A Tissue-Engineering Technique for Vascularized Laryngotracheal Reconstruction

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Objective: To perform laryngotracheal reconstruction (LTR) using a vascularized neotracheal segment.

Design: A neotracheal segment was created within the sternocleidomastoid muscle. An anterior cricoid split procedure was performed using a pedicled, vascularized neotracheal segment. Results were compared with a control group that underwent anterior cricoid split using standard (avascular) autografted cartilage. Cross-sectional area, cartilage viability, extrusion, mucosalization, and wound healing were compared between groups.

Subjects: Sixteen female New Zealand white rabbits.

Interventions: Eight animals underwent placement of a cartilage-wrapped silicone implant into the sternocleidomastoid muscle. After 2 weeks, the silicone implant was removed, leaving a fibrovascular “foreign body” capsule and the interwoven autografted cartilage. The neotracheal segment was trimmed to create an anterior graft for LTR. The remaining animals underwent standard anterior graft LTR using autografted auricular cartilage. The reconstructed segments were harvested for comparison at 2 and 4 weeks.

Results: All reconstructed animals survived the postoperative period. No significant differences in stenosis rates or mucosalization were noted between groups. Two animals in the standard LTR group had microabscess formation, and no graft extrusions were encountered.

Conclusion: A pedicled neotracheal graft can be used for anterior cricoid split procedures in rabbits.

The surgical repair of subglottic stenosis remains one of the most challenging aspects of airway management. The repair has traditionally been achieved using cartilage interposition grafts in cricoid split procedures. Reported complications of this surgery include restenosis, graft failure, migration, infection, and donor site morbidity. The ideal tissue for reconstruction would consist of an autologous graft with pliability, elasticity, and mucosal lining similar to that of native trachea. The graft must resist stenosis and tolerate implantation with minimal risk of infection, extrusion, migration, or failure.

Tissue engineering involves the creation or re-creation of viable tissue constructs though cell culturing, tissue prelamination, or tissue prefabrication. Prelamination involves the layering of different tissues to create the desired structure. Prefabrication involves the manipulation of blood supply to tissues. In many cases, tissue engineering can manipulate bioimplantables and existing living tissue to mimic a native structure. Significant advances have been made in the tissue engineering of blood vessels, skin, esophagus, and trachea. The trachea specifically proves to be an interesting area of study in the tissue-engineering arena. It is a relatively simple organ that functions mainly as an air conduit, with a mucosal surface that serves to protect against infection and aid in mucous clearance.

This study used tissue-engineering techniques to create a prefabricated and prelaminated autogenous tracheal segment. A portion of this segment was used as a pedicled graft in an anterior cricoid split procedure. Restenosis rates, migration, degree of epithelialization, cartilage viability, and granulation tissue formation are compared with a group undergoing anterior cricoid split using simple autologous cartilage graft.
METHODS

Sixteen adult New Zealand white female rabbits were used as subjects. The study protocol was approved by the Southern Illinois University laboratory animal care and use committee protocol. All animals received humane care, and all surgical procedures were performed under dissociative anesthetic and intramuscular sedation using sterile technique and perioperative penicillin. The animals in this study were not intubated and the procedures were performed under spontaneous respiration.

The 16 animals were divided into 2 groups (each, n=8). Group 1 (neotracheal) animals underwent 2 procedures 14 days apart. In the first procedure, autografted auricular cartilage was harvested with perichondrium intact and sliced into thin strips resembling cartilage rings. Then, 3 to 5 strips of cartilage were wrapped around a silicone stent measuring 1.5 cm in length by 5 mm in diameter. The silicone stent was required to maintain the shape of the cartilage and to provide a well-vascularized foreign body capsule around the cartilage. The cartilage strips were secured in position using 6-0 fast-absorbing gut sutures (Figure 1).

The right sternocleidomastoid muscle (SCM) was exposed, and a single longitudinal incision was made in the fascia. The muscle was dissected longitudinally sufficiently to allow the graft to be placed within the muscle belly. The muscle belly, which acted as a vascular carrier for the tracheal construct, was closed around the implant using 5-0 fast-absorbing gut sutures. The segmental blood supply to the SCM was maintained only in the inferior aspect of the muscle. The superior vascular pedicles to the SCM were divided to encourage a “delay phenomenon” enhancement of blood supply to the muscle through the inferior vasculature so that the entire muscle/cartilage construct could be reliably moved only on the inferior blood supply at a later time. Two weeks were allowed to pass, and the SCM was reexposed and the silicone implant removed, leaving the cartilage rings and the foreign body fibrovascular capsule that had developed (Figure 2). The neotracheal segment was then sectioned and a “boat graft” was fashioned for the anterior cricoid split procedure (Figure 3).

The graft was a tapered graft measuring 7 mm in length and 3 mm in width. Care was taken to trim the graft to permit maximal muscle bulk to be preserved with the graft tissue, thereby maintaining blood supply and tissue bulk to aid in closure. Next, the neotracheal segment and SCM pedicle were mobilized and prepared for anterior interposition extending from the cricoid through the fourth tracheal ring. The graft was sutured into position using 7-0 nylon interrupted sutures, and the overlying muscular pedicle was preserved and loosely sewn into the pretracheal fascia using absorbable sutures to aid in overclosure of the graft. At this point, the neotracheal group was further subdivided into 2 groups of 4 animals. Four tracheal specimens were harvested at 2 weeks (2-week neotracheal group) and the remaining 4 at 4 weeks (4-week neotracheal group) after operation.

The 8 animals in the control group underwent a single-staged procedure in which auricular cartilage was harvested with perichondrium intact. A graft measuring 7 mm in length and 3 mm in width was fashioned for interposition into the subglottic trachea. The graft was sutured in place using 7-0 nylon sutures in an interrupted fashion, and the pretracheal fascia was closed using absorbable suture material. As in the neotracheal group, 4 animals were euthanized at 2 weeks (2-week standard LTR) and 4 at 4 weeks (4-week standard LTR) after operation.

Figure 1. Silicone implant wrapped in cartilage strips.

Figure 2. Neotracheal construct following stent removal.

Figure 3. Neotracheal construct sectioned for creation of a boat-shaped patch graft.
The cross-sectional areas of the native tracheal segment were evaluated. The cross-sectional areas in the operated segment were subtracted from areas of native segment (nonoperated). With this method, each animal served as its own control for cross-sectional area. This was done to avoid overinterpretation of the cross-sectional area and underrepresentation of luminal granulation tissue formation. Granulation tissue was scored as mild if the underling fibrovascular tissue was less than 7 times the thickness of the epithelium. Moderate designation was given to granulation tissue growths greater than 7 times the thickness of the epithelium but less than one third of the epithelial thickness. Severe designation was given to granulation tissue formation greater than one third of the epithelial thickness but less than one half. Epithelium was scored as either present or absent. Cross-sectional areas were statistically analyzed using paired t test and 4-factor analysis of variance.

Table 1. Degree of Necrosis

<table>
<thead>
<tr>
<th>Time</th>
<th>Technique</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Standard LTR</td>
<td>Severe</td>
<td>Mild</td>
<td>Mild</td>
<td>Mild</td>
</tr>
<tr>
<td>4 wk</td>
<td>Neotrachea</td>
<td>Moderate</td>
<td>Mild</td>
<td>Mild</td>
<td>Mild</td>
</tr>
<tr>
<td></td>
<td>Standard LTR</td>
<td>Severe</td>
<td>Severe</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Abbreviation: LTR, laryngotracheal reconstruction.

Data were collected including cartilage viability, cartilage necrosis, cross-sectional area, percentage of mucosalization, granulation tissue, graft extrusion and/or migration, and microabscess formation. Cartilage viability was determined by identification of eccentrically located nuclei within the chondrocytes present in the cartilage stroma. The presence of any viable cartilage was scored as a viable graft. Cartilage necrosis or reabsorption was qualitatively scored as mild, moderate, or severe by a single-blinded observer. The airway cross-sectional area was determined by analyzing the lumen at its most stenotic point throughout the graft site. A microcomputer imaging device (MCID, Imaging Research Inc, London, Ontario) was used to determine the cross-sectional area of the specimen. Granulation tissue formation within the lumen was qualitatively scored as mild, moderate, or severe by a single-blinded observer. Graft extrusion or prolapse was determined by examining the specimen for telescoping or overt disruption of the graft at any of the interposed margins. Microabscess formation was also evaluated as being either present or absent. Cross-sectional areas were statistically analyzed using paired t test and 4-factor analysis of variance.

RESULTS

All animals survived their procedures and returned to their regular diet within 48 hours. None of the animals required surgical revisions nor did they experience any postoperative sequelae with the exception of mild wound complications. Wound complications were carefully recorded for each group. There were no wound complications with the neotracheal group. However, 3 animals in the standard LTR group developed subcutaneous air. Two animals required removal of sutures for decompression.

Viable chondrocytes were seen along the perichondrium in all cartilage autografts in this study. Similarly, all grafts exhibited some degree of chondrocyte death in their central portions. The degree of cellular viability appeared to correlate with the method used. The degree of necrosis was qualitatively scored by a single-blinded observer, and the results are given in Table 1.

The cross-sectional areas of the native tracheal segment and the operated segment (at the narrowest portion) were evaluated. The cross-sectional areas in the operated segment were subtracted from areas of native segment (nonoperated). With this method, each animal served as its own control for cross-sectional area.

No difference in area existed between the 2- and 4-week groups when time was evaluated independent of method. Similarly, no difference was detected when the data were evaluated based on technique independent of time. When all groups were evaluated independently (paired t test), a significant difference was detected in the 2-week standard LTR group with a 60% decrease in the cross-sectional area of the lumen (P = .006). The percentage of decline in total airway area in all groups is graphically represented in Figure 4.

Analysis of variance findings suggests an interaction between week and method (P = .07). This interaction likely occurred due to the findings in the 2-week standard LTR group.

The percentage of mucosalization for each evaluated slide was recorded by a blinded observer and averaged for each group. This semiquantitative method did not yield a significant difference between the neotracheal and standard LTR groups. The averages for each group were within 6 percentage points of one another with an average 70% to 76% of all surfaces covered for each group. Significant intergroup variability was observed, and poor mucosalization appeared to be loosely correlated with the granulation tissue formation. Qualitatively, the mucosa appeared similar among each of the groups. The histologic slides seen in Figure 5 represent the 2-week neotracheal group. The epithelium is characteristic of that seen throughout all specimens in this study. Note the underlying fibrous stroma and capsule and the viable cartilage autograft seen in this slide.

Granulation tissue formation was evaluated independently of the cross-sectional area. This was done to avoid overinterpretation of the cross-sectional area and underrepresentation of luminal granulation tissue formation. Granulation tissue was scored as mild if the underlying fibrovascular tissue was less than 7 times the thickness of the epithelium. Moderate designation was given to granulation tissue growths greater than 7 times the thickness of the epithelium but less than one third of the airway diameter. These data are summarized in Table 2.
Graft extrusion was assessed based on collapse of the graft, either into or out of alignment with the surrounding normal trachea. Only 1 specimen exhibited graft extrusion; this animal was in the 4-week standard LTR group. Microabscess formation was seen in 1 animal (4-week neotracheal group) near a small piece of nylon suture. The microabscess was present extraluminally in the soft tissue of the neck.

**COMMENT**

Surgical treatment of congenital laryngotracheal stenosis has been achieved in a variety of different ways. Anterior cricoid and posterior cricoid split procedures remain the most popular techniques for reconstruction. Unfortunately, many patients undergo reintubation, postoperative tracheostomy, or multiple operations to achieve eventual extubation or decannulation. For the more severe stenosis, treatment with LTR has been less successful.\(^2,3\) Cricotracheal resection is an option for the treatment of more severe subglottic stenosis. Reported complications are exertional dyspnea, dysphonia, inability to decannulate, recurrent laryngeal nerve damage, anastomotic dehiscence, restenosis, and prolapse of the arytenoid cartilage.\(^4,6\) Cricotracheal resection is a more extensive procedure than LTR, requiring tracheal mobilization. Overall surgical success based on decannulation rates varied from 85% to 97%.\(^4,7\) Reconstruction with combined myocutaneous flaps and costal cartilage or artificial materials has been suggested for severe laryngotracheal stenosis and large tracheal defects.\(^8-11\) Reported flap advantages in some studies were the muscle bulk and skin lining that resist infection and tolerate pressure, tension, and shearing motions.\(^11,12\) Other studies used vascularized perichondrium and cartilage for reconstruction of cervical tracheal defects.\(^13,14\) Watson et al\(^13\) designed a free flap of rib and attached pleura, which provided adequate support and a functional conduit in a canine model.

Others have used regional pedicled flaps for reconstruction. Liu and Wen\(^15\) and Tovi and Gator\(^16\) performed tracheal reconstruction with a sternocleidomastoid myoperiosteal flap. Their patients were successfully decannulated and tolerated normal exercise. Additionally, the sternohyoid myocutaneous rotary door flap provided adequate rigid support for extensive LTR without the requirement of skeletal support.\(^12,17,18\) While each represent novel approaches, none of these techniques has emerged as a dominant technique.

Rainer et al\(^19\) have used foreign body type capsule for a number of tissue-engineering techniques including creation of an epithelial-lined capsule and neocartilage formation within a preformed capsule. The capsule is extremely vascular and augments the blood supply to the cartilage grafts. In the present study, a similar fibrovascular capsule was used to incorporate the autografted cartilage into a single construct for use in tracheal reconstruction. The capsule proved to be simple to create and reliable to construct. No local or systemic complications were encountered while creating the constructs, and none of the attempts at creation failed. The SCM proved to be a good muscle to use, with a relatively long pedicle and a caliber large enough to support introduction of the graft into its fascial envelope. After

### Table 2. Degree of Granulation Tissue Formation

<table>
<thead>
<tr>
<th>Time</th>
<th>Technique</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>4 wk</td>
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</tr>
<tr>
<td></td>
<td>Standard LTR</td>
<td>Mild</td>
<td>Mild</td>
<td>Mild</td>
<td>Mild</td>
</tr>
</tbody>
</table>

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![Figure 5. A, Neotracheal segment, cartilage, fibrovascular tissue, and epithelium present (original magnification ×20); B, respiratory epithelium (original magnification ×40).](https://archotol.jamanetwork.com/)
the graft was placed into the SCM, the superior portion of the muscle was stripped of its blood supply to promote the delay phenomenon and encourage a dominance in blood supply from the inferior portion.

Using a pedicled graft has several potential advantages. First, the autografted cartilage can obtain vascularization in preparation for entry into the tracheal lumen. This preformed blood supply could help limit infectious complications and graft migration, while providing adequate substrate for rapid epithelialization. Second, it has been observed that epithelium can and often does grow well over a fibrovascular base. The foreign body capsule in our model likely replicates the fibrovascular base discussed by Jacobs et al. Interestingly, the standard LTR autografted (avascular) cartilage in our study developed a similar-appearing fibrovascular capsule surrounding the entire graft. It stands to reason that the neotracheal segment simply had this fibrovascular base formed a priori. This preformed fibrovascular base could potentially enhance wound healing. Third, the attached muscle, which accompanies the graft into its position in the trachea, has an occlusive feature that renders the anastomosis less susceptible to leaking than a traditional cartilage autograft.

We suspect that the muscle bulk available with the pedicle provided or enhanced wound healing in our study. The only wound complications encountered were subcutaneous air trapping, which occurred only in the standard LTR group. It should be noted that the standard LTR group had the available fascia and soft tissue loosely closed over the graft, but the skin incisions were closed tightly (to prevent the animals from licking their wounds and developing dehiscence and infection). The tight skin closure and relative lack of soft tissue for closure may have contributed to the development of air trapping. The available muscle bulk associated with the neotracheal segment should be considered a potential benefit of this technique.

Cartilage necrosis in our study occurred more commonly in the standard LTR group. The small numbers present in the study limit the conclusions that can be drawn. However, cartilage necrosis was seen more commonly in the standard LTR specimens, and this appeared to be related to time. In the study by Jacobs et al., a 4.4% necrosis rate at 2 weeks and a 25% necrosis rate at 4 weeks following standard cartilage interposition in the trachea was noted. This number increased to 39% at 10 weeks following surgery. In our study, the neotracheal cartilage was autografted 2 weeks earlier than the standard LTR group, potentially making these grafts more likely to exhibit necrosis. That this was not observed may be a significant component of granulation tissue formation in this study. Only 1 animal in the 4-week groups and 3 animals in the 2-week groups had moderate granulation tissue formation. Granulation tissue formation likely has a multifactorial etiology and may depend on interindividual variability. Granulation tissue formation has been correlated with superficial infection, gastroesophageal reflux disease, aspiration, and a number of uncontrolled conditions not controlled for in our study. Therefore, this technique should be tested in a larger animal model. Additionally, extended follow-up studies are needed to determine the ultimate performance of the neotracheal construct. Increasing the number of interval histologic examinations of the neotracheal tissues could help elucidate microscopic tissue differences between the techniques to a greater extent than that of the present study.

Our study introduces an elegant technique for tracheal tissue engineering. We believe that this technique may be of use for total tracheal reconstruction as well as for LTR. The fibrous capsule has the benefit of being universally available, easy to create, and very reliable. The combination of this technique with the use of auricular or thyroid cartilage in humans could potentially limit donor site morbidity. Ultimately, epithelialization of the con-

Epithelialization noted in our study is similar to that reported in other studies. Jacobs et al. noted 53% ± 13% (mean ± SD) epithelialization at 2 weeks and 100% epithelialization at 4 weeks. While most of the animals in the 4-week groups exhibited 100% epithelialization, there were outliers that pulled the group averages to around 70% to 75%. The outliers occurred with equal distribution between groups. Theoretically, the vascularized construct might have been better able to encourage epithelialization with its enhance blood supply, but this was not observed in our study. Perhaps this occurred due to the need for deposition of a rudimentary basement membrane, which is created by the surrounding epithelium. It is possible that basement membrane deposition represents the rate-limiting step in the process of epithelialization. In all the specimens examined, the quality of the epithelium was good, with all cellular elements represented.

The cross-sectional area in the most stenotic segments of the grafts did not differ from native trachea significantly with the exception of the 2-week standard LTR group. At first glance it may seem that this represents a relative failure of the graft. One would expect the graft to have increased the lumen size significantly. In the study by Jacobs et al., 5-kg New Zealand white rabbits underwent a similar procedure and the results were essentially the same as those found in our study. We suspect that the stenosis rates in the study by Jacobs et al. and the present study are likely a product of the small tracheal dimensions and the airway model. The focus of the present study was to compare 2 methods. In so doing, no difference was recognized between standard LTR and neotracheal reconstruction.

Granulation tissue formation was evaluated separately for each animal. However, there did not seem to be a significant component of granulation tissue formation in this study. Only 1 animal in the 4-week groups and 3 animals in the 2-week groups had moderate granulation tissue formation. Granulation tissue formation likely has a multifactorial etiology and may depend on interindividual variability. Granulation tissue formation has been correlated with superficial infection, gastroesophageal reflux disease, aspiration, and a number of uncontrolled conditions not controlled for in our study. Therefore, this technique should be tested in a larger animal model. Additionally, extended follow-up studies are needed to determine the ultimate performance of the neotracheal construct. Increasing the number of interval histologic examinations of the neotracheal tissues could help elucidate microscopic tissue differences between the techniques to a greater extent than that of the present study.

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Tissue engineering using a prefabricated tracheal construct has been demonstrated using a simple and reliable technique. This technique compared favorably with standard LTR using auricular cartilage in the rabbit model. Further study is necessary to determine the future role for this technique of tracheal reconstruction.

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