Biofilm-infected wounds in a dog

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Case Description—A 4-year-old spayed female Mastiff was evaluated for treatment of chronic nonhealing pressure wounds over both elbow regions resulting from attempts at hypertrophic callus excision.

Clinical Findings—The wound bed granulation tissue was mottled red and yellow with hyperemic, rolled epithelial edges. The right wound communicated with a large fluid pocket along the thoracic wall. The dog had an inflammatory leukogram with a left shift.

Treatment and Outcome—The wounds were debrided, and tissue specimens were collected for histologic evaluation, microbial culture, and bacterial identification by means of molecular diagnostic techniques. The left wound was closed immediately. Calcium alginate rope with silver was packed into the right wound. Vacuum-assisted closure was applied for 6 days. Debridement was repeated, and a thoracodorsal axial pattern flap was used to cover the wound. Systemic treatment with antimicrobials was initiated, and pressure over the elbow regions was relieved. Bacterial biofilms were identified histologically in tissue specimens from both wounds. *Staphylococcus intermedius, Staphylococcus epidermidis,* and *Streptococcus canis* were cultured and identified by 16S rRNA fragment sequencing. Pyrosequencing identified multiple bacterial species and no fungal organisms. Both wounds healed successfully.

Clinical Relevance—Biofilms are implicated in infected orthopedic implants in veterinary patients; however, this is the first report of a bacterial biofilm in chronic wounds in a dog. In human wound care, extensive debridement is performed to disrupt the biofilm; a multimodal treatment approach is recommended to delay reformation and help clear the infection. In this case, biofilm reformation was prevented by systemic treatment with antimicrobials, by reducing local pressure on the wounds, and by wound closure. (*J Am Vet Med Assoc* 2014;244:699–707)

A 4-year-old spayed female Mastiff was evaluated at the Purdue University Veterinary Teaching Hospital for treatment of nonhealing wounds over both elbow regions. The dog had a history of atopy and pyoderma and sustained a left cranial cruciate ligament rupture at 2 years of age. Nine months later, the dog developed hypertrophy of the callus over the right elbow region that subsequently cracked and developed a purulent discharge. The hypertrophic portion of the callus was ablated with a carbon dioxide laser. Over the next 14 months, the callus was treated by intermittent oral administration of antimicrobials with no resolution of signs. Because of a lack of response to medical treatment and development of a similar problem over the left elbow region, both calluses were surgically excised. Both incisions dehisced between 4 and 7 days after surgery. The open wounds were managed by the application of honey, wet-to-dry bandages, and elbow pads. The dog was referred for evaluation and advanced wound care 16 days after surgical excision. At the time of referral, the dog was receiving deracoxib (2.5 mg/kg [1.1 mg/lb], PO, q 24 h), tramadol (1.7 mg/kg [0.8 mg/lb], PO, q 12 h, as needed), and ciprofloxacin (21 mg/kg [9.5 mg/lb], PO, q 24 h).

Abbreviation

VAC	Vacuum-assisted closure

At the time of initial evaluation, the dog was quiet with a depressed attitude. Hydration status was adequate, and body temperature (39.0°C [102.2°F]), pulse rate (128 beats/min), and respiratory rate (36 breaths/min) were within reference range limits. The dog weighed 59.7 kg (131.3 lb) and was assigned a body condition score of 6 of 9.[a] No abnormalities were detected on thoracic auscultation or abdominal palpation. Joint effusion, medial buttress, crepitus, cranial drawer movement, and cranial tibial thrust were found on palpation of the left stifle joint. Both wounds over the elbow regions were open, and the wound bed granulation tissues were mottled red and yellow, rough, and friable. The epithelial edges were thickened, hyperemic, and rolled (**Figure 1**). There was marked cellulitis of the entire right forelimb that communicated with a large, subcutaneous, fluid-filled pocket extending from the right axilla to the right ventrolateral aspect of the thoracic wall.

A CBC revealed that the dog had an inflammatory leukogram with a toxic left shift and a stress component, including leukocytosis (18,000 leukocytes/

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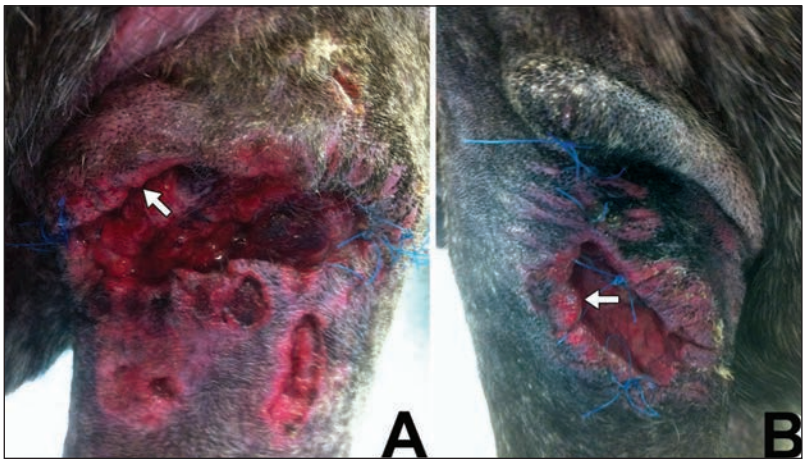

Figure 1—Photographs of chronic nonhealing pressure wounds over the left (A) and right (B) elbow regions of a 4-year-old spayed female Mastiff resulting from attempts at hypertrophic callus excision. The granulation bed in both wounds was uneven, mottled, and friable. The wound edges were thickened, and epithelium was growing underneath the wound edges (arrow).

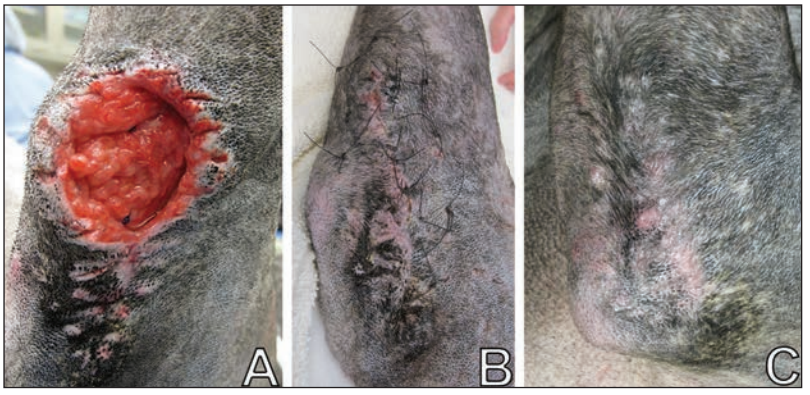

Figure 2—Photographs of the wound over the left elbow region after removal of loose sutures and before debridement (A), 6 days after closure (B), and 1 month after closure once the wound had healed (C).

μL; reference range, 6,000 to 17,000 leukocytes/μL), neutrophilia (15,300 neutrophils/μL; reference range, 3,000 to 12,000 neutrophils/μL), band neutrophilia (900 band neutrophils/μL; reference range, 0 to 300 band neutrophils/μL), and lymphopenia (700 lymphocytes/μL; reference range, 1,000 to 5,000 lymphocytes/μL). Serum biochemical analysis revealed high BUN (41 mg/dL; reference range, 7 to 32 mg/dL) and creatinine (2.10 mg/dL; reference range, 0.50 to 1.50 mg/dL) concentrations and high alkaline phosphatase activity (261 U/L; reference range, 20 to 157 U/L). Lateral and caudocranial radiographs of the elbow joints revealed a soft tissue defect at the caudal aspect of the right elbow joint, but both the right and left olecranon were normal radiographically with no evidence of osteomyelitis. All other structures also appeared radiographically normal.

For initial wound care, the dog was placed under general anesthesia; bilateral hanging limb preparations were performed with the dog in dorsal recumbency. By use of sterile technique, the wounds were aggressively debrided and copiously lavaged with sterile lactated Ringer's solution. Both wounds were then evaluated for the possibility of primary closure. Cefazolin (22 mg/kg [10 mg/lb], IV) was administered following induction of anesthesia and was subsequently administered every 2 hours during the procedure. During sharp debridement, tissue specimens were obtained for histologic evaluation, molecular diagnostic bacterial and fungal identification, standard microbial culture and antimicrobial susceptibility testing, and biochemical identification of bacteria.

Following debridement, the defect over the left elbow region measured 4 × 2 cm. The skin of the wound over the left elbow region could be brought together with minimal tension, so the wound was closed (**Figure 2**). A closed-suction drain[b] was placed in the wound bed, and the wound edges were apposed with 2-0 polydioxanone suture for the subcutaneous tissues and 2-0 nylon suture for the skin. Suture loops were placed around the incision with 2-0 nylon for a tie-over bandage. Silver-impregnated foam padding[c] was placed over the incision and held with umbilical tape. The foam padding was covered, and a VAC system[d] was applied to the wound for 24 hours.

A large sinus tract extended medially from the caudolateral aspect of the wound over the right elbow region into a subcutaneous pocket located at the right axilla and along the right side of the thorax. Because of the severity of the cellulitis in the limb, the large size of the wound, and the presence of the fluid-filled axillary pocket, the wound was aggressively debrided and left open to facilitate drainage and promote healthy granulation. After debridement, the wound measured 8 × 6 cm.

The pocket was copiously lavaged with sterile lactated Ringer's solution and then was packed with calcium alginate rope with silver.[e] Sterile silver-impregnated foam[c] was cut to size and placed into the wound bed, and then a VAC system was applied to the wound. The VAC system was initially set at –125 mm Hg with continuous suction. After 48 hours, both the cellulitis in the right forelimb and the fluid in the axillary pocket had resolved. The VAC settings were then changed to intermittent suction (0 to –100 mm Hg) to better promote granulation tissue formation.[1,2]

A foam pipe insulation bandaging technique described by Pavletic[3] was used to reduce pressure on the elbow regions. Hydromorphone (0.05 mg/kg [0.02 mg/lb], SC, q 4 h) was administered starting at extubation for 12 hours after surgery. The dog was treated with ampicillin-sulbactam (22 mg/kg [10 mg/lb], IV, q 8 h) and tramadol (3.4 mg/kg [1.5 mg/lb], PO, q 8 h). The VAC system and drain were removed from the left side after 24 hours because of minimal fluid production from the surgical site. The VAC system on the right side was maintained for 6 days. By use of aseptic technique, the foam was changed daily for the first 2 days and then

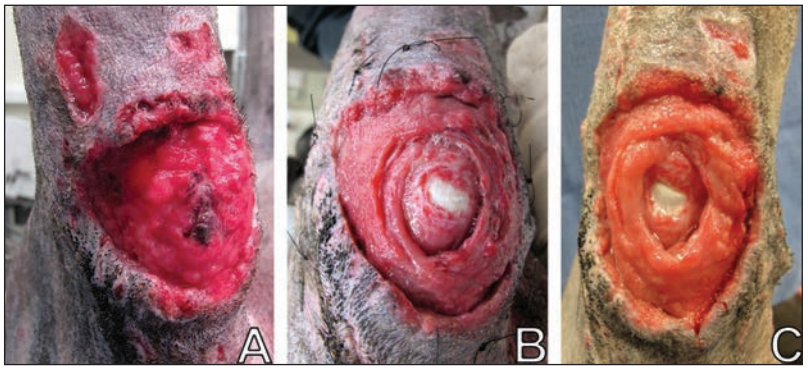

Figure 3—Photographs of the wound bed over the right elbow region treated with VAC therapy after debridement obtained immediately (A), 2 days (B), and 6 days (C) after debridement.

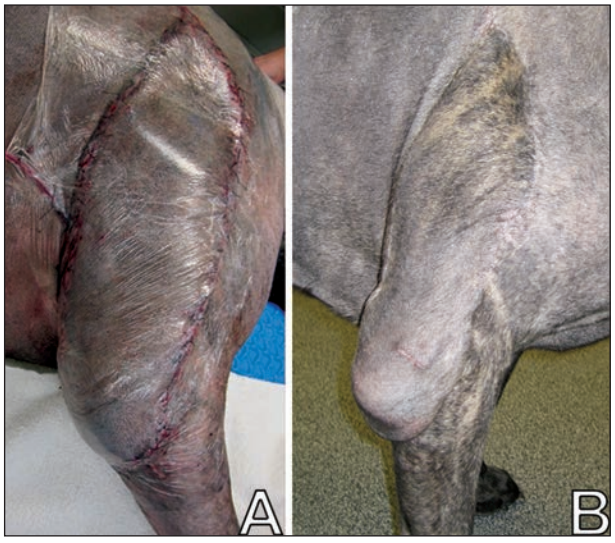

Figure 4—Photographs of the thoracodorsal axial pattern island flap, which was used to close the wound over the right elbow region, immediately after surgery (A) and 1 month after surgery (B) once healed.

again on the fifth day after initial wound care. The calcium alginate rope with silver was removed from the subcutaneous axillary pocket on the third day.

On the sixth day after initial wound care, the granulation bed of the wound over the right elbow region was considerably thicker and appeared healthy (**Figure 3**). The dog was placed under general anesthesia, sharp debridement of the wound bed was repeated, and tissues were collected again for microbial culture. To close the wound over the right elbow region, a right thoracodorsal axial pattern flap was performed (**Figure 4**).

The thoracodorsal artery was located caudal to the shoulder with a sterile pencil Doppler probe, and the flap margins were marked. The margins of the flap were then incised, and the flap was elevated with sharp dissection, maintaining the subcutaneous tissues with the flap. A pedicle of fat was preserved around the vascular pedicle to prevent twisting and occlusion of the thoracodorsal artery and vein. A bridging incision was made between the flap and the wound bed, and the flap was then rotated approximately 180° in a caudal direction around the vascular pedicle to cover the wound over the elbow region without tension.

Two Jackson-Pratt closed-suction drains were placed prior to closure. The edges of the flap were closed in a 2-layer closure with 2-0 polydioxanone suture for the subcutaneous tissues and 2-0 nylon suture for skin. A light bandage was placed over the flap, and the foam pipe insulation bandages were again placed on both forelimbs.

A fentanyl continuous rate infusion (2 µg/kg/h [0.9 µg/lb/h], IV) was given for 12 hours, and oral administration of tramadol was resumed for pain management. Ampicillin-sulbactam administration was continued for another 5 days and then was switched to enrofloxacin (10 mg/kg [4.5 mg/lb], PO, q 24 h) on the basis of microbial culture and antimicrobial susceptibility test results. The enrofloxacin administration was discontinued after 3 days because of gastric upset and vomiting, and an injection of cefovecin (8 mg/kg [3.6 mg/lb], SC, once) was administered. The bandage over the flap was changed daily for the first 3 days and then was replaced by a clear occlusive bandage.[f] The medially placed Jackson-Pratt drain was removed on day 3, and the laterally placed Jackson-Pratt drain was removed on day 10. The dog was discharged from the hospital on day 18 after admission and was seen for bandage changes every 2 days.

A superficial pressure sore developed at the distal end of the flap and produced a green, citrus-smelling discharge. Bandaging materials placed between the elbow region and the pipe insulation to cushion the area were too thick and were causing increased pressure and friction; thus, all padding was removed. The pressure sore started to dry and appeared less red within 2 days after removal of the excessive bandaging. The dog was custom-fitted with therapeutic elbow pads,[g] and within 1 week, the pressure sore at the distal end of the flap was completely healed. Both incisions continued to heal without complication.

For histologic evaluation, tissue specimens obtained during the initial wound debridement had been immediately placed in neutral-buffered 10% formalin, processed, and sectioned. Tissue sections were stained and examined under a light microscope. Sections of tissue from the affected wounds were ulcerated and partially covered by necrotic cellular debris, blood, and numerous bacterial colonies (**Figure 5**). Numerous microcolonies of gram-positive cocci bacteria formed a distinct layer near the surface of the wound. Deep to the layer of bacteria was a thick layer of neutrophils. The subjacent tissue was composed of a thick bed of immature granulation tissue that was poorly vascularized and was variably infiltrated by mononuclear and polymorphonuclear inflammatory cells admixed with various quantities of blood and proteinaceous edema fluid.

Tissue specimens collected from both wound beds during initial wound debridement were submitted for microbial culture. *Staphylococcus intermedius* and a β-hemolytic *Streptococcus* sp were initially isolated from both wounds. *Staphylococcus intermedius*, an α-hemolytic *Streptococcus* sp, and methicillin-resistant

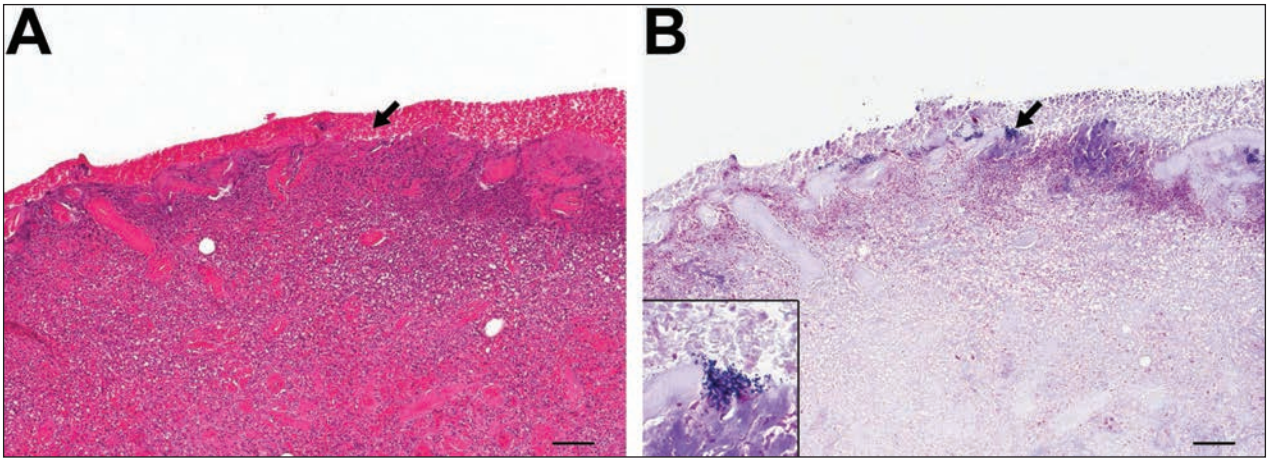

Figure 5—Photomicrographs of a section of the wound biofilms. A—The ulcerated surface of the wound and the associated granulation tissue bed are apparent. The granulation bed is poorly vascularized and has a marked infiltration of neutrophils, particularly at the wound surface. H&E stain; bar = 100 µm. B—Numerous gram-positive bacterial microcolonies are visible along the surface of the granulation bed (beneath the superficial serocellular crust [arrow]). Gram stain; bar = 100 µm. Insert—Higher magnification of the bacterial colonies in panel B.

Table 1—Number (%) of sequences as determined by pyrosequencing and phenotypic information for microbial populations in chronic nonhealing pressure wounds over both elbow regions of a 4-year-old spayed female Mastiff resulting from attempts at hypertrophic callus excision.

Bacteria	Right wound	Left wound	Gram stain result	Aerotolerance
Listeria monocytogenes	4,393 (96.86)	9,900 (99.02)	Positive	Facultative anaerobe
Listeria (unknown species)	6 (0.13)	24 (0.24)	Positive	Facultative anaerobe
Listeria innocua	0	2 (0.02)	Positive	Facultative anaerobe
Simonsiella spp	6 (0.13)	1 (0.01)	Negative	Aerobe
Propionibacterium spp	14 (0.30)	1 (0.01)	Positive	Anaerobe
Serratia spp	4 (0.08)	0	Negative	Facultative anaerobe
Ralstonia (unknown species)	4 (0.08)	0	Negative	Facultative anaerobe
Ralstonia spp	87 (1.91)	58 (0.58)	Negative	Facultative anaerobe
Lactobacillus spp	0	6 (0.06)	Positive	Facultative anaerobe
Sphingobium spp	1 (0.02)	0	Negative	Aerobe
Streptococcus spp	8 (0.17)	0	Positive	Facultative anaerobe
Saccharibacter spp	1 (0.02)	0	Negative	Anaerobe
Shigella sonnei	2 (0.04)	0	Negative	Facultative anaerobe
Delftia tsuruhatensis	0	5 (0.05)	Negative	Aerobe
Laceyella sacchari	4 (0.08)	0	Positive	Aerobe
Methylobacteriaceae (unknown genus)	1 (0.02)	0	Unknown	Unknown
Alteromonadales (unknown family)	1 (0.02)	0	Unknown	Unknown
Listeria spp	1 (0.02)	0	Positive	Facultative anaerobe
Escherichia spp	2 (0.04)	0	Negative	Facultative anaerobe

Staphylococcus epidermidis were isolated from tissue specimens collected on day 6 from the right wound.

For molecular diagnostic bacterial and fungal identification, tissue specimens obtained from both wound beds during initial wound debridement had been placed in PBS solution and immediately frozen at −20°C for future analysis. The DNA was extracted and purified from each sample with a commercial DNA extraction kit,[h] following the protocol of the manufacturer. Deoxyribonucleic acid was eluted with 100 µL of elution buffer and tested for PCR-amplifiable DNA with 16S rRNA-specific primers.

The 16S eubacterial primers 530F and 1100R were used for amplifying the 600–base pair region of 16S rRNA.[4] The 50-µL PCR mixture contained 5 µL of DNA template, 0.5 µL of each primer (100mM), 25 µL of a commercial PCR mixture,[i] and 19 µL of free water. The PCR amplification was performed in a thermal cycler[j] with the following cycle conditions: initial denaturing step (1 cycle at 95°C for 5 minutes), 40 cycles (denaturing at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute), and final extension step at 72°C for 5 minutes.

Detection of PCR-amplified product was performed by electrophoresis on a 0.7% (wt/vol) agarose gel stained with ethidium bromide. Bands of DNA stained with ethidium bromide were observed after exposure of the gel to UV light. Amplified PCR products were extracted from the gel with a commercial gel extraction kit.[k] The purified unknown DNA fragments were sent for nucleotide sequencing[l] at the Purdue Genomics Facility with forward and reverse primers.

With the aid of the bioinformatics alignment program of 2 genetic databases,[m,n] 16S rRNA sequences were analyzed. *Streptococcus canis* was identified as a species with a high degree of similarity in both databases.

To further characterize the bacterial and fungal diversity within chronic, nonhealing wounds in this dog, 16S rRNA gene and small subunit-coding sequence pyrosequencing for bacterial and fungal organisms was performed. Extracted DNA samples were sent to a commercial laboratory[o] for bacterial and fungal tag-encoded amplicon pyrosequencing[p] and data processing as described.[5] A greater variety of bacterial species was identified in tissue debrided from the right wound than the left wound. *Listeria monocytogenes* was the most prevalent organism in both wounds, accounting for 96.86% of all organisms in the right wound and 99.02% of all organisms in the left wound. A *Streptococcus* sp was identified in the right wound but not in the left wound. No *Staphylococcus* spp were identified with this method. Additionally, no fungi were identified in the extracted DNA samples. The identified genera of the bacteria found with pyrosequencing in the DNA samples extracted from the right and left wounds, the number and percentage of sequences corresponding to a given genus, Gram staining properties, and the aerotolerant (aerobic, anaerobic, or facultative anaerobic) nature of the genus were summarized (**Table 1**).

At the time of follow-up via a telephone conversation 1 year after surgery, the owner reported that both the incision over the left elbow region and the right thoracodorsal axial pattern flap had completely healed without complication. The dog was wearing the therapeutic elbow pads regularly, and there had been no recurrence of the hypertrophic calluses or infection. The owner was pleased with the cosmetic and functional outcomes of the case.

Discussion

The case described in this report is the first to demonstrate important histologic findings from wound biopsy specimens that suggest the presence of biofilm in chronic wounds in a dog. Furthermore, as has been found in chronic wounds in humans, molecular diagnostic techniques revealed a broader spectrum of microorganisms than did standard microbial culture techniques.

Bacteria have distinct phenotypic growth patterns: a free-living planktonic form and a sessile biofilm form.[6] A biofilm is defined as an adherent community of bacteria that is surrounded and protected by an exopolysaccharide matrix consisting of polysaccharides, extracellular DNA, proteins, and lipids.[6,7] Infecting bacteria divide until a critical number of bacteria is reached, at which point interbacterial chemical communication, called quorum sensing, takes place.[7,8] This communication causes a change in gene expression that results in adhesion of the bacteria to the underlying substrate, proliferation into microcolonies in the form of a tower or mushroom, excretion of the exopolysaccharide matrix, and an overall decrease in metabolic activity, compared with planktonic bacteria. The exopolysaccharide matrix serves as a shield against environmental insults such as desiccation and UV radiation, and in a living organism, it also prevents the host immune response and antimicrobials from reaching the bacteria within.[7] The slow to absent growth and genotypic changes of bacteria deep within the biofilm interfere with the mechanisms of action of several antimicrobial drug classes, including β-lactam and aminoglycoside antimicrobials.[6,9] Because of these characteristics, biofilms are notoriously hardy and re-form quickly after disruption. Biofilms disseminate by releasing surface bacteria in planktonic form into the surrounding environment.

Bacterial biofilms have been implicated as a major cause of delayed healing in chronic wounds in humans,[6,7,10] including in pressure ulcers similar to those seen in the dog of this report. Biofilms have been described in chronic wounds of distal aspects of the limbs in horses and in orthopedic implant infections and osteomyelitis in dogs.[11–14] The role of biofilm in chronic infection in veterinary patients is otherwise ill defined. Bacteria capable of forming biofilms have been isolated from microbial cultures of skin and wounds in dogs, but they have not been histologically identified within a wound.[15,16]

Pressure ulcers form as a result of chronic compression of the soft tissues that overlie a bony prominence, causing ischemia-reperfusion injury to the soft tissues.[7] Consequently, the tissues become necrotic, and if a suture line is present, dehiscence may occur. The reason that biofilms form preferentially on compromised wound beds rather than intact skin is as yet unknown, but a combination of necrotic tissue, poor vasculature supply, and other host issues such as systemic illness and immune compromise likely creates a perfect environment for chronic infection.

Healing in humans and loose-skinned animals is markedly different, in that loose-skinned animals, such as dogs, heal primarily by wound contraction. Because of this, chronic wound infections, namely biofilm-infected wounds, are rarely a problem in small animal patients. The limbs, however, do not benefit from an abundance of loose skin; therefore, wounds must rely more on granulation and epithelialization to heal. This aspect of healing, along with the repeated trauma and constant movement to which a wound over the elbow region is subjected, results in a situation more like that seen in chronic pressure sores in humans. It is possible that in these situations, biofilm-infected wounds may be overlooked in small animal patients.

Biofilm-infected wounds were first suspected in the dog of this report because of similarities in the appearance of these wounds with that described in biofilm-infected chronic wounds in humans. In humans, biofilms cannot be seen on gross examination of a wound but should be suspected in any wound with delayed healing (> 3 weeks). Rather than the classical signs of inflammation (pain, erythema, edema, heat, and purulence), chronic wounds in humans with signs of increasing pain, friable granulation tissue, foul odor, and wound breakdown support a higher index of suspicion for infection in chronic wounds.[17] In the case described in the present report, the wounds had poor-quality, highly friable granulation tissue and had repeated incisional dehiscences, and a foul odor was emanating from the wounds. Pain specifically at the wound was difficult to assess because of the presence of generalized cellulitis in the right forelimb.

To identify biofilms in the chronic wounds of the dog of this report, biopsy specimens were obtained from the wound beds at the time of debridement for histologic

evaluation. With conventional light microscopy, biofilms of humans appear as discrete aggregates and microcolonies of bacteria along the wound bed when stained with H&E and Gram stains.[18] The bacterial microcolonies are often surrounded by neutrophils and occasionally macrophages. The tissue specimens obtained from the wound beds of the dog of this report at initial debridement had these same characteristics. Many colonies of cocci bacteria formed a distinct layer near the surface of the wound. Deep to the layer of bacteria was a thick layer of neutrophils. The underlying granulation tissue was poor in quality with decreased vascularity.

Treatment of chronic wounds in humans has advanced considerably in the last few years, largely because of the development of biofilm-based wound care regimens.[8,19] Previously, chronic wounds in humans failed to completely resolve even when treated with antimicrobials that were selected on the basis of conventional microbial culture and antimicrobial susceptibility testing of wound swabs. Standard microbial culture techniques are based on planktonic bacterial growth and antimicrobial susceptibility patterns and do not reflect the actual antimicrobial susceptibility of the biofilm bacteria. Indeed, bacteria in biofilms have been shown to have minimum inhibitory concentrations to antimicrobials that were 100 to 1,000 times as great as those of the planktonic phenotype of the same species.[20] Most systemically administered antimicrobials never accumulate tissue concentrations high enough to reach the minimum inhibitory concentration necessary to kill biofilm bacteria.

Bacteria within a biofilm are more susceptible to antimicrobial treatment for a period up to 24 hours when the biofilm is disrupted.[8,19,21–24] The most effective way to disrupt a wound biofilm is to remove it through sharp debridement. Therefore, aggressive and frequent sharp debridement of the wound bed has become the mainstay of biofilm-based wound care in humans. In addition to debridement, the recommended multifaceted approach to wound biofilms also includes selection of topically and systemically administered antimicrobials on the basis of both standard microbial culture and molecular diagnostic techniques, the use of selective biocide agents (eg, ionic silver, nanoparticle silver, or cadexomer iodine) and antibiofilm agents (eg, lactoferrin, xylitol, or the quorum sensing inhibitor hamamelitannin), and appropriate dressing of the wound, in addition to correction of the underlying etiology of the wound.[8,19,25,26]

Because of suspicion for biofilm-infected wounds in the dog of the present report, preemptive antibiofilm treatments were initiated that were modified from biofilm-based wound care recommendations for humans. Specifically, repeated wound debridement, long-term administration of antimicrobials, topical administration of biocides, and appropriate treatment of the underlying etiology of the wound were initiated. Molecular diagnostic results were not available at the start of treatment. None of the antibiofilm agents have been approved for use in dogs, and some, such as xylitol, may be toxic; therefore, these agents were not used for this case.

The initial antimicrobial choice for the dog of the present report was empirical and was based on the types of bacteria typically found in cutaneous wounds of dogs (ie, *Staphylococcus* spp and *Streptococcus* spp). This choice was later confirmed by antimicrobial susceptibility test results. Over the course of treatment, antimicrobials were changed because the patient could not tolerate orally administered medications. All antimicrobial choices were made on the basis of antimicrobial susceptibility test results. Despite recommendations to use topically administered antimicrobials that are able to reach higher concentrations at the site of infection, systemic use of antimicrobials was chosen because the dog had signs of sepsis. Sepsis in cases of chronic wounds occurs when bacteria are disseminated from the biofilm and enter the circulation. A VAC was used to strengthen the wound bed and improve the character of the granulation bed. Silver-impregnated VAC foam was used to apply the biocide silver to the wound.

Because the wounds in the dog of the present report were located directly over the pressure points of the elbow joint, it was imperative to address this factor to achieve wound healing. To that extent, the VAC was combined with reduction of pressure over the elbow regions. Vacuum-assisted closure is used to treat chronic wounds in humans and has proven beneficial in aiding removal of large amounts of wound exudate, promoting growth of granulation tissue, and stabilizing the wound bed by promoting thickening of the underlying tissues.[27,28] A recently reported study[27] revealed increased bacterial numbers without overt infection in acute wounds in dogs treated with VAC. Because of this finding, some clinicians avoid the use of VAC in infected wounds. The effect of VAC on bacterial loads in chronic wounds has not been evaluated; however, 1 study[29] has demonstrated that the use of VAC substantially decreased the thickness of *Pseudomonas* biofilms in an in vitro model. It is not apparent from that study[29] whether the biofilm was simply compressed by negative pressure or whether the actual bacterial load in the biofilm was decreased.

The main reason for use of the VAC system in the dog of the present report was to remove the large amount of effusion from the right wound and to promote a healthier and more stable granulation bed in the open wound. It also allowed for easier bandaging of the open wound. A healthy granulation bed provides better circulation to the wound that can help to fight infection and promote healing. Additionally, use of silver-impregnated foam as the contact layer for the open wound took advantage of the antibacterial effects of nanoparticle silver, as recommended by Ngo et al,[29] and may have helped to reduce bacterial bioburden in the wound.

Culture of an α-hemolytic *Streptococcus* sp and methicillin-resistant *S epidermidis* in addition to the previously cultured *S intermedius* from the healthy-appearing granulation bed at day 6 supports the finding that bacterial count may increase during VAC but that overt infection does not result. Continued culture of bacteria from the wound underscores the importance of repeated debridement in the treatment of chronic wounds.

The technique described by Pavletic[3] of use of foam pipe insulation to reduce pressure over the elbow regions was useful in addressing the underlying pressure point etiology of the wounds in the dog of this report. The bandages were inexpensive, easy to apply, and allowed access to the wounds for dressing changes. Use

of leftover foam from the VAC system application also may have helped to cushion the healing incisions without placing excessive pressure on the elbow regions. One problem encountered after closure of the wound over the right elbow region with the thoracodorsal axial pattern flap was development of a new superficial area of pressure necrosis at the center of the distal end of the flap that most likely developed because of overpacking of the space between the elbow region and the pipe insulation. The lesion resolved once the amount of packing was decreased to allow a space between the bandage material and the pipe insulation.

One reason that chronic wounds do not heal is the lack of a robust blood supply to bring healing factors and immune system components to an infected wound. Even though the immediate area of the wound over the right elbow region in the dog of the present report did not allow for primary closure, it was possible to take advantage of nearby loose skin to bring a direct blood supply to the wound bed, an advantage that is not often available for treatment of chronic wounds in humans. Management of chronic, biofilm-infected wounds in small animals may therefore differ from that in humans because of the abundance of loose skin in many locations that can be used to create local flaps that can restore blood supply to the wound bed. To that end, a thoracodorsal axial pattern flap was chosen to provide definitive closure of the wound over the right elbow region. An axial pattern flap based on the thoracodorsal artery and vein is advocated for closure of wounds over the elbow region that cannot be closed by other techniques.[3] The length of thoracodorsal axial pattern flaps is flexible and allows the flap to cover the wound without tension. In addition to provision of a direct cutaneous blood supply to the flap, thereby increasing the chance of flap survival, and to the wound, thereby improving wound perfusion, the flap also positions a cushion of subcutaneous fat over the bony olecranon.

For the dog of the present report, the combination of antibiofilm techniques and definitive wound closure allowed both wounds over the elbow regions to completely heal by 3 weeks after surgery, with no additional dehiscence and no loss of flap viability. The superficial pressure sore over the distal end of the flap completely resolved within 1 week following application of custom-fitted therapeutic elbow pads.[g] At 1 year after treatment, the dog was doing well at home, and the owner was pleased with the final outcome.

Standard microbial culture techniques are optimized to identify common pathogens such as *Staphylococcus* spp, *Streptococcus* spp, *Pseudomonas* spp, and gram-negative coliform bacteria. They are much less reliable for the identification of anaerobic and slow-growing bacterial species that are key to the tenacity of biofilms. Dowd et al[4] estimated that standard microbial culture techniques only identify 10% of all bacteria within a wound. On the other hand, molecular diagnostic techniques, such as pyrosequencing and PCR assay combined with denaturing gradient gel electrophoresis when the 16S rRNA primer that is unique to all prokaryocytic organisms is used, will isolate bacterial DNA from the host DNA in tissues. Because DNA will remain present in tissues, molecular methods can identify anaerobic organisms that may not grow on conventional microbial culture. Also, by identifying DNA rather than live organisms, molecular methods bypass the difficulty in culturing slow-growing pathogenic organisms such as *Corynebacterium* spp or fungal organisms. Current technology allows for a turnaround time as short as 24 hours for molecular diagnostic techniques, if sequencing equipment is readily available.

Studies[4,30] comparing the types of wound bacteria identified by standard culture techniques versus molecular diagnostic techniques consistently show that a greater overall number of bacterial species are isolated by molecular techniques than standard culture methods. As demonstrated by James et al[10] and Dowd et al,[4] molecular diagnostic methods have also been shown to occasionally miss species that were isolated by standard microbial culture. Thus, for human wound care, it is now recommended to use a combination of conventional microbial culture and antimicrobial susceptibility testing and molecular diagnostic techniques when selecting both topical and systemic antimicrobial treatments.[4]

By combining the results of conventional culture techniques and molecular diagnostic techniques performed on tissue specimens (as opposed to surface swabs) that were debrided from the wound, a more complete picture of the biota of the wound can be formed. Conventional microbial cultures are useful for selection of systemic treatment with antimicrobials, with the goal of reducing planktonic spread of the biofilm bacteria. Molecular diagnostic techniques have allowed for more tailored topical treatment of wound biofilms. A commercial laboratory[o] offers full bacterial DNA sequencing for wounds with a current turnaround time of 3 to 5 days for clinical specimens at a cost of $150.00/sample.[q] They provide a detailed report showing the type and quantity of bacterial species identified within the wound. On the basis of proprietary bioinformatics for antimicrobial susceptibility, they then use this information to create targeted topical treatment with antimicrobials for that wound. With use of such targeted treatment following debridement, wounds were shown to heal 2 to 5 times as fast as wounds that underwent standard-of-care treatment.[25]

Microbial culture results for the dog of the present report were interesting. Conventional microbial culture of tissue obtained from both wounds during the first debridement grew strains of the common wound pathogens *S intermedius* and a β-hemolytic *Streptococcus* sp that were found to be intermediately susceptible on antimicrobial susceptibility testing from both elbow regions. On 16S rRNA fragment analysis of DNA extracted from amplified PCR products, the *Streptococcus* sp was identified as *S canis*. However, 16S rRNA sequencing directly from clinical samples as a diagnostic tool has been limited to infections that are predominantly monobacterial, so only the dominant bacterial sequence could be identified, raising concern that other bacterial species may not have been detected. Microbial culture of tissue from the second wound debridement of the wound over the right elbow region raised the question as to whether the bacteria were newly invading pathogens or were present but not cultured previously.

In the present case, the reason DNA was submitted for pyrosequencing was to determine whether chronic

wounds in this dog had similar polymicrobial populations to those reported for wounds of humans and horses. Bacterial diversity, as found by molecular diagnostic techniques, has not yet been reported for chronic wounds in dogs. Thus, to characterize the diversity of bacteria within these wounds, DNA was submitted for 16S rRNA gene pyrosequencing and small subunit assay for bacterial and fungal organisms. The most prevalent bacterial species in both wounds was *L monocytogenes*. The importance of this finding is unknown, given that *L monocytogenes* is not recognized as a common wound pathogen in humans or in dogs. The DNA submitted for sequencing had previously been extracted from the wound tissues because fresh tissue specimens were not available. Although strict isolation protocols were followed, it is possible the samples became contaminated by saprophytic organisms at some point between DNA extraction and arrival at the commercial laboratory. In the future, more meaningful results may be obtained by use of the laboratory's sample submission kit, thereby reducing the risk of contamination and possibly maximizing the amount of extracted bacterial DNA.

The remainder of the bacteria identified by pyrosequencing were similar to those obtained in studies[4,12,30,31] of chronic wounds in both humans and horses, indicating that biofilms infecting wounds in dogs are also polymicrobial. When comparing conventional culture and molecular diagnostic results of the case described in the present report, it was interesting to note that no *Staphylococcus* spp were identified either by 16S rRNA sequencing or by pyrosequencing. Most likely, the methods used to sequence the bacterial DNA either did not extract or did not amplify the *Staphylococcus* DNA that was present in the wound tissue specimen. The reason for this failure was not evident. Potentially, the tissue specimen used for DNA extraction was obtained from deeper within the wound and did not contain *Staphylococcus* spp that would have been present nearer the surface. Such discrepancies between conventional microbial culture and molecular diagnostic test results have been previously reported for wounds of humans, underlying the observation that no single diagnostic technique can reliably identify all bacterial species within a wound.[4] Until molecular methods become more advanced, the best treatment decisions should be based on the combined results of both bacterial identification techniques.

Bacterial sequencing results were obtained well after clinical resolution of the chronic wounds for the dog in the present report; therefore, DNA analysis did not play a role in the treatment choices. Possible contamination of the specimens also confounded interpretation of the pyrosequencing results. Concurrent submission of debrided tissues for DNA sequencing and for conventional microbial culture with antimicrobial susceptibility testing may be useful in developing a treatment plan that includes topical administration of antimicrobials.

a. Nestle Purina Body Condition System, Néstle Purina PetCare, St Louis, Mo.
b. TLS Surgical Drainage System, Stryker, Kalamazoo, Mich.
c. GranuFoam Silver Dressing, KCI, San Antonio, Tex.
d. KCI Vet, San Antonio, Tex.
e. ACTICOAT Absorbent, Smith & Nephew, London, England.
f. 3M Tegaderm Transparent Film Dressing, 3M Co, Saint Paul, Minn.
g. DogLeggs Therapeutic & Rehabilitative Products, Reston, Va.
h. DNeasy Blood & Tissue Kit, Qiagen, Valencia, Calif.
i. GoTaq Green Master Mix, Promega Corp, Madison, Wis.
j. Bio-Rad thermal cycler, BioRad Laboratories Inc, Hercules, Calif.
k. Gel Extraction Kit, Qiagen, Valencia, Calif.
l. ABI 3137XL low-throughput capillary machine, Applied Biosystems Inc, Foster City, Calif.
m. GenBank [database online]. Bethesda, Md: National Institutes of Health. Available at: www.ncbi.nlm.nih.gov/genbank/. Accessed Apr 23, 2012.
n. RDP, release 10, Center for Microbial Ecology, Michigan State University, East Lansing, Mich. Available at: rdp.cme.msu.edu/index.jsp. Accessed Aug 26, 2013.
o. Research and Testing Laboratory LLC, Lubbock, Tex.
p. FLX-Titanium, Life Sciences, Foster City, Calif.
q. Kennedy JP, Vice-President of Development, Southeastern Medical, Savannah, Ga: Personal communication, 2013.

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From this month's *AJVR*

Comparison of the cardiorespiratory effects of a combination of ketamine and propofol, propofol alone, or a combination of ketamine and diazepam before and after induction of anesthesia in dogs sedated with acepromazine and oxymorphone

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Objective—To evaluate the cardiorespiratory effects of IV administration of propofol (4 mg/kg), ketamine hydrochloride and propofol (2 mg/kg each; K-P), or ketamine hydrochloride (5 mg/kg) and diazepam (0.2 mg/kg; K-D) before and after induction of anesthesia (IoA) in dogs sedated with acepromazine maleate and oxymorphone hydrochloride.

Animals—10 healthy adult Beagles.

Procedures—Each dog was randomly allocated to receive 2 of 3 treatments (1-week interval). For instrumentation prior to each treatment, each dog was anesthetized with isoflurane. After full recovery, acepromazine (0.02 mg/kg) and oxymorphone (0.05 mg/kg) were administered IV. Fifteen minutes later (before IoA), each dog received treatment IV with propofol, K-P, or K-D. Cardiorespiratory and arterial blood gas variables were assessed before, immediately after, and 5 minutes after IoA.

Results—Compared with findings before IoA, dogs receiving the K-P or K-D treatment had increased cardiac output, oxygen delivery, and heart rate 5 minutes after IoA; K-P administration did not change mean arterial blood pressure or stroke volume and decreased systemic vascular resistance. Propofol decreased mean arterial blood pressure and systemic vascular resistance immediately after IoA but did not change heart rate, cardiac output, or oxygen delivery. All treatments caused some degree of apnea, hypoventilation, and hypoxemia ($Pa_{O_2} < 80$ mm Hg).

Conclusions and Clinical Relevance—In dogs, K-P treatment maintained mean arterial blood pressure better than propofol alone and increased heart rate, cardiac output, or oxygen delivery, as did the K-D treatment. Supplemental 100% oxygen should be provided during IoA with all 3 treatments. (*Am J Vet Res* 2014;75:231–239)

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