

Laser-induced transepidermal elimination of dermal content by fractional photothermolysis

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Abstract. The wound healing process in skin is studied in human subjects treated with fractional photothermolysis. *In-vivo* histological evaluation of vacuoles formed over microthermal zones (MTZs) and their content is undertaken. A 30-W, 1550-nm single-mode fiber laser system delivers an array of 60 μm or 140 μm $1/e^2$ incidence microbeam spot size at variable pulse energy and density. Treatments span from 6 to 20 mJ with skin excisions performed 1-day post-treatment. Staining with hematoxylin and eosin demonstrates an intact stratum corneum with vacuolar formation within the epidermis. The re-epithelialization process with repopulation of melanocytes and keratinocytes at the basal layer is apparent by 1-day post-treatment. The dermal-epidermal (DE) junction is weakened and separated just above zones of dermal coagulation. Complete loss of dermal cell viability is noted within the confines of the MTZs 1-day post-treatment, as assessed by lactate dehydrogenase. All cells falling outside the irradiation field remain viable. Content within the epidermal vacuoles stain positively with Gomori trichrome, suggesting a dermal origin. However, the positive staining could be due to loss of specificity after thermal alteration. Nevertheless, this dermal extrusion hypothesis is supported by very specific positive staining with an antihuman elastin antibody. Fractional photothermolysis creates microthermal lesions that allow transport and extrusion of dermal content through a compromised DE junction. Some dermal material is incorporated into the microepidermal necrotic debris and shuttled up the epidermis to eventually be exfoliated through the stratum corneum. This is the first report of a nonablative laser-induced transport mechanism by which dermal content can be predictably extruded biologically through the epidermis. Thus, treatment with the 1550-nm fiber laser may provide the first therapeutic option for clinical indications, including pigmentary disorders such as medically recalcitrant melasma, solar elastosis, as well as depositional diseases such as mucinosis and amyloidosis.

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Keywords: ablation; dermatology; exfoliation; Fraxel; infrared; melasma; pigmentation; resurfacing; tattoo; texture; vacuole.

Paper 05307SSR received Oct. 10, 2005; revised manuscript received Jan. 9, 2006; accepted for publication Jan. 25, 2006; published online Aug. 16, 2006.

1 Introduction

The skin is a complex metabolic organ with unique structure and function.¹ It is composed of three primary layers, namely the epidermis, dermis, and subcutis. Within the epidermis, a further architectural delineation occurs with the most immature cell layer located at the base, the so-called basal layer.² It is this layer that actively divides to provide regenerative capacity following any epidermal tissue injury. Under normal conditions, the basal layer cells migrate upward over the course of two weeks to create the stratum spinosum and stratum granulosum. An additional two weeks elapse before those cells are exfoliated from the stratum corneum, the nonviable

selectively permeable barrier component of skin. Thus, exfoliation is a constitutive function of healthy normal skin.

In addition to exfoliation of dead epidermal cells, the skin is also capable of removing dermal content through a viable epidermis. This process, known as transepidermal elimination, allows the disposal of foreign material aberrantly implanted in skin.³ The mechanisms by which this transport system is activated remain unknown. This process at times goes awry, leading to several pathological skin conditions such as Kyrle's disease, elastosis perforans serpiginosa, reactive perforating collagenosis, acquired perforating dermatosis, chondrodermatitis nodularis helices, and perforating folliculitis.⁴⁻⁷ These diseases share in common the physiological function of tran-

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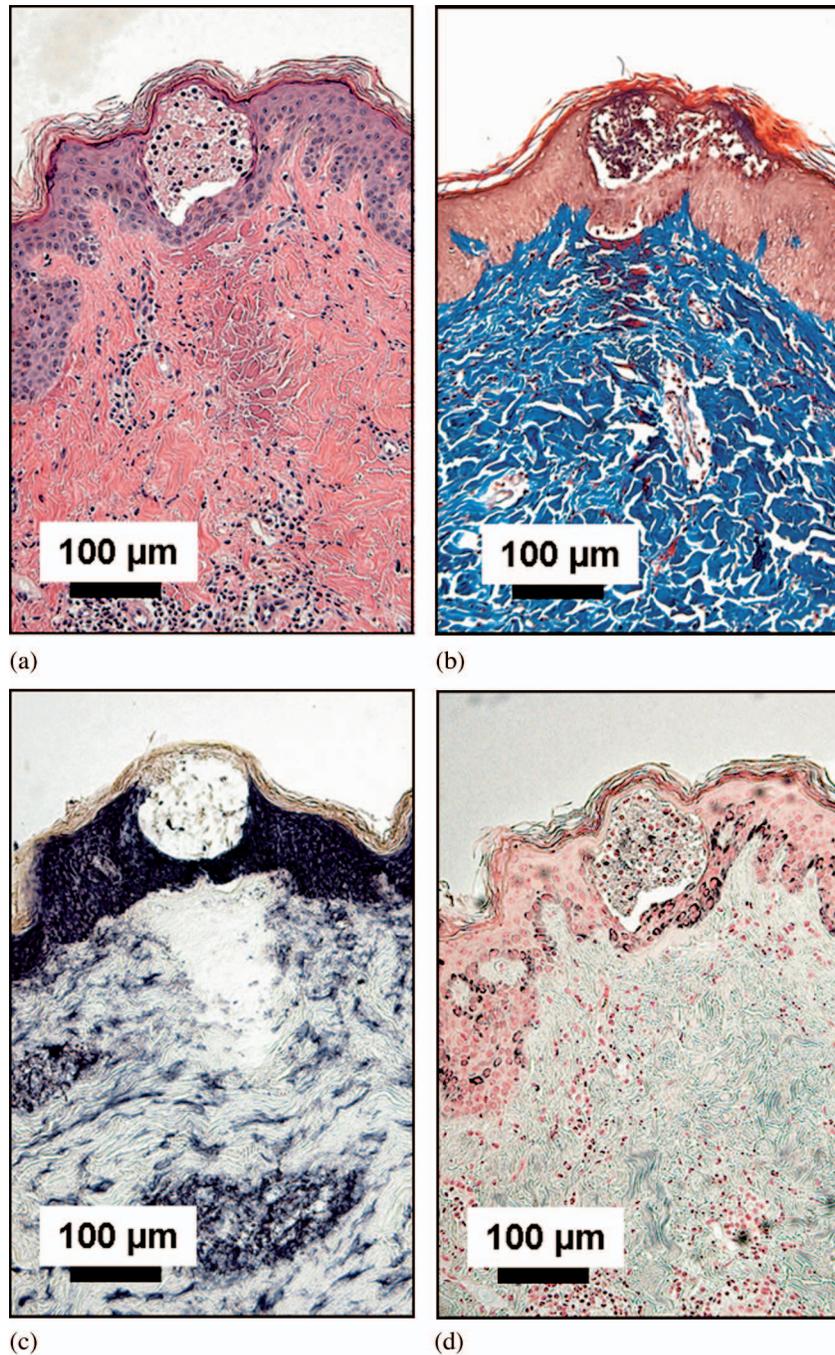


Fig. 1 Histology of human abdominal skin treated with the Fraxel[®] SR laser at 20 mJ with 60- μm $1/e^2$ incidence microbeam spot size, excised 1-day postirradiation. The series of images represent (a) paraffin-embedded H&E, (b) Gomori trichrome, and (d) Fontana Masson and (c) frozen-embedded LDH stained sections. The epidermal vacuole overlies the thermal wound.

sepidermal elimination, albeit triggered by a stimulus that leads to a pathological state.

We postulated that this physiological mechanism would potentially be advantageous in unwanted conditions such as the presence of pigment in the dermis, a problem commonly seen by dermatologists treating melasma, tattoos, and postinflammatory hyperpigmentation. Often times, patients express significant frustration due to a lack of effective therapeutic options for these pharmacologically recalcitrant clinical entities. Similarly, a multiplicity of lasers has been utilized with-

out predictable reliability.^{8,9} Recent reports by Tannous and Astner, and Rokhsar and Fitzpatrick, however, demonstrated improved efficacy in the treatment of therapy-resistant melasma using a fractional photothermolysis approach.^{10,11} The mechanism by which successful treatment was achieved in these reports was not defined.

We previously characterized the healing process up to one-week postfractional photothermolysis.^{12,13} Immediately following treatment, we observed well-defined, quasi-cylindrical thermal coagulation zones extending into the dermis, with in-

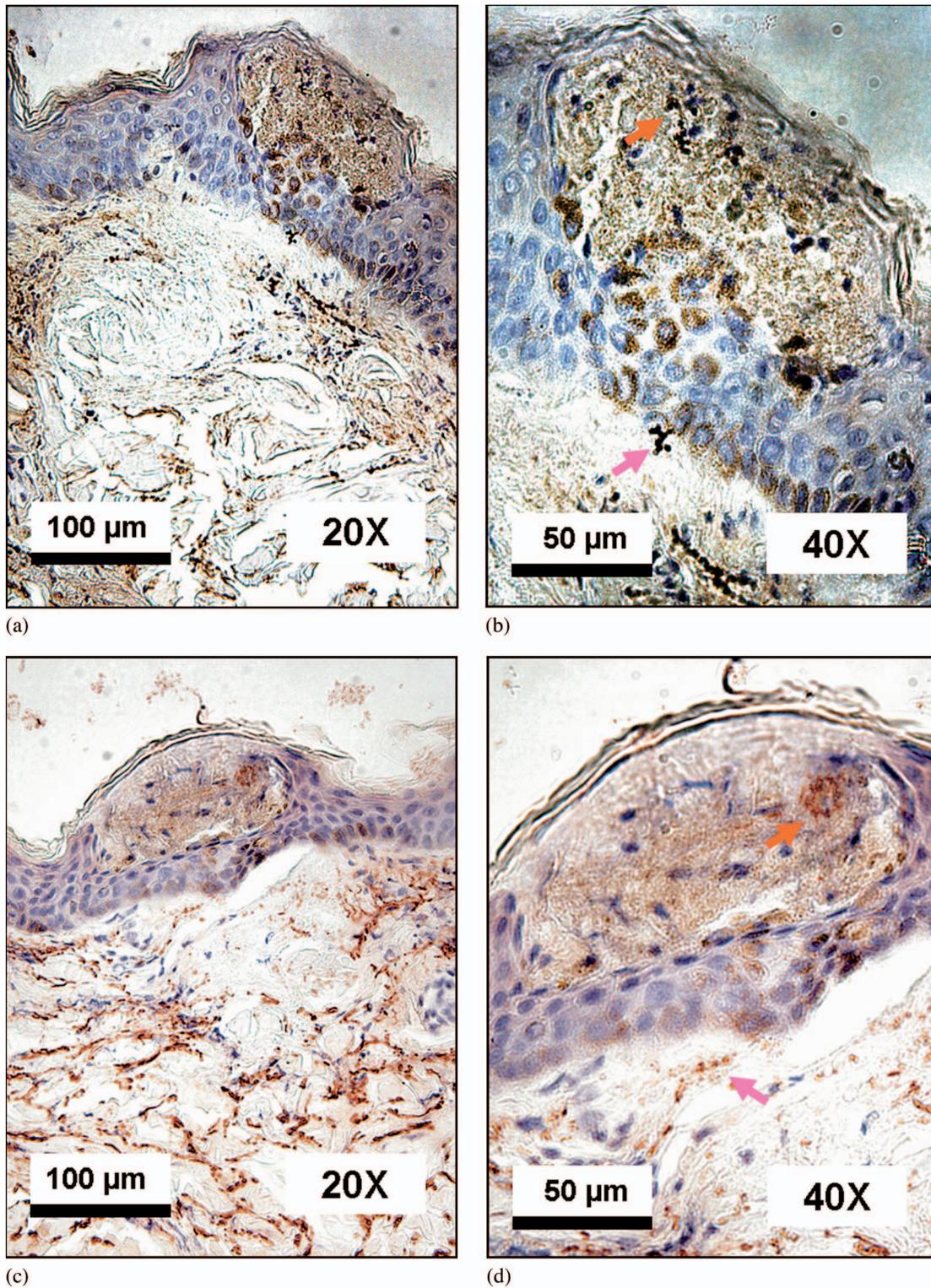


Fig. 2 Histology of human abdominal skin treated with the Fraxel[®] SR laser at 20 mJ and 140- μ m $1/e^2$ incidence spot size, and excised 1-day post-treatment. The samples were paraffin embedded, treated with antihuman elastin antibody, and stained with (a) and (b) DAB or (c) and (d) Novared. Stained elastin appears brown to black (DAB) or red to orange (Novared) in color. Red arrows represent elastin fibrils contained within the epidermal vacuole, and pink arrows show similar staining of dermal elastin fibrils.

tralesional loss of cell viability. Histological analysis also revealed intraepidermal vacuolar formation. The content of these vacuoles has not previously been characterized.

We hypothesized that a portion of the vacuolar content was dermal in origin and could be extruded through the epidermis based on our previous work that revealed transmigration of the vacuole up the epidermis.¹³ This study sought to determine whether or not a transepidermal shuttle could be activated by treatment with a 1550-nm fiber laser utilizing fractional photothermolysis. We report for the first time the successful activation of a transepidermal elimination mechanism induced by a mid-infrared fiber laser. The objective of this work is to histologically characterize this transport system and more clearly define the origin of its contents.

2 Materials and Methods

Two healthy subjects of Fitzpatrick skin type II were treated on the abdomen, with the 1550-nm Fraxer[®] SR laser system (Reliant Technologies Incorporated, Palo Alto, California) one day prior to abdominoplasty. An institutional review board approved the study protocol and both subjects were consented prior to participation in the study. Hair within the test sites was removed by shaving and topical anesthesia was locally administered an hour prior to laser treatment. Each laser treatment covered approximately 12 cm². The laser handpiece was operated in user mode, allowing real-time deposition of a fixed density of laser microbeams on the skin within a range of handpiece velocities.

The array of microbeams was single-mode Gaussian beams, either 60 or 140 μm in diameter at the $1/e^2$ incidence beam waist. The first subject received treatment from the smaller diameter microbeams, and the second subject received treatment from the larger diameter microbeams, both at various pulse energy levels. Pulse energies ranged from 6 to 20 mJ, with four passes at 250 microthermal zones (MTZs) per cm² to 16 passes at 125 MTZs per cm² made to produce final spot densities of 1000 to 2000 MTZs per cm² (i.e., 250 MTZ/cm²/pass \times 4 = 1000 MTZ/cm²; 125 MTZ/cm²/pass \times 16 = 2000 MTZ/cm²). In general, the higher the pulse energy, the lower the final spot density. Excision of the treated abdominal skin was performed during the abdominoplasty at 1-day post laser treatment.

Immediately following surgical excision, each 12 cm² patch of treated skin was further excised from the abdominal skin. Both paraffin sections and frozen sections were performed with tissue across the entire 12-cm² patch. For frozen sectioning, the samples were embedded in Optimal Cutting Temperature Compound (IMEB Incorporated, San Marcos, California). For paraffin sectioning, samples were fixed in 10% v/v neutral buffered formalin (VWR International, West Chester, Pennsylvania) overnight and embedded in paraffin blocks. The frozen samples were sectioned into 12- μm -thick slices and stained with hematoxylin and eosin (H&E) and lactate dehydrogenase (LDH), while the paraffin-embedded samples were sectioned into 10- μm -thick slices and stained with H&E, Gomori trichrome,¹⁴ and Fontana Masson. Elastin staining was performed according to a previously published protocol.¹⁵ 50 lesions were selected at random from the histological sections of each treated sample from the 12-cm² patch at each pulse energy. These lesions were imaged and

recorded using a Leica[®] DM LM/P microscope and a DFC320 digital camera (Leica Microsystem, Cambridge, United Kingdom). The number and percentage of lesions with vacuoles that stained positively with elastin were recorded for each spot size and pulse energy.

3 Results

All laser exposures produced a constant pattern of well-spaced MTZs. Figures 1(a) through 1(d) show examples of histological sections obtained from abdominal skin processed with a variety of stains 1-day post fractional photothermolysis. Using a laser pulse energy of 20 mJ, 60- μm incidence microbeam spot size, and spot density of 2000 MTZ/cm², a clearly demarcated dermal lesion representing collagen coagulation or denaturation was detected by H&E [Fig. 1(a)].^{16,17} As expected, intact dermal collagen was stained blue-green, while coagulated dermal collagen stained red by Gomori trichrome¹⁴ [Fig. 1(b)]. An epidermal vacuole overlying the thermal lesion was evident [Figs. 1(a) and 1(b)]. Some of the content within the vacuole, however, seemed to exhibit a similar color contrast as that of dermal coagulated tissue [Figs. 1(a) and 1(b)]. LDH staining indicated that the vacuolar content lacked viability [nonviable zone is not stained by Nitro Blue Tetrazolium Chloride; Fig. 1(c)]. To test for the presence of melanin in the vacuole, we used Fontana Masson. Figure 1(d) demonstrates darkly stained granules within the vacuole, consistent with melanin.

Although each vacuole appeared to contain both epidermal and dermal material, we attempted to more clearly identify the different components of each vacuole. To distinguish between dermal and epidermal origin, we elected to immunohistochemically stain tissue sections to search for the presence of elastin within the laser-induced epidermal vacuole. Figure 2 demonstrates tissue specimens stained with mouse antihuman elastin antibody at 1-day post-treatment at a pulse energy of 20 mJ and spot size of 140 μm . The elastin appeared black when tissue sections were developed with 3, 3'-diaminobenzidine (DAB).¹⁵ No staining was observed when the primary antibody was omitted, suggesting the black stained material was specifically elastin. As expected, elastin was clearly identified just beneath the dermal-epidermal (DE) junction in the dermis [Fig. 2(a)] as well as in the media layer of deeper medium-sized arterioles. Note that elastin staining was detected within the dermal coagulation zone, confirming its increased resistance to thermal denaturation. Remarkably, elastin staining was absent throughout the epidermis, except within vacuoles [Fig. 2(a)]. This is clearly depicted in a higher magnification image [Fig. 2(b)]. To ensure that the material stained was not melanin, we also processed tissue sections with Vector[®] Novared [Fig. 2(c)], which stained the elastin fibrils a bright orange to a dark red color. Figure 2(d) shows a higher magnification image of another vacuole stained with Novared with an identical morphology to material located just beneath the DE junction. This data strongly suggested that dermal material was present with the epidermal vacuole.

We observed a tendency toward a larger vacuole-to-lesion ratio using the 60- μm spot size treatment. However, the incidence spot size and the resulting vacuolar dimension did not appear to affect the transport of elastin through the DE junction. Both (60 and 140 μm) modes of treatment produced

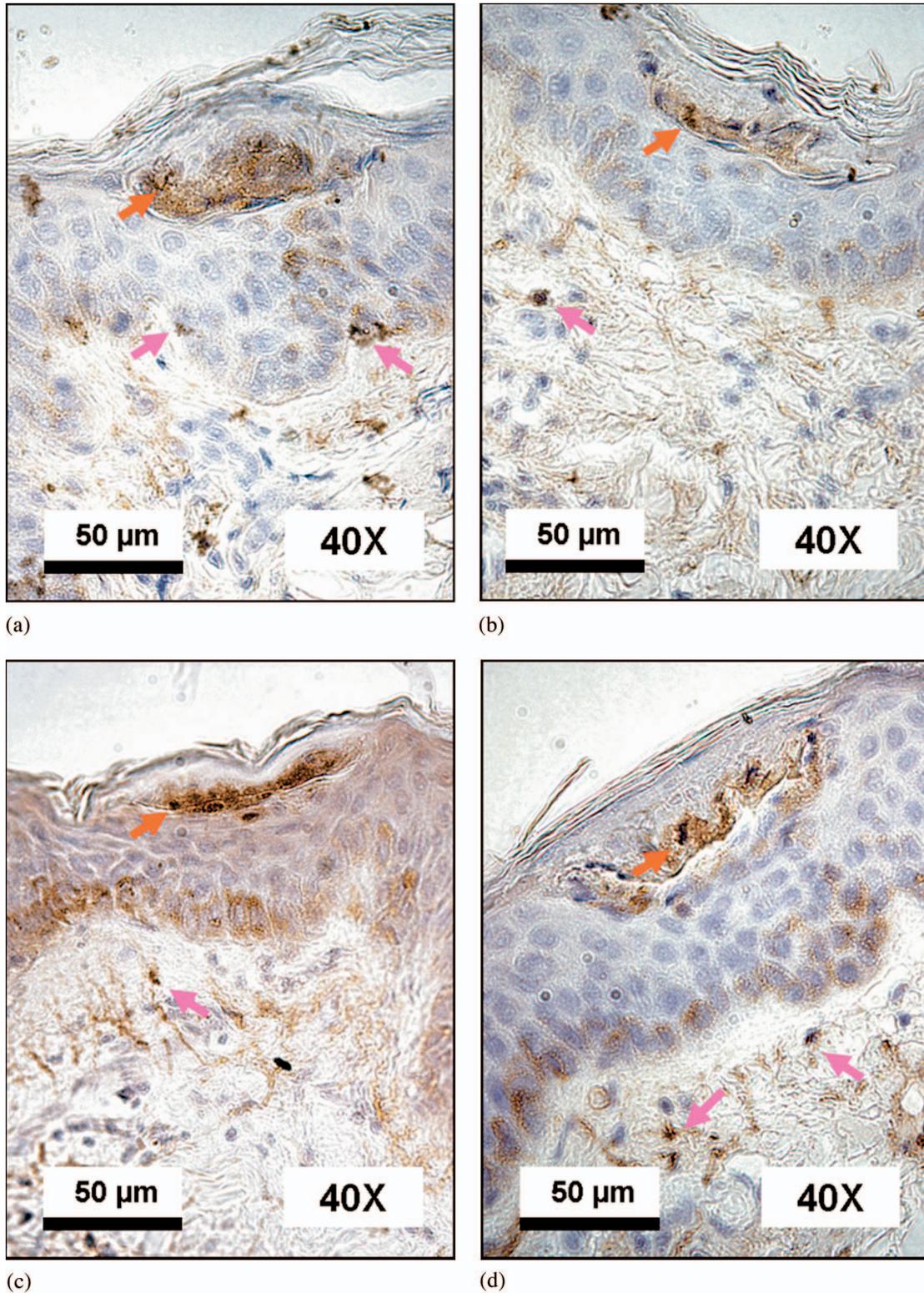


Fig. 3 High magnification images of histology of human abdominal skin treated with the Fraxel[®] SR laser at 6 and 10 mJ at both 60- μm and 140- μm $1/e^2$ incidence spot sizes, excised 1-day post-treatment. Respectively, they are (a) 6 mJ, 60- μm spot size, (b) 10 mJ, 60 μm spot size, (c) 6 mJ, 140 μm spot size, and (d) 10 mJ, 140 μm spot size. The samples were paraffin embedded and then stained for elastin, as described in the materials and methods in Sec. 2. Red arrows represent elastin fibrils contained within the epidermal vacuole, and pink arrows show similar staining of dermal elastin fibrils.

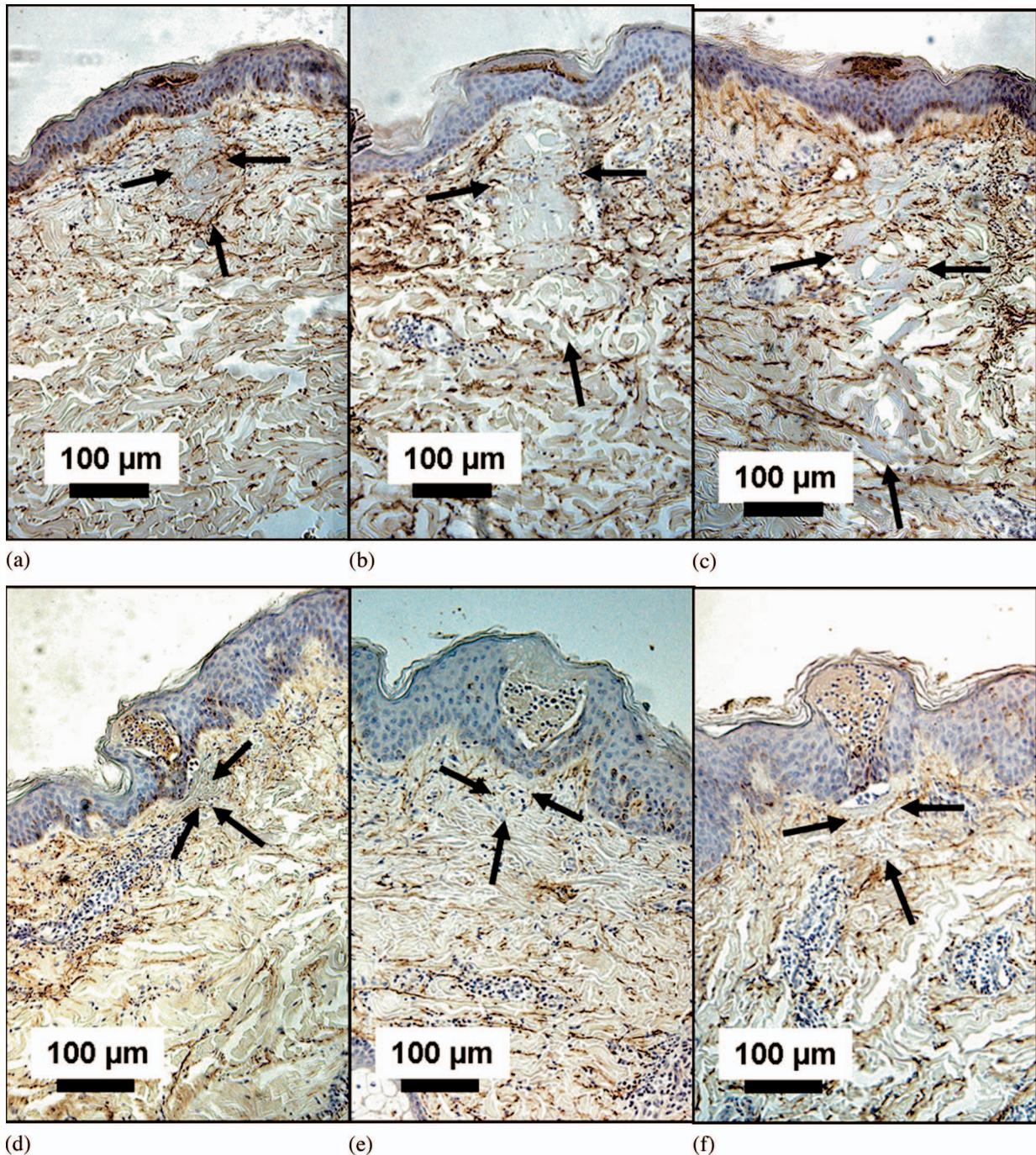


Fig. 4 Histology of human abdominal skin treated with the Fraxel[®] SR laser at 6, 12, and 20 mJ at both 60- μm and 140- μm $1/e^2$ incidence spot sizes, excised 1-day post-treatment. Respectively, they are (a) 6 mJ, (b) 12 mJ, and (c) 20 mJ at 140- μm spot size, and (d) 6 mJ, (e) 12 mJ, and (f) 20 mJ at 60- μm spot size. Each sample was paraffin embedded, treated with antihuman elastin antibody, and stained with DAB. The black arrows outline the hyalinized or denatured dermal regions that define the MTZs. Note the much deeper lesions created by the 140- μm spot size, and the higher vacuoles-to-lesion ratio of the 60- μm spot size lesions at their respective pulse energies. All the lesions presented here contain elastin within the vacuoles, though not obvious at this level of magnification.

consistent transfer of elastin from the dermal compartment into the epidermal vacuole. Similar results were obtained when the pulse energy was reduced by up to 70%. Epidermal vacuoles stained positively for elastin at pulse energies of 6 and 10 mJ for both 60- μm [Figs. 3(a) and 3(b)] and 140- μm [Figs. 3(c) and 3(d)] incidence spot sizes. Further

testing revealed consistent transfer of elastin from the dermal compartment into epidermal vacuoles following fractional photothermolysis treatment at pulse energies spanning 6 to 20 mJ and spot sizes of either 60 or 140 μm (Table 1). The 60- μm spot size treatments demonstrated that $82 \pm 5\%$, while the 140- μm spot size treatments demonstrated that $80 \pm 4\%$

Table 1 Assessment of the percent of lesions with epidermal vacuoles that stained positively for elastin using 60- μm and 140- μm $1/e^2$ incidence spot sizes at selected treatment energies.

Pulse energy (mJ)	Spot size (μm)	Number of lesions assessed	Number of vacuoles indicating presence of elastin	Percent vacuoles indicating presence of elastin (%)
6	60	50	38	76
	140	50	41	82
10	60	50	43	86
	140	50	41	82
12	60	50	40	80
	140	50	37	74
20	60	50	43	86
	140	50	41	82

of the lesions assessed in the study contained vacuoles that stained positively for elastin.

4 Discussion

In this work, we uncovered for the first time a laser-dependent transepidermal transport system capable of eliminating dermal material by definitively identifying elastin in epidermal vacuoles created by treatment with fractional photothermolysis. This phenomenon was consistently observed with the laser operated at an incidence $1/e^2$ microbeam spot size of 60 or 140 μm with variable pulse energies and densities.

A typical treatment with the Fraxel SR laser system (140- μm $1/e^2$ incidence spot size) produced a pattern of microscopic thermal wounds that extended from approximately 200 to 700 μm into the dermis¹² [Figs. 4(a)–4(c)]. At 60- μm $1/e^2$ incidence spot size, however, the apparent thermal lesion depths for the corresponding pulse energies were much shallower [Figs. 4(d)–4(f)]. Because the pulse durations for both incidence spot sizes were on the order of 0.4 to 1.5 ms, it was likely that the laser was operating in the thermal confinement region. Interestingly, lesions of the 60- μm spot size appeared more disruptive to the epidermis. The difference in the dynamics of laser-tissue interaction may be due to the difference in the incidence radiant exposure (J/cm^2) or the irradiance (W/cm^2) levels; i.e., the irradiances of the 60- μm spot size were at least five fold higher than those of the 140- μm spot size at any given pulse energy. At the irradiance levels of the 140- μm spot size, most of the photothermal energy deposition into the skin was likely below the threshold temperature for vaporization, efficiently heating and coagulating the epidermis and dermis. Though also operating in the photothermal regime, the higher laser irradiance levels for the 60- μm spot size may have resulted in rapid vaporization of a major portion of the epidermis, thermomechanically coupling the disruption of epidermal debris during the laser pulse duration. The results of epidermal disruption created a highly scattering medium for subsequent photon deposition, therefore drastically reducing the fluence (J/cm^2) delivered

into the dermis, creating shallower lesions, as shown in Figs. 4(d)–4(f). The hypothesis was that the smaller and more disruptive 60- μm spot size was more efficient in weakening the DE junction, and hence allowing easier transepidermal elimination of dermal contents. However, our results indicated that the probability of dermal elastin extrusion for a given number of lesions (Table 1) is not significantly different, independent of spot size and pulse energy. There has not been any statistical analysis of the volume of elastin removed, nor have any studies been performed on the extrusion of other types of dermal contents (i.e., melanin, collagen, dermal necrotic debris, etc.). More detailed experiments and analyses are warranted to further understand the differences in the dynamics of laser-tissue interaction and the eventual biological response observed herein.

The progression from various histochemical stains to an immunohistochemical stain in our histologic analyses was necessary to definitively conclude that the transepidermal transport mechanism illustrated in this work was not only eliminating material of epidermal but also of dermal origin. Conventional histochemical stains such as H&E, LDH, Gomori trichrome, and Fontana Masson are pH-dependent. Through thermal alteration of epidermal and dermal cells and the extracellular matrix by laser irradiation, these stains may lose their specificity.⁸ Although some material in the epidermal vacuole suspiciously resembled that seen in the dermis as stained by H&E and Gomori trichrome, we could not conclusively determine the origin of the vacuolar content. Moreover, even with the Fontana Masson stain allowing us to visualize accumulation of melanin within the vacuole, it was impossible to pinpoint the origin of the melanin. Since the treated tissue was derived from the abdomen, a sun-protected site, the likelihood was that the vacuolar melanin was mainly of epidermal origin and would not allow us to definitively conclude that the transepidermal elimination mechanism was capable of removing material of dermal origin. We surmised that a thermally resistant molecule located in the dermis but not the epidermis would best serve to test this possibility.^{19,20} The thermal profile of collagen suggested that immunohistochemical techniques targeting collagen also might not offer adequate specificity.^{17,18} Elastin, on the other hand, appeared to be more heat stable than collagen.^{21,22} Indeed, laser-treated tissue exposed to antihuman elastin antibody showed positive staining. The positive staining was located in both the spared and treated sections of the dermis, and more interestingly, within the epidermal vacuole itself. This confirmed our suspicion that dermal content was being transported through the epidermis to eventually be exfoliated.

A previous study by Richert and Bridenstine elucidated a transepidermal exfoliation process of dermal content 18 to 22 days after ablative laser skin resurfacing.^{23,24} However, the VerHoeff-van Gieson stain used in that study is also pH-dependent, leaving it susceptible to loss of specificity to any thermally altered cellular or extracellular material. Furthermore, it is not surprising to identify dermal content in the exfoliated material post- CO_2 skin resurfacing, as the ablative laser removes the entire epidermis and stratum corneum, exposing the dermis during the wound healing process. To date, there has been no known laser-dependent biological dermal elimination process utilizing a nonablative laser modality, which maintains the stratum corneum's barrier function im-

mediately post irradiation and throughout the wound healing process. Our observation is significant because it implies that a biological elimination process can be activated that is capable of removing coagulated dermal tissue, necrotic debris, or other depositional material. This transport system exploited the presence of a weakened DE junction induced by the fractional photothermolysis treatment. Since the Fraxel® SR laser system only treats a small portion of the overall skin surface area, rapid healing can be achieved, and coagulated epidermal tissue can quickly be exfoliated through the stratum corneum. Thus, the stratum corneum serves a dual role, maintaining barrier function to prevent problems such as microbial contamination and infection as well as permitting exfoliation of treated tissue. Unlike most laser resurfacing procedures,^{25,26} wherein epidermal and dermal components are removed immediately by laser ablation, the mechanism by which fractional photothermolysis removes dermal material takes advantage of normal physiological functions of the epidermis, mainly exfoliation. Since the exfoliated material is not strictly epidermal and includes papillary dermal components, we decided to coin this mechanism of action as laser-induced transepidermal “biological resurfacing” or “biological ablation” of the dermis. This biological resurfacing modality minimizes the adverse clinical side effects associated with ablative skin resurfacing,²⁷ and avoids the questionable clinical efficacies of laser treatment often seen in nonablative dermal remodeling procedures.^{28–32}

Even though this was a preliminary study and treatments of only two subjects were the basis of our results, the authors felt that the consistency of these results warrants reporting. This study has led to the discovery of a potentially important biological transport mechanism, which can be activated by fractional photothermolysis. Further testing on a higher number of subjects is necessary to confirm this transepidermal exfoliation mechanism. In addition, this work only discussed the elimination of dermal elastin; other components such as hemoglobin or porphyrin ring structure, which is quite thermally stable, can also be investigated. While implying that other dermal contents may be extruded in a similar manner, there is no direct evidence that this is the case. Further experiments are required to elucidate the use of this mechanism for various clinical indications. However, the authors foresee the potential of clinical indications that may be addressed with this laser-induced transepidermal elimination modality, which include but are not limited to recalcitrant melasma, Hori’s macule, tattoo, postinflammatory hyperpigmentation, and other pigmentary disorders. It may also address photoaging and other dermal depositional disorders such as solar elastosis, mucinosis, amyloidosis, and scars.

5 Conclusions

Fractional photothermolysis provides physicians with a modality that promotes deep dermal remodeling and improves superficial repigmentation, retexturing, and retoning of skin that is comparable to most existing ablative skin resurfacing and nonablative dermal remodeling modalities, while avoiding adverse clinical side effects with minimal or no downtime post laser treatment. Our results provide the first definitive evidence of a transepidermal biological transport mechanism capable of transferring dermal constituents into an epidermal

vacuole to eventually be exfoliated through an intact stratum corneum. This discovery may potentially serve as a useful treatment for a number of difficult to treat dermatological conditions.

Acknowledgments

We thank G. Scott Herron for conceptualizing the use of fractional photothermolysis for the elimination of dermal content. We thank Brad Hauser, Kerrie Jiang, Heather Tanner, and Zakia Rahman for their assistance in carrying out the experiments described in this report, and Len DeBenedictis and Rox Anderson for their guidance and for sharing their considerable insights.

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