Optimizing Fluence and Debridement Effects on Cutaneous Resurfacing Carbon Dioxide Laser Surgery

Noah Kawika Weisberg, MD; Timothy Kuo; Berhooz Torkian; Lou Reinisch, PhD; Darrel L. Ellis, MD

Objective: To develop methods to compare carbon dioxide (CO₂) resurfacing lasers, fluence, and debridement effects on tissue shrinkage and histological thermal denaturation.

Design: In vitro human or in vivo porcine skin samples received up to 5 passes with scanner or short-pulsed CO₂ resurfacing lasers. Fluences ranging from 2.19 to 17.58 J/cm² (scanner) and 1.11 to 5.56 J/cm² (short pulsed) were used to determine each laser’s threshold energy for clinical effect. Variable amounts of débridement were also studied.

Main Outcome Measures: Tissue shrinkage was evaluated by using digital photography to measure linear distance change of the treated tissue. Tissue histological studies were evaluated using quantitative computer image analysis.

Results: Fluence-independent in vitro tissue shrinkage was seen with the scanned and short-pulsed lasers above threshold fluence levels of 5.9 and 2.5 J/cm², respectively. Histologically, fluence-independent thermal depths of damage of 77 µm (scanner) and 25 µm (pulsed) were observed. Aggressive débridement of the tissue increased the shrinkage per pass of the laser, and decreased the fluence required for the threshold effect. In vivo experiments confirmed the in vitro results, although the in vivo threshold fluence level was slightly higher and the shrinkage obtained was slightly lower per pass.

Conclusions: Our methods allow comparison of different resurfacing lasers’ acute effects. We found equivalent laser tissue effects using lower fluences than those currently accepted clinically. This suggests that the morbidity associated with CO₂ laser resurfacing may be minimized by lowering levels of tissue input energy and controlling for tissue débridement.

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MATERIALS AND METHODS

TISSUE SOURCES

Human skin obtained as excess tissue from reduction mammoplasties or abdominoplasties was used for all in vitro experiments. Tissue was placed on saline-moistened gauze, double wrapped in aluminum foil, sealed in an airtight bag, and stored at 4°C for 2 days or less, or frozen at −20°C. All tissue was used within 2 months of freezing. All experiments were performed at room temperature.

A plastic template was used to cut 2.0×0.5-cm sections of tissue by scalpel incision. More than 80 tissue samples from at least 5 sources were used in this investigation. Tissue samples from at least 2 sources were used for each data point. Three samples from each source were used for each data point. Therefore, each data point consisted of 6 samples, treated and measured after each laser pass for 5 passes, making each value a least-squares fit of 30 measurements.

LASER PARAMETERS

A Sharplan SilkTouch CO2 laser (Sharplan, Allendale, NJ) was the continuous-wave scanner laser used in these experiments. The 125-mm handpiece was connected to a Sharplan 1060 CO2 laser and set between 1 and 8 W with a 200-millisecond repeated scan. The “scan size” reported by the manufacturer for this handpiece is 3.7 to 4.0 mm. The scan size (as measured by burning on a tongue depressor and measuring the image on ×25 magnification with a surgical microscope) was a 3.4-mm spiral-scanning pattern. Laser fluences were calculated from the intensity as measured with a Power One energy meter with an LM-10 detector head (Coherent, Palo Alto, Calif). The porcine skin was used to confirm the applicability of our results to live subjects.

RESULTS

Tissue contracted linearly with the number of passes for both lasers, as previously described. The short-pulsed laser within its typical operating range of 250 to 500 mJ gave a maximum of 3.6% shrinkage per pass with a threshold of 220 mJ when aggressive debridement was used (Table 1 and Figure 1, A). Without debridement, the maximum shrinkage per pass was 2.3% per pass with a threshold of 990 mJ.

Within the scanner laser’s typical operating range of 5 to 8 W, the observed plateau shrinkage per pass was 5.1% with a threshold of 2.7 W when aggressive debridement was used (Figure 1, B). This 5.1% shrinkage plateau per pass is seen below the energy settings typically used clinically with the scanner laser. When simple rehydration of the tissue without debridement was done, the observed plateau of shrinkage per pass was 2.4% with a threshold of 6 W. Using a 4×4 gauze for debridement increased the plateau of shrinkage to nearly 9% per pass (data not shown). The threshold did not measurably change from 2.7 W.

Because results obtained in vitro may differ markedly from the clinically observed in vivo results on patients, we confirmed our in vitro human skin sample results by doing in vivo resurfacing experiments with live pigs. Figure 2, A, shows the data for the porcine skin shrinkage with the short-pulsed laser using debridement between passes. The maximum shrinkage in the pig model was 2.0%, and the threshold was 340 mJ. The maximum shrinkage observed was only slightly lower than the maximum shrinkage of 3.6% seen in the human skin model. Results seen with the scanner laser in the pig model, also using debridement between passes, are shown in Figure 2, B. Here, we observed a maximum shrinkage level of 3.7% per pass with a threshold of 3.3 W for the pig skin contraction. Again, this is slightly lower than the maximum shrinkage of 5.1% observed in the human skin experiments.

Morphometric evaluation of the histological specimens was performed on the skin sections stained for collagen using Gomori trichrome. Collagen denaturation was observed by dermal tincture change. The amounts of coll...
a saline-moistened cotton swab (as above) and blotted dry prior to the next pass.

In Vivo Studies

Piglets weighing 4 kg were used for the experiments. India ink tattoo dots placed 1.0 cm apart served as markers for contraction of the skin. The axes of the dots were oriented longitudinally on the animals. The 1.0 × 0.5-cm area inside the marks was irradiated with the lasers. The laser energy fluences, debridement techniques, and tissue and data analysis used were the same as for the in vitro experiments.

TISSUE SHRINKAGE

Tissue shrinkage was evaluated by measuring the linear distance change between 2 centrally placed India ink tattoos 1.0 cm apart. To measure shrinkage, the tissue was digitally photographed before irradiation and subsequent to each laser pass and debridement. The digital photographs were taken with a video camera (model MKC-301A, Ikegami, Tokyo, Japan) mounted to the sideport of a surgical microscope with a 400-mm focal length lens (Carl Zeiss Inc, Thornwood, NY). The camera was interfaced with the built-in video port of a microcomputer (MAC 840 AV, Apple Computer Inc, Cupertino, Calif). Distances were measured with Photoshop 2.0 software (Adobe, Mountain View, Calif).

CALCULATIONS

The relative distance measured between the tattooed dots on the tissue was plotted vs the number of laser passes. These data were usually linear and were fit to a straight line. The slope of the line (S) gave the tissue shrinkage per laser pass for each laser fluence. Measured tissue shrinkage or depth of thermal damage (see “Histological Change” section) was graphed as a function of irradiation using the Henderson-Hesselbalch 2-state equation to obtain a best-fit line for the observed data points.

\[ S = \frac{S_{\text{max}}}{[1 + 10^{(I - I_{1/2})/I_{1/2}}]} \]

where \( S \) indicates tissue shrinkage or depth of thermal damage as a function of the laser irradiation (I); \( S_{\text{max}} \), maximum tissue shrinkage or maximum depth of thermal damage; I, the laser fluence or intensity or energy; and \( I_{1/2} \), the fluence or intensity or energy where one half of the maximum shrinkage or depth of thermal damage is measured. The threshold value (\( I_{T} \)) was defined as the point where 90% of \( S_{\text{max}} \) is reached: \( I_{1/2} = 1.95 \times I_{T} \).

We used this 2-state model to describe the data because they appeared to follow a sigmoid curve. This model implies a 2-state situation in which (in state 1) the intensity of the laser is not enough to cause shrinkage or thermal damage, or (in state 2) the laser intensity is sufficient to induce shrinkage or thermal damage. The data were fit to the mathematical model using MacCurve Fit software (V.1.0.7, Kevin Raner, Victoria, Australia). This program finds the best-fit parameters and the SEs of the parameters.

HISTOLOGICAL CHANGE

Tissue samples for histological analysis received 3 passes with the respective lasers because this correlates with most clinical CO2 laser resurfacing in humans. Debridement was done between each laser pass on all the histological samples with sterile saline solution and a cotton swab. Tissue specimens were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with either hematoxylin-eosin or Gomori trichrome for light microscopic evaluation. Analysis was performed on a light microscope (Vnox AH-2, Olympus, Lake Success, NY). Morphometric analysis was performed with planar morphometry software (Southern Micro Instruments, Atlanta, Ga).

A comparison of collagen denaturation in vivo with a threshold fluence similar to what was observed with tissue shrinkage (Table 1 and Table 2). Interestingly, the threshold for collagen denaturation was lower than the threshold for cutaneous contraction in vivo with both lasers, but the opposite was observed in vitro.

The plateau depth of collagen denaturation observed in the human breast skin treated with the short-pulsed laser (Figure 4, C and D) for comparison. We observed that the depth of collagen denaturation plateaus above a threshold fluence similar to what was observed with tissue shrinkage (Table 1 and Table 2). Interestingly, the threshold for collagen denaturation was lower than the threshold for cutaneous contraction in vivo with both lasers, but the opposite was observed in vitro.

The plateau depth of collagen denaturation observed in the human breast skin treated with the short-

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**Table 1. Fit Parameters of Equation for Each Laser Using In Vitro Human Skin**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Pulsed Laser Debrided</th>
<th>Not Debrided</th>
<th>Scanned Laser Debrided</th>
<th>Not Debrided</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin shrinkage</td>
<td>( I_{1} = 2.5 \pm 0.2 \text{ J/cm}^2 ) ( (220 \pm 20 \text{ mJ}) )</td>
<td>( I_{1} = 11 \pm 2 \text{ J/cm}^2 ) ( (990 \pm 320 \text{ mJ}) )</td>
<td>( I_{1} = 5.9 \pm 0.4 \text{ J/cm}^2 ) ( (2.7 \pm 0.2 \text{ W}) )</td>
<td>( I_{1} = 13 \pm 5 \text{ J/cm}^2 ) ( (6 \pm 2 \text{ W}) )</td>
</tr>
<tr>
<td></td>
<td>( S_{\text{max}} = 3.6% \pm 0.1% \text{ per pass} )</td>
<td>( S_{\text{max}} = 2.3% \pm 0.8% \text{ per pass} )</td>
<td>( S_{\text{max}} = 5.1% \pm 0.1% \text{ per pass} )</td>
<td>( S_{\text{max}} = 2.4% \pm 0.5% \text{ per pass} )</td>
</tr>
<tr>
<td>Skin thermal denaturation</td>
<td>( I_{1} = 3.5 \pm 0.4 \text{ J/cm}^2 ) ( (320 \pm 40 \text{ mJ}) )</td>
<td>...</td>
<td>( I_{1} = 9.1 \pm 1.0 \text{ J/cm}^2 ) ( (4.2 \pm 0.7 \text{ W}) )</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>( S_{\text{max}} = 25 \pm 2 \text{ \mu m} )</td>
<td>...</td>
<td>( S_{\text{max}} = 77 \pm 5 \text{ \mu m} )</td>
<td>...</td>
</tr>
</tbody>
</table>

*See the “Calculations” section of the text for an explanation of the fit parameters of the equation. Plus-or-minus values are SEs.*
pulsed laser was 25 µm (Figure 5, A); the threshold was 320 mJ (Table 1). The scanner laser produced considerably more thermal change. The observed plateau depth of collagen denaturation was 77 µm, with a threshold of 4.2 W (Figure 5, B).

Collagen denaturation observed in vivo in the porcine model was similar to that observed for the in vitro human skin model. For the short-pulsed laser, the plateau depth of collagen denaturation was 35 µm (Table 2). The observed plateau depth of collagen denaturation for the scanner laser was 80 µm. These depths of collagen denaturation were within experimental error in comparing the in vitro and in vivo models.

**COMMENT**

Our study revealed several interesting observations about cutaneous laser resurfacing. Most intriguing was that these lasers have a threshold energy requirement for both cutaneous contraction and thermal denaturation. Graphs
of these studies demonstrate a best fit with the Henderson-Hesselbalch 2-state equation. This implies a 2-state laser-tissue interaction where the laser energy is either insufficient (state 1) or sufficient (state 2) to cause tissue shrinkage or thermal damage. Applying too little energy would be predicted to have an inadequate clinical effect. Once the energy fluence threshold is reached, our study shows that additional energy does not appear to enhance the laser’s acute effect. In fact, additional energy may increase the potential for undesirable outcomes such as hypertrophic scarring, pigmentary change, and persistent postoperative erythema, since more energy is being deposited into the tissue with little or no apparent gain. This excess laser energy may result in increased thermal damage that is not measured acutely with histological methods such as those used in this study, or may result in increased tissue ablation (which we cannot accurately measure at present), leading to a deeper dermal injury.

Many studies have compared the clinical efficacy and adverse effects of various CO₂ resurfacing lasers. However, previous studies have not compared the threshold effects of the different resurfacing lasers as done in this study. We found that the scanner and short-pulsed lasers showed cutaneous shrinkage thresholds at energy fluences of 5.9 and 2.5 J/cm² in vitro and 7.3 and 3.7 J/cm² in vivo, respectively. We also found the threshold irradiance (intensity) for consistent depth of thermal change in the dermis using the scanner laser to be 5.0 kW/cm² (3.5 W), while ablation was measured.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Pulsed Laser</th>
<th>Scanned Laser</th>
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<tbody>
<tr>
<td>Skin shrinkage</td>
<td>3.7 ± 0.4 J/cm²</td>
<td>7.3 ± 0.9 J/cm²</td>
</tr>
<tr>
<td></td>
<td>(340 ± 40 mJ)</td>
<td>(3.3 ± 0.4 W)</td>
</tr>
<tr>
<td>Smax per pass</td>
<td>2.6% ± 0.2%</td>
<td>3.7% ± 0.2%</td>
</tr>
<tr>
<td>Thermal denaturation</td>
<td>2.9 ± 0.8 J/cm²</td>
<td>5.6 ± 2 J/cm²</td>
</tr>
<tr>
<td></td>
<td>(260 ± 80 mJ)</td>
<td>(2.5 ± 1.0 W)</td>
</tr>
<tr>
<td>Smax = 5 ± 10 µm</td>
<td></td>
<td>80 ± 6 µm</td>
</tr>
</tbody>
</table>

*See the “Calculations” section of the text for an explanation of the fit parameters of the equation. Plus-or-minus values are SEs.

Figure 4. Histological sections of in vivo porcine tissue treated with 3 passes of the respective lasers at various fluences (Gomori trichrome stain, all sections photographed at ×50) (bar = 100 µm). A, Pulsed laser subthreshold (100 mJ); B, pulsed laser above threshold (500 mJ); C, scanner laser subthreshold (1 W); D, scanner laser above threshold (8 W). Each point is the average of 10 measurements per slide, with 2 pigs used for the experiment. Arrows indicate the lower level of collagen denaturation as denoted by tincture change.

Table 2. Fit Parameters of Equation for Each Laser Using In Vivo Porcine Skin With Debridement Between Passes

Figure 5. Depth of histological thermal damage of in vitro human skin as determined by tissue morphometry on Gomori trichrome–stained slides plotted vs fluence. A, Pulsed laser (energy in millijoules at top of graph); B, scanner laser (intensity in watts at top of graph). Each point is the average of 10 measurements per slide, with 2 different samples used for each experiment. The fit parameters—1/2 (I 1⁄2) and T (IT)—are indicated by arrows.
as the removal of tissue. Kamat et al. reported an energy fluence of 4 J/cm² as the lower limit for histological changes observed secondary to irradiation energy using a continuous-wave CO₂ laser. Our data with the scanner and short-pulsed CO₂ lasers also correspond well to their data.

Our threshold values correspond with the accepted operating range of the TruPulse laser of 250 to 500 mJ with an energy fluence of 2.78 to 5.56 J/cm². Our measured depth of collagen change was 25 to 35 µm with the TruPulse laser. This is slightly less than the 50- to 100-µm change observed when nitroblue-tetrazolium chloride staining was used by Smith et al. Staining sensitivity differences may account for this variance. The clinical operating range of the SilkTouch laser is from 5 W to 8 W, with a corresponding energy fluence of 10.99 J/cm² to 17.58 J/cm², well above our observed 7.3 and 5.6 J/cm² in vivo thresholds for shrinkage and thermal denaturation, respectively. The use of higher energy fluences than necessary with resurfacing CO₂ lasers may explain some of the observed undesirable clinical adverse effects.

Energy fluences required for threshold clinical effect with resurfacing lasers would be predicted to be higher than our observed in vitro study due to differences in live tissue such as the cooling effect of blood flow. Our in vivo data using porcine skin confirmed this expectation. We found 35% and 18% higher fluence thresholds for contraction with the pulsed and scanner lasers, respectively. We also observed a 28% and 27% reduction in the amount of shrinkage per pass in porcine skin (in vivo) compared with human skin (in vitro) for the pulsed and scanner lasers, respectively. This is also expected, as the in vivo skin is surrounded by normal skin that offers resistance to contraction, in contrast to the in vitro skin, which lacks this resistance. Our human skin in vitro model approximated the in vivo acute effects of resurfacing lasers sufficiently well to be helpful for the prediction of clinical cutaneous effects with various resurfacing lasers.

Methods of debridement may cause wide variations in the effectiveness of CO₂ laser resurfacing. For instance, we found that debridement with a saline-soaked gauze pad was better than using cotton swabs, which in turn was better than simple rehydration. Our data establish the concept that vigorous debridement of the desiccated tissue between laser passes will increase the amount of shrinkage obtained per pass and decrease the threshold energy fluence required for adequate shrinkage. This concept requires clinical testing for correlation with the cosmetic results.

In summary, our data predict that the clinical effects of the CO₂ resurfacing lasers will be similar when operated at their respective threshold levels. Clinical adverse effects may be decreased by operating at these lower energy fluences. Clinical studies substantiating the threshold levels for each resurfacing laser are therefore necessary. For purposes of comparison, we suggest that clinical laser resurfacing studies should use lasers at the calculated threshold levels, and control for the amount of debridement.

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REFERENCES