Prefabrication of Composite Grafts for Long-Segment Tracheal Reconstruction

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Objective: To investigate the prefabrication of vascularized mucosa-lined composite grafts intended to replace circumferential tracheal defects.

Design: Plane grafts composed of ear cartilage and full-thickness oral mucosa were revascularized by the laterothoracic fascia. The use of meshed vs nonmeshed mucosa to improve the epithelial coverage was examined. We also investigated the creation of a vascular bed over the cartilage and the subsequent application of meshed mucosa. Macroscopic aspects, viability, and degree of mucosal lining were analyzed.

Subjects: Twenty male New Zealand white rabbits.

Interventions: Ten animals underwent placement of auricular cartilage under the laterothoracic fascia. Intact (group 1) or meshed mucosa (group 2) was applied over the fascia and protected by a silicone sheet. After 3 weeks, prefabricated grafts were removed for comparison. In 10 other animals, a sheet of perforated cartilage was placed under the laterothoracic fascia. Two weeks later, 5 grafts (group 3) were harvested. The remaining 5 grafts were re-opened for mucosal application over the cartilage and re-vascularized for 3 additional weeks (group 4).

Results: Vascularized plane grafts were obtained in all groups. Mucosal lining increased significantly with meshed mucosa (14%-68%; mean, 40%) compared with nonmeshed mucosa (3%-15%; mean, 10%) (P=.008). Induction of a vascular bed over perforated cartilage was achieved, but survival of secondary implanted mucosa was variable.

Conclusions: A reliable technique to prefabricate composite grafts with cartilaginous support and mucosal lining is presented. The use of meshed mucosa significantly improves epithelial coverage.

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LONG-SEGMENT TRACHEAL STENOsis remains a challenging condition to treat. Failed treatments for subglottic and/or tracheal stenosis, often aggravated by tracheal damage induced by the tracheostoma and cannula, are the most frequent causes. Occasionally, congenital stenosis or tracheal malalignancies may also warrant an extensive tracheal resection.

Tracheal resection with end-to-end anastomosis represents the treatment of choice when the stenosis encompasses less than 50% of the tracheal length.1,2 Reconstruction of longer tracheal defects is still an unsolved problem.

Congenital tracheal stenosis with circular tracheal rings is the main cause of primary long-segment stenosis in children and may involve up to 80% of the tracheal length.2,3 However, it remains a rare condition, with fewer than 200 cases reported in the literature.4,5 The lack of a common established therapy is evident by the wide variety of therapeutic approaches, including balloon dilation,6 pericardial or costal cartilage patch tracheoplasties,7-9 slide tracheoplasty, and tracheal autografts. Slide tracheoplasty, described in 1989 by Tsang et al10 and subsequently modified by Grillo,11 and tracheal autografts, described by Backer et al,12 are very promising techniques, but no long-term results are yet available. Augmentation tracheoplasty with insertion of a cartilaginous, periosteal, or, more commonly, a pericardial patch is rarely uneventful. Results are variable because of the strong induction of granulation tissue, graft disruption with lumen obstruction, and recurrence of stenosis.5,7,9-12 Slide tracheoplasty or tracheal autografting seem to be the most appropriate initial surgical procedures. In case of failure with recurrent stenosis, tracheal homografting has been performed with some success.13 The tracheal homograft is then used as an ante-
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only includes half or two thirds of the tracheal circumference; the residual mucosa of the posterior tracheal wall helps reepithelialize the graft from the tracheal side.

However, in case of malignancies and acquired long cicatrical stenosis without residual normal mucosa, a circumferential replacement of the whole trachea is often necessary. The reepithelialization process may then be compromised and often leads to recurrent stenosis.

Since 1990, Delaere and colleagues have been working on different models of composite autografts for the reconstruction of laryngotracheal defects. They successively demonstrated the importance of sufficient vascularization, a cartilaginous framework, and a mucosal lining to obtain primary healing without excessive granulation tissue formation and scarring. A double-stage procedure proved its superiority; heterotopic revascularization of the graft was followed by orthotopic transplantation. The second step had to be performed within 16 to 20 days after heterotopic implantation. After 3 weeks, the luminal aspect of the grafts became infected with consecutive loss of the grafted elements. Their studies were developed in a rabbit model and adapted to humans with good results, but the grafted segments remained limited in size and never encompassed the whole circumference of the trachea.

Many studies have been done on the development of an ideal model of circumferential tracheal reconstruction. A variety of prosthetic and tissue grafts (omentum, esophagus, jejunum, perichondrium) have been used, but with only limited success. Graft ischemia, immune rejection, anastomotic dehiscence, and stenosis were the major reasons of failure.

Our study investigates the prefabrication stage of composite autografts intended for circumferential tracheal reconstruction. The grafts are made of the 3 elements previously described: vascularized fascia, autogenous ear cartilage, and oral mucosa. Different graft designs were studied and their viability and structural properties were analyzed after a revascularization period in a heterotopic position.

Twenty New Zealand white rabbits weighing 3 to 4.5 kg were used in this experimental study. All of them were certified to be free of specific pathogens. The animals were divided into 4 groups of 5 rabbits each. One graft design was used in each group. All composite grafts, consisting of oral mucosa and autologous cartilage in contact with a vascularized fascia, were prefabricated and revascularized in the laterothoracic region.

The laterothoracic fascia flap is located on the lateral chest wall of the rabbit and measures 15 cm in the craniocaudal axis and 5 cm in the ventrodorsal axis. It consists mainly of muscular and connective tissue and contains the laterothoracic artery and vein, emerging from the axillary vessels. The cartilage is provided by the rabbit ear where thin and flat sheets can be harvested. Oral mucosa can be obtained easily from the rabbit cheek, but only a limited area is available.

Throughout the duration of the experiments, the animals were housed in separate quarters in an environmentally controlled facility and fed with a standard rabbit diet. They all received antibiotics (cefazolin, 50 mg/kg, intramuscularly every 48 hours) for 7 days. They were also given pain medication (butorbital sodium). Animals were painlessly euthanized with an overdose of pentobarbital sodium. All experiments were conducted in accordance with protocols approved by the Ethics Committee for Animal Experiments of our institution.

In group 1 animals, a composite graft that included the distal part of the laterothoracic fascia, a sheet of auricular cartilage, and a patch of oral mucosa was created. The sheet of auricular cartilage (5-8 cm²) was placed and sutured under the fascia. During the same procedure, the patch of oral mucosa (2-3 cm²) was grafted on the fascia’s outer surface, opposite the cartilage (Figure 1A). Mucosa lined only a small area of the graft’s surface, defined by the size of the cartilage.

In group 2 animals, the graft was similar to that of group 1 but the patch of mucosa was first meshed before implantation using a mesh-graft device. The dimension of the genuine mucosal patch was comparable to that of group 1 (2-3 cm²). Its surface was increased by a 1:1.5 ratio with the mesh-graft device (Figure 1B), thus covering up to 50% of the graft’s surface.

Figure 1. Graft models. A, Group 1: 1-step procedure. The cartilage sheet (d) is sutured under the fascia (c) separated from the underlying muscle (e). The mucosal patch (b) is grafted on the outer surface of the fascia and covered with a silicone sheet (a). B, Group 2: 1-step procedure. Similar graft design. The mucosal patch (b) is meshed before implantation. C, Group 3: 1-step procedure. The cartilage sheet (d) is perforated before implantation under the fascia (c). After 2 weeks, granulation tissue (f) colonized the inner surface of the grafted cartilage creating a vascular bed. D, Group 4: 2-step procedure. First experimental step is identical to group 3; in the second experimental step, meshed mucosa (b) is grafted over the granulation layer (f), 15 days after the first operation.
For group 3, the cartilage sheet was perforated with many rectangular windows and sutured under the dissected fascia. No mucosa was inserted. Granulation tissue coming from the fascia was expected to grow through the windows and colonize the inner surface of the grafted cartilage, thus creating a vascular bed for mucosal grafting in a second stage (Figure 1C).

For group 4, a 2-step procedure was performed. The first experimental step was similar to that described for group 3. In the second stage, a patch of oral mucosa was meshed and sutured to the inner aspect of the cartilage, opposite the fascia. A 3-component graft was obtained, the mucosa being attached directly to the sheet of cartilage (Figure 1D).

**OPERATIVE TECHNIQUE**

The rabbits were placed in the supine position. Anesthesia was induced by administration of xylazine hydrochloride (1 mg/kg intramuscularly) and ketamine hydrochloride (60 mg/kg intramuscularly) and maintained with a halothane-oxygen mixture through a 3.5-mm (inner diameter) orotracheal tube. Fentanyl citrate (5 µg/kg intravenously) was used for analgesia during the operative procedure. A full-thickness oral mucosal patch (1.5 × 2 cm) was harvested from the oral cavity and immersed in a diluted iodine solution until it was used for grafting. The oral defect was closed with 5.0 polyglactin 910 (Vicryl) sutures. In groups 2 and 4, the mucosa was meshed with a mesh-graft device (Aesculap, Tüttlingen, Germany) with an expansion ratio of 1:1.5. A sheet of auricular cartilage (up to 2 × 4 cm) was dissected from the overlying skin after a longitudinal incision was made and removed with its intact perichondrium. The ear skin was closed with 5.0 Vicryl sutures. In groups 3 and 4, multiple small rectangular windows (1.5 × 5 mm) were cut in the cartilage sheet before grafting, using a metallic template (leaving 3 mm between each window).

After shaving and scrubbing of the laterothoracic wall with iodine solution, the skin was incised and dissected from the underlying fascia. The laterothoracic vessels were first located and a 4-cm incision was made through the fascia, parallel to the laterothoracic vessels. Fascia was separated from the muscle, creating a suitable space to insert the cartilage sheet by suturing it on the inner surface of the fascia (groups 1-4) with 5.0 Vicryl sutures.

In groups 1 and 2, a mucosal patch, intact or meshed, was sutured on the outer surface of the fascia opposite the cartilage. A silicone sheet (3 × 5 × 0.2 cm) was fixed over it for protection. The fascia and the overlying skin were then closed and the graft was left in place for 21 days before removal.

In group 3, no mucosa was inserted and the 2-component graft with perforated cartilage was removed after 15 days. Histological study then determined if a sufficient vascular bed had been created for the subsequent mucosal grafting.

In group 4, the first experimental step was similar to that for group 3. Fifteen days later, a harvested mucosal patch was meshed and added to the graft on the inner surface of the cartilage. A silicone sheet was sutured over the mucosa to avoid friction with the underlying muscle. The graft was left in place for 21 more days.

**EVALUATION**

After the appropriate revascularization period, the grafts were removed from the laterothoracic area. Gross examination was first performed. The grafts were then fixed in 4% formalin, embedded in paraffin, transversely sectioned (precisely every 4 mm), and stained with hematoxylin-eosin. The same procedure was followed for control cartilage taken from the ears of 2 animals. Histological slides were analyzed with respect to viability and dimensions of the different graft components. All slides were reviewed by an experienced pathologist. The surface of the graft was defined in all groups by the size of the cartilaginous sheet. The graft area lined with mucosa was calculated on the histological slides and expressed as a ratio of the graft surface. The mucosalized area corresponded to the length of mucosal lining on each slide multiplied by the distance (4 mm) to the next section.

Differences of mucosal coverage between groups 1 and 2 were analyzed using the Wilcoxon rank sum test (Stata 8.2; Stata Corp., College Station, Tex). A nonparametric test was performed because of small sample sizes and unequal variances between groups. Statistical significance was expressed as a 1-sided exact P value.

**RESULTS**

All 20 animals survived the follow-up period until they were euthanized. At the beginning of the study, 2 animals died during the induction of anesthesia. The donor sites (auricular cartilage and oral mucosa) healed without any complication. The oral defect was completely reepithelialized after 3 weeks. No local infection was encountered in either operative site. Seromas of variable sizes developed around the grafts in almost every animal.

**GROUP 1: MUCOSA–FASCIA–CARTILAGE**

Macroscopic evaluation of all 5 specimens showed relatively thin (4-8 mm) and flat structures with a shrunken mucosal patch bulging in the middle of the graft. The cartilage shape was preserved, and the 3 layers were adherent to each other. All specimens demonstrated similar histological patterns (Figure 2A). Living cartilage and mucosa were observed. The cartilaginous framework was intact and the presence of normal nuclei inside almost all of the chondrocytes attested to their viability. Moreover, a variable amount of new-grown cartilage was found on the edges of all grafts. Control materials from native ears showed identical histological patterns. A thick layer of highly vascularized granulation tissue was identified between the mucosa and fascia. The mucosa did not show any sign of necrosis. The extent of the mucosal lining ranged from 3% to 15% (mean, 10%) of the graft surface with a mean graft size of 5.6 cm² (Table).

**GROUP 2: MESHED MUCOSA–FASCIA–CARTILAGE**

All of the grafts were macroscopically thin and flat with the mucosa well applied on the surface; the 3 components were well bonded to each other. Histological analysis of all 5 specimens was comparable and showed living cartilage and mucosa (Figure 2B). Less granulation tissue was observed between mucosa and fascia. Islets of full-thickness meshed mucosa were scattered on the fascia. The gaps between the islets were filled with mucosal regrowth, consisting of 1 or more layers of squamous epithelial cells. This newly grown mucosa was also characterized by a flat junction to the submucosa (absence of papillae). The surface lined with mucosa ranged from 14% to 68% (mean, 40%) of the graft surface and was significantly higher than in group 1 (P = .008). The mean graft size was 5.8 cm² (Table).
GROUP 3: FASCIA–FENESTRATED CARTILAGE

In each case, little seroma had formed between the cartilage and the underlying muscle. The macroscopic evaluation showed strips of cartilage well adherent to the fascia. Living cartilage strips were covered by a thin layer of translucent tissue (Figure 2C). The gaps between the strips of cartilage were filled with a highly vascularized tissue that extended into a thin layer covering the cartilage. The mean thickness of this new-grown tissue (measured under the cartilage) was 0.4 mm. The mean graft size was 6.1 cm².

GROUP 4: FASCIA–FENESTRATED CARTILAGE–MUCOSA

Similar macroscopic aspect to that of group 3 was achieved 15 days after cartilage insertion. On removal of the graft, 4 rabbits presented a laterothoracic lump at the grafting sites. Macroscopic analysis showed a fibrotic mass located above the fascia and filled with a caseous content. The underlying grafts were distorted. Histologically, the alignment of the cartilaginous strips was lost and an excessive proliferation of granulation tissue was present around the cartilage graft. Small mucosal islets were totally embedded in this neofomed tissue and showed necrotic features. Nevertheless, viable mucosal lining was still observed over a very limited area in each case. One animal healed without any local complication, and the graft

**Figure 2.** Histological analysis. A, Group 1: shrunken mucosal patch (a) overlying a thick layer of granulation tissue (b) grown from the fascia (c). B, Group 2: islet of full-thickness meshed mucosa (a) with mucosal regrowth (a') lining a thin layer of granulation tissue (b). Viable cartilage (d) is adherent to the fascia (c). C, Group 3: strips of perforated cartilage (a) are surrounded by highly vascularized granulation tissue (b) grown from the fascia (c). D, Group 4: meshed mucosa (a) with lateral regrowth (a') overlying a thin layer of granulation tissue (b). Viable strips of cartilage (d) are aligned above the fascia (c) (hematoxylin-eosin, original magnification ×20).

**Comparison of Nonmeshed and Meshed Mucosa**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Graft Size, mm²</th>
<th>Mucosal Cover, mm²</th>
<th>Mucosal Cover, %</th>
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<tbody>
<tr>
<td>Group 1 (Nonmeshed)</td>
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<tr>
<td>1</td>
<td>384</td>
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<tr>
<td>Mean</td>
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<td>56.2</td>
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</tr>
<tr>
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<tr>
<td>Mean</td>
<td>576</td>
<td>228</td>
<td>39.6</td>
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</table>

**GROUP 4: FASCIA–FENESTRATED CARTILAGE–MUCOSA**

Similar macroscopic aspect to that of group 3 was achieved 15 days after cartilage insertion. On removal of the graft, 4 rabbits presented a laterothoracic lump at the grafting sites. Macroscopic analysis showed a fibrotic mass located above the fascia and filled with a caseous content. The underlying grafts were distorted. Histologically, the alignment of the cartilaginous strips was lost and an excessive proliferation of granulation tissue was present around the cartilage graft. Small mucosal islets were totally embedded in this neofomed tissue and showed necrotic features. Nevertheless, viable mucosal lining was still observed over a very limited area in each case. One animal healed without any local complication, and the graft
Circumferential tracheal reconstruction has only rarely been performed in clinical practice.13 Segmental resection of up to 50% of the tracheal length with primary end-to-end anastomosis is the treatment of choice, but the management of long-segment tracheal stenosis remains unsolved. Theoretically, the replacement of a tube designed to allow air passage to the lungs seems to be a simple procedure. However, the existence of numerous different surgical methods attests to the difficulties of creating a neotracheal segment. Allograft transplants are not to be considered until a safe long-term immune modulation is available. The use of synthetic materials is associated with induction of granulation tissue, risk of infection, and extrusion. A composite graft of autogenous tissue prevents most of these complications.

The trachea consists of 3 histologically different layers, each with its specific function: a tunica mucosa, a tunica fibro-musculo-cartilaginous, and a tunica adventitia. Composite grafts designed to copy this original framework have been investigated in only a few experiments, but the results are promising.15

We studied different models of 3-component grafts in order to mimic the original tracheal histological characteristics. Cartilage harvested from the rabbit’s ear is a suitable scaffold for the prevention of airway collapse. Graft models without epithelial lining undergo a secondary intention healing with granulation tissue overgrowth and wound contraction leading to stenosis.22 Furthermore, bare cartilage directly exposed in the airway rapidly undergoes necrosis.23 Other types of epithelium may substitute for pseudostratified ciliated epithelium in segmental tracheal replacement without interfering with the mucociliary function.24 Oral mucosa from the cheek of the rabbit is a convenient substitute, and will subsequently be replaced by respiratory epithelium growing from both ends of the graft.17 In long-segment replacements, the blood supply from the anastomosis is not sufficient to prevent necrosis of a free graft. A proper vascular carrier is thus required for the viability of the graft. The laterothoracic fascia flap fulfills this function perfectly in the rabbit model. A close and immobile contact between the vascular bed and the other 2 components is of utmost importance for the revascularization process. This cannot be achieved in the upper airway as the graft moves with each respiration and swallowing act. Therefore, a first stage of heterotopic revascularization of the graft in the laterothoracic area has been experienced.

Different graft models were prefabricated and revascularized in heterotopic position to determine a suitable model for a further tracheal replacement. In groups 1 and 2, the fascia located in the middle of the graft was in close contact with both mucosa and cartilage. In groups 3 and 4, the grafts were designed to mimic the tracheal histology exactly, with cartilage between mucosa and fascia.

In all groups, adequate revascularization was confirmed by the viability of both cartilage and mucosa. Grafted cartilage was histologically similar to the control cartilage of native ears and even showed multiple foci of newly grown cartilage at its extremities. The presence of perichondrium on both sides did not seem to isolate the cartilage from direct nutrient supply.25 Perichondrium also helped avoiding infections and necrosis when free grafted cartilage was directly exposed to the airflow.26 The submucosal layer showed an abundant vascular network arising from the fascia. Mucosa was firmly adherent to the latter and did not show necrotic features. Groups 2 and 4 even demonstrated newly grown epithelium that merged from the section margins of the native mucosa. It consisted of a few layers of epithelial cells regularly spread over the vascular bed. The surface of the graft lined with mucosa was significantly larger in group 2 than in group 1. Meshed mucosa enabled 14% to 68% of the graft surface (mean, 40%) to be covered, compared with 3% to 15% (mean, 10%) with intact mucosa. This difference is explained by the mesh expansion ratio of 1:1.5 and by the amount of mucosal regrowth observed between the meshes and toward the periphery of the graft. In group 1, contraction of the fascia probably induced the shrinkage of the mucosal patch and subsequent absence of regrowth. To our knowledge, no previous report has mentioned the use of meshed grafted mucosa to improve the epithelial coverage of prefabricated grafts intended to replace airway segments. Some investigators have experienced the efficiency of strips or small patches of mucosa scattered over the graft’s surface, but with variable results.17,19,27 Grafting of meshed oral mucosa can be considered as an efficient and easy way to expand the surface of mucosal coverage.

A 2-step procedure was investigated in groups 3 and 4. The windows created through the cartilage allowed the growth of highly vascularized tissue, thus forming an adequate support for the subsequent mucosal application. If applied over an unperforated cartilage, the mucosa will rapidly undergo ischemic necrosis. This first step was achieved successfully for all rabbits in groups 3 and 4. However, mucosal application over the deep cartilage surface was associated with some technical difficulties. This may explain the variable results in group 4. The excessive fibrotic reaction observed in 4 of 5 animals is possibly due to the successive surgical traumas of a 2-step procedure. The caseous content of the fibrotic mass may be related to the neighborhood of active mammary glands embedded in the fascia. Histologically, this active proliferating tissue surrounded the mucosal islets, distorted its architecture, and then prevented its growth and viability. However, we obtained limited areas of living mucosa in each graft. One animal presented the expected result with adequate graft architecture and a satisfactory mucosal coverage. This confirms the feasibility of the 2-step procedure, even if some technical improvements are still required. Male rabbits will also be considered for future trials to avoid the presence of active mammary glands.

Currently, we are investigating the possibility of prefabricating grafts around a silicone tube to create a pipe intended to replace a tracheal segment. The prefabricated tube situated on the distal end of the fascia will be transferred...
to the neck region by way of a rotated pedicled flap. In humans, autologous composite grafts may be considered with a similar approach. Large areas of oral mucosa and different fascia flaps (antebrachial, deep thoracodorsal, serratus, temporoparietal) are available. Auricular or costal autologous cartilages are easy to obtain with little morbidity. However, both an insufficient amount of cartilage and difficulty shaping it represent major drawbacks. Therefore, allogenic tracheal cartilage would be an outstanding option as long as antigenicity can be reduced or abolished. Alternate ways to manage immunogenicity are now available, such as cryopreservation, radiation therapy, or chemical treatment. They may be of interest in prefabricating graft models with allogenic cartilages.

CONCLUSIONS

A 3-layer composite graft has been prefabricated in different designs mimicking the original tracheal framework. A free graft of oral mucosa survives either over vascularized fascia or when applied over a prefabricated vascular bed. The mesh-graft technique is an efficient way to increase the surface of mucosal coverage without interfering with its viability. The living cartilage obtained in all cases will constitute the rigid scaffold needed to prevent airway collapse for circular reconstructions. Double-layer models encompass the needed characteristics for an optimal tracheal repair. Thus, further experimental trials will be undertaken to evaluate their ability to replace circumferential tracheal segments as described herein. Composite grafts can be considered as a promising way to reconstruct long-segment airway defects.

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