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Ex vivo histological study on the effects of non-fractional ER:YAG laser for wrinkle therapy

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The Er:YAG laser-assisted wrinkle therapy is based on both superficial tissue ablation and tissue remodeling via stimulation of the collagen synthesis. Non-fractional or fractional laser handpieces perform this treatment modality, and there is no consensus in the literature about their effectiveness. This pilot ex vivo study aimed to evaluate the histological changes after wrinkle treatment with non-fractional Er:YAG laser with air/water cooling. Prior to planned facial skin excision, the pilot patient received laser irradiation in intervals (0-14-30 days) on a linear wrinkle divided into three equal sections. The parameters used were 40mJ/10Hz with air/water spray. The treated skin zone was removed and evaluated histologically. The observed histological changes for all studied intervals of treatment revealed satisfactory healing processes without side effects. This pilot ex vivo study demonstrates that the conventional non-fractional Er:YAG laser is effective and safe, and can be adjusted successfully in wrinkle therapy.

Key words: Er:YAG laser, non-fractional, wrinkle, histology

Introduction

Er:YAG laser skin therapy (including treatment for wrinkles) involves controlled tissue ablation and subsequent tissue remodeling. Histologically, this translates to the removal of damaged skin layers, collagen stimulation, and new collagen formation, leading to wrinkle reduction and skin rejuvenation [8]. The extent of ablation and thermal damage can vary depending on laser parameters like fluence and pulse duration, influencing the healing process and clinical outcomes [2, 9]. Er:YAG laser penetrates to an average depth of 2-5 μm per J/cm^2 and the residual necrotic layer does not exceed 10-15 μm [1]. The fractional Er:YAG laser is reported to be effective in resurfacing skin, yielding similar results to non-fractional Er:YAG lasers, which involve a more continuous ablation pattern, which may

require longer healing times and have a higher risk of side effects [4]. However, the different outcomes after procedures with fractional and non-fractional Er:YAG have been discussed in various publications, and not only in favor of the fractional lasers [1]. The specific goal of the procedure – skin rejuvenation versus wrinkle reduction - is decisive when choosing a laser. The rejuvenation therapy usually covers a broad skin area, in contrast to the wrinkle "elimination", which is focused on a minor linear defect. The controversies in the literature and the fact that only a few articles include human subjects have stimulated our team to conduct this pilot ex vivo study, evaluating the histological effects of non-fractional Er:YAG laser on wrinkle therapy [11, 14].

Materials and Methods

This pilot study included one healthy female volunteer aged 64 years with Fitzpatrick skin type II. The study was performed according to principles of good clinical practice and the Declaration of Helsinki. The enrolled patient was referred for a facial lift procedure to the Clinic of Plastic Surgery (Uni Hospital, Sofia, Bulgaria), and the area for laser treatment was carefully selected to be within the borders of the planned skin excision. The patient was informed about the goal of the study, and informed consent was signed. In order to evaluate histologically the wound healing process in vivo, a facial area of human skin with presented wrinkles was selected and treated with an Er:YAG laser system (LiteTouch™, Light instruments Ltd., Israel) using a non-fractional esthetic handpiece with air/water cooling. The parameters were as follows: 40mJ/10Hz, air/water spray (level 2). The selected linear wrinkle was divided into three equal sections, and each one was irradiated in a homogeneous pattern within three consecutive laser passes. No anesthesia was used during the treatment. Three laser sessions without overlap were performed consecutively before the skin excision – the first section was irradiated 30 days before, the second section was irradiated 14 days before, and the last section was irradiated immediately before the skin excision. The patient was instructed to avoid sun exposure, use of products containing acids, make-up, and any abrasive processes for the skincare routine for the first few days before and after the laser irradiation. Protective ointment was prescribed (Eucerin Aquaphor Protective Ointment).

Histological evaluation

Immediately following excision, each sample was fixed in 10% buffered neutral formalin overnight and then embedded in paraffin. The samples were sectioned, stained with Hematoxylin and Eosin, and examined under a light microscope using 4X and 10X magnifications.

Results

The histological sample representing the skin area immediately after laser irradiation revealed a well-controlled ablation penetrating approximately 120 μm deep through the epidermis and near the underlying papillary dermis. A thin area of coagulation with a more basophilic appearance surrounding the ablation area was observed (**Fig. 1a**).

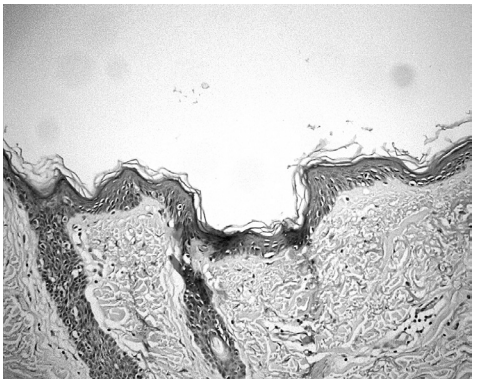

Fig. 1. A

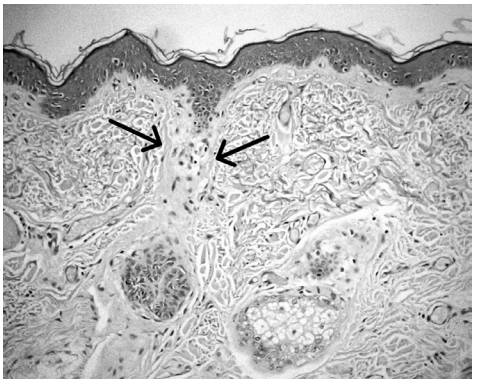

Fig. 1. B

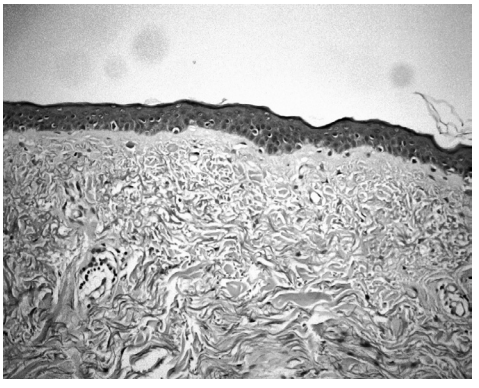

Fig. 1. C

Fig. 1. *Histological findings in human skin after non-fractional Er:YAG laser irradiation immediately after the treatment (**a**), 14 days after the treatment (**b**), and 30 days after the treatment (**c**). Hematoxylin/eosin, 10× magnification.*

Fourteen days after the laser treatment, re-epithelialization with a hyperproliferative epithelium and normal maturation, manifested with the presence of stratum corneum and orthokeratosis, was observed (**Fig. 1b**). Under the area of re-epithelialization, the smaller area of subepidermal fibrin and a few neutrophil infiltrations could be observed (**indicated with arrows**). Thirty days after the treatment, complete re-epithelialization with normal maturation of the epidermis was observed, manifested by normal-width orthokeratosis, the presence of stratum corneum, and stratum granulosum. Below the epidermis, the presence of multiple fibroblasts and evidence of glomeruloid angiogenesis (**Fig. 1c**).

Discussion

The Er:YAG lasers, with a wavelength of 2940 nm, are highly absorbed by water in the skin. This absorption leads to rapid vaporization and removal of the epidermis and superficial dermis. The depth of ablation is controllable by adjusting laser parameters, allowing precise targeting of wrinkles. Ablative techniques are still considered the most effective methods for improving photodamaged skin, but are associated with

a prolonged recovery time and high risk of side effects [10]. Thus, the minimally invasive or even non-ablative (the ablation is microscopically presented but its volume is clinically insignificant) methods are gaining popularity in modern rejuvenation therapy. The mechanisms behind this dermal remodeling are the minimal trauma (micro ablation) combined with mild heating of the dermis caused by the laser, affecting both the microcirculatory complex and the fibroblasts' activity [6]. The Er:YAG laser used in the current study demonstrated an ablation depth of approximately 120 μm through the epidermis and near the underlying papillary dermis, which corresponds with the micro ablation techniques. In such a situation, only the epidermis is affected, while deep dermal structures are heated. The expected thermal effects, however, leading to both beneficial collagen denaturation and potentially undesirable thermal damage, must be controlled adequately. Residual thermal damage, characterized by vacuole formation and severe inflammation, was not observed in the current study. Proper laser settings and cooling techniques can minimize the thermal damage and promote more effective collagen remodeling. The Er:YAG laser used in the current study delivers controlled thermal energy to the skin without significantly ablating its outer layer. In combination with air/water spray, this controlled heating stimulates the production of new collagen, leading to a tightening effect and wrinkle reduction. This noninvasive methodology is a basis of many different therapies such as laser vaginal tightening, stress urinary incontinence therapy, treatment of snoring [5, 7, 12].

The other point of controversy in the literature is the effects of non-fractional versus fractional Er:YAG lasers used for skin therapies. The non-fractional lasers are considered much more invasive in contrast with the fractional Er:YAG lasers which create microscopic "columns" of tissue ablation, leaving surrounding tissue almost intact for faster healing and potentially reduced side effects [13]. The non-fractional Er:YAG lasers involve a more continuous ablation pattern, which may require longer healing times and have a higher risk of side effects [3]. The main misinterpretation however is the primary goal of the therapy. The skin resurfacing and rejuvenation therapies cover a significantly larger treatment area than the wrinkle "elimination", which is focused on a tiny linear defect(s) requiring a precise and controlled approach. In this context, the non-fractional Er:YAG lasers are the only reasonable choice, but they must be used carefully, operating in subablative mode and with proper cooling.

Conclusion

This ex vivo histological study demonstrates that the conventional non-fractional Er:YAG laser is effective and safe, and can be adjusted successfully for wrinkle therapy. However, additional clinical research with multiple patients is required to verify this modality as a routine. Additionally, the histological findings are correlated with clinical improvements in skin texture, wrinkle depth, and overall appearance, but the duration of these improvements can vary, and multiple treatment sessions may be necessary for optimal and sustained results. In this context, the long-term clinical effects also must be studied.

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