Original Contribution

Micro-island damage with a nonablative 1540-nm Er:Glass fractional laser device in human skin

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Summary

Background and objectives Fractional photothermolysis produces micro-islands of thermal injury to the skin while preserving areas among treated tissue sites in order to promote wound healing. Histological changes associated with single and multiple passes of the 1540-nm Er:Glass fractional laser were examined using *in vivo* human skin.

Methods and materials Panni of five abdominoplasty patients were treated intraoperatively with a Fractional Lux1540 erbium glass laser system at various laser parameters, with single and multiple passes. Biopsies were removed and examined using standard histological stains.

Results Deep coagulated columns of collagen separated by regions of unaffected tissue were observed at variable fluence parameters. A direct correlation between the depth of penetration of the coagulated microcolumns and increasing energies was observed. Micro-islands of coagulation were $\sim$250 μm in diameter and separated by $\sim$800 μm of unaffected tissue. With multiple passes, significantly more disruption of the dermal–epidermal junction (DEJ) occurred at higher fluences. In contrast to the controlled fractional columns observed with single-pass treatments, nonuniform coagulated columns were distributed randomly throughout the tissue when instituting multiple passes over the same treatment region.

Conclusion Micro-islands of thermal damage were observed at variable energy parameters. Pathological changes within the skin were clearly dependent on amount of energy and number of passes of the laser treatment. Significantly more superficial damage, accompanied by disruption of the DEJ was observed with multiple passes when compared with single pass at similar fluences. However, with multiple passes, depth of thermal injury did not increase with increasing energies but did disrupt the micro-island array observed with single-pass fractional treatments.

Keywords: esthetic surgery, nonablative lasers, photoaging of the skin

Introduction

Ablative and nonablative resurfacing procedures are two common treatment modalities used for skin resurfacing and remodeling. Unpredictable clinical outcomes and limitations pertaining to variable skin penetration, side effects, skin type, and inconsistency of results have plagued many current laser devices and associated

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procedures. Ablative resurfacing procedures have shown consistent results of skin rejuvenation at the cost of significant side effects. Nonablative resurfacing decreases the morbidity associated with more aggressive ablative procedures but has failed to reproduce the consistent skin enhancement observed with single-pass ablative resurfacing (CO2, Erbium).[1–5]

The theory of micro-island damage and fractional photothermolysis as described by Manstein *et al.*[6–9] has stimulated the development of new laser devices and technology. The incorporation of fractional devices into the ablative and non-ablative arena has inspired a new and exciting approach to laser surgery, leading to many unanswered questions about optimal collagen remodeling and wound repair. With fractionated laser treatments, columns of coagulated tissue or microthermal zones are separated by unaffected skin areas that, in theory, promote and accelerate tissue healing. Relatively little scientific validation regarding the acute histopathological skin changes and wound healing responses with regard to fractional laser treatments has been reported in the literature.[8–10]

The 1540-nm fractional erbium glass laser (Fr1540) is a non-ablative laser that is capable of deep tissue penetration.[11–15] The Fr1540 utilizes the fractional methodology and distributes micro-arrays of energy in distinct foci throughout the treated region. The near-infrared wavelength, which is preferentially absorbed by water, allows the option of treating superficial photo-induced wrinkles or focusing on deeper rhytids extending up to 1–2 mm from the skin surface. Minimal absorption by melanin at this wavelength allows deeper penetration of photons and thermal energy (400 μm to 2 mm). The Er:Glass 1540-nm Aramis (Quantel Medical, Clermont-Ferrand, France) non-ablative system has been extensively evaluated clinically; however, few studies regarding acute histopathological skin changes following its use have been reported.[11–16]

The effect and relationships among optical energies, wavelengths, and pulse durations in tissue can be studied *in vivo*. The purpose of this study was to perform a histopathological evaluation of the degree and depth of microcoagulation columns of collagen in human skin after *in vivo* single- and multiple-pass treatments with the Fr1540 laser device at various fluences and pulse widths.

Materials and methods

Five abdominoplasty patients of the senior author (JMK) were treated with the Lux1540 non-ablative fractional laser system (Fr1540) (Palomar Medical Technologies, Inc., Burlington, MA, USA). The laser treatments were administered over the abdomen, immediately prior to the start of the operation. There were 50–70 treatment spots per patient depending on the amount of tissue planned for excision. The study was approved by the Institutional Review Board (IRB) of the University of Texas Southwestern Medical Center. Appropriate informed consent regarding all potential risks, objectives, and technical details were obtained for each participant.

The Fr1540 laser system contained an erbium glass laser with a microlens array that created a periodic arrangement of individual microbeams delivered to the skin through a simultaneously cooled sapphire glass window held at 17 °C. The handpiece contained a 10-mm-diameter window with a 1-mm pitch array (microbeam density = 100 microbeam/meter squared). The laser emitted a 1540-nm-wavelength pulse with a well-defined grid of microbeams separated by 1 mm from the center of each individual beam. Microbeam energies (18–100 mJ) at various pulse widths (10–30 ms) were examined following single- and multiple-pass treatments. Multiple passes consisted of: (1) treating repeatedly over the same spot (3–10 times) with rotation of the handpiece by approximately 45° prior to the delivery of each treatment or (2) re-treating over the previously treated spots, without rotation of the handpiece, in a sequential stamping fashion (2–3 times). There was a 2- to 3-s delay between firing of the device before each pass in the multi-pass group. The number of treatment spots per patient varied depending on the amount of tissue planned for excision. There was a minimum of three treatment spots for a given parameter per patient.

Punch biopsies (8 mm) were obtained directly after tissue excision, approximately 4 h post-laser treatment. Sections were placed in 10% neutral-buffered formalin, and placed on a shaker for 24 h to ensure adequate fixation. After rinsing in 70% ethanol solution, the biopsies were embedded in paraffin, cut in serial horizontal/longitudinal sections (4–6 μm), and mounted on poly-L-lysine slides and stained. All slides were reviewed with a board-certified pathologist.

Histological analysis

A standard staining protocol was used for hematoxylin and eosin (H&E) and Masson's trichrome.[17,18] Sections were viewed with bright field and polarized light to highlight collagen damage via loss of birefringence of the damaged areas. The depth of microcolumns observed at each fluence with the single- and multiple-pass treatments were recorded using a standardized micrometer. Multiple sections (n = 3+) were evaluated at each fluence.

Statistical analysis

Mean and SEM were calculated from three or more independent observations as calculated by standard software program (Excel 2003; Microsoft Inc., Redmond, Washington, USA).

Results

Following single-pass treatments, well-delineated, coagulated collagen columns extended vertically through the papillary and into the reticular dermis at all fluences (18–100 mJ). Column depths increased proportionally with incremental increases in fluence. Microbeam energies of 25–30 mJ produced superficial columns (~150–400 μm), whereas significantly deeper penetration into the tissue was observed with fluences of 90–100 mJ (~800–1000 μm). For every millijoule of increased energy, the depth of coagulation increased roughly by a factor of 10 μm (~10 mJ/100–150 μm) (Table 1; Figure 1).

Collagen within the columns exhibited a more intense hyalinized staining with both the H&E and Masson's trichrome compared with the unaffected surrounding epidermis, dermis and dermal appendages. Lower magnification illustrated spatial distribution and depth of individual columns in relation to unaffected dermis. Microcolumns of thermal damage were uniformly distributed approximately 800–1000 μm apart, with each lesion approximately 150–225 μm in diameter. Under polarized light, damaged columns presented as black empty spaces in contrast to the bright yellow birefringence of the surrounding unaffected collagen (Figure 2). Dermal adnexae and microvasculature within columns showed distortion of nuclei as a sign of thermal injury, but remained structurally intact. Distortion of nuclei within the endothelial lining was also observed without evidence of coagulation or acute thrombosis of the vessel.

Disruption of the dermal–epidermal junction (DEJ) was observed within each treatment column as basal cells of the epidermis exhibited marked streaming of nuclei, vacuolization and separation from the papillary dermis. However, the stratum corneum overlying affected areas maintained structural integrity. Dermal separation from epidermis was exaggerated with higher fluences as shown in Figure 3.

With multiple passes, higher energies caused increased DEJ disruption similar to single-pass treatments. However, multiple-pass treatments created an extensive separation of the epidermis from the dermis over a larger surface area. With multiple passes, the fractionated microcolumns overlapped and blended, creating a random pattern of coagulation throughout the dermis and produced significant deep epidermal destruction. Fluence did not directly correlate with tissue penetration as was observed with single-pass treatments. The more passes of the handpiece over a defined region dramatically increased the affected surface area, producing a nonuniform coagulated zone of injury focused around the DEJ of the treatment site. As with single-pass treatments, the integrity of the stratum corneum remained intact across all multiple pass treatments (Figure 4).

Table 1 Treatment settings.

No. of passes	Energy (mJ)	Pulse width (ms)
Single	18	15, 30
	28	15, 30
	30	15, 30
	34	15, 30
	50	15, 30
	55	15, 30
	58	15, 30
	66	15, 30
	70	15, 30
	82	15, 30
	90	15, 30
	100	15, 30
Multi-pass (sequential stamping)	50	15
	55	15
	58	15
Multi-pass (rotational stamping)	30	15, 30
	50	15, 30
	70	15, 30
	100	15, 30

Discussion

Our results demonstrate the histopathological changes in the skin produced by variable fluences with the Fr1540 laser system using single- and multiple-pass techniques. Coagulated columns were observed in the epidermis and dermis at all fluences of laser treatment (18–100 mJ). When performing single-pass treatments, as with the Fraxel device (Reliant Technologies, Inc., Palo Alto, CA, USA),[7,9] well-delineated columns of coagulation penetrated into the dermis with isolated disruption of the DEJ contiguous with each coagulation column.

When compared with single-pass treatments, multiple-pass treatments, which are recommended for all fractional laser devices, created an increased broad-based destruction of the DEJ over a larger surface area.

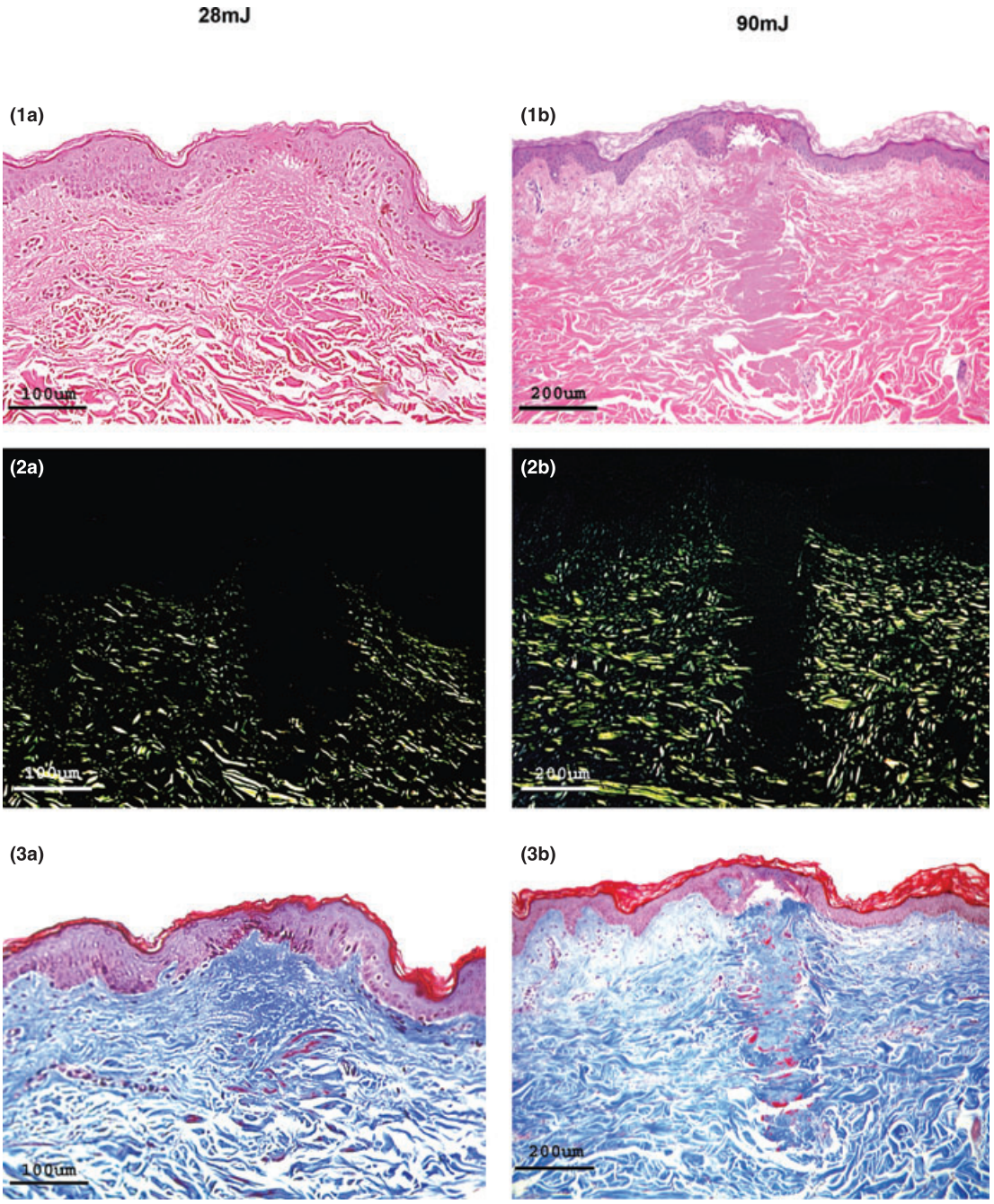

Figure 1 Histopathological sections stained with hematoxylin and eosin (H&E) (1a,b) and Masson's trichrome (3a,b) at low and high fluences after a single pass of the fractional 1540 handpiece. Illumination of the H&E sections with polarized light (2a,b) showed the loss of birefringence within the denatured column. The coagulated columns penetrated approximately 250–300 μm with 28 mJ and a pulse width of 15 ms (1a–3a) and 800–1000 μm with 90 mJ using the same pulse width (1b–3b).

The higher energy multiple-pass treatments demonstrated a more superficial treatment than anticipated for their respective fluences that may be attributed to the potential absorption and concentration of energy or heat at the DEJ. From the observations of the results, it was hypothesized that the DEJ may be a particularly vulnerable area within the skin on account of the higher concentration of edema in that area within the

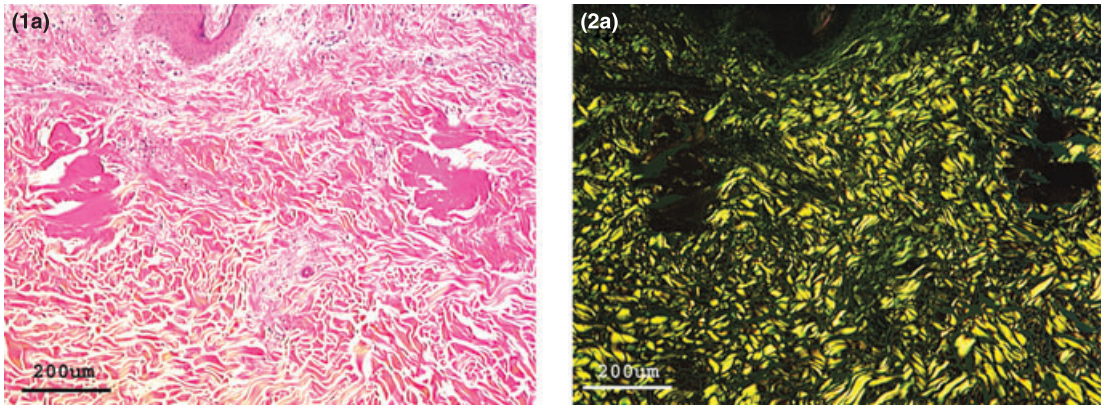

Figure 2 Horizontal histopathological sections stained with H&E (1a) and viewed with polarized light (2a) demonstrating the spatial distribution of microcolumns after single-pass fractional treatments. The microcolumns of thermal damage were uniformly distributed approximately 800–1000 μm apart, with each lesion approximately 150–225 μm in diameter.

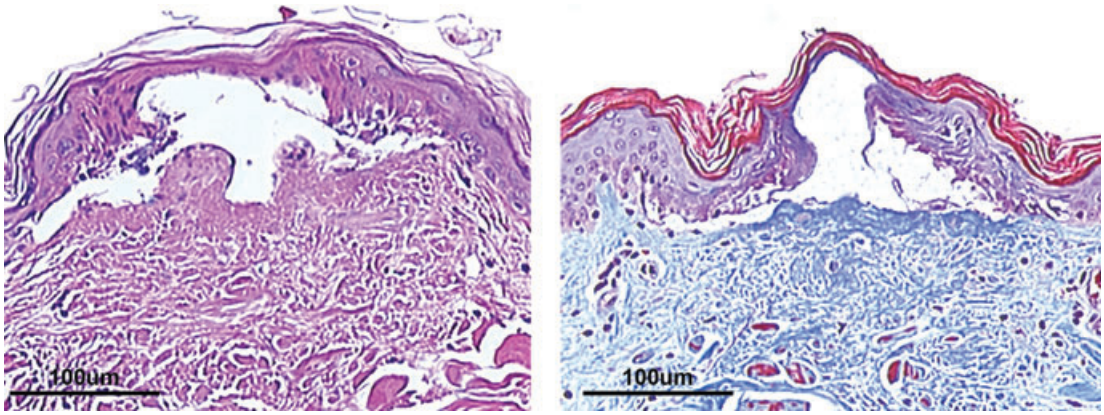

Figure 3 Histopathological sections stained with hematoxylin and eosin (H&E) and Masson's trichrome. Destruction of the deep epidermis and separation of the epidermis from the underlying papillary dermis within the coagulated microcolumn at 70 mJ/15 ms. Note that the stratum corneum is intact.

microcolumns of injury following treatment. The heat that distributed throughout the damaged DEJ with each pass of the laser may have increased the damage to the basement membrane of the epidermis and superficial papillary dermis. The biomechanical properties of the DEJ are a complex system depending on multiple cell–cell and cell–extracellular matrix interactions. The vulnerability of this area to the FR1540 system warrants further investigation. To the best of our knowledge, our results on acute histopathological skin changes to multi-pass fractional treatments have not been reported in the literature.

Higher microbeam energies created deeper columns and disruption of the epidermis and DEJ. Energy fluences of 90 mJ penetrated as deep as 800 μm to 1 mm into the dermis. The coagulated column depth increased proportionally with incremental changes in fluence (~10–15 μm/mJ), but showed some variability. Dermal appendages and microvasculature remained anatomically intact, but demonstrated evidence of thermal damage and streaming of nuclei without total destruction.

Each microcolumn was separated by approximately 800 μm to 1 mm as expected from the micro-array arrangement of the handpiece. As described by Manstein *et al.*, the pattern and density of the microthermal zones are paramount in tailoring the aggressiveness of a treatment.[6,9,10] By manipulating the density of the microcolumns, a more aggressive treatment may be achieved in a single treatment. However, when retreating or passing over a treated area multiple times, a

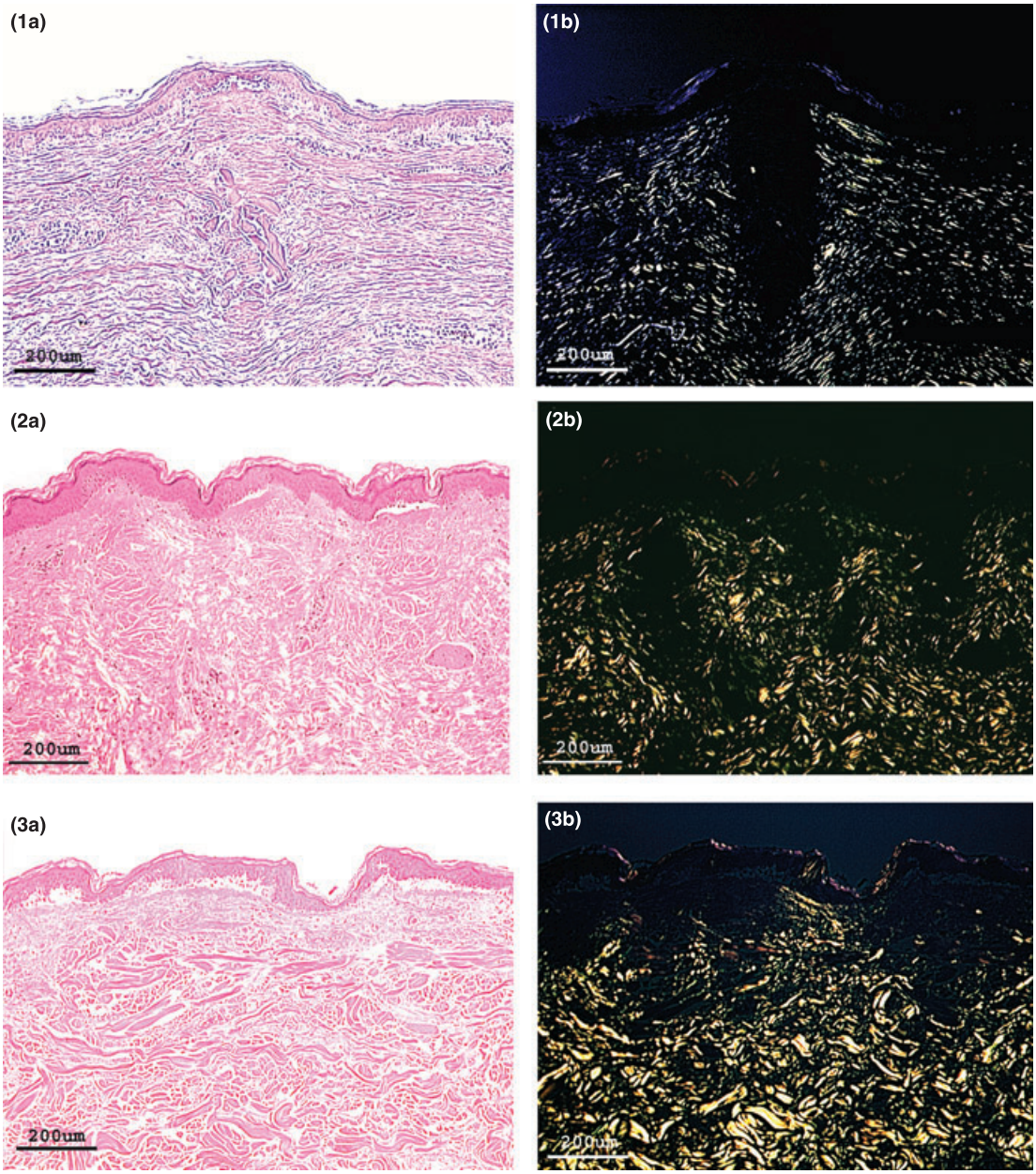

Figure 4 Longitudinal histopathological sections stained with hematoxylin and eosin (H&E) and viewed with bright field and polarized light. Sections demonstrate the increased surface area of treatment with a single pass (1a,b), three passes (2a,b), and eight passes (3a,b). All sections were treated with 50 mJ and a 15-ms pulse width. Note the increasing disruption of the epidermal basement membrane and dermal–epidermal junction with the increasing number of passes. The stratum corneum remained anatomically intact.

significant overlap of the pulse beams and blending of the columns was inevitable, causing bulk damage of the treatment site. This technique created a nonuniformity or random pattern to the laser column distribution throughout the treated region. Our results suggest that when retreating over the same area multiple times the uniformity of depth and distance between columns of coagulated tissue become inconsistent and unpredictable (Figure 5). The concept of an overlapped or random fractional treatment raises several questions. If 'fractional' effects are blunted and even eliminated after multiple passes, how critical is this concept for accelerated wound healing? Would it be more consistent and effective to focus the density of the microcolumns

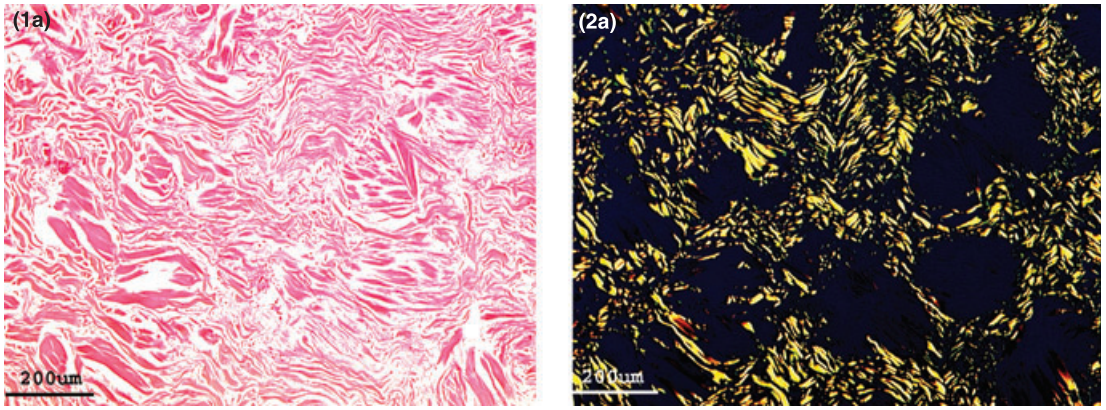

Figure 5 Horizontal histopathological section stained with hematoxylin and eosin and viewed with bright field (1a) and polarized light (2a) demonstrating the nonuniform distribution of microcolumns with multiple-pass treatments (eight passes). Note the overlapping and blending of the coagulated zones.

within each treatment spot? Increasing the density of the microbeams may allow for the target site to be treated more homogenously, avoiding the random nature seen with multiple passes. The balance between wound healing, neo-collagenesis, coagulation, and remodeling for optimal skin tightening and rejuvenation with fractional technology warrants further investigation.

It has been suggested that microcolumn separation may also be dependent on fluence and skin temperature.[12] It is important to note the role of contact cooling with fractional nonablative laser treatments and the relationship to microthermal damage and depth and width of the columns.[19,20] The Fr1540 device used a protective contact sapphire cooling plate held to 17 °C. A more aggressive protective cooling temperature may decrease damage to the DEJ providing more adequate epidermal protection. The histological characteristics and alteration in non-ablative fractional laser treatments with aggressive cooling is in process.

This study is not without limitations and is not a clinical report. A demonstration of the histopathological damage profile of abdominal skin with single and multiple passes of the Fr1540 device in an acute setting was presented. The clinical application of the Fr1540 has focused on facial rejuvenation and remodeling. Facial skin is populated with a significantly larger number of hair follicles, glandular tissue, and vasculature when compared with abdomen skin. The reaction of facial skin to the Fr1540 laser may differ significantly from the tissue reactions observed in the abdominal skin of abdominoplasty patients. Further research is required with regard to coagulation depth, penetration and microcolumn distribution for facial laser rejuvenation procedures. All specimens were harvested 4 hr post-laser treatment which provided insights into acute tissue response. Future studies evaluating fibroblast and keratinocyte necrosis and apoptosis, as well as collagen deposition and remodeling over various time intervals through the wound healing process are currently underway.

Conclusion

A thorough histopathological evaluation provides physicians with invaluable information regarding limitations and end points of specific laser devices. Many of the questions with fractional photothermolysis are yet to be addressed. Further study is needed to evaluate skin responses to fractional photothermolysis over time and its correlation with clinical practice. With this *in vivo* model, human skin reaction and remodeling over time with single- and multiple-pass treatments was investigated. Cellular markers and labeling involving wound repair, neo-collagenesis, and apoptosis/necrosis at various time intervals following ablative and nonablative treatments are currently underway. Detailed histopathological information assessing the acute damage profile to various laser devices will assist clinicians to tailor laser treatments appropriately to various skin pathologies, providing the safest and most efficacious skin resurfacing and remodeling treatment for their patients in the clinical setting.

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References

1 Hruza GJ, Dover JS. Laser skin resurfacing. *Arch Dermatol* 1996; **132**: 451–5.
2 Airan LE, Hruza G. Current lasers in skin resurfacing. *Facial Plast Surg Clin North Am* 2005; **13**: 127–39.
3 Dover JS, Hruza GJ. Laser skin resurfacing. *Semin Cutan Med Surg* 1996; **15**: 177–88.
4 Dover JS, Hruza GJ, Arndt KA. Lasers in skin resurfacing. *Semin Cutan Med Surg* 2000; **19**: 207–20.
5 Fitzpatrick RE, Goldman MP, Satur NM, Tope WD. Pulsed carbon dioxide laser resurfacing of photo-aged facial skin. *Arch Dermatol* 1996; **132**: 395–402.
6 Manstein D, Herron GS, Sink RK, Tanner H, Anderson RR. Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. *Lasers Surg Med* 2004; **34**: 426–38.
7 Hantash BM, Mahmood MB. Fractional photothermolysis: a novel aesthetic laser surgery modality. *Dermatol Surg* 2007; **33**: 525–34.
8 Hantash BM, Bedi VP, Chan KF, Zachary CB. *Ex vivo* histological characterization of a novel ablative fractional resurfacing device. *Lasers Surg Med* 2007; **39**: 87–95.
9 Hantash BM, Bedi VP, Kapadia B, Rahman Z, Jiang K, Tanner H, Chan KF, Zachary CB. *In vivo* histological evaluation of a novel ablative fractional resurfacing device. *Lasers Surg Med* 2007; **39**: 96–107.
10 Laubach HJ, Tannous Z, Anderson RR, Manstein D. Skin responses to fractional photothermolysis. *Lasers Surg Med* 2006; **38**: 142–9.
11 Rico PJ, Johnson TE, Mitchell MA, Saladino BH, Roach WP. Median effective dose determination and histologic characterization of porcine (Sus scrofa domestica) dermal lesions induced by 1540-nm laser radiation pulses. *Comp Med* 2000; **50**: 633–8.
12 Dahan S, Lagarde JM, Turlier V, Courrech L, Mordon S. Treatment of neck lines and forehead rhytids with a nonablative 1540-nm Er:glass laser: a controlled clinical study combined with the measurement of the thickness and the mechanical properties of the skin. *Dermatol Surg* 2004; **30**: 872–9, Discussion 879–880.
13 Fournier N, Dahan S, Barneon G, Diridollou S, Lagarde JM, Gall Y, Mordon S. Nonablative remodeling: clinical, histologic, ultrasound imaging, and profilometric evaluation of a 1540 nm Er:glass laser. *Dermatol Surg* 2001; **27**: 799–806.
14 Fournier N, Dahan S, Barneon G, Rouvrais C, Diridollou S, Lagarde JM, Mordon S. Nonablative remodeling: a 14-month clinical ultrasound imaging and profilometric evaluation of a 1540 nm Er:Glass laser. *Dermatol Surg* 2002; **28**: 926–31.
15 Lupton JR, Williams CM, Alster TS. Nonablative laser skin resurfacing using a 1540 nm erbium glass laser: a clinical and histologic analysis. *Dermatol Surg* 2002; **28**: 833–5.
16 Nouri K, Ballard CJ. Laser therapy for acne. *Clin Dermatol* 2006; **24**: 26–32.
17 Lillie RD, Pizzolato P, Donaldson PT. Hematoxylin substitutes: gallein as a biological stain. *Stain Technol* 1974; **49**: 339–46.
18 Towers B. A modification of Masson's trichrome stain which differentiates in colour between striated and smooth muscle. *J Physiol* 1953; **3**: 23P–4P.
19 Simanovskii DM, Mackanos MA, Irani AR, O'Connell-Rodwell CE, Contag CH, Schwettman HA, Palanker DV. Cellular tolerance to pulsed hyperthermia. *Phys Rev* 2006; **74**: 011915.
20 Laubach H, Chan HH, Rius F, Anderson RR, Manstein D. Effects of skin temperature on lesion size in fractional photothermolysis. *Lasers Surg Med* 2007; **39**: 14–8.

