An extracellular matrix scaffold for esophageal stricture prevention after circumferential EMR

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Background: EMR is an accepted treatment for early esophageal cancer and high-grade dysplasia. One of the limitations of this technique is that extensive mucosal resection and endoscopic submucosal dissection may be required to obtain complete removal of the neoplasm, which may result in significant stricture formation.

Objective: The objective of the current study was to evaluate the efficacy of an endoscopically deployed extracellular matrix (ECM) scaffold material for prevention of esophageal stenosis after circumferential EMR.

Design: Ten mongrel dogs were subjected to surgical plane anesthesia and circumferential esophageal EMR by the cap technique. In 5 animals, an ECM scaffold material was endoscopically placed at the resection site; the remaining 5 animals were subjected to circumferential esophageal EMR without ECM placement. Follow-up endoscopy was performed at 4 and 8 weeks; necropsy with histologic assessment was performed at 8 weeks.

Setting: Animal laboratory.

Interventions: Circumferential esophageal EMR by the cap technique, followed by endoscopic placement of an ECM scaffold material.

Main Outcome Measurements: Degree of esophageal stricture and histologic assessment of remodeled esophageal tissue.

Results: All 5 control dogs had endoscopic evidence of esophageal stenosis. Three required early euthanasia because of inability to tolerate oral intake. Incomplete epithelialization and inflammation persisted at the EMR site in control animals. Endoscopic placement of an ECM scaffold material prevented clinically significant esophageal stenosis in all animals. Histologic assessment showed near-normal esophageal tissue with a lack of inflammation or scar tissue at 8 weeks.

Conclusions: Endoscopic placement of an ECM scaffold material prevented esophageal stricture formation after circumferential EMR in this canine model during short-term observation. (Gastrointest Endosc 2009;69:289-96.)

Endoscopic treatment of Barrett's esophagus with high-grade dysplasia and early esophageal cancer has gained acceptance in the last decade as an effective therapeutic option. EMR is being performed commonly in this setting for both diagnostic and therapeutic purposes. In

experienced hands, hemicircumferential mucosal resection can be performed, and after a period of healing, additional resections can be performed to achieve complete removal of affected and at-risk tissue.[1,2] Full circumferential EMR in a single setting has led to significant stricture formation, which limits the wide acceptance of this practice.[3]

Tissue engineering and regenerative medicine approaches, such as the use of biologic scaffolds[4] or cell-based therapies,[5,6] are targeted toward the reconstruction of structurally and functionally normal tissues. Such approaches have the potential to circumvent the complications of scarring and stricture that are limitations for selected surgical procedures in the esophagus, such as EMR. Biologic scaffold materials composed of an extracellular matrix (ECM) derived from porcine small intestine and urinary bladder

have been shown to promote site-specific, constructive tissue remodeling in preclinical and clinical applications for a variety of body systems, including the esophagus.[4,7]

The purpose of the current study was to evaluate the feasibility and efficacy of endoscopic deployment of an ECM scaffold for prevention of esophageal stenosis after circumferential EMR.

MATERIAL AND METHODS

EMR and scaffold insertion

Ten healthy adult mongrel female dogs (20.6 kg ± 0.7 kg for control group and 20.3 kg ± 2.0 kg for ECM treatment group) were subjected to circumferential esophageal EMR from 25 cm to 30 cm as measured from the dental arch. With the dog under general anesthesia, cap-assisted EMR was performed with a therapeutic endoscope (EG-3430, Pentax Medical, Montvale, NJ) and a commercially available kit (EMR Kit, Olympus America, Center Valley, Pa). Piecemeal mucosal resections were sequentially performed until a 5-cm circumferential resection was completed (Fig. 1). A tubular device of ECM derived from the porcine urinary bladder (UBM) was then endoscopically placed in 5 dogs (treatment group). The 5 remaining dogs were allowed to heal without treatment (control group).

The UBM-ECM biologic scaffold material was prepared as previously described and configured into a tubular shape.[4,7] In brief, porcine urinary bladders were harvested from market-weight pigs (approximately 110-130 kg) immediately after death. 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 1.0 nanomol/L saline solution. 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% (vol/vol) peracetic acid (σ), 4% (vol/vol) ethanol, and 96% (vol/vol) deionized water for 2 hours. The UBM-ECM material was then washed twice for 15 minutes with phosphate-buffered saline solution (pH = 7.4) and twice for 15 minutes with deionized water.

Tubular scaffolds were fabricated to match the anatomy of the canine 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 a total of 4 complete revolutions (ie, a 4-layer tube).[7] The constructs were then placed into plastic pouches and attached to a vacuum pump (model D4B, Leybold, Export, Pa) with a condensate trap in line. The constructs were subjected to a vacuum of 710 to 740 mm Hg for 10 to 12 hours to remove the water and

Capsule Summary

What is already known on this topic

- One of the limitations of esophageal endoscopic mucosal resection (EMR) is that extensive resection and submucosal dissection may be required to obtain complete removal of a neoplasm, resulting in significant stricture formation.

What this study adds to our knowledge

- Endoscopic placement of an extracellular matrix scaffold material in dogs undergoing circumferential esophageal EMR prevented clinically significant esophageal stenosis in all.
- Histologic assessment showed near-normal esophageal tissue with a lack of inflammation or scar tissue at 8 weeks.

form a tightly coupled multilaminate construct. After each device was removed from the mandrel, they were terminally sterilized with ethylene oxide.

The endoscopic placement of the ECM device is shown schematically in Figure 2. The tubular scaffold was hydrated in a saline solution bath for 5 minutes and then placed over a 30-mm achalasia balloon (Cook Endoscopy Achalasia balloon, Wilson-Cook Medical, Winston-Salem, NC). The UBM-ECM device was constrained with two 4-0 silk sutures with surgeon's knots that would release when the balloon was inflated. A 0.035-inch wire (Jagwire, Boston Scientific, Natick, Mass) was endoscopically placed into the dog's stomach. The balloon was then passed over the wire and positioned under endoscopic guidance with the UBM-ECM bridging the length of the mucosal resection. One milliliter of a degradable, lysine-derived urethane (LDU) surgical adhesive (TissuGlu, Cohera Medical, Pittsburgh, Pa) was then injected through a 6F endoscopic guiding catheter (Oasis stent introduction system, Wilson-Cook Medical) between the esophageal wall and the UBM-ECM in 2 separate strips on opposite sides of the device to prevent slippage. The balloon was then manually inflated to full capacity, expanding the scaffold against the esophageal wall. Balloon inflation was maintained for 15 minutes before deflation and removal, leaving the UBM-ECM scaffold in place within the esophagus (Fig. 3).

Postoperative 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. All dogs were given oral prophylactic antibiotics consisting of cephalothin/cephalexin (35 mg/kg) twice daily for 7 to 9 days. Intravenous acepromazine (0.1 mg/kg) and butorphanol (0.05 mg/kg) were
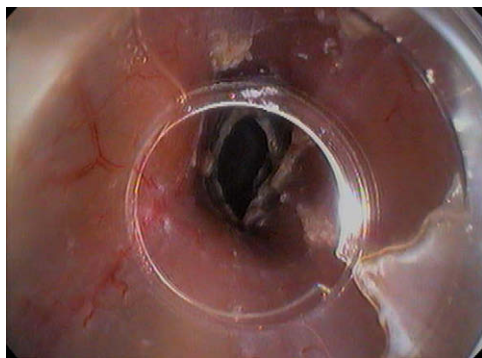
Figure 1. Endoscopic image of the 5-cm circumferential mucosal resection. There are a few small areas of mucosa remaining that are scattered over the length of the defect.

administered for 2 days, followed by subcutaneous or intramuscular buprenorphine (0.01 to 0.02 mg/kg) every 12 hours thereafter as needed for analgesia. All dogs were also given omeprazole 20 mg daily. Oral intake began 36 hours after surgery. Dogs were fed from an elevated/raised platform. Daily nutritional requirements were calculated and divided into 3 separate feedings. Gruel/soft food was provided for 1 week postoperatively followed by a gradual change to solid food over the ensuing 2-week period. The dogs were weighed weekly and housed in a run measuring approximately 10 × 14 ft to allow freedom to ambulate. Endoscopic examinations were conducted 1 month postoperatively and immediately preceding euthanasia at 2 months to evaluate esophageal mucosal appearance and stricture.

All animal procedures were performed in compliance with the 1996 *Guide for the Care and Use of Laboratory Animals* and approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Morphologic/histologic assessment

Immediately after euthanasia, the scaffold placement site and the native esophageal tissue proximal and distal to the scaffold placement site were harvested. The excised segment was split longitudinally and the exposed mucosal surface was examined and photographed for dimensional measurements. The luminal circumference of the esophagus was measured 3 cm proximal to the superior edge of the remodeled site and in the middle of the graft to determine the extent of stenosis. Results for the 2 groups were expressed as percent reduction of the circumference between the remodeled site and the proximal normal tissue (mean ± SD).

The excised tissue was pinned to corkboard in a flattened position and immersed in 10% neutral buffered formalin. The specimen was trimmed longitudinally including both normal and remodeled tissue, sectioned, and stained with both hematoxylin-eosin and Masson's trichrome stains. The areas examined included the native tissue, the proximal and distal interfaces between the remodeled area and the native tissue, and the middle region of the remodeled area.

Statistical analysis

On the basis of examination of the distributions of data for the 2 groups, it was felt that the data appear to be normally distributed. Thus, the statistical approach used to compare the results was the parametric t test (n = 5 per group). The hypothesis tested was that the treatment of the EMR defect with UBM-ECM would cause less reduction in the circumference compared with no treatment. It is recognized that data from individual test animals were subjected to multiple statistical analyses (ie, circumference reduction and weight). The comparison of reduction in luminal circumference in the experimental versus control groups was taken as the primary statistical analysis, which did not involve multiple testing. All other statistical tests are considered to be secondary with their P values stated uncorrected for repeated measures and should be taken as descriptive only.

RESULTS

Three of 5 control dogs (60%) had esophageal stricture with inability to tolerate oral intake, requiring euthanasia between postoperative weeks 2 and 3. The 2 remaining control dogs were electively killed 2 months after surgery. The animals showed a decrease in weight of 0.5 (0.6) kg (2.4% decrease), which was not statistically different from the weight at the time of surgery (P = .15). Immediately preceding euthanasia, endoscopy revealed incomplete epithelialization of the EMR site with associated inflammation. The esophageal stricture prevented passage of an 18F endoscope in the 3 dogs euthanized prematurely. The remaining 2 animals had a noticeable but clinically insignificant stricture (Fig. 4A). The mean reduction in the luminal circumference of the esophagus for the control group was 64.8% (17.5%), with a range of 35.6% to 80.2% (Fig. 5). Histologic examination showed a lack of continuous epithelial layer in the 3 animals that had the poor clinical outcome and in the 1 animal that survived until elective euthanasia. Histologic examination of these specimens showed a chronic, active inflammatory response with an accumulation of polymorphonuclear and mononuclear cells scattered diffusely throughout the area of EMR (Fig. 6). The final animal had nearly complete coverage with an immature epithelial layer with focal areas of epithelial erosion and organized submucosal tissue with an accumulation of mononuclear cells (Table 1).

The dogs in the UBM-ECM treatment group showed a weight gain of 0.8 (2.0) kg (3.5% increase) during the postoperative course until they were electively killed 2 months after surgery. This weight change was not statistically different from that of the control group (P = .43). Endoscopy at 1 month showed a normal-appearing esophageal mucosal surface in the entire area where the device was implanted. No evidence of stricture was found either at the proximal or distal transition between the device and
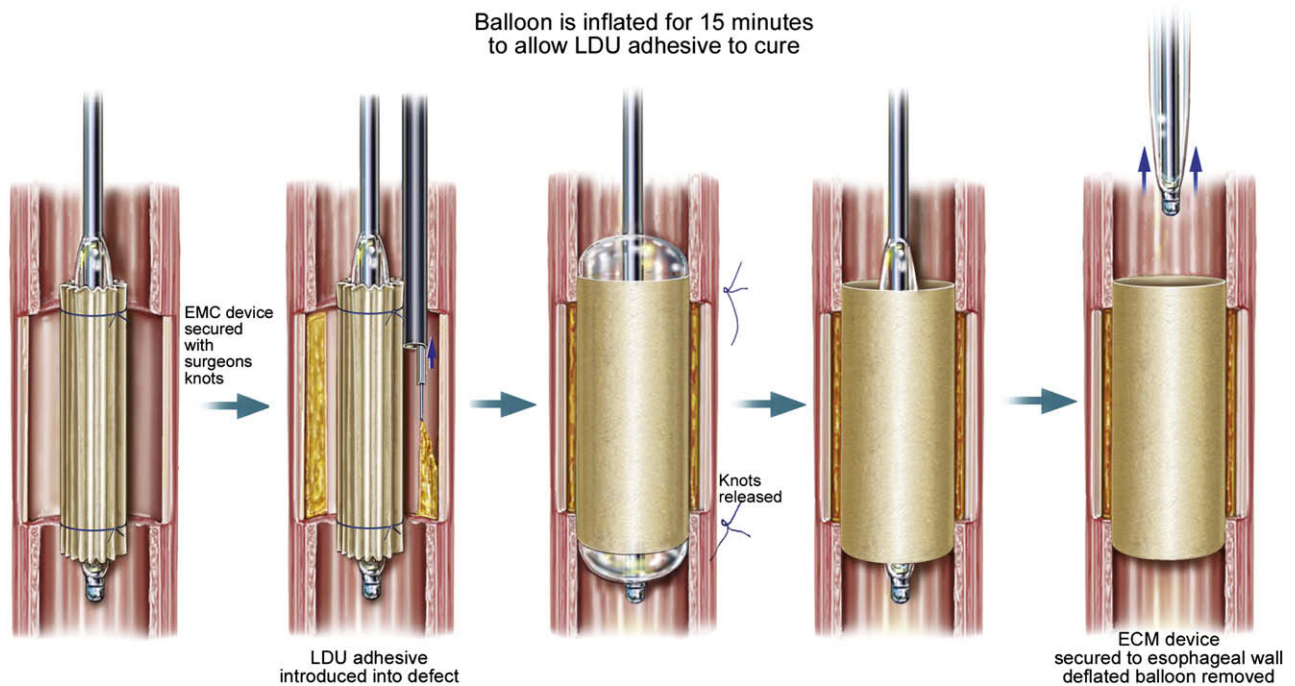
Balloon is inflated for 15 minutes
to allow LDU adhesive to cure

EMC device
secured
with
surgeons
knots

LDU adhesive
introduced into defect

Knots
released

ECM device
secured to esophageal wall
deflated balloon removed

Figure 2. Schematic of the surgical deployment of UBM-ECM device with an achalasia balloon and delivery of the surgical adhesive.

the normal esophagus. There was no gross evidence of active inflammation, erosion, scarring, or necrosis in the remodeled tissue region (Fig. 4B). The transition between native and remodeled esophagus could be identified only by the lack of the folds that characterize a mature esophageal lining. The mean reduction in luminal circumference was less for the ECM-treated group compared with the control group (45.6% [16.9%] vs 64.8% [17.5%], $P = .041$) (Fig. 5).

Histologic assessment of the ECM-treated group showed a continuous intact mucosal layer consisting of a stratified epithelium on an intact basement membrane. Rete pegs were not as evident in the remodeled area as in native esophageal epithelium, a finding that is consistent with previous studies.[4,7] The submucosal layer showed organized connective tissue with numerous blood vessels but no glandular structures. There was an absence of inflammatory cells in the remodeled esophageal segment, and there was no morphologic evidence of either the ECM device remnant or residual surgical adhesive 2 months after surgery (Fig. 6) (Table 1).

DISCUSSION

Treatment of early esophageal neoplasia with EMR is rapidly evolving with excellent efficacy and low rates of bleeding and perforation.[1,2,8-10] There is concern for stricture formation if more than 75% of the esophageal mucosal circumference is resected in a single setting,[11] with small series reporting stricture rates of 70% to 80% with

Figure 3. Endoscopic image of the UBM-ECM device deployed over the EMR.

circumferential EMR.[12,13] The current study in a dog model showed that a UBM-ECM scaffold derived from porcine urinary bladder, deployed endoscopically after circumferential EMR, facilitated esophageal mucosal remodeling without stricture formation. The remodeled tissue consisted of a completely epithelialized lumen with a dense, organized collagenous submucosa and normal-appearing muscularis externa. These results are consistent with those of previous investigations of UBM-ECM for reinforcement of esophageal anastomoses and reconstruction of a full circumferential esophageal resection.[4,7] UBM-ECM in the presence of colocalized autologous muscle tissue was shown to form functional esophageal tissue when used for repair of a 5-cm full circumferential segmental resection in a surgical canine model.[7] The remodeled tissue showed mature epithelialization, a distinct submucosal layer
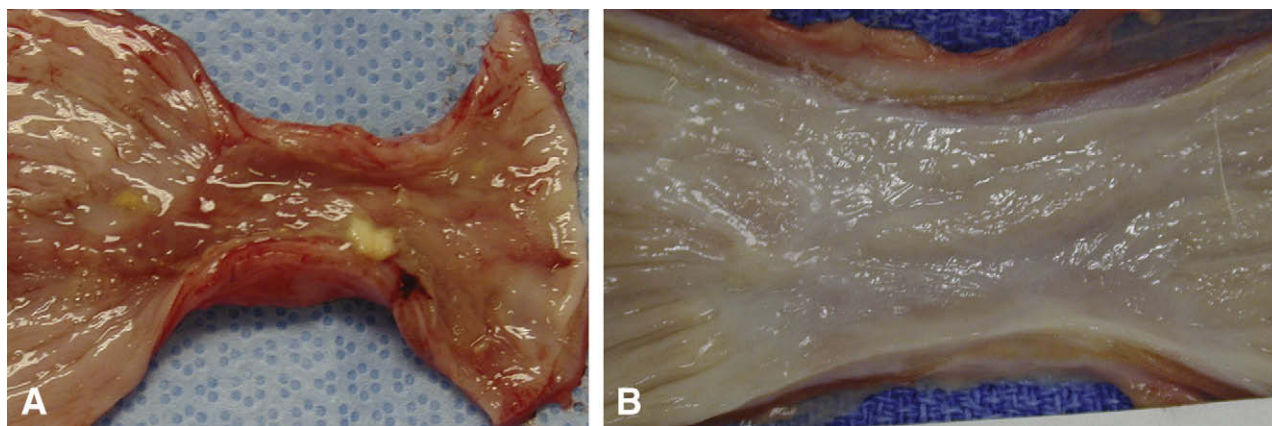
Figure 4. Gross view of the remodeling EMR areas at 2 months after surgery. The control (**A**) shows pronounced stricture with reductions in the circumference and the length of the injury site. In contrast, the EMR site treated with UBM-ECM shows a smooth mucosal surface and limited circumferential and longitudinal reduction (**B**).

characterized by dense, organized collagenous tissue, and an organized, appropriately oriented skeletal muscle layer that was integrated into the adjacent normal muscle tissue.[7] In another study in which UBM-ECM was used for reinforcement of esophageal anastomoses in a canine model, placement of the ECM by an open esophageal transection model reduced stenosis compared with primary repair.[4]

The mechanisms by which ECM scaffolds promote site-specific tissue remodeling are not fully understood, but recent studies have increased our understanding of the remodeling events. ECM scaffolds such as UBM-ECM and small-intestinal-submucosa–ECM are minimally processed to remove cellular material while retaining important biochemical constituents[14] such as glycosaminoglycans and growth factors that maintain their bioactivity.[15-17] Removal of the cellular material alters the profile of lymphocyte and macrophage phenotype in response to the biomaterial from a proinflammatory phenotype to an accommodative remodeling response.[18] Non-cross-linked ECM scaffold materials degrade rapidly after implantation with complete disappearance from the implant site by 60 to 90 days.[19,20] Several in vitro studies have shown that degradation of ECM scaffolds leads to the release of matricryptic peptides that exhibit bacteriostatic, angiogenic, and chemotactic properties.[21-23] In vivo, ECM scaffold materials have shown resistance to intentional bacterial contamination in preclinical studies[24-27] and spontaneous contamination in the clinical setting.[28] There is also evidence that ECM scaffolds recruit a population of bone marrow progenitor cells to the site of remodeling and that those cells persist and become part of the new tissue.[29-31] Stated differently, ECM scaffolds change the default process of wound healing.

Early epithelialization and the minimization of the associated proinflammatory response is thought to be critical to prevent stricture formation in hollow organs.[32] ECM scaffolds have been successfully used to reconstruct a number of epithelial organs in addition to the esophagus,
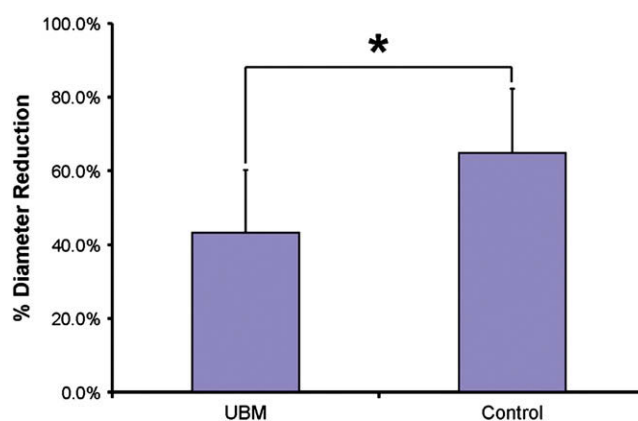

Figure 5. Graph of the reduction in circumference (mean ± SD) for the EMR site with and without treatment with UBM-ECM. The reduction in the circumference was greater for the control group ($P < .05$).

including blood vessels, the urinary bladder, and the heart.[33-37] Selected ECM scaffolds, such as UBM-ECM, can be processed so that a basement membrane surface is preserved,[38,39] which is the ideal substrate for epithelial cell growth and differentiation.[40]

Preliminary attempts to secure the UBM-ECM scaffold at the site of EMR included the use of endoscopic clips and a self-expanding silicone stent. Both approaches resulted in migration of the scaffold into the stomach, similar to the results of a previous study in which an ECM scaffold derived from porcine small intestinal submucosa was wrapped around an esophageal stent.[41] The use of a surgical adhesive to secure the tubular ECM device prevented scaffold migration. The LDU adhesive (TissuGlu, Cohera Medical, Pittsburgh, Ps) cures in the presence of moisture and covalently bonds to tissue.[42] On curing, the adhesive has considerable elasticity so it can deform with the esophagus and the scaffold. The elasticity and deformability are important considerations because there is evidence that the site-appropriate mechanical environment is
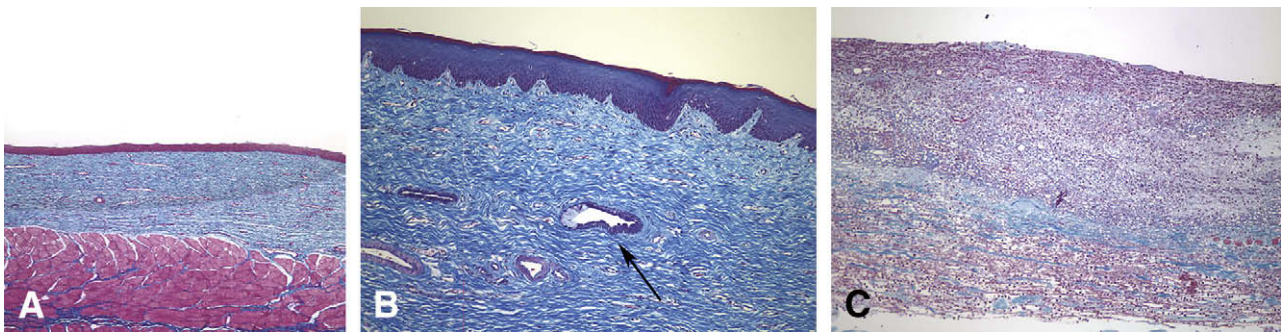
Figure 6. Microscopic photograph showing the histologic findings of ECM remodeled scaffold after 60 days. Note the organized tissue in all layers of the esophagus (**A**, Masson's trichrome, orig. mag. ×40), with a complete squamous epithelium (**B**, Masson's trichrome, orig. mag. ×100) and some glandular structure formation *(arrow)*. Control dogs with poor clinical outcome showed a massive inflammatory infiltrate with scar tissue formation (**C**, Masson's trichrome, orig. mag. ×100).

TABLE 1. Summary of histologic findings for the control and UBM-ECM treated EMR

	Control	UBM-ECM
Epithelialization	Lack of continuous epithelial layer in 4 animals. The remaining dog had nearly continuous, immature epithelial layer with focal areas of erosion.	Continuous intact mucosal layer consisting of a stratified epithelium on an intact basement membrane.
Cellularity	Abundance of chronic, active inflammatory cells, including polymorphonuclear cells and mononuclear cells in the animals that lacked an epithelial layer. The remaining dog showed accumulation of mononuclear cells in the submucosal layer.	Absence of inflammatory cells in the remodeled esophageal segment.
Organization	Disorganized fibrotic collagenous tissue formation in the animals that lacked an epithelial layer. The remaining dog showed organized collagenous tissue in the submucosal tissue.	Organized collagenous, connective tissue with numerous blood vessels but no glandular structures.

critical for constructive remodeling of an ECM scaffold.[7,19,43,44] The LDU adhesive degrades into benign products including lysine (57% by mass) and minor amounts of carbon dioxide (7%), alcohol (2%), and low-molecular-weight polyols (eg, glycerol). It is not clear whether the adhesive provides any specific biologic benefit for the site-appropriate remodeling response observed. Previous studies have shown that there is a minimal host response to the adhesive in vivo,[42] so it is unlikely that the adhesive itself contributes directly to the remodeling response.

Recent studies have shown that most commercially available ECM scaffolds still retain small fragments of donor DNA,[45] but there is no evidence that such DNA remnants can be incorporated into host cells. Peracetic acid, the chemical used for removal of cellular material from UBM-ECM, has been shown to effectively eliminate any viral load.[46] Furthermore, previous studies have shown that, even with direct coculture of human cells with porcine DNA, only 0.22% of the cells contained porcine DNA immediately after exposure and that no DNA was detectable by 4 weeks.[47] This result strongly suggests that any viral DNA present in the ECM could not transmit disease to the host.

Several cell-based technologies have been investigated in attempts to eliminate stricture formation after EMR procedures. In an animal model, autologous cell sheets composed of oral epithelial cells were endoscopically placed to cover a 5-cm hemicircumferential mucosal resection.[6] The transplanted cell sheets were able to adhere to the underlying muscle layers at the resection site and successfully provide an intact, stratified epithelium without evidence of stricture formation. Another study evaluated the injection of keratinocytes, cultured from buccal mucosa, into EMR defects. The keratinocyte injection promoted re-epithelialization without scarring or stricture formation.[5] A combined approach using both ECM scaffolds and cell-sheet technologies could further enhance the regeneration of esophageal tissue after injury or EMR.

For clinical application of the current ECM-based approach, it is conceivable that a 1-step deployment system could be developed where the ECM scaffold is preconstrained over a collapsed 20-mm esophageal balloon dilator and then covered by a sheath. With a double-channel endoscope, this prepackaged deployment system could be advanced through the therapeutic channel and positioned endoscopically. The covering sheath would be

withdrawn, the adhesive injected through the alternate channel, and the balloon inflated under endoscopic visualization. Balloon expansion time could also be potentially reduced with additional use of endoscopic clips applied to the proximal end to provide device anchoring.

In summary, an endoscopically deployed UBM-ECM scaffold induced site-specific remodeling of esophageal tissue without stricture formation at the site of circumferential EMR. Given the relatively simple deployment technique, use of this scaffold could advance the goal of stricture prevention after EMR. The acellular nature of the scaffold and relative simplicity of the approach should facilitate rapid clinical translation and may be of benefit to the patient population with Barrett's esophagus.

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