Open wound management of traumatic wounds presents a unique, laborious, and costly clinical problem. The difficulty in delineating a specific treatment for wound management is likely due to the complexity of wound healing itself, which involves a variety of processes, including angiogenesis, inflammation, cell proliferation, matrix deposition, and tissue remodeling. Furthermore, these events often rely on other complicated pathways, such as hemo-
stasis, inflammation, granulation tissue formation, epithelialization, neovascularization, collagen synthesis, and wound contraction. The field of open wound management continues to evolve as additional cellular or signaling targets are identified and new topical dressings are manufactured to promote wound healing. Topical dressings, including hydro-
gel, alginate, and antimicrobial dressings, promote a specific function or support a particular phase of wound healing. Hydrogel can be used to keep wound surfaces moist to promote epithelialization, and alginate dressings, such as calcium alginate, can be used to promote granulation tissue formation. Antimicrobial dressings, such as silver-impregnated products, can be used in infected wounds. Wounds should be assessed by a veterinarian with a thorough understanding of wound healing in order to identify the phase of wound healing and determine which topical dressing should be used to help promote that specific phase. A wound dressing that could promote wound healing at all stages may simplify wound management and accelerate wound healing. Nitric oxide (NO) plays a crucial role in wound healing. It is known to regulate wound healing with...
unique functions through several pathways, including inflammation, angiogenesis, cell proliferation, differentiation, apoptosis, matrix deposition, and remodeling. It may act in every phase of wound healing. After the initial break in skin, the production of NO is upregulated at the wound site, where it works as a biocide, exhibiting broad-spectrum antibacterial activity against gram-positive and gram-negative bacteria. As wound healing progresses, NO promotes granulation tissue formation through the stimulation of angiogenesis and cell proliferation. Finally, NO has signaling functions to aid in re-epithelialization and collagen maturation required for remodeling.

Despite the integral role that NO plays throughout all phases of wound healing, to our knowledge there are no studies assessing the use of an NO-based wound dressing for open wound management of traumatic wounds in dogs. The purpose of this study is to describe the use of a novel wound dressing that delivers NO directly to the wound bed in dogs with naturally occurring traumatic wounds.

Methods

Eligibility criteria

This prospective clinical trial was performed at the Colorado State University Veterinary Teaching Hospital. The study was approved by the Colorado State University Clinical Review Board (protocol #3921). Dogs that were presented to the Urgent Care Service or the General Surgery Service for treatment of a traumatic wound were evaluated for enrollment from January 2023 through January 2024. Inclusion criteria included a full-thickness traumatic wound that occurred within 5 days of presentation with a minimum loss of 2 cm of skin or subcutaneous pocketing. Owner consent was obtained for enrollment if the primary clinician determined that open wound management was indicated. Dogs were required to be seen through the General Surgery Service at the Colorado State University Veterinary Teaching Hospital for bandage changes and continued wound management. Dogs were excluded if the primary clinician assessed them to have systemic disease that put them at risk with repeated sedation, if hemorrhage from the wound was not controlled following vessel ligation or after 5 minutes of direct pressure (uncontrolled hemorrhage), or if joint tissue or bone was exposed. Dogs were withdrawn from the study if they had any clinical signs consistent with a reaction to the novel contact layer, including progressive irritation or erythema of the skin or wound edges that were in contact with the product, unexpected pain that was not explained by the wound itself, or hemorrhage from the wound after application of the wound dressing. Dogs could also be withdrawn at the primary clinician’s discretion if there were any additional concerns.

Wound management

At the initial visit, demographic data collected included age, breed, sex/neuter status, body weight, date of initial presentation, comorbidities, and medications prescribed. Wound-specific data collected included date and duration of wound, number of wounds, location of wounds, and etiology of the wound. If the wound occurred within the previous 24 hours, the duration of the wound was assigned as 1 day. For abscesses that ruptured that day, the duration of the wound was assigned as 1 day. The etiology of the wound was classified as (1) laceration, (2) abscess, (3) bite wound, (4) unknown, and (5) other.

During the initial visit, dogs were sedated at the clinician’s discretion, and the wound was clipped, the periwound skin was cleaned with 2% chlorhexidine gluconate and 70% isopropyl alcohol (BD ChloraPrep Hi-Lite Orange 3-mL applicators; CareFusion), and the wound was lavaged with 0.9% saline delivered with a 1-L pressure bag pressurized to 300 mm Hg. Wounds were flushed until all visible debris was removed; the total volume of lavage administered was at the discretion of the primary clinician. Wound cultures were performed at the discretion of the primary clinician and may have been collected at initial presentation, during subsequent wound management, at the time of closure, or after closure due to wound healing complications.

The novel NO wound dressing (Restore; Noxsano) is available in 2 different delivery systems: a pad and a dehydrated gel precursor. All products were used according to manufacturer guidelines. The pad (5 X 7 cm or 5 X 12 cm) comes in a clear, sterile package (Figure 1). To activate the pads, 15 or 30 mL (based on pad size) of sterile 0.9% saline was placed in the clear package containing the pad and allowed to absorb for 1 minute. The gel precursor (Figure 1) is packaged in a 5-mL syringe, which was activated by drawing up sterile 0.9% saline to the 5-mL mark on the syringe and shaking vigorously for 1 minute.

The formulation used was determined by the primary clinician. The size of the wound, anatomic location of the wound, and the presence of undermining or pocketing of the wound were considered in determining which formulation was used. After applying the wound dressing, the wound was bandaged with either a tie-over bandage or soft-padded bandage. A variety of secondary dressings, such as Copa (Kendall Covidien), Telfa (Covidien), 3M Ioban (3M), and saran wrap (S.C. Johnson & Son) could be used. The time-to-recheck examination for bandage change with the General Surgery Service was determined by the primary clinician.

During recheck examinations, patients were sedated, and the primary surgery clinician determined whether additional open wound management or wound closure was appropriate. If open wound management was still required, the wound was lavaged and debrided at the discretion of the primary surgery clinician, the novel NO wound dressing was applied, and a bandage was replaced. At any point during wound management the primary clinician could elect to close the wound.

Antibiotics, cultures, and follow-up

Information regarding antibiotic use and wound cultures was collected, including the type, dosage,
and duration of antibiotic use. If a wound culture was performed, the time of culture, culture results, susceptibility profile, and whether a change was made to the antimicrobial therapy was documented. Follow-up information included date and type of closure, whether the dog was removed from the study and why, complications associated with the use of the novel wound dressing, complications associated with wound healing/closure, and time to last follow-up. Complications associated with the use of the wound dressing were described as erythema of the skin, discoloration of the tissue, increased quantity and opaqueness of wound fluid, and those directly related to the handling and use of the product. Complications associated with wound healing and closure were described as failure of the wound to contract, failure of the wound to develop granulation tissue, opaque/purulent drainage from the incision, seroma formation, and dehiscence. Dogs could be removed from the study at any time if the clinician believed the wound was not healing as expected. Dogs were excluded from follow-up data if they were removed from the study prior to wound closure.

Statistical analysis
Statistical analysis was performed using JMP Pro (version 17; SAS Institute Inc). Categorical variables were expressed as frequency, and continuous data were expressed as median and range.

Results
Study population
Twenty-four dogs and 30 wounds were included in the study. Twenty dogs had 1 wound, 3 dogs had 2 wounds, and 1 dog had 4 wounds. The median age of dogs was 6.4 years (range, 1.2 to 12.6 years), and the median weight was 27.3 kg (range, 6.4 to 46.7 kg). Eleven of the 24 (45.8%) dogs were spayed females, 9 (37.5%) were castrated males, 3 (12.5%) were intact males, and 1 (4.2%) was an intact female. The most frequent breeds were Labrador Retrievers (5), mixed-breed dogs (5), and Jack Russell terriers (2). There was 1 each of the following breeds: American Pit Bull Terrier, Australian Shepherd, Beagle, Border Collie, Boxer, Brittany Spaniel, Cardigan Welsh Corgi, Coonhound, German Wirehaired Pointer, German Shorthair Pointer, Great Pyrenees, and Miniature Australian Shepherd.

Comorbidities
There were 2 dogs with comorbidities that could have potentially affected wound healing. One dog had recently been diagnosed with a urinary tract infection and finished a course of antibiotics 3 days prior to presenting with a wound. The second had concurrent systemic illness that could have affected wound healing, including septic shock, fluid overload, anemia, a history of a protein-losing nephropathy, and a mixed hepatopathy.

Wound etiology and duration
Thirty traumatic wounds were included in the study and were classified as a laceration (12), a bite wound (9), an abscess (4), unknown (3), and other (2). The 2 wounds classified as other were from self-trauma of a benign ulcerated mass and an incisional dehiscence following closure of a traumatic wound. The median duration of the wound prior to presentation was 1 day (range, 1 to 4 days). The duration of the wound was 1 day for 18 of 24 (75%) dogs, 2 days for 5 of 24 (20.8%) dogs, and 4 days for 1 of 24 (4.2%) dogs.

Antimicrobial therapy
All 24 dogs were prescribed empirical antimicrobial therapy. Seven dogs (29.2%) were hospitalized...
and were treated with ampicillin sulbactam 30 mg/kg, IV, 3 times daily (median duration, 3 days; range, 1 to 6 days) prior to starting oral antimicrobial therapy. Two of the 7 (28.6%) hospitalized dogs were also treated with enrofloxacin (10 mg/kg, IV, once daily). For 23 of 24 (95.8%) dogs, the initial oral antibiotic prescribed was amoxicillin trihydrate/clavulonic acid (median dose, 14.7 mg/kg, twice daily; range, 11.2 to 20.6 mg/kg) for a median of 10 days (range, 4 to 38 days). One dog was prescribed clindamycin (6.4 mg/kg, twice daily) for 35 days. In 4 of 24 (16.7%) cases, antibiotics were changed once, and in 1 of 24 (4.2%) cases, antibiotics were changed twice. Antimicrobial adjustment was based on a resistant bacterial culture in 4 of 5 dogs.

**Culture results**

Wound cultures were performed in 11 of 24 (45.8%) dogs. Eight (33%) dogs had 1 culture performed, and 3 (12.5%) dogs had 2 cultures performed, for a total of 14 cultures. Of the 14 cultures performed, 6 were performed at initial presentation, 2 were performed during open wound management, 2 were performed at the time of wound closure, and 4 were performed due to a complication after closure.

**Bandages, time to closure, and type of closure**

The pad formulation of the product was used in 15 of 30 wounds, and the gel formulation was used in 15 of 30 wounds. In one dog with multiple wounds, both the gel and pad formulations were used. Time between bandage changes ranged from 1 to 8 days (median, 2 days). All dogs and all wounds had the novel NO wound dressing placed in their wound until wound closure was deemed appropriate (Figure 2). Wound closure was performed in 27 of 30 (90%) wounds; no wounds were left to heal by second intention. The median time to wound closure was 6 days (range, 2 to 42 days). In 22 of 27 (81.4%) wounds, secondary wound closure was performed after the formation of granulation tissue. In 5 of 27 (18.5%) wounds, delayed primary closure was performed. One wound was reconstructed with punch grafts after the formation of granulation tissue 12 days after initial presentation. Following punch grafts, the wound was managed with hydrophilic foam bandages until epithelialization 31 days later. Closure time was not reported in 2 wounds. One wound was removed from the study due to joint exposure after the third bandage change, and another wound was lost to follow-up during open wound management.

**Figure 2**—Wound progression from one dog following a dog bite wound to the ventral cervical region. The wound was debrided at the time of presentation (A–C), and the pad formulation was placed within the wound (D) and secured with a bandage. Open wound management was performed using the pad formulation, and representative images are from day 6 (E), day 10 (F), day 19 (G), and day 43 before (H) and after (I) wound closure.
after the fourth bandage change. These 2 wounds were excluded from closure and follow-up data.

Complications associated with novel wound dressing

Graying of tissues was noted in 5 wounds during bandage change (Figure 3). The gel formulation was used in all 5 cases. All wounds with graying of tissues healed without complication. In 1 dog, the seal of the pad ruptured during bandage change, releasing the contents of the pad into the wound. This wound healed without complication, and there was no apparent complication associated with pad rupture. There were no apparent skin or tissue reactions attributed to the product requiring withdrawal from the study. No clinician determined wound healing to be delayed or inadequate resulting in the need to adjust open wound management protocol to a different topical dressing.

Complications associated with wound healing/closure

Four wounds (4/28) from 4 dogs (4/22) experienced complications following wound closure. Incisional dehiscence occurred 10 days following wound closure in one dog. The wound was debrided and reclosed 4 days later. Although no additional topical wound management was performed, the dog was seen for continued wound drainage and delayed healing for an additional 52 days. One dog had prolonged open wound management due to a pharyngeal tear that communicated with the wound and failed to heal with closure of the pharyngeal mucosa. Approximately 3 weeks after presentation, contamination of the wound with saliva and regurgitation was no longer noted, and presumably the pharyngeal tear had healed by second intention. Wound closure was performed 42 days following the initial trauma. This dog had partial dehiscence 4 days following wound closure and was treated with the placement of a single skin staple. One dog developed dehiscence 3 days following wound closure, and purulent exudate was noted along the incision. The wound was opened, lavaged, debrided, cultured, and reclosed. Culture results revealed that antimicrobial therapy was appropriate, and no additional intervention occurred other than prolonged antibiotic therapy.

Follow-up

Twenty-two dogs and 28 wounds were followed after wound closure, with a median follow-up time of 15 days (range, 4 to 91). Wound healing was confirmed in 19 dogs with 25 wounds with either a physical examination at the time of suture removal or phone follow-up with the referring veterinarian or owner. Three dogs (13.6%) were lost to follow-up prior to suture removal at a median time of 5 days (range, 4 to 7 days). For the 3 dogs lost to follow-up, there were no concerns for healing complications at the time of the last visit. The 2 dogs with comorbidities were followed up at 11 and 14 days after wound closure. Sutures were removed, and both wounds were considered clinically healed.

Discussion

In this study, the novel NO wound dressing was found to be easy to use, there were no complications directly attributed to the product that clinically affected the dogs, and all wounds that were available for follow-up went on to heal. At no point did a clinician decide that a wound was not progressing and that it needed to be removed from the study. One dog was removed from the study due to joint exposure, which was an exclusion criterion of the
studied. The effects of the novel NO contact layer on cartilage are unknown, and therefore this dog was removed and excluded from the closure data.

The novel NO wound dressing has 2 formulations: a pad and a dehydrated gel precursor. In both formulations, NO is generated through the reduction of sodium nitrite in the presence of sterile saline. The amount of saline required for the activation of each formulation is indicated in the manufacturer guidelines. The outer layer of the pad formulation is made of a nonwoven, viscose polyester, and the interior contains a superabsorbent polymer that absorbs water and maintains moisture in the pad, allowing the generation of NO to continue. There are several factors to consider when deciding which formulation to use, including the size of the wound, the anatomic location of the wound, and the presence of undermining or pocketing of the wound. In this study, many of the wounds had large amounts of undermining and pocketing, and in these cases, the pad formulation was used for its ability to pack into the wound. According to the manufacturer product guidelines, if the gel or pad remains wet, it can continue to produce NO for 7 days, although their recommendation is to change dressings every 2 to 4 days. In this study, the frequency of bandage changes was determined by the primary clinician. In some cases with more dynamic wounds, bandage changes were completed more frequently, whereas in others, the frequency of bandage changes exceeded the manufacturer-recommended 2 to 4 days. None of the wounds that had the product in place for more than 4 days experienced wound healing complications. Based on the findings of this study, the bandage can be changed at any time until 7 days after placement.

One observation regarding the use of the gel formulation was that in some cases, it left a gray hue on the wound bed. This was more commonly noted in wounds that did not have granulation tissue present. This is suspected to be due to the water binder in the gel formulation, which is different from the pad formulation. The material in the gel formulation is bioresorbable, and depending on the type of tissue in the wound bed, the product could potentially be resorbed into the tissues, creating the gray hue. All of the wounds that developed a gray hue healed without complication, so it would be important to note and exclude this gray hue as devitalized tissue. In one case, the sealed portion of the pad perforated, and the inner contents of the pad formulation were released onto the wound bed. There were no apparent complications associated with this, and the wound went on to heal without complication.

There were no complications directly attributed to the product that clinically affected the dogs. It is possible that complications were missed and falsely attributed to the trauma. For example, the skin surrounding wounds is commonly erythematous in the first few days after trauma. Although attributed to trauma, it is possible that this may have been related to the product. However, there was no progression of periwound erythema documented in any wounds. In one case, the product was used overlying the carotid artery, the vagosympathetic trunk, trachea, and exposed pharyngeal mucosa. Although the pharyngeal mucosa failed to heal after 3 closure attempts, it eventually went on to heal without any known complications to the mucosa or the exposed carotid artery and vagosympathetic trunk. Pharyngeal tears can be challenging to heal, particularly in the face of a contaminated wound, constant motion, and intermittent regurgitation. The authors suspect that the delayed healing is secondary to the location of trauma and ongoing contamination from regurgitation yet cannot rule out that the NO wound dressing impacted healing.

Dehiscence occurred in 3 of the 28 wounds that underwent wound closure. There are numerous factors that influence the healing of traumatic wounds, so it is difficult to determine the cause of dehiscence in these cases. In one dog, dehiscence was minor and only required a single staple to be placed. The cause of this complication was thought to be related to tension. In one dog, the owner noted that the dog could lick at its incision even with the E-collar in place. Compliance issues may have contributed to dehiscence and prolonged wound healing in this dog, which took 91 days. In the third dog, dehiscence occurred 3 days after wound closure. The wound was opened, debrided, cultured, and reclosed. The expectation was that the wound was infected, but culture results revealed that the antimicrobial therapy was appropriate, and the wound went on to heal normally.

A recent study by Hamil et al. found that 22% of dogs with acute traumatic wounds treated with empiric antibiotic therapy went on to develop wound infections following wound closure, and 9.4% developed wound breakdown. This is similar to our study, where 10.7% of wounds developed wound breakdown. Only 2 (7.1%) wounds in our study had clinical signs consistent with wound infection following closure. NO is known to have antimicrobial activity, so it is possible that the NO wound dressing led to a lower infection rate in our study. However, cultures were not performed in all wounds at the time of closure, so this could be an underestimation.

There were several limitations to this study, many associated with the difficulties in subjectively assessing wounds. As in all clinical studies of wounds, it is impossible to say whether the wound healed as expected or in the expected time frame. This is a clinical study, so there was no control group. Although there were no apparent complications associated with the product’s use during wound management, local irritation or erythema that was attributed to trauma could have been associated with the product. This study relied on the clinician’s assessment of the wound to determine whether wound healing was progressing. The level of experience of the surgery clinic varied from a surgery resident to a boarded surgeon. Median follow-up time was 15 days, so it is possible that a wound healing complication developed beyond the last follow-up time. Additionally, none of the wounds in this study healed by second.
intention, so the role of NO described in promoting healing by second intention could not be assessed. Finally, while NO has been described to have a role in antimicrobial action and wound debridement, this study did not have a large number of grossly, heavily contaminated wounds, so the role of NO in these processes could not be assessed. NO has been shown to inhibit or kill microbes when NO donor compounds were directly administered in vitro. Additionally, biofilm regulation has been documented in the presence of exogenously applied NO. Wound cultures were not performed in all cases, and timing was not standardized; therefore, conclusions regarding the novel wound dressing’s impact on preventing or treating infection cannot be made. According to a recent study, routine bacterial culturing of acute traumatic wounds is not predictive of subsequent wound infection and was therefore not considered a significant limitation in this study. Lastly, this study did not test for the presence of NO in the wound nor did it evaluate the dose and duration of NO delivery. This should be considered in future studies.

The novel NO wound dressing was easy to use and was well tolerated by dogs during open wound management for acute traumatic wounds throughout the inflammatory, debridement, and repair phases of wound healing. Multiple formulations provide many options that suit both superficial and large pocketed wounds. There were no apparent complications directly attributed to the use of the novel NO wound dressing that clinically affected the dogs. All wounds improved with the use of NO wound dressing and went on to heal following wound closure, with low clinically apparent infection rates. The use of NO wound dressing may simplify decision making in open wound management in a practice where the inventory of numerous products for different phases of wound healing is limited. Additional studies on the efficacy of this product in chronic wounds are warranted.

Acknowledgments

The authors would like to acknowledge Maggie Yates for her dedication and support of case enrollment, patient management, and maintenance of study documents and photos.

Disclosures

Financial support for the study was provided by Noxsano.

Noxsano was not involved in study design, case enrollment, case management, data or statistical analysis, or manuscript preparation. No AI-assisted technologies were used in the generation of this manuscript.

Funding

This study was supported by Noxsano, and all wound dressings were provided by Noxsano.

ORCID

K. Zersen https://orcid.org/0000-0003-0327-8537

References