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Endoscopic Management of Tissue-Engineered Tracheal Graft Stenosis in an Ovine Model

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Abstract

Objective—To evaluate the safety and efficacy of bronchoscopic interventions in the management of tissue-engineered tracheal graft (TETG) stenosis.

Study Design—Animal research study.

Methods—TETGs were constructed with seeded autologous bone marrow-derived mononuclear cells on a bioartificial graft. Eight sheep underwent tracheal resection and orthotopic implantation of this construct. Animals were monitored by bronchoscopy and fluoroscopy at 3 weeks, 6 weeks, 3 months, and 4 months. Bronchoscopic interventions, including dilation and stenting, were performed to manage graft stenosis. Postdilation measurements were obtained endoscopically and fluoroscopically.

Results—Seven dilations were performed in six animals. At the point of maximal stenosis, the lumen measured $44. \pm 8.4 \text{ mm}^2$ predilation and 50.7 ± 14.1 postdilation by bronchoscopy ($P = 0.3517$). By fluoroscopic imaging, the airway was $55.9 \pm 12.9 \text{ mm}^2$ predilation and $65.9 \pm 22.4 \text{ mm}^2$ postdilation ($P = 0.1303$). Stents were placed 17 times in six animals. Pre- and poststenting lumen sizes were $62.8 \pm 38.8 \text{ mm}^2$ and $80.1 \pm 54.5 \text{ mm}^2$ by bronchoscopy ($P = 0.6169$) and $77.1 \pm 38.9 \text{ mm}^2$ and $104 \pm 60.7 \text{ mm}^2$ by fluoroscopy ($P = 0.0825$). Mortality after intervention was 67% with dilation and 0% with stenting ($P = 0.0004$). The average days between bronchoscopy were 8 ± 2 for the dilation group and 26 ± 17 in the stenting group ($P = 0.05$). One hundred percent of dilations and 29% of stent placements required urgent follow-up bronchoscopy ($P = 0.05$).

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Conclusion—Dilation has limited efficacy for managing TETG stenosis, whereas stenting has a more lasting clinical effect.

Keywords

Tissue-engineered trachea; TETG; tissue engineering; tracheal stenosis; tracheal stenting; tracheal dilation

INTRODUCTION

Few therapeutic options exist for patients with tracheal defects involving over 50% of the airway length in adults and 30% in children. Options including tracheal replacement with autologous tissue, artificial materials, and transplantation have been limited by infection, graft collapse, vascular erosion, and the need for lifetime immunosuppression.¹ Tissue engineering offers the potential to generate a conduit to replace the diseased segment with healthy tissue that can be customized, nonimmunogenic, and resistant to infection.

Due to the high morbidity and mortality associated with long-segment airway disease, there have been several published cases involving compassionate use of tissue-engineered tracheal grafts (TETGs).² The few studies that address the postoperative course in humans illuminate that graft stenosis is the most common postoperative complication, frequently requiring bronchoscopic interventions such as dilation and stenting.^{3–5} Stenosis, therefore, represents a large obstacle to the clinical application of TETGs. The development of strategies to prevent and treat this complication continues to be an area of active investigation.

In the clinic, tracheal stenosis commonly results from iatrogenic causes such as tracheal intubation, tracheostomy, transplantation, or trauma.⁶ Endoscopic interventions such as dilation, stenting, or steroid injections can be used as primary interventions or as an adjunct following open laryngotracheoplasty.⁷ Failure to maintain airway patency despite endoscopic and open techniques may require stenting.⁸ The need for stenting is more common with active inflammation, malignancy, or tracheomalacia.⁷ Use of stenting in benign strictures should only be considered after the exhaustion of other options because stent migration, retained secretions, and granulation tissue development is often unavoidable.⁹

The clinical experience with TETGs has thus far been fraught with complications and controversy.^{4,5,10} Although the use of endoscopic interventions has been reported, the outcomes and response to these techniques have not been detailed. We previously described an ovine model of TETG implantation using a nanofiber scaffold that had previously been implanted in human recipients.^{11,12} Our pilot study demonstrated viability of this construct; however, similar to clinical experiences, stenosis was the most common complication observed. In this phase of the study, we sought to examine the response of TETG-related stenosis to conventional endoscopic interventions using an ovine model.

MATERIALS AND METHODS

Animal Care and 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 postoperatively. 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 U.S. Department of Agriculture regulations outlined in the Animal Welfare Act.

Scaffold

Scaffolds (Fig. 1a) were manufactured by Nanofiber Solutions, Inc. (Columbus, OH). The grafts were electrospun using a 20% polyethylene terephthalate and 80% polyurethane mixture and three-dimensional (3D) printed polycarbonate rings using methods previously described.^{11,12} The scaffold was sterilized using gamma irradiation.

Implantation

Sedation/Anesthesia—After receiving a dose of butorphanol/midazolam (0.2/0.5 mg/kg IM), sheep (Dorset) underwent induction using ketamine (5–10 mg/kg) with diazepam (0.02–0.08 mg/kg) or propofol (4 mg/kg). The anesthetic plane was maintained using isoflurane (1%–5% in oxygen) and/or propofol (20–40 mg/kg/hr).

Bone Marrow Harvest—The animals were placed in a lateral recumbent position and a Jamshidi needle (Carefusion Inc, Franklin Lakes, NJ) was used to aspirate bone marrow from the iliac crest.

Mononuclear Cell Isolation and Graft Seeding—Mononuclear cells were isolated using a closed disposable filtration system, as previously described.¹³ In brief, the marrow is injected into the closed disposable system, passing through the filter (Fig. 1b). The mononuclear cells are trapped within the filter, and a 2% saline solution is used to flush the cells from the filter. The mononuclear cell concentrate is vacuum-seeded onto the graft using a custom-made porous mandrel (Fig. 1c).

Preoperative Imaging—The animal was placed in a dorsal recumbent position. Prior to implantation, rigid bronchoscopy and 3D fluoroscopic imaging was performed, and images of the native airway were obtained, as described below.

Implantation—Eight sheep underwent implantation of tissue-engineered trachea grafts. Following sterile preparation, a midline neck incision was made. Dissection was continued until the trachea was isolated circumferentially for an adequate length. After placement of stay sutures, a 3-cm segment starting at the superior border of the third tracheal ring was resected. An endotracheal tube (ETT) was inserted in the distal airway for ventilation, and the oral endotracheal tube was retracted into the proximal airway. The tracheal graft was anastomosed using interrupted 3-0 polydioxanone suture (Ethicon, Somerville, NJ). Prior to completion of the distal anastomosis, the oral endotracheal tube was advanced through the

proximal anastomosis and into the distal airway. Additional sutures were placed as needed to achieve an air-tight anastomosis, and fibrin glue was used to reinforce the suture lines. Wound closure was performed using braided absorbable suture. Rigid bronchoscopy and 3D fluoroscopic imaging were repeated to evaluate the anastomotic sites. Postoperatively, the animals were monitored daily. They received 1 week of prophylactic antibiotics (cefazolin 50 mg/kg) and 3 days of analgesia (buprenorphine 0.015 mg/kg and banamine 1.1–2.2 mg/kg).

Postimplantation Bronchoscopy

Following implantation, animals underwent planned surveillance rigid bronchoscopy with 3D fluoroscopy at 3 weeks, 6 weeks, 3 months, and 4 months. Urgent bronchoscopy was performed for respiratory distress, increased work of breathing, or biphasic stridor at rest. Sedation and anesthesia were performed in a similar fashion to the implantation. Bronchoscopic and fluoroscopic imaging of the anastomoses was obtained prior to intervention. Early in the study, stenosis was managed with dilation only, and stenting performed when there was failure of the dilation, as demonstrated by recurrence of stenosis. Due to the results of the first three animals, stents were subsequently placed when stenosis was observed.

Dilation—If the animals had significant airway stenosis (> 50% luminal narrowing), the site of stenosis was dilated using either endotracheal tubes or balloon dilation (Acclarent, Irvine, CA). With ETT dilation, the rigid bronchoscope was advanced across the graft and the sequential sizes of endotracheal tubes (sizes 8.5 – 10) were guided over the bronchoscope in Seldinger fashion. Endotracheal tubes were left in place for 60 seconds, while ventilation continued. With balloon dilation, the device (14–16mm) was advanced across the stenotic segment. The inflation device (Acclarent) was used to inflate the balloon to a pressure of 8 to 10 atm. The balloon was left in place for 60 seconds, or until oxygen saturation dropped below 90%. Imaging and bronchoscopic documentation were then repeated.

Stenting—Prior to stent placement, balloon dilation was performed as above, if needed, to allow placement of the stent. Stent size (14 mm × 50 mm and 16 mm × 60 mm; Hood Laboratories, Pembroke, MA) was determined by endoscopic airway measurements. The stent was placed using Pilling Jackson Alligator grasping forceps (Teleflex, NC), which was used to grasp the proximal end of the stent. If required, the bronchoscope was passed through the lumen of the graft and the stent was advanced over the scope. Appropriate stent placement was defined as spanning the proximal and distal anastomoses, including stenotic regions. On follow-up bronchoscopy, stents were evaluated for migration and the anastomotic sites were imaged (Fig. 2).

Airway Sizing

Bronchoscopic Images—The optical forceps (spoon-shaped, round, 10350L) were mounted on a 5.5 mm diameter/50 cm length Hopkins Straight Forward 0 Degree Telescope (10320AA, Storz Tuttlingen, Germany). Calibration was performed by placing a ruler at the end of the instrument and obtaining an image (Fig. 2a). As the forceps are a fixed distance

from the camera lens, the measurements at this point can then be determined. Pictures were taken with the forceps placed at the level of the anastomoses. The images were processed using Image J (v1.49, NIH, Bethesda, MD) to obtain the lumen cross-sectional area as previously described.¹⁴

Fluoroscopic Images—Three-dimensional fluoroscopic images were obtained using the Infinix C-arm (Toshiba, Japan). These images were processed to obtain cross-sectional images (Fig. 2c) that were measured using Vitrea Enterprise Suite Imaging Software (Vital, Minnetonka, MN) to obtain the lumen cross-sectional area.¹⁴

Statistical Methods

Comparisons of minimum TETG lumen cross-sectional area between pre- and postballoon dilation, as measured by either bronchoscopy or 3D fluoroscopy, were performed by two-tailed paired parametric *t* tests. Minimum lumen diameters before and after stent placement (at time of next follow-up imaging), as measured by either bronchoscopy or 3D fluoroscopy, were compared in a similar fashion. The efficacy of either stenting or balloon dilation, defined by days until next intervention, was determined by Mantel–Cox Log rank and Gehn–Breslow–Wilcoxon tests. Contingency analyses comparing mortality after intervention or requirement for urgent return to the OR between dilation and stenting groups were performed via two-sided Chi-square tests. For all analyses, *P* values ≤ 0.05 were considered statistically significant. Statistical analyses were performed with GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA).

RESULTS

Dilation of TETG-Related Stenosis

Six animals underwent seven incidences of balloon dilation with immediate follow-up imaging and bronchoscopic measurement. Two (28.5%) of these interventions were urgent, whereas the remainder were surveillance bronchoscopies. Three animals underwent isolated balloon dilation without subsequent stent placement. Of these sheep, one animal died during anesthesia from an unknown cause, one was euthanized due to severe respiratory distress, and the third underwent stent placement at subsequent bronchoscopy.

The minimal luminal diameter was encountered at the proximal anastomosis in three animals (43%), but all subjects showed some degree of narrowing at both anastomoses. The average area at the point of maximal stenosis predilation was $44.6 \pm 8.4 \text{ mm}^2$ by bronchoscopic measurement and $55.9 \pm 12.9 \text{ mm}^2$ by fluoroscopy. Postdilation, luminal area was $50.7 \pm 14.1 \text{ mm}^2$ by bronchoscopy and $65.9 \pm 22.4 \text{ mm}^2$ by fluoroscopy. On paired analysis (Fig. 3), no significant difference was noted between the pre- and postdilation measurements for either bronchoscopy ($P = 0.3517$) or fluoroscopy ($P = 0.1303$).

Stenting of TETGs

Six animals underwent stent placement for stenosis. Of these animals, stents were placed anywhere between one and five times, accounting for 16 individual placements. Stenosis was noted at the proximal anastomosis 50% of the time ($n = 8$). On initial bronchoscopy,

minimal luminal area was $62.8 \pm 38.8 \text{ mm}^2$ by bronchoscopy and $77.1 \pm 38.9 \text{ mm}^2$ by fluoroscopy. At the following procedure, bronchoscopic measurements were $80.1 \pm 54.5 \text{ mm}^2$ and $104 \pm 60.7 \text{ mm}^2$ by fluoroscopy. There was no significant difference between the initial and follow-up bronchoscopic measurements ($P = 0.6169$) or fluoroscopy ($P = 0.0825$) (Fig. 4).

Clinical Outcomes

Two animals (67%) within the dilation cohort were either euthanized or died following intervention (Fig. 5). There was no mortality among the animals that underwent stenting. No other complications were noted among either group as a direct result of the interventions. All surviving animals within the dilation group ($n = 2$) required urgent return to the operating room for bronchoscopy, whereas only 29% ($n = 5$) of the stented animals progressed to this point. The average time to reintervention was 8 ± 2 days among the dilation group and 26 ± 17 days following stent placement. Stent migration was noted following 29% ($n = 5$) of stent placements.

DISCUSSION

Stenosis is the most common complication of TETG implantation in both animal models and clinical trials.²⁻⁴ Although there are limited descriptions of the outcomes and postimplantation interventions in patients who have received TETGs, two reports document multiple bronchoscopies with associated dilations, steroid injections, and stenting. One of these cases demonstrates that these interventions decreased in frequency with time.⁴ It is unclear the exact reason for the decrease in dilation requirements. It may relate to the maturation of the respiratory epithelial layer within the airway, leading to improved mucociliary clearance, decreased stasis of secretions, and an overall decrease in inflammation. Although bronchoscopic interventions are well described for use in the native trachea, the impact and efficacy of these interventions has not been closely examined following tissue-engineered tracheal graft implantation.

Clinically, dilation has been shown to be effective in anywhere from 50% to 70% of patients with laryngotracheal stenosis and is typically the first option in management.¹⁵⁻²⁰ Although dilation has been used in the handful of patients who received TETGs, the current study reveals that dilation is relatively ineffective, as demonstrated by the lack of change in luminal area on either bronchoscopy or fluoroscopy. Additionally, the average length of time until the animal required subsequent intervention was only 8 days. Although animals did experience an immediate relief of symptoms, this response was short-lived and inadequate for clinical use. The difference in TETG-related stenosis response to dilation may be multifactorial. Possible etiologic factors could include an extended inflammatory reaction, graft- or host-related biomechanical forces, the innate biomechanical properties of the nanofiber scaffold, or foreign body reaction to the scaffold. Based on these concerns and previous results regarding chronic inflammation,⁷ it follows that dilation would not be the treatment of choice in TETGs.

Although stenting did not show statistical significance in terms of airway size pre- and postdilation, there was a significant difference in the length of time between interventions

and the need for urgent intervention. An average interval of 27 days between interventions was seen with stenting, compared to 8 days with dilation alone. Additionally, 60% of these interventions were for surveillance and the length between interventions was likely shorter than if the animals had been allowed to declare themselves symptomatically. The lack of changes between the pre- and post-stenting sizing may be related to several factors. One issue is the small sample size that makes it difficult to capture differences in luminal sizing. Also, graft infolding at the posterior wall following removal of the stent may lead to luminal narrowing at measurement.

The results of this study are limited by several factors. The first is limited power, which is a common issue in large animal research. There was some difference in the pretherapy luminal area between these two groups (dilation: $44.6 \pm 8.4 \text{ mm}^2$, stent: $62.8 \pm 38.8 \text{ mm}^2$), which may bias the comparisons of outcomes between the two groups. The second limitation involves the method of dilation performed. Maintenance of an anesthetic plane allowing spontaneous ventilation was challenging in an ovine model. Consequently, the ability to accurately place an occluding balloon dilation catheter, dilate effectively, and resume ventilation without significant oxygen desaturation proved difficult in this model. Over the course of the study, we noted an improved ability to dilate while maintaining ventilation by using the serial endotracheal tube dilation method, but a disadvantage of this method is the potential for a shearing-type force on the tissues. Another potential limitation is that only a few animals were treated with isolated dilation, which limits the conclusions that can be drawn when comparing the two therapies. Although our initial aim was to follow a protocol similar to that used in pediatric airway reconstruction, it quickly became apparent that stenosis encountered in this model was not responsive to dilation alone. After the initial failures, the decision was made to stent all significant stenosis unless there were signs of gross epithelialization.

We experienced a 30% incidence of stent migration, which in part may be due to the use of silicone stents. We selected a silicone tubular stent for its tissue compatibility, ease of manipulation for surveillance bronchoscopies, lack of mesh to prevent neotissue ingrowth, and variety of sizes available. In a previous study, silastic stents were linked to worsened granulation tissue when compared to Teflon stents.²¹ Other types of stents, such as covered metal stents, may offer advantages in terms of dislodgement and recurrent stenosis. The study is also limited by the short follow-up period of 4 months. It may be that with a longer observation period, there would be a similar decrease in required interventions as seen in clinical trials.³

Finally, the large-animal protocol has inherent differences from human airway reconstruction surgery. In human tracheal surgery, postoperative care often involves heavy sedation with mechanical ventilation, providing an airway stent, assisting with clearance of mucus, and controlling movement. Additionally, patients receive enteral feeding via tube to avoid aspiration, anti-reflux medication, and antibiotic therapy. Due to the constraints of the sheep model, the animal environment, and research facilities, many of these variables cannot be directly controlled; therefore, we cannot estimate the effect they have on the surgical outcomes.

Because many different prototypes of tissue-engineered tracheas are being explored, each with variable characteristics, response to endoscopic interventions may vary. However, based on clinical results involving the various iterations, stenosis remains the most common complication observed and the predominant hurdle for clinical translation from the bench to the bedside. For this reason, close evaluation of the response of these grafts in a preclinical model provides a foundation for the algorithms that should be used to manage complications in the clinical population. Based on our results, stenting offers improved outcomes with TETG-associated airway stenosis when compared to dilation alone.

CONCLUSION

Both dilation and stenting are feasible following TETG implantation; stenting is more effective for clinical management of stenosis with use of our graft.

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J.J. is a cofounder of Nanofibers Solutions, Inc., the company that provided the grafts for the study. C.K.B. is on the scientific advisory board of Cook Biomedical.

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Fig. 1.

Methods: graft preparation and implantation. (a) polyethylene terephthalate/polyurethane graft prior to seeding (7.5 cm in length, 20 mm in diameter). (b) Filtration device with bone marrow (arrow) processed through filter (*). The mononuclear cells are trapped within the filter, whereas the remaining cell types are collected in the lower bag (arrow head). The mononuclear cells are collected by flushing the filter with a hypertonic solution. (c) The graft is seeded with the mononuclear cell solution using vacuum seeding and can then be implanted into the animal (d). Preimplantation (e) and postimplantation (f), bronchoscopy is performed. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

Fig. 2.

Methods: airway measurement. Airways were measured using two techniques. In the first, calibration using a standard bronchoscopic instrument (a) allowed measurement of the airway luminal area using a readily available software program (b). Airway luminal measurements were also obtained using three-dimensional fluoroscopic imaging (c). Arrow = surgical clip; star = orogastric tube. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

Fig. 3. Acute impact of balloon dilation. Paired analysis of luminal area at the narrowest segment pre- and postdilation using both bronchoscopy ($P = 0.3517$) and 3D fluoroscopic images ($P = 0.1303$). There is no significant difference in the luminal area using either method. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

Fig. 4. Impact of stenting on luminal area. Bronchoscopic (a) and 3D fluoroscopic (b) measurements of the minimal luminal area. Although there is no significant difference between the pre- and poststent measurements (Bronch $P = 0.6169$, Fluoro $P = 0.0825$), there is a trend toward significance within the 3D fluoroscopic group. Bronch = bronchoscopic; Fluoro = fluoroscopic. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

* n=3 (One animal died intra-operatively after balloon dilation. One animal was electively euthanized. The remaining animal underwent stent placement at second intervention.)

Fig. 5.

Bronchoscopic interventions outcomes: Dilation versus stenting. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]