

In Vivo Histological Evaluation of a Novel Ablative Fractional Resurfacing Device

Basil M. Hantash, MD, PhD,^{1,2} Vikramaditya P. Bedi, MS,² Bhumika Kapadia, BS,² Zakia Rahman, MD,^{1,2} Kerrie Jiang, NP,² Heather Tanner, MS,² Kin Foong Chan, PhD,^{2*} and Christopher B. Zachary, MBBS, FRCP³

¹Stanford University School of Medicine, Stanford, California 94305

²Reliant Technologies, Inc., Mountain View, California 94043

³University of California, Irvine, California 92697

Background and Objectives: A novel carbon dioxide (CO₂) laser device employing ablative fractional resurfacing was tested on human skin *in vivo* for the first time.

Study Design/Materials and Methods: An investigational 30 W, 10.6 μm CO₂ laser system was focused to a $1/e^2$ spot size of 120 μm to generate an array of microscopic treatment zones (MTZ) in human forearm skin. A range of pulse energies between 5 and 40 mJ was tested and lesion dimensions were assessed histologically using hematoxylin & eosin. Wound healing of the MTZ's was assessed immediately-, 2-day, 7-day, 1-month, and 3-month post treatment. The role of heat shock proteins was examined by immunohistochemistry.

Results: The investigational CO₂ laser system created a microscopic pattern of ablative and thermal injury in human skin. The epidermis and part of the dermis demonstrated columns of thermal coagulation that surrounded tapering ablative zones lined by a thin eschar layer. Changing the pulse energy from 5 to 30 mJ resulted in a greater than threefold increase in lesion depth and twofold increase in width. Expression of heat shock protein (hsp)72 was detected as early as 2 days post-treatment and diminished significantly by 3 months. In contrast, increased expression of hsp47 was first detected at 7 days and persisted at 3 months post-treatment.

Conclusion: The thermal effects of a novel investigational ablative CO₂ laser system utilizing fractional resurfacing were characterized in human forearm skin. We confirmed our previous *ex vivo* findings and show for the first time *in vivo*, that a controlled array of microscopic treatment zones of ablation and coagulation could be deposited in human skin by varying treatment pulse energy. Immunohistochemical studies of heat shock proteins revealed a persistent collagen remodeling response lasting at least 3 months. We successfully demonstrated the first *in-vivo* use of ablative fractional resurfacing (AFRTM) treatment on human skin. Lasers Surg. Med. 39:96–107, 2007.

© 2007 Wiley-Liss, Inc.

Key words: fractional photothermolysis; infrared; lasers; CO₂; Er:YAG; collagen; MTZ; MEND; wound healing; heat shock; epithelialization

INTRODUCTION

The gold standard for facial skin tightening remains ablative laser surgery. However, both the CO₂ and the “hot” erbium:yttrium-aluminum-garnet (Er:YAG) lasers can be associated with prolonged post operative healing, including delayed reepithelialization, persistent erythema, delayed and permanent hypopigmentation, and the potential for scarring particularly when treating areas off-the-face [1]. Because of these problems with traditional laser resurfacing, non-ablative lasers are now preeminent in utilization, but they have never reached, nor are ever likely to reach, the same end result as their predecessors, the CO₂ and Er:YAG lasers. Despite tremendous progress in the field of laser medicine, the exact mechanism by which ablative resurfacing achieves clinical wrinkle reduction remains poorly understood and continues to be the topic of intense investigation.

Although the optical and thermal properties of skin at the far infrared wavelengths have previously been characterized, the dynamics of laser–tissue interaction including the ablation and heat diffusion processes remain poorly understood. Furthermore, the biological wound healing response to laser irradiation in the far infrared spectrum has remained largely unexplored. Several reports have demonstrated significant delays in wound healing when compared to scalpel incisions [2–4]. This is consistent with the persistent erythema and delayed pigmentary alteration that has been observed clinically post-treatment [4,5]. Zweig et al. [6] speculated that the delayed healing was secondary to the large thermal damage zone induced by the ablative treatment, rendering the coagulated collagen “mummified” in the dermis for several months.

Recently, our group discovered fractional photothermolysis (FP), a novel mechanism of skin treatment that

*Correspondence to: Kin Foong Chan, PhD, Reliant Technologies, Inc., 464 Ellis Street, Mountain View, CA 94043, USA.
E-mail: kchan@reliant-tech.com

K.F.C. has disclosed a potential financial conflict of interest with this study.

Accepted 31 October 2006

Published online 26 February 2007 in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/lsm.20468

generates an array of micro-thermal zones of thermal injury in the epidermis and dermis [7]. Using a near-infrared fiber laser source, we were able to demonstrate thermal coagulation of collagen at depths approaching 700 μm at various tunable spatial frequencies [7,8]. The advantage of FP over traditional devices using selective photothermolysis (SP) is greater safety with preserved clinical efficacy. This has been confirmed by several independent studies to date [9,10]. Dermatologists have enjoyed success in treating Asian skin as well as conditions such as melasma, photoaging, fine wrinkles, poikiloderma, striae, and scars [7,10–22]. Thus, the advent of FP has revolutionized the treatment modality associated with non-ablative laser.

In an attempt to overcome the broad zones of thermal damage typical of current ablative treatment modalities, our group recently developed a novel ablative resurfacing device that utilized the concept of fractional resurfacing producing zones of ablation and coagulation [23]. In ex-vivo human skin, freshly isolated from the abdomen, a similar array of microscopic treatment zones (MTZs) was successfully created. In order to further characterize how the resultant MTZs affect human skin in-vivo, we treated the forearm of human subjects with an investigational ablative fractional resurfacing (AFRTM) system and histologically assessed the wound healing response at various intervals post-treatment.

MATERIALS AND METHODS

The study protocol was approved by an institutional review board and all subjects were consented prior to participation in the study. Twenty-four healthy subjects of Fitzpatrick skin types II–IV were treated on the forearm with an investigational 30 W, CO₂ laser system (Reliant Technologies, Inc., Mountain View, CA) in order to assess the wound healing response of human skin in-vivo, post AFRTM treatment. The CO₂ laser system has a beam quality with an M^2 value of less than 1.2. The laser beam was delivered through multiple deflective and refractive elements and focused to a diffraction-limited $1/e^2$ spot size

of approximately 120 μm in diameter at incidence to the skin in order to deposit a microscopic array of laser beams across the surface.

Topical anesthesia was administered locally prior to laser treatment. The forearm of each subject was first cleansed with alcohol, after which a 23% lidocaine 7% tetracaine ointment was topically applied on the intended treatment sites and occluded for approximately 30–45 minutes. The topical anesthesia was wiped off before the treatment was administered. No OptiGuide Blue was used as optical (velocity) tracking was disabled; the laser handpiece was operated in the manual mode allowing deposition of a constant density of MTZs with the subject's forearm held stationary [24]. Each laser treatment site covered approximately 1.5 cm by 1.0 cm, at pulse energy ranging from 5 to 40 mJ with a single pass. A spot density of 400 MTZ/cm² creating an interlesional distance of approximately 500 μm was used for pulse energies of 5–30 mJ, while 100 MTZ/cm² was used for 40 mJ. Pain was assessed immediately after treatment using a Visual Analog Scale (VAS), with scores ranging from 0 to 100. A total of 24 subjects received multiple treatments at varying pulse energies prior to biopsy excisions that were made immediately, 2 days, 7 days, 1 month, and 3 months post-treatment. The biopsy schedule is outlined in Table 1.

Immediately following excision, each biopsy sample was fixed in 10% v/v neutral buffered formalin (VWR International, West Chester, PA) overnight and then embedded in paraffin. The samples were sectioned into 5–10 μm thick slices, and stained with hematoxylin and eosin (H&E), hsp72 antibody, or hsp47 antibody. A minimum of 10 lesions from the histological sections of each biopsy sample were imaged and recorded using a Leica[®] DM LM/P microscope and a DFC320 digital camera (Leica Microsystem, Cambridge, UK). Lesion dimensions were determined using a proprietary Visual Basic computer program (Reliant Technologies, Inc.) [24] and were based on H&E stained sections. The lesion dimensions represent the maximum depth and width of the outermost border of the coagulation zones.

TABLE 1. Biopsy Schedule of an IRB Approved Study of an Investigative AFRTM Device

Q (mJ)	Biopsy post-treatment # of biopsies ($n = \#$ of lesions evaluated)				
	0 day	2 days	7 days	1 month	3 months
5	6 ($n = 60$)	6 ($n = 60$)	—	1 ($n = 10$)	—
10	7 ($n = 70$)	—	3 ($n = 30$)	—	—
20	2 ($n = 20$)	5 ($n = 50$)	3 ($n = 30$)	2 ($n = 20$)	2 (no visible lesions)
30	3 ($n = 30$)	—	—	2 ($n = 20$)	—
40	—	—	—	—	2 (no visible lesions)

A full series of immediately through 3-month post-treatment biopsies were only available at 20 mJ. Immediately post-treatment biopsies from 5 mJ through 30 mJ were available except for at 40 mJ.

RESULTS

We first set out to determine the effects of AFRTM treatment on human forearm skin at varying pulse energies immediately post-treatment (Fig. 1) and at various time intervals (Figs. 2–4) post-treatment. Images in Figure 2 depict the wound healing from 2-day to 3-month post treatment using H&E stain. Figure 3 illustrates the upregulation of hsp72 immediately through 3-month post-treatment (Fig. 3B–F), with Figure 3A being the untreated control sample. Accordingly, Figure 4 describes the upregulation of hsp47 immediately through 3-month post-treatment (Fig. 4B–F), with Figure 4A being the untreated control sample.

As shown in the H&E-stained images in Figure 1A–D, the AFR treatment led to immediate ablation of the epidermis and dermis. The tapering shape of ablative zones ranged from 71 to 121 μm in width and 210 to 659 μm in depth for the pulse energies of 5 to 30 mJ. The ablative

zone was lined by a very thin layer of eschar, and on occasion, contained a serum exudate and red blood cells, none of which was found to be extravasated in the dermis (Fig. 1). Our histology results suggested that adequate hemostasis was achieved when utilizing ablative fractional resurfacing, and was partially due to the surrounding thermal coagulation zone (33 to 75 μm). Lesions measuring 138 to 270 μm in width and 298 to 993 μm in depth with an interlesional distance of approximately 500 μm were detected for the pulse energies tested (Table 2). In general, the lesion depth increased more than threefold, while a less than twofold increase in width was detected for 5 to 30 mJ. The dimensions of the ablative zones followed the same trend; and the coagulation zones slightly more than doubled in thickness from 5 mJ to 30 mJ. We also stained for heat shock markers, hsp72 and hsp47, but detected no significant difference in expression post-treatment compared to untreated samples (Figs. 3B and 4B).

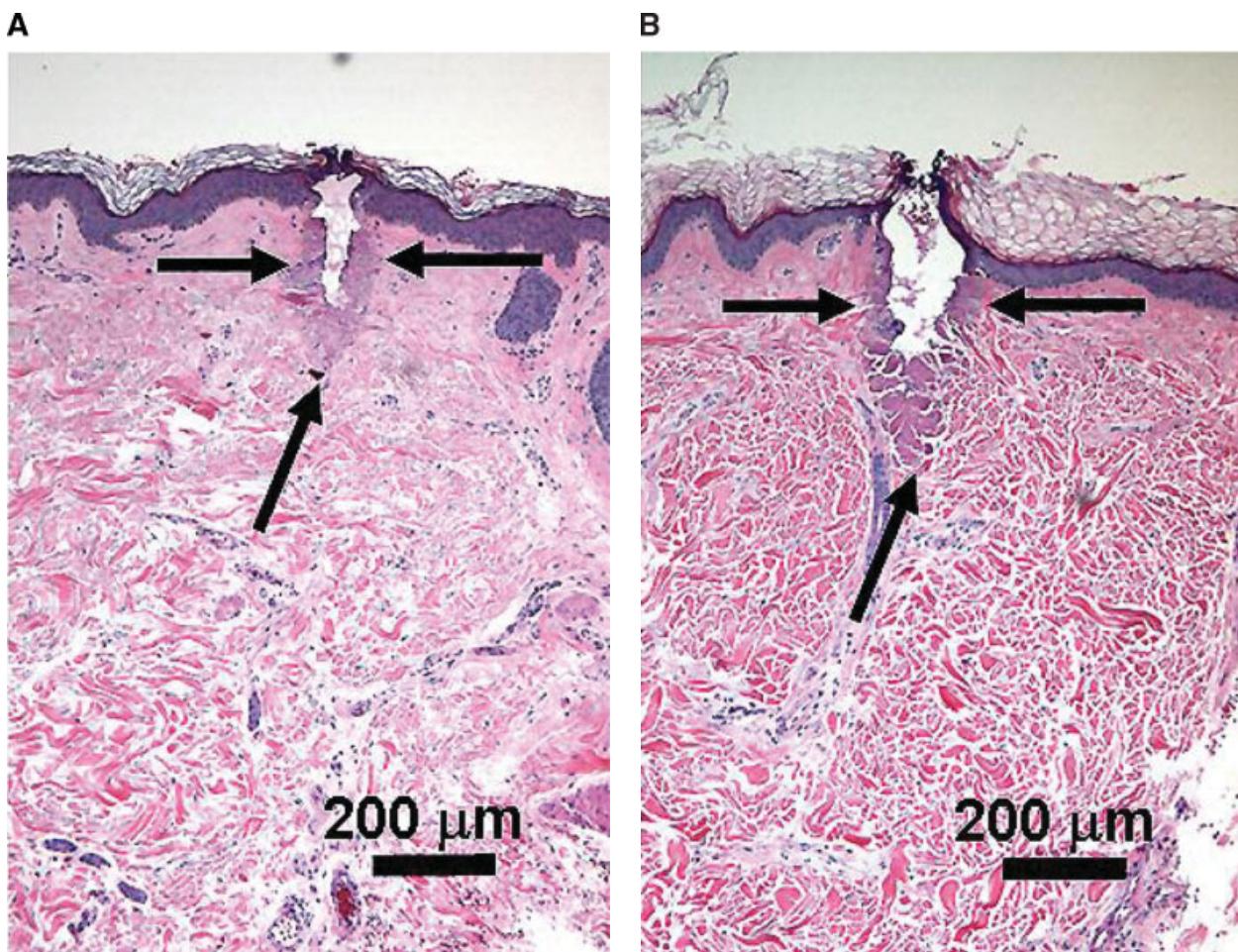


Fig. 1. AFRTM micro-lesions produced *in vivo* and biopsied immediately post-treatment at (A) 5 mJ, (B) 10 mJ, (C) 20 mJ, and (D) 30 mJ, show increase in the depth of ablative zones with increasing pulse energies. Each ablative zone was surrounded by a layer of coagulation zone that promotes hemostasis and tissue shrinkage. Histology images shown here were stained with H&E; optical magnification: 5 \times .

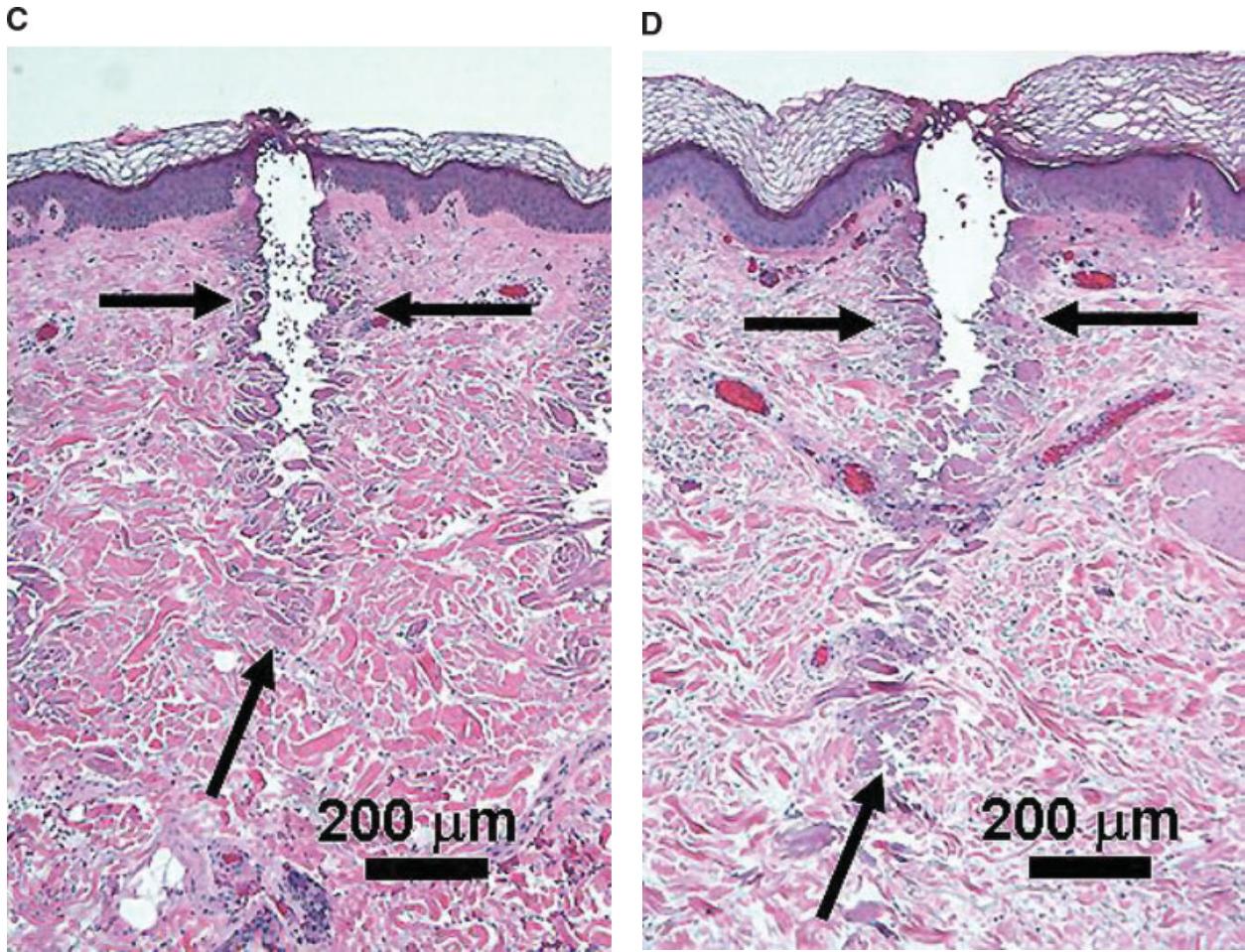


Fig. 1. (Continued)

By 48 hours post-treatment, the ablative zone was completely replaced by invaginating epidermal cells as demonstrated in Figure 2A. The MTZ surrounded the newly invaginated epidermal tissue, although the basement membrane remained partially disrupted as evidenced by basal layer vacuolar change. Microscopic exudative necrotic debris (MEND) was found at the level of the stratum corneum, typical of treatments using fractional resurfacing [23]. At the 48-hour time point, staining with anti-human hsp72 antibody revealed increased expression throughout the epidermis and around each coagulation zone but was conspicuously absent within the micro-lesion and necrotic debris (Fig. 3C). In contrast, no change in expression of hsp47 was found relative to baseline (Fig. 4A,C).

By 7 days post-treatment, MEND exfoliation was evident with residual material at the very superficial aspect of the stratum corneum (Fig. 2B). H&E staining of the coagulation zone appeared diminished, but close inspection of the dermis revealed an increase in the number of spindle cells; this most likely indicated the continued presence of fibroblast activity, consistent with ongoing dermal remodeling (Fig. 2B). Hsp72 expression remained

elevated throughout the epidermis and areas adjacent to the micro-lesion (Fig. 3D), while only slight increase in hsp47 expression in the basal epidermal layer and dermis surrounding micro-lesions was observed (Fig. 4D).

By 1 month post-treatment, the MEND was replaced by normal stratum corneum and was no longer detectable. The epidermal invagination had significantly regressed (Fig. 2C). In addition, the space vacated by the regressed epidermis (i.e., ablative zone) within the MTZ was replaced by newly synthesized collagen. H&E staining of the coagulation zone surrounding the original ablative zone was diminished but relatively well demarcated, indicating a slow but continuous dermal remodeling process. Both collagen within the original ablative zone and coagulation zone appeared haphazard. Not surprisingly, spindle cells remained abundant around and especially within the dermal zone of thermal coagulation at this stage (Fig. 2C). Interestingly, hsp72 remained positive throughout the epidermis and dermis and outlined individual MTZs (Fig. 3E). Hsp47 followed a strikingly similar pattern in the dermis although staining of the epidermis remained restricted to the basal layer (Fig. 4E).

At 3 months post-treatment, H&E staining showed no definitive evidence of micro-lesions, with only rare areas in the dermis resembling "old" lesions (Fig. 2D). Hsp72 expression had significantly declined but persisted at a level greater than baseline (Fig. 3F). Remarkably, hsp47 expression has increased and become more diffuse at 3 months consistent with continued collagen synthesis and remodeling (Fig. 4F).

Figure 5A depicts a horizontal cross-section of a typical AFRTM micro-lesion at a depth of 350 µm from the surface of the skin. A clear zone of annular collagen denaturation was observed to surround the micro-lesion. This was confirmed by a cross-polarized image of the same lesion shown in Figure 5B, with loss of birefringence within the collagen denaturation (coagulation) zone.

DISCUSSION

We recently developed a non-ablative laser device for treatment of human skin using a novel concept similar to

fractional photothermolysis [7]. This method allows for higher energy treatments in the target tissue while sparing the surrounding non-target tissue. Our non-ablative fractional resurfacing (NFRTM) technique does not spare the epidermis within the laser beam path, thus also fractionally coagulating the superficial layer to promote rapid re-epithelialization and cell-turnover, hence biologically resurfacing the skin [23]. By only irradiating a particular fraction of the total cross-sectional surface area, we and others discovered that efficacy could be preserved and/or enhanced while improving the side effect profile [9]. We further extended the use of fractional resurfacing to ablative laser wavelengths, specifically focusing on 10,600 nm.

In the latter study, we developed a prototype device using a CO₂ laser and characterized its effects on ex vivo skin when functioning in fractional mode [25]. Microscopic arrays of ablative and thermal coagulation zones were successfully generated using a range of laser parameters.

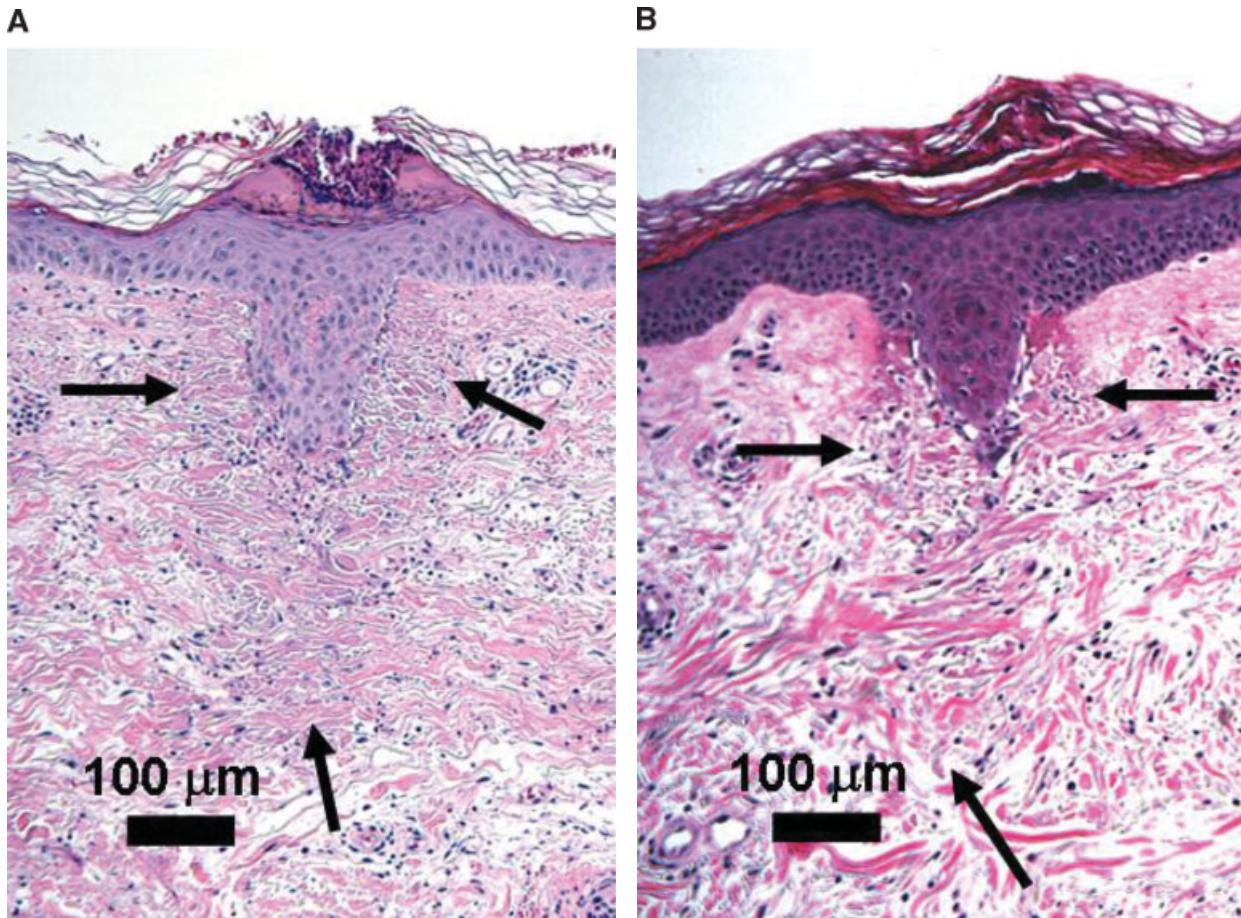


Fig. 2. AFRTM micro-lesions produced *in vivo* at 20 mJ and biopsied (A) 2-day, (B) 7-day, (C) 1-month, and (D) 3-month post-treatment. The histology images show the process of wound healing with invagination of the epidermis into the ablative zone and complete re-epithelialization within 24 hours (A), a sustained coagulation zone that can still be demarcated up to 1 month post-treatment indicating long-term remodeling

process (C), and a regressed epidermal invagination with replacement of new collagen within the original ablative zone (C and D). Micro-lesions shown here may not be sectioned across the right plane indicating the nominal lesion depths. Histology images shown here were stained with H&E; optical magnification: 10×.

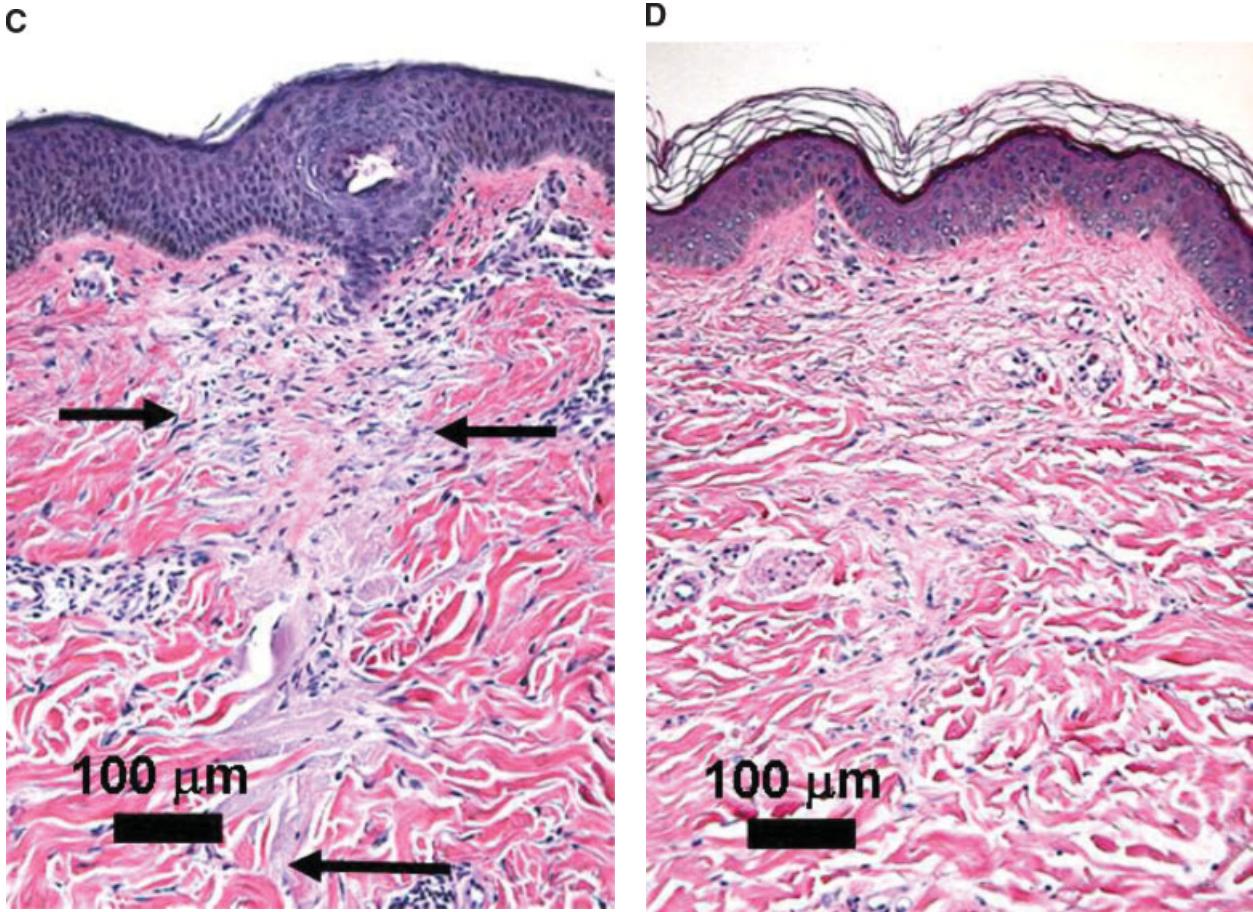


Fig. 2. (Continued)

Since the seminal study was performed on ex-vivo skin, we were not able to understand the wound healing response of the AFR™ treatment nor could we determine the side effect profile of this novel ablative treatment device. We therefore examined the effects of ablative fractional resurfacing on in-vivo human forearm skin for the first time by examining the wound healing response up to 3 months post-treatment.

Similar to the lesions observed in ex-vivo experiments, our data revealed micro-lesions involving a tapering ablative zone in the skin lined by a thin layer of eschar surrounded by a thermal coagulation zone, together constituting the MTZ (Fig. 1A–D). Lesion dimensions for similar laser parameters were not significantly different when comparing results of ex-vivo and in-vivo treatments [25]. For example, at a pulse energy of 10 ± 1 mJ, the lesion depth was 439 ± 70 μm versus 434 ± 64 μm for in-vivo and ex-vivo, respectively; similarly, the lesion width was 184 ± 15 versus 193 ± 11 for in-vivo and ex-vivo treatments, respectively [25]. Although lesion dimensions correlated well between in-vivo and ex-vivo treatments, no information about the wound healing response was garnered from our previous ex-vivo studies.

Histological data obtained in the current study was illuminating. Analysis with H&E staining revealed some

occasional serum exudate and red blood cells within the ablative zones, although the latter were not found extravasated in the dermis. Clinical responses, such as serous oozing and punctuate bleeding, varied widely among the subject participants within each treatment parameter set (i.e., equivalent pulse energy and spot density setting); however, such post-treatment responses resolved within 24 hours for the majority of study subjects. In fact, clinical purpura was never observed during follow-up and was not evident histologically at 2, 7, 30, or 90 days post-treatment (Figs. 1 and 2). We hypothesized that the width of the thermal coagulation zone appears to be within an optimal range necessary for achieving a hemostatic effect. Taken together, this data and other clinical reports [26] suggest that AFR™ treatment provides sufficient hemostasis and that oozing may resolve within 12–24 hours post-treatment. Discussions of clinical results and observations including pain, discomfort, and other effects are beyond the scope of this manuscript and will be presented in a separate article along with results from clinical studies on the face and neck [26].

Interestingly, ablative fractional resurfacing demonstrated much more rapid re-epithelialization when compared to its non-fractional predecessors, whether powered by erbium or CO₂ lasers. By 48 hours, most subjects

demonstrated complete re-epithelialization (Fig. 2A) with restoration of the basement membrane apparent no later than 7 days post-treatment (Fig. 2B). This advantage over non-fractional ablative devices partially explains the apparent reduction in patient downtime, such as persistent erythema and infection risk. Unlike non-fractional CO₂ laser treatments, which may result in post-treatment erythema lasting several months [27,28], erythema subsided within 4–7 days after AFR™ treatment for all study patients. Furthermore, no clinical infections were observed in any subject on follow-up examinations after treatments on the forearm. We theorize that the rapid healing re-epithelialization demonstrated histologically is critical to abrogating the risk for prolonged downtime and bacterial infections commonly associated with the current ablative laser devices [28,29].

Even though current ablative CO₂ lasers suffer from increase complication rates relative to non-ablative lasers, investigators have consistently reported unparalleled

clinical efficacy for treatment of deep rhytides and consider it the gold standard for skin tightening. Although the exact mechanism underlying this benefit remains poorly understood, a number of studies have implicated a prolonged and vigorous wound healing response as a key factor [4]. To evaluate the wound healing response of ablative fractional resurfacing, we turned our attention to the study of heat shock proteins. Previous work has established hsp72 as an early (hours) responder to thermal damage in skin [30]. On the other hand, hsp47 appears to play a more important role in long-term wound healing by acting as a procollagen chaperone [30,31] promoting neocollagenesis. As expected, hsp72 expression was upregulated significantly at 48 hours post-treatment and peaked between 2 and 7 days later (Fig. 3C–D). On the other hand, increased hsp47 expression was delayed, first apparent at 7 days post-treatment and remained elevated at least for 3 months (Fig. 4D–F). In support of this, we noted persistence of spindle cells in the dermis consistent with an ongoing fibroblastic response

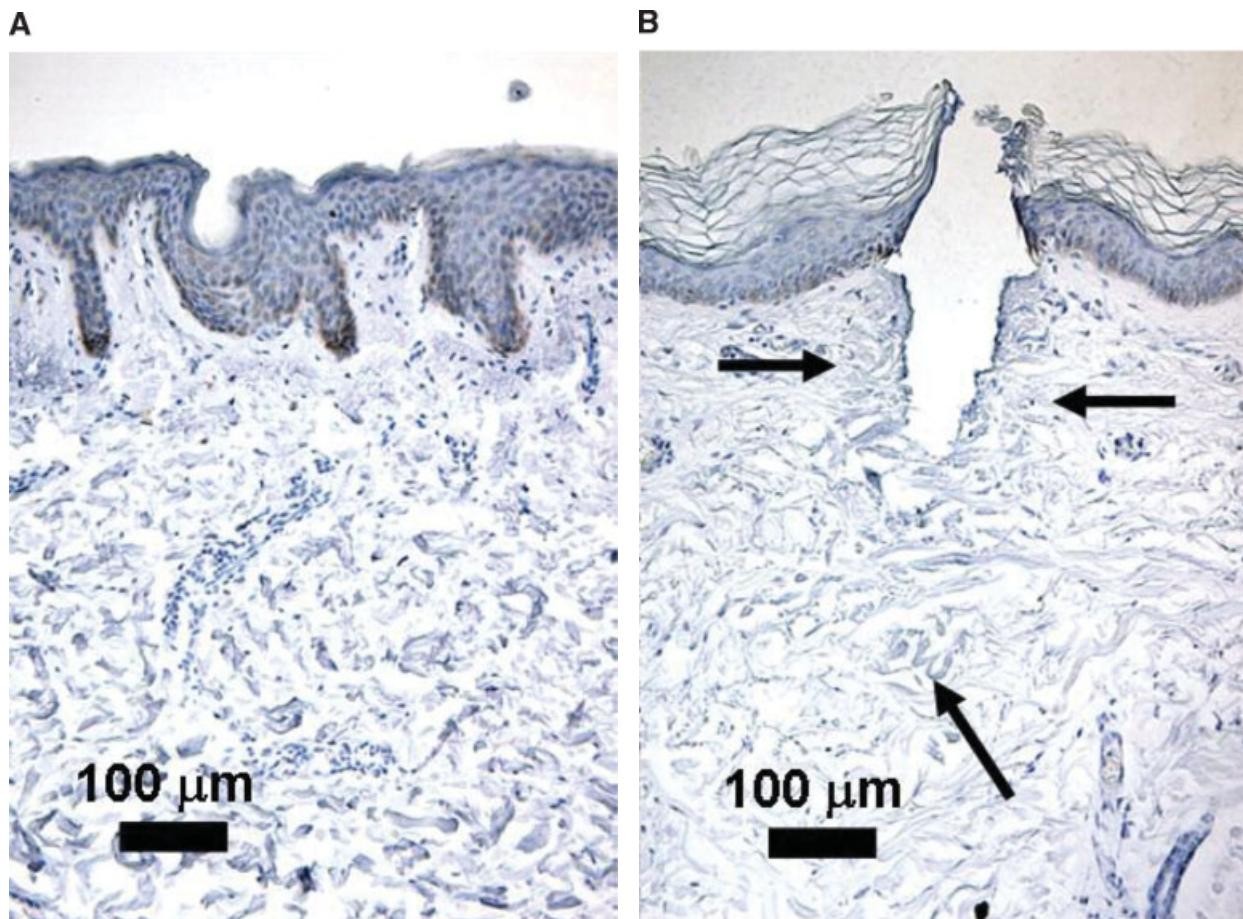


Fig. 3. Immunohistochemistry of human skin using anti-human hsp72 antibody, showing a baseline expression of hsp72 (brown stain) above the basement membrane in an untreated or control sample (A). In vivo human skin treated with the AFR™ device at 20 mJ were biopsied (B) immediately, (C) at 2-day, (D) at 7-day, (E) at 1-month, and (F) at 3-month post-

treatment illustrating changes in the micro-lesions during the wound healing process. Increase in hsp72 expression peaked at 2- to 7-day post-treatment in the dermis. Micro-lesions shown here may not be sectioned across the right plane indicating the nominal lesion depths; optical magnification: 10×.

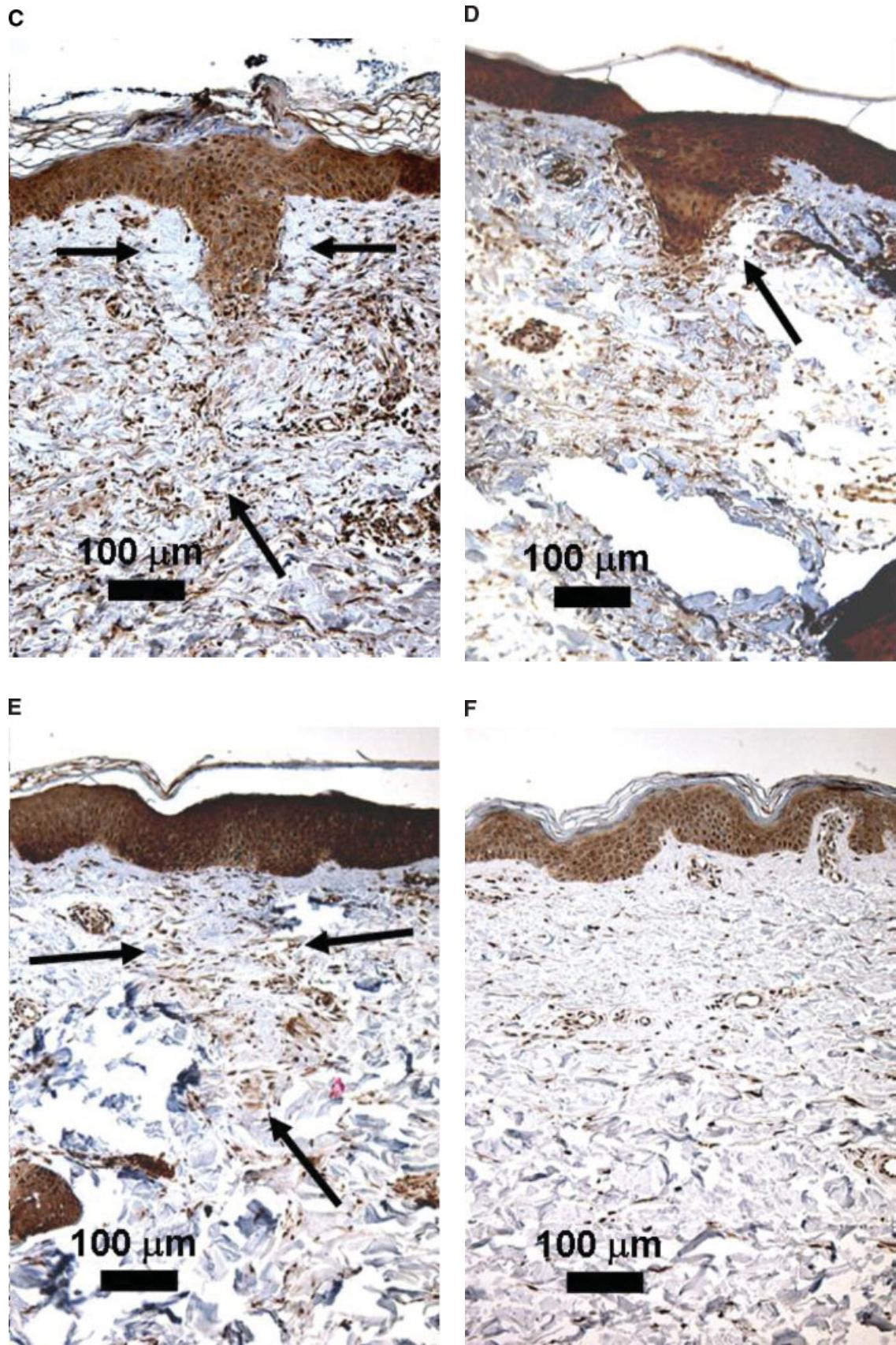


Fig. 3. (Continued)

(Fig. 2C–D). In particular, expression of hsp47 became diffuse in the dermis at 3 months post-treatment, indicating that activation of fibroblasts was occurring in both treated and untreated tissue (Fig. 4F). Although we did not assess for neocollagenesis immunohistochemically, we believe the results of our hsp47 experiments provide strong evidence that AFRTM treatment leads to long-term dermal remodeling with increased collagen synthesis. Furthermore, this study lends further support for the notion that treatment of only a fraction of the total skin can lead to dermal remodeling in a substantially larger proportion of skin beyond the immediate zones of denatured collagen. This critical phenomenon thus mitigates the need for higher risk bulk tissue treatments that often result in long-term side effects such as scarring and permanent hypopigmentation.

The AFRTM treatment also removed a relatively large volume of dermal tissue to depths that conventional CO₂

and Er:YAG laser resurfacing were previously incapable of reaching without causing untoward side effects. As our results indicated, dermal tissue as deep as $659 \pm 69 \mu\text{m}$ at 30 mJ can be removed through ablative fractional resurfacing (Table 2) with a general trend towards greater increases in depth than width with increasing pulse energy. This implies that the aspect ratio of the ablative zone (i.e. ablative depth-to-width ratio) increases with rising pulse energies, a phenomenon not observed with non-ablative fractional resurfacing (NFRTM) treatment using the 1550 nm fiber laser which maintained a relatively constant aspect ratio of 5 [24]. Thus an advantage of the AFRTM modality versus the NFRTM modality may be its potential to remove more unwanted and deep dermal material, such as solar elastosis, through activation of a transepidermal elimination pathway recently shown to contribute to dermal remodeling post-fractional resurfacing [23]. This mechanism was previously reported to

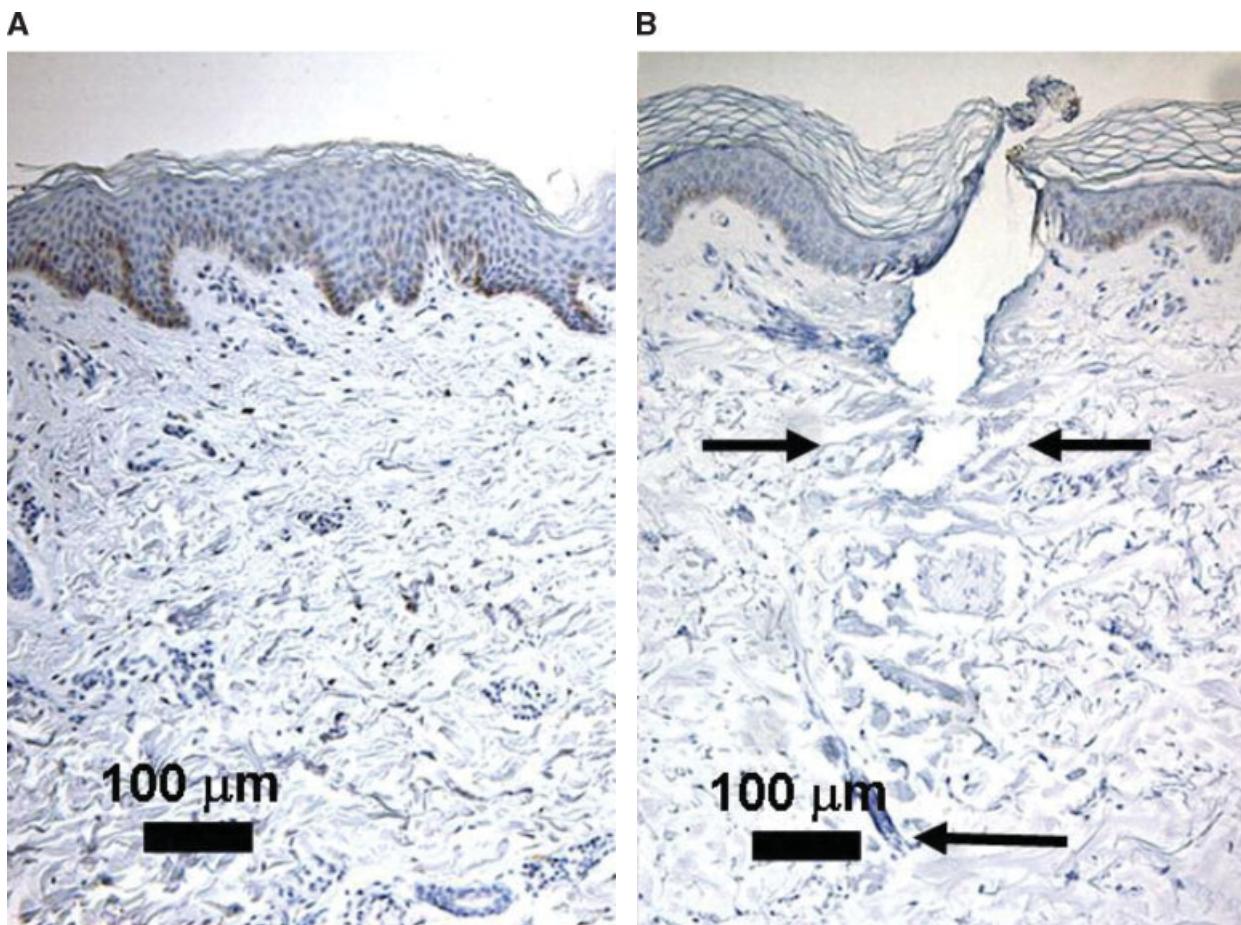


Fig. 4. Immunohistochemistry of human skin using anti-human hsp47 antibody, showing a baseline expression of hsp47 (brown stain) above the basement membrane in an untreated or control sample (A). In vivo human skin treated with the AFRTM device at 20 mJ were biopsied (B) immediately, (C) at 2-day, (D) at 7-day, (E) at 1-month, and (F) at 3-month post-treatment illustrating changes in the micro-lesions during the

wound healing process. Upregulation of hsp47 expression was noticeable at 7-day post-treatment in the dermis, and continued to increase and persisted at least up to 3-month post-treatment. Micro-lesions shown here may not be sectioned across the right plane indicating the nominal lesion depths; optical magnification: 10×.

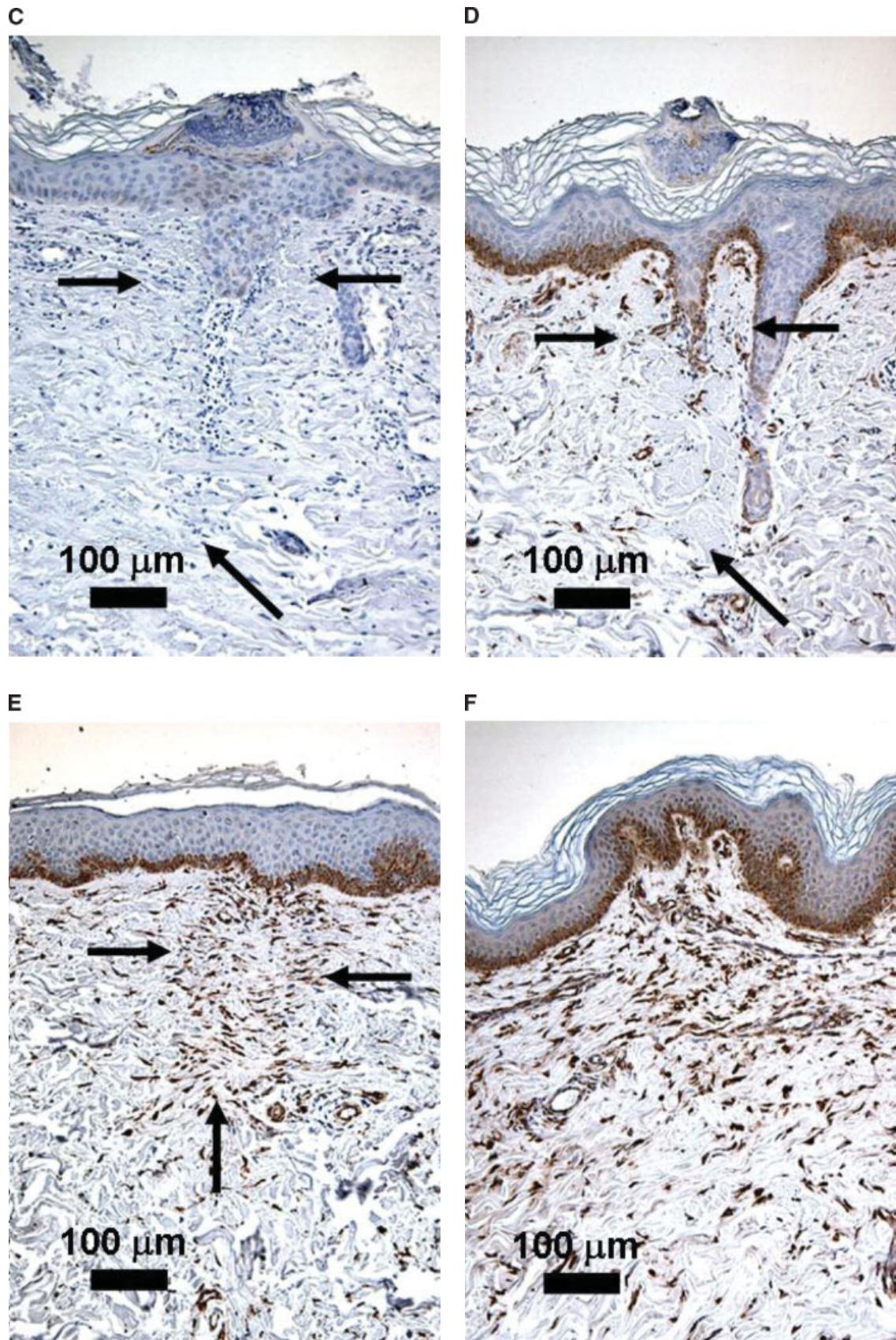


Fig. 4. (Continued)

TABLE 2. Dimensions of the Ablative and Coagulation Zones of Micro-Lesions Created by the Investigative AFRTM Device From 5 to 30 mJ

Q (mJ)	Immediately post treatment lesion characterization				
	Maximum lesion depth (μm)	Maximum lesion width (μm)	Maximum ablative depth (μm)	Maximum ablative width (μm)	Thickness of coagulated zone (μm)
5	298 ± 48	138 ± 20	210 ± 67	71 ± 17	33 ± 11
10	439 ± 70	184 ± 15	286 ± 76	95 ± 17	44 ± 13
20	778 ± 57	218 ± 10	560 ± 86	110 ± 18	54 ± 8
30	993 ± 77	270 ± 23	659 ± 69	121 ± 16	75 ± 13

The maximum lesion depths and widths include the coagulation zones plus the maximum ablative depths and widths, respectively.

become activated after CO₂ irradiation [32,33]. Eventually, the excavated dermal tissue was replaced with new collagen in the natural haphazard orientation (Fig. 2D).

Previous studies of CO₂ ablative lasers have emphasized the important role of the thermal coagulation zone in effecting tissue shrinkage [34]. In addition to detecting a similar zone of thermal coagulation, we also observed that this zone formed an annular ring around the micro-lesions created by AFRTM treatment (Fig. 5). Although tissue shrinkage was not directly assessed in the forearm study, we speculate that the annular configuration of the thermal coagulation zone may provide additional tissue shrinkage benefits due to favorable tensile vectors exerted by collagen shortening in a 3-dimensional manner. The degree of tissue shrinkage may be significant with the additive contraction of hundreds of thousands of AFRTM MTZs. Hence, the level

of tissue shrinkage may be modulated by the pulse energy (i.e. depth of micro-lesion) or the treatment spot density. If true, the AFRTM modality has strong implication for applications in dermatoplastic surgery. Further studies are warranted to quantify the degree of shrinkage both short-term and long-term produced by the AFRTM modality.

CONCLUSION

Preliminary results through histochemistry and immunohistochemistry indicated rapid re-epithelialization of micro-lesions created by the AFRTM modality, significantly reducing downtime and morbidity as compared to other ablative resurfacing techniques. Our results also revealed a favorable long-term wound healing process of the AFR treatment. Diffuse upregulation of hsp47 expression provided conclusive evidence that neocollagenesis was

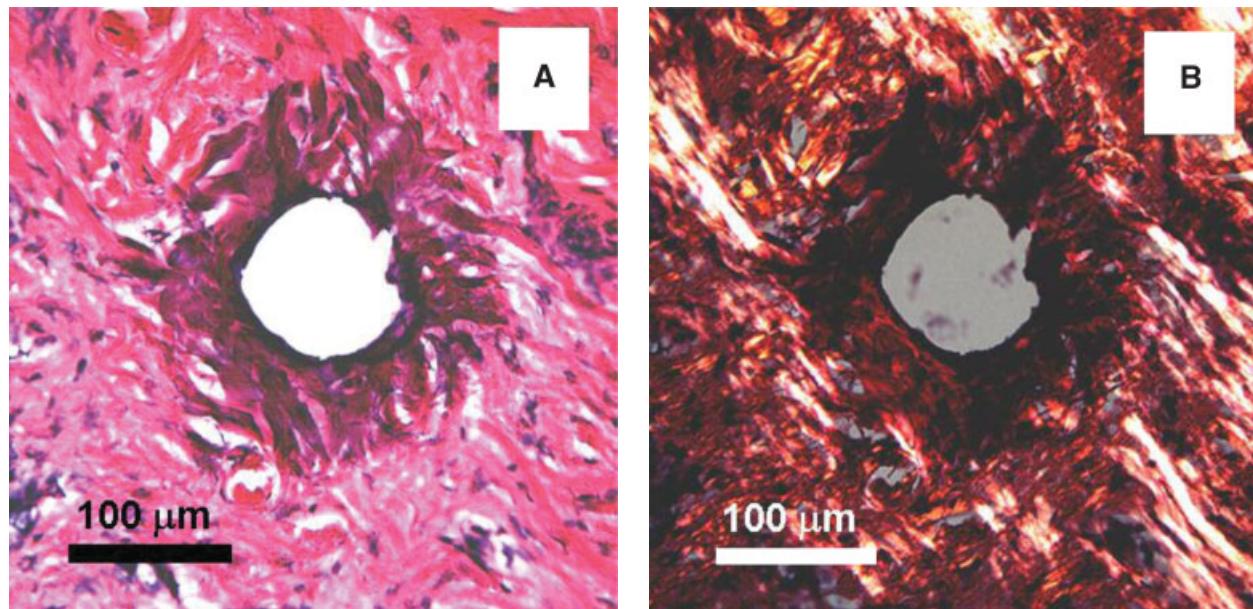


Fig. 5. An H&E-stained horizontal cross-section image of an AFRTM micro-lesion in the papillary dermis under (A) light and (B) cross-polarized microscopy, showing an ablated zone surrounded by an annular coagulation zone. The cross-polarized image indicates the loss of birefringence, confirming the denaturation of the collagen matrix within the coagulation zone.

persistent for a minimum of 3 months post-treatment, ensuring long-term generalized dermal remodeling that may rival conventional CO₂ and Er:YAG laser resurfacing.

REFERENCES

- Zachary CB. Modulating the Er:YAG laser. *Lasers Surg Med* 2000;26(2):223–226.
- Hall RR. The healing of tissues incised by a carbon-dioxide laser. *Br J Surg* 1971;58(3):222–225.
- Kamat BR, Carney JM, Arndt KA, Stern RS, Rosen S. Cutaneous tissue repair following CO₂ laser irradiation. *J Invest Dermatol* 1986 Aug; 87(2):268–271.
- Ross EV, Grossman MC, Duke D, Grevelink JM. Long-term results after CO₂ laser skin resurfacing: A comparison of scanned and pulsed systems. *J Am Acad Dermatol* 1997;37 (5 Pt 1):709–718.
- Bernstein LJ, Kauvar AN, Grossman MC, Geronemus RG. The short- and long-term side effects of carbon dioxide laser resurfacing. *Dermatol Surg* 1997;23(7):519–525.
- Zweig AD, Meierhofer B, Muller OM, Mischler C, Romano V, Frenz M, Weber HP. Lateral thermal damage along pulsed laser incisions. *Lasers Surg Med* 1990;10(3):262–274.
- 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(5):426–438.
- Khan MH, Sink RK, Manstein D, Eimerl D, Anderson RR. Intradermally focused infrared laser pulses: Thermal effects at defined tissue depths. *Lasers Surg Med* 2005;36(4):270–280.
- Fisher GH, Geronemus RG. Short-term side effects of fractional photothermolysis. *Dermatol Surg* 2005;31:1245–1249.
- Fisher GH, Kim KH, Bernstein LJ, Geronemus RG. Concurrent use of a handheld forced cold air device minimizes patient discomfort during fractional photothermolysis. *Dermatol Surg* 2005;31:1242–1244.
- Chan H. Effective and safe use of lasers, light sources, and radiofrequency devices in the clinical management of Asian patients with selected dermatoses. *Lasers Surg Med* 2005; 37:179–185.
- Rokhsar CK, Fitzpatrick RE. The treatment of melasma with fractional photothermolysis: A pilot study. *Dermatol Surg* 2005 Dec; 31(12):1645–1650.
- Tannous ZS, Astner S. Utilizing fractional resurfacing in the treatment of therapy-resistant melasma. *J Cosmet Laser Ther* 2005;7(1):39–43.
- Behroozan DS, Goldberg LH, Dai T, Geronemus RG, Friedman PF. Fractional photothermolysis for treatment of surgical scars: A case report. *J Cosmet Laser Ther* 2006;8: 35–38.
- Behroozan DS, Goldberg LH, Glaich AS, Dai T, Friedman PM. Fractional photothermolysis for treatment of poikiloderma of civatte. *Dermatol Surg* 2006;32:298–301.
- Glaich AS, Rahman Z, Goldberg LH, Friedman PF. Fractional resurfacing for the treatment of hypopigmented scars: A pilot study. *Dermatol Surg* 2006. In press.
- Glaich AS, Goldberg LH, Friedman RH, Friedman PF. Fractional photothermolysis for the treatment of post-inflammatory erythema resulting from acne vulgaris. *Dermatol Surg* 2006. In press.
- Weiss RA, Weiss MA, Beasley KL, Munavalli G. Our approach to non-ablative treatment of photoaging. *Lasers Surg Med* 2005;37:2–8.
- Bernstein L, Kim KH, Fisher GH, Geronemus R. Treatment of striae distensae with fractional photothermolysis [abstract]. *Lasers Surg Med* 2005;36(suppl 17):32.
- Rokhsar CK, Rahman Z, Avram M, Fitzpatrick R. The treatment of acne and other scars. *J Am Acad Dermatol* 2006. Submitted.
- Rahman Z, Tanner H, Jiang K. Treatment of atrophic scars with the 1550 nm erbium fiber fractional laser. *Lasers Surg Med* 2006;38 (suppl 18):24.
- Rahman Z, Tanner H, Herron S, Jiang K. Comparison of high energy versus low energy treatment for resurfacing with the 1550 nm erbium fiber fractional laser. *Lasers Surg Med* 2006; 38 (suppl 18):62.
- Hantash BM, Bedi VP, Sudireddy V, Struck SK, Herron GS, Chan KF. Laser-induced transepidermal elimination of dermal content by fractional photothermolysis. *J Biomed Opt* 2006;11(4): 041115; 1–9.
- Bedi VP, Chan KF, Sink RK, Hantash BM, Herron GS, Rahman Z, Struck SK, Zachary CB. The effects of pulse energy variations on the dimensions of microscopic thermal treatment zones in fractional photothermolysis. *Lasers Surg Med* 2007;39(2):145–155.
- Hantash BM, Bedi VP, Chan KF, Zachary CB. Ex vivo histological characterization of a novel ablative fractional resurfacing device. *Lasers Surg Med* 2007;39(2):87–95.
- Rahman Z, Tanner HE, Chan KF, Zachary CB, Jiang K, Bedi VP. Resurfacing of the face and neck using a novel fractional CO₂ Laser. *Dermatol Surg* 2007; In preparation.
- Bernstein LJ, Kauvar AN, Grossman MC, Geronemus RG. Scar resurfacing with high-energy, short-pulsed and flashscanning carbon dioxide lasers. *Dermatol Surg* 1998; 24(1):101–107.
- Schwartz RJ, Burns AJ, Rohrich RJ, Barton FE, Byrd HS. Long-term assessment of CO₂ facial laser resurfacing: Aesthetic results and complications. *Plast Reconstr Surg* 1999;103:592–601.
- Sriprachya-Anunt S, Fitzpatrick RE, Goldman MP, Smith SR. Infections complicating pulsed carbon dioxide laser resurfacing for photoaged facial skin. *Dermatol Surg* 1997; 23:527–536.
- Keagle JN, Welch WJ, Young DM. Expression of heat shock proteins in a linear rodent wound. *Wound Rep Reg* 2001;9(5): 378–385.
- Razzaque MS, Foster CS, Ahmed AR. Role of collagen-binding heat shock protein 47 and transforming growth factor-beta1 in conjunctival scarring in ocular cicatricial pemphigoid. *Invest Ophthalmol Vis Sci* 2003;44(4):1616–1621.
- Woo TY, Rasmussen JE. Disorders of transepidermal elimination. Part 2. *Int J Dermatol* 1985;24(6):337–348.
- Richert SM, Bridenstine J. Transepidermal elimination of elastic fibers after carbon dioxide laser resurfacing. A report of two cases. *Dermatol Surg* 1998;24(2):275–278.
- Ross EV, McKinlay JR, Anderson RR. Why does carbon dioxide resurfacing work? *Arch Dermatol* 1999;135:444–454.