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# Designing a tissue-engineered tracheal scaffold for preclinical evaluation

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ABSTRACT

*Objective:* Recent efforts to tissue engineer long-segment tracheal grafts have been complicated by stenosis and malacia. It has been proposed that both the mechanical characteristics and cell seeding capacity of TETG scaffolds are integral to graft performance. Our aim was to design a tracheal construct that approximates the biomechanical properties of native sheep trachea and optimizes seeding with bone marrow derived mononuclear cells prior to preclinical evaluation in an ovine model.

*Methods:* A solution of 8% polyethylene terephthalate (PET) and 3% polyurethane (PU) was prepared at a ratio of either 8:2 or 2:8 and electrospun onto a custom stainless steel mandrel designed to match the dimensional measurements of the juvenile sheep trachea. 3D-printed porous or solid polycarbonate C-shaped rings were embedded within the scaffolds during electrospinning. The scaffolds underwent compression testing in the anterior-posterior and lateral-medial axes and the biomechanical profiles compared to that of a juvenile ovine trachea. The most biomimetic constructs then underwent vacuum seeding with ovine bone marrow derived mononuclear cells. Fluorometric DNA assay was used to quantify scaffold seeding.

*Results:* Both porous and solid rings approximated the biomechanics of the native ovine trachea, but the porous rings were most biomimetic. The load-displacement curve of scaffolds fabricated from a ratio of 2:8 PET:PU most closely mimicked that of native trachea in the anterior-posterior and medial-lateral axes. Solid C-ringed scaffolds had a greater cell seeding efficiency when compared to porous ringed scaffolds (Solid: $19 \times 10^4$ vs. Porous: $9.6 \times 10^4$ cells/mm$^3$, $p = 0.0098$).

*Conclusion:* A long segment tracheal graft composed of 2:8 PET:PU with solid C-rings approximates the biomechanics of the native ovine trachea and demonstrates superior cell seeding capacity of the two prototypes tested. Further preclinical studies using this graft design *in vivo* would inform the rational design of an optimal TETG scaffold.

## 1. Introduction

Long-segment airway disease in the pediatric population continues to be a challenging clinical and surgical problem. Congenital causes such as tracheobronchomalacia, high-grade laryngotracheal clefts, complete tracheal rings, and tracheal agenesis are rare but can be life threatening. Other conditions such as trauma, malignancy, or infection may also result in long-segment defects in need of reconstruction [1]. The lack of suitable materials and/or tissues for long-segment airway reconstruction within the pediatric population makes these conditions particularly difficult to manage.

Despite the evolution of open and endoscopic techniques for managing laryngotracheal stenosis, no current treatment exists for defects that exceed 30% of the airway in children. Attempts at airway

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replacement with artificial material, autologous tissue transplant, or cadaveric transplantation have been limited by chronic infection, graft collapse, vascular erosion, or the need for life-long immunosuppression [2,3]. Tissue engineering offers an alternative that may mitigate many of these concerns. However, the clinical performance of tissue-engineered tracheal grafts (TETGs) has yet to meet its theoretical potential. Isolated case reports describing the compassionate use of tissue-engineered tracheal grafts have used a variety of techniques and materials including decellularized cadaveric grafts and biosynthetic materials. In each case, significant complications have occurred requiring multiple hospitalizations and additional procedures [3–6].

Stenosis is one of the most commonly reported complications associated with the use of TETGs both clinically and in animal models [3]. Many factors may contribute to stenosis, including quantity of cells seeded, cell type(s), scaffold composition, and immunologic modulators. In 2015, our team described an ovine model that evaluated a biosynthetic scaffold that had previously been used in the clinical realm. All animals in this study suffered from stenosis and deterioration of respiratory function [7]. These grafts were designed to have supraphysiologic biomechanical properties due to concerns regarding malacia, however, it has been demonstrated in other organ systems that a mismatch in the biomechanical properties of graft and native tissue may lead to stenosis. The purpose of this study was to examine the impact of alterations in the composition of our prototype TETG on the biomechanical properties and seeding capacity of the scaffold, with the goal of identifying a candidate design worthy of further preclinical pursuit.

## 2. Material and methods

### 2.1. Animal care/ethics statement

Nationwide Children's Hospital's (Columbus, OH) Institutional Animal Care and Use Committee reviewed and approved the protocol (AR13-00071). Representatives of the Animal Care staff monitored all animals pre-, intra-, and post-operatively. Care was in accordance with humane care standards published by the Public Health Service, National Institutes of Health (Bethesda, MD) in the Care and Use of Laboratory Animals (2011), and USDA regulations outlined in the Animal Welfare Act.

### 2.2. Scaffold fabrication

Scaffolds were manufactured by Nanofiber Solutions, Inc. (Hilliard, OH) as previously described [7]. A custom mandrel composed of stainless steel was designed to match the dimensions of native juvenile sheep trachea (lumen diameter = 20 mm). Polymer nanofiber precursor solutions were prepared by: 1) dissolving 8% polyethylene terephthalate (PET) in 1,1,1,3,3,3-hexafluoroisopropanol and heating the solution to 60 °C and by 2) dissolving 3% polyurethane (PU) in 1,1,1,3,3,3-hexafluoroisopropanol. Once cooled, the solutions were combined to create 2:8 or 8:2 PET:PU co-polymer solutions. The PET/PU solutions were electrospun onto the custom-designed mandrel utilizing a 20-gauge blunt tip needle, a high-voltage DC power supply set to 14 kV, and a 15 cm tip-to-substrate distance (Fig. 1a). Solid or porous 3-D printed C-rings composed of polycarbonate were embedded into the graft during the process of electrospinning. (Fig. 1b–d). The completed scaffold was then lyophilized overnight to remove residual solvent and sterilized using ethylene oxide prior to further evaluation.

### 2.3. Micro-computed tomography

A solid or porous ringed 2:8 PET:PU TETG was scanned in the Bruker Skyscan1172d (Kontich, Belgium) micro computed tomography scanner. A 27 μm voxel resolution scan (33 kV, 198 μA, no filter, 0.6° rotation per projection, 6 frames averaged per projection, and 40 ms exposure time) and a 6.82 μm voxel resolution scan (33 kV, 198 μA, no filter, 0.25° rotation per projection, 6 frames averaged per projection, and 1200 ms exposure time) were acquired of the TETG specimens. The 27 μm voxel resolution reconstruction (no smoothing, ring artifact correction 1, beam hardening 0%) and the 6.82 μm voxel resolution reconstruction (no smoothing, ring artifact correction 5, beam hardening 0%) were completed using NRecon (Kontich, Belgium). 3D segmentation of the TETG was done using Synopsys Simpleware ScanIP (Mountain View, CA).

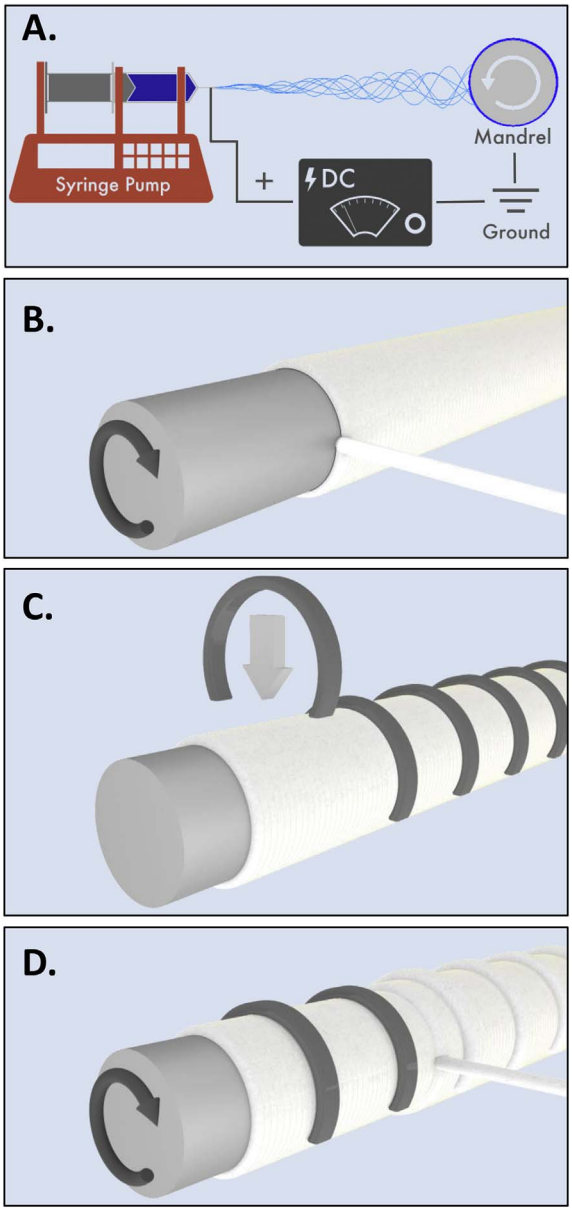


**Fig. 1.** TETG scaffold fabrication schematic.
(A) Electrospinning tubular scaffolds involves a syringe pump a set distance away from a grounded, rotating mandrel. A high voltage DC (Direct Current) source accelerates and extrudes nanofibers from the syringe tip forming a Taylor cone. Nanofibers are deposited into the rotating mandrel in a layered fashion. In this study, once 50% of the graft thickness was spun (B) 3D-printed polycarbonate C-rings were manually placed onto the scaffold (C), atop of which the remaining graft thickness was spun.

### 2.4. Scanning electron microscopy (SEM)

Solid ringed 8:2 and 2:8 PET:PU scaffolds and a porous ringed 2:8 PET:PU scaffold were sectioned, mounted, and gold sputter coated. SEM

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