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Imaging of the Irradiation of Skin With a Clinical CO₂ Laser System: Implications for Laser Skin Resurfacing

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Background and Objective: Several published reports describe the benefits of using the carbon dioxide laser for cutaneous resurfacing. The mechanisms on which skin resurfacing work are still not completely understood. This study was performed to obtain quantitative and qualitative information describing the thermal response of skin during high-energy, short-pulsed CO₂ laser irradiation.

Study Design/Materials and Methods: A Tissue Technologies TruPulse CO₂ laser was used to irradiate an in vivo rat model. The laser parameters that were used were a 100- μ s pulsewidth, a 1-Hz repetition rate, a 3 mm $\times$ 3 mm square spot size, and 2.4 J/cm² and 3.9 J/cm² radiant exposures. A 3–5 μ m thermal camera was used to obtain temperature information during irradiation. Single spots were irradiated with one pulse, and the temperature-time history was obtained. In a different experiment, 15 pulses were applied to single spots, and both thermal and video images were obtained.

Results: Irradiation with one pulse at 2.4 J/cm² and 3.9 J/cm² led to peak temperatures >100°C. The temperature relaxation time was ~25–150 ms. Multiple-pulse irradiation at 2.4 J/cm² led to a slight rise in the peak temperature with each pulse. At 3.9 J/cm², the peak temperature increased with successive pulses until pulse 10, after which the peak temperature oscillated between 300 and 400°C. Video images showed concurrent burning events that occurred during pulses 10–15.

Conclusion: Temperatures >100°C were measured during CO₂ laser irradiation of skin. Pulse stacking can lead to peak temperatures approaching 400°C and to tissue charring with as few as three stacked pulses. It is crucial for the physician to

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manipulate the laser handpiece at parameters with which he or she can avoid pulse overlap. *Lasers Surg. Med.* 23: 185–193, 1998. © 1998 Wiley-Liss, Inc.

Key words: ablation; carbon dioxide laser; pyrolysis; skin resurfacing; thermography

INTRODUCTION

The theoretical advantages of the pulsed CO₂ laser for skin resurfacing are that it: (1) ablates tissue precisely (10 μm per pulse at a radiant exposure of 5 J/cm² [1]); (2) leaves a minimal zone of residual thermal necrosis ($\sim 50 \mu\text{m}$) when operated at high irradiances ($>1,000 \text{ W/cm}^2$) and short exposure times [2,3]; (3) seals small nerve endings, which may lead to a reduction in postoperative pain [4]; (4) seals small lymphatics, resulting in less postoperative edema [5]; (5) increases the operative speed [6]; and (6) seals small blood vessels, leading to decreased hemorrhage, improved vision in the surgical field, and less postoperative bruising and swelling.

The short-pulsed aspect of these resurfacing lasers is derived from the idea of selective photothermolysis [7]. An assumption is made that if the pulsewidth of the laser (τ_p) is less than the thermal relaxation time (τ_r), then heat is confined to the target during the laser pulse [8,9], and heat conduction occurs only after the pulse ends. The thermal relaxation time is defined as the time necessary for the temperature distribution of the irradiated target to decrease by 63%. For CO₂ laser irradiation of skin, τ_r is estimated to be between 695 μs and 950 μs [2,9].

The optical-thermal response of skin to laser resurfacing is complicated by the need for multiple passes in order to obtain the desired clinical effect. In this report, we examine the response of a single spot to irradiation with multiple pulses. We hypothesize that pulse energy sufficient enough to remove a few microns of skin leaves a thin, thermally altered layer of tissue. The thermal response of subsequent pulses will depend on any residual temperature increase from the preceding pulse, the physical condition and integrity of the surface, and the local hydration level of the exposed tissue.

Quantitative and objective data can provide a better understanding of the thermodynamic mechanisms underlying skin resurfacing. This study examines the thermal response of skin to CO₂ laser irradiation in terms of an *in vivo* study performed on a rat model. The implications of the

thermographic and video images that were obtained during pulsed CO₂ irradiation are addressed and discussed. The effects of pulse stacking are examined both quantitatively and qualitatively.

MATERIALS AND METHODS

Animal Model

In vivo experiments were performed on “Fuzzy” rats (Sprague Dawley strain), which are immunologically competent rats that do not produce coarse hair but are covered with fine undercoat hair. They were anesthetized with a mixture of Ketamine and Rompun (4:3 ratio, 0.1 mL/100 g body weight). The hair on the backs and flanks of the rats was removed by applying a depilatory cream (Nair) with cotton-tipped applicators and then by wiping with dry gauze.

During the experiments, the rats were kept on a heating pad in order to counteract the hypothermic effects of the anesthesia and of the absence of body hair. The depth of anesthesia was monitored constantly by checking heart rate, breathing rate, and the toe-pinch response.

After the experiments were completed, the rats were euthanized in a carbon dioxide chamber according to the required procedure of The University of Texas at Austin Institutional Animal Care and Use Committee.

Laser System

The laser system used for the experiments was the TruPulse CO₂ laser (Tissue Technologies, Albuquerque, NM). It is characterized by: (1) the emission of relatively short pulses ($\tau_p = 65\text{--}125 \mu\text{s}$) and (2) a 3 mm $\times$ 3 mm square spot size at the focus with a uniform irradiance profile over the entire spot (called the “Mesa Mode”). An articulated arm served as the delivery system for the TruPulse laser, and a 3.5-inch focal length lens focused the beam to a spot size of 3 mm $\times$ 3 mm at the treatment focal plane. For ease of use, the tip of the laser handpiece was in the treatment focal plane.

The energy output of the laser was measured with a joulemeter (Labmaster, Coherent Laser

Group, Palo Alto, CA) placed at the focus of the laser handpiece. The radiant exposure was calculated by dividing these energy measurements by the spot area (9 mm²). A pulse duration of 100 μ s was used for all of the experiments.

Thermal Camera

Surface temperatures were measured with a 3–5 μ m band-limited thermal camera (Model 600L, Inframetrics, Billerica, MA) composed of a HgCdTe detector and two oscillating mirrors that scanned the camera's field of view (FOV) horizontally and vertically. To reduce the effects of thermal noise, the detector was cooled with liquid nitrogen to -196°C . The radiant energy that the camera detects was converted to a voltage; this voltage was displayed as a grayscale or false color image on the video display of the camera.

The thermal camera imaging mode was the fast line scan. In this setting, the vertical oscillating mirror was frozen into place, and the horizontal scanning mirror scanned repeatedly across the same line in the center of the camera's FOV. The time for each scan across this line was 125 μ s, and 256 samples were taken across this line. Using this mode, a time-temperature history of a single line was obtained.

To reduce the FOV of the thermal camera, a 3 $\times$ telescope and a 9.5-inch focal distance close-up lens were attached to the camera. With the camera placed at a distance of 9.5 inches from the treatment plane, the camera's FOV was $\sim 3\text{ cm} \times 3\text{ cm}$. Internal calibration of the thermal camera compensated for the presence of the external optics. For our experiments, an emissivity of 1.0 was assumed [10].

Basic Experimental Setup

A diagram of the setup used in these experiments is shown in Figure 1. A heating pad was placed on a lab jack, and an anesthetized "Fuzzy" rat was placed on top of the pad. The thermal camera was placed 9.5" from the rat. The handpiece of the laser was measured and removed. A spacer of the same length was attached to the articulated arm; the tip of the applicator was placed in the same plane as the treatment focal plane. The handpiece was removed because its relatively large size blocked a significant portion of the camera's FOV during irradiation; the spacer was much smaller in size.

The thermal images were recorded with a Super VHS recorder (Diamond Pro, Mitsubishi, Japan) and were digitized and processed on a PC

equipped with a frame grabber. Microsoft Excel 97 and Kaleidagraph Version 3.08 software packages were used to convert the grayscale values into temperatures. To aid in the analysis of the videotapes, a frame counter was used to label each individual frame.

A fan was used to blow the ablation plume away from the scene during irradiation. The fan speed and the distance between the rat and fan were constant. The fan ensured that the measured peak temperatures were not due to the high temperatures of the ablation ejecta [11].

Temperature Response to a Single Pulse

A single pulse was applied to in vivo rat skin. Pulse energies of 215 mJ and 350 mJ (corresponding to radiant exposures (H_0) of 2.4 J/cm² and 3.9 J/cm², respectively) were used. The surface temperatures were measured with the thermal camera, and the temperature decay as a function of time was obtained. Prior to irradiation of the rat skin, burn paper was irradiated, and the camera was moved on its swivel mount so that its FOV was centered on the location of pulse impact. This ensured that the measured temperatures were obtained from the center of the laser spot [12].

Temperature Response to Multiple Pulses

To determine the peak temperatures due to pulse stacking on a single spot, 15 pulses were applied to a single location on the rat skin. The laser was set at a repetition rate of 1 Hz. No wiping was performed between pulses, and no extra time (besides the 1 second between pulses) was provided for the skin to cool. Radiant exposures of 2.4 J/cm² and 3.9 J/cm² were used. The peak temperature after each pulse was measured with the thermal camera.

Video Imaging of CO₂ Laser Ablation

A CCD camera (XC-75, Sony, Japan) was used to image the ablation of rat skin during multiple pulse exposure of a selected 3 mm $\times$ 3 mm site. The CCD camera provided images at a standard rate of 30 frames per second. The setup was identical to the one shown in Figure 1, with the CCD camera used in place of the thermal camera; no temperature measurements were taken during this set of experiments. A frame counter simplified analysis of the videotapes of these images.

The laser was set at a repetition rate of 1 pulse per second, and radiant exposures of 2.4 J/

Fig. 1. Schematic of the experimental setup used to measure surface temperatures. For video imaging, the thermal camera was removed from the setup and was replaced by a CCD camera.

cm² and 3.9 J/cm² were used. A total of 15 pulses was applied to the rat skin, and laser irradiation of the skin was performed in an uninterrupted fashion.

Typical ablation events such as ablation plume formation, burning, incandescence, pyrolysis, char formation, and carbonization were examined [13–15]. The onset of these events was correlated with the corresponding temperature events observed with the thermal camera.

RESULTS

The initial temperature of the rat skin varied between 22°C and 25°C. These temperatures were obtained by analyzing the thermal camera images that were generated prior to the onset of irradiation.

Temperature Response to a Single Pulse

Representative examples of the temperature response of in vivo rat skin to single ablative pulses are shown in Figure 2 for radiant exposures of 2.4 J/cm² (Fig. 2a) and 3.9 J/cm² (Fig. 2b). The peak temperatures were >100°C, and the peak temperatures associated with 3.9 J/cm² irradiation were consistently higher than those associated with 2.4 J/cm². The time at which the peak temperature occurred was at the end of the laser pulse (100 μs). For these radiant exposures, the temperature relaxation time (τ), the time required for the temperature to decrease from a maximum value to 37% of the maximum, was ~20–40 ms.

Temperature Response to Multiple Pulses

The mean peak temperatures measured as a function of pulse number are illustrated in Figure

Fig. 2. Peak temperature measured during single pulse irradiation of in vivo rat skin. Radiant exposures of (a) 2.4 J/cm² and (b) 3.9 J/cm² were used. The temperature relaxation time (see text) was 20–40 ms.

3 for radiant exposures of 2.4 J/cm² (Fig. 3a; $n = 15$) and 3.9 J/cm² (Fig. 3b; $n = 15$). Irradiation with 2.4 J/cm² pulses led to only a slight monotonic increase in peak temperatures with each successive pulse. The peak temperatures obtained during irradiation with 3.9 J/cm² pulses were much different. The temperature recorded after the first nine pulses increased with pulse number, but the change in peak temperature with successive pulses varied significantly. After the tenth pulse, the maximum temperature dropped; peak temperatures after pulses 11–15 changed in an oscillatory fashion as nucleation sites in the 3 mm $\times$ 3 mm area burned [14].

Video Imaging of CO₂ Laser Ablation

A CCD camera was used to obtain real-time images of CO₂ laser ablation of in vivo rat skin during multiple-pulse irradiation of a fixed position. Representative images of ablation produced with the first three pulses are shown in for radiant exposures of 2.4 J/cm² (Fig. 4a) and 3.9 J/cm² (Fig. 4b). Note that charring occurred upon the third pulse impact with 3.9 J/cm² pulses but was absent during irradiation with 2.4 J/cm² pulses until after five to eight pulses.

For both radiant exposures, a distinct popping sound was audible upon laser pulse impact on the skin; the magnitude of the sound was noticeably louder for 3.9 J/cm² pulses. Upon irradiation with the first pulse, debris that is blown away by the fan was seen. During irradiation of the skin with 2.4 J/cm², no pyrolytic events were visualized. With a radiant exposure of 3.9 J/cm², burn-

ing was seen after the tenth pulse (Fig. 5a), and the region of burning tissue increased in size with successive pulses (Fig. 5b–e).

The temperature events and corresponding ablation events that occurred during irradiation are shown in Figure 3.

DISCUSSION

The surface temperature of in vivo rat skin during CO₂ laser irradiation was measured with a band-limited thermal camera for single and multiple pulses. The ablation process was imaged with a CCD camera during multiple pulse application.

An excellent description of the features and limitations of Inframetrics thermal cameras was provided by Torres et al. [16]. They noted that the measured temperatures are underestimated for target sizes <2 mm. In these experiments, the laser spot size was 3 mm $\times$ 3 mm; thus spot size considerations were not applicable to these results.

Thermal cameras detect thermal emission from a finite volume of tissue [10]. If a significant thermal gradient exists within this layer of tissue, then the measured peak surface temperature will be less than the actual peak surface temperature if the measurement occurs before heat conduction “washes out” the gradient. Thus the peak temperatures presented here represent a lower bound on the actual peak temperature at the surface of the tissue.

Two radiant exposures (2.4 J/cm² and 3.9 J/

Fig. 3. Peak temperature response of single spot to multiple pulses. Radiant exposures of (a) 2.4 J/cm² and (b) 3.9 J/cm² were used. The repetition rate was 1 Hz. No wiping was performed between pulses. In (a), the onset of charring did not lead to a marked deviation in temperature change per pulse; the curve is smooth throughout. Also the beginning of grossly visible charring was variable (started at pulses 5–8). In (b), the onset of carbonization led to a sharp increase in temperature increase per pulse. Charring began always with the third pulse. Burning could be seen during pulses 10–15; note the fluctuation in peak temperatures.

cm²) were used for all experiments. These radiant exposures were mild compared to typical clinical radiant exposures of 3.5 J/cm² to 7.1 J/cm² [17].

CO₂ ablation of skin is considered to be largely water dominated [15,18]. Although water has a boiling point of 100°C at atmospheric pressure, peak temperatures above 100°C were measured. The high rates of heat generation due to the high absorption of CO₂ laser light lead to a rapid superheating of the tissue water with a large increase in subsurface pressure. When this pressure exceeds the tensile strength of the tissue, an explosive event occurs in which tissue particles are ejected outwards from the tissue surface. Thus the temperatures we measured are not unreasonable or unexpected [13,15].

Brugmans et al. [12] noted that the time needed for the cooling of in vitro tissue after a single nonablative CO₂ laser pulse was ~200 ms. After irradiation of in vivo rat skin with a single ablative CO₂ laser pulse, the time for total cooling of the skin was on the order of 25–150 ms (Fig. 2); thus in vivo skin cooled slightly faster than in vitro tissue. Convective effects due to blood flow, extracellular water, and local metabolism increase the rate of temperature decay in in vivo systems.

The effects of pulse stacking compromise the concept of high-energy, short-pulsed laser vaporization and result in an extended zone of thermal damage and in elevated temperatures due to cumulative thermal events. In general, temperature

superposition results from the act of stacking pulses on a single spot with the interpulse time being less than the total cooling time and/or with the presence of ablation debris between pulses. The time between pulses was 1 second. Since the measured temperature relaxation time for single pulse irradiation was 20–40 ms, as shown in Figure 2a, the elevation in temperature at $t = 3\tau$ (60–120 ms after the pulse) was ~5°C. Thus the temperature superposition effects were minimal. This was confirmed by examining the skin temperature prior to each pulse.

Each pulse impact removed a certain amount of tissue, and associated with this event was the appearance of desiccated nonvaporized tissue debris on the surface of the skin. The debris acted as a heat sink and became superheated when irradiated with successive laser pulses. Also, successive pulses led to a displacement of the tissue water and to a subsequent decrease in thermal conductivity [15]. These effects accounted for the large increase in measured peak temperature with successive pulses (Fig. 3).

The events that characterize ablation were viewed visually and thermographically during multiple pulse irradiation of a single site. Irradiating with a radiant exposure of 2.4 J/cm² led to a slight increase in temperature with each of the 15 pulses. The onset of charring was observed at various times (after pulses 5–8) during irradiation. The increase in temperature decreased with pulse number and the peak temperature during

(a)

2.4 J/cm²

(b)

3.9 J/cm²

Fig. 4. CCD camera images of in vivo rat skin after the first three pulses during multiple pulse irradiation of a single spot. Radiant exposures were (a) 2.4 J/cm² and (b) 3.9 J/cm². The repetition rate for both cases was 1 Hz. The areas of interest are enclosed in the white box superimposed on each image. In (a), images are shown just prior to irradiation and just after each of the first three pulses. The irradiated site appears whiter with each successive pulse, but no charring is evident. In (b), charring is present in the central portion of the irradiation spot after the third pulse.

Fig. 5. CCD images of irradiation of in vivo rat skin with pulses 10–14 (images a–e, respectively) during multiple pulse irradiation of a single spot. The radiant exposure was 3.9 J/cm², and the repetition rate was 1 Hz. Nucleation sites can be seen in each image, with the size of the burning site and the number of focal spots of burning increasing with successive pulses. Each image was taken immediately after each pulse impact.

multiple pulse irradiation appeared to plateau at about 200°C.

In contrast, irradiation with 3.9 J/cm² pulses led to a quite different temperature-pulse number history (Fig. 3b). After the third pulse, char tissue was noticed immediately on the skin surface (Fig. 4b); this was in contrast to the absence of char formation immediately after the third pulse with 2.4 J/cm² pulses (Fig. 4a). With the onset of charring, the change in peak temperature with pulse number increased (Fig. 3b). With the 10th pulse impact on the skin, focal tissue burning was visible (Fig. 5a). With each successive pulse, the burning became more widespread, but distinct foci at which burning occurred were still evident (Fig. 5b–e). During these burning events, the temperature oscillated around 350°C (Fig. 3b). This was due to the apparent randomness of the location of the nucleation sites; a given burning event may or may not have registered on a given thermal camera scan. This also explains the large error bars seen in the plot of peak temperature versus pulse number during pulses 10–15 (Fig. 3b).

In essence, this study presents a best-case scenario for the deleterious effects of pulse stack-

ing during skin resurfacing. The radiant exposures of 2.4 J/cm² and 3.9 J/cm² and the repetition rate of 1 Hz were on the low end or below clinically used laser parameters ($H_0 = 3.5\text{--}7.1$ J/cm², 5–11 Hz). In general, the peak temperature increased with an increase in pulse number. At 3.9 J/cm², as few as three stacked pulses resulted in charred tissue and peak temperatures >300°C. With higher repetition rates and radiant exposures, it is likely that nonspecific thermal events would occur with fewer pulses. Further studies need to be performed with other combinations of clinically relevant laser parameters.

The ablation events seen during pulsed and CW laser irradiation of tissue were described by several authors [13–15,18]. The “popcorn effect” is the characteristic trait of ablation; the explosive ejection of tissue due to elevated subsurface pressures occurs with a “popping” sound during an ablation event. In our experiments, the cracking sound was very audible upon laser pulse impact on the skin with both radiant exposures. LeCarpentier et al. [13] irradiated porcine aortae with a CW argon laser and noted that ~5 seconds after explosions (ablation) occurred, the surface tem-

perature of the aortae specimens increased to a temperature range of 350–450°C, and pyrolytic events (burning, carbonization) occurred as nucleation sites formed on the tissue surface. In our experiments involving pulsed CO₂ laser light, irradiation with both radiant exposures led to charring before burning occurred; with 2.4 J/cm² pulses, burning did not occur after 15 successive pulses at 1 Hz. Irradiation with a radiant exposure of 3.9 J/cm² resulted in burning after the tenth pulse; burning occurred 9 seconds after the onset of ablation (first pulse). Tissue burning occurred at distinct nucleation sites on the tissue surface (Fig. 5). Thus the ablation process induced by pulsed CO₂ laser irradiation was similar to that of a CW laser if the pulsed laser irradiated the same spot with free-running pulses.

In summary, the surface temperatures of in vivo fuzzy rat skin were measured during pulsed CO₂ laser irradiation with a band-limited thermal camera. With single low radiant exposure pulses, peak temperatures >100°C were measured. The time necessary for the skin surface temperature to return to baseline was on the order of tens of ms. With multiple-pulse superposition of a single site, pulse stacking led to measured surface temperatures in the 200–300°C range and to nonspecific thermal events such as charring and, with several pulses, burning. These pyrolytic events result in increased thermal damage. In clinical practice, it is extremely important for the physician to use laser parameters with which he or she is comfortable in order to minimize pulse overlap and subsequent harmful effects.

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