Injuries or diseases that result in full-thickness skin loss occur commonly in veterinary medicine. Clinicians usually manage such wounds by primary closure, rotational flaps, axillary pattern flaps, negative pressure treatment, laser therapy, or by second intention healing with dressings and bandages, depending on the severity and extent of these wounds. Less commonly, they perform free grafting with autogenous, allogeneic, or xenogenic tissue to temporarily or permanently close large skin defects, but these have higher rates of complications, such as donor site morbidity, infection, rejection, or transmission of infectious agents. Investigators have applied synthetic, bioelectric, and biological wound dressings to augment wound healing with varying success. Synthetic wound dressings are readily available, procured at a reasonable cost, and have long-term storage possibilities. Biological wound dressings can become incorporated into the wound bed, providing a scaffold that promotes adhesion and migration of fibroblasts and keratinocytes. Biological dressings potentially contain multiple factors, such as growth factors and cytokines, that might help wounds heal faster reducing patient morbidity and costs.

OBJECTIVE
To compare the differences in the healing of surgically created full-thickness wounds in dogs treated with a novel extracellular matrix (ECM) dressing as compared with a standard wound management protocol and to investigate the effect of antibiotics in these 2 populations.

ANIMALS
15 purpose-bred Beagles, 8 female spayed and 7 males neutered, operated on, and monitored between March 14, 2022, and April 18, 2022.

METHODS
Four 2 X 2-cm full-thickness skin wounds were created on the trunks of each dog. The right-sided wounds were treated with the novel ECM wound dressing, and the left-sided wounds served as the controls. Wound planimetry and qualitative wound scores were obtained at 12 time points. Wound biopsies for histopathologic assessment of wound repair and wound inflammation were obtained at 6 time points.

RESULTS
Wounds treated with ECM had higher percent epithelization at days 7, 9, 12, and 18 postoperatively (P < .001) and better histologic repair scores (P = .024) than wounds treated by the standard protocol. Subjective wound assessment scores of wounds treated with ECM did not differ from those treated by the standard protocol at any time point.

CLINICAL RELEVANCE
Wounds treated with the novel ECM dressing epithelialized more rapidly than wounds treated by a standard protocol.

Keywords: wound, Wharton’s jelly, extracellular matrix, epithelialization, contraction
In human wound care, collagen-based wound dressings followed by an epidermal autograft, or bioengineered bi-layered skin substitute provide an excellent wound dressing.\textsuperscript{13–16} Investigators have recently examined new, less expensive techniques utilizing sterilized fish skin (tilapia, cod) which have led to faster epithelization, fewer painful bandage changes, and decreased need for narcotics.\textsuperscript{16–18} Veterinary clinicians have used biological wound dressings such as allogeneic peri toneum, amnion, omentum, collagen dressings, porcine small intestinal submucosa, and extracellular matrix (ECM) products to treat open wounds in dogs and horses with mixed success.\textsuperscript{2,19,20} Currently, multiple collagen products exist in the veterinary market, some of which are reported to contain active growth factors, such as fibroblast growth factor-2 (FGF-2), transforming growth factor β (TGFβ), and vascular endothelial growth factor (VEGF), making these materials bioactive constructs that can promote tissue remodeling.\textsuperscript{2} More recently, investigators have developed dressings incorporating mesenchymal stem cells (MSC).\textsuperscript{21,22}

Dressings and gels with MSCs, or substances that attract MSCs, have the advantage of applying multipotent cells directly into the wounds and reports indicate increased angiogenesis, epithelization, and decreased scar formation.\textsuperscript{21,22} Mesenchymal stem cells and ECM, harvested from numerous anatomical locations including bone marrow, adipose tissue (ASC), and Wharton's jelly of the umbilical cord, all display similar immunophenotypic profiles. Mesenchymal stem cells from adipose and bone marrow have been previously investigated in veterinary wound models but Wharton’s jelly also conveys potential benefits if implanted in an easily applied wound dressing. Wharton’s jelly is a gelatinous substance that lies between the amniotic epithelium and umbilical vessels. It contains high levels of proteoglycans and collagen and few mesenchymal stem cells.\textsuperscript{23} Surgeons have successfully used Wharton’s jelly seeded on the human acellular amniotic membrane to treat chronic, nonhealing diabetic ulcers in clinical human patients.\textsuperscript{24} Reports of the use of Wharton’s jelly for skin wounds in veterinary patients are limited to case reports describing successful treatment of chronic nonhealing wounds in 2 dogs and 1 horse.\textsuperscript{25,26}

We sought to compare the effect of a novel ECM wound dressing with a standard wound management protocol for experimental full-thickness wounds in dogs. The primary objective was to compare wound healing rates as defined by epithelialization and contraction. The secondary objective was to compare subjective wound healing characteristics such as pain, quantity and quality of wound discharge, etc. A third objective was to determine the effect, if present, of antibiotics on wound healing in these groups. We hypothesized that this novel ECM dressing would provide an environment for enhanced cell migration and angiogenesis, epithelialization, and contraction, leading to faster healing times for wounds, and that antibiotics would have no effect.

### Methods

#### Animals

Fifteen adult purpose-bred beagles (8 female spayed and 7 male castrated) with a mean age of 369.4 days (± 2.5 days) and a mean body weight of 10.4 kg (± 2.0 kg) were included in the study and housed individually. The number of dogs was determined based on previous wound studies.\textsuperscript{2–5} All dogs underwent a physical examination and blood work consisting of a complete blood count and serum biochemistry shortly before the study. The study was approved by the Institutional Animal Care and Use Committee at Cornell University (IACUC 2021-0055).

#### Surgical procedure

On day 0, four 2 X 2 cm full-thickness wounds were created on the trunk (2 on each side) of each dog, as described by Schallberger et al.\textsuperscript{1} Dogs were premedicated with hydromorphone 0.1 mg/kg and acepromazine 0.02 mg/kg IV and induced under general anesthesia with propofol up to 6 mg/kg IV. After wound creation and recovery from anesthesia, they were administered an additional dose of hydromorphone 0.1 mg/kg IV and carprofen 2.2 mg/kg SC. After wound creation, the right-sided wounds were covered in a single layer of the novel ECM dressing (Sanatela Matrix; Sanatela Medical; Figure 1). The left-sided wounds acted as a control with only a non-adherent pad (Telf; Covidien) placed over the wound. Hydrogel dressing (Curage; Tyco Healthcare) was placed on all 4 wounds before nonadherent dressings were applied. Cranial wounds on both sides were used for tissue biopsies and caudal wounds on both sides were used for wound planimetry. The trunk was initially covered in a cross-your-heart style bandage (the primary contact layer of the wound was ECM dressing followed by a nonadherent pad or nonadherent pad alone, followed by cast padding, followed by cling gauze, then followed by adhesive wrap as the outer layer) to keep the wounds clean and prevent self-traumatization for the first 7 days. Postoperatively each subject was administered carprofen 2.2 mg/kg and pregabalin 2 mg/kg by mouth every 12 hours for 7 days for analgesia. For the duration of the study, each subject received 3–10 mg/kg trazodone by mouth every 8 to 12 hours to facilitate rest. After 7 days, nonadherent pads were applied to each wound over ECM dressing (right side) or over no treatment (left side)_affixed by sutures, and the dogs were placed in custom-made t-shirts. Five dogs were randomized to receive oral cephalaxin 38 to 43 mg/kg by mouth every 12 hours for the first 7 days postoperatively. The dose was calculated to approximate a 22 mg/kg dose every 8 hours.

#### Wound assessments

Wounds were studied for 32 days. Bandage changes for planimetry/wound evaluation were performed on days: 0, 2, 4, 7, 9, 12, 14, 18, 21, 24, 28, and 32. Bandages were inspected daily and changed as needed (torn, strike-through noted) or according to the schedule. Dogs were sedated with
dexmedetomidine 5 mcg/kg IV for all wound evaluations. After sedation, the bandages or t-shirts were removed, and each wound was assessed by qualitative measurements (performed by N.B. or K.K.). All bandage changes were performed using an aseptic technique. Qualitative measurements included a wound characterization score: wound bed appearance, quantity, and quality of discharge, peri-wound skin appearance, and discomfort of the patient (Table 1). After the assessment, the wounds were gently lavaged with saline and the peri-wound skin was cleaned with sterile saline. The wound bed was evaluated to determine if ECM dressing needed to be reapplied according to the manufacturer’s instruction; if no remaining dressing was visible, a new sheet of ECM dressing was cut to size and reapplied to the wound. Hydrogel was re-applied at each wound assessment. Subjective wound characterization scores and planimetry were always performed on the caudal wounds to prevent biopsy sites from disturbing the data.

Planimetry
All caudal wounds were photographed for planimetry with a digital SLR Nikon N7500 resulting in digital photographs of (4,288 X 2,848 pixels). A measurement scale was included in each picture to allow for calibration of the wound in the image processing software (Image J software, U. S. National Institutes of Health, https://imagej.nih.gov/ij/, 1997–2018.). Wound areas were color-coded and the following 3 areas were measured by 2 investigators blinded to the wound site or treatment (N.B., S.R.): open wound area, defined as pre- or granulation bed, the epithelialized area, defined as an area with new epithelial tissue present, the total wound area, defined as the sum of the latter 2 areas (Figure 2). All wound areas were calculated in square centimeters. Percent contraction was calculated with respect to the original wound size as follows (% contraction day N = 100 - ([total wound area day N/original wound area day0] X 100) and percent epithelialization of the total wound area as calculated as follows (% epithelialization day N = area of epithelium day N/total wound area day N X 100).

Histopathology
Biopsies for histopathology were obtained from cranial wounds on days: 0, 4, 7, 14, 21, and 28.

Table 1—Subjective wound score system.

<table>
<thead>
<tr>
<th>Variable score</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid color</td>
<td>Clear Serous</td>
<td>Pink/red Serosanguinous</td>
<td>Brown Sanguinous</td>
<td>Yellow Purulent</td>
<td>Green Purulent ooz</td>
<td>Purulent with tissue liquefaction</td>
</tr>
<tr>
<td>Wound fluid</td>
<td>Serous</td>
<td>Serosanguinous</td>
<td>Dry and clean</td>
<td>Dry and stained</td>
<td>Wet (&lt;4 layers wet)</td>
<td>Strike-through</td>
</tr>
<tr>
<td>Secondary layer</td>
<td>Dry and clean</td>
<td>Periwound edema</td>
<td>Periwound oedema</td>
<td>Periwound ulceration</td>
<td>Periwound induration</td>
<td>Periwound discoloration</td>
</tr>
<tr>
<td>Periwound</td>
<td>Normal skin</td>
<td>Moist (&lt;4 layers wet)</td>
<td>Maceration + Healthy red and granular</td>
<td>Maceration +++ Purple and friable Irregular</td>
<td>Desiccation + Pale and fibrous Exuberant</td>
<td>Exuberant</td>
</tr>
<tr>
<td>Hydration status</td>
<td>Normal None</td>
<td>Maceration + Healthy red and granular</td>
<td>Dark red and granular</td>
<td>Maceration +++ Purple and friable Irregular</td>
<td>Maceration ++ Purple and friable Irregular</td>
<td>Exuberant</td>
</tr>
<tr>
<td>Granulation tissue</td>
<td>None</td>
<td>Maceration + Healthy red and granular</td>
<td>Dark red and granular</td>
<td>Maceration +++ Purple and friable Irregular</td>
<td>Maceration ++ Purple and friable Irregular</td>
<td>Exuberant</td>
</tr>
<tr>
<td>Quality Discomfort</td>
<td>Smooth None</td>
<td>Mild irritation (twitches, turns head)</td>
<td>Healthy red and granular</td>
<td>Maceration ++ Purple and friable Irregular</td>
<td>Maceration ++ Purple and friable Irregular</td>
<td>Exuberant</td>
</tr>
</tbody>
</table>

Subjective wound score system using qualitative measurements of wound and periwound characteristics to compare surgically created wounds of 15 dogs treated with a novel Extracellular Matrix dressing vs a control treatment between March 14, 2022, and April 18, 2022.
Biopsies were performed using a 4 mm diameter Baker’s punch in a prescribed order. The biopsy site locations were systematically and diametrically positioned at the wound edges, to include the migrating epithelium. On day 4 the biopsy was obtained from the craniodorsal aspect of the wound, on day 7 the caudoventral aspect, on day 14 the cranioventral aspect, on day 21 the caudodorsal aspect, and on day 28 the midpoint of the cranial wound edge. Collected skin tissue was fixed in 10% neutral-buffered formalin, processed for light microscopy, and stained with hematoxylin and eosin (H&E) and Masson’s trichrome to highlight the connective tissue. Histopathologic analysis of wound biopsies was performed by 1 board-certified pathologist (A.D.), blinded to the wound site or treatment. Histopathology scores were calculated as previously described\(^1\) and included a histologic acute inflammation score (HAIS; range = 0 to 12) and histologic repair score (HRS; range = 0 to 9). The HAIS consisted of 4 components: neutrophilic cellular infiltration, degree of edema, hemorrhage, and necrosis, evaluated on the H&E stain. The HRS consisted of 3 components: fibroblast proliferation, collagen density, and neovascularization by combined evaluation of the H&E and Masson’s trichrome stains. Each component was scored as 0 = none, 1 = mild, 2 = moderate, and 3 = severe.

### Statistical analysis

We examined 5 response variables: total wound area (cm\(^2\)), % contraction, % epithelialization, HAIS, and HRS. We examined all variables using a 2-factor repeated measures ANOVA, with time and treatment both as within-subject factors and applying a Greenhouse-Geisser correction for sphericity. We also included an interaction term of treatment*time. Post hoc comparisons for within-subject factors were performed using Tukey’s honestly significant difference tests. Statistics were performed using the jamovi project (2022) jamovi (Version 2.3.18); retrieved from https://www.jamovi.org. The level of significance was set at \( P < .05 \).

### Results

All dogs were enrolled in the study between March 14, 2022, and April 18, 2022, and had normal preoperative physical exams and blood work. No intraoperative or anesthetic complications occurred during wound creation. The dogs were continually active, and the initial bandages had to be modified by day 7 due to disruption of the external layers. All wounds healed by the conclusion of the study.

### Histopathologic data

The HAIS and HRS improved over time for both the wounds treated with ECM and by the standard protocol (\( P < .001 \)). This finding is consistent with the phases of wound healing and validates the histopathologic data. We observed no difference in the HAIS score between wound treatments at any time point. However, ECM-treated wounds had higher HRS scores over time than control wounds, consistent with greater histologic repair in the ECM-treated wounds (\( P = .024 \); Figures 3 and 4).

![Figure 3](image-url) — Scatter plot with line graph of HRS score over time for ECM and control wounds. ECM-treated sides (solid line) had higher overall HRS score than control sides (dashed line; \( P = .024 \)), but values did not differ significantly at any specific time point. Vertical bars represent 95% confidence intervals of the estimated marginal means. HRS = Histology repair score; ECM = Extracellular matrix; CONT = Control.

### Wound planimetry

We failed to observe any differences between ECM-treated and the control wound contraction or wound area at any time point. However, the ECM-treated wounds showed greater epithelialization than the control wounds at days 7 (\( P < .001 \)), 9 (\( P = .015 \)), 12 (\( P = .02 \)), and 18 (\( P = .01 \); Figure 5).

![Figure 2](image-url) — Planimetry photos from 1 dog of an ECM-treated wound at day 18 as described in Figure 1. Open wound area (OWA) was calculated as the tissue within the yellow line while the epithelialized area (EA) is outlined in red. The total wound area (TWA) was defined as the sum of the latter 2 areas. OWA = 0.7 cm\(^2\), EA = 1.4 cm\(^2\), TWA = 2.1 cm\(^2\).

### Wound score data

Both ECM-treated and the control wounds developed granulation tissue by day 4. Subjectively, the ECM-treated wounds displayed more irregular granulation beds (12/15 [80%]) on day 7 through
day 14 (11/15 [73%]) than control wounds (7/15 [46%] and 9/15 [60%], respectively). This difference was resolved by day 21. Both sets of wounds also showed higher values in wound fluid characteristics (score of ≥4) between days 12 and 14. We found no effect of antibiotics on the presence or characteristics of fluids. All wounds appeared well hydrated, the peri-wound skin appeared normal throughout the study, and dogs exhibited no pain on the removal of bandage material or light manipulation.

**Discussion**

Our study evaluated the effect of a novel ECM dressing on the healing characteristics of experimental wounds in dogs. When undergoing a patented material processing technique that removes all cells, the remaining acellular ECM contains natural chemical and mechanical signals that support MSC colonization and replication. Such ECM dressings should recruit MSCs to an injury site to colonize the wound and assist healing, thereby providing an advantage over pure collagen dressings (e.g., porcine small intestinal submucosa, fish skin). Although we failed to observe any effect of the ECM dressing on the inflammatory phase of healing, the ECM-treated wounds did exhibit more robust epithelialization and histological evidence of wound repair, as measured by fibroblast proliferation, collagen density, and neovascularization.

The ECM-treated wounds showed similar degrees of inflammation as the control wounds, as measured by HAIS. This indicates the ECM did not induce a greater inflammatory response with treatment, similar to what was found when a hyaluronic acid-based gel product was investigated for the use of full-thickness skin wounds in dogs. These products are acellular and contain, at best, minimal quantities of proinflammatory substances; thereby, generating no augmented inflammatory response. This differs from a similar study investigating topical porcine small intestinal submucosa for the treatment of acute full-thickness wounds in dogs, which showed an increased inflammation score at multiple time points during treatment, an effect the authors attributed to the interspecies differences in collagen. However, we observed an effect of the ECM on the histologic repair score, consistent with improved fibroblast proliferation, collagen density, and neovascularization compared to the control wounds. This is in contrast to the studies investigating a hyaluronic acid-based gel and porcine small intestinal submucosa, which failed to demonstrate a difference in repair scores throughout treatment.

Our findings are similar to a previous study of chronic canine wounds treated with Wharton’s Jelly-derived mesenchymal stem cells, where the posttreatment biopsy showed increases in fibroblasts, neovascularization, and mononuclear cells consisting of macrophages, plasma cells, and lymphocytes. The novel ECM dressing we investigated is reported to contain an abundance of extracellular matrix components (including collagen, fibronectin, hyaluronic acid, and sulfated proteoglycan) as well as several growth factors, including insulin-like growth factor 1 (IGF-1), FGF, TGF-β, epidermal growth factor (EGF), and...
platelet-derived growth factor (PDGF). All of these components could contribute to the enhanced repair scores seen in the ECM-treated wounds, either by directly providing the components to the wound (such as collagen), or by recruitment of cells by the growth factors present in the ECM.

We failed to observe any difference in contraction or wound area between the 2 wound treatments over time. This is somewhat surprising, given that contraction of the wound is primarily controlled by myofibroblasts, which other investigators have shown to be upregulated in mouse wounds treated with the decellularized extracellular matrix, and even more so with decellularized extracellular matrix derived from umbilical tissue, similar to the matrix investigated in our study. This could be explained by a difference in models (dogs vs mice) or the specific matrices used in each study. The ECM-treated wounds in our study exhibited greater epithelization than control wounds at numerous time points. In contrast, wounds treated with hyaluronic acid gel or porcine small intestinal submucosa wounds showed decreased epithelization compared with the control for the majority of the study periods. Epithelization is facilitated by the health of the granulation tissue in the wound and the ability of the leading edge of epithelial tissue to dissect the ECM and migrate across the wound bed. The ECM dressing in our study improved the health of the granulation tissue, as indicated by the histologic repair score, which could explain the improved epithelization we observed. The ECM also provides a source of proteins important in epithelial cell migration, such as fibronectin, and growth factors, such as EGF and (TGF-βI) that stimulate epithelial cell proliferation. Increased epithelization could lead to faster healing times and decreased scar formation, both of which would be clinically relevant to patients and owners. Decreased scar tissue formation is advantageous from a cosmetic and functionality perspective as scar tissue is less flexible and prone to ongoing injury. Wounds that heal by the second intention are not only cosmetically unappealing but the tissue is less flexible and prone to ongoing injury. Wounds that heal by the second intention are scar tissue is less flexible and prone to ongoing injury.

The subjective wound data was very similar between all wounds. We found no differences in the time to the first appearance of granulation tissue and similar wound fluid characteristics over time, even in dogs administered antibiotics. While the granulation bed was irregular in more ECM-treated wounds for a longer period of time, the histologic scores during this time did not differ and there were no clinical effects seen in the dogs.

Limitations of our study include the lack of monitoring aerobic culture results on these wounds as performed in other similar studies. While these were surgically induced wounds, the effects of this novel ECM dressing on infection prevention or treatment are unknown and should be evaluated in future studies. However, no wounds appeared grossly infected, no histological evaluations revealed intra-cellular bacteria, and antibiotics in a select number of dogs failed to improve outcomes. This may be partially explained by the effect of cephalexin on keratinocytes, as investigated by White et al, who showed that cephalexin significantly increases the production of IL-8, a proinflammatory cytokine, by canine keratinocytes in vitro. Interleukin 8 helps recruit inflammatory cells to wounds and would lead to higher HAIS scores and a longer inflammatory phase. Cephalexin was chosen as it is an excellent antibiotic for skin contaminants, which was felt to be the most likely source if an infection was to occur. Another limitation is the short-term nature of this study. Biopsies performed at a later date (60- or 90-days post wounding) might have shown differences in continued remodeling of the tissue.

In conclusion, this study supports the use of novel ECM dressing to enhance histologic repair and epithelialization in acute, full-thickness trunk wounds in dogs at the dosage, frequency, and timing of administration used in this study. This novel ECM dressing would be most useful once granulation tissue appears in a wound bed to speed the rate of epithelialization and decrease scar tissue formation. There does not appear to be an advantage to its use in the early inflammatory phase of healing; however, it does not appear to inhibit this phase of wound healing. This novel ECM dressing appears to be a biocompatible wound dressing with no complications or significant negative effects reported during topical application. Future work assessing its use in clinical patients in the late inflammatory/debridement and repair phases is indicated, especially in situations where enhanced epithelialization and a smaller scar are desired, such as distal limbs.

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None reported.

Disclosures
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