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Bridging the Gap: Using 3D Printed Polycaprolactone Implants to Reconstruct Circumferential Tracheal Defects in Rabbits

David S. Chan, MD; Nathalie Gabra, MD, FRCSC; Ayesha Baig, MD; John J. Manoukian, MD, FRCSC; Sam J. Daniel, MD, MSc, FRCSC

Objective: 1) To assess the feasibility of reconstructing 2-cm-long circumferential tracheal defects with a 3D printed polycaprolactone (PCL) implant in rabbits. 2) To evaluate endoscopic, histologic, and functional characteristics of a PCL tracheal implant over time.

Methods: Ten New Zealand rabbits were included in this study. A 2-cm-long 3D printed PCL tracheal implant was created. All rabbits underwent surgical excision of a 2-cm-long cm segment of cervical trachea, which was reconstructed with the implant. Rabbits were sacrificed at the following time points: 0, 4, 5, 6, and 7 weeks postoperatively. At these time points, a rigid bronchoscopy was performed, and blinded evaluators calculated the percentage of airway stenosis. The tracheas were then harvested and prepared for histologic analysis.

Results: All rabbits survived to their date of sacrifice except for one. Rabbits were euthanized between 0 to 54 days postoperatively with a median of 30 days. All rabbits developed significant granulation tissue with an average percentage stenosis of 92.3% ± 6.1%. On histology, granulation was present with extensive neovascularization and mixed inflammatory cells. There was re-epithelialization present on the luminal surface of the PCL implant near the anastomoses but absent at the center of the implant.

Conclusion: This study demonstrates that our 2-cm-long 3D printed PCL tracheal implant can be used to reconstruct a tracheal defect of equivalent size in a New Zealand rabbit model in the short term. However, significant granulation tissue formation limits long-term survival. Further research is warranted to limit the granulation tissue overgrowth.

Key Words: PCL (polycaprolactone), tracheal reconstruction, 3D-printed.

Level of Evidence: NA

INTRODUCTION

The trachea is a vital organ that can be affected by various medical conditions. Congenital and acquired tracheal pathology can cause significant symptoms and even death. Conditions include tracheomalacia, tracheal stenosis from prolonged intubation, autoimmune conditions, and cancers that arise from or invade the trachea. Occasionally, segmental tracheal resection may be necessary as part of the treatment plan. Surgical options to repair the trachea following a segmental resection can be very challenging and subject patients to high operative risks. In a short defect, primary anastomosis is possible with or without a suprahyloid release. Currently, a slide tracheoplasty that carries a mortality rate ranging between 5% to 30%.1,2 is the conventional option for tracheal stenosis and longer segment tracheal defects. Other options when such techniques have failed or are not feasible include tracheal stents or mediastinal tracheostomies.3 Interestingly, Delaere et al. and the Leuven Tracheal Transplant Group have recently described six successful cases of tracheal allotransplantation in human patients for the use of long-segment defects. A trachea from a deceased donor was placed and wrapped in the recipient’s forearm fascia. After several months, an orthotopic transplantation was performed of the trachea with a vascularized forearm free flap.4,5

Multiple authors have also investigated different combinations of polymers, stem cells, and growth factors in the hopes of designing a tracheal substitute to reconstruct tracheal defects in animal models. Because of its favorable characteristics, polycaprolactone (PCL) is one of the polymers that has gained popularity for its evaluation in airway reconstruction in animal models. It has strong tensile properties, a low melting point, and allows for cellular growth along its surface. PCL can also easily be 3D printable, which allows for a customizable solution to reconstruct tracheal defects.6 PCL has also successfully been used in select human pediatric cases as external tracheal splints for severe tracheomalacia.7,8

The use of stem cells has gained popularity in organ regeneration in the last few decades, the trachea included. PCL has been embedded with different types of
stem cells, including epithelial cells, bone marrow stem cells, chondrocytes, and human turbinate mesenchymal stem cells. Tracheal defects reconstructed with PCL have ranged in size from partial wall defects to full circumferential (360°) defects in animal models. Partial reconstructed defects using PCL, regardless of the cellular additive, seem to have good results with appropriate cellular regeneration and minimal granulation tissue formation at the level of the implant. On the other hand, complete circumferential defects reconstructed with PCL in the majority of cases were limited by the significant granulation tissue formation, especially with longer PCL implants, regardless whether stem cells were used.

Interestingly, few studies have compared PCL implants with biologic additives to bare PCL implants in the reconstruction of circumferential tracheal defects in animal models. Also, the true clinical value for stem cells applied to a nonvascularized scaffold is unknown. We believe it is imperative to evaluate the baseline reaction to the bare PCL implant to be able to adequately assess adding biologic factors to it or as using it as a scaffold for a vascularized flap.

Thus, the objectives of this study are twofold: 1) to assess the feasibility of reconstructing 2-cm-long circumferential tracheal defects with a 3D printed PCL implant in New Zealand rabbits; and 2) to evaluate endoscopic, histologic, and functional characteristics of a PCL tracheal implant over time.

MATERIALS AND METHODS

**Implant**

A 3D model was designed with the Autodesk AutoCAD software 2018, (Autodesk, San Rafael, California) (Fig. 1A) and then 3D-printed (Fig. 1B) using the Prusa i3 MK2 desktop printer (Prusa Research, Prague, Czech Republic) with 1.75-mm-thick PCL 3D filament. The implant was designed to approximate the tracheal size of a 3.2 kg rabbit with the following dimensions: height of 20 mm, inner diameter of 5.27 × 7 mm, and a wall thickness of 0.9 mm.

**Animals and Surgical Implantation**

Ten New Zealand rabbits were used for our animal model. All protocols were performed in accordance with the guidelines of the Animal Care Ethics Committee of the McGill University Health Center in Montreal, Quebec, Canada. The rabbits were premedicated with a combination of ketamine and midazolam and then induced and maintained with isoflurane. They were then intubated, placed in a supine position, and shaved on the anterior surface of their neck and thorax. Rabbits were prepped and draped to maintain sterility. A midline vertical incision was made, and the strap muscles were divided down the midline until the trachea was exposed. Careful blunt dissection was performed circumferentially around the trachea until it was completely freed from the esophagus (Fig. 2A). A stay suture was placed in the distal cervical trachea to keep it from retracting into the thorax. The trachea was incised, and a second endotracheal tube was placed into the distal trachea for ventilation...
At this time, a 2 cm segment of the cervical trachea was resected proximally.

Anastomosis of the implant to the native trachea was done with 5-0 PDSII (Ethicon, Somerville, NJ) simple interrupted sutures at the proximal end, ensuring that the knots were on the external surface of the trachea. We then removed the second endotracheal tube in the distal trachea, and the original endotracheal tube was advanced until it was through the PCL implant and into the distal trachea. The distal segment was then anastomosed with interrupted 5-0 PDSII sutures (Ethicon, Somerville, NJ) around the endotracheal tube (Fig. 2C). The strap muscles were approximated with 4-0 Vicryl (Ethicon, Somerville, NJ), and the skin was closed with interrupted 4-0 Prolene stitch (Ethicon, Somerville, NJ).

The rabbits were extubated and brought to the animal care facility, where they remained in their isolated cage and monitored daily for respiratory distress, weight changes, and adequate food intake with their regular care. They received enrofloxacin antibiotics for 1 week as well as meloxicam for pain postoperatively for 3 days. Prolene (Ethicon, Somerville, NJ) skin sutures were removed on postoperative day 7.

**Evaluation**

To progressively analyze the implant over time in the short term. Rabbits were divided into five groups, with two rabbits in each group, which corresponded to the time points at which the rabbits were evaluated by bronchoscopy (0 [control], 4, 5, 6, 7 weeks postoperative). Following bronchoscopy, rabbits were euthanized and their tracheas harvested.

**Bronchoscopy**

Rabbits were sedated with ketamine, xylazine, and acepromazine prior to undergoing rigid bronchoscopy with spontaneous ventilation. Bronchoscopy was performed with a 0° 2.7 mm x 175 mm rigid endoscope, and photographs of the airway were taken above the proximal anastomosis. The grade of stenosis for each rabbit was calculated as a percentage of narrowing of the tracheal lumen using the photographs. Using ImageJ software v1.3 (National Institutes of Health, Bethesda, MD), two reviewers were asked to manually outline the lumen of the stenosed trachea and the lumen of the native trachea separately using the freehand selection tool, thereby computing the relative areas. A similar technique has been described in previous studies.10,11 This process was repeated three times, and an average was taken. We determined the percentage of stenosis by calculating the luminal area of the stenosed trachea area over the estimated area of the nonstenosed trachea. Finally, the average of the percentage of stenosis was taken between the 2 reviewers. All reviewers performed well over 20 rigid bronchoscopies in human patients, which meets the training requirements to be competent as per the American Thoracic Society, American College of Chest Physicians, and the European Respiratory Society.12 It was decided to grade the stenosis based on images as opposed to the more traditional clinical methodology of sizing the airway. This was done because the airway narrowing in the rabbits is often quite small and we did not want to risk disrupting the granulation tissue to preserve the histologic features.

**Histology**

Tracheal segments were fixed in 10% neutral formalin solution and then processed by dehydration through ethanol, cleared in xylene, and embedded in paraffin blocks by our histopathology department. Standard 4-μm-thick axial segments were taken at 5 points along the specimen, one section on each side of the anastomosis as well as one section in the center of the PCL implant. Each segment underwent hematoxylin and eosin staining and was analyzed by a pathologist.

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Fig. 3. Examples of photographs taken during rigid bronchoscopy. (A) Rabbit 4: 4 weeks postoperatively. (B) Rabbit 9: 7 weeks postoperatively.

**TABLE I. Rabbit Characteristics.**

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**Fig. 3.** Examples of photographs taken during rigid bronchoscopy. (A) Rabbit 4: 4 weeks postoperatively. (B) Rabbit 9: 7 weeks postoperatively.
RESULTS

Ten New Zealand male rabbits weighing 2.64 to 3.35 kg underwent surgical implantation of the PCL implant. Rabbits were euthanized between 0 and 54 days postoperatively, with a median of 30 days (Table I). Rabbit 6 died of an aspiration pneumonia on postoperative day 5. Bronchoscopy was performed on all rabbits except for rabbit 6. All rabbits were found to have significant stenosis at the proximal anastomosis site, and whitish fibrinous material was noted on the luminal surface of the PCL implants (Fig. 3A–3B). The average percentage of stenosis following surgery ranged between 83% and 98% (Table II), with a mean of 92.3% ± 6.1%. The Spearman rank demonstrated a strong correlation between the two reviewers (Rho = 0.988). There was no apparent trend in the percentage of stenosis over time ($r^2 = 0.0019$).

All tracheas were grossly examined once they were harvested. The PCL implants appeared to be well incorporated to the native rabbit tracheas without any evidence of dehiscence or collapse. Histopathological analysis revealed granulation tissue with extensive neovascularization in the tracheal tissue around the PCL implant in all rabbits after 4 weeks (Fig. 4A–4B). Mixed inflammatory cells, including neutrophils, lymphocytes, histiocytes, eosinophils, and plasma cells were identified (Fig. 4C). There was submucosal hyperplasia caused by proliferation of fibroblasts; foci of cartilage were also noted but likely produced by fibroblast-like cells. Re-epithelialization was present on the luminal surface of the PCL implant, mostly noted near the anastomoses (Fig. 4D) but not at the center of the implant. On average, epithelium was mixed type (columnar, columnar with goblet cells, and stratified squamous epithelium). Based on the qualitative evaluation of the slides, it was difficult to perceive a significant difference in histology between postoperative weeks 4 to 7.

DISCUSSION

A New Zealand rabbit model was used for this study for several reasons. Some authors argue that it is an ideal model for tracheal reconstruction due to its anatomical similarity to humans and the availability of tracheal segments for grafting. The results from this study suggest that the PCL implant is well integrated into the native rabbit trachea, with a high degree of stenosis and significant inflammatory response. Future studies may focus on optimizing the implant design and surgical technique to further improve outcomes.
model for tracheal surgery because of its similarities to a human infant trachea in terms of structure and size. Rabbits have a long cervical trachea that is easily surgically accessible. In addition, New Zealand Rabbits are easily obtained for research purposes and relatively inexpensive to purchase and house. Rabbits also have a more diverse genetic background compared to other animals, which approximates that of humans. PCL implants have recently been used to reconstruct different-sized tracheal defects in animal models, with few studies evaluating complete circumferential reconstructions. To our knowledge, the longest circumferential tracheal reconstruction in a rabbit model with a PCL implant has been 2 cm in longitudinal length, which matches the size of our implant. The total length of the rabbit trachea ranges between 5 to 6 cm; therefore, a 2 cm implant corresponds to a reconstruction of 30% to 40% defect. This length of resected trachea usually corresponds to the limit at which primary anastomosis can no longer be performed in the pediatric population.

Despite not having any cellular components to our implants, the results in our study are similar to those in some studies in the literature. For example, Kaye et al. impregnated a 2 cm, 270° PCL scaffold with hyaline chondrocytes prior to tracheal reconstruction in six rabbits. The subjects were divided into two equal groups that were sacrificed at 3 or 6 weeks postoperatively. Their average intraluminal stenosis was 83%. In our study, we calculated an average stenosis of 92%; however, our end points were later, which may have contributed to further stenosis. We also noted that this can start to occur prior to 4 weeks post-PCL implantation. Gao et al. reconstructed tracheal defects in rabbits using a 1.6 cm chondrocyte-treated PCL implant in two groups, which differed in lengths of time spent suspended in the chondrocyte culture. In 75% of the rabbits, the cause of death was determined to be caused by granulation tissue formation, with a mean survival time of 14 and 22 days in each group. Lin et al. implanted a shorter, 1-cm-long PCL construct seeded with chondrocytes in six rabbits. Their rabbits had a mean survival time of 52 days. Similarly, all rabbits experienced narrowing of their airway secondary to granulation tissue with luminal diameters ranging between 1 and 3 mm.

Airway stenosis secondary to granulation tissue formation appears to be the limiting factor when circumferential tracheal reconstruction is performed using PCL regardless whether biologic materials are added to the implant. Granulation formation likely occurs because of the disruption of ciliated tracheal mucosa that is necessary to clear pathogens and foreign materials. Inhaled microorganisms and other microparticles, if not cleared, may induce a continuous inflammatory reaction, which will lead to the formation of granulation tissue and subsequent airway stenosis. In addition, the presence of the PCL implant and the sutures used to secure the implant may contribute to a foreign body reaction and therefore further granulation tissue.

Following human airway surgery, patients are often treated with systemic corticosteroids to control edema, inflammation, and to reduce granulation tissue formation. They may remain intubated in the intensive care, and often a repeat bronchoscopy, with or without intervention on granulation tissue/stenosis, is performed soon after—neither of which was performed as part of this study. It was decided to forego the use of corticosteroids to better understand the innate reaction to the PCL implant. Another limitation is the fact that the PCL implants were not customized to the tracheal size of each rabbit; instead, a standard size was used. Occasionally during surgery, this meant that the diameter of the PCL implants did not coincide with that of the rabbit’s native trachea. This mismatch in diameter may have impeded the migration of epithelium cells, which may also have contributed to granulation tissue formation.

PCL is known for its tensile strength; however, the evaluation of long-term structural integrity of PCL as a tracheal substitute has proven to be difficult because granulation tissue and airway stenosis limit animal survival. To our knowledge, Tsukada et al. performed the only study that could demonstrate survival of over 1 year in 5 of 11 of their canine subjects after circumferential tracheal defects were reconstructed with copolymer of PCL combined with L-lactide. However, this was supported by a titanium stent and covered with a vascularized omental flap.

Synthetic tracheal implants, especially circumferential ones, are also limited by the fact that they would not grow with the subject; therefore, the implant itself would be a source of airway stenosis regardless of granulation tissue formation. Theoretically, if this type of implant was to be used in pediatric cases, repeated surgeries could be required to replace it with a larger implant if respiratory symptoms were to arise.

CONCLUSION

3D printed PCL implants have the potential to be used in reconstructing long-segment circumferential tracheal defects. This current study has allowed us to create a baseline evaluation of the PCL implant without any additives. It was demonstrated that it is feasible in the short term, although granulation tissue and stenosis at the anastomoses are the limiting factors that occur prior to 4 weeks of implantation. Further studies would be necessary to tackle this problem. Trials investigating the use of systemic or local anti-inflammatory drugs to reduce the innate reaction against the implant may be beneficial to reduce the amount of granulation tissue formation and allow for longer term evaluation of the implants.

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BIBLIOGRAPHY


