# Reinforcement of Esophageal Anastomoses With an Extracellular Matrix Scaffold in a Canine Model

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*Background.* The gastric pull-up procedure, a standard intervention after radical esophagectomy, is associated with high morbidity and mortality due to leaks and stricture. A previous preclinical study showed that an extracellular matrix (ECM) scaffold with autologous muscle tissue could be used to repair a complete circumferential defect in the cervical esophagus. The aim of the present study was to determine if healing of end-to-end anastomoses of the esophagus could be improved by reinforcement with an ECM scaffold.

*Methods.* Twelve female mongrel dogs underwent a complete transection of either the cervical esophagus (n = 6) or the gastroesophageal junction (n = 6). A portion of the endomucosa at the anastomotic site was resected and replaced with an ECM scaffold in contact with the subjacent muscle and the muscle was anastomosed. The measured end points included macroscopic and microscopic evaluation and quantification of the esophageal diameter at the anastomotic site.

*Results.* No anastomotic leaks or systemic complications were observed in the ECM-treated animals. Morphologic findings in both groups showed complete mucosal covering of the surgery site. The remodeled esophageal tissue showed angiogenesis and complete epithelialization. Intact, organized layers of muscle tissue were present between the native muscularis externa and the submucosal layer and effectively bridged the transected ends.

*Conclusions.* The ECM scaffold altered the default mechanism of esophageal repair. Scar tissue formation with associated stricture was virtually eliminated, and the esophageal healing response was characterized by the replacement with structurally normal tissue layers. These findings suggest that the high morbidity rate associated with esophagectomy procedures may be reduced by this ECM augmentation procedure at the anastomotic site.

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Regenerative medicine approaches using extracellular matrix (ECM) scaffolds derived from the porcine small intestinal submucosa and urinary bladder (UBM) have been shown to promote site-specific constructive tissue remodeling in preclinical [1–3] and clinical applications [4–6], including musculoskeletal reconstruction, lower urinary tract repair, vascular replacement, and dermatologic wound repair. A recent study showed that an ECM scaffold in the presence of autologous muscle cells induced constructive remodeling of esophageal tissue after creation of a full circumferential defect in a canine model [1]. This ECM scaffold approach could also potentially benefit patients undergoing reconstructive surgery of the esophageal tract by reinforcing surgical anastomoses, a more conservative application that may have immediate clinical translation.

The most commonly accepted approach for restoring food transit after radical esophagectomy is a "gastric pull-up", which involves mobilizing the stomach through the mediastinum after shaping it into a tube and anastomosing it to the remaining cervical esophagus [7]. When the stomach is not available for anatomic or pathologic reasons, a colonic or small bowel interposition can be used [8]. Although these procedures are successful for some patients, they are associated with a high degree of morbidity, including anastomotic leakage, scarring, and stricture [9, 10].

Mobilization of the abdominal organs into the mediastinal cavity compromises the blood supply to these tissues, which decreases the healing capacity of the tissue and contributes to complications at the anastomotic site [11]. Anastomotic leakage is one of the most common complications and is considered an independent risk factor in the prognostic outcome [12]. Attempts to modify the surgical technique to reduce the incidence of leakage have had limited success [13, 14]. Another common complication is postoperative scarring and stricture of the anastomosis, which can require endoscopic dilation in up to 50% of patients [7].

The purpose of the present study was to evaluate the use of an ECM scaffold in the remodeling of the anastomotic site in two locations in a canine model: the cervical esophagus and the gastroesophageal junction. We hypothesized that the reinforcement of an end-to-end anastomosis with a UBM-ECM scaffold would minimize scar tissue with stricture and promote a constructive, site appropriate remodeling response.

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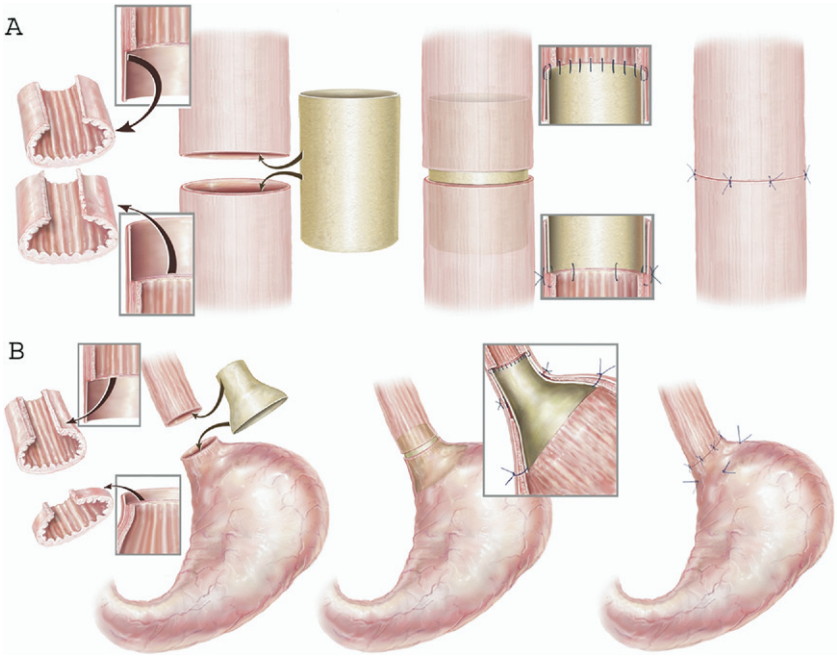


*Fig 1. Schematic of the surgical procedures. Dogs were divided in two groups. (A) Group 1 underwent complete esophageal section at the mid-cervical region, and the endomucosal layer was resected on both proximal and distal ends (left). A urinary bladder matrix (UBM) tubular scaffold was telescoped inside the esophagus (center) and an end-to-end anastomosis was completed on top of the device (right). (B) Group 2 underwent complete esophageal resection at the gastroesophageal junction, and the endomucosa was resected on the proximal esophageal end (left). A UBM funnel-shaped scaffold was telescoped inside (center) and an end-to-end anastomosis was completed on top of the device (right).*

## Material and Methods

### *Overview of Experimental Design*

Twelve healthy adult female mongrel dogs, weighing between 17 and 24 kg, were divided into two equal groups of 6 dogs. In group 1, the cervical esophagus of each dog was completely transected and anastomosed in an end-to-end fashion. In group 2, the gastroesophageal junction was completely transected and anastomosed in an end-to-end fashion. The anastomotic sites in both groups were reinforced with a UBM-ECM scaffold that was placed on the mucosal surface of the anastomosis. Eight additional dogs were used as controls in which no UBM-ECM scaffold was placed. All animal procedures were performed in compliance with the *Guide for The Care and Use of Laboratory Animals* (National Institutes of Health, 1996).

All dogs survived until elective euthanasia was performed at prescribed time points (range, 30 to 180 days) or until stricture formed that prevented swallowing. The measured end points included esophageal function and morphologic characteristics of the anastomotic site.

### *Group 1: Cervical Esophageal Anastomosis*

The 6 dogs in this group underwent a complete transection of the esophagus in the mid-cervical region, with surgical resection of 1.5 cm of the full circumferential endomucosa from both ends of the transection site. The endomucosa was defined as the epithelium, basal lamina (basement membrane), and lamina propria, plus the tunica submucosa, leaving only the muscularis externa. A tubularized form of UBM-ECM scaffold was sutured to the ends of the remaining endomucosa, and the ends of the abluminal muscularis externa were anastomosed (Fig 1A). Two control dogs underwent the same surgical procedure without the UBM-ECM scaffold placement (Table 1).

### *Group 2: Gastroesophageal Junction Anastomosis*

The 6 dogs in this group underwent a complete transection of the gastroesophageal junction to assess the remodeling of the UBM-ECM in a relatively harsh environment (ie, the environment to which the anastomosis is subjected after a gastric pull-up procedure). The endomucosa for a 1.5-cm length on the esophageal side of the transection was resected and a funnel-shaped UBM-ECM scaffold was sutured proximally to the free end of the endomucosa and then sutured distally through the stomach wall (Fig 1B). Three control dogs underwent a complete transection of the gastroesophageal junction that was repaired without endomucosal resection or UBM-ECM scaffold placement. Another 3 control dogs underwent transection of the gastroesophageal junction, followed by endomucosal resection of the esophageal segment without UBM-ECM scaffold placement (Table 1).

### *UBM-ECM Device Preparation*

Porcine urinary bladders were harvested from market weight pigs (approximately 110 to 130 kg) immediately

*Table 1. Overview of Experimental Design*

| Experimental Group | Site of End-to-End Anastomosis | Endomucosal Resection | ECM Scaffold |
|---|---|---|---|
| CE (group 1, n = 6) | CE | Yes | Tubular shape |
| Control for group 1 (n = 2) | CE | Yes | No scaffold |
| GEJ anastomosis (group 2, n = 6) | GEJ | Yes | Funnel shape |
| Control for group 2 (n = 3) | GEJ | Yes | No scaffold |
| Control for group 2 (n = 3) | GEJ | No | No scaffold |

CE = cervical anastomosis; ECM = extracellular matrix; GEJ = gastroesophageal junction.

after sacrifice. Residual external connective tissues, including adipose tissue, were trimmed and all residual urine was removed by repeated washes with tap water. The urothelial layer was removed by soaking the material in 1 N saline. The tunica serosa, tunica muscularis externa, tunica submucosa, and most of the muscularis mucosa were mechanically delaminated from the bladder tissue. The remaining basement membrane of the tunica epithelialis mucosa and the subjacent tunica propria, collectively termed UBM, were then decellularized and disinfected by immersion in 0.1% (v/v) peracetic acid (σ), 4% (v/v) ethanol, and 96% (v/v) deionized water for 2 hours. The UBM-ECM material was then washed twice for 15 minutes with phosphate-buffered saline (pH = 7.4) and twice for 15 minutes with deionized water.

The scaffolds were fabricated with two different shapes to specifically match the anatomy of either the cervical esophagus or the gastroesophageal junction. For group 1, tubular shaped scaffolds were fabricated to match the anatomy of the cervical esophagus. Briefly, multilayer tubes were created by wrapping hydrated sheets of UBM around a 22-mm perforated tube/mandrel that was covered with umbilical tape for four complete revolutions (ie, a four-layer tube) [1].

For group 2, funnel shaped scaffolds were fabricated to match the anatomy of the gastroesophageal junction. A custom mold was constructed in the shape of a funnel, with a perforated straight tube (25-mm diameter) connected to a bowl (70-mm radius of curvature). Before the ECM was placed on the mold, the tube was wrapped with umbilical tape and the bowl was wrapped with cheesecloth to facilitate removal of the UBM after vacuum pressing. Hydrated sheets of UBM-ECM were wrapped around the mold until 4 to 6 layers were present. Both the esophageal and the gastroesophageal constructs were then placed into plastic pouches and attached to a vacuum pump (Model D4B, Leybold, Export, PA) with a condensate trap inline. The constructs were subjected to a vacuum of 710 to 735 mm Hg for 10 to 12 hours to remove the water and form a tightly coupled multilaminate construct. After removal of each device from the mandrel, it was terminally sterilized with ethylene oxide.

### *Surgical Procedures*

Each animal was anesthetized by intravenous administration of sodium thiopental and a surgical plane of anesthesia was maintained by intubation and inhalation of isoflurane in oxygen. The surgical area was shaved and prepared with standard draping for aseptic surgery.

#### *Group 1*

A midline cervical incision was made and tissue layers were dissected to isolate the esophagus. A complete transection of the esophagus was made, and 1.5 cm of the endomucosa was resected from cut ends of both the proximal and distal segments. A tubular UBM-ECM scaffold was telescoped within the remaining muscularis mucosa and sutured to the proximal mucosal end with two short running sutures of either Prolene 5-0 (Ethicon, Somerville, NJ) or PDS 5-0 (Ethicon), and to the distal end with four separate stitches equally spaced about the circumference. Finally an end-to-end anastomosis of the muscularis mucosa was performed with separate stitches of PDS 3-0. The cervical wound was closed by layers with Vicryl 3-0 (Ethicon). The same procedure was repeated for the control dogs without implanting the scaffold.

#### *Group 2*

A midabdominal incision was made, the triangular ligament of the liver was sectioned, and the diaphragm hiatus was dissected to expose the gastroesophageal junction. A complete transverse section of the gastroesophageal junction was made, and 1.5 cm of the esophageal endomucosa and a 5-mm ring of gastric mucosa were resected. The funnel shaped UBM-ECM device was placed within the luminal position at the point of the anastomosis between the structures. The tubular segment of the device was anastomosed to the esophageal mucosa with two short running sutures of PDS 5-0, and the flared portion was fixed to the gastric wall with four separate stitches of PDS 4-0 (Fig 2). The gastroesophageal end-to-end anastomosis was completed by restoring the muscularis externa with separate 3-0 PDS sutures.

The abdominal cavity was closed by layers with Vicryl 2-0, Prolene 1-0, and PDS 3.0 respectively. A chest tube was placed on the left side of the thorax because the pleural cavity was opened during hiatus dissection.

The same procedure was repeated for control dogs, with the exception that no device was implanted, and in three animals, the esophageal mucosa was not removed as described in Table 1.

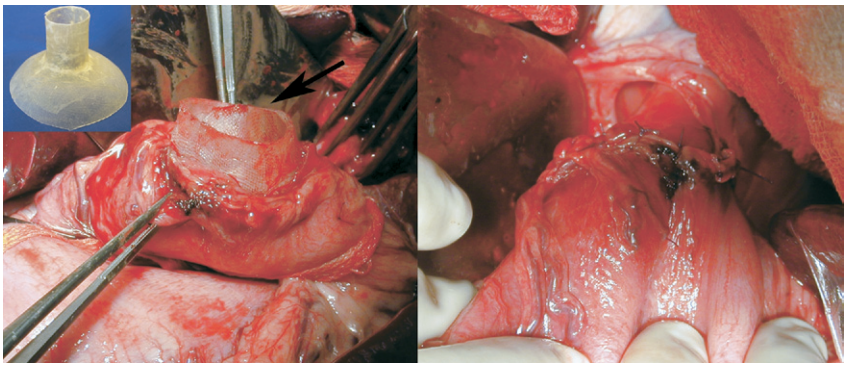

*Fig 2. Macroscopic photograph of the urinary bladder matrix funnel-shaped scaffold (inset) designed to match the anatomy of the gastroesophageal anastomosis. The flare part of the device was mounted first on the gastric end (left), the tubular part (arrow) was telescoped later inside the esophagus, and finally, the end-to-end anastomosis between the esophagus and the stomach was completed on top (right).*

### *Postsurgical Care*

The dogs were recovered from anesthesia, extubated, and monitored in the recovery room until they were resting comfortably in a sternal position. The dogs were kept in a cage overnight and returned to their larger run housing on postoperative day 1. They were given oral prophylactic antibiotics consisting of cephalothin/cephalexin (35 mg/kg) twice daily for 7 to 9 days. The dogs also received intravenous acepromazine (0.1 mg/kg) and butorphanol (0.05 mg/kg) for 2 days, followed by subcutaneous or intramuscular buprenorphine (0.01 to 0.02 mg/kg) every 12 hours thereafter for analgesia as needed. The group 2 dogs (including controls) were given 20 mg of omeprazole daily.

Dogs were fed from an elevated/raised platform. The dog's daily nutrition was calculated and divided into two to three daily feedings. Gruel/soft food was provided for 1 week postoperatively. The animals were reintroduced to solid food over a 2-week period, with oral intake starting 36 hour after surgery. The dogs were weighed weekly and housed in a run measuring approximately 10 × 14 ft to allow freedom to ambulate. Endoscopic examinations were conducted at approximately monthly intervals to evaluate esophageal structure.

### *Morphologic Examination*

Immediately after euthanasia, the scaffold placement site, including native esophageal and gastric tissue both proximal and distal to the graft site, was harvested. The excised sample was opened by making a longitudinal incision in the distal-to-proximal direction. The exposed mucosal surface was examined, and the tissue was then immersed in 10% neutral buffered formalin.

The luminal circumference of the esophagus was measured 4 to 5 cm proximal to the anastomotic level and at the anastomotic site to determine the degree of stricture. Results were expressed as percent reduction of the circumference between the anastomotic site and the proximal normal tissue. Tissue was trimmed longitudinally across the anastomosis, sectioned, and stained with both hematoxylin and eosin and Masson's trichrome stains. The areas examined included the native tissue, the proximal and distal anastomoses of the scaffold, and the mid-scaffold region.

## Results

### *Clinical Outcomes*

All dogs in both groups (including controls) recovered well from the surgical procedure and had a favorable clinical outcome immediately after surgery. After oral intake was reestablished, the dogs in group 1 had no signs of leak or infection in the cervical wound, except one of the control dogs had an untreatable leak at the anastomosis level that required euthanasia. In group 2, there were no signs of abdominal distress, except in one of the control dogs had an episode of fever and lethargy 48 hours after the surgery that was successfully treated with antibiotics and conservative care. One dog in group 1 had an untreatable stricture of the proximal anastomosis that required euthanasia at day 39. In group 2, one of the control dogs had an untreatable stricture at day 14 that required euthanasia. All the other dogs had an uneventful recovery, showing normal appetite, normal hydration, normal weight, and normal activity.

### *Endoscopic Examination Results*

GROUP 1. The endoscopic examination of the dogs in this group showed the formation of a normal appearing mucosal surface in the area where the device was implanted. No signs of stricture were found at the level of the anastomosis through endoscopic examination. The surface transition from the distal segment of the device to the normal native esophagus showed no difference in morphology, and the anastomotic lines were difficult to detect and required identification of the sutures. The transition from the proximal native esophagus to the proximal end of the device had a mild scarring that did not prevent passing the 6F endoscope and did not affect the clinical outcome in 5 of 6 dogs. There was no evidence for active inflammation, erosion, scarring, or necrosis in the rest of the remodeled UBM-ECM scaffold.

The endoscopic examination of the control dogs showed adhesions at the level of the mucosal resection

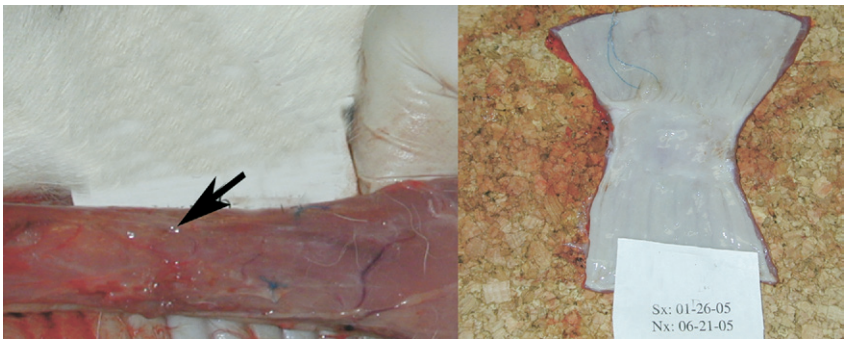


*Fig 3. Macroscopic photograph of cervical anastomosis reinforced with urinary bladder matrix scaffold. In the external view (left), the site of the native anastomosis was difficult to distinguish by eye (arrow). The union of the scaffold to native tissue can be determined from the presence of the Prolene sutures. In the inner surface (right), complete epithelialization was observed with no signs of stricture. There was a lack of folds in the remodeled area compared with native tissue.*

and lack of epithelialization, with a severe inflammatory reaction at the anastomosis level in the dog that had the leak at day 7.

GROUP 2. The endoscopic examination of the dogs in this group showed a complete epithelialization of the anastomotic site with a normal appearing transition from esophageal mucosa to the gastric folds at approximately 1 month. Mild redness was evidenced in some spots, likely due to increased reflux of gastric contents after surgical manipulation of the gastroesophageal junction. The anastomotic site was not detectable, and there were no signs of stricture at that level. The proximal anastomosis of the UBM-ECM scaffold also had an increased inflammatory reaction and mild scarring that did not affect the clinical outcome in any of the dogs. This scarring was more pronounced in 1 of the 6 dogs and was dilated endoscopically to prevent further complications.

The control dogs that did not undergo endomucosal resection had an increased inflammatory response in the area of the anastomosis, and clear scar tissue formation was visible between the esophageal and the gastric segments. The control dogs that underwent endomucosa resection had an irregular mucosal covering with scattered foci of glistening, shiny surface mixed with a reddened, eroded surface at the level of the anastomosis. Several mild adhesions were present at the anastomotic site that were easily disrupted with the scope and were clinically asymptomatic, except for one dog that had an untreatable stricture at day 14 and the scope could not pass through the stenosis.

### *Macroscopic Appearance*

GROUP 1. The anastomotic area showed complete remodeling of the endomucosal and muscular layers with minimal scarring. In several dogs, the site where the complete section had been made was difficult to find and was detected only by identifying the sutures (Fig 3). Scarring was evident on the abluminal surface between the two edges of the muscular layer. The remodeled device could be identified by the lack of folds that characterize a mature esophageal lining. A completely epithelialized luminal layer was evident, however, and the transition with the native tissue was very smooth. There were no macroscopic differences between dogs in group 1 and the control dogs that had an uneventful recovery. In the control dog that had a leak, a hole of 3/8 of the circumference was evident at the anastomosis level. In this group, there were no noticeable differences in circumference reduction, although it was slightly more severe in the control dogs (25.6% ± 10.9% for UBM-ECM treated versus 30.1% ± 0.2% for controls; Fig 4).

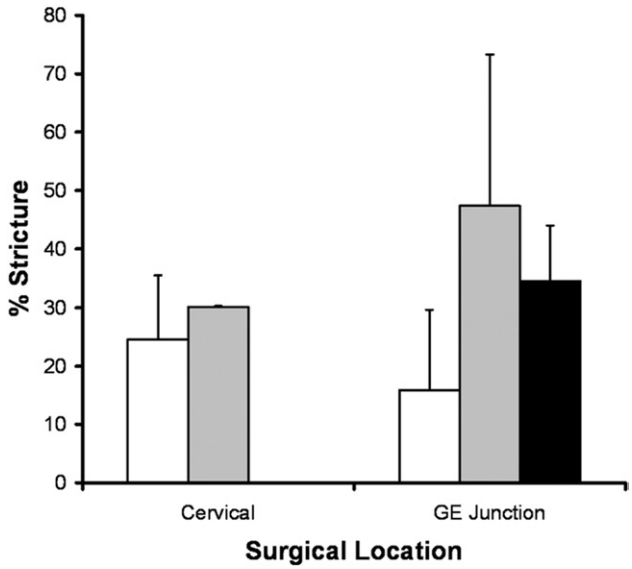


*Fig 4. Percentage reduction in the circumference of the esophagus at the anastomotic levels compared with the normal esophagus (4 to 5 cm proximal to the implant). No differences were observed between the urinary bladder matrix (UBM) treated group and the control in the cervical esophagus. At the level of the gastroesophageal junction, the control group without endomucosal resection had an increased reduction of the circumference that approached significance ($p = 0.057$) compared with the treated group. The control group with endomucosal resection (experimental control) showed a greater reduction of the circumference than both the clinical control and the treated group, although there was considerable variability. Data represent means ± SD. □ = UBM treated; ▩ = experimental control; ■ = clinical control.*

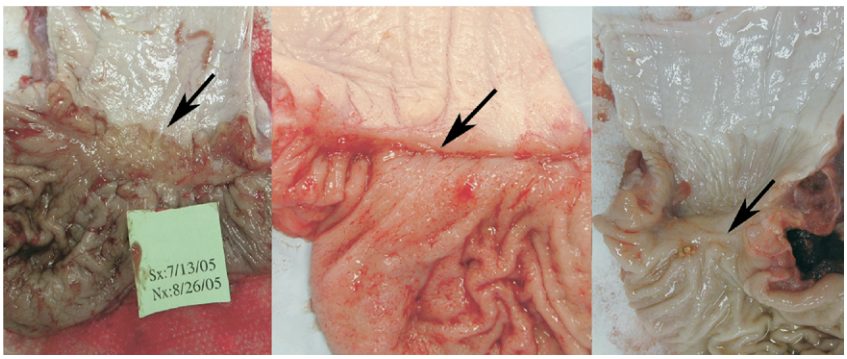


*Fig 5. Macroscopic photograph of gastroesophageal anastomosis. The group treated with urinary bladder matrix (left) had a normal appearance at the transition between esophageal and gastric mucosa (arrow), without gaps, and was completely epithelialized in the area of the endomucosal resection. The control group in which there was no endomucosal resection (center) showed a clear gap at the anastomosis level (arrow). The control group with endomucosal resection but no scaffold (right) had no gaps at the anastomotic level, but the area where the endomucosa was resected was only covered by a thin epithelial layer and there was a stricture ring at the anastomotic level (arrow).*

GROUP 2. As in group 1, the anastomotic area was completely remodeled with a continuous mucosal layer and almost no signs of scarring (Fig 5). In contrast, the control dogs in which the endomucosa was left intact and no device was implanted had a clear endomucosal gap at the anastomotic level. The control dogs in which the endomucosa was resected had a stricture at the anastomotic level, and the resected area was covered by a thin epithelial layer that was clearly distinguished from the adjacent normal mucosa (Fig 5). The control dogs had a noticeable reduction of the circumference at the anastomosis level, with a reduction of 47.4% ± 25.9% with endomucosal resection and 34.5% ± 9.5% without endomucosal resection. The reduction in the circumference tended to be less for the UBM-treated group (15.9% ± 13.7%), although significance was not detected. The comparison of the UBM-treated group with the control group with endomucosal resection yielded a $p = 0.159$ due to the substantial variability observed in the control group. The UBM-treated group approached statistical significance compared with the control group without endomucosal resection ($p = 0.057$; Fig 4).

### *Microscopic Examination*

GROUP 1. Histologic examination showed a continuous intact mucosal layer from the proximal to the distal native esophagus across the anastomotic site where the scaffold was placed. The mucosa was characterized by a stratified squamous epithelium upon an intact basement membrane. Rete pegs of the epithelium were not as pronounced as those in the adjacent native esophageal mucosa (Fig 6). The muscularis externa at the anastomosis site showed bands of organized collagen with numerous islands of skeletal muscle cells that appeared to be independent from the muscle fascicles of the adjacent native esophagus. The tissues from the control dog that had an uneventful recovery also had a continuous intact mucosal layer but also had a subjacent accumulation of polymorphonuclear and mononuclear inflammatory cells, characteristic of chronic active inflammation. The

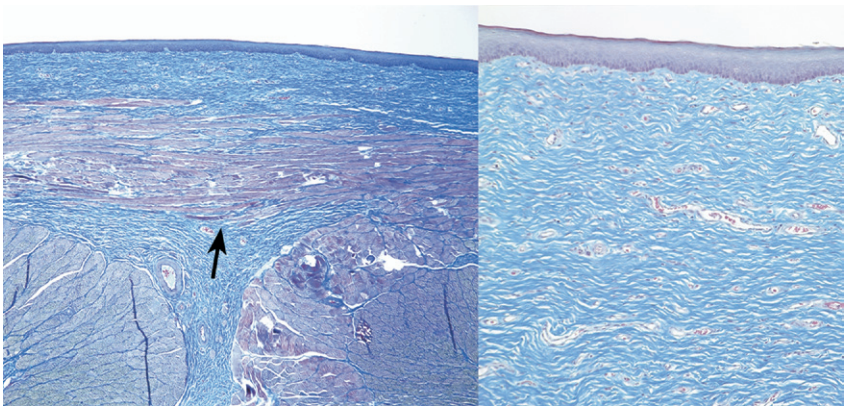

*Fig 6. Histologic appearance of cervical anastomosis. The urinary bladder matrix-extracellular matrix device was completely incorporated and a submucosal layer with organized collagen, with no inflammatory reaction or fibrosis, was observed. The remodeled scaffold was completely epithelialized, and a new muscle tissue layer was regenerated between the muscularis externa and the submucosal layer (arrow) (trichrome stain; original magnification ×4 [left]) and ×10 [right]).*

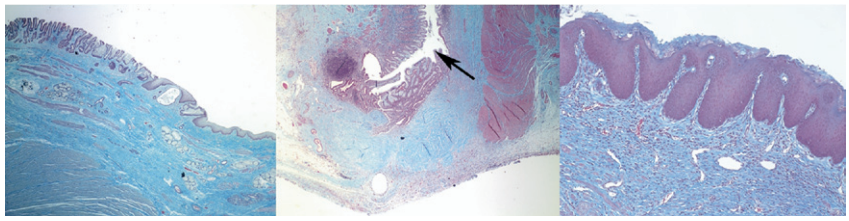

*Fig 7. Histologic appearance of gastroesophageal anastomosis. The treated group (left) showed a normal epithelial transition with an organized submucosal layer and continuity with the underlying muscular layer. The control group with no endomucosal resection and with no extracellular matrix scaffold (center) had a distinct invagination of the mucosa at the level of suture placement (arrow) and an accumulation of scar tissue. The control group with endomucosal resection and with no scaffold implantation (right) showed no invagination of the mucosa, but rather a collection of dense collagenous connective tissue with an abundance of inflammatory cells subjacent to the neoepithelium that indicated a hyperplastic response (trichrome stain; original magnification ×4 [left, center], and ×10 [right].*

dog that had a leak at day 7 showed a clear lack of epithelialization with a pronounced inflammatory response.

GROUP 2. Histopathologic examination of dogs that received the UBM-ECM device showed a normal transition from esophageal to gastric epithelium, with no signs of active or chronic inflammation in the subjacent tissue (Fig 7). The repair site between the transected edges of the muscle tissue was very narrow and filled with partially organized collagen. The submucosal tissue consisted of organized connective tissue with an abundant capillary supply. There was an absence of inflammatory cells in any of the remodeled scaffold materials that were examined beyond 4 weeks after surgery. The tissues from the control dogs in which the endomucosa was resected but no UBM-ECM devices were placed showed signs of chronic and active inflammation beneath a transitional neoepithelium that had a hyperplastic response with accumulated debris on the luminal surface (Fig 7). In the dog that had the untreatable stricture, the epithelialization was incomplete, and a variable amount of dense, disorganized, fibrous connective tissue (scarring) was present.

The tissues from the control dogs with no endomucosal resection showed a clear tissue retraction, with scarring at the level of the anastomosis and a considerable amount of fibrous connective tissue that formed a large anastomotic gap (Fig 7). A typical foreign body reaction was always evident and limited to the tissues immediately surrounding the sutures in both groups.

No evidence of the ECM material could be identified in any of the dogs in both groups, consistent with complete degradation of the scaffold [15].

## Comment

Surgical procedures involving the esophagus are associated with increased morbidity and mortality rates [7, 9, 10, 16]. The present study showed that the xenogeneic ECM bioscaffold derived from porcine urinary bladder altered the normal healing response to site-specific constructive remodeling of esophageal tissue when used as a reinforcement of native esophageal anastomosis. The constructive remodeling response observed in this study replaced the usual scarring process observed in surgical anastomoses of the digestive tract in a clinical setting.

It was interesting to note that the control dogs in which the endomucosa was resected had a better healing response than the controls where the endomucosa was left intact, despite an increased inflammatory infiltrate. The dogs that had the UBM scaffold as reinforcement showed a near normal macroscopic and microscopic appearance, with absence of inflammation or detrimental scar tissue. This difference between the control dogs and the UBM-ECM treated dogs was more noticeable at the level of the gastroesophageal junction, suggesting that ECM scaffolds promote constructive remodeling despite the harsh environment caused by gastric reflux [17, 18].

We have previously shown that when an ECM scaffold is placed in direct contact with autologous muscle tissue, even relatively small amounts (ie, 30% circumference), the reconstitution of the esophagus is ad integrum [1]. Furthermore, that study also showed that the mechanical behavior of the UBM scaffold changed during the remodeling process from a very stiff, relatively noncompliant material to a tissue that had characteristics similar to native esophageal tissue.

Similar remodeling responses using ECM bioscaffolds have been seen in other body locations [2, 3]. Numerous reports of lower urinary tract reconstruction including the urinary bladder, urethra, and ureter show similar replacement of the ECM scaffold by an epithelial cell population, submucosa, and smooth muscle cell layer. In these locations, the presence of a muscle cell population in contact with an ECM scaffold enhanced but was not essential for remodeling without scar tissue formation [19].

Replacement of muscular body wall structures with sheets of skeletal muscle mixed with collagenous connective tissue and adipose tissue have been reported with an ECM scaffold derived from porcine small intestinal submucosa [3, 6]. ECM scaffolds have now been used in more than 500,000 patients worldwide for numerous clinical applications.

Although the mechanisms of esophageal scarring and stricture formation are largely unknown, they are likely related to the presence of a harsh environment (ie, distal esophagus with gastric reflux), proteolytic enzymes, and molecular mediators of inflammation that lead to fibro-

blast proliferation and contractility [20–23]. These mechanisms are likely aggravated when a leak occurs due to an even more aggressive inflammatory response and infection.

There are a number of reasons that UBM-ECM scaffold may enhance the normal esophageal wound-healing response. Naturally occurring ECM scaffolds have shown resistance to infection after deliberate contamination with bacteria or gastrointestinal contents [24–26]. This antimicrobial property exhibited in vivo is due to the presence of small peptides (5 to 16 kDa) that are formed during the degradation of an ECM scaffold. These and similar peptides have shown resistance to gram-positive and gram-negative bacteria in in vitro [27], and have also exhibited chemoattractant properties [28]. It is thought that the chemoattractant properties of the degradation products promotes the recruitment of bone marrow derived cells to the site of ECM remodeling that, in turn, respond to local mechanical and biochemical signals that direct these cells down a tissue-specific differentiation lineage [29, 30]. UBM-ECM has also been shown to retain an intact basement membrane that supports the rapid confluent growth of epithelial cells [31]. Such cells are considered to be important for the successful development of a luminal structure [32].

Attempts to improve the outcome of esophagogastric anastomoses have included modification of the surgical technique from hand sewing to mechanical staples. However, the nonanatomic position of the gastric tube in the gastric pull-up, the lack of adequate blood supply, and the acidic environment of the stomach drastically hamper the healing potential, often leading to catastrophic complications [9]. The use of UBM-ECM scaffolds to alter the normal healing response might decrease the overall morbidity and mortality associated with esophageal procedures.

The ability to withstand the harsh esophageal environment can also be useful to attempt a less-invasive repair of esophageal fistulae or perforations where total resection is usually required. A recent report using ECM scaffolds as a reinforcement of mechanical sutures in jejunal anastomoses in pigs showed increased leak pressure, suggesting the potential of this approach to prevent undesired outcomes [33]. Furthermore, current minimally invasive therapies for Barrett disease, such as endomucosal resection or photodynamic therapy, could be improved by the use of UBM-ECM scaffolds to prevent the scar healing and stricture associated with those procedures [34].

One limitation of the present study is that the gastric pull-up was not mimicked and the healing response was not assessed under the tension at the anastomotic site, nor was the compromised blood supply that exists in the clinical setting. However, ECM scaffolds are commonly used in load-bearing applications with site-appropriate remodeling [2, 3]. Furthermore, previous studies suggest that ECM scaffolds induce an aggressive angiogenesis in the remodeling tissue [28, 35].

Another limitation is that the low number of control dogs prevented a powerful statistical analysis. This decision was from the results of our previous study, in which all control animals (complete circumferential resection without repair with UBM-ECM scaffold) experienced a severe, untreatable stricture with convincing results [1]. Therefore, repeating a large number of controls for this study was not in accordance of the Animal Welfare Regulations as suggested by our local Internal Animal Care Use Committee.

In summary, the findings of the present study suggest that it is possible to reinforce the surgical anastomoses of native esophagogastric tissue and decrease postoperative morbidity using a regenerative medicine approach with a biodegradable ECM scaffold. The ECM scaffold promotes remodeling to site-specific esophageal tissue rather than the scar tissue that is usually associated with wound healing in mammals. The experimental configuration was specifically intended to address surgical anastomoses that are created during the gastric pull-up procedure, but a similar reinforcement approach may also be applicable to partial resection of the esophagus (ie, mucosectomy in Barrett disease), which also tends to result in scar tissue formation and esophageal stenosis [36, 37]. This conservative approach could rapidly be translated to the clinical setting and serve as the basis for future esophageal replacement or reconstruction using a regenerative medicine approach.

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