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In Vivo Histopathologic Comparison of the Acute Injury Following Treatment With Five Fractional Ablative Laser Devices

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Abstract

Background: Both erbium (Er:YAG) and carbon dioxide (CO_2) devices are commonly-used, efficient laser systems for aggressive skin resurfacing procedures. The devices each have different adjustable parameters (density, spot size, number of pulses, pattern, etc) and utilize variable energy capabilities to tailor individual treatments depending on the skin pathology and goals of treatment. Overall, the consensus has been that multiple-pass erbium treatments needed for efficacious wrinkle reduction had similar downtime and comorbidity to the traditional CO_2 treatments. Unfortunately, there were limited data comparing the histological differences and changes throughout the wound-healing process over time between the two treatment methods.

Objectives: The authors compare the difference in injury following treatment with five novel fractional ablative laser systems in vivo. Differences in damage pattern, treatment depth, and degree of surrounding cellular injury following treatment with each device at common clinical settings are evaluated in a side-by-side histopathologic comparison.

Methods: Prior to planned excisional surgery, the panni of 20 abdominoplasty patients were treated with five novel ablative fractional carbon dioxide or Er:YAG laser systems at various clinical parameters, in accordance with the manufacturers' treatment guidelines. After tissue removal two to four hours later, the skin was biopsied and processed for histopathologic evaluation. Specimens were stained with hematoxylin and eosin, along with a terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay, to highlight the degree of irreversible cellular injury.

Results: The acute damage profile differed between the fractional Er:YAG and CO_2 devices with respect to depth of penetration and extension of coagulation surrounding the microcolumns. The damage pattern was dependent on the parameters set with each device (eg, fluence, pulses, density, pulse width). The TUNEL-stained sections demonstrated more collateral cellular injury surrounding the ablated columns with the CO_2 devices than with the Er:YAG systems.

Conclusions: Following treatment with the fractional Er:YAG and CO_2 devices, deep tissue injury with various coagulative and ablative properties was observed, and it was confirmed that carbon dioxide and erbium devices result in different patterns of injury. As such, each may be better suited for different clinical situations. It is important for practitioners to understand the limitations of a specific device, as well as the tissue injury following a given treatment pattern or protocol, to appropriately tailor their treatment algorithm for a given patient. This extensive histopathologic evaluation of the acute characterization of injury across devices is helpful in clarifying the differences/similarities in laser-tissue interaction following treatment in an in vivo human model.

Keywords

laser, ablative, fractional, histology, injury

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Fractional ablative laser treatments have become significant and frequent procedures in many aesthetic surgery practices. These procedures target intracellular water to vaporize tissue in the treated region. Through the wound-healing process, the ablated microcolumns are replaced with a more youthful, robust collagen and epidermal surface, leading to a decrease in the appearance of facial rhytids and an improved skin texture and contour. The fractional tissue injury is theorized to hasten recovery and decrease the potential for complications observed following treatment with older resurfacing procedures.

Both the erbium (Er:YAG) and carbon dioxide (CO_2) devices are efficient laser systems for aggressive skin

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resurfacing procedures. The devices each have different adjustable parameters—such as density, spot size, number of pulses, and pattern—and utilize variable energy capabilities in order to tailor individual treatments depending on the patient's skin pathology and goals of treatment. Traditional CO_2 ablative laser systems achieved excellent skin tightening and rejuvenation at the cost of significant healing and downtime, with the potential for hyper/hypopigmentation, prolonged erythema, and scarring. Most of the morbidity associated with these procedures has been attributed to their thermal injury and heating components.[1-5] Er:YAG resurfacing devices were introduced with the hope of providing a laser treatment that could achieve consistent and similar skin tightening without the significant downtime and potential complications seen with CO_2 lasers. The Er:YAG 2940 wavelength also targets intracellular water, but has 12 to 16 times the absorption of water compared to the carbon dioxide laser, resulting in a purer ablation with minimal deposition of heat into the surrounding tissue.[6-8] Unfortunately, to achieve skin tightening and wrinkle reduction similar to CO_2 laser treatments, numerous passes and overlap are required with the Er:YAG systems.[9-14]

About 10 years ago, an excellent article by Khatri et al[10] compared the clinical differences between Er:YAG and CO_2 multiple-pass ablative resurfacing in a split-face study with six months of follow-up. The study concluded that, at equal fluencies, the Er:YAG was able to achieve a depth and clinical result similar to the carbon dioxide device only when a higher number of treatment passes was used. The goal of achieving a similar clinical result with less downtime and faster healing with the Er:YAG device was not attained. Overall, the consensus has been that the multiple-pass erbium treatments needed for efficacious wrinkle reduction had similar downtime and comorbidity as that which followed the traditional CO_2 treatments.[10-12,14] Unfortunately, there were limited data comparing the histological differences and changes throughout the wound-healing process over time between the two treatment methods.

Solid scientific validation of new technologies is often inadequate and, due to market forces and pressures, products are commonly rushed to the marketplace prematurely. This is the first side-by-side comparison of the histopathologic acute pattern of injury with five novel Er:YAG and CO_2 devices in human skin in vivo.

METHODS

Twenty abdominoplasty patients who presented to the senior author (JMK) were treated with Active FX or Deep FX (Lumenis Ltd., Yokneum, Israel), Pro-Fractional Erbium (Sciton, Palo Alto, California), Palomar Fractional 2940 (Palomar Medical Technologies, Burlington, Massachusetts), or Fraxel re:pair CO_2 (Reliant Technologies, Inc., Mountain View, California) lasers. All patients included in the study were Fitzpatrick skin phototypes I to IV. Skin types V to VI were excluded.

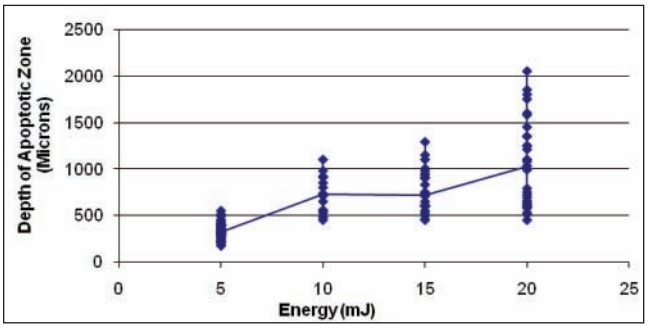

Figure 1. Scatter plot representation of energy versus depth following treatment with the Deep FX device.

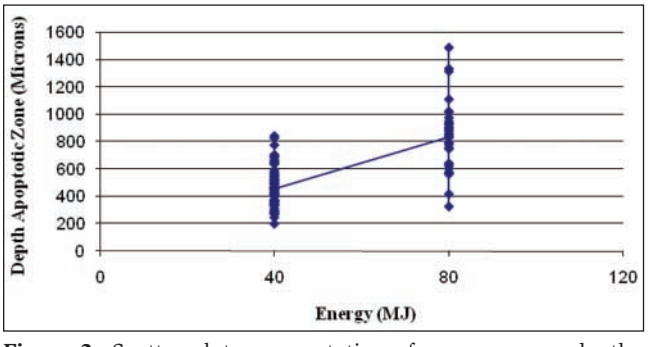

Figure 2. Scatter plot representation of energy versus depth following treatment with the Fraxel re:pair CO_2 device.

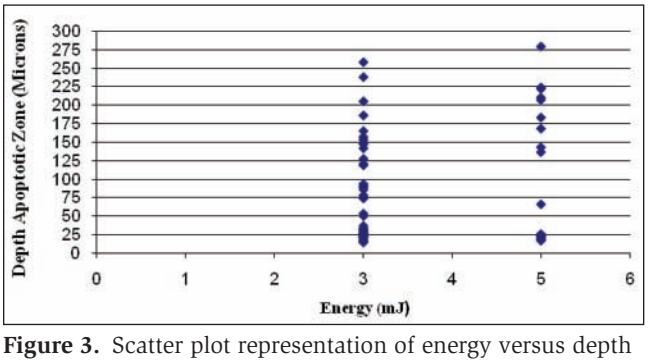

Figure 3. Scatter plot representation of energy versus depth following treatment with the Palomar Fractional 2940 device.

The abdomen of each patient was treated just prior to the start of the surgical procedure. All laser procedures were performed by the senior author (JMK). Each treatment parameter (energy, pulses, density, pulse repetition rate) evaluated was performed in triplicate on each subject, resulting in approximately 40 to 60 treatment areas per patient. The parameters evaluated for each device in the study are outlined in Figures 1-5. The number of treatment spots varied slightly from patient to patient, depending on the amount of tissue planned for excision. Parameters were selected based on the clinical experiences of the senior author (JMK). The study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center.

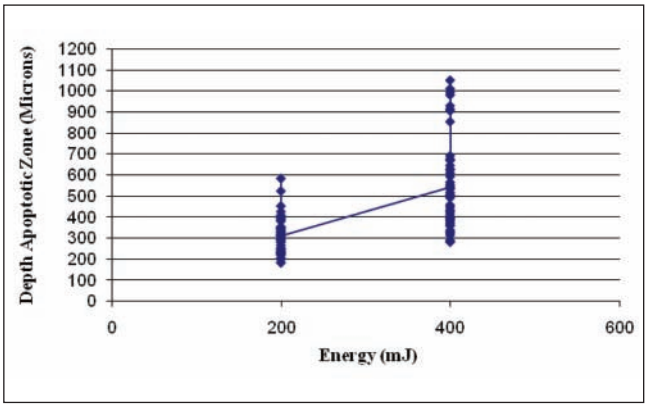

Figure 4. Scatter plot representation of energy versus depth following treatment with the Pro-Fractional Erbium device.

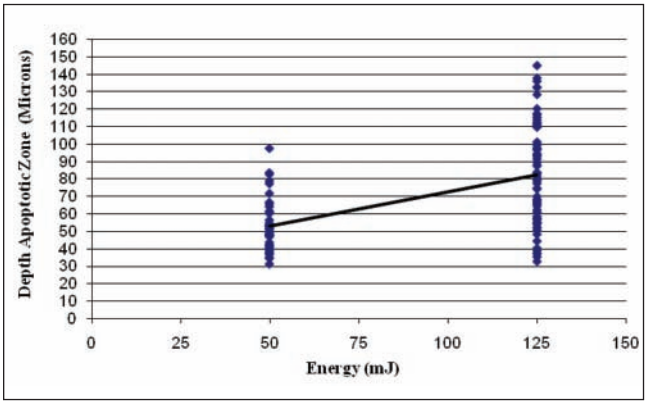

Figure 5. Scatter plot representation of energy versus depth following treatment with the Active FX device.

Appropriate informed consent regarding all potential risks, objectives, and technical details was obtained from each participant.

Punch biopsies (8 mm) were obtained from the treated sites following surgical excision of the pannus, approximately two to five hours following laser treatment. Sections were placed in 10% neutral buffered formalin and then deposited on a shaker for 24 hours. After being rinsed in 70% ethanol solution, the biopsies were processed, embedded in paraffin, cut in serial longitudinal sections (4-6 µm), and mounted on poly-L-lysine slides. Multiple serial sections (10-15) of each specimen were processed in order to obtain accurate representation of the damage profile in each treatment sample.

Histopathologic Evaluation

Hematoxylin and Eosin

Slides were stained with standard hematoxylin and eosin protocol; unstained contiguous sections were stained with the immunofluorescent terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) method to identify irreversibly damaged nuclei within the treatment areas.

Terminal Deoxynucleotidyl Transferase-Mediated Deoxyuridine Triphosphate Nick End Labeling

The assay was performed with the TUNEL kit from Promega Corporation (Madison, Wisconsin).[15] Slides were incubated at 56°C for 15 minutes and deparaffinized in xylene, hydrated in graded ethanol solutions, and equilibrated in normal saline for five minutes and then in phosphate-buffered saline (PBS) for an additional five minutes. The sections were fixed in 4% paraformaldehyde for 15 minutes and washed in PBS; they were then permeabilized with 20 µg/mL of proteinase K (Promega) for eight minutes at room temperature and prepared with 1:500 dilution of 10 mg/mL stock from the kit.

Sections were then washed in PBS and postfixed in 4% paraformaldehyde, washed in PBS again, and set in 100 µL of equilibration buffer. The slides were incubated flat in a humid chamber for five to 10 minutes. The terminal deoxynucleotidyl transferase (TdT) reaction mix (45 µL equilibration buffer, 5 µL nucleotide mix, and 1 µL TdT enzyme) was prepared during the equilibration step and protected from light. Then, 50 µL of the TdT reaction mix was applied to each slide. Plastic coverslips were applied before incubation in a humid chamber protected from light for one hour at 37°C. The slides were washed in 2 × SSC (Promega), rinsed and washed in PBS, and counterstained with propidium iodide (Invitrogen Molecular Probes, Eugene, Oregon). They were then washed in double distilled water and coverslipped with Vectashield (Vector Labs, Burlington, California).

Fluorescent Microscopy

The slides were evaluated with fluorescence excitation microscopy. TUNEL-positive nuclei demonstrated a bright green fluorescence using the ~470-nm (FITC) fluorescence filter. Mouse thymus was the positive control. Review and photography of all histologic preparations were carried out on a Leica DM2000 photomicroscope (Leica Microsystems, Inc., Bannockburn, Illinois) equipped with bright-field, epifluorescence, and incident angle dark-field illumination and measured with a standardized optic micrometer. All sections were reviewed by a board-certified pathologist.

Statistical Analysis

The depths of the microcolumns of injury in the skin specimens stained with the fluorescent TUNEL assay were measured with a standardized ocular reticle micrometer by three blinded observers and recorded accordingly. At least 25 to 30 individual microablation columns were analyzed and recorded at each laser parameter. The mean and standard deviation for each were recorded and plotted along a scatter plot graphical distribution using a standard software program (Microsoft Excel, 2003).

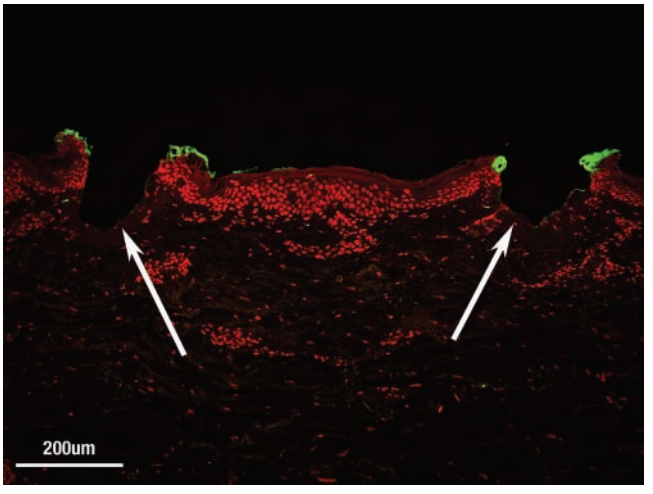

Figure 6. TUNEL-stained histologic skin section following treatment with the Palomar Er:YAG 2940 device at 5 mJ and 300 sec. Note the clear "punched out" ablation craters without any identifiable surrounding cellular injury.

RESULTS

Fractional laser injury was identified in all hematoxylin and eosin and TUNEL sections treated with the various ablative devices. The contiguous TUNEL-stained sections assisted in highlighting the amount of cellular injury surrounding the ablation columns.[16]

Following treatment with the Er:YAG devices, microcolumns of ablation approximately 150 to 200 µm wide were evident, penetrating in a conical fashion from the epidermal surface into the underlying papillary and reticular dermis. There was minimal tissue coagulation surrounding the ablated channels with either of the Er:YAG systems (Figure 6). The TUNEL stain confirmed the limited cellular injury surrounding the ablated microcolumns. The hematoxylin and eosin and TUNEL-stained sections showed evidence of the collapsing of the microablation columns and/or refilling of the areas with the collagen fibrils of the surrounding dermis. The TUNEL stain was instrumental in identifying the width and depth of cellular injury that lined the borders of the collapsed microcolumns.

The Palomar 2940 has the ability to add a coagulative component to the ablative system. When the coagulative mode was evaluated, an increase in tissue coagulation was observed surrounding the ablation microcolumns as compared to the sections treated with the pure ablative mode (Figure 7). Following treatment with the fractional carbon dioxide devices, a greater degree of coagulation surrounding the microcolumns of ablation was observed than in the skin sections treated with the purely ablative Er:YAG devices. Two of the devices (Active and Deep FX) are marketed to utilize a single-pass treatment, whereas the third system uses a multipass sweeping technology (Fraxel re:pair). The Active FX device resulted in a wide, superficial ablation injury that only penetrated to the papillary dermis, regardless of treatment fluence. The TUNEL-stained sections highlighted the amount of collateral cellular injury surrounding each of the ablation craters, providing a clearer picture of the total extent of injury to the treated region (Figure 8). Additionally, with the Active FX device, collateral thermal injury was more extensive in sections treated with slower repetition rates when compared to areas that were treated with faster repetition rates at similar energies.

Following treatment with the super-short-pulsed deep CO_2 laser systems (Deep FX and Fraxel re:pair), deep channels of ablation extending from the epidermal surface to the deep reticular dermis were observed (Figure 9). Following treatment with the Deep FX system, higher energies and multiple-pulse treatments demonstrated deeper and wider regions of TUNEL-positive cells surrounding the microablation columns, extending up to 4 mm from the epidermal surface. Increased treatment densities resulted in a proportional increase in TUNEL-positive cells between the microcolumns of ablation. At a density (MTZ/cm^2) greater than or equal to 4, TUNEL-positive cells were identified homogeneously across the entire tissue section.

The ablation injury following treatment with the super-short pulse Fraxel re:pair system was similar to the Deep FX system, with the ablation microcolumns penetrating from the epidermis into the underlying papillary and reticular dermis, with a wider plume of thermal cellular injury surrounding the ablation channels. The ablation columns were approximately 150 to 175 µm wide, tapering into the dermis at variable depths depending on the fluence selected. The Fraxel re:pair device works in a scanning, brushing fashion, requiring multiple passes that lay down the microablation columns over the treatment surface. Depending on the density selected, the number of microcolumns per unit of surface areas changes. Blending and overlapping of the microcolumns, with varying distance between the areas of injury with each subsequent treatment pass, created a random pattern of injury to the treatment region (Figure 10).

Figures 1-5 demonstrate the different depths of tissue penetration observed with each device in a side-by-side scatter plot graphic representation at various clinical parameters. Each depth was plotted as a single point on the graph. Approximately 50 different microcolumns were measured with the microscopic ocular reticle micrometer at the respective clinical laser parameter. The Deep FX and Fraxel re:pair devices demonstrated the deepest tissue penetration among the devices examined in the study. At 20 mJ and a single pulse, the Deep FX device demonstrated tissue injury up to 2 mm from the tissue surface.

DISCUSSION

The amount of fractional injury following treatment with the Er:YAG and CO_2 devices is markedly different. The CO_2 systems cause increased thermal injury and heating of the tissue that extends far beyond the borders of the ablative microchannels. In our study, the TUNEL stain was very helpful in identifying the extent of cellular injury and necrosis in the surrounding tissue that was not apparent

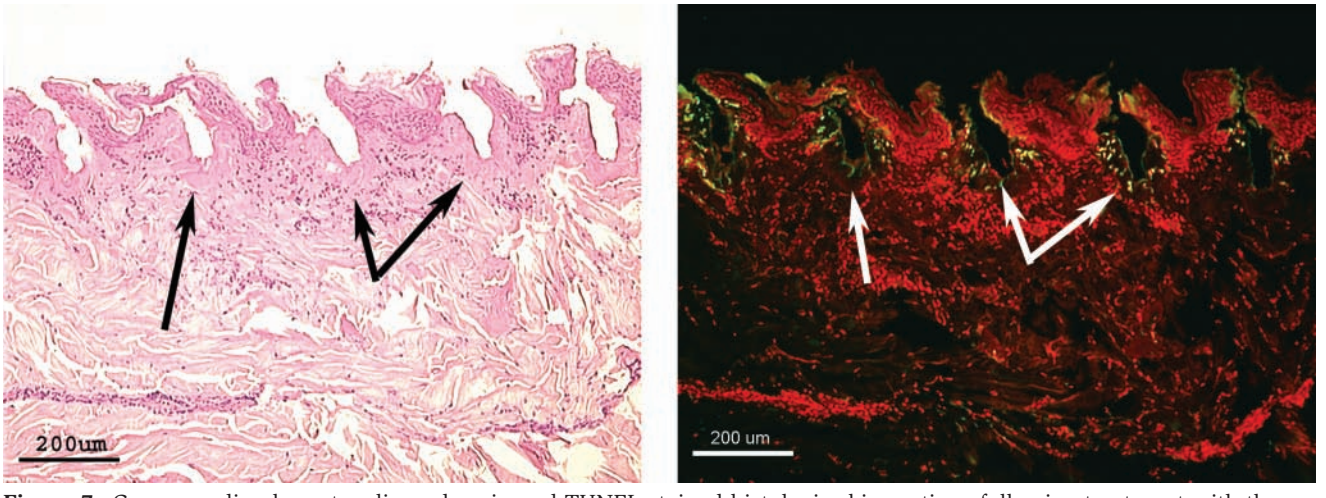

Figure 7. Corresponding hemotoxylin and eosin and TUNEL-stained histologic skin sections following treatment with the Palomar 2940 system at 5 mJ/300 sec with a "piggy back" coagulative pulse of 5 mJ/3 ms. When the coagulative mode was evaluated a small increase in tissue coagulation was observed surrounding and between the ablation microcolumns, as demonstrated by the positive TUNEL-stained nuclei in the section.

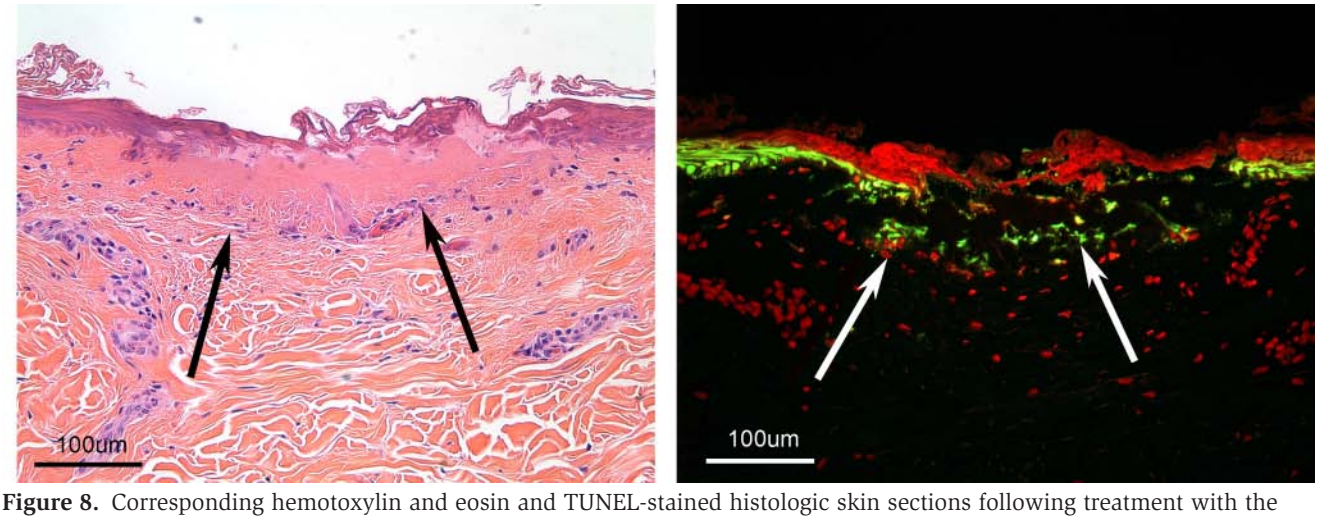

Figure 8. Corresponding hemotoxylin and eosin and TUNEL-stained histologic skin sections following treatment with the Active FX system. Note the bright yellow/orange cells of the epidermis in the TUNEL-stained sections highlighting the affected cells within the treated section. The TUNEL technique allowed for the identification of cellular injury that was imperceptible with standard H&E.

with standard hematoxylin and eosin; in general, it is a useful adjunct for objectively identifying the total damage pattern following laser treatment.[16] Future studies evaluating the degree of apoptosis versus necrosis and extent of sublethal thermal injury surrounding the microcolumns are currently being explored by our group. The damage pattern at the cellular level and the cellular response to laser injury will assist in further understanding the wound-healing process and regeneration of tissue within the area of treatment. The optimal ablation/coagulation ratio, depth of treatment, and density per unit surface area for skin remodeling and rejuvenation are just a few of the many undetermined parameters in the field of fractional ablative laser injury. A histologic damage profile from the inciting laser injury is essential to understanding the wound-healing and remodeling process of the treated region. Basic science and clinical research studies designed to improve our understanding of fractional wounding and wound healing are currently under way at institutions across the county.

The histologic evaluation of these devices provides useful information that can assist in planning clinical treatment algorithms. For example, our histologic damage pattern illustrated the superficial ablation following treatment with the Active FX device, which should be employed only for superficial resurfacing and pigment. Deeper skin pathologies such as ice-pick acne scars and deep rhytids would need to be approached with deeper penetrating devices, such as the Deep FX, Fraxel re:pair, or higher energy Er:YAG systems. The deep devices in this report all showed evidence of deep fractional tissue injury. However, the clinical benefit of increasing the depth of injury to the reticular

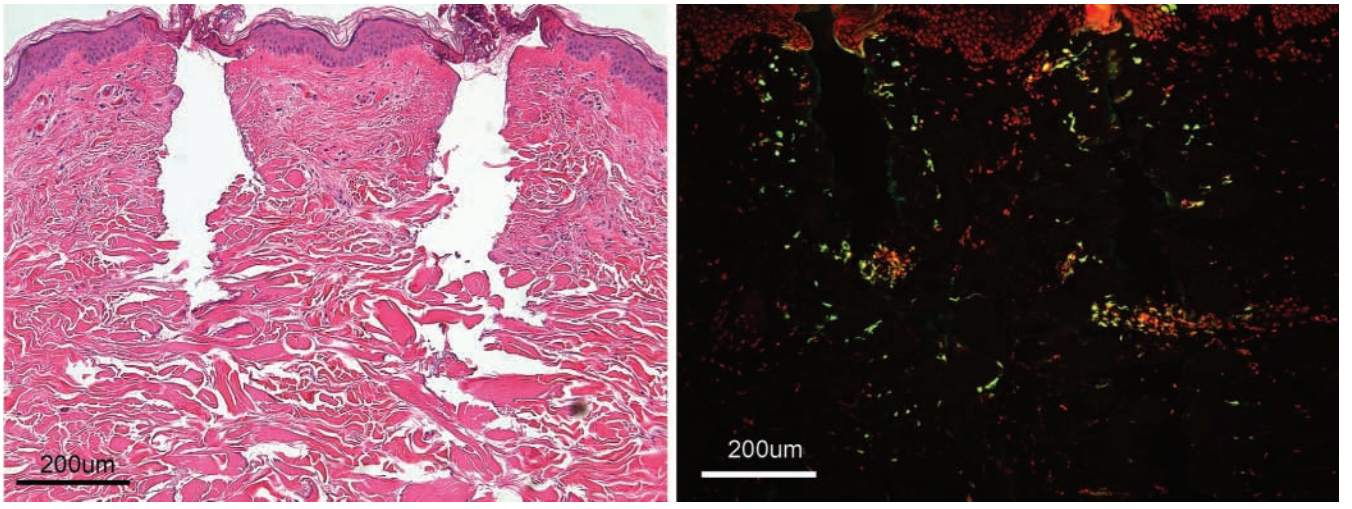

Figure 9. Corresponding hemotoxylin and eosin and TUNEL-stained histologic skin sections following treatment with the Fraxel RE:pair device at 80 mJ and 200 MTZ/cm^2. The carbon dioxide devices created much wider regions of ablation with an extending rim of coagulated or injured collagen highlighted by the TUNEL positive nuclei.

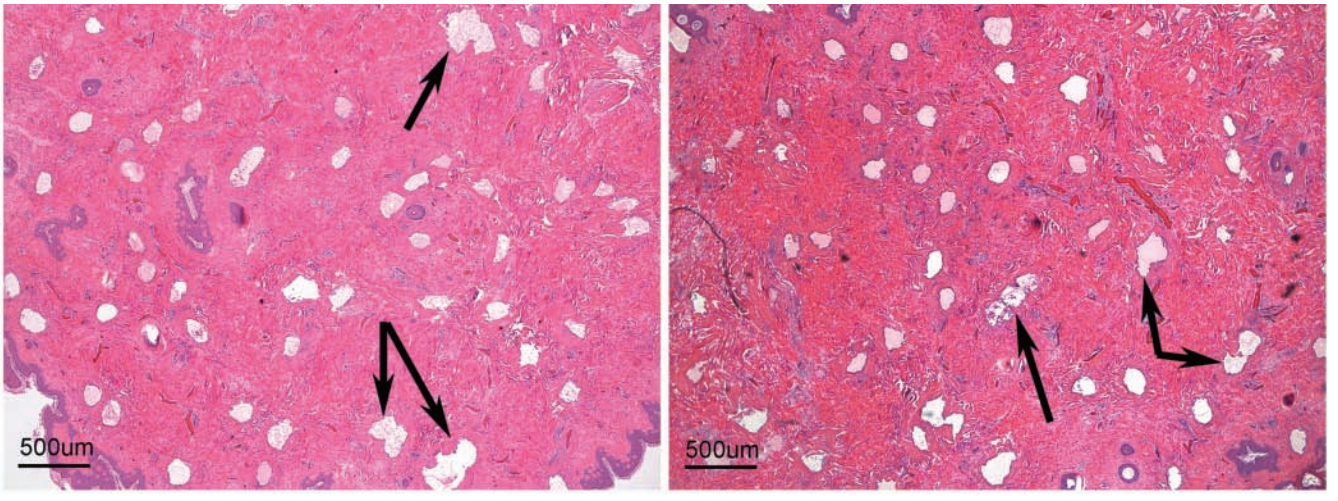

Figure 10. Hemotoxylin and eosin histopathologic skin sections demonstrating the nonuniform distribution of ablation injury with the Fraxel RE:pair device on account of the multiple passes or sweeps of the device with the treatment (arrows).

dermis in the skin is still unknown. Superficial dermal injury may be all that is needed to achieve the desired improvement in skin architecture. At this time, optimal energy parameters and depth of injury for a desired clinical outcome remain to be established. This study provides histologic evidence of the depth of skin injury achieved with leading ablative devices to assist in providing tangible evidence regarding tissue injury in human skin in vivo.

Although fractional erbium and carbon dioxide resurfacing procedures are becoming more popular with laser specialists, fractional ablative technology and laser tissue interaction are still in the preliminary stages of development and understanding.[8,17-23] The true advantages and optimal treatment parameters for these devices are currently unknown. Some of the newer Er:YAG devices, such as the Palomar 2940 evaluated in this report, have a "coagulative mode" that uses subablative energies (less than 0.7 J/cm^2) in order to add a heating or thermal component to the purely ablative Er:YAG lasers in an effort to emulate the heating benefit observed with CO_2 systems.[19] The thermal, longer millisecond pulse "piggybacks" on the shorter, microsecond ablative pulse in order to add the extra heating component to the purely ablative pulse. The subablative or thermal component allows for a similar injury pattern to the one seen with the fractional carbon dioxide devices. It is postulated that the thermal addition to laser injury assists in tightening of the skin, but the actual etiology of skin tightening through neocollagenesis versus microburn contracture is still unclear. It is likely that both play a part in the skin tightening and potential benefit of thermal injury with laser treatments.

It is important for practitioners to understand the limitations of a specific device and understand the tissue injury resulting from a given treatment pattern or protocol in order to appropriately tailor their treatment algorithm for a given patient. For example, solar elastosis, or

sun-damaged skin, has a different skin pathology and may benefit from a different resurfacing approach than the deep, fine rhytids associated with chronic smoking. Understanding skin damage profiles following treatment of different resurfacing devices allows the physician to select a specific device to maximally treat a particular area of skin to maximize the treatment effect of a given device. It is also important to mention the benefit and necessity of posttreatment skin care, sun protection, and antiviral prophylaxis, which should not be ignored and can considerably reduce posttreatment comorbidity and infection.[5,8]

Our study is not without limitations. We emphasize that the acute histopathologic changes illustrated in this report provide information only on the immediate tissue response following treatment with these devices, and the results do not constitute a clinical report. Clinically, resurfacing procedures are performed on facial or neck skin, not on abdominal skin. Facial skin is densely populated with hair follicles, sebaceous glands, and blood vessels. Therefore, facial skin may react differently to laser treatment than abdominal skin. Also, it is important to note the thickness of the abdominal skin in this study (more than 5-6 mm). Facial skin is significantly thinner, and this should be considered when planning laser treatments at aggressive energy settings.

Besides the anatomic tissue differences, inherent obstacles must be taken into account when evaluating laser skin interactions histologically. Due to processing and microtome sectioning, precise tissue measurements following laser treatment may not be a true representation of the actual tissue injury in vivo. Paraffin-embedded tissue sections require dehydration of the tissue samples, which causes shrinkage. This is an important point to consider when evaluating microcolumn lesion depth and width.[16-18] Also, due to the conical shape of the laser injury, slight sectioning angles may dramatically affect identification of the true depth of a microcolumn. To overcome this problem, multiple serial sections were cut, numerous columns were measured, and treatments were performed in at least triplicate to provide an accurate representation of the tissue injury at a given treatment parameter. As discussed, along with hematoxylin and eosin staining, the TUNEL method was a helpful adjunct in evaluating the extent of injury, due to its ability to label the irreversibly damaged cells surrounding the area of injury.[15-18] However, keratinocytes of the epidermis and fibroblasts within or surrounding an obvious area of injury did not always fluoresce with the TUNEL-positive signal. Natural thermal tolerance from cell to cell, as well as complete denaturation and destruction of chromosomes, should be taken into account when viewing the TUNEL-stained sections.

CONCLUSIONS

Fractional ablative technologies have a very promising future in laser resurfacing and are an exciting option for patients seeking nonoperative facial rejuvenation. Both the Er:YAG and CO_2 systems are capable of deep tissue penetration and tissue injury, but practitioners must take care to fully understand the limitations and indications for each specific device, selecting treatment protocols appropriately, because each may be better suited for different clinical conditions. Surgeons should also keep in mind that long-term, objective clinical outcomes data regarding histopathologic skin architecture changes following treatment with the different fractional devices are still largely unknown. Fractional laser resurfacing procedures are still in the preliminary stages of development and, if not performed safely and appropriately, can result in catastrophic complications. Future studies looking into the clinical and histologic benefits from Er:YAG and CO_2 fractional resurfacing throughout the wound-healing and skin remodeling process are needed. This information will assist laser surgeons in developing and tailoring treatment algorithms for different skin pathologies in order to attain the best possible aesthetic outcomes for their patients.

Disclosures

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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