

# Correlation of Histological Findings of Single Session Er:YAG Skin Fractional Resurfacing With Various Passes and Energies and the Possible Clinical Implications

Mario A. Trelles, MD, PhD,<sup>1</sup> Mariano Vélez, MD,<sup>1</sup> and Serge Mordon, Ph.D.<sup>2\*</sup>

<sup>1</sup>*Instituto Medico Vilafortuny, FUNDACION ANTONI DE GIMBERNAT, Cambrils, Spain*

<sup>2</sup>*INSERM & Lille University Hospital, Lille, France*

**Background and Objectives:** Ablative fractional resurfacing shows promise for skin resurfacing and tightening and also to improve treatment of epidermal and dermal pigmentary disorders. This study aimed at determining any correlation between epidermal ablation and effects on the dermis when using an Er:YAG laser in ablative fractional resurfacing mode.

**Materials and Methods:** Ten female subjects participated in the study, mean age 52 years, Skin phototypes: 1 Fitzpatrick type II; 8 type III and 1 type IV. The degree of wrinkles (Glogau scale II or III) was similar in all cases. The laser used was the *Pixel* Er:YAG system (Alma Laser™, Israel) which delivers the laser beam via a hand-piece equipped with a beam splitter to divide the 2,940 nm beam into various microbeams of 850 μm in diameter in an 11 mm × 11 mm treatment area. Using a constant energy of 1,400 mJ/cm<sup>2</sup>, on a test area of 4 cm × 2 cm. Two, 4, 6, and 8 passes on the preauricular area of the face were evaluated immediately after treatment. In all cases, the handpiece was kept in the same position, and rotated slightly around its perpendicular axis between passes, then moved on to the next spot. Biopsies were performed and tissue samples were routinely processed and stained with hematoxylin and eosin (H&E).

**Results:** No patient reported any noticeable discomfort, even at 8 passes. The histological findings revealed that, independent of the degree of the wrinkles, more laser passes produced more ablative removal of the epidermis. Residual thermal damage (RTD) with 2 laser passes was not observed but with 4 and 6 passes increased thermal effects and vacuole formation in the epidermal cells were noticed. With 8 laser passes, total epidermal removal was seen together with frank RTD-related changes in the upper part of the papillary dermis.

**Conclusion:** In this study, we have demonstrated that high density fractional Er:YAG laser energy in a single session with multiple passes targeted not only the skin surface with elimination of the epidermis, but could also achieve heat deposition in the upper dermis. When performing ablative fractional resurfacing with an Er:YAG laser, treatment of varying degrees of damage could be achieved by varying the number of passes. *Lasers Surg. Med.* 40:174–177, 2008. © 2008 Wiley-Liss, Inc.

**Key words:** laser surgery; fractional resurfacing; epidermis; residual thermal damage

## INTRODUCTION

Ablative Laser resurfacing with the CO<sub>2</sub> and/or the Er:YAG is an effective therapeutic method for treating the signs of skin ageing, and is still the gold standard in severe photoaged skin [1–3]. However, the excellent results obtained are often associated with an unacceptably long patient downtime, and ablative resurfacing is not complication-free [4]. Fractional resurfacing has been proposed as an alternative ablative method of treatment because it produces less aggressive photothermal side effects allowing for faster recovery of tissue and much shorter downtime. The Er:YAG laser at the wavelength of 2.94 μm is very strongly absorbed in water. Multiple passes could thus offer selective partial or total removal of the epidermis, while allowing the build-up of thermal damage in the upper dermis. This study was designed to determine the possible correlation between various laser settings with a fractional Er:YAG system, which could lead to epidermal ablation and deliver thermal effects into the dermis, thus offering the potential to induce collagen synthesis and remodeling to rejuvenate photoaged skin. Based on these observations and because of the precise vaporization characteristics of the Er:YAG wavelength, highly predictive elimination of the epidermis and delivery of RTD to the dermis could be monitored closely to ensure more precise efficacious clinical results in skin rejuvenation, while limiting side effects and decreasing downtime.

## MATERIALS AND METHODS

Ten female subjects, mean age of 52 years, were treated on the preauricular area of the face. Skin phototypes were 1 Fitzpatrick type II; 8 type III and 1 type IV. The degree of wrinkles according to the Glogau scale was similar in all

\*Correspondence to: Serge Mordon, Ph.D., INSERM U703-IFR 114, Pavillon Vancostenobel, Lille University Hospital, CH&U, 59037 Lille, France. E-mail: mordon@lille.inserm.fr

Accepted 6 December 2007

Published online in Wiley InterScience  
(www.interscience.wiley.com).

DOI 10.1002/lsm.20607

cases (degrees II and III). All patients, independently of their degree of wrinkles were treated with the laser in one single session, but with a varied number of passes.

Details of the study were explained to each patient and all signed a form of informed consent for biopsies and the use of the microscopic photographs. The study was approved by the Antoni de Gimbernat Foundation Ethics Committee.

The laser used was the *Pixel Er:YAG* system (Harmony<sup>®</sup> platform, Alma Laser<sup>™</sup>, Israel) which delivers the laser beam via a hand-piece equipped with a beam splitter which divides the 2,940 nm beam into microbeams of 850  $\mu\text{m}$  each in diameter. The window of the laser handpiece covers an 11 mm  $\times$  11 mm area of treatment with 49 microbeams. The system has three treatment pulse length programmes: Short (1 milliseconds pulse length), Medium (1.5 milliseconds pulse length) and long (2 milliseconds pulse length). The repetition rate was 2 Hz—2 shots per second, so approximately 500 milliseconds between passes.

The laser operates at a fixed power and selection of energy for fractional resurfacing depends on the pulse length. The selected pulse was programmed for the Long mode and an energy of 1,400 mJ/cm<sup>2</sup> (corresponding to the whole 11 mm  $\times$  11 mm area of treatment). Previous trials carried out at our Centre showed that this energy was most effective to achieve results in tissue [5].

Due to the relatively small area of 4 cm  $\times$  2 cm selected for testing, no anaesthesia was applied and the skin surface was previously cleaned with regular soap and gently rinsed. Then, laser energy was fired various times over each treatment spot to give the equivalent of 2, 4, 6, and 8 passes. At each treatment spot, the laser handpiece was

rotated slightly around its perpendicular axis between shots. Immediately after, 4 punch biopsies of 1 mm were taken from each patient (2 biopsies from each preauricular area). An extra biopsy was taken for analysis of tissue condition before laser test. Tests were done using a different number of laser passes at each point on every patient, following the same sequence, so that every patient served for analysis of effects of various laser passes (2, 4, 6, and 8 passes) but using the same laser settings.

Once the laser had fired all its designated shots, the hand-piece was moved to the next area to pulse the number of passes according to the protocol. When tests were finished, samples were embedded in 25% formalin for microscopic analysis. The tissue samples were routinely processed and stained with hematoxylin and eosin (H & E), and were sent for analysis, comment and photographic documentation of the epidermal and dermal architecture by an independent pathologist blinded to the number of passes delivered to each specimen.

## RESULTS

Figure 1 shows the histological progression associated with increased number of passes compared with the unirradiated baseline. Typically, the histological findings revealed that, independent of the degree of wrinkles, more laser passes led to more epidermal elimination. Residual thermal damage (RTD) with 2 laser passes was not observed (Fig. 1B) but with 4 and 6 passes an increase in heat effects and vacuole formation was observed in the epidermis (Fig. 1C,D), and some signs of mild RTD in the dermis, greater in 1d than in 1c, as a result of heat diffusion

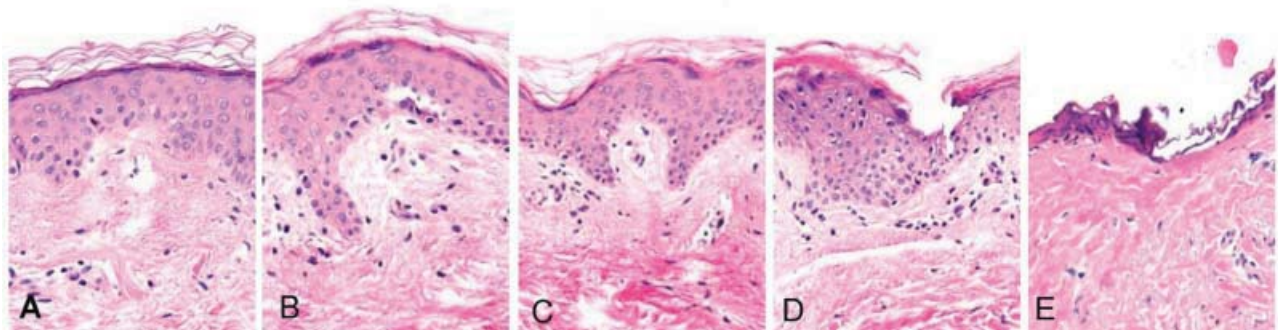


Fig. 1. **A:** Skin  $\times$  125, HE stain, baseline before treatment with long pulse programme, 1,400 mJ total energy density in an treated area of 11 mm  $\times$  11 mm (49 Er:YAG laser microbeams). **B:** Skin  $\times$  400, HE stain, immediately after 2 laser passes. The handpiece was slightly rotated between shots so that on the surface a doubled number (49  $\times$  2 = 98) microbeams has been stacked on the skin, but in slightly different positions. Notice some elimination of the stratum corneum and total absence of any residual thermal damage (RTD) as seen by completely normal dermal architecture. **C:** Skin  $\times$  125, HE stain, after 4 laser passes. The first few epidermis cell layers have been eliminated. A fine zone of mild RTD is present in the

dermis, secondary to the heat generated in the epidermis, together with signs of epidermal vacuolization. **D:** Skin  $\times$  125, HE stain, after 6 laser passes. Treatment with 6 passes shows more ablation and homogenous vacuolization of the epidermis with superficial thermal effects in the dermis, that is, RTD caused by conducted heat, comprising a layer of eosinophilic tissue giving way to normal-looking dermal architecture. **E:** Skin  $\times$  125, HE stain, after 8 laser passes. Total epidermis removal with evident RTD in the superficial dermis comprising a layer of frank collagen coagulation in the superficial dermis, followed by tissue with clear signs of eosinophilia.

from the epidermis. With 8 laser passes, total epidermal removal was seen and frank thermal changes in the upper part of the papillary dermis could be identified as an area of RTD seen from the very superficial layer of the dermis downwards as a change from basophilic (i.e., coagulated tissue) to eosinophilic changes in the morphological aspect of the collagen fibers. There was a loss of interfibrillary spaces due to thermally mediated microshrinkage of the dermal collagen and the loss of ground substance. These changes were present in all of the 10 patients.

## DISCUSSION

When using a prototype fractional handpiece (Sciton Profile Er:YAG system), Pozner et al. [6] have demonstrated on 60 patients that erythema was extremely mild and healing was much faster than traditional erbium resurfacing. In this study, we have demonstrated that high density Er:YAG laser microbeams achieved by various passes target not only the skin surface with elimination of epidermis, but also produced photothermally related effects in the dermis. Repeated laser passes accumulated heat in the epidermis and the thermal gradient increased, conducting heat to the underlying dermis, concomitant with gradual ablation of the epidermis. The dermis was thus affected by heat diffusion although the epidermis was the first target for the Er:YAG laser, because of the water absorption associated with the Er:YAG wavelength, and the different tissue water content between the epidermis and dermis. Once the epidermal 'window' was created, the Er:YAG beam directly targeted the dermis, with its much higher water content, creating a very superficial zone of frank coagulation and adding to the overall residual thermal damage zone. This region of dermal RTD results in inflammation which in turn induces fibroblast-driven collagen neo-formation at the time that the dermal wound repair takes place. Absorption of the 2,940 nm wavelength of the Er:YAG laser by the skin takes place with subablative energies carried by every microbeam, and though the output power was constant, as was the pulse width (Long pulse setting), the delivery of multiple laser passes over the same treatment area at a high repetition rate, with slight rotation of the treatment head between passes, increased the density of pulses on the skin surface. This pulse repetition did not give the epidermis time to cool down, thus building up heat production which diffused into the dermis [7,8]. It is likely that the increased heat profile and RTD thus stimulated tissue inflammation and, consequently, set in train the wound healing process, resulting in new collagen formation and eventual remodeling.

Multiple long pulse passes of 2 milliseconds of Er:YAG laser energy exceed the skin thermal relaxation time and, therefore, the combination of heat effect and selectivity was lost. The direct thermal effect occurred in the dermis in a preferential manner because of the water content; therefore effects were seen not only in the epidermis but also in the dermis, where there was a 'doubled' effect caused by direct absorption of the Er:YAG energy through the newly created epidermal window, and because of heat propaga-

tion inwards from the beam repeatedly targeting the epidermis.

Histologically, a microshrinkage of collagen fibers was noticed in the superficial dermis as a possible consequence of its higher water content index, when compared to the somewhat less-hydrated epidermis where the appearance of vacuoles was noticed. The combination of effects obtained in both skin layers ensured the instigation a photothermal damage-mediated wound repair process.

The histological findings (Fig. 1) strongly suggested a relationship between increased epidermal elimination, diffused heat to the dermis and clinical RTD effects which would possibly produce a stronger tissue reaction, collagen formation and a more positive response in more photoaged skin as for example, the amelioration and even removal of wrinkles.

A well-selected programme based on the pulse length mode of the laser used in the present study and increased energy deposited through increasing the number of laser passes can be implemented, so that this ablatively based treatment could probably cope with more severe skin ageing characteristics than the nonablative approach. Heating of the upper dermis, together with the eliminated epidermis, resulted in better clinical and aesthetic results obtained for the skin [9]. RTD is histologically represented by the reorganization of the new collagen fibers, with good linear alignment, the so-called Grenz layer running under the dermoepidermal junction and firmly attached to the basement membrane. This is the key factor for wrinkle elimination, as noticed in patients treated before [10,11].

## CONCLUSIONS

Er:YAG laser fractional resurfacing was achieved in 2 milliseconds pulses, at an energy of 1,400 mJ over 49 microbeams in an 11 mm×11 mm treatment area per shot. Progressive epidermal elimination was produced according to the number of laser shots or passes, slightly rotating the handpiece around its perpendicular axis between shots. Effects were also seen in the dermis due not only to direct Er:YAG absorption through the epidermal window, but also due initially to heat propagation inwards from the sequentially treated epidermis. Clinically speaking, the effects achieved can rejuvenate the skin, and more positive effects are obtained with more passes. The repeated pulses of subablative energy carried by the microbeams also exceed the coefficient of thermal relaxation time of the target epidermis, thus adding to the heat stacking effect. The Histological findings showed a correlation between epidermal elimination and an increased number of laser passes, thus increasing the degree of residual thermal damage.

## ACKNOWLEDGMENTS

The authors declare no financial or other interest in the Company or laser systems mentioned in this study. The report given in this study is registered in the academic activities of the FUNDACION ANTONI DE GIMBERNAT year 2007.

## REFERENCES

1. Fitzpatrick RE, Goldman MP, Sriprachya-Anunt S. Resurfacing of photodamaged skin on the neck using the Ultrapulse carbon dioxide laser. *Lasers Surg Med* 2001;28:145–149.
2. McDaniel DH, Ash K, Lord J, Newman J. The erbium:YAG laser: A review and preliminary report on resurfacing of the face, neck and hands. *Aesthet Plast Surg* 1997;17:157–164.
3. Trelles MA, García-Solana L, Calderhead RG. Skin resurfacing improved with a new dual wavelength Er:YAG/CO<sub>2</sub> laser system: A comparative study. *J Clin Laser Med Surg* 1999; 17:99–104.
4. Trelles MA, Allones I, Luna R. One-pass resurfacing with a combined-mode erbium:YAG/CO<sub>2</sub> laser system: A study in 102 patients. *Br J Derm* 2002;146:473–480.
5. Trelles MA, Levy JL, Pardo L, Knotoes V, Soria C. Complication du relissage laser CO<sub>2</sub>: une revue de 728 patients. *J Med Esth Chir Derm* 2000 XXVII:107;169–174.
6. Pozner JN, Glanz S, Goldberg DJ. Fractional erbium resurfacing: Histologic and early clinical experience. *Lasers Surgery Med* 2007;39, S19:73.
7. Trelles MA. Red light-emitting diode (LED) therapy assisted healing improves results of facial skin fractional resurfacing. 16TH European Academy of Dermatology and Venereology Congress, May 2007.
8. Trelles MA, Mordon S, Benítez V, Levy JL. Er:YAG laser resurfacing using combined ablation and coagulation modes. *Dermatol Surg* 2001;27:727–734.
9. Miller LD. The erbium laser gains a role in cosmetic surgery. *Biophoton Int* 1997(May/June);38–42.
10. Trelles MA, Soria C, Pardo-Mateu L, Chamorro JJ, Makaron T, Buezo O. renovación anatómica y estética de los labios, con ayuda del láser. *Cir Plas Iberolatinoam* 1998;24(1):63–71.
11. Trelles MA. Laser ablative resurfacing for photorejuvenation based on more than a decade's experience and 1200 patients: Personal observations. *J Cosmetic Dermatol* 2003; 2–13.