejuvenating severely photodamaged facial skin, but it is associated with long-term sequelae-related patient downtime. Recently, fractional resurfacing has been introduced in the armamentarium of the dermatologist's equipment that offers an alternative to laser ablative resurfacing, designed to decrease the photothermal side effects, while still achieving good results, with faster healing and significant reduction in downtime.[5]

The novel concept of fractional photothermolysis was introduced to the market as an answer to the need for effective, yet low risk, resurfacing techniques. Unlike conventional ablative and nonablative lasers, fractional ablative and nonablative photothermolysis treats only a fraction of the skin, leaving the undamaged surrounding tissue as a reservoir of viable tissue, permitting rapid epidermal repair.[16]

Fractional laser is used to resurface the epidermis and, at the same time, to heat the dermis to promote safely the formation of new collagen. The untreated healthy skin remains intact and actually aids the repair process, promoting rapid healing with only a day or two of downtime. It induces a wound healing response in the dermis by the creation of MTZs of thermal damage. The thermally damaged collagen inside the MTZs is completely replaced with new collagen.[17]

Debate continues on the use of multiple treatment sessions or one single treatment session. *Trelles et al.,*[5] used a single session of fractional Er:YAG for facial rejuvenation at a fluency of 1400 mJ/cm^2, and 8 passes demonstrating that the clinical assessment was 93%, they reported good or very good improvement in the degree of their wrinkles, with a satisfaction index of 83%.

Meanwhile, the present study has revealed that clinical results after multiple sessions of fractional Er:YAG laser resurfacing were rated as: very good (40%), good (30%), and fair (30%), with complete healing occurring within 2–4 days after laser session and improved skin texture. No complications were reported after fractional resurfacing. This is in agreement with the clinical observations and histological findings of *Dierickx et al.,*[18] who demonstrated that microfractional ablative treatment with 2940 nm erbium lasers resulted in safe and effective wrinkle reduction with minimal patient downtime. Meanwhile, the variable number of required sessions, from 3 to 5 sessions, was mainly determined by the clinical response and opinion of each volunteer in the study.

It is worth to mention that all volunteers had not experienced any pigmentary changes despite of the dark skin type of Egyptian patients (Fitzpatrick skin types III-V), which may be also attributed to patient's compliance in using sun screen and careful skin protection.

In the present work, improvement in the fine wrinkles and textural changes was more pronounced rather than static wrinkles, especially in the crow's feet area. This goes with *Walgrave et al.,*[19] *and Ling*[20] who reported improvement in photoaged skin in overall surface texture, including fine lines and skin dyschromia after fractional resurfacing.

After fractional Er:YAG laser, there is an improvement in epidermal appearance that becomes well organized with increased cell layers.[5] The histological and histometrical examination revealed improved organization of the epidermis with significantly increased thickness after fractional resurfacing ($P < 0.001$).

Dermal extracellular matrix components in adult skin are composed of collagen type I and type III in addition to glycosaminoglycans and elastic fibers.[21] Reduction in fibrillar (type I and III) collagen is a characteristic feature of chronologically aged skin and is enhanced by photodamage.[22] Dermal collagen synthesized by fibroblasts is normally composed of 80–85% type I collagen and 10–15% type III collagen.[23] Anchoring fibrils are composed mainly of collagen type VII and contribute to the stabilization of dermo-epidermal junction. These fibrils are severely reduced by the process of photodamage.[24]

The current study demonstrated a significant quantitative increase in concentration of newly formed collagen ($P = 0.006$) and collagen types I ($P < 0.001$), III ($P < 0.001$), and VII ($P = 0.001$) with improved collagen consistency and organization after fractional Er: YAG laser resurfacing. This is in agreement with *Laubach et al.*[6] who reported that thermally damaged collagen inside the MTZs is completely replaced with new collagen after 3 months and demonstrated an immunohistochemical evidence of increased collagen III production 7 days after fractional photothermolysis. Moreover, both ablative and fractional Er:YAG laser resurfacing for facial rejuvenation increased collagen I, III, and VII concentrations.[9] Therefore, neocollagen formation, in and around MTZs, is an expected wound healing response.

The present objective quantitative histological analysis of various types of collagen gives further evidence that multiple fractional Er:YAG laser sessions stimulates newly formed collagen of different types (collage type I, III, and VII) up to 6 months after the sessions had ended.

In contrast to the large bulk of collagen in the dermis, elastic fibers compose only 1–2% of the dry weight of sun protected skin.[25] The histologic hallmark of photoaging is dermal elastosis which largely consists of thickened, tangled, and ultimately granular amorphous elastic structures.[26] There is replacement of the normal dermal matrix of collagen, elastin, and glycosaminoglycans by large bundles of coarse elastotic material with decreased collagen.[27,28]

Elastin is synthesized as a precursor, tropoelastin, with a characteristic composition.[9] Quantitatively, the relative amount of elastin in photoaged facial skin shows a gradual and significant increase with aging, particularly in old ages.[28]

The present work demonstrated a significant quantitative decrease in old abnormal elastin ($P < 0.001$) with paradoxical effect on tropoelastin (newly formed elastin), which increased significantly ($P < 0.001$) after fractional Er:YAG resurfacing. These results are contradictory to those previously reported by *Laubach et al.*[6] who observed no significant qualitative change (using Van Gieson stain) in the elastic tissue before and after treatment. This contradiction can be attributed to the quantitative evaluation of elastin and tropoelastin in the present study rather than subjective qualitative estimation of elastic tissue.[6]

Conclusion

Fractional Er:YAG laser resurfacing showed significant effects on the epidermis and dermal collagen, elastin, and tropoelastin with better improvement of skin texture and fine wrinkles. Multiple sessions are effective in rejuvenation of the aging face with high safety, short downtime, and no adverse effects. Multiple sessions increase the clinical and histological improvement with stimulated new collagen formation (collagen type I, III, and VII) up to 6 months after treatment. However, no statistically significant difference is encountered according to the number of sessions. This could be attributed to the small number of volunteers treated and different degree of photodamage encountered in each individual volunteer, resulting in different outcome.

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