Circumferential Three-Dimensional–Printed Tracheal Grafts: Research Model Feasibility and Early Results

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Background. Methods for tracheal graft research have presented persistent challenges to investigators, and three-dimensional (3D)–printed biosynthetic grafts offer one potential development platform. We aimed to develop an efficient research platform for customizable circumferential 3D-printed tracheal grafts and evaluate feasibility and early structural integrity with a large-animal model.

Methods. Virtual 3D models of porcine subject tracheas were generated using preoperative computed tomography scans. Two designs were used to test graft customizability and the limits of the construction process. Designs I and II used 270-degree and 360-degree external polycaprolactone scaffolds, respectively, both encompassing a circumferential extracellular matrix collagen layer. The polycaprolactone scaffolds were made in a fused-deposition modeling 3D printer and customized to the recipient’s anatomy. Design I was implanted in 3 pigs and design II in 2 pigs, replacing 4-ring tracheal segments. Data collected included details of graft construction, clinical outcomes, bronchoscopy, and gross and histologic examination.

Results. The 3D-printed biosynthetic grafts were produced with high fidelity to the native organ. The fabrication process took 36 hours. Grafts were implanted without immediate complication. Bronchoscopy immediately postoperatively and at 1 week demonstrated patent grafts and appropriate healing. All animals lived beyond a predetermined 1-week survival period. Bronchoscopy at 2 weeks showed significant paraanastomotic granulation tissue, which, along with partial paraanastomotic epithelialization, was confirmed on pathology. Overall survival was 17 to 34 days.

Conclusions. We propose a rapid, reproducible, resource-efficient method to develop various anatomically precise grafts. Further graft refinement and strategies for granulation tissue management are needed to improve outcomes.


Efforts to develop circumferential tracheal replacement grafts present ongoing challenges to investigators. And because previous tracheal replacement methods using prosthetic grafts, allotransplantation, xenotransplantation, and autologous tissue reconstruction have all met with significant barriers to routine use in clinical settings [1-5], new platforms for graft development are needed. Investigators have more recently turned toward tissue-engineering techniques to develop bioengineered grafts, including early work using decellularized native tracheal grafts [6-8]. However, limited donor availability and the complex decellularization process hamper the research necessary to overcome challenges related to graft malacia, granulation tissue formation, stenosis, and stent-dependence [9].

Biosynthetic scaffolds offer an alternative research model to decellularized native grafts and are not constrained by decellularization process and donor availability issues. They can potentially provide early structural support and then degrade over time, allowing neotissue formation [9]. Several previous studies, using a wide variety of materials, have investigated biosynthetic repair of partial tracheal defects in various animal models [10-21], and a few studies have investigated circumferential graft models [22-25], without clearly demonstrating superiority of any specific approach [9]. Recent work has shown survival up to several weeks using resource- and time-intensive techniques, including circumferential cell-seeded nanofiber scaffolds [26] and acellular tissue-stent biocomposite tracheal grafts [27]. We set out to develop an efficient research platform using three-dimensional (3D) printing.
to fabricate and study various customizable circumferential tracheal replacement designs in a large-animal model. Ideally, this platform would facilitate quick graft design and construction, be easily customizable to patient size and anatomy, offer wide variability in potential materials used, and provide a venue to efficiently study tracheal granulation tissue formation.

In this pilot study, we investigated combining an extracellular matrix (ECM) dermal collagen layer with a 3D-printed polycaprolactone (PCL) frame to establish a process for creating customized circumferential biosynthetic tracheal grafts. ECM is a readily available biocompatible collagen-rich scaffold derived from natural sources that can support epithelialization and neovascularization [28] but lacks the structural rigidity to resist circumferential compression. PCL is a relatively rigid biocompatible polymer [29, 30] that can resist external airway compression and can be customized to graft recipient’s anatomy using commercially available 3D printing technology. Building on our previous work repairing partial tracheal defects [31] and then capitalizing on the complementary properties of ECM and PCL, our primary goals here were to evaluate the technical feasibility of a platform to rapidly develop customized biosynthetic tracheal grafts to facilitate further research, and secondarily, to evaluate early (7-day) structural integrity in a porcine model.

Material and Methods

Graft Design and Customization

Chest computed tomography (CT) imaging of the porcine subjects was used to generate virtual models of the trachea in Vitrea software (Vital Images Inc, Minnetonka, MN) to determine the dimensions of the graft. Two graft designs were trialed in this study. In group I, the graft design incorporated an external PCL scaffold wrapping 270 degrees (Fig 1A) around a circumferential ECM dermal collagen layer. The design in group II used a 360-degree external PCL scaffold (Fig 1B) around an ECM dermal collagen layer. The inner diameter of the graft ranged from 11 to 14 mm, depending on the anatomy of the porcine subject. These design variations were used to test the customizability and limits of the construction process, to reflect small differences between pig and human anatomy, and finally, to assess for preliminary differences in graft integrity and durability.

3D Printing of Graft Components

The PCL components of the tracheal graft constructs were designed in SOLIDWORKS (Dassault Systèmes, Waltham, MA), based on tracheal dimensions obtained by CT scans of the cervical trachea for each subject. Stereolithography (.STL) files of the designed segments were imported into MakerWare software (MakerBot Industries, Brooklyn, NY) and printed in a MakerBot Replicator 2 (MakerBot Industries) 3D printer. The 3D-printed PCL segments were extruded at 150°C. The PCL segments were then allowed to cool and crystallize at room temperature before sterilization.

Graft Preparation Before Implantation

The 3D-printed PCL segments were sterilized in 10% hydrogen peroxide and washed three times using 1X sterile phosphate-buffered saline. Decellularized 2-mm-thick bovine dermal ECM (Surgimend; Integra LifeSciences, Plainsboro, NJ) was hydrated in warm cell growth media and then secured to the PCL using 4-0 poly-(ethylene tetraphthalate) sutures (Ethicon, Somerville, NJ; Fig 2).

In Vivo Model and Animal Care

Juvenile female Yucatan miniature pigs were used in this model, with a wide variation in size and weight (18 to 50 lbs), which provided variability in tracheal anatomy and the requisite graft dimensions. The Institutional Animal Care and Use Committee of Icahn School of Medicine at Mount Sinai, New York, NY, approved the protocol for animal care. Experiments were conducted in compliance with the “Guide for the Care and Use of Laboratory Animals” recommended by the United States National Institutes of Health.

Surgical Implantation, Surveillance, and Evaluation

Each animal was sedated and intubated and placed supine on the operating table. The neck was scrubbed with alcohol and povidone iodine and draped in a sterile fashion. After a cervical midline incision and separation of strap muscles, adequate exposure of the upper trachea was obtained. A 4-ring segment was removed (Fig 3) and reconstructed with the 3D-printed tracheal graft. Cross-table ventilation was used during the creation of the proximal anastomosis.

Once the proximal anastomosis was complete, the endotracheal tube was readvanced through the 3D-printed tracheal graft into the distal trachea, and the distal anastomosis was completed over the endotracheal tube. The anastomoses were performed using interrupted 4-0 poly-(ethylene tetraphthalate) sutures. An airtight seal was assured before the strap muscles were approximated. A Penrose drain was placed, and the incision was closed in layers.

Animals were extubated in the operating room, recovered in a monitored setting, and subsequently moved to standard pens in the animal facility. Perioperative antibiotics and pain medications were managed and administered by the veterinary team. The drain was removed on postoperative day 2 or 3.

Flexible bronchoscopy and balloon tracheoplasty (as needed) were performed under sedation while monitoring graft integration and airway patency weekly. Based on our primary objective to assess feasibility, as well as on previous reports of survival from ranging from 3 to 7 days in pigs implanted with 3D-printed PCL tracheal splints [32] and the history of stent dependence for bioengineered airways, we determined 7 days as the minimum successful survival for this pilot study. Tracheas were harvested at death or study end point.
Results

Graft Design and Construction
Two graft designs were constructed for implantation, as previously described. The 3D printing process yielded a precise PCL scaffold that did not require further processing, apart from sterilization, before the PCL was secured to the ECM layer. Neither graft design proved more difficult to construct than the other. The complete fabrication process of these grafts, beginning with CT imaging, continuing through construction, and including time for sterilization, can be completed in 36 hours. Figure 2 shows a graft after construction and before implantation.

Outcomes Summary
A total of 5 cervical tracheal segment replacements (group I, n = 3; group II, n = 2) are reported here. The grafts of both designs were implanted without perioperative or immediate postoperative complication and without the need for perioperative stenting. All animals lived beyond the predetermined 7-day minimum successful survival period. Animal 1 died of pneumonia on postoperative day (POD) 30 (confirmed at necropsy). Animal 2 died on POD 17 from respiratory failure as a result of granulation tissue at the distal anastomosis. Animal 3 was euthanized on POD 18 due to failure to thrive. Animals 4 and 5 died on POD 24 and 34, respectively, secondary to granulation tissue formation and secretions. Outcomes are summarized in Table 1.

Surveillance Bronchoscopy Evaluation and Balloon Tracheoplasty
Immediate postoperative bronchoscopy demonstrated a patent graft in good position without collapse (Fig 4A). All animals had patent airways at 1 week postoperatively, with appropriate anastomotic healing and a patent airway (Fig 4B). There was some mucus, but no malacia. Significant granulation tissue formation was observed by the end of the second week after implantation (Fig 4C).
Balloon tracheoplasty was performed in animals 2 and 3 in Table 1.

Gross and Histologic Evaluation of Explanted Grafts
On explant, the grafts were well incorporated with the native tissue. Granulation tissue was seen in all cases. Fibrinous material was seen covering the luminal surface of the graft, and cytology revealed a paucicellular sample of plump spindle cells, few ciliated epithelial cells, and few goblet cells with abundant mucinous material. The PCL tracheal segment remained intact. Histology confirmed significant granulation tissue with active fibroblasts, extensive vascularization, and abundance of neutrophils (Fig 5A). Necrotic foci were also noted abutting the external scaffold between the scaffold and newly formed granulation tissue. Epithelialization of the lumen was weakly noted near the anastomotic sites (Fig 5B) but not the center of the graft.

Comment
In this study, we developed an approach to design, produce, and test anatomically precise circumferential 3D-printed tracheal grafts and used a large-animal model to test feasibility. Our graft design is a hybrid, combining the 3D-printed PCL scaffold with an inner layer of ECM. ECM provides a biocompatible environment for cell attachment, proliferation, and differentiation that has been routinely used in tissue reconstruction in other clinical settings. We previously demonstrated repair of anterior tracheal defects in a porcine model using a similar ECM [31]. PCL is a relatively rigid, biodegradable, biocompatible polymer that can be customized to graft recipient’s anatomy using readily available 3D printing technology [29, 30] and can give structural integrity to the circumferential tracheal graft.

The process for graft construction described here is easily reproducible, time efficient, and maximizes the use of resources, making it feasible for potential clinical use. The porcine subject airway dimensions were obtained from preoperative CT images. These dimensions were used to create an appropriately sized virtual model of the proposed graft structure, which was then used to 3D print the PCL frame. 3D printing offers a remarkably resource-efficient method to create customized constructs of various materials, and the low relative cost of this technique has been described previously for other applications [33]. Standard suture techniques were used to secure the ECM to the PCL frame. This pilot graft construction process, from initial imaging to graft implantation, can be completed in approximately 36 hours.

The graft designs used in this study were varied for multiple reasons. In group I, the graft design combined a 270-degree external PCL scaffold around an internal circumferential ECM dermal collagen layer. Group II differed, in that the external PCL scaffold encompassed the entire 360-degree circumference. Of note, the cartilaginous tracheal rings in humans are C shaped and extend approximately 270 degrees around the tracheal circumference (similar to the graft used in group I), whereas pig tracheas are more cartilaginous [34], and the rings extend nearly 360 degrees, with a very narrow

![Fig 4.](image-url)

(A) Surveillance bronchoscopy performed immediately after implantation demonstrates a patent lumen. (B) Bronchoscopy performed 1 week postoperatively demonstrates a patent lumen with appropriate healing and some mucus. (C) Bronchoscopy performed 2 weeks postoperatively shows significant granulation tissue formation.
membranous attachment posteriorly (similar to the graft used in group II). These design variations were used to test the customizability and limits of the construction process as well as to assess for differences in graft performance and durability in vivo. Neither graft design proved more difficult to construct than the other, and the ability to easily adjust the graft design demonstrates the primary advantage of using 3D printing technology.

Feasibility of this technique was shown, as all animals survived and the 3D-printed PCL scaffolds maintained their structural integrity beyond the preset period of 7 days. However, there were significant challenges and limitations encountered with the grafts in this pilot study, which contributed to the observed causes of death. Survival among group I was 30, 18, and 17 days, and survival in group II was 34 and 24 days. This sample size was too small to make definitive determinations about design superiority, but both designs clearly encountered significant issues with granulation tissue formation and secretion clearance.

Granulation tissue formation was significant, especially by POD 14 (Fig 4C). This granulation tissue was not adequately responsive to balloon dilation, and other endoscopic techniques using energy or cryotherapy were not yet available in our research setting. Future work will capitalize on the expeditious, efficient nature of the method described here to study both existing and new local and systemic methods for preventing and managing tracheal granulation tissue formation.

Because this was a preliminary study testing feasibility, we used acellular grafts to replace the circumferential segments of trachea we removed, without cell seeding or attempting preimplantation epithelialization. Compromised mucociliary clearance after tracheal replacement causes retention of secretions at the site of anastomosis, resulting in infection and airway obstruction. Furthermore, although in vivo epithelialization is possible for partial acellular grafts due to migration of surrounding epithelium, this is likely not sufficient when large circumferential segments of trachea are replaced. A functional epithelial lining of the tracheal graft is necessary for adequate clearance of airway secretions, and this was clearly a limitation in this pilot study using an acellular graft.

One possible technique for preimplantation graft epithelialization was recently described by Butler and colleagues [35], who demonstrated a method to culture and populate large numbers of functional airway basal epithelial onto a tracheal scaffold model. This is an area of ongoing investigation. However, the aim of our work described here was first to demonstrate that anatomically accurate circumferential tracheal grafts can be reliably produced using image-guided 3D printing and can maintain early postimplantation structural integrity without immediate perioperative stenting in a large-animal model.

In conclusion, 3D-printed circumferential tracheal grafts can repair segments of trachea in a porcine model in the short-term. Our approach offers a rapid, reproducible, and resource-efficient technique to create and test anatomically precise grafts of various designs. Ongoing investigations, capitalizing on the expeditious and efficient nature of the model described, are focused on developing and testing various tissue-engineered tracheal grafts as well as studying tracheal granulation tissue formation.

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