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A novel method of esophageal lengthening in a large animal model of long gap esophageal atresia

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ABSTRACT

Purpose: Long gap esophageal atresia remains a significant treatment challenge. We aimed to create the first large animal model of long gap esophageal atresia to test a degradable esophageal lengthening device.
Methods: The distal esophagus was divided 2 cm above the gastroesophageal junction in 6 minipigs. A polycaprolactone (PCL) spring device was secured inside the distal esophageal segment, and the end was oversewn. Nonexpanding PCL tubes served as controls. An esophagogastric anastomosis was created to restore continuity. After 4 weeks, the distal esophageal pouch was analyzed.
Results: The distal esophageal pouch of experimental animals increased in length from 1.9 to 4.5 cm. Control animals demonstrated no change. When comparing lengthened to native esophagus, there was no difference in the thickness of muscularis mucosa or muscularis propria. Mechanically lengthened esophagus showed mild to moderate superficial inflammation and fibrosis. There were no differences in the number of myenteric or submucosal ganglia.
Conclusion: We created the first porcine model of long gap esophageal atresia and lengthened the distal esophagus with an internally placed device. This model may be used to explore novel therapies in the management of long gap esophageal atresia.

Esophageal atresia (EA) and tracheoesophageal fistula (TEF) are major congenital malformations that affect 1:3500 live-born infants [1]. The described subtypes are based on extent of the atresia and location of the TEF. Type A, or pure EA, accounts for approximately 8% of all EA/TEF [2]. Management of pure EA depends largely on the length of gap between the proximal and distal atretic ends of the malformed esophagus. While children with small gap EA can be managed with a single surgical procedure, long gap atresias pose a significant problem because of the length of esophagus needed for repair.

Current surgical management of long gap EA is widely varied. Interposition grafts using the stomach, colon, and jejunum have been described, however most patients have significant long-term sequelae requiring additional procedures [3]. Most agree that preservation of the native esophagus is best. To achieve this, there are several mechanical lengthening techniques described. Regardless of the method used, children are subject to significant morbidity and no single technique is ideal. Major complications include anastomotic leak, stricture, infection, dysphagia, and gastroesophageal reflux disease [4,5]. Patients consequently suffer long hospitalizations and have significant oral aversion.

Gastrointestinal tissue engineering has been a focus of investigation with the goal of improving therapies for both short bowel syndrome and EA. In regard to short bowel syndrome, recent animal models of distraction enterogenesis, or intestinal lengthening by mechanical means, have been promising [6,7]. These models utilize internal propulsion force, rather than external traction, to achieve lengthening. Given the current clinical use of distractive force in esophageal tissue, there may be a role for mechanical lengthening using similar devices in the treatment of long gap EA. Presently, there are no practical animal models of pure EA. Our goal is to create a viable large animal model of long gap EA for the purpose of testing a novel, internally placed, distal esophageal lengthening device.

1. Materials and methods

Animal experimental protocols were approved by the Institutional Animal Care and Use Committee (Protocol #20808-01) and complied with all established regulations. Male Yucatan minipigs (S&S Farms, Ramona, CA) weighing between 8 and 14 kg were used. All materials used in device fabrication were FDA approved for use in humans.

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1.1. Device fabrication

Polymer springs made from polycaprolactone (PCL) were fabricated using a 3.8% (w/w) PCL (Lactel, Birmingham, AL) solution mixed in chloroform and spray-coated onto a spinning 7 mm stainless steel mandrel to form polymer tubes. PCL tubes measuring 1.5–2.0 cm were laser cut into spirals, stretched and heat set at 50 °C for 1 hour to form final PCL springs. Target spring constants were extrapolated from stress versus strain curves of rat intestinal tissue and previously used spring constants from successful mechanical lengthening of rat intestine [6]. Spring constants were measured using an Instron electromechanical testing system (Instron, Norwood, MA). PCL spring devices were compressed and placed into gelatin capsules (Torpac Inc., Fairfield, NJ) then coated with cellulose acetate phthalate as we have previously described [8]. The capsule allows compression of the device and the polymer coating delays full expansion until several days after it is secured in the esophagus.

1.2. Surgical procedure

Animals were anesthetized with inhaled oxygen and 2.0% isoflurane. The abdomen was entered through an upper midline laparotomy incision and the stomach was identified and eviscerated. The esophagus was mobilized and ligated 1.5–2.0 cm above the gastroesophageal junction (GEJ) to create a distal esophageal pouch. To restore enteric continuity, a gastrotomy was made in the gastric cardia and a tension-free anastomosis using 3-0 Maxon suture (Covidien, Mansfield, MA) in simple interrupted fashion was created between the distal end of the proximal esophagus and the stomach (Fig. 1). In experimental animals, the encapsulated PCL spring device was placed into the distal esophageal pouch and the open end was closed with a 3-0 polypropylene running suture ($n = 4$). A large vessel loop was wrapped around the gastroesophageal junction (GEJ) and clipped to secure the device inside the distal esophageal pouch. PCL tubes measuring 2 cm in length were placed into the distal esophageal pouch of control animals ($n = 2$). For the first postoperative day, animals were started on a low residue diet (TestDiet®, Purina, Richmond, IN) and water, then advanced to a regular diet. They were observed closely for signs of distress. Animal weights were recorded weekly.

1.3. Tissue analysis

Euthanasia was performed after 28 days and gross tissue was examined. The length and circumference of the distal esophageal stump were measured and recorded. The GEJ was examined for evidence of stenosis. The native esophagus from each animal was also harvested and measured for comparison. Tissues were fixed in 10% zinc formalin for 24 hours, then representative sections were sampled and embedded in paraffin blocks. Four-micrometer sections were mounted on slides and stained with hematoxylin and eosin (H&E), Sirius red special chemical stain, and immunohistochemical stains for smooth muscle actin (SMA) and S100. Monoclonal mouse anti-SMA antibody (Cell Marque Corp., Rocklin, CA), diluted at 1:3000 and polyclonal rabbit anti S100 antibody (Cell Marque Corp., Rocklin, CA) diluted at 1:400 were visualized by horseradish immunoperoxidase staining using a BenchMark XT automated immunohistochemistry/in situ hybridization slide staining system (Ventana Medical Systems, Inc., Tucson, AZ) with external controls. All four stains were performed following standard institutional operating procedures. All microscopic evaluation was performed under bright field microscopy (Nikon Instruments, Inc., Melville, NY) and slides from at least 4 representative sections per tissue block were examined. An initial unblinded analysis was followed by a blinded review by a board certified anatomic pathologist. Histologic architecture was examined qualitatively on H&E stain. Degree of inflammation seen on H&E, and degree of fibrosis seen on Sirius red stain were graded semiquantitatively (none, mild, moderate, severe) with comparison of lengthened to native esophagus. Muscularis mucosa and muscularis propria thickness measurements were quantified using microscopic length measurements of representative areas recorded with the use of a reticle on anti-SMA antibody localized tissue. Anti-S100 localized ganglia cells counts were visualized under fluorescent light microscopy and recorded per unit area of submucosa and muscularis propria as the number of cells per 5-μm diameter high power field (HPF). At least 50 HPFs per sample were examined and then mathematically converted to a cell count per square millimeter.

1.4. Statistical analysis

Data were expressed as means ± standard deviations. Statistical significance was determined using paired and unpaired Student's t-tests where appropriate.

2. Results

All animals survived with an average weight gain of 164 ± 57 grams per day. The distal esophageal pouch of experimental animals increased in length from 1.9 ± 0.3 cm to 4.5 ± 0.7 cm ($p < 0.05$) (Fig. 2). Control animals had no significant change in esophageal length (2.0 ± 0 cm to 2.1 ± 0.1 cm, $p = 0.5$). The native GEJ exhibited normal histology without evidence of stricture in all animals. Histologic examination of the distal esophageal pouch revealed the presence of stratified squamous esophageal epithelium and all layers of esophageal architecture were present (Fig. 3). When comparing lengthened to native esophagus there were no differences in the thickness of muscularis mucosa (0.73 ± 0.65 mm versus 0.65 ± 0.19 mm, $p = 0.78$) or muscularis propria (2.35 ± 0.73 mm versus 2.53 ± 0.74 mm, $p = 0.42$). Mechanically lengthened esophagus showed mild to moderate superficial inflammation. Sirius red staining revealed a mild to moderate degree of fibrosis in lengthened tissues. When comparing S100 immunohistochemical staining of lengthened to native esophagus, there were no

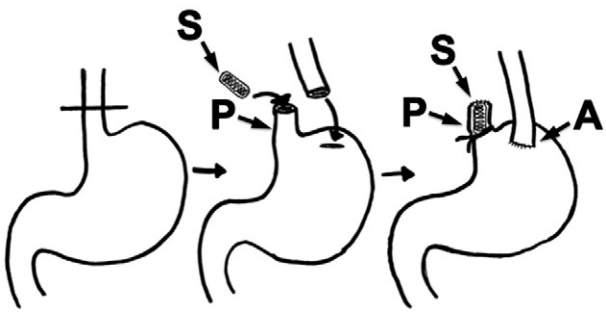

Fig. 1. Schematic representation of the surgical methods and procedure. P = distal esophageal pouch; S = spring device; A = esophagogastric anastomosis.

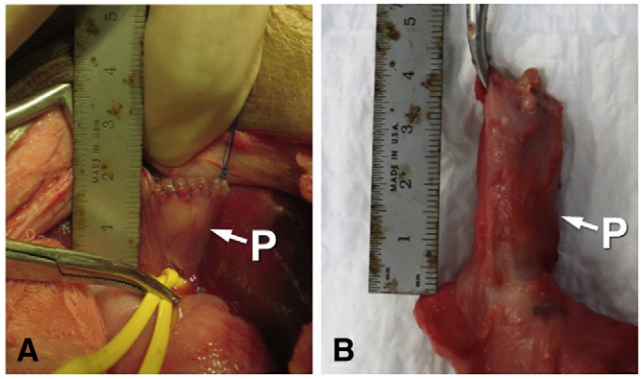

Fig. 2. Photographs of (A) initial placement of the device into the distal esophageal pouch (P), and (B) lengthened distal esophagus 4 weeks after device placement.