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Biomimetic and synthetic esophageal tissue engineering

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ABSTRACT

Background/Purpose: A tissue-engineered esophagus offers an alternative for the treatment of pediatric patients suffering from severe esophageal malformations, caustic injury, and cancer. Additionally, adult patients suffering from carcinoma or trauma would benefit.
Methods: Donor rat esophageal tissue was physically and enzymatically digested to isolate epithelial and smooth muscle cells, which were cultured in epithelial cell medium or smooth muscle cell medium and characterized by immunofluorescence. Isolated cells were also seeded onto electrospun synthetic PLGA and PCL/PLGA scaffolds in a physiologic hollow organ bioreactor. After 2 weeks of *in vitro* culture, tissue-engineered constructs were orthotopically transplanted.
Results: Isolated cells were shown to give rise to epithelial, smooth muscle, and glial cell types. After 14 days in culture, scaffolds supported epithelial, smooth muscle and glial cell phenotypes. Transplanted constructs integrated into the host's native tissue and recipients of the engineered tissue demonstrated normal feeding habits. Characterization after 14 days of implantation revealed that all three cellular phenotypes were present in varying degrees in seeded and unseeded scaffolds.
Conclusions: We demonstrate that isolated cells from native esophagus can be cultured and seeded onto electrospun scaffolds to create esophageal constructs. These constructs have potential translatable application for tissue engineering of human esophageal tissue.

1. Introduction

Esophageal atresia occurs in 1:3000–1:5000 live births in the United States [1]. For many of these patients a primary anastomosis, either immediate or delayed, remains a feasible option. However, in long gap esophageal atresia, the most severe form, esophageal replacement by gastrointestinal transposition may be necessary [2]. Besides esophageal atresia, caustic injuries to the esophagus also add to the demand for esophageal replacement, with an estimated 5000–15,000 caustic ingestions occurring per year in the United States. This can be a devastating injury depending on the length of the esophagus that becomes necrotic [3]. Finally, esophageal carcinoma occurs in 500,000 adults annually [4]. Surgical resection remains the mainstay of treatment, often leading to complete esophageal resection. While a variety of reconstructive options are available to replace the esophagus, these procedures are associated with substantial short and long-term complications such as stenosis and dysmotility, and there is a consensus among most pediatric surgeons that better postoperative outcomes are achieved through preservation of native esophageal tissue [5,6]. Unfortunately, conventional organ transplant is not always a suitable option, especially in the pediatric population where there is already a shortage of appropriately sized donor tissue [7]. A tissue-engineered esophagus made from either natural or synthetic biomaterials may therefore offer a real alternative to conventional treatments for severe esophageal disease.

In order for a tissue-engineered construct to achieve functional properties, it must mimic the architecture of the native tissue [8]. De-cellularization of native tissue is widely recognized in many organs including the lung, kidney, heart, and blood vessels [9–12]. The process of de-cellularization removes cellular elements while

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preserving the native extracellular matrix (ECM), surface topography, and biomechanical properties of the native tissue. Theoretically, these acellular scaffolds have reduced immunogenicity and can support cell adhesion, proliferation, chemotaxis, and differentiation [9–12]. However, due to the lack of appropriately sized cadaveric donors, generating size-specific scaffolds using electrospinning of natural polymers may lend itself more to personalized medicine. A tissue-engineered scaffold can be constructed using natural polymers already FDA approved for use in skin, bone and cartilage applications [13]. Previous studies have been published utilizing either synthetic poly (lactic-co-glycolic acid) (PLGA) or polycaprolactone (PCL)-based scaffolding for esophageal tissue engineering [14–19]. In addition, recent studies have utilized isolated epithelial cells or organoid units generated from fetal esophageal tissue [14–16]. Our study is novel in that it is the first to enrich both smooth muscle cells and epithelial cells from native tissue and seed them in 3 dimensions on the appropriate surfaces to mimic a normal esophagus.

The esophagus is a hollow conduit with stratified squamous epithelium, submucosa, and outer longitudinal and inner circular muscle layers. Optimally, a tissue-engineered scaffold should incorporate into native tissue, enable the passage of food, propagate peristalsis, and withstand the acidic pH of gastric fluid. The ability to synthesize a tubular scaffold that supports the adhesion and proliferation of biomimetic cells lends itself to the creation of a relevant tissue-engineered esophagus. Identification of an appropriate cell source is essential. Ideally cells will be autologous, phenotypically resemble esophageal cells, and ultimately enable restoration of function. One method is to expand cells from the native organ in culture and use this population to seed a scaffold for implantation. Herein we describe the isolation of allogenic cells from a rat esophagus which were then seeded onto synthetic matrix in a physiological bioreactor. Segments of these re-seeded scaffolds were orthotopically implanted into a rat and demonstrated integration with the native tissue. A schematic of the steps required to generate a synthetic scaffold for implantation is presented in Fig. 1.

2. Methods

2.1. Scaffolding

2.1.1. Electrospinning PLGA and PLGA/PCL scaffolds

The following procedure was adapted from a previously published protocol [20]. PLGA (Mw = 50–75 kg/mol, 85:15 lactide/glycolide) (Evonik Wallingford, CT) was dissolved in a 80/20 mix of dichloromethane (DCM) (Fisher Scientific, Pittsburgh, PA) and dimethylformamide (DMF) (Fisher Scientific, Pittsburgh, PA) to result in a concentration of 25% w/v. The solution was loaded into a 5 mL plastic syringe with a 22 gauge needle and was pumped at a flow rate of 1.0 ml/h using a syringe pump. A high voltage power source was used to apply 15 kV between the needle and a flat piece of aluminum foil. Scaffolding was either spun onto glass coverslips or was spun onto tin foil for generating three dimensional scaffolds. Three-dimensional scaffolds were created by rolling the flat PLGA sheet around a 5 French nasogastric tube and sealing the ends with clinical grade TISSEEL Fibrin Sealant (Baxter Healthcare, Westlake Village, CA). This size nasogastric tube was chosen as it was appropriately sized for an adult Sprague Dawley rat.

2.1.2. Resection of esophageal tissue

10–20 week-old Sprague–Dawley rats (Charles River, Wilmington, MA) were euthanized in accordance with University of Connecticut Health Center IACUC approved protocol (ACC#100621-0416). A midline incision was made from the lower abdomen to the throat and a median sternotomy was performed. A 5 French nasogastric catheter was inserted through the mouth and advanced to the terminal end of the esophagus. The entire length of the esophagus was carefully dissected and rinsed in phosphate-buffered saline (PBS) with Primocin (InvivoGen, San Diego, CA 1:500). This tissue was then either de-cellularized or utilized for harvesting native cells.

2.1.3. Esophagus de-cellularization for morphologic comparison with synthetic scaffolds

The following procedure was adapted from a previously described protocol [21]. De-cellularization of harvested esophagi was completed in a small animal hollow organ bioreactor from HART (Harvard Apparatus Regenerative Technology). Both ends of the organs were fixed to cannulas using 5–0 Prolene sutures (Ethicon). All de-cellularization steps were completed at 37°C and esophagi were subjected to rotational mechanical agitation at a speed of 3 revolutions per minute. The lumen of the esophagi was filled with 4% sodium deoxycholate (SDC) (Sigma–Aldrich) and the entire organ was bathed in 4% SDC for 24 h. Esophagi were then treated with 0.4 mg/mL DNase-I (Sigma–Aldrich) with 1M NaCl in PBS for 12 h. Tissues were rinsed for 8 h in deionized water (DI) containing Primocin (InvivoGen, San Diego, CA). De-cellularized esophageal scaffolds were used in this study exclusively as a control for SEM.

2.2. Cell source

2.2.1. Isolation of epithelial and smooth muscle cells

The following procedure was adapted from a previously described protocol [22]. Esophageal tissue was harvested from

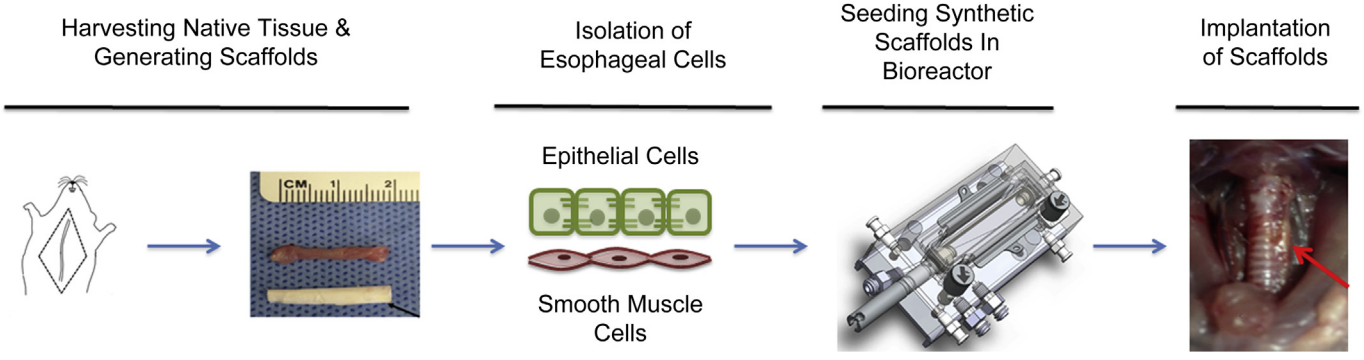

Fig. 1. Schematic of steps required to generate a synthetic esophageal scaffold for *In vivo* implantation. Epithelial cells were seeded in the lumen of the synthetic scaffold and the smooth muscle cells were seeded on the abluminal surface.