Carbon Dioxide Laser Resection of a Distal Carpal Pilomatricoma and Wound Closure Using Swine Intestinal Submucosa in a Dog

A carbon dioxide laser was used to incise around, dissect, and remove a 2-cm intradermal mass from the left carpus of an 8-year-old, spayed female wheaten terrier. The wound was partially closed, resulting in a 3-cm diameter circular defect with extensor tendons exposed. A swine intestinal submucosa graft was utilized to cover the remaining defect. The graft was removed 5 days later, revealing a healthy granulation tissue bed covering previously exposed tendons with minimal wound margin retraction. The remaining wound was allowed to heal by contraction and epithelialization that was complete by 5 weeks postoperatively. The mass, a pilomatricoma, had not recurred at the last follow-up contact 18 months after surgery. Pilomatricoma, laser application, swine intestinal submucosa grafting, and postoperative wound management are discussed.

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Introduction

An intradermal mass below the elbow or stifle often poses difficult management options. Adequate surgical excisions frequently leave wounds impossible to close without the assistance of skin flaps,[1] grafts,[2] or second-intention healing.[3] Conventional sliding, rotational, and Z-plasty flaps usually are not appropriate for skin closure in the extremities because of skin tension and immobility that precludes tissue shifting.[2] Remaining options for closure include indirect tube flaps, body wall pouch flaps, full- or split-thickness skin grafts, and seed or strip grafts.[2,4] Indirect tube flaps and body wall pouch flaps require much time and multiple surgical procedures to complete.[2,4] Full-thickness, split-thickness, seed, and strip grafts require adequate vascular supply from a healthy granulation tissue bed at the recipient site, and a second wound donor site is also required.[2] Exposure of bone or tendon within a wound hinders development of an adequate granulation tissue bed necessary for grafting or second-intention wound healing.[2,4] Motion and lack of adequate soft-tissue support structures associated with bone and tendon exposure in a wound may disrupt or restrict the formation of a microvascular bed necessary for the formation of healthy granulation tissue. The lack of a granulation tissue bed can delay wound healing, resulting in increased time to achieve wound resolution.[2]

Carbon dioxide (CO_2) lasers are rising in popularity for soft-tissue surgery because of their ability to incise most soft tissues with minimal lateral thermal damage combined with excellent hemostasis.[5] Carbon dioxide lasers emit light with a wavelength of 10,600 nanometers (nm) that is highly absorbed in water, a major component of soft tissue.[5,6] This focused energy absorption, when combined with appropriate power density and rapid delivery to tissue, results in clean incisions with minimal (typically <0.1 mm) lateral thermal tissue damage.[5,7] Laser resection results in dramatically reduced hemorrhage and less disruption of

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the excisional site through decreased tissue handling compared to conventional surgical excision. Improved hemostasis and decreased tissue handling result in excellent visualization of lesion margins, allowing closer lesion excision. Minimal resection around lesions results in smaller excision areas, allowing for more options for closure and faster wound resolution.

Swine intestinal submucosa (SIS) has been utilized *in vivo* for xenographs in multiple species and tissue types.[8-11] Swine intestinal submucosa is an acellular, collagen-based matrix, which contains selected cytokines and signaling molecules.[12] Swine intestinal submucosa is primarily composed of type I collagen, is degradable and resorbable *in vivo*,[13] and illicits no adverse immunological response.[11,12] Swine intestinal submucosa provides a scaffold in wounds that promotes a rich and rapid vascular response with a resultant resistance to infection.[12,14]

The purpose of this report was to demonstrate the utility of a CO_2 laser to afford a conservative excision of a distal extremity mass with minimal hemorrhage and tissue trauma. This, in combination with the application of an SIS xenograft, eliminated the need for a skin graft and minimized the time needed to achieve second-intention wound healing.

Case Report

A 16.8-kg, 8-year-old, spayed female wheaten terrier was presented to the soft-tissue surgical referral service for the treatment of a dermal mass on the left thoracic limb distal to the carpus. The mass was first noticed 4 months previously by a groomer. One month prior to presentation, the referring veterinarian examined the mass, noted it to be cystic, and drained it by aspiration. Referral was elected after the cyst consolidated and rapidly developed into a solid, ulcerated mass. The dog had a history of urinary incontinence and was being medicated with phenylpropanolamine (25 mg, per os [PO], *q* 12 hours); she was also being supplemented with chondroitin sulfate (500 mg, PO, *q* 12 hours) for ancillary treatment of a luxated patella. All appropriate prophylactic care, including vaccinations, were current.

Presenting physical examination revealed a 1 × 3-cm mass located in the subcutaneous tissue of the right thorax, just caudal to the elbow. This mass was soft and freely movable. Fine-needle aspiration cytopathology was suggestive of a lipoma. The suspected lipoma was not addressed at this time. A firm, elevated, 2-cm dermal mass with a small central surface erosion was noted over the dorsolateral surface of the metacarpus of the left thoracic limb [Figure 1]. This mass was freely movable and appeared confined to the dermis. Fine-needle aspiration cytopathology yielded a diagnosis of cystic epithelial neoplasia with mild to moderate features of malignancy and evidence of mild inflammation. This presentation was suggestive of a basal cell/follicular cell tumor with possible squamous differentiation. Excisional biopsy was chosen for the initial treatment of the distal limb mass, because of the low malignancy rate associated with follicular cell tumors, along with owner concerns regarding amputation.

The day following admission, atropine sulfate (0.04 mg/kg body weight, intramuscularly [IM]), xylazine (5 mg/kg body weight, IM), and morphine (0.5 mg/kg body weight, IM) were administered as preanesthetics. A left lateral saphenous intravenous (IV) catheter was placed for intraoperative and postoperative management. During anesthesia, lactated Ringer's solution was given IV at the rate of 170 mL per hour for the first hour, and then 85 mL per hour thereafter. Thiopental sodium (10 mg/kg body weight, IV) was used for induction of anesthesia, and isoflurane was used for maintenance of inhalant anesthesia. With the dog in right lateral recumbency, the entire distal left thoracic limb from the elbow was aseptically prepared for surgery.

Palpation of the mass during aseptic preparation revealed a second, smaller, adjacent mass at the proximolateral aspect of the first. A CO_2 laser[a] with a 0.4-mm tip set at 6 watts average power in continuous super-pulse mode was defocused and used to ablate and seal the central erosion on the surface of the primary mass. The laser was then refocused on the dermis and used to make pinpoint incisions at 2- to 3-mm intervals to outline the masses and define the area of excision [Figure 2]. These pinpoint incisions were then connected to completely incise through the skin and subcutaneous tissues [Figure 3]. The subcutaneous tissue below the masses was carefully incised with the laser while the excisional portion of the masses was retracted medially, exposing the metacarpal tendons beneath [Figure 4]. The masses were removed *en bloc* and submitted for histopathological evaluation. Once hemostasis was ensured, closure of the defect was performed.

The proximolateral aspect of the wound was closed with a single, subcuticular, horizontal mattress suture using *3-0* glycomer 631[b] [Figure 5]. Four layers of single-ply SIS[c] were used to cover the remaining wound and exposed tendons. The SIS was sutured to the surrounding skin margin in a simple continuous pattern using *3-0* glycomer 631 [Figure 6]. A bandage was placed from just above the elbow to the digits. A nonadhering dressing[d] covered the wound, followed with two rolls of cast padding,[e] then two rolls of stretch bandage.[f] Strips of heat-conforming material[g] were used to make a splint that continued down the ventral aspect of the leg. Stretch bandage and elastic tape[h] were then utilized to incorporate the splint into the bandage.

Postoperatively, the dog was recovered in the intensive care unit (ICU) and was continued on morphine (0.5 mg/kg body weight, IV, *q* 4 hours) for the first 24 hours. Intravenous lactated Ringer's solution (25 mL per hour, IV) was continued while in ICU. Phenylpropanolamine (25 mg, PO, *q* 8 hours) and chondroitin sulfate (500 mg, PO, *q* 24 hours) were administered as before surgery for the previously mentioned problems. The bandaged limb was monitored hourly for signs of soiling. The exposed toes of the bandaged limb were monitored hourly for swelling, hypothermia, and discoloration.

The dog was removed from the ICU and placed in the general ward on the second day after surgery. She was

Figure 1—Photograph of a 2-cm mass located on the dorsolateral surface of the left metacarpus of an 8-year-old wheaten terrier.

Figure 2—Laser pinpoint incisions outline a left metacarpal mass and an adjacent proximolateral smaller mass immediately prior to excision in the dog from Figure 1. The arrow points to a laser-ablated erosion on the surface of the mass.

Figure 3—Laser excision margin for the mass in Figure 2 was created by connecting laser pinpoint incisions.

given carprofen[i] (37.5 mg, PO, *q* 12 hours) for the next 5 days for continued pain management. The bandage and splint were removed on the third day after surgery to evaluate the wound. At that time, the SIS was intact, and there was minimal swelling and drainage [Figure 7]. The bandage and splint were replaced as previously described. During bandage change on the fifth postoperative day, the SIS was beginning to liquefy. The remaining SIS was removed, revealing a healthy granulation tissue bed that completely covered the previously exposed tendons [Figure 8]. The bandaging technique previously described was demonstrated to the owner. The owner was instructed to perform bandage changes every 2 to 3 days until such time that epithelialization was completed over the new granulation tissue bed. The dog was released to her owner with instructions for re-examination in 3 weeks.

Figure 4—Photograph of the remaining wound bed and exposed metacarpal tendons following excisional biopsy of a left dorsal metacarpal mass from Figure 3. Charred area (arrow) represents the minimal use of electrosurgery to control hemorrhage.

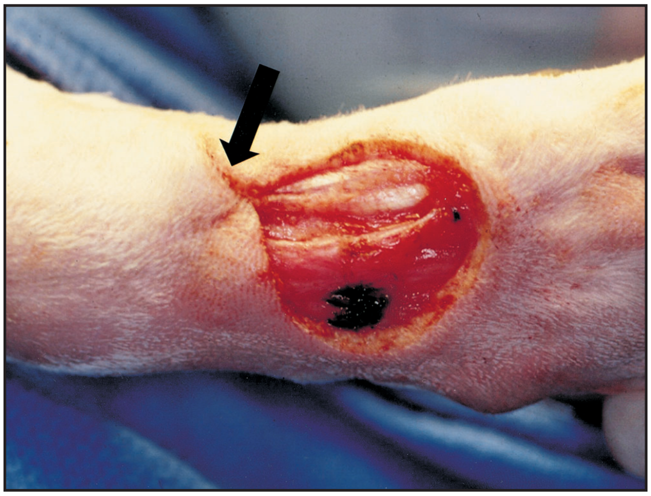

Figure 5—Partial closure (arrow) of a left dorsal metacarpal wound bed from Figure 4 with *3-0* glycomer 631.

The incised margins of the resected tissue were painted with india ink, and the tissue was placed in a 10% neutral-buffered formalin solution and submitted for histopathological evaluation, which yielded a diagnosis of pilomatricoma, consistent with the presurgical cytopathology. This multilobular mass was composed of cystic areas lined by layers of basal epithelial cells. The lining cells had multifocal areas of squamous differentiation. Three to four mitotic figures were noted per 400× field. The lumens of the cysts were filled with large rafts of ghost cells and keratin. Cysts were sometimes ruptured and induced an inflammatory reaction of mononuclear cells with scattered, multinucleated giant cells. The inflammatory reaction from the ruptured cysts contributed to the lesion size. The neoplastic tissue extended to one surgical border identified by india ink stain.

Due to scheduling conflicts, the owner was not able to return for the 3-week postoperative examination. Thirty-three days postoperatively, contact was made with the owner who reported the surgical site as "99% healed" with no evidence of tumor regrowth. The bandage was removed, and the bandage changes discontinued. The owner reported that, at one point, the patient had removed her bandage and chewed out the remaining *3-0* glycomer 631 suture, but this seemed to only result in a minor setback in healing time. Six months postoperatively, the owner reported complete resolution of the wound with no indication of localized tumor regrowth. Eighteen months postoperatively, the owner reported that the dog was free of local tumor regrowth.

Figure 6—Final placement of a swine intestinal submucosa (SIS) graft over the dorsal left metacarpal wound bed from Figure 5. The perimeter of the SIS is attached to the wound margins with a simple continuous suture pattern using *3-0* glycomer 631.

Figure 7—Appearance of a swine intestinal submucosa (SIS) graft and left dorsal metacarpal wound bed 3 days after excisional biopsy.

Figure 8—Appearance of healthy granulation tissue in the left dorsal metacarpal wound bed following swine intestinal submucosa (SIS) graft removal 5 days after excisional biopsy.

Discussion

Aspiration cytopathology revealed a cystic epithelial neoplasm of a basal cell/follicular cell origin. Histopathological examination of the excised mass confirmed it to be a pilomatricoma. A pilomatricoma arises from hair follicles. Hair follicle tumors account for approximately 5% of all skin tumors in the dog.[15] They are extremely rare in other species and account for <1% of all skin tumors in the cat.[15] Hair follicle tumors can appear in four histopathological types. The majority are trichoepitheliomas (80%) and pilomatricoma (20%), with rare occurrences of tricholemmomas and trichofolliculomas.[15]

Pilomatricoma, sometimes referred to as a calcifying epithelioma, is a tumor of mature dogs; the reported average age of patients is 5.5 years.[16] Kerry blue terriers and poodles have been reported to be predisposed to development, with no sex predilection demonstrated.[15] Pilomatricoma involves the dermis and subcutis, usually limited to the shoulders and extremities.[16] It appears as a solid, solitary, well-circumscribed mass ranging from 1 to 10 cm in diameter. Pilomatricoma is usually freely movable over the underlying structures; has thin, hairless overlying skin; and is frequently ulcerated. One-fourth are reported to be cystic, one-third are hyperpigmented, and all may be gritty on a cut surface from mineral deposition.[15]

Pilomatricoma consists of variably shaped masses of epithelial cells.[16] Two distinct cell types, basophilic and shadow cells, are present.[16] Basophilic cells are small, deeply staining cells with scant amounts of cytoplasm and indistinct borders.[16] Basophilic cells resemble hair matrix cells found in the bulbs of actively growing hairs.[16] Shadow cells are fully keratinized, have a distinct border with a central unstained area at the site of the nucleus, and stain faintly eosinophilic with hematoxylin and eosin.[16] In areas where progression from basophilic to shadow cells is seen, an intermediate cell type is present that has undergone partial keratinization and still possesses a basophilic nucleus.[16] In recently developed tumors, the basophilic cells predominate; but over time, these tumors progress to a shadow cell predominance with few basophilic cells.[16] Calcification occurs within the areas of the shadow cells.[16] Usually a portion of the tumor stroma contains a foreign-body giant cell reaction.[16] Pilomatricoma is a benign, slow-growing tumor that tends to be noninvasive and nonmetastatic.[16] Complete surgical excision carries an excellent prognosis with very low incidence of local recurrence, even though it may appear to have histopathological evidence of malignancy.[16]

Carbon dioxide lasers have been utilized in medicine for over 25 years. Carbon dioxide lasers produce a monochromic, collimated, coherent light energy at the wavelength of 10,600 nm.[5-7] This wavelength of light energy is highly absorbed by water, making it an excellent choice for soft-tissue applications.[6,7] Laser energy reacts with tissue through four basic mechanisms: reflection, absorption, scatter, or transmission.[5] The laser energy produced by the CO_2 laser is absorbed by water within soft tissue and is converted to thermal energy.[5,7]

The CO_2 laser energy is highly absorbed by the water within soft tissue, resulting in thermal overheating and vaporization of that tissue. Due to the high level of absorption, very little energy is reflected, scattered, or transmitted to the surrounding tissue, resulting in minimal lateral thermal damage. Vaporization seals small vessels, reducing hemorrhage and thereby limiting, but not always eliminating, the need for electrocoagulation and its associated thermal tissue damage.

The CO_2 laser can result in increased collateral thermal injury if applied to tissue inappropriately. Laser energy applied to target tissue over an extended period of time leads to heat diffusion into surrounding tissue and results in an increase in collateral thermal damage. The magnitude of this heat diffusion can be influenced by both the surgeon and the mode of laser energy delivery.[17-19] Vaporization of tissue results in ejection of a plume of steam and debris, carrying much of the thermal energy accumulation within that tissue.[20] Low laser settings result in increased contact time to achieve target tissue vaporization and allow increased time for heat diffusion to surrounding tissue. The time required for diffusion of heat through tissue is referred to as the thermal relaxation time.[18,20] This time is variable depending on the tissue composition and the wavelength of laser energy applied.[18-20]

Continuous super-pulse mode was chosen for the application of energy to the target tissue in this case study. Continuous super-pulse mode allowed the delivery of high peak power pulses in short pulse lengths in a continuous stream of micropulses that averaged over time to the displayed power setting.[21] Continuous super-pulse mode allowed tissue recovery between micropulses, as the micropulse duration was shorter than the tissue thermal relaxation time. Thermal energy escaped primarily through vaporization with minimal collateral thermal diffusion. Continuous super-pulse mode allowed dermal excision with minimal collateral tissue damage.[18,19]

Laser energy is produced in a collimated beam of light. Most CO_2 lasers use mirrors to transmit the collimated beam to the handpiece. The CO_2 laser in this application utilizes a hollow reflective wave-guide to deliver the laser energy to the handpiece.[7] As a result of this reflection down the wave-guide, the laser energy exits the handpiece as a diverging beam.[7] Different tips placed at the end of the handpiece are utilized to reflect and refocus the beam to a precise focal plane.[7] By defocusing or distancing the handpiece beyond that focal plane, the laser beam becomes more divergent, decreasing the power density and vaporizing ability. Defocusing the laser allowed the surgeon to ablate the tumor surface without complete vaporization and to thermally seal the ulcerated surface of the tumor, reducing the opportunity for wound seeding.

In the past, CO_2 laser use has been limited by its cumbersome size and delivery mechanism. Recent advances in technology have made CO_2 lasers more user friendly in size, application, and cost. Carbon dioxide lasers enable the surgeon to point and cut without distorting tissue, which is a significant advantage over conventional scalpel excision and electrocoagulation. Controlled hemostasis and minimal tissue distortion allow excellent visualization during resection and enable the surgeon to excise a more conservative margin of normal tissue than with conventional excision, while at the same time ensuring appropriate gross tumor margins.

Reduced excision contributes to faster resolution with fewer complications. One must always be aware of the increased chance of recurrence by leaving tumor behind from encroached borders following inadequate excision. In this case study, it was noted on histopathological examination that the tumor extended to the margins of the excision. Eighteen months postoperatively, the owner reported no regrowth of the mass. Laser ablation of the tumor's surface creates a seal, reducing potential wound contamination from exfoliation of tumor cells. Laser excision results in vaporization of the margins of the excised tissue, resulting in concurrent ablation of the wound margins and possible curative marginal resection; this is less likely with conventional surgical excision.[22] Vaporization and ablation may result in cleaner margins with a reduced incidence of local tumor regrowth.[22] However, laser resection with ablation is no substitute for obtaining adequate excisional margins.

Upon completion of the excision in the case presented here, a partial closure of the surgical wound was performed. Due to the extent and location of the excision, routine closure of the remaining surgical wound was not feasible. Options for the remaining wound closure were limited to skin grafting or second-intention healing. Sliding, rotational, or Z-plasty flaps were not considered because of the limited amount of skin available at the carpus. Full-thickness, split-thickness, seed, or strip grafts could not be utilized until a granulation tissue bed covered the exposed tendons within the wound.[2] A SIS xenograft was chosen to facilitate and optimize early formation of a granulation tissue bed.

Swine intestinal submucosa has been extensively researched for its utilization in tissue engineering.[11] Swine intestinal submucosa is a primarily type I collagen-based biomaterial prepared from the small intestine of swine. Swine intestinal submucosa contains bioactive growth factors including fibroblast growth factor-2 (FGF-2), transforming growth factor-beta (TGF-β), and vascular endothelial cell growth factor (VEGF).[12,23] Swine intestinal submucosa used topically can enhance granulation tissue formation and angiogenesis.[9,24]

Granulation tissue forms with normal second-intention wound healing; however, SIS was incorporated as a graft to optimize conditions for rapid revascularization and granulation. In this case, SIS was utilized as a biological bandage as well as a template for stimulation of angiogenesis and formation of a granulation tissue bed over the existing wound. Suturing the SIS to the skin margins reduced skin margin retraction and provided a matrix for granulation tissue formation over the exposed tendons. Five days postoperatively, the SIS matrix was noted to be dissolving and liquefying. This process occurred as the collagen matrix experienced biological engineering changes, becoming incorporated into the host tissue.[11] At this point, the SIS had performed the necessary function for wound healing by providing a biological matrix for revascularization, resulting in the formation of the granulation tissue bed beneath. The SIS was removed, allowing the option of either skin grafting or healing by wound contracture and epithelialization. In this case, the wound was allowed to resolve by second-intention healing, and a second surgical procedure was avoided.

Bandaging techniques likely contributed to the success of rapid granulation tissue formation and second-intention wound healing. Incorporating the splint within the bandage resulted in immobilization of the exposed tendons. Immobilization allowed the SIS matrix to stimulate angiogenesis and capillary formation with structures below, resulting in the granulation tissue bed formation. Further protection of the vascular supply to the granulation tissue bed through immobilization contributed to the rapid epithelialization. The soft-padded bandage over the wound assisted in maintaining SIS contact during initial formation of the granulation tissue bed and offered protection to that granulation tissue bed during wound contracture and epithelialization.

Conclusion

There are limited options for closure of wounds created by surgical excision of distal extremity masses. These masses often require excisions that result in large wounds with limited skin available for closure. Laser excision may allow marginal resection and smaller resultant wounds compared to conventional scalpel excision.

The formation of a healthy granulation tissue bed is necessary for successful second-intention wound healing or skin grafting. Distal extremity wounds often have decreased soft-tissue structures with bone and tendon exposure, limiting angiogenesis and slowing granulation tissue formation. The SIS matrix contributed the scaffold and growth factors necessary to create optimal conditions to stimulate granulation tissue formation.

This case study illustrates the advantages of utilizing laser resection in combination with an SIS graft for resection of epithelial tumors of distal extremities. The laser excision contributed to a smaller resection with minimal tissue damage. The SIS contributed a scaffold for angiogenesis and granulation tissue formation over the exposed tendons. These two factors together allowed expedient wound closure through second-intention healing without the need for additional surgery.

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[a] Accuvet Novapulse LX-20SP; Lumenis (Formerly Luxar), Bothel, WA
[b] Biosin; United States Surgical Corporation, Norwalk, CT
[c] BioSISt; Cook Veterinary Products, Spencer, IN
[d] Release nonadhering dressing; Johnson & Johnson, Arlington, TX
[e] Specialist cast padding; Johnson & Johnson, Raynham, MA
[f] Conform stretch bandage; Kendall, Mansfield, MA
[g] Hexalite; Veterinary Specialty Products, Boca Raton, FL
[h] Conform elastic tape; Kendall, Mansfield, MA
[i] Rimadyl; Pfizer Animal Health, New York, NY

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