Comparison of first-intention healing of carbon dioxide laser, 4.0-MHz radiosurgery, and scalpel incisions in ball pythons (*Python regius*)

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Objective—To evaluate first-intention healing of CO_2 laser, 4.0-MHz radiowave radiosurgery (RWRS), and scalpel incisions in ball pythons (*Python regius*).

Animals—6 healthy adult ball pythons.

Procedures—A skin biopsy sample was collected, and 2-cm skin incisions (4/modality) were made in each snake under anesthesia and closed with surgical staples on day 0. Incision sites were grossly evaluated and scored daily. One skin biopsy sample per incision type per snake was obtained on days 2, 7, 14, and 30. Necrotic and fibroplastic tissue was measured in histologic sections; samples were assessed and scored for total inflammation, histologic response (based on the measurement of necrotic and fibroplastic tissues and total inflammation score), and other variables. Frequency distributions of gross and histologic variables associated with wound healing were calculated.

Results—Gross wound scores were significantly greater (indicating greater separation of wound edges) for laser incisions than for RWRS and scalpel incisions at all evaluated time points. Necrosis was significantly greater in laser and RWRS incisions than in scalpel incision sites on days 2 and 14 and days 2 and 7, respectively; fibroplasia was significantly greater in laser than in scalpel incision sites on day 30. Histologic response scores were significantly lower for scalpel than for other incision modalities on days 2, 14, and 30.

Conclusions and Clinical Relevance—In snakes, skin incisions made with a scalpel generally had less necrotic tissue than did CO_2 laser and RWRS incisions. Comparison of the 3 modalities on the basis of histologic response scores indicated that use of a scalpel was preferable, followed by RWRS and then laser. (*Am J Vet Res* 2013;74:499–508)

In reptiles, advanced surgical modalities such as CO_2 laser and RWRS are often used for skin incisions, removal of masses, and reproductive surgery.[1–3] A CO_2 laser (light amplification via stimulated emission of radiation) produces infrared light with a wavelength of 10,600 nm that is highly absorbed by intracellular and extracellular water molecules, resulting in ablation and vaporization of tissue.[4–7] Radiowave radiosurgical instruments use electron waves at a specific radio frequency (1.5 to 4.0 MHz) to cause ionic agitation in the cells at the tip of the electrode when the electrode is in contact with tissue, resulting in heating of the tissue with vaporization of tissue fluid, thus causing incision and coagulation.[8–10] There are fundamental differences in the ways CO_2 lasers and RWRS units generate thermal energy (65° to 75°C), but both vaporize intracellular water to cut and coagulate tissue. With either modality, heat is produced; this heat causes collagen denaturation and tissue shrinkage with thrombosis of associated blood vessels, which manifests as improved incisional hemostasis.[11] However, the thermal energy produced by each device also dissipates into the surrounding tissues or environment in the form of heat, which may delay healing and increase the risk of dehiscence.[12] In the few studies[13–18] comparing the use of CO_2 laser, radiosurgery, and scalpel for various procedures in domestic and nondomestic animals, none of these modalities have been consistently shown to be superior; although all are commonly used and advocated, there are no reports of studies comparing first-intention wound healing of CO_2 laser, RWRS, and scalpel incisions in reptiles.

Reptilian skin is similar to mammalian skin, and its histologic composition has been described.[19] However, in contrast to mammals, the skin of members of the

Abbreviations

GLM	Generalized linear model
RWRS	Radiowave radiosurgery

Received March 28, 2012.
Accepted July 2, 2012.

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The authors thank Janet Pezzi and Steve Tinkel for technical assistance.

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order Squamata (snakes and lizards) is dry and largely devoid of glands. Scales found in lizards and snakes represent a folding of the epidermis and vary in size and shape among species. The dermis consists of mesenchymal tissue, which includes nerves, blood vessels, and lymphatic vessels.[20] The epidermis is composed of the outer keratinized stratum corneum, intermediate zone, and inner or deeper stratum germinativum and is separated from the underlying dermis by a basement membrane. The dermis is adhered to the underlying muscle with a small amount of loose connective tissue. Mammalian skin undergoes continual sloughing of the outer keratinized layers with basal proliferation and differentiation of the stratum germinativum occurring without any quiescent periods. In contrast, squamate skin has periods of quiescence and activity with intermittent shedding (ecdysis). During ecdysis, the stratum germinativum undergoes mitosis to form a new intermediate zone and acellular stratum corneum.[21,22] The wound-healing sequence in squamates is similar to that of mammals with an initial vascular and cellular inflammatory response, fibroplasia and new collagen production, reepithelialization, and dermal and epidermal maturation.[21] Wound healing in reptiles is slower than in mammalian and avian species and is affected by wound orientation, nutritional and health status of the patient, immune status, and provision of species-specific environmental conditions.[21,23–26]

The objective of the study reported here was to compare first-intention healing and tissue reactions of incisions made with CO_2 laser, 4.0-MHz RWRS, and a scalpel in ball pythons (*Python regius*). We hypothesized that incisions created with the scalpel would have the least degree of histologic reaction and result in the most consistent first-intention wound healing, compared with the other 2 modalities.

Materials and Methods

Animals—Six healthy adult female ball pythons were obtained from a commercial snake breeder in the United States. Snakes were housed individually in plastic containers with paper substrate, a hiding box, and access to water ad libitum. Every 7 to 10 days, they were offered a thawed mouse of appropriate size for their body diameter. The room was maintained at a mean ± SD daytime temperature of 29.4 ± 2.2°C and nighttime temperature of 27.7 ± 1.2°C for the duration of the study. A 12-hour photoperiod was provided for all snakes. Each animal had a physical examination with weight and snout-to-vent length recorded; a blood sample (0.5 mL) was collected for determination of hematologic and plasma biochemical variables, and fecal examination for parasites was performed. Snakes were acclimated to their environment for 7 days prior to the beginning of the study. At the conclusion of the study, the snakes were adopted by private individuals. The study was approved by the University of Tennessee Institutional Animal Care and Use Committee.

Anesthesia and surgery—Food was withheld from snakes for 7 days prior to anesthesia. All snakes were administered a combination of buprenorphine[a] (0.01 mg/kg), ketamine[b] (10 mg/kg), and midazolam[c] (0.2 mg/kg) IM 30 minutes prior to induction of anesthesia. A surgical plane of anesthesia was induced and maintained with isoflurane[d] (2.5%) in oxygen via an uncuffed endotracheal tube connected to a nonrebreathing (Bain) anesthesia system. Intermittent positive pressure ventilation was performed at 6 to 8 breaths/min. Heart and respiratory rates, absence of the righting reflex, and response to noxious stimulus (tail pinch) were monitored and recorded throughout anesthesia. During anesthesia and surgical procedures, each snake was placed on a conductive fabric warming blanket[e] set to a temperature of 43°C, with a pillowcase between the blanket and the snake. All surgeries were performed in an aseptic operating room with an ambient temperature of 29.4°C.

Both lateral aspects of each snake, extending from 2 cm caudal to the level of the heart to 2 cm cranial to the level of the vent, were aseptically prepared with alternating washes of 4% chlorhexidine scrub and sterile saline (0.9% NaCl) solution and draped with sterile Huck surgical towels. Twelve 2-cm full-thickness skin incisions (4 each via CO_2 laser, RWRS, and scalpel) were made in each snake on day 0 (**Figure 1**). Incisions were performed by 1 surgeon (RTH). All incisions were

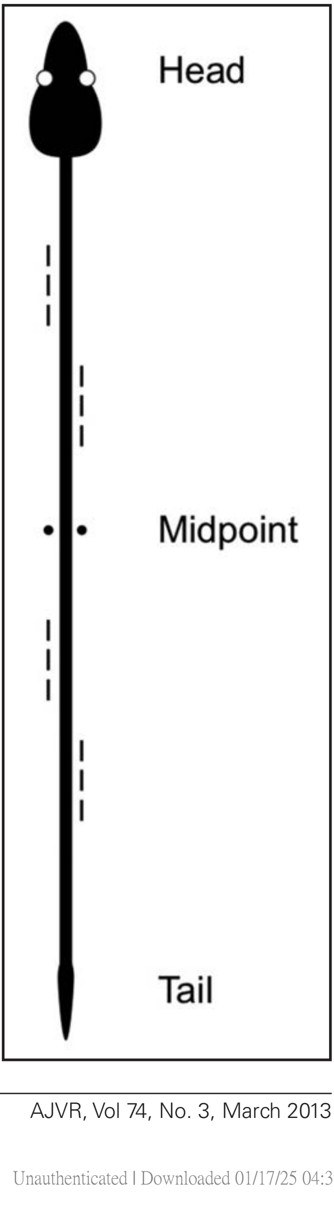

Figure 1—Schematic drawing of a snake indicating locations (lines parallel to dorsal midline) of 12 skin incisions made via CO_2 laser, RWRS, and scalpel in 6 healthy adult ball pythons (*Python regius*) in a study to compare first-intention healing and tissue reactions at incisions made with the 3 modalities. For each modality, four 2-cm, full-thickness skin incisions/snake were made. In each group of 3 incisions, the 3 modalities are represented in randomized order and location. From each snake, 1 control biopsy sample was collected from either the left or right lateral body wall at the midpoint of the body length (circles) on the day incisions were made (day 0).

made parallel to the dorsal midline between the second and third scale rows dorsal to the ventral scales. The laser[f] incisions were made (following laser safety precautions) with a 0.4-mm gold tip at 9-W output on a continuous setting with a focused beam and a distance of approximately 3 mm between the tip and the skin surface. The RWRS incisions were made with a 0.007-mm wire electrode and 4.0-MHz dual frequency radiosurgery unit[g] at a digital setting of 25 (55 W). Scalpel incisions were made with a No. 15 scalpel blade (**Figure 2**).

Starting on the left or right side of the snake, one 2-cm full-thickness skin incision was made via each modality with a space of 2 cm between incisions. Another series of 3 incisions was made on the contralateral side, and this was repeated twice, so that 4 groups of 3 incisions/snake were made. The order of modalities used was randomized for each group of 3 incisions via a block randomization method. To prevent excess tension on the skin when biopsy samples were collected, the 2 groups of 3 incisions made on the ipsilateral side were separated in a craniocaudal direction by > 12 cm, and contralateral incision groups were staggered between the right and left sides. All incisions were immediately closed with sterile surgical skin staples[h] (Figure 2). As a control for subsequent histologic evaluations, a single 1 × 1-cm full-thickness biopsy sample of normal skin was obtained on day 0 from each snake at the midpoint of the body, 2 to 3 scale rows dorsal to the ventral scales, on the left or right side (3 snakes each). Control biopsy sites were closed with sterile skin staples, and biopsy specimens were placed in neutral-buffered 10% formalin solution on cardboard in cassettes and submitted for histologic evaluation.

After completion of the surgical procedures, recovery of snakes was monitored until they were alert, the righting reflex was present, and they were moving on their own. For analgesia, buprenorphine[a] (0.01 mg/kg, SC) was administered immediately after surgery, 12 hours after surgery, and then as needed (q 8 to 12 h). Snakes were evaluated 3 times daily for the first 3 to 4 days for signs of discomfort or pain following surgical procedures, then twice daily thereafter until the end of the study period (day 60). The need for additional analgesia was determined on the basis of unusual body posture, increased or decreased activity level, abnormal response to external stimuli (response to handling), change in behavior, and increased respiratory rate and effort.

Gross observations—Incision and biopsy sites were evaluated daily until all sites were completely healed (day 60). Swelling, crusting, discharge, and dehiscence were used as gross indicators of wound healing or infection. Three observers (RTH, PAS, and JPS) evaluated each incision site independently and assigned a gross wound score using a 4-point scale (0 to 3 [0, absence of swelling, crusting, discharge, and dehiscence; 1, mild swelling, mild crusting, mild discharge, and < 1 mm of skin edge separation; 2, moderate swelling, moderate crusting, moderate discharge, and 1 to 2 mm of skin edge separation; and 3, severe swelling, severe crusting, severe discharge, and > 2 mm of skin edge separation]). The mean of the observer scores was recorded daily until each incision site was biopsied (up to

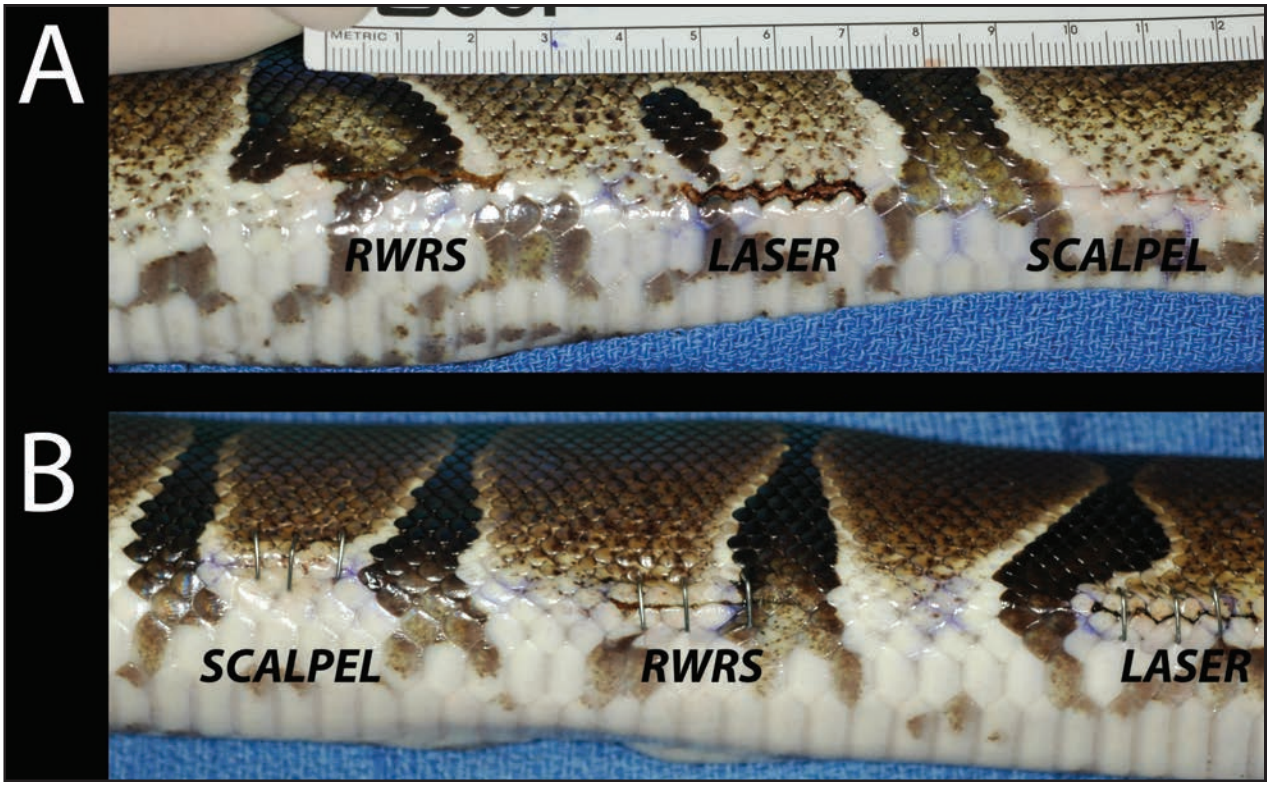

Figure 2—Photograph of skin incisions made via CO_2 laser, RWRS, and scalpel in a healthy Ball python. Twelve incisions (4/modality; A) were made in each snake and immediately closed with surgical steel staples (B).

day 30); statistical analysis was performed for days that biopsies were performed.

Biopsy and histologic evaluation of skin samples—For each incisional modality, 1 full-thickness 1 × 1-cm skin biopsy sample/snake was collected on postoperative days 2, 7, 14, and 30. Snakes were premedicated with buprenorphine[a] (0.02 mg/kg, IM) and midazolam[c] (0.2 mg/kg, IM). For local anesthesia, 0.3 mL of 2% lidocaine solution[i] diluted with 0.15 mL of sodium bicarbonate solution[j] was divided among the 3 biopsy sites and administered SC. Biopsies were performed with a No. 15 scalpel blade and DeBakey thumb forceps by means of sharp dissection to separate the skin from underlying muscle. Biopsy specimens were obtained from the center of 1 incision for each of the 3 modalities and included incised skin and intact skin from each side of the incision. Biopsy samples obtained from all sites at each time point were randomly selected (block randomization method). Biopsy sites were closed with 3-0 poliglecaprone 25 suture[k] in a horizontal mattress pattern. Skin samples were placed in neutral-buffered 10% formalin solution and submitted for histologic evaluation. Thirteen 1 × 1-cm skin biopsy specimens (including the day 0 control sample) were collected from each snake during the study period. Following skin biopsy procedures, recovered snakes were returned to their enclosures and examined daily for general health and healing of incision and biopsy sites.

Fixed skin specimens were trimmed, routinely processed, embedded in paraffin, sectioned at 5 µm, and stained with H&E and Masson trichrome stains. A board-certified pathologist (KMN) blinded to the surgical modality and postoperative interval evaluated all specimens. Sections were examined via light microscopy to evaluate necrosis, fibroplasia, and inflammation. Necrosis was defined as coagulated, hypereosinophilic (necrotic) dermal collagen, or loss of differential staining with retention of cellular architecture (coagulation necrosis) of the epithelial cells in the epidermis. A representative width of necrotic tissue on 1 side of the wound bed was measured. On histologic examination, fibroplasia was characterized by increased numbers of plump spindle cells (fibroblasts) separated by small amounts of collagen. Fibroplastic tissue was measured in Masson trichrome–stained sections and was measured across the apposed sides of the wound bed. Measurements used to assess the width of necrotic and fibroplastic tissue were performed in the most representative section for each site. Inflammation was subjectively scored on the basis of severity of granulocytic (heterophil) infiltrates into the wound bed and the severity of perivascular infiltrates of lymphocytes and macrophages within examined sections. The following scores were assigned: 0 = no infiltrates, 1 = mild infiltrates, 2 = moderate infiltrates, and 3 = dense infiltrates. The scores for granulocytic wound bed infiltrates and perivascular infiltrates were summed to create a total inflammation score, which ranged from 0 to 6. A histologic wound score was assigned to each sample according to degree of severity for width of necrotic tissue (0 = absence of necrosis, 1 = 1 to 15 µm, 2 = 16 to 99 µm, and 3 = ≥ 100 µm) and fibroplastic tissue (0 = absence of fibroplasia, 1 = 1 to 25 µm, 2 = 26 to 74 µm, and 3 = ≥ 75 µm). These scores were combined with the total inflammation score to calculate a histologic response score for each biopsy sample.

Skin sections were evaluated for the presence or absence of organized granulomas, bacteria within granulomas, and surface bacteria. For purposes of analysis, a numeric assessment value was assigned for the absence (0) or presence (1) of granuloma formation as well as bacteria absent or present on the skin surface or within granulomas (0, 1, and 2 respectively). The numeric assessment values for surface bacteria and bacteria within granulomas for each biopsy sample were combined to yield a total wound bacteria score.

Degree of healing was subjectively evaluated on the basis of the degree of epithelial migration from the wound margin. A score from 0 to 3 (0 = absent, 1 = 1% to 49% healing, 2 = 50% to 99%, and 3 = 100%) was assigned; incisions with a score of 3 were considered completely healed.

Statistical analysis—The percentages and frequency distributions of the gross and histologic variables assessed were computed for each day and compared among the 3 incisional modalities. Because the study data included repeated measurements of each variable, GLMs with repeated measures were fit to the data for each of the gross and histologic measures of interest. The least squares means were then computed within the GLMs. Additionally, contrast statements were used within the GLMs to investigate and identify differences in each of the gross and histologic variables under investigation. All calculations were performed with statistical software.[l] Values of $P < 0.05$ were used to identify significant differences among the incisional modalities.

Results

Animals—All snakes were determined to be healthy on the basis of physical examination, and no snake had clinical evidence of dermatologic disease. Hematologic and plasma biochemical variables were within laboratory reference intervals for ball pythons at the investigators' institution. Results of fecal examination were negative for endoparasites. Snakes weighed a mean ± SD of 0.79 ± 0.04 kg, and mean ± SD snout-to-vent length was 89.5 ± 3.4 cm. All snakes recovered from anesthesia and surgical procedures without complications and were behaving normally the following day and throughout the study. No additional analgesia was required for any snake. Control biopsy samples of the skin from all snakes were considered normal on histologic evaluation (**Figure 3**). By 30 days after the last skin biopsy (day 60), all biopsy sites had healed uneventfully with subjectively minimal scarring. All snakes underwent normal ecdysis, and no retained skin was detected at the surgical sites.

Gross observations—Skin incisions performed with the CO_2 laser and the RWRS unit resulted in no visible hemorrhage. Scalpel incisions caused minimal hemorrhage of ≤ 1 drop of blood/incision. Throughout the study period, no swelling, crusting, or discharge was detected grossly for any of the incisions evaluated, and all incisions were free of exudate or macroscopic signs of infection; therefore, gross wound scores were

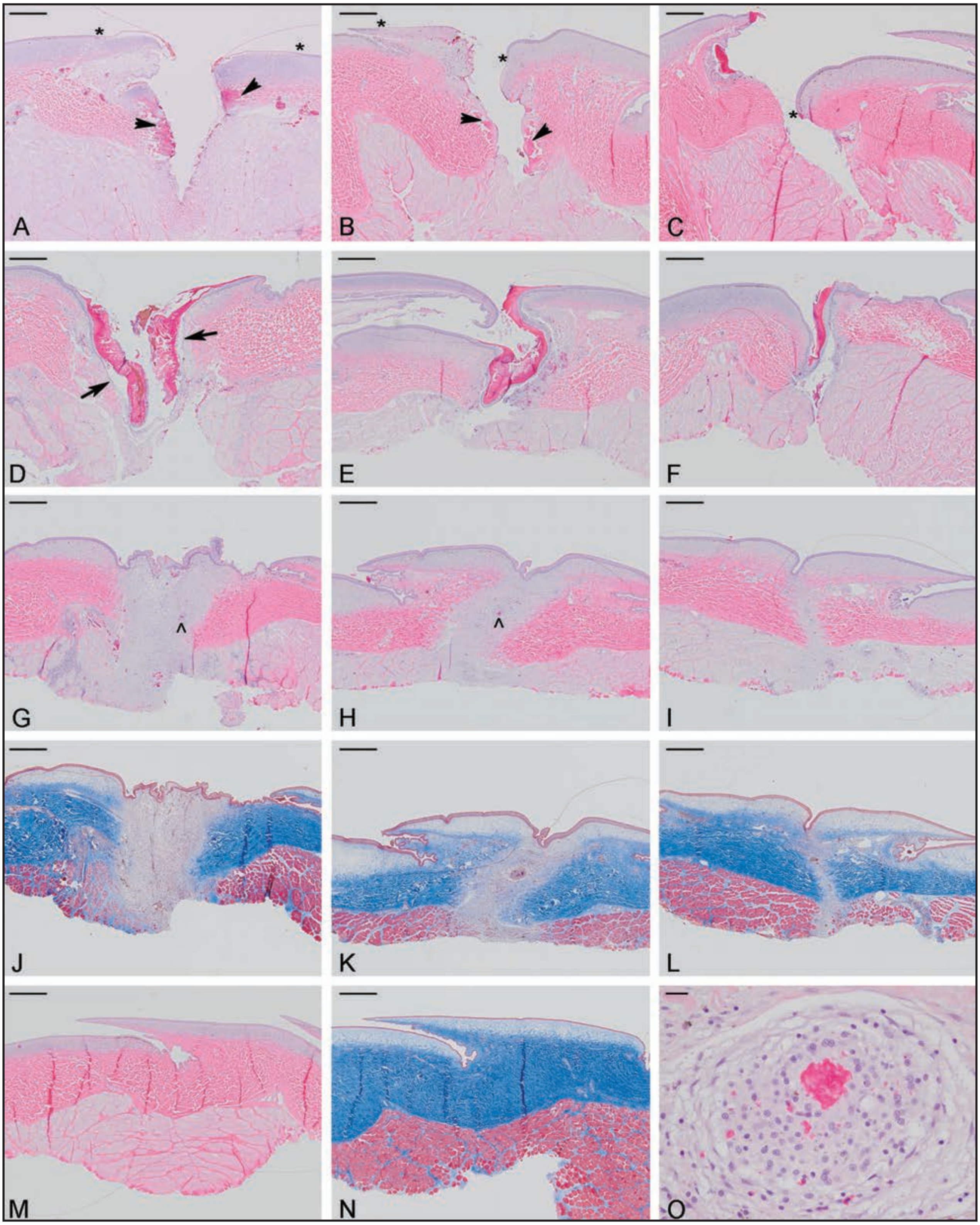

Figure 3—Photomicrographs of the skin of a ball python at various time points following surgical incision via CO_2 laser, RWRS, and scalpel, with control biopsy samples obtained from the same snake on day 0 (the day incisions were made). On day 2, no evidence of epithelial migration indicative of wound healing is seen for incisions made via laser (A), RWRS (B), or scalpel (C). Edges of epidermal necrosis (asterisks) and dermal collagen necrosis (arrowheads) are indicated. On day 14, notice partial healing of the laser incision (D) and complete healing of the RWRS (E) and scalpel (F) incisions; a thick crust is evident over the incision site on all skin surfaces. Arrows indicate the leading edge of epidermal reepithelialization. On day 30, healing is complete and the crust is resolved for laser (G), RWRS (H), and scalpel (I) incisions; carets indicate granulomas in the wound bed. The degree of fibroplasia on day 30 is greater for the laser incision (J), compared with incisions made via RWRS (K) and scalpel (L). Sections of a day 0 control biopsy sample (panels M and N) and higher magnification of a wound-associated granuloma (O) are shown. For panels A through I, M, and O, H&E stain; for panels J through L and N, Masson trichrome stain. Bar in panels A through N = 500 μm. Bar in panel O = 20 μm.

based solely on width (ie, edge-to-edge skin separation) of dehiscence. Laser incision lines typically had greater separation of skin edges, compared with RWRS and scalpel incisions, and had brown discoloration and carbonization at the skin edges consistent with char; these findings persisted throughout the study period. When dehiscence occurred in laser incisions, it involved the entire incision line; therefore, when biopsy samples were collected, the dehisced area was included. Grossly, healing of laser incisions was subjectively delayed, compared with the RWRS and scalpel incisions.

Least squares mean gross wound score was significantly ($P \leq 0.001$) greater for laser incisions, compared with RWRS and scalpel incisions, for all days (**Table 1**). No significant difference in this variable was detected between RWRS and scalpel incisions for any of the time points evaluated. The least squares mean gross wound score for RWRS and scalpel incisions indicated that neither modality resulted in values significantly different from zero for any time point, whereas that for laser incisions was always significantly ($P < 0.001$) different from zero (range, 0.71 ± 0.07 to 1.5 ± 0.10). Grossly, other than dehiscence, no adverse effects associated with the incisional modality were observed for any snake, and no snakes required medical intervention during the study period.

Histologic findings—For all modalities, areas of necrosis in biopsy samples were associated with coagulated, hypereosinophilic dermal collagen or coagulation necrosis of epithelial cells in the epidermis (Figure 3). Most incisions developed a thick serocellular crust (eschar) composed of serum and necrotic cellular debris. When laser and RWRS were used, tissue necrosis was most severe at day 2 and decreased by day 30, whereas when a scalpel was used, necrosis was most severe at day 7 (Table 1). Compared with scalpel incision sites, laser incision sites had significantly more necrosis on days 2 ($P = 0.007$) and 14 ($P = 0.005$). Use of RWRS resulted in significantly greater necrosis, compared with

Table 1—Measurements of necrotic and fibroblastic tissue and subjective surgical wound scores for CO_2 laser, RWRS, and scalpel incision sites in the skin of 6 healthy adult ball pythons (*Python regius*).

Time point and variable	Incisional modality CO_2 laser	 RWRS	 Scalpel
Day 2			
Necrotic tissue (µm)	108.3 ± 20.5^{a}	112.5 ± 20.5^{a}	10.4 ± 20.5^{b}
Fibroplastic tissue (µm)	0	0	0
Subjective score			
Total inflammation	3.3 ± 0.3^{a}	2.5 ± 0.3^{a}	2.7 ± 0.3^{a}
Histologic response	6.0 ± 0.4^{a}	5.0 ± 0.4^{a}	3.5 ± 0.4^{b}
Degree of healing	0	0	0
Gross wound	0.71 ± 0.07^{a}	3.7 × 10^{-17} ± 0.07^{b}	3.7 × 10^{-18} ± 0.07^{b}
Day 7			
Necrotic tissue (µm)	30.0 ± 5.0^{a,b}	36.7 ± 5.0^{a}	16.7 ± 5.0^{b}
Fibroplastic tissue (µm)	2.1 ± 3.6^{a}	6.3 ± 3.6^{a}	8.3 ± 3.6^{a}
Subjective score			
Total inflammation	4.5 ± 0.4^{a}	3.7 ± 0.4^{a}	3.3 ± 0.4^{a}
Histologic response	6.5 ± 0.6^{a}	6.0 ± 0.6^{a}	5.0 ± 0.6^{a}
Degree of healing	1.2 ± 0.2^{a}	1.3 ± 0.2^{a}	0.8 ± 0.2^{a}
Gross wound	0.94 ± 0.09^{a}	0 ± 0.09^{b}	0 ± 0.09^{b}
Day 14			
Necrotic tissue (µm)	40.4 ± 7.5^{a}	25.0 ± 7.5^{a,b}	6.3 ± 7.5^{b}
Fibroplastic tissue (µm)	48.3 ± 8.9^{a}	36.3 ± 8.9^{a}	25.0 ± 8.9^{a}
Subjective score			
Total inflammation	3.8 ± 0.5^{a}	3.5 ± 0.5^{ab}	2.8 ± 0.5^{b}
Histologic response	8.0 ± 0.6^{a}	6.8 ± 0.6^{a}	4.8 ± 0.6^{b}
Degree of healing	1.7 ± 0.3^{a}	1.7 ± 0.3^{a}	2.5 ± 0.3^{b}
Gross wound	1.50 ± 0.1^{a}	0.08 ± 0.1^{b}	1.5 × 10^{-16} ± 0.1^{b}
Day 30			
Necrotic tissue (µm)	4.2 ± 2.5^{a}	2.1 ± 2.5^{a}	4.2 ± 2.5^{a}
Fibroplastic tissue (µm)	106.3 ± 16.2^{a}	66.7 ± 16.2^{ab}	25.0 ± 16.2^{b}
Subjective score			
Total inflammation	4.2 ± 0.4^{a}	4.0 ± 0.4^{a}	3.7 ± 0.4^{a}
Histologic response	7.3 ± 0.4^{a}	6.8 ± 0.4^{a}	5.3 ± 0.4^{b}
Degree of healing	2.8 ± 0.3^{a}	2.5 ± 0.3^{a}	3.0 ± 0.3^{a}
Gross wound	0.83 ± 0.14^{a}	0.16 ± 0.14^{b}	5.5 × 10^{-19} ± 0.14^{b}

Data are reported as least squares means ± SE. In total, 24 samples were collected from incision sites for each modality (4 biopsy samples/incision type/snake [1 each on days 2, 7, 14, and 30, where day 0 = the day of skin incision surgery]). Total inflammation score was the sum of scores for granulocyte infiltrates into the wound bed (0 to 3) and perivascular infiltrates of lymphocytes and macrophages (0 to 3), where 0 = no infiltrates and 3 = dense infiltrates. Histologic response score was the sum of scores assigned for measurement of necrotic tissue (0 [none detected] to 3 [≥ 100 µm]) and fibroplastic tissue (0 [none detected] to 3 [≥ 75 µm]) and the total inflammation score. Degree of healing was scored on the basis of epithelial migration from the wound margin (0 [absent] to 3 [100%]). Gross wound scores were based on width of dehiscence (ie, edge-to-edge skin separation; 0 [none] to 3 [> 2 mm]).

[a,b]Within a time point, means of a variable with different superscript letters are significantly ($P < 0.05$) different between modalities.

Table 2—Presence of granulomas, surface bacteria, bacteria within granulomas, and total wound bacteria (surface bacteria or bacteria within granulomas) for CO_2 laser, RWRS, and scalpel incision sites (n = 24/modality) in the skin of 6 ball pythons.

Modality	Granulomas	Surface bacteria	Bacteria within granulomas	Wound bacteria
CO_2 laser	6 (25)	16 (67)	1 (4)	17 (71)
RWRS	9 (38)	13 (54)	7 (29)	20 (83)
Scalpel	1 (4)	12 (50)	0 (0)	12 (50)
Total	**16 (22)**	**41 (57)**	**8 (11)**	**49 (68)**

Data are No. (%) of samples.

use of a scalpel, on days 2 (*P* = 0.005) and 7 (*P* = 0.01). There was no significant difference in necrotic tissue measurements between laser and RWRS incision sites for any time point evaluated or among laser, RWRS, and scalpel incision sites on day 30.

During the course of the study, areas of necrosis were gradually replaced with fibroplastic tissue, as confirmed with Masson trichrome staining (Figure 3). No fibroplasia was present in any samples on day 2 (Table 1). On days 7 and 14, there were no significant differences in the degree of fibroplasia among modalities. However, on day 30, measurement of fibroplastic tissue was greatest in the laser incision sites and was significantly (*P* = 0.003) greater than that in scalpel incision sites.

All 3 incisional modalities caused inflammation in the skin sections examined on days 2, 7, 14, and 30 (Table 1). On day 14, the total inflammation score was significantly (*P* = 0.032) greater in laser incision sites than in scalpel incision sites.

The histologic response score was apparently lower for scalpel incision sites than for laser and RWRS sites at all time points, and significant differences were detected on days 2, 14, and 30 (Table 1). At these 3 time points, histologic response scores were not significantly different between laser and RWRS incision sites. However, on day 2, both laser (*P* < 0.001) and RWRS (*P* = 0.017) had significantly higher histologic response scores than did scalpel incisions. Similar results were observed on day 14 (*P* = 0.002 and *P* = 0.028 for laser and RWRS, respectively) and day 30 (*P* = 0.004 for laser and *P* = 0.017 for RWRS).

Of 72 biopsy samples evaluated, 41 (57%) had surface bacteria, 16 (22%) had incision-associated granulomas, and 8 (11%) had bacteria within granulomas (**Table 2**; Figure 3). Whereas granulomas developed in only 1 scalpel incision site, these were detected in 6 and 9 laser and RWRS incision sites, respectively. Use of a scalpel resulted in a significantly (*P* = 0.007) lower total wound bacteria score (0.5 ± 0.13), compared with results of RWRS (1.0 ± 0.13), when the scores were combined for all days.

No evidence of epithelial migration was present for any incisional modality on postoperative day 2 (Figure 3). On day 14, the degree of healing, as characterized by epithelial migration, was scored significantly (*P* = 0.029) greater for scalpel incisions, compared with laser or RWRS incisions (Table 1). No statistical evaluation of healing time was performed, but interestingly, by day 14, 4 of 6 laser incisions and 3 of 6 RWRS incisions were ≥ 50% healed. In contrast, 3 of 6 scalpel incisions were completely healed and 6 of 6 were ≥ 50% healed on day 14. By day 30, all 6 biopsied scalpel incisions were healed with complete epithelial migration, compared with 5 of 6 laser and 5 of 6 RWRS incisions.

Discussion

A consistent pattern of wound healing was detected in ball pythons of the present study, as was reported in a study[21] evaluating cutaneous wound healing of unsutured incisions and excisional wounds made with a scalpel blade in common garter snakes (*Thamnophis siralis*). Wound healing in reptiles is affected by surgical technique and orientation of the incision as well as by nutritional and health status of the patient, immune status, and provision of species-specific environmental conditions, including appropriate temperature and humidity.[21,23–26] As recommended in a previous report,[21] to provide optimal environmental conditions for healing, the ambient environmental temperature of snakes in the present study was kept close to the upper optimal body temperature for ball pythons during the day (29.4°C) and night (27.7°C). Additionally, incisions were made in a craniocaudal direction, parallel to the lines of skin tension, to reduce the effect of tension on incisional healing.[23] Every attempt was made to incise the skin between scales because incisions made in this manner rather than through the scales reportedly heal more quickly.[27] Subjectively, it was easier and faster to make skin incisions with the RWRS than with either the CO_2 laser or scalpel. Skin staples were chosen to close the incisions because stainless steel is the least reactive and strongest monofilament suture material available.[28–30] Skin staples also result in eversion of the skin, which allows skin edges to be apposed for primary healing.[26]

The main advantages gained with the use of laser and RWRS, compared with a scalpel, are their hemostatic properties, which result in sealing of lymphatics and small-diameter blood vessels. In the present study, hemorrhage from skin incisions made via scalpel was minimal, negating the necessity of these modalities for this purpose. Disadvantages of both laser and RWRS include thermal collateral penetration beyond the targeted tissue site with resulting tissue damage and potentially delayed healing.

To reduce lateral heat generation, any time RWRS is used, general principles of radiowave surgery should be followed, including high-frequency RWRS unit use, selection of the smallest appropriate electrode, minimal contact time with tissues, and an appropriate power setting. The power setting should be sufficient to vaporize and incise the tissues without charring or sparking; therefore, it should be increased if resistance to cutting (drag) is encountered or decreased if sparking or charring occurs.[8] The ideal power setting for incising snake skin is unknown. The power setting in the study reported here was chosen on the basis of experience with the RWRS unit for mammalian, avian, and reptile surgery at the investigators' institution. Incisions were easily and quickly made with the RWRS at a digital power setting of 25 (55 W) without tissue drag, sparking, or charring. The power setting chosen in this study was higher than that used in previous studies for skin incisions in iguanas[17] and pigeons[18] but similar to the current used in another study[31] for making porcine skin incisions. As for incisions in other species, RWRS power settings require adjustments according to the nature of the tissue and species of reptile.

The CO_2 laser is primarily used as a surgical scalpel because of its ability to make precise incisions, and it also has the advantage of reduced lateral thermal injury (0.05 to 0.2 mm width) with minimal tissue penetration in comparison with diode lasers (0.3 to 0.6 mm)[4,32–34]; however, this modality still has the potential to create collateral thermal damage. Reported depths of thermal damage range from < 0.1 mm[35–37] to 0.5 mm.[38] Lasers can be used to incise tissue, coagulate vessels ≤ 1 mm in diameter, seal lymphatics, and control intraoperative blood loss and postoperative edema.[4,34] Laser effects on tissue depend on power density and time of exposure. With low power density, there is increased thickness of the thermal necrosis zone because a longer time period is required to vaporize the tissue and more heat is transferred to the surrounding tissue. The longer the time interval used to deliver sufficient energy into the tissue to reach the ablation threshold, the greater the transmission of thermal energy into adjacent tissues. Power density decreases exponentially as the spot diameter increases. Therefore, a focused beam setting, small laser tip, appropriately high-energy setting to allow tissue vaporization without charring, and rapid laser movement over the tissue should be used to make surgical incisions with the CO_2 laser.

The CO_2 laser beam delivery can be in continuous-wave or pulsed mode; the rate of energy delivery is constant during laser activation in continuous-wave versus short pulses of peak energy in pulsed mode.[6,34] Incisions in rat skin made with a pulsed laser have been reported to heal at a rate similar to that achieved with scalpel incisions and to have reduced wound healing delay in comparison with continuous-wave laser incisions.[39] No studies comparing the use of continuous-wave versus pulse mode for making skin incisions in reptiles have been reported, and the ideal setting for skin incisions in reptiles with the CO_2 laser is unknown. The same surgeon performed all incisions in the present study to maximize consistency of speed and distance from the tissue, reduce collateral tissue damage, and decrease incisional variability. A 0.4-mm gold tip and 9-W continuous wave delivery were selected on the basis of recommendations for chelonian and iguana skin surgery,[2] reported protocols for making canine skin incisions,[12,16] and experience with the CO_2 laser for mammalian, avian, and reptile surgery at the investigators' institution.

Healing of incisions made with a surgical laser is reportedly delayed in comparison with scalpel incisions.[31,40–43] In a study[31] comparing CO_2 laser, RWRS, and scalpel modalities for making porcine skin incisions, the laser and RWRS incisions healed more slowly than did those created via scalpel, consistent with the findings in ball pythons of the present study. Although results of another study[44] in dogs indicated improved healing of incisions created via CO_2 laser, compared with those made via scalpel or RWRS, the results varied for different tissue types (uterine tissue vs skin).

In the present study, degree of first-intention healing was lower in CO_2 laser and RWRS incisions, compared with scalpel incisions on days 14 and 30, but this was only statistically significant on day 14. Potential reasons for a lesser degree of healing of laser and RWRS incisions early in the study period include increased tissue necrosis and collateral tissue damage and decreased fibrin clot formation as a result of increased intraoperative hemostasis.

Laser incisions in the present study had brown discoloration and carbonization at the skin edges consistent with char, and the laser incision edges were separated to a greater degree than incisions made via RWRS or scalpel. This finding persisted throughout the study period, which is in contrast to results of a study[45] in dogs that evaluated skin incisions made with continuous-mode CO_2 laser and scalpel, in which, after 4 days, there was no visible difference between the 2 types of incision. The persistence of char along the scale edges and wider appearance of laser incisions throughout the present study period have been attributable to the intermittent skin shedding of squamates, compared with continual sloughing of the outer keratinized skin layers in mammals. All snakes underwent ecdysis during the study period with lessening of the brown discoloration at the scale edges; however, the discoloration did not completely resolve, and it was possible to grossly identify the site of laser incisions throughout the study period. Additionally, laser incisions had a significantly greater degree of dehiscence, compared with those made via RWRS and scalpel on all days evaluated (days 2, 7, 14, and 30, where day 0 was the day of surgery). The RWRS and scalpel incision sites appeared grossly similar throughout the study period and often could not be differentiated.

Little difference was noted in the histologic appearance of the laser and RWRS incision sites for all time points evaluated and in their patterns of healing. On the basis of histologic findings of this study, neither laser nor RWRS was superior to a scalpel for making skin incisions in snakes, and the wounds made by these modalities were indistinguishable from each other as determined via histologic examination. Combining necrosis, fibroplasia, and inflammation scores to calculate a histologic response score, the least squares mean values for laser and RWRS incision sites were significantly different from that for scalpel incision sites on days 2, 14, and 30. These findings indicate that use of a scalpel resulted in the least tissue damage and inflammation of the 3 modalities. In the present study, the total inflammation score was highest for laser incision sites on all days, but a significant difference was only detected on day 14 between laser and scalpel incision sites. There was no significant difference in measurement of necrotic or fibroplastic tissues, total inflammation score, or histologic response score between laser and RWRS incision sites for any time point evaluated. This is in contrast to a study[18] evaluating skin incisions in green iguanas (*Iguana iguana*), in which RWRS was found to cause significantly less necrosis and collateral tissue damage than CO_2 laser immediately following incision. Unfortunately, that study[18] did not investigate wound healing for both incisional modalities.

In the present study, incision-associated granulomas were detected more frequently following use of the laser and RWRS than following use of a scalpel to create incisions. To the authors' knowledge, development of wound granulomas in healthy snakes in the absence of foreign material has not been reported. Surgical skin staples were used for incisional closure because surgical steel is the least reactive suture material available. Staples were used to close the incisions; therefore, granuloma development in the present study appeared to be independent of suture material use. Investigators of another study[46] evaluated histologic reactions to commonly used suture materials, including stainless steel skin staples and cyanoacrylate tissue adhesive, in the skin and muscle of ball pythons. They found no significant difference in granuloma formation between incisions that were closed with staples and those left to heal by second intention (negative control) or between incisions closed with tissue adhesive and the negative control group. On the basis of those findings,[46] cyanoacrylate tissue adhesive could have been used for incisional closure in the present study instead of staples. It is possible that granulomas detected in the present study developed in response to the surface bacteria, but this is unlikely because surface bacteria were detected in 12 of 24 (50%) scalpel incision sites, compared with 13 of 24 (54%) RWRS sites. Even though there was histologic evidence of crusting, surface bacteria, and granuloma formation, no incisional complications, other than dehiscence, occurred with any of the modalities evaluated, and first-intention wound healing progressed uneventfully with no gross evidence of infection.

Results of the present study suggest that CO_2 laser and RWRS may not be clinically practical for the sole use of making skin incisions because no substantial benefit was found for either modality, compared with use of a scalpel. Also, equipment cost is greater and additional specific safety measures are required for the use of laser and RWRS modalities. Carbon dioxide laser units are typically more expensive (3 to 10 times the cost of radiosurgical units) and require more stringent safety measures than RWRS, including the use of protective eyewear, evacuation of surgical vapors, covering of windows, and protection of the patients' eyes.[9,47] Radiosurgical units necessitate surgical vapor evacuation, but no other specific safety precautions are required. Radiosurgery application may be more diverse than that of CO_2 laser because RWRS can be used in fluid environments and for endoscopic procedures, and a larger selection of electrodes and devices are available.[9] The ability of CO_2 laser and RWRS to create bloodless incisions was not clinically important in study reported here but may be beneficial for other surgical applications.

In the present study, skin incisions in snakes were safely made with via CO_2 laser, RWRS, and scalpel, with similar histologic effects, minimal scarring, and complete healing. Comparison of the 3 modalities on the basis of histologic response scores indicated that use of a scalpel was preferable, followed by RWRS and then laser. Further studies are needed to elucidate the healing of laser incisions made with super-pulse mode versus continuous mode, determine ideal settings for CO_2 laser and RWRS for skin and muscle incisions in various reptile species, and determine whether the lesser degree of first-intention wound healing detected early in the present study following use of the laser and RWRS is clinically important.

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a. Buprenex, Reckitt Benckiser Healthcare Ltd, Hull, Yorkshire, England.
b. Ketaset, Fort Dodge Animal Health, Fort Dodge, Iowa.
c. NOVAPLUS, Hospira Inc, Lake Forest, Ill.
d. IsoFlo USP, Abbott Laboratories, North Chicago, Ill.
e. HotDog, Augustine Biomedical Design, Eden Prairie, Minn.
f. Nova-Pulse CO_2 laser LX-20SP Luxar Co, Bothell, Wash.
g. Surgitron, Ellman International, Oceanside, NY.
h. Royal 35W, United States Surgical, Norwalk, Conn.
i. Lidocaine 2%, Hospira Inc, Lake Forest, Ill.
j. Sodium bicarbonate 8.4%, Hospira Inc, Lake Forest, Ill.
k. Monocryl, Novartis Animal Health, Greensboro, NC.
l. Stata Statistical Software, Release 12, StataCorp LP, College Station, Tex.

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