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Er:YAG Laser Skin Resurfacing Using Repetitive Long-Pulse Exposure and Cryogen Spray Cooling: I. Histological Study

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Background and Objective: To evaluate histologically the characteristics of repetitive Er:YAG laser exposure of skin in combination with cryogen spray cooling (CSC), and its potential as a method of laser skin resurfacing.

Study Design/Materials and Methods: Rat skin was irradiated in vivo with sequences of 10 Er:YAG laser pulses (repetition rate 20 Hz, pulse duration 150 or 550 μ s, single-pulse fluence 1.3–5.2 J/cm²). In some examples, CSC was applied to reduce epidermal injury. Histologic evaluation was performed 1 hour, 1 day, 5 days, and 4 weeks post-irradiation.

Results: A sequence of ten 550- μ s pulses with fluences around 2 J/cm² resulted in acute dermal collagen coagulation to a depth of approximately 250 μ m, without complete epidermal ablation. CSC improved epidermal preservation, but also diminished the coagulation depth. Four weeks after irradiation, neo-collagen formation was observed to depths in excess of 100 μ m.

Conclusion: Dermal collagen coagulation and neo-collagen formation to depths similar to those observed after CO₂ laser resurfacing can be achieved without complete ablation of the epidermis by rapidly stacking long Er:YAG laser pulses. Application of CSC does not offer significant epidermal protection for a given dermal coagulation depth. *Lasers Surg. Med.* 28:121–130, 2001.

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Key words: collagen denaturation; dynamic cooling; repetitive laser exposure; skin remodeling; thermal damage

INTRODUCTION

The Er:YAG laser is a primary tool for ablation of superficial skin lesions in dermatology. Due to the extremely high absorption coefficient of its mid-infrared radiation in water ($\mu_a \sim 1000 \text{ mm}^{-1}$), the Er:YAG laser induces minimal thermal injury to the underlying tissue [3]. At commonly utilized parameters, the zone of residual thermal damage (RTD) is typically 20–50 μ m deep. This results in faster skin re-epithelialization as compared to deeper penetrating lasers, such as the CO₂, [3–6], which induce a 60–150 μ m thick zone of RTD [7,8].

The Er:YAG laser is used also in cosmetic surgery for laser skin resurfacing (LSR). This procedure rejuvenates

the skin and significantly improves rhytides in properly selected patients. It has been noted, however, that rhytid improvement achieved after Er:YAG LSR is, in general, less pronounced compared with wounding to equivalent depth with the CO₂ laser [6]. This can be attributed to the difference in injury induced by the two lasers. A number of studies have demonstrated increased wound healing factors and subsequent neo-collagen formation following thermal, as compared to primarily ablative laser damage [9–11]. It has been proposed that dermal collagen coagulation of 70 μ m or deeper is required to optimize the clinical outcome of LSR [12–14].

Regardless of the laser used (Er:YAG or CO₂), LSR is primarily an ablative procedure and results in complete epidermal removal. The open wound puts the patient at risk for bacterial, viral and fungal infections, and may result in skin dyspigmentation or scarring. Re-epithelialization is generally faster after Er:YAG LSR as compared to CO₂ [15], but the patient must still endure 5–7 days of intensive wound care.

A few recent attempts at non-ablative LSR have been variably successful, but none have demonstrated rhytid improvement equivalent to that observed after CO₂ or Er:YAG LSR. Goldberg [16] investigated clinical and histological improvement in 10 patients after four treatments with the 1.32- μ m Nd:YAG laser in combination with cryogen spray cooling (CSC). Biopsy specimens demonstrated some collagen thickening in the papillary dermis, but histological changes did not extend deeper into the dermis. Ross et al. [17] recently treated human patients with an Er:glass laser (1.54 μ m) in combination with a contact cooling device. They were able to achieve epidermal preservation with slight tinctoral change in dermal collagen to depths of 400–1,300 μ m. In some specimens, an increased number of fibroblasts was noted after 2 months. However, the authors concluded that optimal cosmetic results would require a more pronounced thermal injury at

Preliminary results of this study were presented at the 20th Annual Meeting of the ASLMS, Reno, NV, April 2000 [1,2].

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depths less than 400 μm . Goldberg and Cutler have explored the use of an intense non-coherent pulse light source for non-ablative resurfacing on humans. While a histologic analysis was not performed, nine of the 30 patients were judged to have substantial improvement and no subjects showed total improvement after 1–4 treatments. None of these studies have demonstrated collagen coagulation and regeneration to the depth and degree sufficient for LSR. Mordon et al. [19,20] recently used an animal model to demonstrate that a sequence of Er:glass laser pulses (at 3 Hz) with the help of a contact cooling device can achieve a subsurface zone of thermal injury at a depth of 200–500 μm , with a completely intact epidermis.

In a recently published study [21] utilizing an in vivo animal model, we demonstrated that deep collagen coagulation without complete epidermal ablation can be achieved by rapid stacking of Er:YAG laser pulses. Coagulation depths up to 260 μm (measured from the epidermal–dermal junction) were observed at pulse fluences around the ablation threshold, which matched well the results of an earlier published numerical model of thermo-mechanical laser ablation of skin [22,23]. At those fluences, the dependence on the number of pulses in the sequence (between 5 and 10) was weak, and the influence of the repetition rate (10 or 30 Hz) was found to be insignificant, in good agreement with predictions of the same theoretical model.

In the present study, we use a constant number of pulses (10) and repetition rate (20 Hz), and investigate systematically the influence of single-pulse fluence and pulse duration on dermal coagulation depth and epidermal damage obtained with repetitive Er:YAG laser irradiation in the same animal model. In an effort to reduce epidermal injury, cryogen spray cooling (CSC) of the skin surface was added for some test sites. Histology specimens were harvested at several intervals up to 4 weeks post-irradiation, in order to monitor the wound healing response and formation of new collagen.

The results demonstrate that repetitive irradiation with an Er:YAG laser can yield dermal collagen coagulation and neo-collagen formation at depths very similar to those observed after CO_2 LSR, while at the same time preventing complete ablation of the epidermis. This may be a viable approach toward minimally ablative Er:YAG LSR.

STUDY DESIGN/MATERIALS AND METHODS

Laser Irradiation and Cryogen Spray Cooling (CSC)

In this study, we utilized a dermatological Er:YAG laser (Fidelis by Fotona, Ljubljana, Slovenia), which offers the user a choice of four pulse durations from 100 μs to nearly 1 ms with a nearly square temporal profile, at repetition rates up to 50 Hz. A manufacturer-provided customization of the system enabled us to generate irradiation sequences with arbitrary pulse durations, repetition rates, and numbers of pulses. With few exceptions, we used sequences of 10 pulses at a 20-Hz repetition rate and two pulse durations; one shorter ($t_p = 150 \mu\text{s}$; full width at half-

maximum), and the other longer (550 μs) than the typical pulse duration of dermatological Er:YAG lasers ($\sim 300 \mu\text{s}$).

A diverging-beam dermatological handpiece was used at the working distance to yield a nominally 3-mm laser spot diameter. The actual fluence (i.e., energy density) values were determined by scanning a 0.36-mm diameter pinhole and a pyroelectric detector (J25LP-Erbi, Molectron, Portland, OR), modified by the manufacturer to accommodate the longer pulse durations, across the laser beam. The pulse fluences presented herewith are the average values in the central 0.7–0.9 mm of the laser spot, which corresponds to the field of view in the presented micrographs (Figs. 3 and 4). The peak fluences were 6–10% higher than the reported values. For a few examples, the laser pulses were attenuated externally with one or two microscope slides. (Note that the fluence values reported in our preliminary presentations were underestimated [1,2], as they were estimated from the manufacturer-specified laser footprint of 2.8 mm. Subsequent measurements of the laser beam profile yielded spot diameters around 2 mm at half-maximum, depending on the laser operation regimen.)

For some lesions, cryogen spray cooling (CSC) was applied in conjunction with laser irradiation in order to minimize epidermal injury. The cryogen used was tetrafluoroethane ($\text{C}_2\text{H}_2\text{F}_4$, R134a), which is FDA approved for use in dermatology. This cryogen has a boiling point at -26°C . However, after atomization by the 0.7-mm diameter nozzle, cryogen spray droplets rapidly evaporate during flight to the skin surface [24,25], and consequently cool to around -50°C [24–28]. A digital delay generator and a function generator (DG535 and DS345 by Stanford Research Systems, Sunnyvale, CA) were used to control the cryogen delivery valve and the laser, such that 6-ms CSC spurts were delivered just prior to, during, or following each laser pulse in the sequence.

Before designing the protocol for the in vivo experiment, transmission of the Er:YAG laser radiation through the cryogen was evaluated. Energy of a laser pulse transmitted through a few millimeter thick layer of liquid cryogen, accumulated in a glass cup after a couple of seconds long spurt, was measured at $80 \pm 2\%$ relative to that through an empty cup (at 2 J/cm^2 ; around 90% at 7 J/cm^2). Temporally resolved transmission signals as detected by an InAs photodiode (J12-18C-RO1M, EG & G Optoelectronics, Salem, MA) through the empty and liquid-cryogen-filled cup are presented in Figure 1 (curves *a* and *b*, respectively). Apart from the undetermined change in Fresnel reflection and possible scattering by cryogen droplets, the laser fluence delivered to the skin is therefore only minimally affected by the short cryogen spurts used in this study.

Animal Model and Histology

Four Sprague-Dawley female rats ($m \sim 300 \text{ g}$) were used as the in vivo animal model, in accordance with a protocol approved by the Institutional Animal Care and Use Committee at the University of California, Irvine. After being housed in a pathogen-free animal facility and fed a base diet and water, the rats were anesthetized with

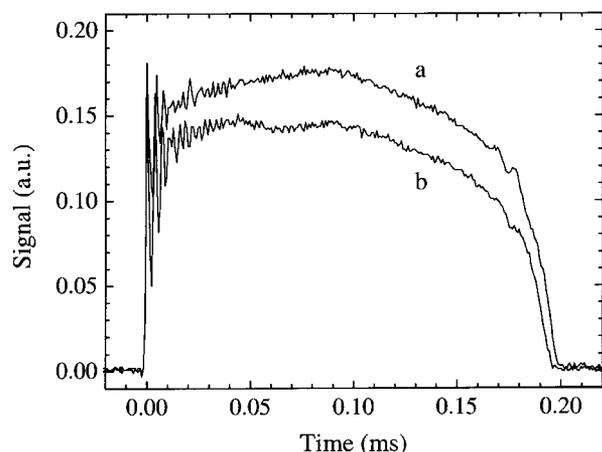


Fig. 1. Temporally resolved transmission of an Er:YAG laser pulse through an empty glass cup [oscilloscope trace (a)] and the same cup containing a few millimeters thick layer of liquid cryogen (b).

an intraperitoneal injection of ketamine (87 mg/kg) and xylazine (13 mg/kg). The hair on each animal's back was trimmed and subsequently removed after topical application of a depilatory lotion (Nair, Carter-Wallace, New York).

A total of 12–19 lesions were induced on each rat using various laser irradiation parameters. A Silvadene cream (PAR Pharmaceutical, Spring Valley, NY) was applied to the wounds in 12–24-hour intervals for 5 days. Four-millimeter punch biopsy specimens were taken acutely (within 1 hour), 1 day, 5 days and 4 weeks post-laser irradiation. The tissue samples were fixed in 10% formalin, embedded in paraffin, sectioned perpendicular to the skin surface and stained with regressive hematoxylin and eosin (H & E). India Ink was used to mark the lesions that were biopsied 4 weeks after exposure.

Three evaluators blinded to the irradiation protocol evaluated the 1-hour and 1-day specimens for depth of collagen coagulation and epidermal preservation. Using an optical microscope with a calibrated reticle eyepiece (Olympus, model BH-2), each evaluator assessed the coagulation depth (measured from the epidermal–dermal junction) in the most affected part of 3–6 histologic sections from each biopsy. The zone of dermal coagulation was identified primarily by hyalinization (glass-like appearance) of collagen fibers, as they become fused due to thermal injury [29,30]. Tinctorial change was also present and helped determine the depth of thermal damage.

Epidermal preservation was graded with six integer values, from 0 for no visible damage, to 5 for completely ablated epidermis – the complete scale description is given in Table I. The 5-day specimens were evaluated for re-epithelialization only, whereas the 4-week specimens were used to determine the depth of neo-collagen formation, a distinct zone of dense and parallel collagen fibril deposition with an increased number of fibroblasts (easily identified by their blue nuclei). The presented results are arithmetic means of all the values reported for the same

TABLE 1. Six-level Scale used to Grade the Epidermal Damage

Epidermal damage	Grade
No visible damage	0
Minimal damage	1
Moderate damage	2
Severe damage	3
Mostly ablated	4
Completely ablated	5

lesion ($n = 9–12$, from the three reviewers and multiple sections), and the error bars represent the standard error of the mean.

RESULTS

Figure 2 presents a photograph of acute laser lesions, induced using various irradiation parameters. Note the variations in general appearance and the absence of bleeding in all lesions. In some examples, CSC was applied just prior to and during (“pre-cooling”), or during and following each laser pulse (“post-cooling”). A complete set of irradiation parameters for these lesions is listed in Table 2.

Figure 3a shows a histological section of non-irradiated skin (control), displaying the reference epidermal thickness ($\sim 13 \mu\text{m}$) and structure of intact dermis in our animal model. Figure 3b presents an acute biopsy, demonstrating deep collagen coagulation extending to approximately $270 \mu\text{m}$ below the epidermal–dermal junction (marked with an arrow). The extent of thermal damage is evidenced by the strongly hyalinized appearance and tinctorial change in this region, distinct from the basophilic stained superficial layer. All results presented in this section were obtained with sequences of 10 laser pulses at a repetition rate of 20 Hz. This specific lesion was induced with $550\text{-}\mu\text{s}$ pulses at a single-pulse fluence of $F = 1.6 \text{ J/cm}^2$ and no CSC. The clearly visible epidermal remnants (in part detached during preparation of the histologic section) demonstrate that the epidermis, while thermally injured, was not completely ablated during laser irradiation. The evaluators graded the epidermal damage as 3.8 ± 0.6 on our 0-to-5 scale (see Table 1). There was essentially no dermal ablation, which accounts for the absence of bleeding in the surgical field. At a higher fluence ($F = 4.3 \text{ J/cm}^2$, $t_p = 550 \mu\text{s}$; Fig. 3c), the epidermis is completely ablated, whereas the coagulation depth is diminished (to $\sim 210 \mu\text{m}$; see the arrow).

When 6-ms CSC spurts were applied just prior to (and through) each laser pulse in the sequence, the epidermis was not completely ablated, despite the relatively high incident fluence (Fig. 3d; $F = 3.4 \text{ J/cm}^2$, $t_p = 550 \mu\text{s}$). The epidermal damage was graded 4.2 ± 0.8 . The dermal coagulation depth was significantly reduced as compared to irradiation without CSC, but still considerable at $140 \pm 10 \mu\text{m}$ (arrow). The collagen coagulation pattern is

rather non-homogeneous in appearance, and the transition between coagulated and normal collagen structure deeper in the dermis is much more gradual than in Figure 3b.

Micrographs of histological sections presented in Figure 4 illustrate the chronology of the wound healing response. One day post-treatment (Fig. 4a; $F = 1.6 \text{ J/cm}^2$, no CSC), histology is essentially identical to that of acute specimens, displaying an intact basophilic staining zone. Little or no inflammatory cells are noted deep to the zone of dermal necrosis. Five days post-treatment, all lesions were completely re-epithelialized (Fig. 4b; same laser parameters as in Fig. 4a). All histology specimens from biopsies taken 4 weeks post-irradiation featured a distinct zone of tightly packed parallel collagen fibrils and an increased number of fibroblasts immediately underlying the epidermis. The depth of this neo-collagen zone was observed to be up to $250 \text{ }\mu\text{m}$ (Fig. 4c; $F = 2.3 \text{ J/cm}^2$, no CSC).

An overview of dermal coagulation depths induced with various treatment parameters is presented in Figure 5a as a function of single-pulse fluence. In the included examples, the irradiation sequences consisted of 10 pulses at a repetition rate of 20 Hz, and the laser pulse duration was either 150 (open squares) or 550 μs (solid circles). In order to complement the data sequence, one histologic sample prepared in our previous study was included in the analysis ($F = 0.8 \text{ J/cm}^2$; marked with a + sign in Figs. 5a, b) [21]. Star symbols mark the examples where CSC was applied in combination with 550- μs pulses. A maximum coagulation depth of $270 \pm 16 \text{ }\mu\text{m}$ was obtained with the 550- μs pulses at 1.6 J/cm^2 and no CSC. The coagulation depth gradually diminishes with increasing fluence values. Less deep coagulation is indicated for the 150- μs pulses. Similarly, the application of CSC significantly reduced the coagulation depth.

A comparison plot of the epidermal damage, graded subjectively from the same histologic sections, is presented in Figure 5b. (See Table 1 for description of the scale). While the large error bars prevent us from drawing statistically significant conclusions, the data suggest that CSC

reduced the epidermal damage, and that the 150- μs laser pulses may cause somewhat more epidermal damage as compared to the 550- μs ones. With the latter, complete epidermal ablation is observed at single-pulse fluences above 3 J/cm^2 .

Figure 6 presents an overview of dermal coagulation depths and epidermal damage, as determined from biopsies taken 1 day post-treatment. Although the data are rather scarce, they seem to match the values and trends observed in acute biopsies. A more detailed discussion of these results in relation to acute histology is presented in the following section.

DISCUSSION

As noted earlier, we have previously demonstrated the feasibility of deep collagen coagulation without complete epidermal ablation by rapidly stacking low fluence Er:YAG laser pulses [21]. This effect results primarily from the very strong absorption of the 2.94- μm radiation in skin, which creates a very high temperature gradient in the superficial interaction layer. As a result, the superficial temperature rise relaxes very rapidly, which leads to a moderate surface temperature increase from pulse to pulse during repetitive laser exposure [12,22,31]. In contrast, deeper in the dermis the deposited heat accumulates due to much slower temperature relaxation in this region. As a result, theoretical models have predicted that collagen coagulation up to 200–300 μm deep could be achieved without exceeding the ablation temperature at the skin surface [12,22]. The present study demonstrates that repetitive Er:YAG irradiation can cause not only deep collagen coagulation, but also subsequent neo-collagen formation, very similar to that observed after CO_2 LSR. Several aspects of the methodology, as well as the results, deserve a further comment.

The thermal damage pattern in most biopsies was found to be quite non-homogeneous. We assessed the collagen coagulation depth and epidermal damage in the most severely affected 0.5 mm of the histological section, presumably corresponding to the center of the laser spot. The

TABLE 2. Laser Irradiation Parameters used to Induce Lesions in Figure 2

Lesion number	Number of pulses	Repetition rate (Hz)	Pulse duration (μs)	Pulse fluence (J/cm^2)	CSC timing
1	10	20	550	1.70	—
2	10	20	550	1.20	—
3	10	20	550	0.85	—
4	20	20	550	0.85	—
20	10	15	550	2.10	—
6	10	20	150	1.20	—
7	10	20	150	0.90	—
8	10	20	150	0.60	—
9	10	20	550	1.70	Pre-cooling
10	10	20	550	1.70	Post-cooling
12	10	20	550	1.20	Pre-cooling
21	10	15	550	2.10	Pre-cooling



Fig. 2. Photograph of acute laser "burn" lesions, induced using various irradiation parameters (laser pulse duration, repetition rate, number of pulses in the sequence, CSC) as listed in Table 2. No bleeding was observed during irradiation.

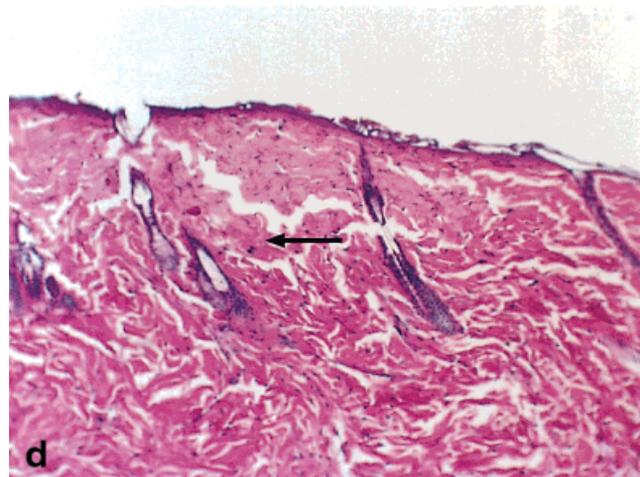
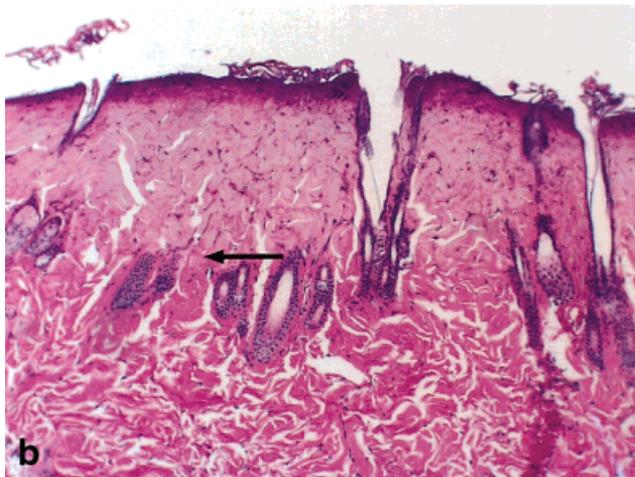
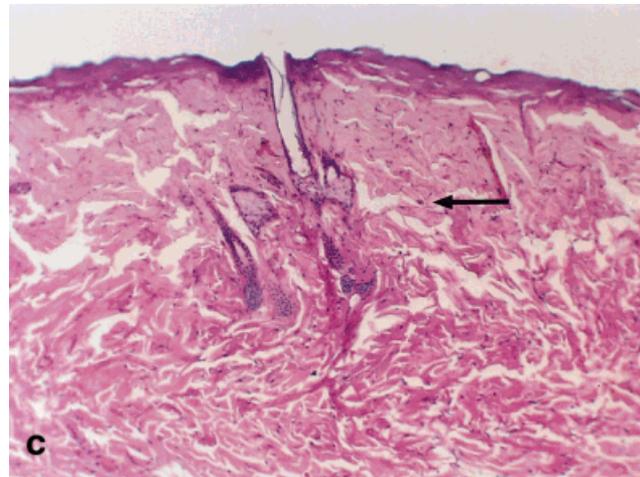
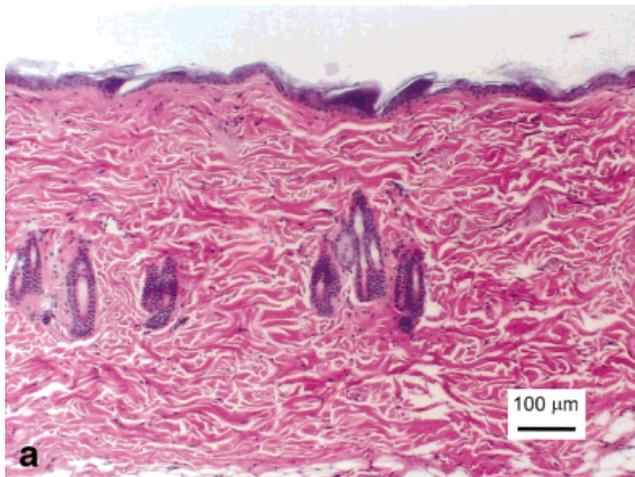


Fig. 3. Histological sections from acute biopsies (regressive H & E stain). The scale of these photographs is indicated by the 100- μm bar in Figure 3a (original magnification: 100 \times). (a) Non-irradiated skin (control); (b) Deep collagen coagulation ($\sim 270 \mu\text{m}$; see the arrow), obtained with a sequence of ten 550- μm pulses (single-pulse fluence $F = 1.6 \text{ J/cm}^2$, repetition rate $\nu = 20 \text{ Hz}$) and no CSC; (c) At a higher fluence ($F = 4.3 \text{ J/}$

cm^2), the epidermis is completely ablated, and the coagulation depth is diminished (to $\sim 210 \mu\text{m}$; arrow); (d) With 6-ms CSC spurts applied just prior to and during each laser pulse, the epidermis is preserved even at a relatively high fluence ($F = 3.4 \text{ J/cm}^2$). The coagulation layer is less thick, but still considerable (arrow).

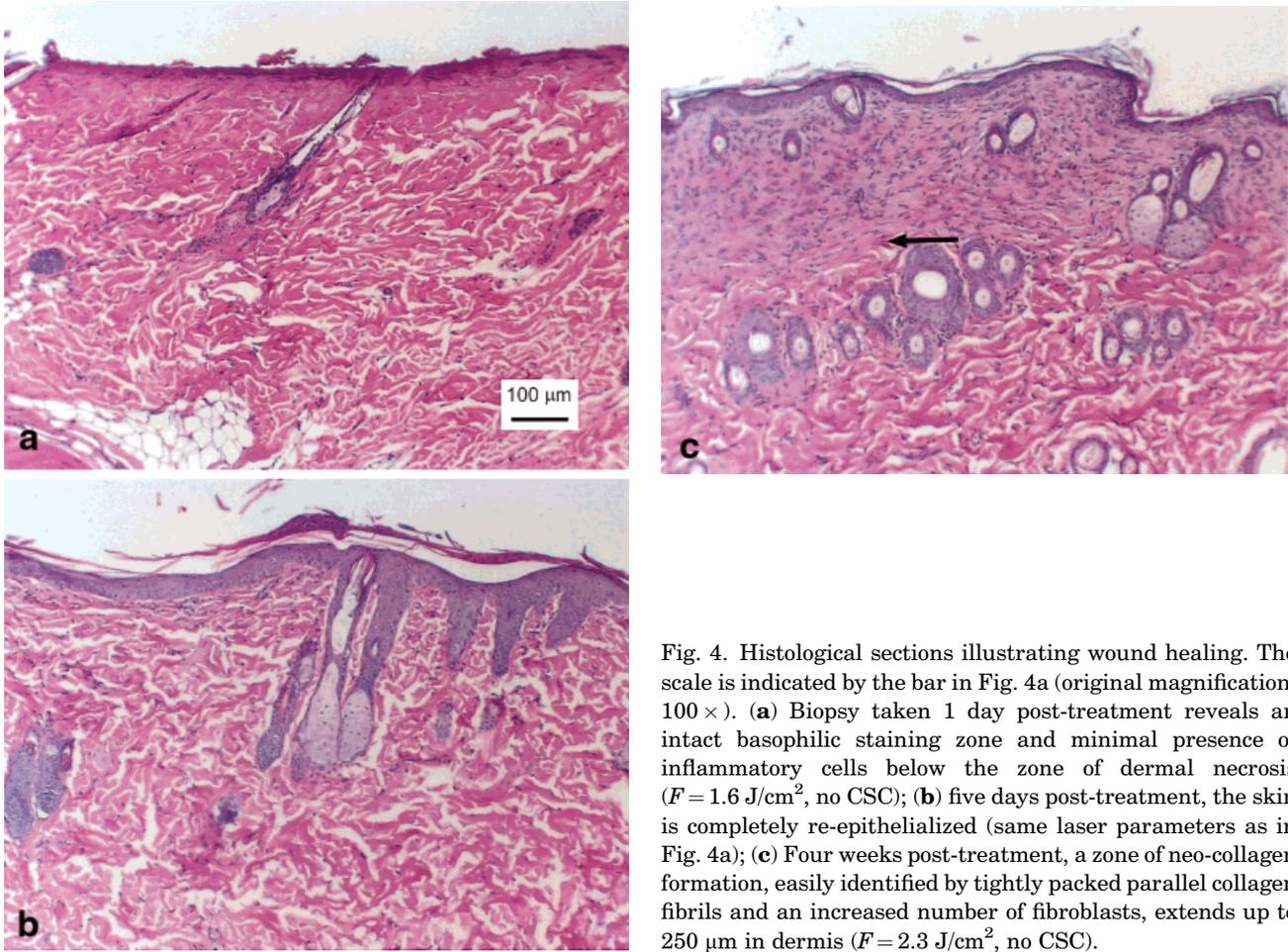


Fig. 4. Histological sections illustrating wound healing. The scale is indicated by the bar in Fig. 4a (original magnification: $100\times$). (a) Biopsy taken 1 day post-treatment reveals an intact basophilic staining zone and minimal presence of inflammatory cells below the zone of dermal necrosis ($F = 1.6 \text{ J/cm}^2$, no CSC); (b) five days post-treatment, the skin is completely re-epithelialized (same laser parameters as in Fig. 4a); (c) Four weeks post-treatment, a zone of neo-collagen formation, easily identified by tightly packed parallel collagen fibrils and an increased number of fibroblasts, extends up to $250 \mu\text{m}$ in dermis ($F = 2.3 \text{ J/cm}^2$, no CSC).

transition between coagulated and normal collagen was gradual with no distinct border between the two zones, in agreement with previous reports involving repetitive laser irradiation [21,32]. As a result, it was difficult to determine the collagen coagulation depth unambiguously. For future studies, it would be very helpful to have an objective measurement of residual thermal damage. Due to our previous discouraging experience [21], we did not use optical polarization microscopy. In ongoing studies at our institution (BLIMC), attempts are underway to correlate histology-determined depths of thermal injury with polarization-sensitive optical coherence tomography (PSOCT) [33,34], and microscopic retardance imaging (LC-PolScope by CRI, Boston, MA).

In earlier studies, a relatively dense band of inflammatory cells (polymorphonuclear leukocytes) underlying the zone of dermal necrosis was observed, and used as a marker of thermal damage depth after CO_2 laser exposure [32,35]. In contrast, our histology 1 day post-treatment was essentially identical to the acute one, having an intact basophilic staining zone and little or no inflammatory cells. The reason for this discrepancy is unclear. Perhaps, our biopsies may have been taken somewhat earlier than those in previous reports and the inflammatory reaction

may not have had enough time to develop. Alternatively, the nature of thermal injury induced by repetitive Er:YAG exposures may be different from that after CO_2 LSR. While it could be argued that a different wound healing response may result in a different clinical result, the proven ability of repetitive Er:YAG laser exposure to induce a significant zone of neo-collagen formation suggests that rhytid improvement would likely be achieved in a clinical situation.

Ross et al. [32] recently reported that, after stacking three CO_2 laser pulses on the same site, tissue necrosis and subsequent neo-collagen formation extended deeper than the acutely hyalinized collagen zone. However, in our study, the maximal depth of neo-collagen formation observed 4 weeks post-repetitive Er:YAG laser exposure was approximately $250 \mu\text{m}$ (Fig. 4c; $F = 2.3 \text{ J/cm}^2$, $t_p = 550 \mu\text{s}$, no CSC). This value is within the experimental error of the collagen coagulation depths assessed from acute and 1 day post-irradiation histologies at similar laser parameters (see Figs. 5a and 6a), which suggests that the latter is a good indicator of subsequent dermal rejuvenation.

The present study provides insight into the influence of pulse fluence and duration on the collagen coagulation depth achieved by repetitive Er:YAG laser irradiation. At

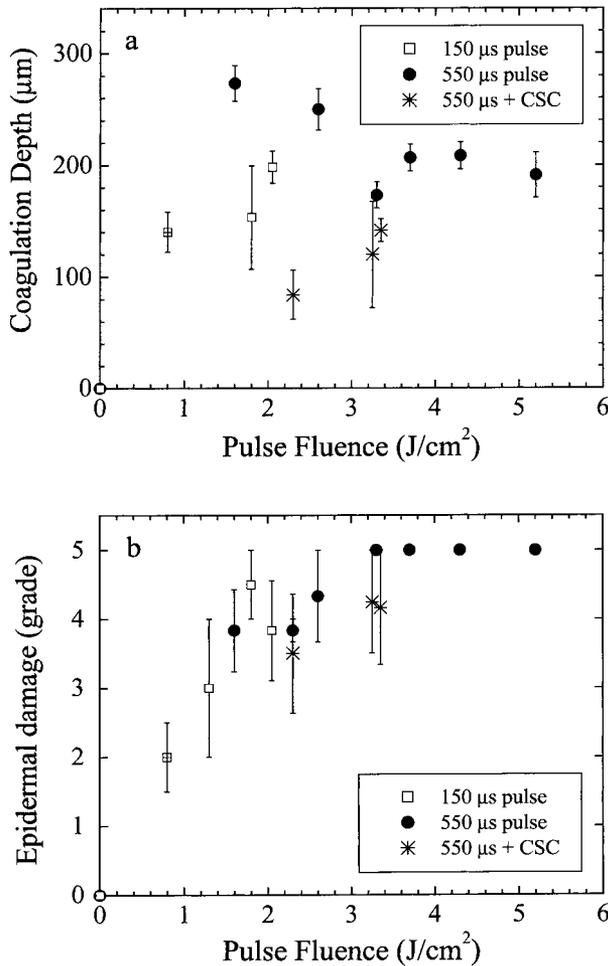


Fig. 5. (a) Acute depth of dermal coagulation (measured from the epidermal-dermal junction) as a function of single-pulse fluence. The irradiation sequence consisted of 10 pulses at repetition rate of 20 Hz, the pulse duration was either 150 (\square) or 550 μs (\bullet). Stars ($*$) represent examples with 550- μs pulses in combination with CSC; and (b) Epidermal damage in the same lesions, graded according to a six level scale (see Table 1 for description). Error bars represent standard errors of the mean.

the 550- μs laser pulse duration (solid circles), the collagen coagulation depth gradually decreases with increasing single-pulse fluence (Fig. 5a). Such a trend was predicted from theoretical considerations of thermo-mechanical laser ablation [36–38], and results from increased ablation efficiency at higher fluences which diminishes the residual thermal damage. With repetitive irradiation, the heat deposition at lower pulse fluences is likely enhanced due to skin desiccation between subsequent laser pulses, which removes the main absorber of the Er:YAG laser radiation [38,39]. Consequently, the optical penetration depth increases, resulting in further diminished ablation efficiency, enhanced deposition of heat, and increased thermal injury. The threshold fluence for skin ablation with the Er:YAG laser is between 0.5 and 1 J/cm^2 [2–4,39], as

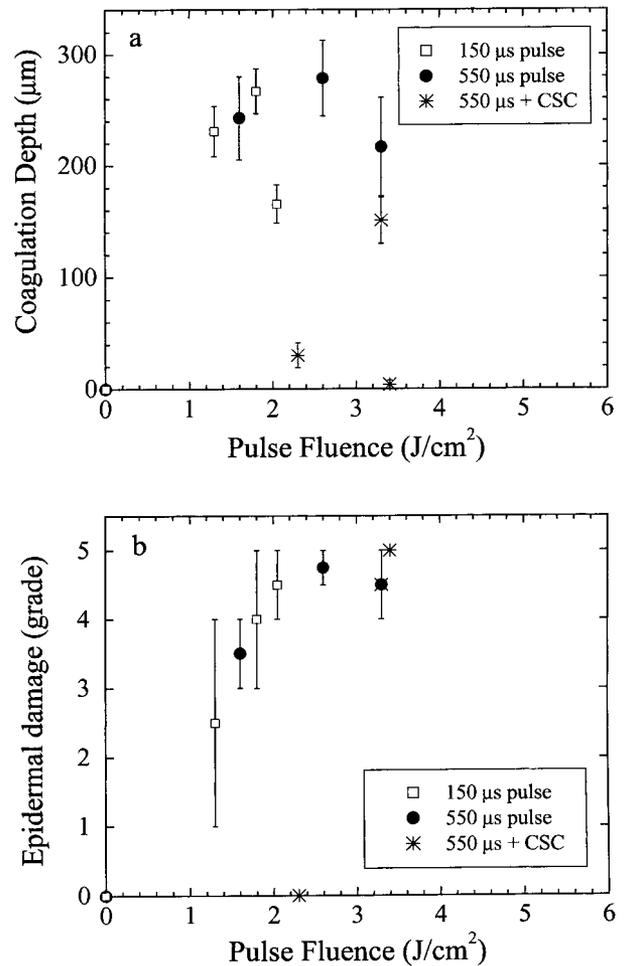


Fig. 6. Same as Figure 5, but from biopsies taken 1 day post-treatment.

evidenced also by the epidermal damage data in Figures 5b and 6b.

For the shorter pulse duration (150 μs), the results obtained from acute biopsies indicate less thermal damage (Fig. 5a, open squares) than with 550 μs . However, 1-day post-treatment histology (Fig. 6a) indicates significantly deeper coagulation depths for the 150- μs pulses, within the range of the results for the longer pulses. Since the coagulation depths with 550- μs pulses do not vary between the acute and 1-day biopsies, we attribute the discrepancy in the 150- μs results to sampling error. In particular, with the laser burn lesions that feature only moderate epidermal damage, it is conceivable that biopsy specimens may have been obtained and/or sectioned slightly off-center. Given the small laser spot size, the coagulation depth could, therefore, be considerably underestimated. In fact, in examples where more than one biopsy was available for the same laser exposure parameters, we sometimes observed large variations in assessed coagulation depth and epidermal damage. As we have described plausible reasons for underestimating the coagulation depth, while we are unaware of any mechanism that would lead to an

artificial appearance of excessive coagulation depth, we have included in the analysis only the biopsies displaying the deepest (average) coagulation depth for a given combination of irradiation parameters.

We therefore conclude that our results do not indicate a significant difference in coagulation depth between pulse durations of 150 and 550 μs , at single-pulse fluences of 1–2 J/cm^2 . This finding is contrary to the notion that longer pulse durations should result in a greater degree of thermal damage. However, a previously published theoretical model of Er:YAG laser irradiation of human skin predicted that below the ablation threshold, the coagulation depth would increase only marginally with pulse duration at constant pulse fluence [12,22]. According to this model, it is primarily the increase in ablation threshold that enables a larger heat deposition at longer pulse durations. We address briefly this effect also in the theoretical consideration in Part II of this article [40].

The application of CSC resulted in somewhat reduced epidermal damage (Fig. 5b) but concomitantly also reduced depth of collagen coagulation (Fig. 5a). Nevertheless, in the example presented in Figure 3d ($F = 3.4 \text{ J}/\text{cm}^2$, $t_p = 550 \mu\text{s}$, 6-ms CSC spurts), the coagulation depth is still considerable at $\sim 140 \mu\text{m}$, while most of the epidermis, albeit damaged, is preserved. This is expected to act as biological dressing, reducing immediate inflammation and shortening the healing time after LSR, as well as reducing the risks associated with ablative LSR modalities. By optimizing the irradiation parameters (fluence and duration of individual laser pulse, number of pulses and repetition rate of the sequence), it might be possible to further reduce epidermal damage while maintaining the same extent of collagen coagulation.

In order to better illustrate the relationship between improved epidermal preservation and reduced dermal coagulation in repetitive Er:YAG laser irradiation complemented with CSC, we plot in Figure 7 the epidermal damage versus the coagulation depth, as observed with various irradiation parameters in this study. The graph shows that, among the tested irradiation parameters, deepest dermal coagulation with respect to epidermal damage was achieved with 550- μs pulses at $F = 1.6 \text{ J}/\text{cm}^2$ and no CSC (the right-most data point in the plot). The data also indicate that significantly deeper dermal coagulation (at similar level of epidermal injury) can be achieved with 550 than with 150 μs pulses – most likely due to the higher ablation threshold at longer pulse durations. Unfortunately, the scarcity of data points in each of the three data sets (150 μs pulse, 550 μs pulse, and 550 μs with CSC) and rather large error bars prevent us from assessing the trends more accurately and reliably. Nevertheless, a large difference can clearly be seen between the data point obtained with the lowest single-pulse fluence ($F = 0.8 \text{ J}/\text{cm}^2$; open square marked with a + sign) and the rest of the data ($F \geq 1.6 \text{ J}/\text{cm}^2$), illustrating the fundamental division between the sub-ablative and ablative irradiation. Based on theoretical considerations that the depth of thermal damage should increase with applied fluence in the first, but decrease in the latter regime,

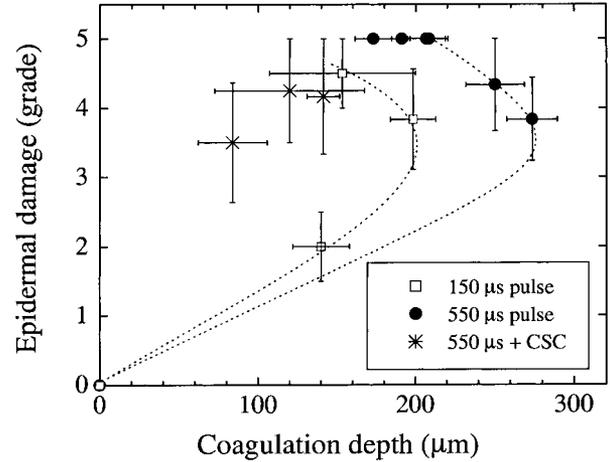


Fig. 7. Epidermal damage (graded in six levels) vs. dermal coagulation depth from the acute biopsies in Fig. 5 (10 pulses, 20 Hz). The laser pulse duration is either 150 (\square) or 550 μs (\bullet). Stars (\ast) represent 550- μs pulses in combination with CSC. The dotted lines are tentative trends for the two datasets without CSC, based on theoretical considerations.

tentative trends are plotted in the graph (for the two pulse durations without CSC), further emphasizing the importance of working close to the ablation threshold in order to maximize dermal versus epidermal thermal injury in LSR.

It can be stated safely, however, that in the treatment modality under study, CSC did not provide a spatially selective protection of the epidermis, while permitting the same thermal effect on the deeper skin layers, as is the case when used in combination with deeper-penetrating wavelengths. This can be attributed to the very superficial absorption of the Er:YAG laser radiation in skin, much shallower than the observed coagulation zone. The majority of dermal damage is therefore caused by diffusion of heat from the interaction volume after the pulse has ended. As the CSC spurts cool the superficial skin layer, they counteract the heating effect of the Er:YAG laser, thus diminishing the coagulative effects in deeper skin layers. A more detailed analysis of this effect, using a simple theoretical model of laser heating and cryogen cooling of skin, is presented in Part II of this article [40].

Based on presented study, which demonstrated collagen coagulation and regeneration to the appropriate depth and degree, we are currently preparing a study of repetitive Er:YAG irradiation as a method of minimally ablative LSR in humans. Rat skin, namely, differs from human skin in that it does not have a well-defined papillary dermis or subepidermal vascular plexus [35]. Further, while the thickness of rat dorsal skin approximates that of human peri-orbital facial skin, the increased thickness of human facial skin in other areas may affect the speed and nature of wound healing. Despite these differences, we are hopeful that repetitive Er:YAG LSR in humans may provide rhytid improvement, healing times and incidence of adverse effects similar or even superior to CO_2 LSR.

CONCLUSION

Irradiation with a sequence of ten 550- μs Er:YAG laser pulses with fluences of 1.5–2.5 J/cm^2 (repetition rate 20 Hz) results in acute dermal collagen coagulation to a depth up to 250 μm , without complete epidermal ablation. The coagulation depth gradually decreases with increasing single-pulse fluence (up to 5.2 J/cm^2). At a constant single-pulse fluence (1–2 J/cm^2), no significant difference in coagulation depth was observed between the 150 and 550 μs pulse durations. However, significantly deeper dermal coagulation at a similar level of epidermal injury can be achieved with 550 than with 150 μs pulses – most likely due to the higher ablation threshold at the longer pulse durations. Application of CSC decreased epidermal damage, but also diminished the coagulation depth, so that spatially selective epidermal protection could not be achieved. Four weeks after irradiation, neo-collagen formation was evident in all biopsies at depths up to 250 μm .

Dermal collagen coagulation and neo-collagen formation to depths similar to those observed after CO_2 laser resurfacing can be achieved without complete ablation of the epidermis by rapidly stacking Er:YAG laser pulses. This presents a possible approach towards minimally ablative LSR which may offer sufficient rhytid improvement while reducing risks associated with traditional LSR modalities.

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