

# Evaluation of various carbon dioxide laser settings on the time and number of laser beam passes required to make a full-thickness skin incision and amount of laser-induced tissue artifact

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## OBJECTIVE

To evaluate the time and number of laser beam passes required to make full-thickness skin incisions and extent of laser-induced tissue artifacts following use of a CO<sub>2</sub> laser at various settings.

## SAMPLE

24 skin specimens from six 5-month-old porcine carcasses.

## PROCEDURES

4 full-thickness skin specimens were harvested from the flank regions of each carcass within 30 minutes after euthanasia and randomly assigned to 4 treatment groups. Three 5-cm-long incisions were made in each specimen with a CO<sub>2</sub> laser (beam diameter, 0.4 mm) set to deliver a continuous wave of energy alone (groups 1 and 2) or in superpulse mode (groups 3 and 4) at 10 (groups 1 and 3) or 20 (groups 2 and 4) W of power. The time and number of passes required to achieve a full-thickness incision were recorded, and extent of laser-induced tissue artifact (as determined by histologic evaluation) was compared among the 4 groups.

## RESULTS

Mean time required to make a full-thickness skin incision for groups 2 and 4 (power, 20 W) was significantly less than that for groups 1 and 3 (power, 10 W). Mean number of passes was lowest for group 2 (continuous wave at 20 W). Extent of laser-induced tissue artifact was greatest for group 4 (superpulse mode at 20 W).

## CONCLUSIONS AND CLINICAL RELEVANCE

Results provided preliminary information regarding use of CO<sub>2</sub> lasers to make skin incisions in veterinary patients. In vivo studies are necessary to evaluate the effect of various CO<sub>2</sub> laser settings on tissue healing and patient outcome. (*Am J Vet Res* 2020;81:514–520)

In veterinary medicine, CO<sub>2</sub> laser technology can be used for skin incision, excision, and ablation.<sup>1</sup> Use of CO<sub>2</sub> laser technology in clinical veterinary practice has increased in popularity during recent years throughout the world. Carbon dioxide laser energy at a wavelength of 10,600 nm is highly absorbed by water, and skin has a high concentration of water. In accordance with the concept of photothermal laser-tissue interactions, photons delivered by a CO<sub>2</sub> laser beam are absorbed and converted into thermal energy, which increases the target tissue temperature.<sup>2,3</sup> Thermal injury of tissue is caused by protein denaturation, which begins at 60°C. When tissue temperature exceeds 100°C, water in the tissue transforms from liquid to steam until the internal tissue pressure surpasses the strength of its collagen network resulting in explosive vaporization.<sup>2</sup> This process converts

solid tissue into gaseous vapor and a smoke plume.<sup>2,3</sup> Ninety-eight percent of CO<sub>2</sub> laser energy is absorbed within 0.1 mm of the target tissue surface; therefore, damage to tissue surrounding the target tissue is minimal, typically ranging from < 0.1 to 0.58 mm.<sup>3–6</sup> Because CO<sub>2</sub> laser energy is associated with minimal tissue penetration and thermal damage, it can be used to precisely cut tissue layer by layer without transmitting energy to the underlying structures.<sup>3</sup> Use of CO<sub>2</sub> laser technology for surgical applications improves visibility of surgical incisions, maintains hemostasis, seals lymphatics and nerve endings, reduces intraoperative wound contamination and postoperative infection risks, and minimizes the risk of tumor seeding, thereby lowering the potential for local tumor recurrence and metastasis.<sup>1,3,7–15</sup>

During use of a CO<sub>2</sub> laser, superheating with incomplete vaporization of target tissue can lead to the formation of a carbonization remnant composed of nonfluid cellular components known as char. Char can readily absorb laser energy and generate radiant

## ABBREVIATIONS

CW Continuous wave  
SPM Superpulse mode

heat, which can result in collateral damage to the surrounding tissue that may cause irritation, delay or lead to abnormal wound healing, and increase the risk of incisional dehiscence.<sup>3,8</sup> Delayed healing is caused by temporary postponement of inflammation, phagocytic resorption, collagen production, and reepithelialization during the early stages of wound repair.<sup>16</sup> Despite the risk of thermal damage to tissues adjacent to the target tissue, investigators of 1 study<sup>4</sup> reported that biopsy specimens obtained by use of a CO<sub>2</sub> laser had uniform char that was restricted to the peripheral margin of the specimens. Further, there was an abrupt demarcation between the char and underlying viable tissue, with little to no intervening zone of coagulation necrosis, and no hemorrhage occurred during the acquisition of the biopsy specimens.<sup>4</sup> The biopsy specimens themselves had minimal CO<sub>2</sub> laser-induced tissue damage; the zone of thermal damage ranged from 0.31 to 0.41 mm and was not expected to interfere with diagnostic evaluation.<sup>4</sup>

An understanding of CO<sub>2</sub> laser equipment settings is required to ensure appropriate use of the technology. The operator selects wave, mode, power, and beam diameter. The most common wave and mode settings used on CO<sub>2</sub> lasers are CW and SPM, respectively. When the CW setting is used alone, the laser output is constant for the entire duration, resulting in heat accumulation within the target tissue.<sup>3</sup> When used incorrectly, the CW setting can lead to an increase in char formation and large zones of thermal necrosis and thermal conduction relative to an equivalent zone of vaporization.<sup>1,3</sup> Use of CW in SPM results in bursts of laser energy with a higher peak power than that generated by CW alone followed by a relaxation period that allows tissue cooling between pulses.<sup>17</sup> This results in efficient tissue ablation over a shorter period of time than the duration of thermal relaxation for tissue, which allows vaporization to occur before the thermal energy can be transferred to surrounding tissues.<sup>2,17-19</sup> Use of the SPM allows the operator to deliver high peak power pulses to the target tissue at appropriate duration and frequency to reduce heat accumulation.<sup>2,18,19</sup>

For CO<sub>2</sub> lasers, power is expressed in watts, focal spot size is determined by the beam diameter, and power density (concentration of energy within an area) is expressed in W/cm<sup>2</sup>. The application of adequate power to a small focal spot size over a specific duration results in optimal vaporization of the target tissue with minimal thermal damage to surrounding tissues. Power density is inversely related to the diameter, distance, and angle of the laser beam. Thus, increasing the focal spot size, distance to the target tissue, and angle of the beam increases the area of the beam and decreases the total power density to the tissue, thereby resulting in tissue coagulation. The ideal focal distance for the CO<sub>2</sub> laser system used in the study reported here is 1 to 2 mm from the target tissue with the beam held perpendicular to the target tissue.<sup>1,3</sup>

A potential drawback to use of a CO<sub>2</sub> laser for surgical applications is that it may extend the time required for incising tissue owing to its limited tissue penetration depth relative to use of a scalpel.<sup>3</sup> However, the additional time required for incising tissue with a CO<sub>2</sub> laser may be offset by the beneficial effects of laser energy on hemostasis.<sup>3</sup> Extended surgery times prolong the duration of anesthesia and increase the risk of morbidity for patients.<sup>20</sup> To our knowledge, only 1 study<sup>21</sup> has been conducted to assess CO<sub>2</sub> laser settings and their effect on incisional depth and width as well as char formation on soft tissue in veterinary patients. Historically, CO<sub>2</sub> laser settings have been chosen anecdotally. The scientific literature contains little information regarding the time required for tissue penetration by use of various CO<sub>2</sub> laser settings while the beam angle, distance, and delivery time to the target tissue are held constant.

The purpose of the study reported here was to evaluate the time and number of laser beam passes (number of passes) required to make a full-thickness skin incision and extent of laser-induced tissue artifacts following use of a CO<sub>2</sub> laser at various settings. We hypothesized that, relative to other settings, use of CW alone at a high power (20 W) would decrease the time and number of passes required to perform a full-thickness skin incision and increase the extent of laser-induced tissue artifacts near the incision site. The information gained from the study could be used to help inform clinical use of CO<sub>2</sub> laser technology for surgical applications in veterinary patients.

## **Materials and Methods**

### **Samples**

The study was conducted at the University of Missouri College of Veterinary Medicine. Skin specimens were obtained from the carcasses of six 5-month-old Duroc-crossbred barrows (median body weight, 123 kg; range, 118 to 143 kg) that were slaughtered for reasons unrelated to the study at the University of Missouri Abattoir. From each carcass, four 10 X 10-cm specimens consisting of skin and subcutaneous tissue were harvested by use of a No. 10 scalpel blade within 30 minutes after euthanasia. Two skin specimens were obtained from the right lateral flank region, and 2 were obtained from the left lateral flank region, with care taken to ensure that all 4 specimens had a similar dermal tissue thickness. Thus, there were a total of 24 skin specimens (6 pigs X 4 specimens/pig) obtained for the study.

Each skin specimen was assigned a number from 1 to 24 in a sequential manner on the basis of harvest order. Thus, specimens 1 to 4 were obtained from pig 1, specimens 5 to 8 were obtained from pig 2, and so on. Immediately following harvest, each specimen was submerged in lactated Ringer solution in a sealed plastic bag<sup>a</sup> that was labeled with the specimen identification number and stored refrigerated at 4°C until experimental manipulation. The study was exempt

from review by the institutional animal care and use committee because it did not involve any live animals and the skin specimens were obtained from the carcasses of pigs that were slaughtered for purposes unrelated to the study.

## Experimental procedures

All study procedures were performed and data collected within 24 hours after skin specimen harvest. A random number generator<sup>b</sup> was used to assign the 4 skin specimens from each pig to 4 treatment groups. The treatment groups were defined by the CO<sub>2</sub> laser settings used for incising the skin specimens. The CO<sub>2</sub> laser settings were CW alone at 10 W for group 1, CW alone at 20 W for group 2, CW with SPM at 10 W for group 3, and CW with SPM at 20 W for group 4.

The same CO<sub>2</sub> laser system<sup>c</sup> was used to perform all skin incisions. For that system, the SPM setting had a peak pulse power of 100 W, pulse duration of 100 to 800 microseconds, and pulse frequency of 160 to 375 pulses/s. The laser beam profile determines how a laser delivers photons across its beam, which influences the distribution of photon energy into the target tissue.<sup>2</sup> For a beam profile with a bell-shaped or Gaussian distribution, the photon concentration is greatest at the center and tapers at the periphery of the beam, creating a deep and narrow incision. Such a distribution is known as a TEM00 profile. For the study reported here, a TEM11 profile was used, and all incisions were made with a beam diameter of 0.4 mm. A TEM11 profile has multiple peaks distributed across the beam tip diameter, which creates a shallower and wider incision than does a TEM00 profile.<sup>2</sup>

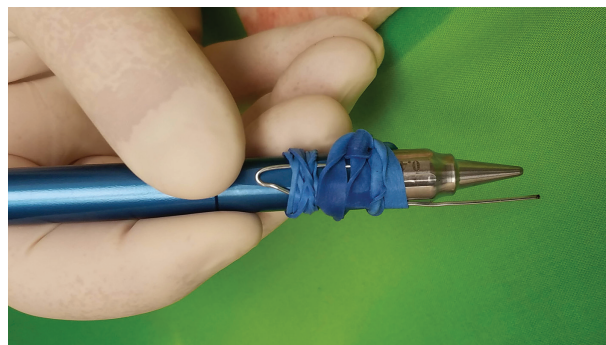
On the day of data collection, skin specimens were removed from the refrigerator and allowed to warm to room temperature (approx 22°C) before experimental manipulation. All subdermal tissue was stripped from each skin specimen, which was then placed on a brightly colored wet cloth to facilitate observation of the point at which a full-thickness incision was achieved with the CO<sub>2</sub> laser. For each specimen, a permanent marker<sup>d</sup> was used to demarcate the location for three 5-cm incisions.

All laser incisions were made by a veterinary surgeon (FAM) with > 25 years of experience using a CO<sub>2</sub> laser in clinical practice. Data were collected and recorded by another investigator (data recorder). Both the surgeon and data recorder remained unaware of (were blinded to) the treatment group assignment for each skin specimen. Prior to initiation of the laser incisions, the data recorder measured the thickness of each specimen with a calibrated metric caliper to ensure that all specimens had a similar thickness. A third investigator (LMA), who was not blinded, was responsible for determining the order in which the specimens were incised and adjusting the CO<sub>2</sub> laser settings. The order in which the skin specimens were experimentally incised was randomized by means of a random number generator.<sup>b</sup> The laser settings were

adjusted for each specimen in accordance with the assigned treatment. The setting screen of the CO<sub>2</sub> laser unit was covered with a cloth to ensure that the surgeon and data recorder remained blinded to the treatment group assignment of each specimen. All personnel wore appropriate laser safety equipment, and a smoke evacuator system<sup>c</sup> was used while the incisions were made.

For each skin section, a full-thickness incision was made at each of the 3 premarked sites. The CO<sub>2</sub> laser was self-calibrated before use on the tissue sections to ensure the same power delivery. Towel clamps were used to create tension on and secure the corners of each specimen. The unblinded (third) investigator used tissue forceps to apply additional tension on the subdermal side of each site as the incision was made. The laser handpiece was held perpendicular to and 2 mm from the skin surface as determined by a premeasured depth guide that was attached to the handpiece (**Figure 1**). The position of the depth guide on the handpiece was checked between specimens to ensure that the appropriate distance between the laser tip and skin was maintained. The speed at which each stroke of the laser beam was delivered to the target tissue was kept constant by the surgeon. In a pilot study in which the depth guide was attached to the laser handpiece, it was determined that the mean speed at which the surgeon passed the CO<sub>2</sub> laser handpiece was 13.5 mm/s for 1 complete pass along an incision and 16.3 mm/s for 2 complete passes (back and forth) along an incision. Those speeds were repeated when the surgeon made 5-cm-long practice incisions in porcine skin specimens prior to the start of data collection for the study reported here.

For each experimental incision, the time (in seconds) and total number of passes (long and short passes combined) required for the surgeon to complete a 5-cm full-thickness skin incision with the CO<sub>2</sub> laser were recorded. Visual observation of the brightly col-



**Figure 1**—Photograph of a CO<sub>2</sub> laser handpiece with a 0.4-mm beam diameter and attached depth guide. The depth guide extended 2 mm beyond the tip of the handpiece and was used to ensure that a consistent distance was maintained between the laser tip and porcine skin specimens in a study conducted to evaluate the time and number of laser beam passes required to make a full-thickness skin incision and the extent of laser-induced tissue artifacts associated with use of a CO<sub>2</sub> laser at various settings.

**Table 1**—Mean  $\pm$  SD time and number of CO<sub>2</sub> laser passes required to achieve a full-thickness incision and the width of laser artifact in porcine skin specimens that were experimentally incised by use of various laser settings.

Treatment group	Time (s)	No. of total passes	No. of long passes	No. of short passes	Width of laser artifact ( $\mu$ m)
1	108 $\pm$ 30 <sup>a</sup>	104 $\pm$ 70 <sup>a</sup>	66 $\pm$ 47 <sup>a</sup>	37 $\pm$ 22	182 $\pm$ 38 <sup>a</sup>
2	51 $\pm$ 7 <sup>b</sup>	43 $\pm$ 19 <sup>b</sup>	23 $\pm$ 5 <sup>b</sup>	19 $\pm$ 7	181 $\pm$ 25 <sup>a</sup>
3	108 $\pm$ 33 <sup>a</sup>	103 $\pm$ 70 <sup>a</sup>	60 $\pm$ 39 <sup>a</sup>	42 $\pm$ 31	149 $\pm$ 30 <sup>a,b</sup>
4	65 $\pm$ 4 <sup>b</sup>	60 $\pm$ 23 <sup>a,b</sup>	34 $\pm$ 18 <sup>a,b</sup>	26 $\pm$ 7	232 $\pm$ 39 <sup>a,c</sup>

Four 10 X 10-cm skin specimens were obtained from the left (n = 2) and right (2) lateral flank regions of each of six 5-month-old Duroc-crossbred barrows (median body weight, 123 kg; range, 118 to 143 kg) that were slaughtered for reasons unrelated to the study (ie, 24 skin specimens were harvested). One skin sample from each pig was randomly assigned to each of 4 treatment groups, which were defined by the CO<sub>2</sub> laser settings used for incising the skin specimens. The CO<sub>2</sub> laser settings were CW at 10 W for group 1, CW at 20 W for group 2, CW with SPM at 10 W for group 3, and CW with SPM at 20 W for group 4. For each specimen, three 5-cm full-thickness skin incisions were made with a CO<sub>2</sub> laser at the assigned settings. The laser handpiece had a beam diameter of 0.4 mm and was held perpendicular to and with the tip 2 mm from the skin surface. A long pass was defined as movement of the laser beam along the entire 5-cm length of the experimental incision site. A short pass was defined as movement of the laser beam a short distance at each end of the incision. The total number of passes was the summation of the long and short passes for each incision. Data for 1 skin specimen assigned to group 4 were excluded from all analyses owing to an error in surgical technique; therefore, values represent the mean  $\pm$  SD for 6 skin specimens in groups 1, 2, and 3 and for 5 skin specimens in group 4.

<sup>a-c</sup>—Within a column, values with different letters differ significantly ( $P < 0.05$ ); the absence of superscript letters indicates the values did not differ significantly within a column.

ored cloth through the incision was used to determine when a full-thickness incision of the skin specimen had been achieved. A long pass was defined as movement of the laser beam along the entire 5-cm length of the experimental incision site. A short pass was defined as movement of the laser beam a short distance at each end of the incision.

For each skin specimen immediately after completion of data collection, the specimen number, treatment group assignment, and time and total number of passes for each incision were marked on the specimen with the CO<sub>2</sub> laser by the unblinded investigator. Then the specimen was fixed in neutral-buffered 10% formalin and submitted for histologic examination by a board-certified veterinary pathologist (DYK). The pathologist was blinded to the definition for each treatment group. The tissue adjacent to each experimental incision at approximately one-third and two-thirds of the incision length was histologically examined. Formalin-fixed tissue specimens were processed for histologic examination in a routine manner, sectioned into 4- $\mu$ m-thick slices, and stained with H&E stain. During histologic examination of each section, a photomicrograph was obtained by use of a digital camera<sup>f</sup> and analyzed by image software<sup>f</sup> to determine the width of laser artifact present. Laser artifact was defined as the complete loss of tissue architecture and the presence of deeply basophilic, acellular, and amorphous material.<sup>4</sup> For each of the 24 skin specimens, the mean width of laser artifact was calculated on the basis of measurements obtained for all histologically evaluated sections and used for analysis purposes.

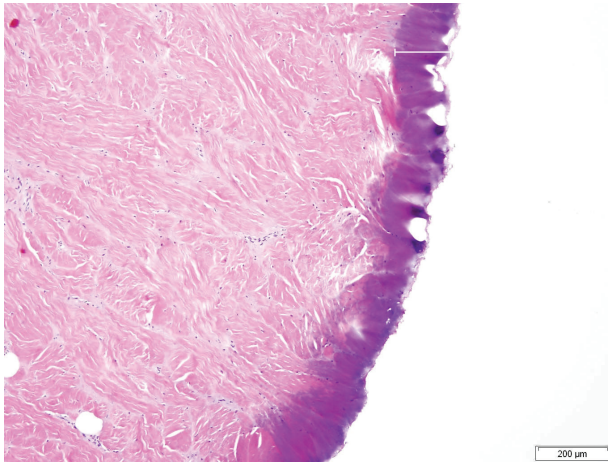
### Statistical analysis

For each of the 24 skin specimens, data from the 3 experimental incisions were combined to calculate

the geometric mean for each variable of interest. Dependent (outcome) variables of interest included tissue segment thickness, time, number of long passes, number of short passes, and total number of passes required to achieve a full-thickness incision with the CO<sub>2</sub> laser and the extent of laser-induced tissue artifact. The data distribution for each outcome variable was assessed for normality by means of the Shapiro-Wilk test, and all variables were normally distributed. Factors associated with each outcome variable were evaluated by means of a 2-way ANOVA for repeated measures. Each model included pulse mode (CW or SPM) and power setting (10 or 20 W) as fixed effects and pig as a random effect to account for the fact that multiple specimens from the same animal were evaluated. The Tukey multiple comparison test was used when post hoc pairwise comparisons were necessary. Values of  $P < 0.05$  were considered significant. All analyses were performed with commercially available statistical software.<sup>8</sup>

### Results

Data for 1 skin specimen assigned to group 4 were excluded from all analyses owing to an error in surgical technique; therefore, analyses were performed with data from 23 skin specimens. The mean  $\pm$  SD skin specimen thickness was 3.7  $\pm$  0.3 mm for group 1 (CW at 10 W), 3.8  $\pm$  0.4 mm for group 2 (CW at 20 W), 3.8  $\pm$  0.3 mm for group 3 (CW with SPM at 10 W), and 3.9  $\pm$  0.3 mm for group 4 (CW with SPM at 20 W); it did not differ significantly ( $P = 0.63$ ) among the 4 groups. Descriptive statistics for the outcome variables were summarized for each treatment group (Table 1). Mean  $\pm$  SD time required to complete a full-thickness incision was significantly lower for groups 2 (51  $\pm$  7 seconds) and 4 (65  $\pm$  4 seconds), compared with that for groups 1 (108  $\pm$  30 seconds) and 3 (108



**Figure 2**—Representative photomicrograph of a section of porcine skin adjacent to an experimental incision made by use of a CO<sub>2</sub> laser with a 0.4-mm beam diameter set to deliver energy in CW with SPM at 20 W of power (group 4). Notice the presence of laser-induced artifact, which was defined as the complete loss of tissue architecture and the presence of deeply basophilic, acellular, and amorphous material, along the edge of the incision. H&E stain; bar = 200  $\mu$ m.

$\pm$  33 seconds). The mean total number of passes and number of long passes required to complete a full-thickness incision for group 2 were significantly lower than those for groups 1 and 3. The mean total number of passes and number of long passes for group 4 did not differ significantly from those for the other 3 groups. The mean number of short passes required to complete a full-thickness incision did not differ significantly ( $P = 0.05$ ) among the 4 groups. Mean  $\pm$  SD laser artifact width for group 4 ( $232 \pm 39 \mu$ m; **Figure 2**) was significantly greater than that for group 3 ( $149 \pm 30 \mu$ m) but did not differ significantly for any of the other pairwise comparisons.

A subjective observation made during the study was that the depth guide attached to the laser handpiece to ensure that a consistent distance was maintained between the laser tip and skin specimen occasionally made contact with the specimen, which resulted in some bumpy passes along the incision and may have affected the mean speed of the handpiece during such passes. In the surgeon's experience, use of the laser handpiece to make a skin incision is much smoother without the attached depth guide.

## Discussion

The objective of the present study was to evaluate the effect of various CO<sub>2</sub> laser settings on the time and number of laser beam passes (number of passes) required to achieve a full-thickness incision and the extent of laser-induced tissue artifact in porcine skin specimens. Regardless of the mode used (CW alone or with SPM), the time required for completion of a full-thickness incision was quicker with a power setting of 20 W than with a power setting of 10 W. In fact, doubling the power setting from 10 W to 20 W decreased the time required to make a full-thickness

skin incision by approximately 50%. That result was expected because use of a higher power (ie, wattage) results in a higher concentration of energy at the laser-tissue interface. Although the experimental skin incisions created in the present study were only 5 cm long, increasing the power setting of a CO<sub>2</sub> laser may help to minimize surgery time in clinical settings when longer incisions are necessary. A decrease in surgery time also decreases the duration of anesthesia and risk for patient morbidity.<sup>20</sup>

In the present study, the group 2 treatment (CW alone at 20 W) was associated with the lowest mean time and lowest mean number of passes to complete a full-thickness skin incision. With the CW-alone setting, laser output is constant for the duration that it is used, which can translate to an increase in its incising ability. These findings supported our hypothesis that use of the CO<sub>2</sub> laser with the CW-alone setting at high (20 W) power would minimize the time and number of passes required to perform a full-thickness skin incision relative to the other laser settings evaluated.

Mean laser artifact was greatest for the group 4 treatment (CW with SPM at 20 W) and was significantly greater than that for the group 3 (CW with SPM at 10 W) treatment. Although the high power setting (20 W) was expected to cause more laser artifact owing to the delivery of a greater amount of thermal energy to the tissue relative to the low power setting (10 W), it was surprising that the mean laser artifact for the group 4 treatment did not differ significantly from that for the 2 CW-alone treatments (groups 1 [CW at 10 W] and 2 [CW at 20 W]). When the CO<sub>2</sub> laser is used in SPM, there is a balance between vaporization and thermal relaxation of tissue, which allows thermal conduction within tissue for the longest possible duration while tissue penetration is maximized, thereby decreasing char formation.<sup>3</sup> Surgical technique may have contributed to the observed differences in laser artifact among the 4 treatment groups of the present study. The depth guide attached to the laser handpiece occasionally caught on a skin specimen as the laser beam was being passed along the incision. The amount of laser energy delivered to the tissue increased during those brief interruptions in beam transit. The surgeon did not remove char from the skin between passes, and the presence of char along the incision line may have prolonged exposure of the adjacent tissue to laser energy. Carbonized char can cause accumulation of heat that will diffuse into the tissue resulting in thermal necrosis. The advantage of CW with SPM over CW alone is that it creates a high-power density of laser energy on the target tissue; however, that advantage is not realized when the laser beam encounters char. Results of the present study failed to support our hypothesis that use of CW alone at 20 W would cause the greatest amount of laser artifact at the incision site, most likely because use of CW with SPM at 20 W resulted in greater char formation than either CW-alone treatment.

One observation in the present study that supported our theory that CW with SPM might be more effective than CW alone in minimizing thermal damage was the fact that the mean laser artifact did not differ between the 2 CW-alone treatment groups but did differ between the 2 CW-with-SPM treatment groups (groups 3 and 4). The mean laser artifact associated with use of the CW with SPM at 10-W setting (group 3) was significantly lower than that associated with use of the CW with SPM at 20-W setting (group 4). Thus, it is possible that decreasing the power when SPM is used might be more effective in minimizing thermal damage to tissues than decreasing the power when CW is used alone. However, evaluation of laser artifact associated with use of a CO<sub>2</sub> laser in the CW setting with and without SPM at incremental power settings would be necessary to validate that theory.

The present study was not without limitations. Results of this study should be interpreted with caution. Variation in technique among surgeons makes it difficult to quantify the angle and speed of the laser beam relative to the skin, which affect the power density of the beam on the target tissue. In an ideal situation, all the experimental skin incisions would have been performed by a machine-operated CO<sub>2</sub> laser. Barring that, the incision protocol was standardized to the extent humanly possible. The same experienced surgeon performed all incisions with the laser handpiece held approximately perpendicular to and 2 mm from the skin surface, and the speed at which the laser beam was passed along the incision site was kept as constant as possible. However, the depth guide attached to the laser handpiece occasionally interfered with passage of the laser beam along the incision site, particularly at the last 5 mm of either end of an incision. This necessitated classification of each pass of the laser beam along the incision site as a long (complete) or short (incomplete) pass. The tension applied to each skin specimen during experimental incision was not objectively measured or regulated. Inconsistency in the amount of tension on the skin specimen during application of the laser beam may have affected the rate at which the edges of the incision separated and extent of char formation. Multiple passes of the laser beam along the incision site were necessary to achieve a full-thickness incision. It is unlikely that the beam followed the same exact track during each pass, and even slight deviation in the focal spot of the beam can decrease the incision depth. Ideally, only a single pass of the laser beam would be necessary to create a full-thickness skin incision because that would eliminate many of the aforementioned technical inconsistencies. For the porcine skin specimens used in this study, the CO<sub>2</sub> laser settings evaluated were insufficient to achieve a full-thickness incision with a single pass of the laser beam. Therefore, a similar study in which the CO<sub>2</sub> laser settings evaluated in this study were applied in a single-pass skin mode is warranted. To help adjust or control for the unavoidable variations in technique

discussed, 3 experimental incisions were performed in each skin specimen by use of the same CO<sub>2</sub> laser settings, and the geometric mean was calculated for each outcome of interest.

Another limitation of the present study was the small sample size. The sample size was small because only 1 surgeon performed all experimental incisions within a limited time period (after skin specimen processing and within 24 hours after specimen harvest). Having only 1 surgeon perform the skin incisions minimized variation in the experimental protocol. A disadvantage of having only 1 surgeon perform all skin incisions, particularly one with > 25 years of experience using CO<sub>2</sub> laser technology, was the potential for surgeon bias. Although the surgeon was blinded to the actual laser settings, he was able to observe the laser-tissue interaction as he was performing each incision and may have unintentionally altered the technique on the basis of his previous experience.

The effects of the CO<sub>2</sub> laser on the fresh porcine skin specimens used in the present study may not translate to other species or in vivo applications. Absorption of laser energy by living tissue may differ from that of ex vivo tissue. In clinical practice, CO<sub>2</sub> laser technology for surgical applications is most commonly used in dogs and cats. The response of canine and feline skin to the CO<sub>2</sub> laser settings evaluated in this study may differ from that of porcine skin. Evaluation of various CO<sub>2</sub> laser settings for surgical applications in live dogs and cats is warranted.

Mode of laser delivery also warrants additional study. For the CO<sub>2</sub> laser used in the present study, the waveguide delivery system had a TEM<sub>11</sub> profile distribution characterized by multiple peaks, which resulted in a wider and more shallow incision, compared with the incision created by use of a TEM<sub>00</sub> profile distribution. The TEM<sub>00</sub> profile distribution delivers a high concentration of photons centrally, which produces a narrow and deep incision.<sup>2</sup> The mode of laser delivery is an important consideration for interpretation and application of the results of the present study. Other CO<sub>2</sub> laser systems, such as an articulated arm and focusing lens, create deeper incisions than the CO<sub>2</sub> laser used in the present study, which will likely translate into a shorter time to achieve a full-thickness skin incision.<sup>22</sup>

In the present study, use of a CO<sub>2</sub> laser at a high power (20 W) resulted in the creation of full-thickness incisions in porcine skin specimens quicker than use of the same laser at a lower power (10 W), regardless of the mode (CW alone or with SPM) used. Use of the CW-alone mode at 20 W was associated with the lowest mean number of laser beam passes over the incision site to achieve a full-thickness incision. Minimizing the time and number of passes required to achieve a full-thickness incision should translate into a shorter overall surgery time, which is generally beneficial for the patient. When the CO<sub>2</sub> laser was used in the SPM mode, decreasing the power from 20

to 10 W decreased the amount of laser-induced artifact in the tissue adjacent to the incision site but significantly increased the time required to achieve a full-thickness incision. Results of this study provided preliminary information regarding use of CO<sub>2</sub> lasers to make skin incisions in veterinary patients, but in vivo studies are necessary to evaluate the effect of various CO<sub>2</sub> laser settings on tissue healing and patient outcome.

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## Footnotes

- a. Ziploc, SC Johnson and Son Inc, Racine, Wis.
- b. Random integer generator, Randomness and Integrity Services Ltd, Dublin, Ireland.
- c. Aesculight LLC, Bothell, Wash.
- d. Sharpie, Newell Brands, Atlanta, Ga.
- e. Edge Systems Corp, Signal Hill, Calif.
- f. Olympus MicroSuite FIVE, Center Valley, Penn.
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