

Histologic Comparison of Canine Skin Biopsies Collected Using Monopolar Electrosurgery, CO₂ Laser, Radiowave Radiosurgery, Skin Biopsy Punch, and Scalpel

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Objective—To compare the histologic appearance of canine skin biopsies collected by use of a scalpel, skin biopsy punch, monopolar electrosurgery, CO₂ laser, and radio wave radiosurgery in fully rectified wave form (RWRS).

Study Design—Experimental, randomized design.

Animals—Healthy adult grayhounds (n = 4).

Methods—Skin biopsies were collected using 5 techniques. Cut margins of biopsy specimens and adjacent peripheral skin were evaluated using light microscopy to compare penetration of the dermis by tissue carbonization (char).

Results—No char occurred in skin specimens collected by biopsy punch and scalpel. Char penetration occurred in all specimens collected by electrosurgery, CO₂ laser, and RWRS. Mean char penetration in skin biopsies collected by RWRS (0.158 mm) was significantly less than for monopolar electrosurgery (0.223 mm) and CO₂ laser (0.215 mm). Mean char penetration in adjacent peripheral skin surrounding biopsies collected by RWRS (0.171 mm) was significantly less than monopolar electrosurgery (0.255 mm) but not less than CO₂ laser (0.215 mm, $P < .07$).

Conclusions—RWRS (blended waves in cut-coagulate mode) caused less lateral thermal damage to canine skin biopsies than monopolar electrosurgery and CO₂ laser and less lateral thermal injury to peripheral skin than monopolar electrosurgery.

Clinical Relevance—Excision of canine skin biopsies with heat-generating devices may not allow reliable histologic interpretation, particularly when assessing margins of small biopsy specimens. RWRS may be less traumatic to canine skin than monopolar electrosurgery and CO₂ laser when used to make incisions.

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INTRODUCTION

NUMEROUS OPTIONS for collection of skin biopsies are available to veterinarians. Traditional use of a scalpel has been replaced in some veterinary practices with technologies that use various forms of energy to cut tissue, specifically electrosurgery, laser, and radio wave radiosurgery (RWRS) devices. Electrosurgery creates heat

in tissue by passing an electric current through the patient's tissues between 2 points of contact (e.g., hand piece and grounding plate). Electrical current resistance within the tissue produces heat from the inside out. The points of contact of the device do not increase in temperature (except by heat transfer from the tissue). Electrosurgery provides a more controlled treatment with less lateral thermal tissue injury than electrocautery.¹

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Lasers work by photostimulation, a process whereby light energy is selectively deposited into a specific optical zone, and photochemical, photothermal, and photomechanical effects on the tissue occur. CO₂ laser energy is selectively absorbed by water. As light energy from the CO₂ laser is absorbed by intracellular and extracellular water molecules, heat is generated resulting in tissue ablation and vaporization.²

RWRS technology delivers low-temperature, high-frequency (4 MHz) radio wave energy to the tissues through a metal tip. The radio wave passes from a hand-held active electrode to a passive electrode positioned very near or beneath the patient. Tissue resistance to the radio wave transmission causes an ionic agitation in the cells at the tip of the active electrode, resulting in molecular friction and subsequent heating of the tissue. Thus, molecular friction within the tissues is the source of the generated heat and not the active electrode tip itself. Factors which increase the precision of tissue dissection with RWRS, while minimizing lateral thermal tissue damage, include the limitation of contact time of the active electrode with tissues, intensity of power, type of electrode, waveform mode, and radio wave frequency.³ One of the perceived advantages of these heat-generating tissue cutting technologies is incisional hemostasis largely because energy produced is converted to thermal energy resulting in tissue coagulation. With any device that creates thermal energy to cut or ablate tissue, heat may be dissipated by diffusion into adjacent tissues (conduction), into the surrounding environment (convection), or into the circulating blood.⁴ The resulting lateral thermal injury to tissues may result in delayed healing and increased risk of wound dehiscence.⁵ This same phenomenon may adversely affect the histologic quality of tissue submitted for microscopic assessment, impair the interpretation for histopathologic diagnosis, and invalidate the evaluation of margins of a biopsy of malignant tissue.

Previous reports have assessed the effects of CO₂ laser on the histologic quality of excised tissues from dogs and humans.⁵⁻¹⁴ Similarly, RWRS has been reported to cause minimal tissue injury to the human uvula and soft palate, canine soft palate, human oviduct, and human skin.¹⁵⁻¹⁸ Although this technology has been available to veterinarians for several years, we are unaware of any data reporting the suitability of RWRS for the excision of skin lesions in dogs.

Our purpose was to compare the histologic appearance of full-thickness skin biopsies and adjacent peripheral skin collected from dogs using 5 different techniques: a #15 scalpel blade, 6 mm skin biopsy punch, monopolar electrosurgery, CO₂ laser, and RWRS. We hypothesized that RWRS, monopolar electrosurgery and CO₂ laser would create the same thermal damage to skin biopsy specimens and to adjacent peripheral skin, and that this

thermal injury would be greater than that created by #15 scalpel blade and 6 mm skin biopsy punch. We tested our hypothesis by comparing the histologic appearance of the cut margins of the biopsy specimens and the adjacent peripheral skin, specifically evaluating the penetration of the dermis by tissue carbonization (char).

MATERIALS AND METHODS

Dogs

Four healthy, adult grayhounds were used studied. Results of physical and dermatologic examinations were considered normal for all dogs.

Surgical Preparation

Dogs were premedicated with atropine, sedated with acetyl promazine and morphine sulfate, induced for general anesthesia with intravenous propofol, and maintained with isoflurane in oxygen. Anesthetized dogs were initially positioned in right lateral recumbency. The left lateral thorax was clipped, aseptically prepared with 2% chlorhexidine scrub, rinsed with saline (0.9% NaCl) solution, and draped.

Specimen Collection

Biopsies were taken from an area of skin on the lateral thorax, centered between the scapular spine cranially, 13th rib caudally, the ventral border of the epaxial muscles dorsally, and the costochondral junction ventrally. Biopsy site locations were measured and marked with a skin pen and template. The centers of all biopsy specimens were spaced 30 mm apart in 2 alternating rows. After 13 biopsies were taken from the left thorax, the dogs were repositioned in left lateral recumbency, the skin on the right lateral thorax was prepared as described earlier, and 12 biopsies were collected from the right thorax.

Thus, 25 full-thickness skin biopsies were collected from each dog with 5 skin biopsies collected by each of 5 different techniques: #15 scalpel blade, skin biopsy punch, monopolar electrosurgery, CO₂ laser, and RWRS. The method of excision was randomly assigned to each site for each dog using a random number generating feature of a commercial software program (Excel, Microsoft Corp, Redmond, WA). Skin biopsy specimens excised with #15 scalpel blade were 6 mm × 24 mm ellipses whereas specimens excised by skin biopsy punch, monopolar electrosurgery, CO₂ laser, and RWRS were 6 mm diameter circles. After collection of all biopsy specimens from 1 side of the thorax, a #15 scalpel blade was used to collect a 10 mm wide perimeter of full-thickness skin around each biopsy site to allow evaluation of treatment effects on the skin immediately adjacent the biopsy. Wounds were not closed and the dogs were euthanatized after tissue collection for reasons unrelated to this study.

Scalpel. A 6 mm × 24 mm elliptical full-thickness skin biopsy was excised using a #15 scalpel blade. DeBakey thumb forceps were used to grasp the specimen and the subcutaneous tissue attachments were cut with Metzenbaum scissors.

Skin Biopsy Punch. The blade of a 6 mm skin biopsy punch (Dermal Biopsy Punch, Miltex Inc, Bethpage, NY) was placed perpendicular to the skin surface and pressure was applied with a twisting motion. After the blade penetrated the subcutis, DeBakey thumb forceps were used to grasp the full-thickness specimen, and the subcutaneous tissue attachments cut with Metzenbaum scissors.

Monopolar Electrosurgery. A 6 mm diameter circular full-thickness skin biopsy was excised using a monopolar electrosurgery (ConMed Excalibur Plus PC, Model 60-62-50-001, Aspen Surgical Systems, Englewood, CO) and a hand piece. The ground plate was placed against the lateral aspect of the dependent thigh. The dial setting was 35 W in cutting mode. After circumferentially penetrating the subcutis, DeBakey thumb forceps were used to grasp and elevate the specimen, and the subcutaneous tissue attachments cut with the electrosurgery hand piece.

CO₂ Laser. A 6 mm diameter circular full-thickness skin biopsy was excised using CO₂ laser (Luxar Nova Pulse 20 W CO₂ laser, Model #LX-20 SP, Luxar Corp., Bothell, WA, presently available as AccuVet, Lumenis Inc, Santa Clara, CA) with a 0.8 mm ceramic tip (Luxar 0.8 mm ceramic tip, Luxar Corp.) and settings of 10 W (W), continuous wave mode. The laser tip was positioned 1–2 mm from the skin surface. Sterile saline (0.9 NaCl) solution moistened gauze sponges were used to remove carbonized debris as needed to facilitate cutting of tissue with the laser. After the laser circumferentially penetrated the subcutis, DeBakey thumb forceps were used to grasp the specimen, and the subcutaneous tissue attachments were divided with the laser. Standard safety precautions for CO₂ laser were implemented and included protective eyewear, laser-safe hydrophobic masks, and use of a smoke evacuator (Luxar AirSafe smoke filtration system, Luxar Corp.) for removal of the laser plume.

RWRS. A 6 mm diameter circular full-thickness skin biopsy was excised using a RWRS unit (ellman Surgitron 4.0 Dual RF, ellman International Inc., Oceanside, NY) with hand piece and a 36-gauge A-series wire tip (ellman A8 bendable electrode, ellman International Inc.). The antenna plate was placed adjacent to the dependent side of the thorax, just caudal to the scapula. The settings were 4 MHz in blend mode (50% cutting mode, 50% coagulation), and wattage varied between 18–39 W. Wattage setting was adjusted based on tactile assessment while cutting. If tissue “drag” was detected while cutting, the power setting was increased. After the tip of the hand piece circumferentially penetrated the subcutis, DeBakey thumb forceps were used to grasp the specimen, and the subcutaneous tissue was divided with RWRS.

Histology

After excision, all tissue specimens were immediately immersed in 10% neutral-buffered formalin solution in individual labeled containers. After fixation, entire biopsy specimens and ~7 mm wide segments of the peripheral skin were embedded in paraffin. All specimens were sectioned at 5 µm and stained with hematoxylin and eosin. The cut margins of each biopsy specimen and its peripheral skin were evaluated under

low- and high-power light microscopy by a pathologist (RR) unaware of collection method. Specimens that were too distorted after processing to allow accurate measurement of char penetration were excluded from analysis.

Char was expected to be recognized as a distinct zone of hyalinization or coagulation with alteration of staining on the cut margins of the specimens (Fig 1A). Specimens were viewed at $\times 40$, then $\times 100$ for measurement of the penetration of char. The depth of char was measured at the location of least width of thermal damage on the margin within the dermis (Fig 1B). This site would have the most perpendicular orientation to the cut margin of the tissue specimen, and thus would be the most representative of char penetration. Penetration of tissue carbonization and coagulation artifact was measured and recorded in millimeters.

Statistical Analyses

Char penetration was analyzed using ANOVA for a repeated measures design with 1 within-subject factor (method) and one within-site within-subject factor (specimen type). Tests of model effects used the 0.05 level of significance. Results were reported as least squares means, standard error based on the pooled estimate of variance from the ANOVA model, and 95% confidence intervals (CI) for the least squares mean. Differences among methods were determined using the least significant difference test. The clinical importance of differences among methods was assessed using CIs for the differences.¹⁹ All calculations were performed using statistical software (SAS System for Windows, Version 9.1, SAS Institute Inc., Cary, NC).

RESULTS

Twenty biopsy specimens were collected using each method. All specimens collected by scalpel and skin punch were evaluated whereas 4 specimens collected by electrosurgery, two by CO₂ laser, and one by RWRS were not considered readable after histologic processing. Thus, char penetration was measured in 16 electrosurgery, 18 CO₂ laser, and 19 RWRS biopsies.

All specimens of adjacent peripheral skin surrounding the biopsy sites collected by scalpel, skin punch, electrosurgery, and CO₂ laser were evaluated. Three RWRS peripheral skin specimens damaged during processing were not evaluated. The margins of the remaining 17 RWRS peripheral skin specimens were readable.

Char

Char was visible at the cut margins as a distinct zone of coagulation with condensation, hyalinization, and loss of fibrillar texture of collagen. Staining varied from increased basophilia to eosinophilia. The epidermis had variable, typically broader, zones of full-thickness necrosis (Fig 1B). The zone of char in the dermis which con-

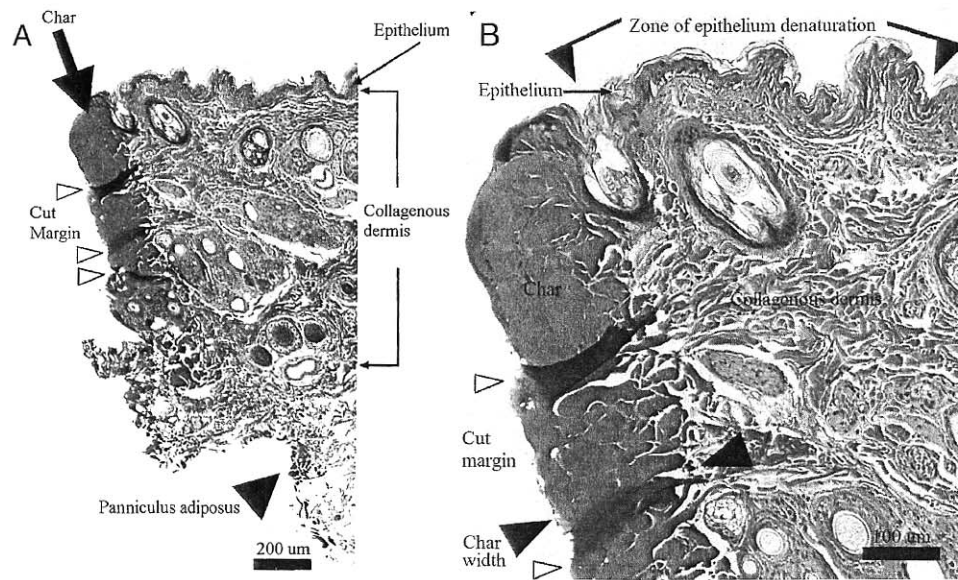


Fig 1. (A) Skin biopsy showing char (large arrow) on the cut margin of the dermis. Note also the decreased char apparent in the deeper panniculus adiposus (black arrowhead). Folding artifacts (open arrowheads) which were typical in the samples collected with monopolar electrosurgery, CO₂ laser, and RWRS ($\times 40$ H&E). (B) same skin biopsy at higher magnification showing broad zone of epidermal coagulation necrosis on the surface (large arrowheads) compared with char in the dermis. Black arrowheads indicate the location of char width measurement. Note folding artifacts (open arrowheads) ($\times 100$ H&E).

tains a higher density of collagen was wider than that in the deeper panniculus. In the panniculus, where adipose tissue is increased and collagen density is decreased, minimal and fragmented zones of carbonization and coagulation artifact were evident. Thermally induced contraction of collagen resulted in variations of tissue density and subsequent folding artifacts along the cut margins after sectioning with the microtome. When viewed at low power ($\times 40$), all but four of the 60 biopsy samples collected with electrosurgery, CO₂ laser and RWRS had easily identifiable folding artifacts in the cut margins (Fig 1A); however, this artifact was identified in none of the 40 biopsies collected by scalpel and skin biopsy punch.

Biopsy Specimens. No char occurred on the margins of skin biopsies excised by scalpel and skin punch. Mean char penetrations in skin biopsies collected by monopolar electrosurgery, CO₂ laser, and RWRS were significantly >0 mm (Table 1). Mean char penetration in specimens

collected by RWRS (0.158 mm) was significantly less than by monopolar electrosurgery (0.223 mm, $P = .01$), and the mean difference (95% CI) between techniques was -0.065 mm (-0.114 mm, -0.015 mm). Mean char penetration in specimens collected by RWRS was significantly less than by CO₂ laser (0.215 mm, $P = .02$), and the mean difference between techniques was -0.057 mm (-0.105 mm, -0.008 mm). However, mean char penetration in specimens collected by monopolar electrosurgery was not significantly different from CO₂ laser ($P = .7$), and the mean difference between techniques was 0.008 mm (-0.042 mm, 0.057 mm).

Peripheral Skin Specimens. No char occurred on the margins of the peripheral skin surrounding biopsies excised by scalpel and skin punch. Mean char penetrations in all peripheral skin specimens collected by electrosurgery, CO₂ laser, and RWRS were significantly >0 mm. Mean char penetration in peripheral skin surrounding

Table 1. Mean Char Penetration (mm) on the Margins of the Biopsy and Adjacent Skin

Technique	Specimen					
	Biopsy			Peripheral Skin		
	N	Least Squares Mean	95% CI	N	Least Squares Mean	95% CI
Monopolar electrosurgery	16	0.223*	(0.180, 0.265)	20	0.255*	(0.215, 0.296)
CO ₂ laser	18	0.215*	(0.174, 0.256)	20	0.215*	(0.174, 0.255)
RSRW	19	0.158*	(0.117, 0.199)	17	0.171*	(0.129, 0.213)

*Within specimen type, means followed by the same letter are not significantly different at the 5% level by the least significant difference test.

N, number of specimens; CI, confidence interval of the mean; RSRW, radio wave radiosurgery.

biopsies collected by RWRS (0.171 mm) was significantly less than in peripheral skin surrounding biopsies collected by monopolar electrosurgery (0.255 mm, $P = .002$), and the mean difference between techniques was -0.084 mm (-0.132 mm, -0.036 mm). Mean char penetration in peripheral skin surrounding biopsies collected by RWRS was not significantly different than CO₂ laser (0.215 mm, $P < .07$), and the mean difference between techniques was -0.044 mm (-0.092 mm, 0.004 mm). Mean char penetration in peripheral skin surrounding biopsies collected by monopolar electrosurgery was not significantly different from CO₂ laser ($P = .08$), and the mean difference between techniques was 0.041 mm (-0.006 mm, 0.088 mm).

The thermal penetration in the biopsies collected by CO₂ laser was the same as that of the adjacent peripheral skin (Table 1). Although, the thermal penetrations in the biopsies collected by electrosurgery and RWRS were less than that of peripheral skin samples, these differences were not significant ($P > .05$).

DISCUSSION

We found that all techniques that used energy-induced heat to incise skin resulted in charring when compared with controls (skin punch, scalpel blade), indicating evidence of peripheral heat penetration and lateral tissue damage. RWRS technology resulted in the least penetration of coagulation artifact for the heat-producing devices we compared. To our knowledge this is the first report to evaluate the suitability of RWRS for excision of skin lesions in dogs.

RWRS

Biopsies of human skin collected with RWRS have been reported to have thermal damage zones of 0.075 mm.¹⁸ RWRS caused minimal (0.3 mm) lateral thermal damage to human oviducts,¹⁷ and has been reported to provide satisfactory results in human patients having uvula and soft palate ablation¹⁵ and in dogs having soft palate resection.¹⁶

CO₂ Laser

Effects of CO₂ laser on the histologic quality of excised tissues has been reported.⁵⁻¹⁴ Margins of cervical biopsies from women collected with CO₂ laser were either difficult to interpret or were unable to be interpreted in 27% of patients.^{6,7} In another study, carbonization artifact ("char") of CO₂ laser excised biopsies resulted in specimens that were not interpretable.⁸ However, Baggish et al⁹ determined that the zone of thermal injury caused by CO₂ laser during cervical biopsy did not result in significant detriment to accurate margin assessment. Thermal

transmission to surrounding tissue by CO₂ laser has been described as minimal,¹⁰ and reported depths of thermal damage range from <0.1 mm¹¹⁻¹³ to 0.5 mm.¹⁴

Rizzo et al⁵ reported that the depth of thermal injury by CO₂ laser delivered at 10–20 W through a 0.8 mm ceramic tip in 6 mm diameter full-thickness canine skin biopsies was 0.31 – 0.41 mm. Although, it was concluded that laser induced artifacts could render small biopsy specimens unreadable, the authors asserted that the zone of thermal damage in 6 mm diameter canine skin biopsies excised by CO₂ laser was "minimal" and would not be expected to interfere with diagnostic evaluation.⁵ Although the zone of thermal reported by Rizzo et al⁵ was much larger than the 0.154 mm zone of lateral thermal tissue injury we obtained with RWRS, our results suggest that accurate interpretation of margins could be hindered if a heat-generating device is used for the excision of cutaneous tissues.

Using electrosurgical machines in continuous waveform and with higher carrier frequencies reduces tissue alterations.²⁰ The manufacturer of the RWRS unit we used recommends that the filtered "cutting" mode be selected when performing biopsy collection. The filtered "continuous wave" waveform is 90% cutting and 10% coagulation. However, bleeding was substantial when the RWRS in filtered ("continuous wave," "cutting") waveform was tested on the dogs' skin at a distant site (the lateral thigh). Because cutting without bleeding is one of the primary clinical advantages of heat-generated tissue incision, we selected the blended mode (50% cut and 50% coagulation) to obtain hemostasis during incision. Use of the blended mode likely increased the charring of the specimens, but did decrease hemorrhage during collection. Interestingly, though the optimal setting (filtered "cutting" mode) was not used, charring artifact in skin incised with RWRS was still less than that in skin incised with CO₂ laser and with monopolar electrosurgery.

We found that lateral tissue damage caused by the CO₂ laser (0.215 mm) was within the range (<0.1 – 0.5 mm) previously reported.^{5,11-14} Selection of a 0.8 mm ceramic tip and 10 W continuous wave were based on another protocol where effects of CO₂ laser on canine skin were compared.⁵ The char produced by the laser may be attributed to excessive time of application⁸ of laser energy to the tissues and the use of a suboptimally sized laser tip. Increased precision and power density and decreased lateral photothermal change may have been obtained by using a smaller diameter (0.3 or 0.4 mm) tip.⁵

Mean depth of char produced by monopolar electrosurgery was significantly greater than that created by CO₂ laser and RWRS excisions. This increased lateral thermal injury associated with monopolar electrosurgery can be attributed to conduction of electric current between the monopolar electrosurgery tip and the ground plate and

the subsequent generation of heat through a wider zone of tissue. The power setting used was selected based on tactile assessment of "smooth" cutting during incision. However, when using monopolar electrosurgery, the cutaneous trunci muscle would contract strongly, making controlled incision difficult, and this may have resulted in more irregular biopsy specimens.

Skin Changes

Changes in normal canine skin caused by routine processing for histologic evaluation have been shown to include a decrease in length (up to 32%) and an increase in thickness (up to 75.8%).²¹ Although we were concerned that changes in skin thickness because of processing artifact might affect the true measurement of char penetration, the relative comparisons between techniques should not be affected.

We did not measure surface epithelium and adnexa because these components had variable, typically broader, zones of denaturation (Fig 1B). These changes in the epidermis may have important clinical relevance when interpreting neoplastic cutaneous lesions or other dermatologic conditions that may disrupt the epidermal architecture.²²

The heat-generating energy devices we used caused deformation of the skin biopsies. Thermal energy causes the contraction of collagen in the dermal tissue, and subsequent shrinking may be irregular. This distortion, along with tissue changes associated with fixation and cutting artifacts by the microtome, may have contributed to some of the specimens having unreadable margins after processing.

When biopsy samples were viewed at low power ($\times 40$), readily identifiable folding artifacts of the cut margins were present in all but four of the biopsy samples collected with the heat-generating methods evaluated. This artifact was not identified in any of the biopsies collected by scalpel and skin biopsy punch. This finding may be attributed to thermally induced collagen contraction and subsequent abrupt changes in tissue density which results in this artifact during sectioning.

Our results study indicate that excision of canine skin biopsies with heat-generating devices causes lateral tissue damage that may interfere with histologic interpretation of specimen margins, especially in small specimens. To assess whether these charring artifacts would interfere with margin evaluation, comparison of several blinded pathologists' interpretations of the margins of tumors excised with these devices are warranted. This study also demonstrates that RWRS used in cut-coagulate mode causes less lateral thermal tissue damage to canine skin biopsies than monopolar electrosurgery and CO₂ laser and less lateral thermal injury to adjacent peripheral skin

than monopolar electrosurgery. Studies comparing the healing of surgical wounds in canine skin created by these techniques are also warranted.

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