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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.

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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: 19×10^4 vs. Porous: 9.6×10^4 cells/mm³, $p = 0.0098$).

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.

Keywords

Tissue-engineered tracheal graft; Electrospinning; Ovine model; Vacuum seeding

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 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 supra-physiologic 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).

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 images were obtained with a Hitachi S-4800 Scanning Electron Microscope at 5.0 kV at 28, 30, or 500× magnifications.

2.5. Uniaxial mechanical testing

Tracheas from Dorset lambs (*Ovis aries* 45–60 kg) were donated from a local abattoir (Columbus, OH). Scaffolds and a native tracheal specimen were then subjected to compression testing in the anterior-posterior and lateral-medial directions, identifying the load (lbs) required to displace a graft to 75% of the luminal diameter (in). No longitudinal forces were applied to the scaffold during testing. Data were represented graphically as load versus displacement curves.

2.6. Bone marrow harvest

After sedation with ketamine (5–10 mg/kg)/diazepam (0.02–0.08 mg/kg), Dorset lambs (*Ovis aries*, $n = 2$) were placed in a lateral recumbent position. The area overlying the iliac crest was sheared and prepped in a sterile fashion. A Jamshidi™ needle (Carefusion Inc., Franklin Lakes, NJ, USA) was used to cannulate the iliac crest and marrow was aspirated into 20 mL syringes containing 2 mL of heparinized saline (200 units/mL).

2.7. Mononuclear cell enrichment and scaffold seeding

The mononuclear cells were isolated from the aspirated heparinized bone marrow using a previously described closed-disposable filtration system (Pall Corporation, Port Washington, NY USA) [8]. Briefly, the marrow is passed through a filter, enriching the mononuclear cell population. Mononuclear cells were harvested by elution from the filter via retrograde perfusion. TETGs were then affixed on a customized porous mandrel (18 mm outer diameter) using Tegaderm™ (3M). The scaffold was then submerged in a 60 mL aliquot of the pooled, enriched mononuclear cell solution and vacuum seeded as previously described [7,8]. To mimic the scenario of immediate delivery to the operating room for implantation in future preclinical studies, seeded scaffolds were not incubated prior to sampling, rather samples were obtained directly after seeding and processed for DNA assay. Grafts composed of 2:8 PET/PU with either solid or porous C-rings were sampled ($n = 4$ /graft type).

2.8. Cell seeding density via DNA assay

DNA assays were performed using PicoGreen® dsDNA reagent (Invitrogen, Carlsbad, CA, USA). Standard curves (Supplementary Fig. 2) were created using an aliquot of the pre-seeding sample and used to determine the quantity of cells seeded on a 25 mm³ segment of scaffold. Graft samples were obtained at the ends of the graft, in the ringed segments, and in the inter-ring segments.

2.9. Statistics

Data were analyzed using GraphPad Prism 7. Data are represented graphically as mean $\pm$ standard error of the mean (SEM). DNA assay data comparing mean cell density between porous and solid ringed scaffolds were compared with an unpaired parametric two-tailed t -test. Cell seeding densities of ring or inter-ring segments between solid and porous ring designs were compared using an ordinary two-way ANOVA followed by Tukey's correction for multiple comparisons. Type I error was controlled at $\alpha = 0.05$ and p values < 0.05 were considered statistically significant.

3. Results

3.1. Scaffold characterization

Hybrid electrospun scaffolds with 3D-printed C-rings resembled native ovine trachea in gross morphology. uCT analysis of prototype designs demonstrated successful incorporation of both solid and porous C-rings within the electrospun nanofiber material with no evidence of graft delamination (Fig. 2) SEM imaging of 8:2 and 2:8 PET:PU scaffolds revealed qualitative differences in the electrospun fiber size and porosity, with 8:2 PET:PU grafts appearing to have larger fiber diameters and greater pore sizes (Supplementary Figs. 1c–f). Pore inter-connectivity between the two polymer blends was comparable. Qualitative comparison of 2:8 PET:PU scaffolds with solid or porous rings indicated that ring type did not grossly affect nanofiber organization (Supplementary Figs. 1e–h). Interestingly, the void space in ring pores was not occupied by electrospun polymer (Supplementary Fig. 1g).

3.2. Mechanical testing

TETG scaffolds created from 8:2 or 2:8 PET:PU copolymer blends and solid or porous rings were subjected to mechanical characterization and compared to native juvenile sheep trachea (Fig. 3) Anterior-posterior (Fig. 3a) or lateral-medial (Fig. 3b) compression of 8:2 or 2:8 PET:PU scaffolds suggested that the mechanical profile of the 2:8 PET:PU copolymer blend yielded the most biomimetic construct, yet both designs demonstrated supraphysiologic strength compared to native trachea. Compression of the solid or porous rings along the anterior-posterior axis indicated that both rings provided supraphysiologic support, and load-displacement profiles between the two ring types were similar (Fig. 3c). Lateral-medial compression indicated a similar mechanical profile between both ring types and native trachea, while the solid ring yielded a supraphysiologic strength profile. The profiles of the porous ring and native trachea were comparable (Fig. 3d).

3.3. Cell seeding

As the 2:8 PET:PU blend yielded a more biomimetic construct, we elected to abandon further analysis of the 8:2 PET:PU scaffold. Grafts with either solid or porous C-rings were evaluated for their cell seeding characteristics. Solid-ringed 2:8 PET:PU grafts had a significantly increased cell density compared to porous ringed 2:8 PET:PU scaffolds ($9.6 \times 10^4/\text{mm}^3$ vs. $19.2 \times 10^4/\text{mm}^3$, $p = 0.0098$, Fig. 4a) after vacuum seeding. There was no significant difference in cell seeding density of the porous or solid-ringed scaffolds when the segments containing rings were compared ($8.4 \times 10^4/\text{mm}^3$ vs. $12.3 \times 10^4/\text{mm}^3$, $p > 0.05$). In the inter-ring segments, however, solid-ringed grafts yielded a significantly greater cell seeding capacity ($10.3 \times 10^4/\text{mm}^3$ vs. $23.3 \times 10^4/\text{mm}^3$, $p < 0.005$) (Fig. 4b). Interestingly, the cell density of the inter-ring segment from solid-ringed grafts was significantly greater than that of either solid or porous-ringed segments ($18.52 \times 10^4/\text{mm}^3$ vs. $12.34 \times 10^4/\text{mm}^3$ vs. $8.4 \times 10^4/\text{mm}^3$, $p < 0.05$ and < 0.005 , respectively) (Fig. 4b).

4. Discussion

Tissue engineering is a multidisciplinary field with the ideal of creating replacement organs and tissues that are accepted by the host and permanently mimic the form and function of

their target. The promise of this field is especially relevant in the pediatric population as synthetic alternatives are plagued by poor durability and the inability to provide growth capacity [9]. Due to the complexity of managing congenital tracheal anomalies, recent reports have embarked upon the characterization and development of TETG scaffolds for use in neonates and children [10,11]. In the field of pediatric otorhinolaryngology, the trachea represents an ideal target for tissue engineering efforts.

We have previously investigated a prototype tracheal scaffold seeded with bone marrow derived mononuclear cells prior to implantation in a lamb model, but encountered graft stenosis in all animals [7]. Prior to embarking upon further critical *in vivo* studies, comprehensive analysis of candidate prototypes is warranted to identify a scaffold that would yield relevant and informative data once applied in an animal model. The purpose of the current study was to modulate two parameters (electrospun polymer ratio and 3D-printed C-ring design) of our previously investigated TETG to identify a mechanically biomimetic construct with advantageous cell seeding capacity.

In the current study, the ratio of PET to PU was inverted (8:2 vs. 2:8) and we found that the mechanical profile of the 2:8 scaffold more closely matched that of the native ovine trachea. Compression in the anterior/posterior axis revealed that the 2:8 PET:PU scaffold closely mimicked the native trachea sample, whereas the 8:2 blend was appreciably supraphysiologic in load bearing capacity. This marked compliance mismatch could have contributed to the graft failures observed in our previous study. In the lateral-medial axis, both 8:2 and 2:8 PET:PU scaffolds had more load bearing capacity than that of the native trachea, however the 2:8 blend more closely approximated the native. We deemed the mechanical profile of the 2:8 PET:PU scaffold to be superior to that of the 8:2 scaffold in approximating the compliance of the native lamb trachea, thus we selected the 2:8 PET:PU design for further testing and abandoned the 8:2 scaffold.

These results may contradict a recent report by Ajallouei et al., where a 70/30 PET/PU polymer blend was found to provide an electrospun scaffold with biomimetic mechanical properties in a rat model [12]. However, electrospinning is a versatile technique allowing rapid and customizable fabrication of polymer constructs [13–16]; thus, modulations in polymer composition, flow rate, applied voltage, and spinneret to mandrel distance can yield vast differences in a construct's properties, possibly explaining the differences between these two results. Additionally, differences may result from inherent biomechanical differences between rat [17] and ovine trachea, suggesting that studies with human tissue would be required to ensure comparative biomechanical properties for human use.

We incorporated the polycarbonate C-rings into our construct by sequential electrospinning, which provided a uniform material with the embedded supports (Fig. 1). Incorporation of additive manufacturing technologies to biosynthetic scaffolds to mimic the cartilaginous rings of the native trachea has been employed in recent TETG designs [18–20], and a primary evaluation metric is a biomimetic mechanical profile. In the current work, we compared the mechanical behavior of our scaffold and ring designs to that of native ovine trachea, to which an optimized TETG scaffold will be applied in future experiments. No evidence of graft delamination was appreciated during normal handling of the constructs, but

was observed when mincing the scaffolds for DNA quantification. Two ring types were tested, namely solid and “porous” designs. The porous ring was created by removing non-essential polycarbonate material from the solid rings via CAD, creating a bridged support (Fig. 2d–f). Interestingly, ring compression in the anterior/posterior axis demonstrated that despite differences in the amount of polycarbonate used in each design, both had roughly equivalent biomechanical profiles. However, in the lateral/medial compression test, the load vs. displacement curve of the porous rings demonstrated slightly less load bearing capacity than the native ovine trachea, whereas the solid rings either matched native (up to roughly 0.03 in of displacement) or surpassed that of the native sample. These results would suggest that a 2:8 PET:PU scaffold with either porous or solid rings would most closely replicate the biomechanical properties of native sheep trachea.

Many factors contribute to the performance of tissue-engineered constructs. While supraphysiologic characteristics may have contributed to the stenosis witnessed in previous trials, many studies have demonstrated the importance of cell dosage to prevent stenosis. Our previous large animal investigation demonstrated a trend towards improved graft performance with increased cell dosage. Therefore, we considered the optimization of scaffold designs to increase seeded cell dose a criteria worthy of investigation when selecting future candidate prototypes for preclinical evaluation. We expected the porous ringed 2:8 PET:PU scaffolds to surpass their solid-ringed counterparts in terms of cell seeding capacity, but were surprised to find the opposite result. Solid ringed 2:8 PET:PU grafts allowed for a significantly greater seeded cell density than the porous ringed scaffolds. To determine the source of this advantage, we grouped samples from each graft type based on whether the specimen was retrieved from a ringed segment or inter-ring segment. There was no difference in the seeded cell density of ringed segments between porous and solid ringed scaffolds, but the inter-ring segment of the porous-ringed scaffolds was observed to have significantly more seeded cells than its porous counterpart of either of the ring segment groups. We interpret these data to suggest that the porous ring did not allow for any greater cell seeding capacity within ringed regions, and some cells may have passed through the pores of the ring and subsequently were not seeded. On the contrary, this phenomenon did not occur with the solid rings, as they may have coerced more cells through the inter-ring segments of the scaffold once the ringed segments reached saturation. Taken together, the biomechanical and seeding capacity analyses suggest that the 2:8 PET:PU solid-ringed scaffold is more biomimetic and supports increased BM-MNC density via vacuum seeding compared to others tested. We intend to continue preclinical investigation of this construct *in vivo* utilizing a juvenile lamb model of TETG implantation.

While the experiments outlined in this report are observational and exploratory, we have validated meaningful *in vitro* standards (DNA assay and compression testing) by which to assess prototypical TETG scaffolds and manipulated polymer blend and C-ring design to create a biosynthetic tracheal construct that approaches these benchmarks. A paradigm of this nature may best be investigated using a computational modeling approach. Indeed, constitutive biomechanical models of the human trachea have already been proposed [21]. Applying these models to assess the effect of modulating TETG design parameters by using initial sets of data to perform sensitivity studies and evaluate the myriad combinations of polymer blends and ring designs, among other scaffold variables (such as porosity, wall

thickness, and degradation rate) *in silico*, may influence future scaffold prototype design. Ideally, an “optimized” TETG could then be validated *in vivo*. We propose that the data presented in this report are a first step toward the rational design of an optimal TETG scaffold that could be translated to the clinical realm.

5. Conclusion

An electrospun TETG scaffold comprised of a 2:8 ratio of PET:PU with solid polycarbonate C-rings offers biomechanical properties similar to that of native trachea, while also permitting appropriate cell seeding capacity. Evaluation of the performance of this biosynthetic construct in a large animal model would inform the rational design of an optimized TETG scaffold.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ijporl.2017.10.036>.

References

1. Munzón GB, Martínez Ferro M. Pediatric tracheal stenosis and vascular rings. *Toraks Cer Bult*. 2012; 3:206–219. <http://dx.doi.org/10.5152/tcb.2012.30>.
2. Grillo HC. Surgery of the Trachea and Bronchi. 2004
3. Chiang T, Pepper V, Best C, Onwuka E, Breuer CK. Clinical translation of tissue engineered trachea grafts. *Ann Otol Rhinol Laryngol*. 2016; 125:873–885. <http://dx.doi.org/10.1177/0003489416656646>. [PubMed: 27411362]
4. Elliott MJ, De Coppi P, Spegginorin S, Roebuck D, Butler CR, Samuel E, et al. Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year followup study. *Lancet*. 2012; 380:994–1000. [http://dx.doi.org/10.1016/S0140-6736\(12\)60737-5](http://dx.doi.org/10.1016/S0140-6736(12)60737-5). [PubMed: 22841419]
5. Gonfiotti A, Jaus MO, Barale D, Baiguera S, Comin C, Lavorini F, et al. The first tissue-engineered airway transplantation: 5-year follow-up results. *Lancet*. 2014; 383:238–244. [http://dx.doi.org/10.1016/S0140-6736\(13\)62033-4](http://dx.doi.org/10.1016/S0140-6736(13)62033-4). [PubMed: 24161821]
6. Hamilton NJ, Kanani M, Roebuck DJ, Hewitt RJ, Cetto R, Culme-Seymour EJ, et al. Tissue-engineered tracheal replacement in a child: a 4-year follow-up study. *Am J Transplant*. 2015; 15:2750–2757. <http://dx.doi.org/10.1111/ajt.13318>. [PubMed: 26037782]
7. Clark ES, Best C, Onwuka E, Sugiura T, Mahler N, Bolon B, et al. Effect of cell seeding on neotissue formation in a tissue engineered trachea. *J Pediatr Surg*. 2016; 51:49–55. <http://dx.doi.org/10.1016/j.jpedsurg.2015.10.008>. [PubMed: 26552897]
8. Kurobe H, Maxfield MW, Naito Y, Cleary M, Stacy MR, Solomon D, et al. Comparison of a closed system to a standard open technique for preparing tissue-engineered vascular grafts. *Tissue Eng Part C Methods*. 2015; 21:88–93. <http://dx.doi.org/10.1089/ten.tec.2014.0160>. [PubMed: 24866863]

9. Kojima K, Vacanti C. Tissue engineering in the trachea. *Anatomical Rec.* 2014; 297:44–50. <http://dx.doi.org/10.1002/ar.22799>.
10. Mansfield EG, Greene VK Jr, Auguste DT. Patterned, tubular scaffolds mimic longitudinal and radial mechanics of the neonatal trachea. *Acta Biomater.* 2016; 33:176–182. <http://dx.doi.org/10.1016/j.actbio.2016.01.034>. [PubMed: 26821338]
11. Teoh GZ, Crowley C, Birchall MA, Seifalian AM. Development of resorbable nanocomposite tracheal and bronchial scaffolds for paediatric applications. *Br J Surg.* 2015; 102:e140–e150. <http://dx.doi.org/10.1002/bjs.9700>. [PubMed: 25627127]
12. Ajallouieian F, Lim ML, Lemon G, Haag JC, Gustafsson Y, Sjöqvist S, et al. Biomechanical and biocompatibility characteristics of electrospun polymeric tracheal scaffolds. *Biomaterials.* 2014; 35:5307–5315. <http://dx.doi.org/10.1016/j.biomaterials.2014.03.015>. [PubMed: 24703872]
13. Bridge JC, Aylott JW, Brightling CE, Ghaemmaghami AM, Knox AJ, Lewis MP, et al. Adapting the electrospinning process to provide three unique environments for tri-layered *in vitro* model of the airway wall. *J Vis Exp.* 2015; 101:e52986. <http://dx.doi.org/10.3791/52986>.
14. Nam J, Huang Y, Agarwal S, Lannutti J. Improved cellular infiltration in electrospun fiber via engineered porosity. *Tissue Eng.* 2007; 13:2249–2257. <http://dx.doi.org/10.1089/ten.2006.0306>. [PubMed: 17536926]
15. Markowski, J., Magiera, A., Lesiak, M., Sieron, A., Pilch, J., Blazewicz, St. Preparation and characterization of nanofibrous polymer scaffolds for cartilage tissue engineering; *J Nanomater.* 2015. p. 1-9. <http://dx.doi.org/10.1155/2015/564087>
16. Zhang Q, Li Y, Lin ZY, Wong KKY, Lin M, Yildirim L, Zhao X. Electrospun polymeric micro/nanofibrous scaffolds for long term drug release and their biomedical applications. *Drug Discov Today.* 2017; 22:1351–1366. <http://dx.doi.org/10.1016/j.drudis.2017.07.007>. [PubMed: 28552498]
17. Jungebluth P, Haag JC, Sjöqvist S, Gustafsson Y, Rodríguez AB, Gaudio CD, et al. Tracheal tissue engineering in rats. *Nat Protoc.* 2014; 9:2164–2179. <http://dx.doi.org/10.1038/nprot.2014.149>. [PubMed: 25122525]
18. Goldstein TA, Smith LP, Smith BD, Grande DA, Zeltsman D. Integrating biodegradable 3-dimensional-printing into tracheal reconstruction. *Ojrm.* 2015; 04:27–33. <http://dx.doi.org/10.4236/ojrm.2015.43004>.
19. Johnson C, Sheshadri P, Ketchum JM, Narayanan LK, Weinberger PM, Shirwaiker RA. In vitro characterization of design and compressive properties of 3D-biofabricated/decellularized hybrid grafts for tracheal tissue engineering. *J Mech Behav Biomed Mater.* 2016; 59:572–585. <http://dx.doi.org/10.1016/j.jmbbm.2016.03.024>. [PubMed: 27062124]
20. Ott, LM., Zabel, TA., Walker, NK., Farris, AL., Chakroff, JT., Ohst, DG., et al. Mechanical evaluation of gradient electrospun scaffolds with 3D printed ring reinforcements for tracheal defect repair; *Biomed Mater.* 2016. p. 1-16. <http://dx.doi.org/10.1088/1748-6041/11/2/025020>
21. Safshekan F, Tafazzoli-Shadpour M, Abdouss M, Shadmehr M. Mechanical characterization and constitutive modeling of human trachea: age and gender dependency. *Materials.* 2016; 9:456–513. <http://dx.doi.org/10.3390/ma9060456>.

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.

Fig. 2.

Micro-computed tomography reconstructions of TETG designs.

3D images rendered in Simpleware of solid (A–C) and porous (D–F) ringed scaffolds. Solid (A) or porous (D) rings were encapsulated in electrospun PET/PU (polyethylene terephthalate/polyurethane) nanofibers in either a 2:8 or 8:2 PET:PU ratio. Final scaffold with solid rings (B) and porous rings (E). High magnification view of a ringed graft segment with either solid (C) or porous (F) rings.

Fig. 3.

Uniaxial mechanical testing of TETG scaffold designs.

Load-displacement curves representing 75% compression of 8:2 and 2:8 PET:PU (polyethylene terephthalate: polyurethane) scaffolds on the anterior-posterior (AP) (A) and lateral-medial (LM) (B) axes in comparison to native ovine trachea. Load-displacement curves of 75% compression of solid and porous rings in comparison to a cartilage ring of the native ovine trachea on the anterior-posterior (AP) (C) and lateral-medial (LM) (D) axes.

Fig. 4.

Cell seeding comparison between porous and solid ringed 2:8 PET:PU scaffolds.

PicoGreen dsDNA (double stranded DNA) assay was utilized to evaluate the effect of ring structure on seeded cell density. Values were interpolated from a standard curve made from serially diluted aliquots of known cell concentrations (manual cell count) from the pre-seeding solution. One curve was generated for each experiment. (A) Comparison of average seeded cell density between porous and ringed scaffolds ($n = 4$ scaffolds per group, each scaffold was sampled $n = 8$ times for analysis). (B) Division of graft samples by ringed or inter-ring segment to evaluate the effect of ring design on homogeneity of scaffold seeding. ns: not significant, * $p < 0.05$, ** $p < 0.005$