# Urinary bladder matrix scaffolds strengthen esophageal hiatus repair

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ABSTRACT

*Background:* Laparoscopic repair of the hiatal hernia is associated with a recurrence rate between 12% and 42% depending on the defect size. Although the impact of hiatal reinforcement on long-term recurrence remains controversial, the main limitation of this approach has been the risk of adverse events related with the use of synthetic materials in the vicinity of the esophagus.

*Methods:* A total of 14 female domestic pigs underwent laparoscopic primary hiatal hernia repair of a simulated defect in the esophageal hiatus. Seven of the hiatal repairs were reinforced with an extracellular matrix (ECM) scaffold, whereas the remaining seven served as primary repair controls. Animals were survived for 8 wk. At necropsy, after gross morphologic evaluation, samples were sent for mechanical testing and histology.

*Results:* The repaired defect site reinforced with ECM scaffolds showed a robust closure of the crura in all cases with a smooth peritoneal-like structure covering the entire repair. Average load at failure of the treated group was found to be significantly stronger than that of the controls (185.8 ± 149.7 g *versus* 57.5 ± 57.5 g, P < 0.05). Similarly, the stiffness was significantly higher in the treated animals (57.5 ± 26.9 g/mm *versus* 19.1 ± 17.5 g/mm; P < 0.01). Interestingly, there was no difference in elongation at failure (7.62 ± 2.02 mm *versus* 7.87 ± 3.28 mm; P = 0.44).

*Conclusions:* In our animal survival model, we have provided evidence that the addition of an ECM to augment a primary hiatal repair leads to tissue characteristics that may decrease the possibility of early failure of the repair. This may translate to decreased recurrence rates. Further study is necessary.

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## Introduction

Laparoscopic repair of the hiatal hernia is associated with a recurrence rate between 12% and 42% depending on the defect size.[1-3] As a result, many surgeons have attempted reinforcement of the esophageal hiatus using a tension-free synthetic mesh, such as those that have been successfully used in the repair of groin hernias and abdominal wall hernias.[3,4] Although the impact of such reinforcement on long-term recurrence remains controversial, the main limitation of this approach has been the risk of adverse events related with the use of synthetic materials in the vicinity of the esophagus. There are several reports of polypropylene or polytetrafluoroethylene mesh esophageal intrusion leading to devastating conditions with disabling symptoms such as dysphagia and food intolerance. Treatment of these complications can present serious surgical challenges.[5-8]

Extracellular matrix (ECM) scaffolds have been recently used for reinforcement of surgical soft-tissue repairs in a wide variety of clinical applications, including hiatal hernia repair. Preclinical and clinical reports describe that these devices are remodeled at variable rates depending on the source of the ECM and placement of the device. Ultimately, the goal is for these scaffolds to be replaced by the patient's own tissue, which would overcome the problem of esophageal intrusion in the case of hiatal hernia repair.

The first clinical reports of the use of ECM scaffolds in this setting of reinforcement of a hiatal hernia repair were encouraging with little or no adverse events and a reduction in the short-term recurrence rate of the hiatal hernia.[9] However, in the long term, the difference in recurrence rate with or without reinforcement tends to dissipate.[10] This finding may be influenced by different factors, with integrity of hiatal closure being just one of them. Detection of the failure mechanism is not always possible in a clinical setting and can only be specified when the patients need reoperation due to that failure, which only happens 3%-5% of the time according to several reports.[11,12] Therefore, the specific contribution of ECM scaffolds to the reinforcement of the hiatal closure remains unstudied. The objective of the present study was to assess the contribution of an ECM scaffold, specifically porcine urinary bladder matrix (UBM; MatriStem Surgical Matrix PSMX; ACell, Inc, Columbia, MD). The contribution of UBM was assessed through investigation of the mechanical properties, gross morphology, and histologic appearance of the esophageal hiatus after primary hiatal hernia repair with and without reinforcement in a preclinical porcine model.

## Materials and methods

A total of 14 female domestic pigs, weighing between 40 and 60 kg, underwent laparoscopic primary hiatal hernia repair of a simulated defect in the esophageal hiatus. Seven of the hiatal repairs were reinforced with an ECM scaffold, whereas the remaining seven served as primary repair controls. Animals were survived for 8 wk. At this time, the scaffolds have a complete disappearance from the implant site, and it was proved by the authors in previous publications.[13] At necropsy, after gross morphologic evaluation, samples were sent for mechanical testing and histology.

All animal procedures were performed in compliance with the 1996 Guide for the Care and Use of Laboratory Animals.

### Surgical procedure

Each animal was preanesthetized with xylazine 20 mg/kg and ketamine 50 mg/kg by intramuscular administration, and anesthesia was maintained by orotracheal intubation (isoflurane and intravenous propofol 5 mg/kg). Then, animals were placed in a supine position, and laparoscopic approach was performed using standard surgical equipment. The retroesophageal space was reached through a window created in the pars flaccida of the lesser omentum. The esophageal hiatus was dissected using harmonic scalpel (Ultracision, Johnson & Johnson) until the esophagus was completely free of attachments. A hiatal defect was created by sectioning the fascia between the crura. Primary repair was performed using 2.0 Prolene (Ethicon; Johnson & Johnson) sutures to the crura. UBM scaffolds were implanted with a U shape in an onlay fashion and secured with titanium tackers (ProTack; Covidien; Fig. 1).

### Postsurgical care

The pigs were recovered from anesthesia, extubated, and monitored in the recovery room until they were resting comfortably in sternal position and were kept in a cubicle specially designed for this kind of animal, were they can stay awake. The pigs were given prophylactic antibiotics consisting in a combination of G penicillin (20.000 UI/kg) and streptomycin (sulfate 2 g) via intramuscular administration. After surgery, the pigs received fentanyl (20 mcg/kg IM) for analgesia as needed and were fed with a high-protein balanced food. Vital signs and wound care were checked every day. The weight was checked every 7 d.

### Gross morphology

Euthanasia was achieved with an anesthesia overdose with xylazine/ketamine by intramuscular administration and a propofol IV bolus.

Through a midline abdominal incision, the hiatal area was carefully inspected before tissue harvesting. Presence of sliding hernias or weak areas at the repair site was recorded. Particular attention was given to esophageal intrusion or strong adhesions to the hiatoplasty. After inspection, the esophageal hiatus and crura were harvested en bloc and submitted to mechanical testing and histologic processing.

### Biomechanical testing

Briefly, 1 strip of tissue from the hiatoplasty was obtained using a 10-mm long by 3-mm wide biopsy punch with a dogbone shape (Fig. 2A and B). The samples were excised taking care of including the center of the hiatoplasty without de prolene sutures and a small portion of each crura. Each test sample was immersed in saline (37°C) bath for tensile testing to failure. The elongation rate was set to 25 μm/s (1.5 mm/
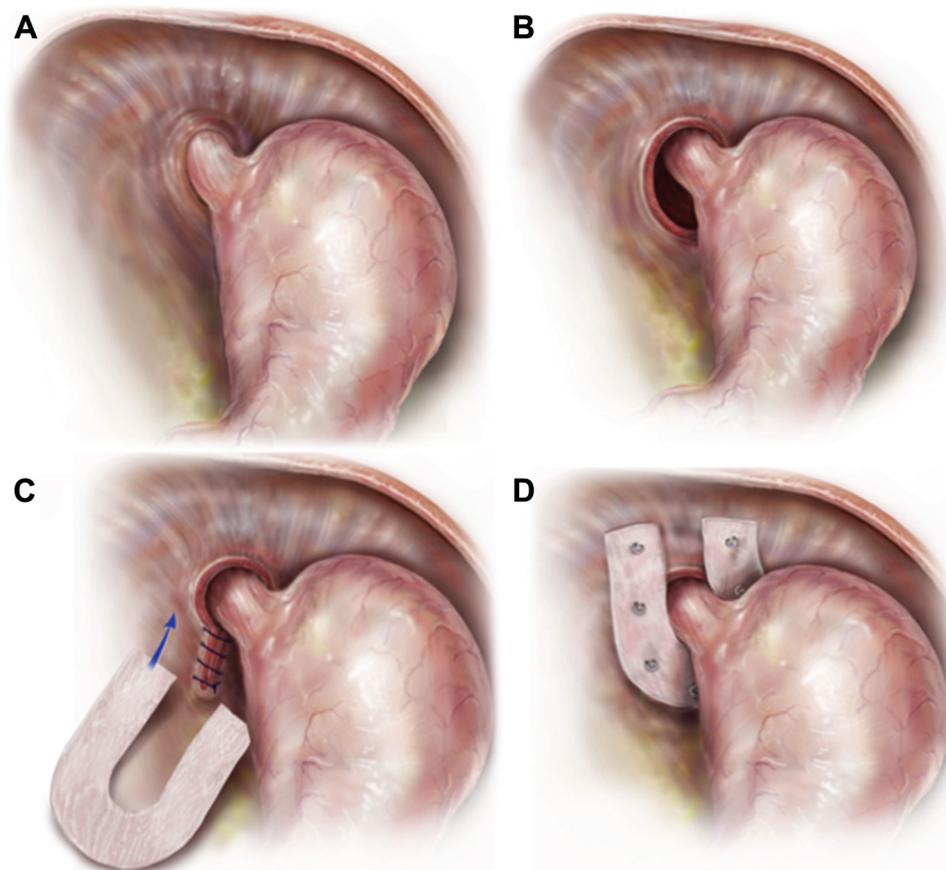
Fig. 1 – Schematic of surgical procedure showing ECM implant and securing method. (A) Normal esophageal hiatus, (B) hiatal defect creation, (C) repair of hiatal crura and ECM placement, (D) Securing of ECM in place by titanium tackers. (Color version of figure is available online.)

min). Stretch was imposed with an accuracy of 10 μm, and strength was measured with a load cell of 10 N (1 kg). Signals of strength and stretching were digitalized at a sampling frequency of 10 Hz with a 12-bit resolution (DVP04ADS module connected to a DVP12SA PLC, Delta Electronics, China). A custom testing device was fabricated in the biomechanics laboratory of the Favaloro University specifically for this study (Fig. 2C). Load (grams) and elongation (mm) were recorded through each test, and a load-elongation curve was plotted. A linear regression was calculated using the linear portion of the curve, from the elbow that indicated end of the collagen recruitment phase until failure. Load at failure, elongation at failure, and stiffness were used for statistical analysis between groups using a one-tailed Student's test considering



Fig. 2 – (A) A 10-mm dog-bone punch. (B) Dog bone tissue sample. (C) Tensile test apparatus. (Color version of figure is available online.)

$P < 0.05$ as significant. Results were expressed as mean ± standard deviation.

## Histology

Samples of hiatal tissue were embedded in paraffin, and 5-μm sections were obtained. Sections were processed for staining with hematoxylin and eosin or Masson's trichrome and photomicrographs were obtained at 40× and 200×.

## Results

### Gross morphology

No firm adhesions were evidenced in the macroscopic analysis in either of the two groups. In the treated group, there was no gross visible evidence of the UBM device by 8 wk after surgery. The repaired defect site reinforced with ECM scaffolds appeared compliant to the chronic movements of the diaphragm. A robust closure of the crura could be noted in all cases with a smooth peritoneal-like structure covering the entire repair. Device area could only be identified by the securing tackers (Fig. 3A). The esophagus was free, with no signs of fibrosis or intrusion.

In contrast, the control group showed weaker tissue at the hiatoplasty level with three animals showing only a thin strip of tissue and a failed hiatoplasty, despite not having developed a hiatal hernia (Fig. 3B).

### Biomechanical testing

Tensile tests were successfully carried out on all 14 specimens. One of the treated specimens was discarded because the load at failure exceeded the load cell range. Average load at failure of the treated group was found to be significantly stronger than the controls (185.8 ± 149.7 g, 95% confidence interval [CI] 28.67-343, interquartile range [IR] 251 *versus* 57.5 ± 57.5 g, 95% CI 4.51-110.54, IR 49, $P < 0.05$). Similarly, the stiffness was significantly higher in the treated animals (57.5 ± 26.9 g/mm, 95% CI 29.69-85.64, IR 43 *versus* 19.1 ± 17.5 g/mm 95% CI 2.92-35.37, IR 26; $P < 0.01$). Interestingly, there was no difference in elongation at failure (7.62 ± 2.02 mm, 95% CI 5.50-9.83, IR 4 *versus* 7.87 ± 3.28 mm, 95% CI 2.020-8.380, IR 4.86; $P = 0.44$; Fig. 4).

### Histology

In the microscopic analysis, full integration of the scaffold to the native tissue was observed in all cases. No foreign body granulomas or giant cells were found in the reinforced tissue. The abdominal side surface of the hiatoplasty was covered by a loose connective tissue, predominantly acellular, layer characteristic of serosal tissue. The body of the hiatoplasty showed that an abundance of collagen fibers appeared organized with little signs of fibrosis. Some scarce areas of mononuclear cells could be seen near the suture lines and in the repaired area. Numerous capillaries and newly formed blood vessels were encountered populating the remodeled area of the scaffold. On the lateral side of the hiatoplasty, striated muscle fibers were evidenced, right at the transition with the native crura.

In contrast, the control animals had a clear gap in the tissue structure where a weaker area was noted. This area was characterized by disorganized loose connective tissue and a strong mononuclear infiltrate suggestive of scar tissue formation (Fig. 5).

## Discussion

This study describes the isolated contribution of UBM scaffolds to the mechanical properties of a hiatal closure as a way to understand the relevance of reinforcement in a clinical setting. The results showed that the repair site consisted of more robust, stronger tissue associated with reinforcement with UBM as compared with the tissue observed after primary repair alone.
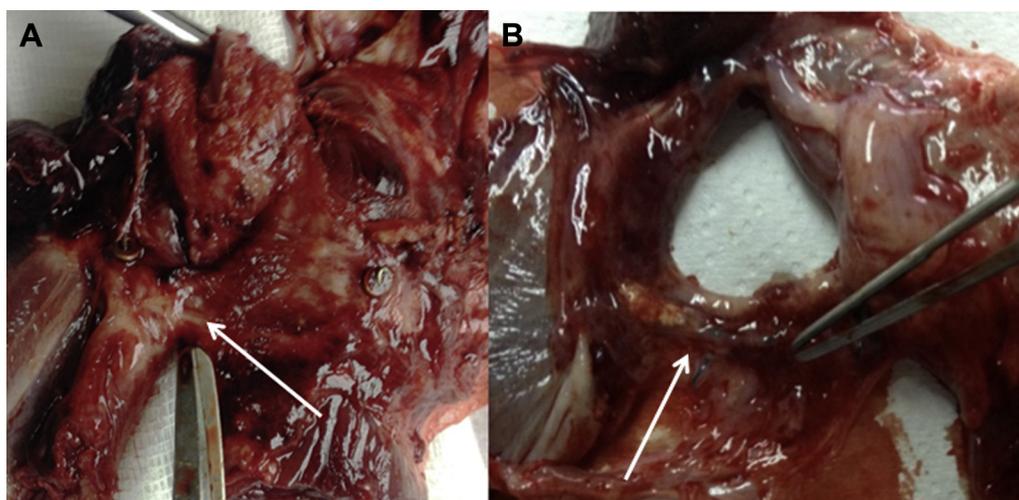


Fig. 3 − (A) Hiatal closure with ECM remodeled after 8 wk (arrow). (B) Defect at the hiatoplasty in no-treatment group (arrow). (Color version of figure is available online.)

**A**



**B**



**C**
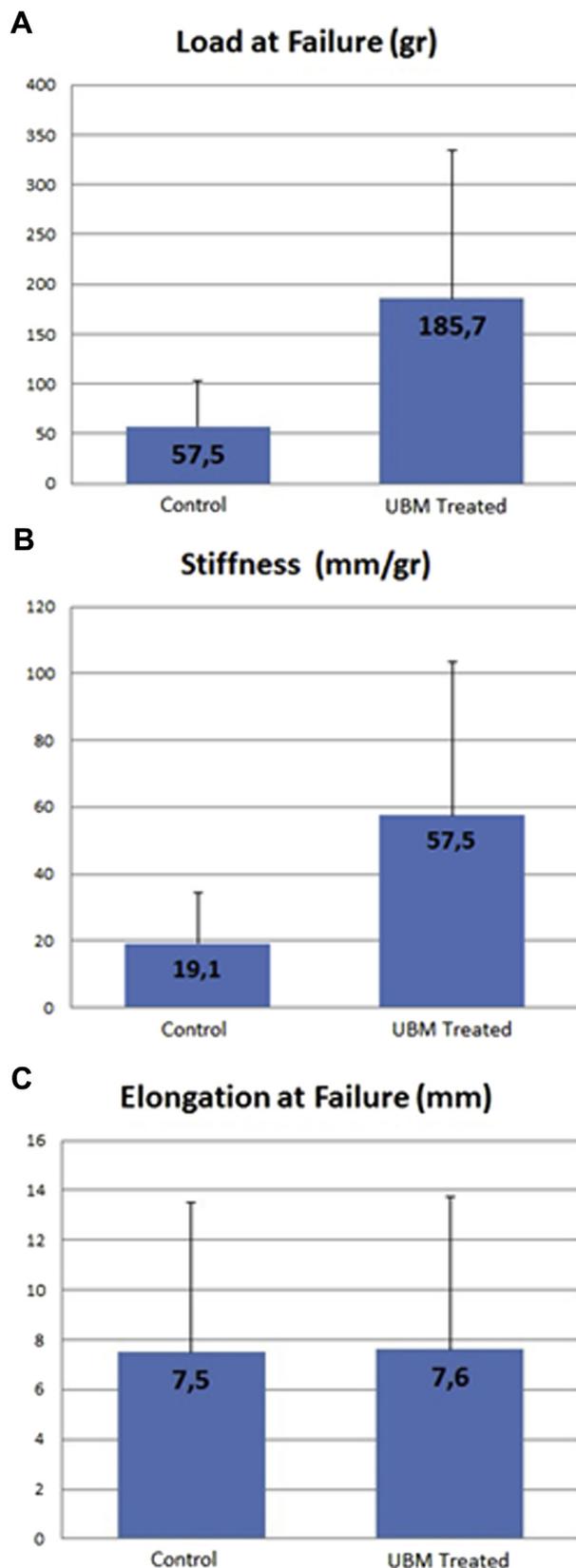


Fig. 4 − **Mechanical properties of hiatal repair. (A) Load to failure. (B) Stiffness. (C) Elongation to failure. (Color version of figure is available online.)**

The tension-free mesh reinforcement technique for hiatal closure has been a topic of great debate for many years. The use of synthetic materials on the hiatus has been strongly discouraged owing to high risk of complications, with the most feared and devastating of all being esophageal erosion and mesh intrusion. Several reports exist showing unacceptable rates of severe complications, including esophageal resections, in up to 25% of cases that require reoperation due to mesh intrusion.[8] The use of synthetic mesh for the repair of the hiatus is also associated with dysphagia due to the significant inflammatory reaction that the body develops in response to the materials. In rabbit model of hiatal hernia repair, two different types of polypropylene mesh were placed on the diaphragm encircling the esophagus. After 3 mo, distinctive mesh shrinkage was observed in all animals, and the meshes lost up to 50% of their original size before implantation.[14]

Biologically derived scaffolds have gained acceptance in hiatal hernia repair because of their lower risk of complications. However, the lack of improvement in recurrence rates of hiatal hernias in the long term has kept the debate active. Oelschlager et al. reported the short-term (6 mo)[9] and long-term (5 y)[10] results of a multicenter study comparing two groups of patients who underwent laparoscopic para-esophageal hernia repair, one group with primary repair of hiatal defect and the other group with hiatoplasty reinforced with a biologically derived prosthesis (Surgisis; Cook Biotech, Lafayette, IN). The primary outcome measured at both time points was incidence of recurrence based on radiologic detection. At 6-mo follow-up, a significant difference in the recurrence rate was observed, with 9% recurrence in the reinforced group versus a 24% recurrence rate in the primary repair group ($P = 0.04$). However, long-term follow-up showed that the recurrence rates were not statistically different, despite a trend favoring the reinforced group. The studies reported no adverse events associated with use of the device supporting the safety of biologically derived scaffolds for reinforcement of hiatal hernias.

It has been widely accepted that there are multiple failure mechanisms for hiatal hernia recurrence with the most common being wrap migration, wrap slippage, and para-esophageal hernia. The inability to clearly isolate and distinguish the failure mechanism in each patient represents an important limitation to understanding the role of the reinforcement in this scenario.[15] It can be argued that slippage is not affected by hiatal closure integrity, and it is one of the most frequent failure modes, which affects the overall recurrence rate published in previous reports. If the use of a mesh can assist in preventing disruption of the hiatal closure, it would still benefit patients with higher risk of failure due to tissue weakness or large defects. There are several reasons to argue that primary suture of the crura is not enough in some hiatal defects. First, the gap is usually wide in large paraesophageal hernias. Second, the muscle tissue of the pillars is weak lacking a fascia of its own, and third, the closure is repeatedly exposed to numerous situations such as valsalva maneuvers, cough, and normal breathing.
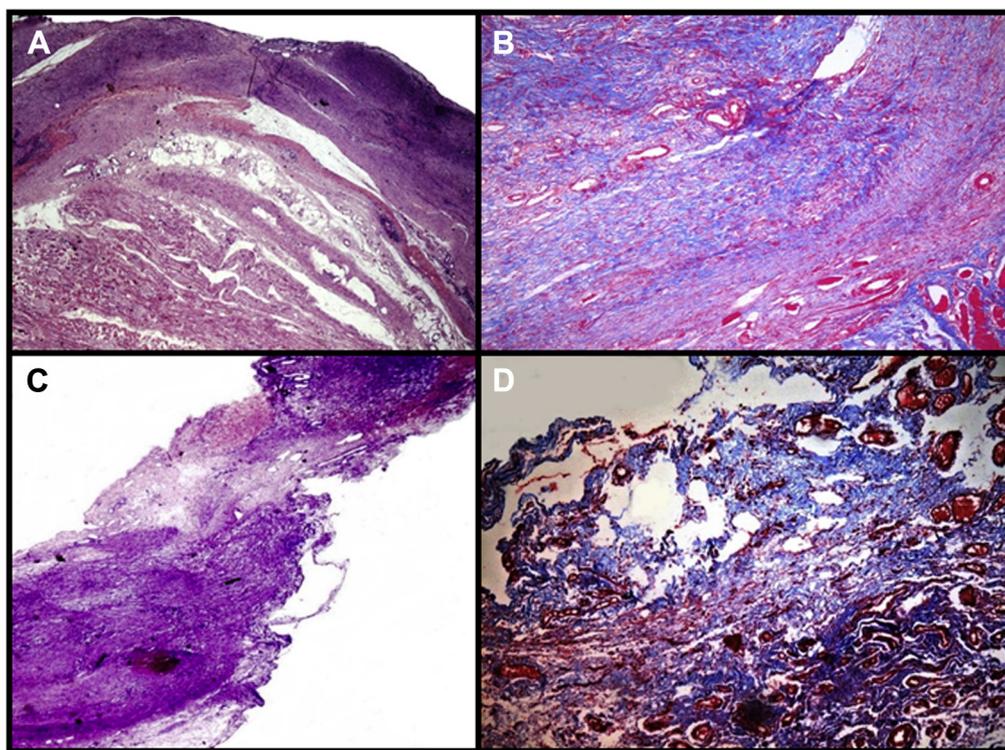
**Fig. 5 — Histology findings (A) Hematoxylin and eosin (H&E) of treated group 40×, (B) Masson's trichrome of treated group, 200×. (C) H&E of control group 40×, and (D) Masson's trichrome of control group 200×. (Color version of figure is available online.)**

The present study provides further knowledge toward the benefit of using mesh reinforcement with ECM scaffolds. This represents, to our knowledge, the first thorough biomechanical assessment of a reinforced hiatal closure. The observation of a more mechanically robust tissue after repair encourages its use in a particular subset of patients that can be at greater risk of hiatal failure. Safety of this procedure is also demonstrated by the absence of adhesions and of foreign body reactions to the material.

ECM scaffolds have been widely used in the clinics for numerous applications including the esophagus. The authors have previously reported successful esophageal repair using ECM scaffolds in both preclinical and clinical settings. In those studies, ECM has been shown to integrate and contribute to remodeling of newly formed tissue that mimicked mechanical properties of host tissue with time.[16,17]

The time point was chosen based on that complete remodeling of ECM scaffolds occurring within the first 4-6 wk, with almost no scaffold remaining at 8 wk. Although some unremodeled scaffold fibers could remain, they would not likely contribute to the mechanical properties. In a study in dogs with ECM scaffolds, tissue harvested at 28 d shows complete reorganization with native tissue with no signs of the graft except a few inflammatory cells that is thought to be crucial in the replacement of the xenogenic collagen into native.[18]

Although the ECM scaffolds used in the present study were not labeled, the histologic examination showed rapid loss of structural identity and rapid integration into host tissues consistent with the findings of the previous studies. This rapid rate of degradation likely contributes to the absence of a foreign body response and the associated fibrous connective tissue that is characteristic of many nonresorbable biomaterials.

Limitations of this study include the short-term follow-up of the animals. Based on previous publications by the authors and colleagues, it is known that, by 8 wk, the scaffolds are mostly remodeled with mechanical properties being influenced mainly by remaining tissue that is stable in time. Also, the lack of radiological or functional end points prevents a stronger conclusion on the clinical impact of the findings.

In conclusion, in our animal survival model, we have provided evidence that the addition of an ECM to augment a primary hiatal repair leads to tissue characteristics that may decrease the possibility of early failure of the repair. This may translate to decreased recurrence rates. Further study is necessary.

contributed to conceiving and designing the study, critical revisions, and approving the final version of the article.

## Disclosure

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