Katholieke Universiteit Leuven
Faculteit der Geneeskunde
Departement Heelkunde en Anesthesiologische Wetenschappen
Centrum voor Experimentele Heelkunde en Anesthesiologie

Tracheal Repair: from Wound Healing Research to Prefabricated Reconstruction

J. Hardillo

Neus- Keel- Oorheelkunde
Gelaat- en Halschirurgie
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Thesis submitted in fulfillment of the requirements for the degree of
"Doctor in de Medische Wetenschappen"

Promotor: prof. Dr. Pierre Delaere

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Table of contents

Abbreviations

ant. def.	anterior defect
blue Microfil®	blue silicone dye
cm	centimeter
CO_2	carbon dioxide
CT	computed tomography
D	distal flap
F	fascia flap
H&E	haematoxylin and eosin stain
Imalgene®	ketamine hydrochloride
kg	kilogram
L	lower mucosal defect
ml	milliliter
mm	millimeter
mm^2	square millimeter
N	number
P	proximal flap
preop	pre-operative
postop	post-operative
p	probability level
SAR	reduction in surface area
SAf	final surface area of reconstruction
SAi	initial surface area of reconstruction
SD	standard deviation
SE	standard error
μm	micrometer
U	upper mucosal defect

Chapter I. Introduction

I.1 Problems in tracheal reconstruction: stenosis of long segments and recurrent stenosis

The preferred surgical approach for tracheal stenosis is resection of the stenotic segment followed by end-to-end anastomosis. In general this may be done safely after resection of up to one half of the trachea in adults (about half the length of the entire trachea) and one third in infants and small children[1].
A tracheal resection (Fig. 1.1) can be performed for tracheal stenosis; a cricotracheal resection[2, 3] can be performed when the cricoid cartilage is also involved in the stenosis ; and a slide tracheoplasty[4] may be used as a primary reconstruction technique for correction of tracheal segments with circular cartilage rings. The advantage of tracheal resection, cricotracheal resection, and slide tracheoplasty is that no graft is necessary and that there is no need for prolonged endotracheal intubation.

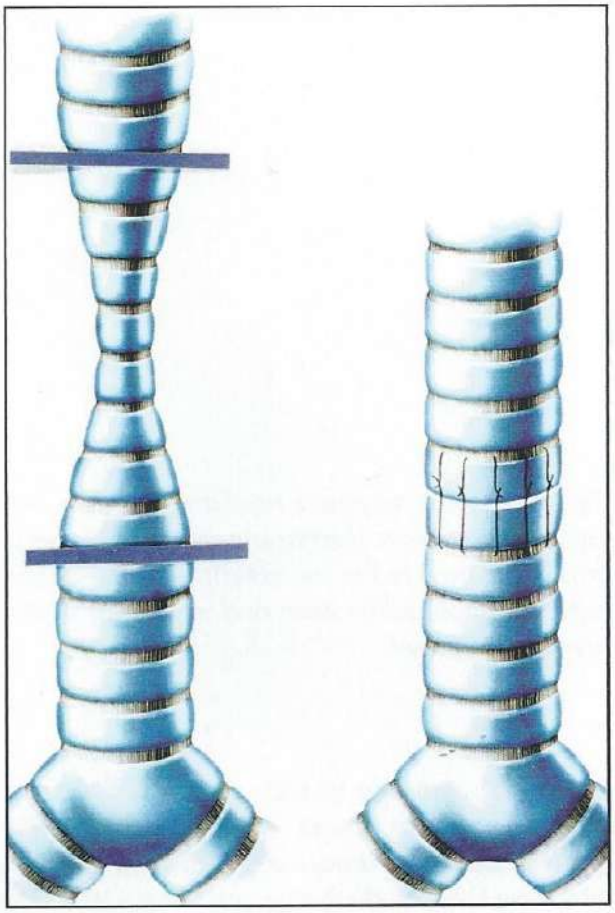


Fig. 1.1. Tracheal stenosis treated by segmental resection and end-to-end anastomosis.

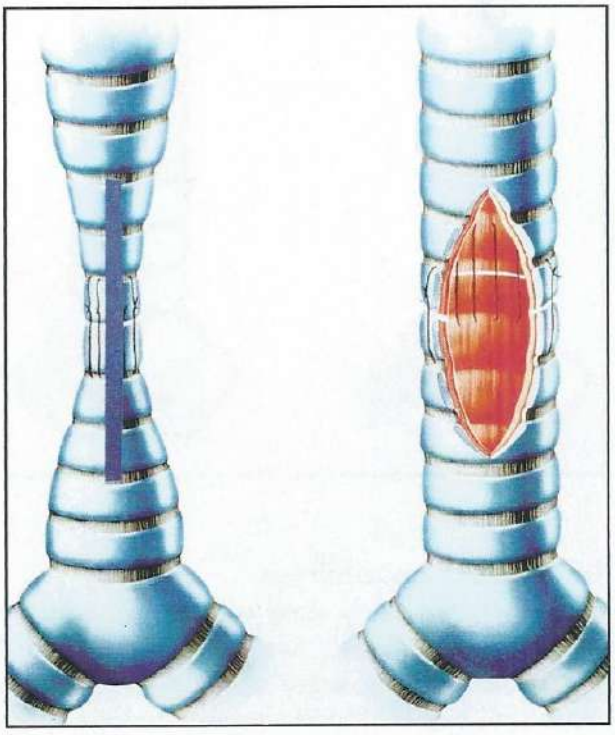


Fig. 1.2. Restenosis after segmental resection. A complication of a segmental tracheal resection is that an anastomotic stricture may develop. These strictures are usually related to excessive tension at the anastomosed site. The restenosis may be treated by longitudinal incision, expansion of the stenotic area and insertion of repair tissue.

Augmentation of the tracheal lumen by inserting local, regional, or distant tissue is necessary when tracheal resection is not possible as for example in long segment stenoses (more than half of the tracheal length) (Fig. 1.3) or in cases of restenosis after tracheal resection (Fig. 1.2).
A stenosis of more than half the tracheal length is rather exceptional. However, because the indications for tracheal resections are growing[5], it can be anticipated that restenosis at the anastomotic site will become a more frequently encountered problem. Restenosis poses a therapeutic problem because further resection is usually not possible and mostly, repair tissue has to be used to augment the problematic airway segment. Tracheal reconstruction by using repair tissue is a second choice solution because no reliable repair tissues are currently available in the clinical situtation.

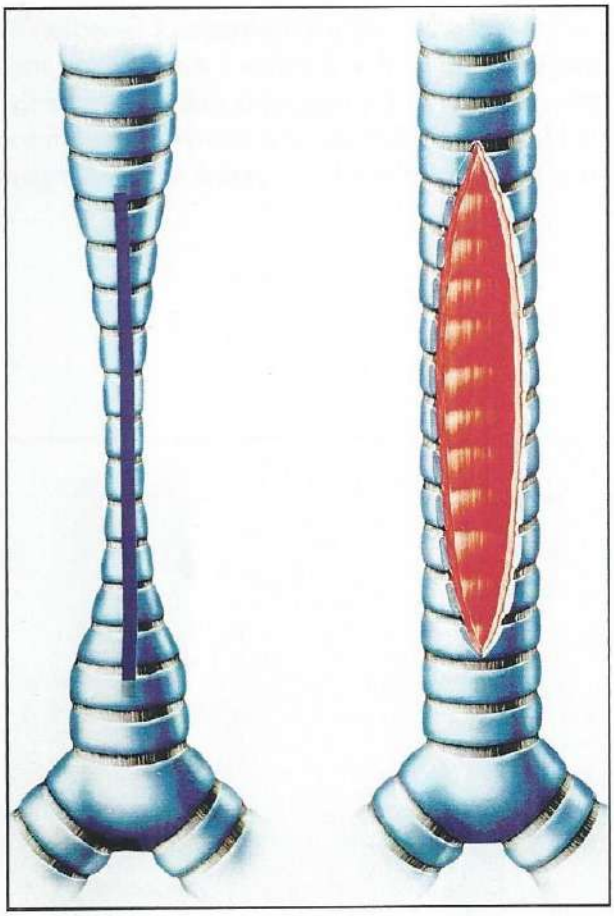


Fig. 1.3. Long segment tracheal stenosis. For an augmentation tracheoplasty, new material has to be inserted in an anterior airway defect after longitudinal incision and expansion of the stenotic segment.

1.2 Current possibilities

The most frequently used reconstructive tissues consist of cartilage grafts[6], pericardium[7], and myocutaneous flaps[8]. Results obtained with these reconstructive tissues are not constant and the choice among these options is difficult since no valuable comparison is available in the literature. Inherent to the rare pathology, these reconstructive possibilities have been applied in series with small number of patients without a clear definition of the repaired defect and with the number of decannulated patients as the only outcome variable.

I.3 Hypothesis-reconstructive concept

We hypothetized that the reconstructive possibilities inside the trachea might be improved if tissue with the combined characteristics of mucosal lining, cartilage support, and vascularity would become available (Fig. 1.4). Experimental evidence for this hypothesis came from our previous experiments on tracheal transplantation[9].

I.3.A Optimal situation: tracheal reconstruction with revascularized tracheal allotransplants

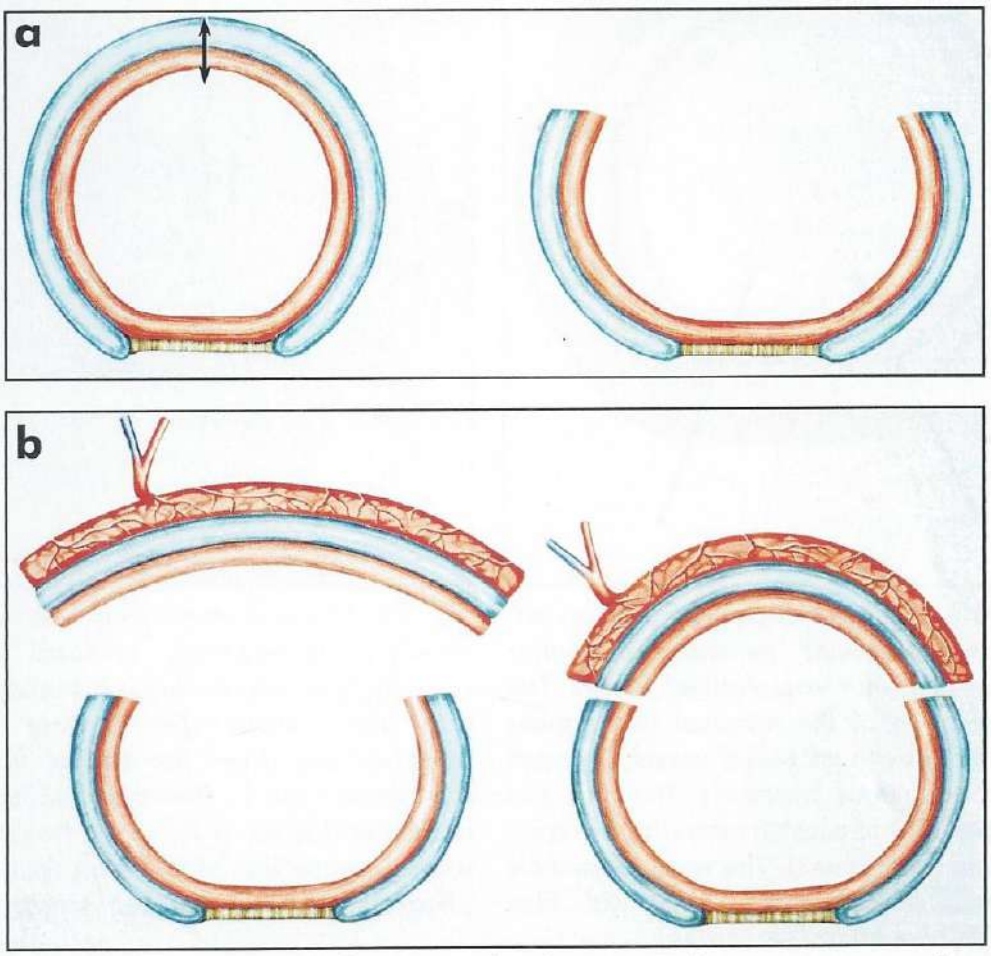


Fig. 1.4. Anterior tracheal defect after longitudinal incision of a recurrent or a long segment tracheal stenosis.
***a**. Anterior tracheal defect after incision (double arrow) and expansion of a recurrent stenosis.*
***b**. Tissue requirements for optimal reconstruction are illustrated when using revascularized allografts. The revascularized allograft gives the reconstruction an optimal cartilage support, the optimal mucosal lining and a good blood supply.*

A tracheal allotransplant can become revascularized in an immunosuppressed animal (rabbit)[9,10]. Full revascularization is obtained after 14 days of heterotopic wrapping in vascularized fascia. An axial section through a revascularized trachea is visible in Fig. 1.5. This revascularized tracheal tube can be transformed into a patch after excision of the membranous trachea and incision of the distal fascia flap. The transformation can be done without interfering with the blood supply to the revascularized trachea because of the distal incision of the fascia flap. The revascularized tracheal allograft patch consists of a vascularized respiratory mucosal lining and a cartilage support and forms the optimal tissue combination for use in an anterior tracheal defect. After reconstruction, the blood supply to the fascia flap and the tracheal patch may be identified after injection of the vascular pedicle of the fascia flap with blue silicone dye (Microfil® ; Canton Bio-medical Products Inc, Boulder, Colo) (Fig.1.5).

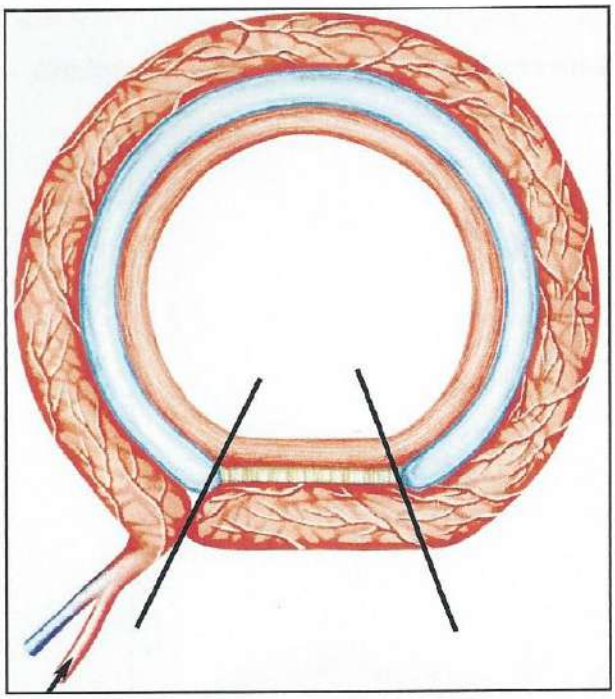


Fig. 1.5. Axial section of revascularized trachea. The trachea becomes revascularized after being wrapped with vascularized fascia. The revascularization of the mucosal lining takes place by outgrowth of blood vessels through the intercartilaginous ligaments. The tube can be transformed into a patch after distal incision of the fascia (black lines). The vascular pedicle of the fascia flap can be injected with blue silicone dye-blue Microfil® (arrow).

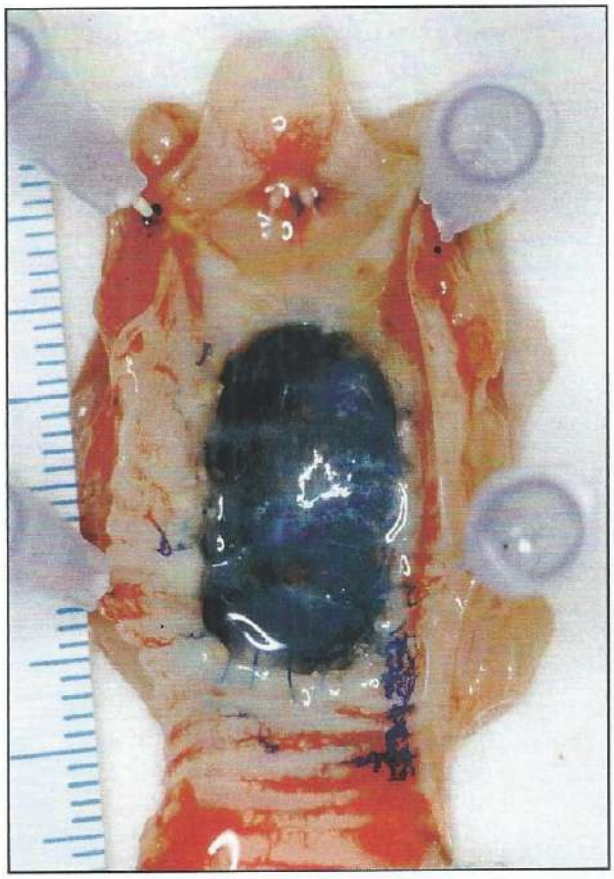


Fig. 1.6. Postmortem reconstruction of anterior airway defect-internal, mucosal view. The respiratory mucosal lining is completely colored with blue silicone dye because it is fully revascularized from the fascial flap around the tracheal patch. The revascularized mucosa is responsible for a primarily healing without wound contraction. A sharp transition between allograft and native trachea is visible.

The reconstructive results after the repair of anterior airway defects with revascularized tracheal allografts are visible in Fig. 1.6 and 1.7.

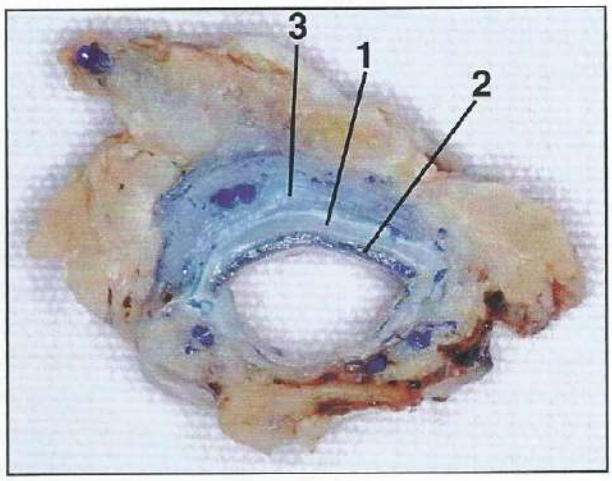


Fig. 1.7. Postmortem reconstruction of anterior airway defect-axial section. The 3 tissue requirements cartilage (1), mucosal lining (2), and vascularization-fascia flap (3) are visible. The elastic cartilage of the allograft gives support to the reconstruction with an anterior expansion of the airway lumen.

Clinically, revascularized tracheal allografts would also provide for the optimal tissue repair when dealing with difficult tracheal stenosis. The continuous administration of immunosuppression necessary to keep the allograft viable forms the main obstacle for clinical use of tracheal allotransplants.

I.3.B Autologous tissues for tracheal reconstruction

In this study we looked for alternatives for the tracheal allotransplant by studying autologous tissues. The most appropriate individual tissues that provide for vascularization, lining, and support were defined experimentally (rabbit) and clinically.

1. Vascularity: Axial perfused fascia flap

As in other areas of reconstructive surgery, the key factor in tracheal reconstruction is a reliable blood supply to the reconstructive tissues. The option was to use the thinnest vascularized tissue sheet which is available. A vascularized fascia flap was used as vascular supply to the reconstructive tissues.
In rabbits, the lateral thoracic fascia (described by Delaere et al.)[10] provides a very reliable fascial connective tissue flap. Our option was to use the radial forearm fascia as a clinical counterpart.

Experimental: Lateral thoracic fascia

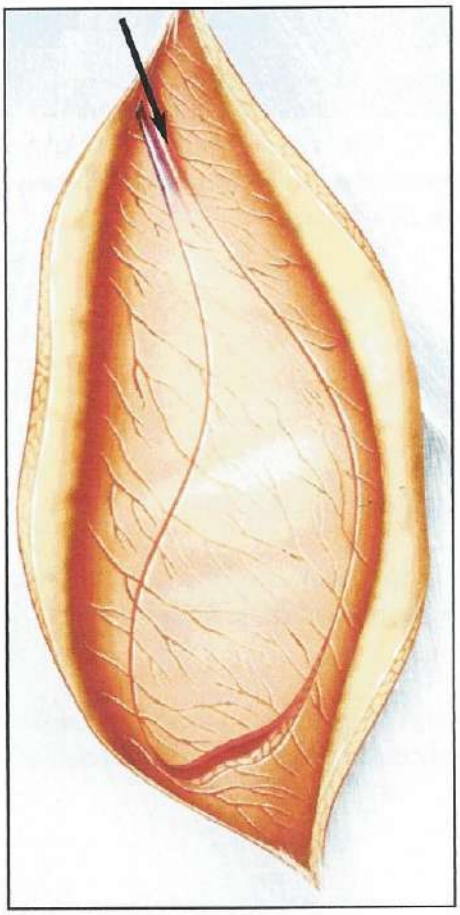


Fig. 1.8. The lateral thoracic fascial flap is situated subcutaneously on the lateral chest wall of the rabbit. The craniocaudal axis measures 15 cm; in ventrodorsal direction the flap is 5 cm wide. The flap consists mainly of a fascial connective tissue containing the vascular network. The flap is supplied by the lateral thoracic vessels which emerge from the axillary vessels. The flap can be rotated to the neck region with preservation of the vascular pedicle. The lateral thoracic artery (arrow) can be injected with blue silicone to visualize the vascularity of the fascial flap.

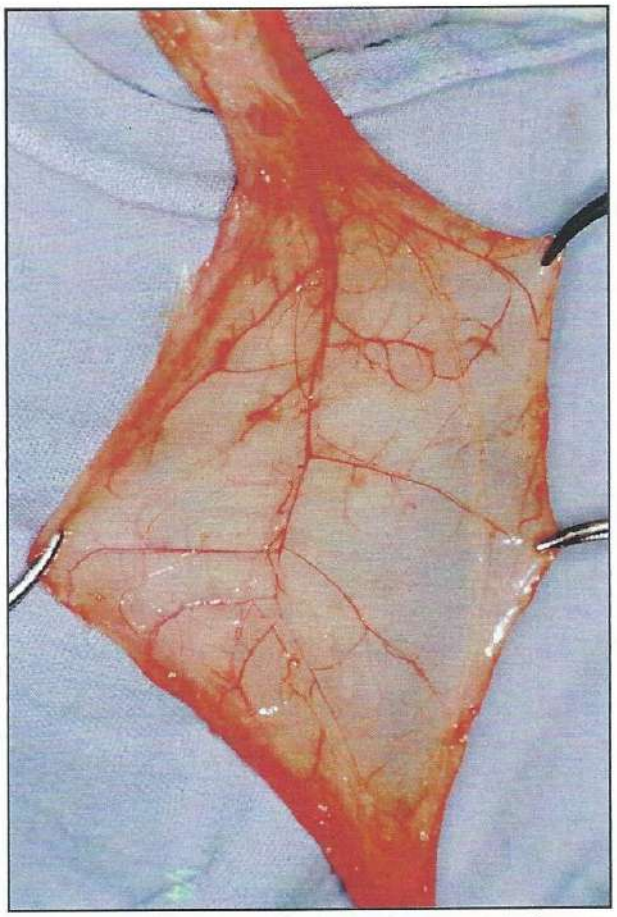


Fig. 1.9. The lateral thoracic fascial flap is shown after dissection. The dorsal branch of the lateral thoracic artery and vein runs in the middle of the flap, axially towards the iliac crest. The fascial tissue is less than 1 mm thick and is extremely pliable

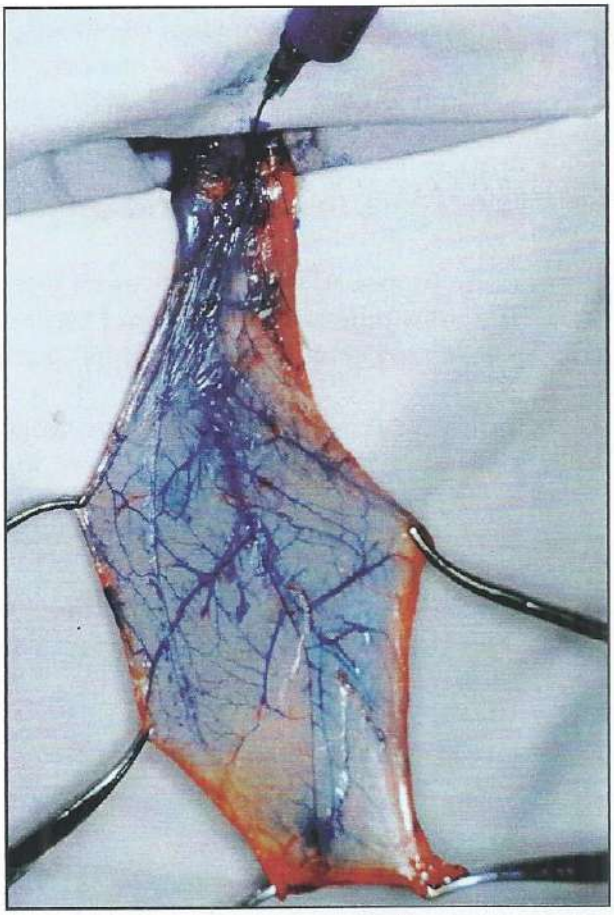


Fig. 1.10. The lateral thoracic fascial flap after injection of the lateral thoracic artery with blue silicone dye-Microfil®.

In this study, the fascial flap was used:

-As vascularized tissue in airway reconstruction
-To revascularize the cartilaginous and mucosal tissue component
-To revascularize segments of autologous trachea.

The amount of vessel outgrowth and revascularization from the fascia flap could be visualized after injection of the fascial vascular pedicle with blue silicone dye (blue Microfil®).

Clinical: Radial forearm fascia

A clinical counterpart was sought for each tissue that was used experimentally in order to make a translation of the experimental results into clinical application (from 'bench to bedside') easier. (Fig. 1.11, 1.12)

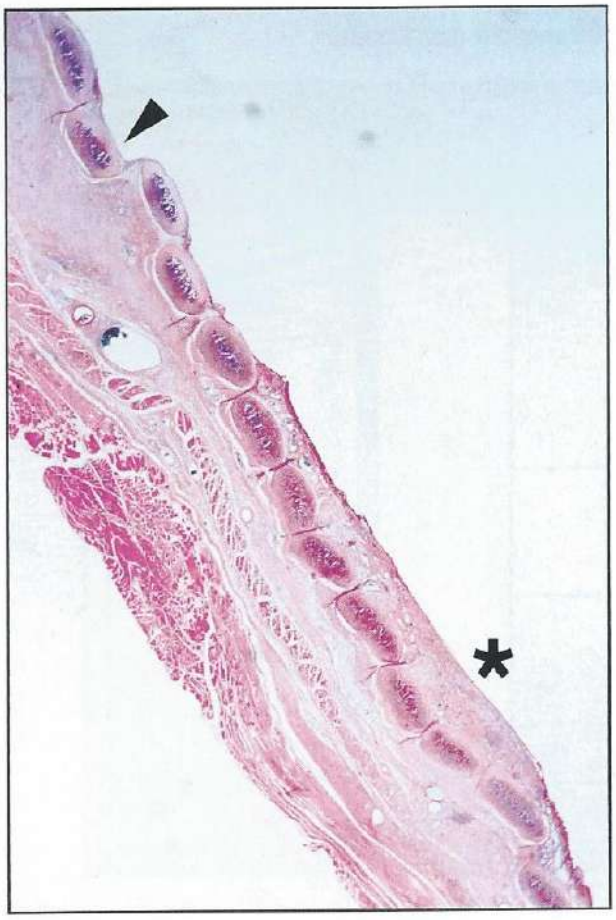
II.3.C Full thickness mucosal defect after mitomycin application

C.1 Anterior patch mucosal defect after mitomycin application (N=7)

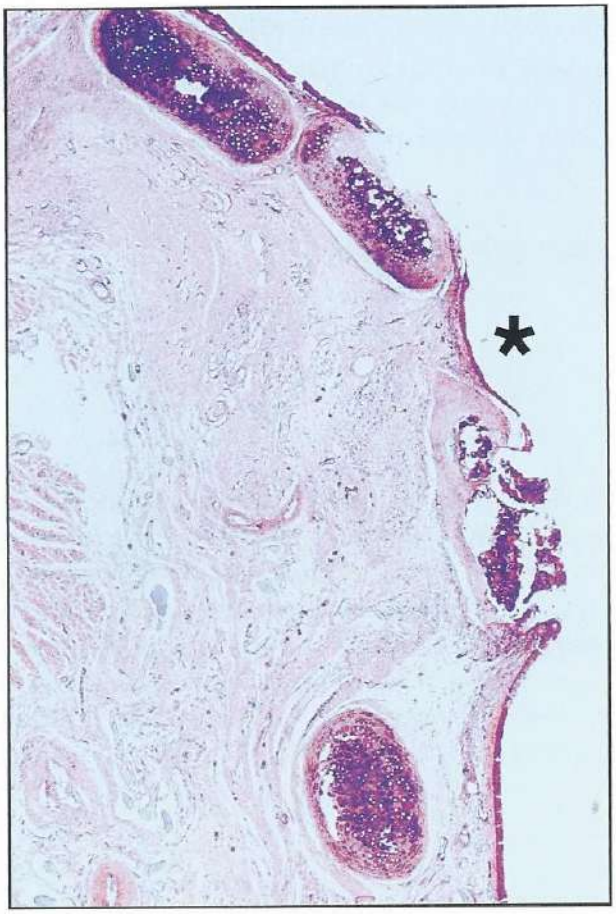
As shown in Fig. 2.9.A and Fig. 2.10, untreated wounds are consistently smaller than the wounds treated with mitomycin. This result is highly reproducible; in 5 separate experiments, a single application of mitomycin produced wounds that were on average 250% larger than controls, representing a 56% decrease in contraction relative to that seen in control healing ($p<0.001$)(Table2.2). Histological examination of the control patches showed a reepithelialized wound with a lamina propria that was slightly thicker than the lamina propria of the normal trachea (Fig. 2.9.B). Histological examination of the mitomycin treated patches showed a blockage of angiogenesis at the sites of the intercartilaginous ligaments. The bare cartilage rings, which remained exposed to the airway lumen, underwent necrosis with loss of support. Two animals were followed for 1 month to evaluate cartilage viability after longer follow-up. Histological examination in these animals revealed cartilage necrosis with respiratory epithelium between the necrotic cartilage rings. In contrast to the process of angiogenesis, migration of respiratory epithelial cells was not inhibited by the mitomycin application (Fig. 2.9.C).

Fig. 2.9. Wound healing of anterior mucosal defects with and without mitomycin application.

A. Macroscopy of treated tracheal segment 14 days after reimplantation. The upper, mitomycin treated, defect is denuded over the majority of the initial defect area whereas the lower defect is nearly completely healed. At the site of the upper defect, granulation tissue formation is completely blocked at the intercartilaginous ligaments.

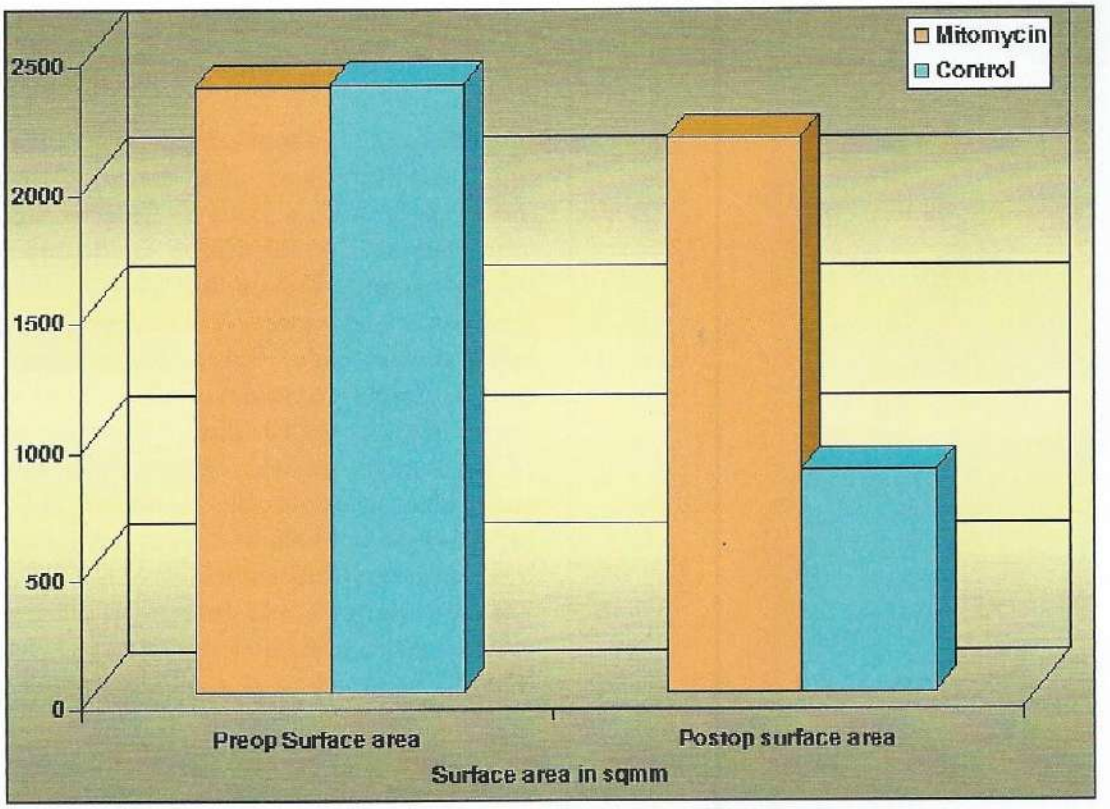


B. Histology on longitudinal section 14 days after reimplantation. The area indicated by an asterisk is the control defect; the area indicated by an arrowhead is the mitomycin treated defect. The control defect underwent some wound contraction with reduction of the initial surface area. The lamina propria of the control defect contains smaller blood vessels and is slightly thicker than the lamina propria of the native trachea. The control defect is completely covered by epithelial cells. The regenerated epithelium is 1 layer thick and has a squamoid differentiation
The mitomycin treated defect has preserved its initial surface area and the cartilage rings are directly exposed to the airway lumen (H&E, original magnification x 4). Note that the external side of the cartilage rings are more eosinophilic (early cartilage necrosis) than the internal side of the cartilage rings.



C. Histology on longitudinal section 1 month after reimplantation. Detail of mitomycin-treated defect in animal suriving for 1 month. The bare cartilage rings underwent partial necrosis and destruction (external side) with loss of support (prolapse inside the lumen). The intercartilaginous space between 2 cartilage rings (asterisk) is lined with respiratory epithelium (H&E, original magnification x10).

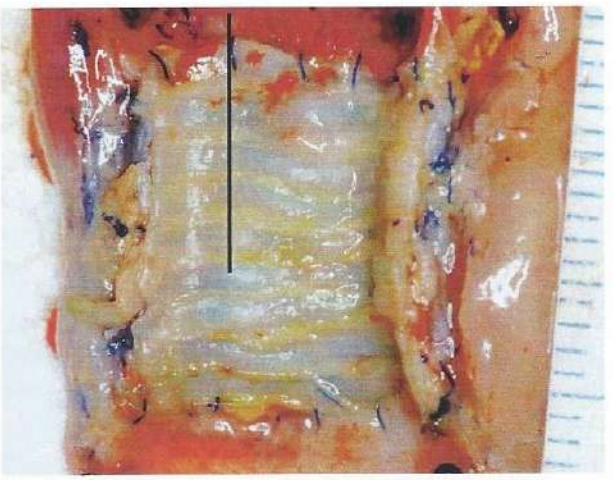
Fig. 2.10. Wound contraction with and without mitomycin application.



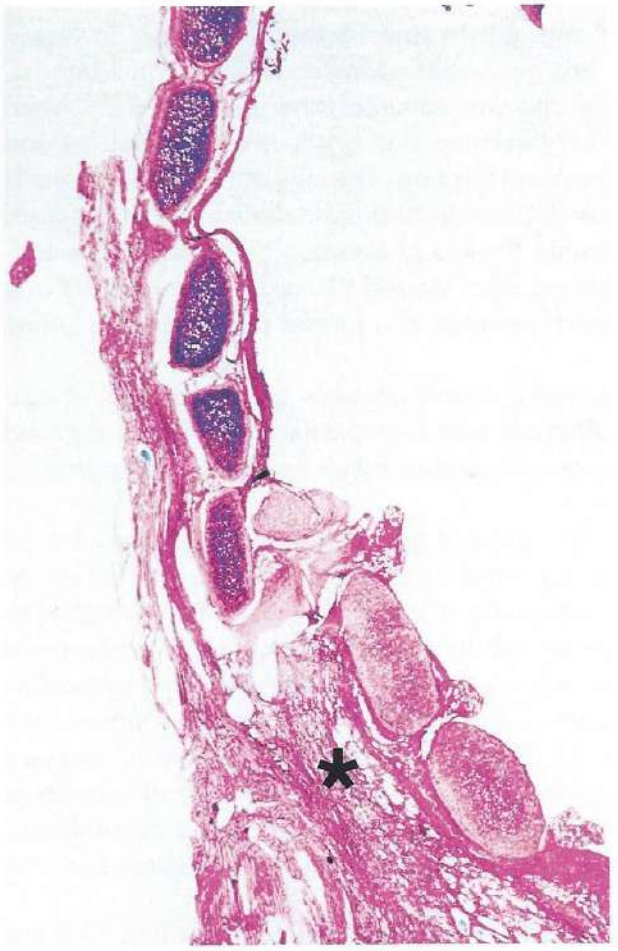
C.2 Circumferential mucosal defect after mitomycin application (N=3)

The 3 animals with the circumferential tracheal defects survived for 10, 13, and 14 days. The animals showed respiratory distress because the bare cartilage underwent necrosis with loss of support. No granulation tissue was formed at the luminal site of the mitomycin treated cartilage segments (Fig. 2.11A, B).

Fig. 2.11. Circumferential mucosal defect after mitomycin application.



2.11.A. Macroscopy of denuded trachea 2 weeks after reimplantation. The fascial flap around the trachea is injected with blue silicone dye . The denuded segment shows bare cartilage rings (with loss of support) and intercartilaginous ligaments without signs of granulation tissue formation. Histology of a longitudinal section (black line) is visible in Fig. 2.11.B.



2.11.B. Histology on longitudinal section. The transition area between intact trachea (normal cartilage rings, vascularized lamina propria, and respiratory lining) and denuded trachea (eosinophilic and airway exposed cartilage rings, no lamina propria, no epithelial lining) is visible. The intensely vascularized fascia around the denuded segment is indicated with an asterisk (H&E, original magnification x 10).

II.4 Discussion

The loss of airway lumen during secondary healing of full thickness mucosal defects is quite extensive. Granulation tissue formation is the main reason for the loss of airway lumen in tracheal wounds with preserved vascularization and cartilaginous support. The granulation tissue is formed by a process of angiogenesis acting through the intercartilaginous ligaments. The proliferation of vascularized connective tissue is blocked when the regenerated lamina propria becomes covered with respiratory epithelial cells. Coverage by respiratory epithelium occurred relatively fast in the small, (.6 x .6 cm) control mucosal defect of the mitomycin treated tracheas. The wounds were completely reepithelialized and the regenerated lamina propria was only slightly thickened in comparison to the lamina propria of the undamaged trachea. This healing pattern, which is characterized by migration and proliferation of respiratory epithelium, is well known in the literature[6,7]. The relatively fast reepithelialization can be explained by an epithelial migration process that originates from the 4 (left, right, upper, lower) sides of a relatively small defect.
The healing pattern is quite different when dealing with larger mucosal defects. Regeneration and migration of respiratory epithelium was much slower in the anterior (0.6 x 1.5 cm) airway defect. In these defects, migrating epithelium has to come from 2 (left, right) sides of the defect. Epithelial regeneration from the upper and lower anastomotic sides will only be possible for the upper and lower extremities of the defect. The center of the scar will have the thickest lamina propria because this area is lastly covered with respiratory epithelium. Loss of airway lumen is due to granulation tissue formation and due to wound contraction forces acting in the direction of the upper surface of the granulating wound (Fig. 2.6.D). Wound contraction is a basic biological process and occurs in an incompletely epithelialized defect. It has been defined as

the mechanism by which the edges of a wound are drawn toward the center due to forces generated within that wound. Wound contraction has been studied intensively for skin wounds[8]. In full-thickness rat skin wounds it was shown that the wound area diminishes by 90% when the wound is left to granulate while wounds with full-thickness skin grafts preserve their wound surface area. Contraction is a vital aspect of open wound healing. The size of the tissue defect is reduced so that a lesser degree of connective tissue deposition and epithelialization is required. Myofibroblasts are the cells thought to be responsible for this phenomenon[8]. The wound contraction forces acting during healing of the anterior mucosal wound (0.6 x 1.5 cm) resulted in a reduction of the curvature of the cartilage rings which resulted in a further loss of airway lumen (Fig. 2.6.D).

In the circumferential mucosal defect, reepithelialization is only possible by new growth of epithelium from the upper and lower anastomosis. Animals with a circumferential defect survived only a short period of 14 days because of excessive granulation tissue formation without signs of reepithelialization.

It has been reported that damage of the perichondrium and the cartilage is necessary for an airway stenosis to develop[1,9]. From the results obtained in this study, we conclude that a full -thickness mucosal tracheal defect will be sufficient for obtaining a loss of airway lumen.Whether or not the healing process will lead to a stenosis depends on the length and on the circumferential extent of the defect. A long, circumferential defect will result in airway stenosis. The circumferential extent of the wound will have a greater impact on the reduction of the airway lumen than the length of the defect. The loss of airway lumen resulting from healing of the anterior mucosal defect (wound measuring 0.6 x 1.5 cm) was extended over a length of 1.5 cm and had no or minimal influence on the respiration of the animals. The loss of airway lumen of a circumferential defect with a length of 0.6 cm would however inevitably lead to respiratory distress because the loss of airway lumen would occur over only 0.6 cm.

The problematic migration of respiratory cells from the wound margins seen in the anterior (0.6 x 1.5 cm) and in the circumferential mucosal defect is in contrast to optimistic reports in the literature concerning the possibility of respiratory epithelial cell migration[10]. Optimistic reports on migration of respiratory epithelium are based on the fast recovery of epithelium in small defects, on the fast recovery of epithelium in superficial mucosal wound healing with preservation of the basement membrane, and on in vitro studies[11].

In our model, topical application of Mitomycin blocked the angiogenic process and inhibited granulation tissue formation at the internal side of the defect. Mitomycin is known to act as an agent for scar inhibition and has gained wide acceptance in ophthalmology[12,13] and otolaryngology[3,4,14] for procedures in which scarring is problematic. In our experimental model we were able to show that the drug acts by blocking angiogenesis but without inhibition of the migration of respiratory epithelium. In our model, mitomycin had a negative influence on airway healing because the bare cartilage rings developed necrosis with loss of support. It seems that mitomycin can safely and effectively be applied on scar tissue in the treatment of airway stenosis. Warning is however necessary when dealing with bare cartilage because of the risk for cartilage necrosis when granulation tissue formation is blocked.

The following conclusions were made concerning the etiology of airway stenosis and the requirements for laryngotracheal repair:

- Full-thickness damage of the respiratory epithelium leads to narrowing and eventual stenosis of the airway lumen.
- Long-term segmental tracheal reconstruction with denuded vascularized tracheal autografts is impossible because of granulation tissue formation in the untreated transplants and because of cartilage necrosis after mitomycin application.
- Restoration of the mucosal component is important in airway reconstruction. Secondary healing leads to reepithelialisation but the granulation tissue formation and the wound contraction have a negative impact on the airway lumen.

Table 2.1. Preoperative and Postoperative surface areas of anterior defects

Anterior defects	preop whole surface area(sqmm)	postop whole surface area (sqmm)	% contraction	preop defect area (sqmm)	postop defect area (sqmm)	% contraction
ANT1	8725.00	6026.41	30.90	3998.50	2162.79	46.00
ANT2	9483.88	7091.32	25.20	4224.00	2080.78	50.70
ANT3	8039.10	6633.18	17.50	4594.52	1349.32	70.60
ANT4	8402.09	7233.01	13.90	4514.03	1808.32	59.90
ANT5	9033.00	7559.67	16.30	4630.45	1498.61	67.60
Average	8736.62	6908.72	20.76	4392.30	1779.97	58.96
Std Dev	557.86	595.11	7.07	271.95	354.42	10.50

Table 2.2 Preoperative and Postoperative surface areas of anterior defects with and without mitomycin application

Groups	preop surface area (sqmm)	postop surface area (sqmm)	% contraction
Mitomycin			
Mito1	2192.87	2137.790	2.51
Mito2	2437.43	2218.040	9.00
Mito3	2402.40	2339.800	2.6
Mito4	2335.26	1986.345	14.90
Mito5	2379.29	2086.210	12.30
Average	2349.45	2153.630	8.00
Std Dev	95.07	133.780	5.60
Controls			
Con1	2245	916.690	59.10
Con2	2449.95	918.580	62.50
Con3	2369.30	848.390	64.70
Con4	2336.11	717.050	69.30
Con5	2408.10	899.720	62.60
Average	2361.69	860.080	63.64
Std Dev	77.89	84.840	3.70

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Chapter III. Tubes of autologous cartilage used as a segmental tracheal replacement: How do they heal?

Abstract

Objectives:

To evaluate the healing process of an autologous cartilage tube used as a segmental tracheal replacement in the rabbit and to predict the progress in tracheal reconstruction that will be made when cartilage tubes would become available by tissue engineering techniques.

Methods:

A piece of auricular cartilage (2.5 cm by 1.6 cm) was tubed and used as a segmental tracheal replacement. Three different cartilaginous tubes were studied:

1.Free cartilage tube used as segmental tracheal replacement (N=5).

2.Cartilage tube wrapped with vascularized fascia (N=2).

3.Cartilage tube wrapped with vascularized fascia and used as segmental tracheal replacement (N=5).

In group 1, a segment (1.6 cm) of the cervical trachea was removed and replaced by the tubed graft of auricular cartilage (1.6 cm). Animals were followed until signs of respiratory distress became apparent. At that time, the cartilage tubes were assessed for remucosalization and cartilage viability.

In a second set of experiments, the blood supply around the cartilage tube was augmented. A piece of auricular cartilage (2.5 cm x 1.6 cm) was sutured to the vascularized fascia in the lateral thoracic area and made into a tube. The cartilage tube was assessed morphologically 1 month after heterotopic revascularization.

In a last set of experiments, the heterotopically revascularized cartilage tubes were transplanted to the trachea with preservation of the established blood supply around the tube.

Results:

Group 1 animals showed respiratory distress after 22.4 days (mean); (SD=8.7) because of cartilage necrosis with loss of airway support. Cartilage graft revascularization and remucosalization was limited to 18.7% (mean); (SD=10.8) of the initial surface area of the cartilage tube. Group 2 animals preserved their full cartilage viability after being wrapped in vascularized fascia. Group 3 animals showed an improved blood supply around the graft but this did not lead to an improved outcome (survival 22.6 days); (SD=4.1); graft revascularization and remucosalization 18.1% (mean); (SD=3.3).

Conclusions:

Tubes of autologous cartilage show a problematic healing when placed inside the airway. Migration of vascularized connective tissue, migration of respiratory epithelium, and preservation of the viability of the cartilaginous graft, is limited to a short segment at the anastomotic sites. Improved vascularization around the cartilage tube improved survival of heterotopically located grafts but had no influence on cartilaginous tube healing when used inside the airway.

III.1 Introduction

Tissue reconstruction of segmental tracheal defects may have a clinical indication in cases of long segment tracheal stenosis in which it is impossible to perform a tracheal resection with end-to-end anastomosis.
Experimental attempts to bridge circumferential tracheal defects were done with tracheal allografts[1], prostheses[2], and autologous[3] tissue. No technique proved fully reliable and it is still impossible to reconstruct circumferential tracheal defects. Recently, tissue engineered tubes of cartilage[4] and bone[5] seemed to hold some promise for circumferential tracheal repair. Tissue engineered cartilage, grown in the shape of cylinders has been used for replacing circumferential defects of the cervical trachea in rats. Cartilaginous and osseous tubes would prevent airway collapse and are supposed to support confluent regrowth of respiratory epithelium from the native trachea[6,7].
In this respect, it has been stated that the ideal composite graft for tracheal replacement should be well vascularized, biocompatible, and nonimmunogenic. Also, the tissue should have intrinsic skeletal support to prevent airway collapse, without the need for stenting, yet still be flexible, and permit rapid epithelialization from the native trachea[5].
It may be possible in the near future to create a hollow tube of autologous cartilage using tissue engineering techniques. However, despite the fact that such a tube might be engineered, it remains a question if these cartilage tubes would succeed in repairing long segmental defects. Do these free unvascularized cartilage grafts pick up sufficient blood supply from the surrounding tissues to survive and will the respiratory epithelium migrate in from quite far away to cover bare cartilage grafts? Our well established experimental rabbit model, developed for laryngotracheal reconstruction[8] and transplantation[1] may be used to answer these important questions. This animal model provides the possibilty to construct a circumferential cartilaginous neotrachea without the need for tissue engineering techniques. A tube consisting of ear cartilage with different patterns of vascular supply was formed. The inner lining of the tube consisted of perichondrium, a tissue that is known to allow for rapid reepithelialization[6]. With this model we were able to reconstruct the cervical trachea of the rabbit without placement of a stent or a tracheostomy.
This experiment was not performed to develop a technique with potential clinical applicability because no similar amounts of cartilage are available in humans. The study was done to answer the following questions:

- Can a circumferential tracheal reconstruction be obtained and maintained with a tube of autologous cartilage?
- Can the healing of the cartilage tube be improved by augmenting the blood supply around the tube?
- What is the capacity of spontaneous respiratory epithelium migration in a cartilage tube?

Answering these questions may help and direct future research on circumferential tracheal replacement.

III.2 Material and methods

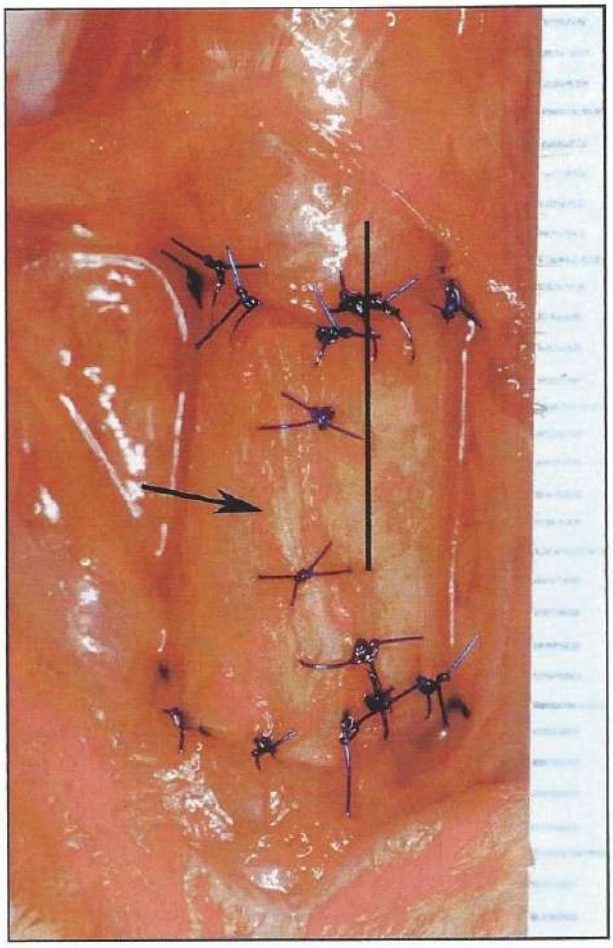
Twelve mature New Zealand white rabbits, with a mean age of 6 months and weighing between 3 and 3.5 kg were used. Rabbits were housed in separate quarters in an environment-controlled facility and fed a standard rabbit diet.

The animals were premedicated with 0.25 mg/kg of Imalgene® and 0.15 mg/kg of Domitor® intramuscularly and anaesthesia was maintained with halothane through mask ventilation. The animals were divided into 2 groups following the degree of vascular supply for the cartilage graft. No additional blood supply was provided for group 1 (N=5) animals while the lateral thoracic fascial flap was used to give additional blood supply to group 2 (N=2) and group 3 (N=5) animals.

III.2.A Group 1. Free cartilage graft used as segmental tracheal replacement (N=5)

The cervical trachea was reached through a midline neck incision and the upper end was dissected over 2 cm from the underlying tissue. A tracheal segment of 1.6 cm was removed. A transverse incision was made over the dorsal surface of the left ear. The perichondrial surface of the auricular cartilage was isolated, and a 25 x 16-mm section of cartilage was excised with the perichondrium left intact. The cartilage graft was sutured into the tracheal defect using Prolene 5-0. After performing the upper and lower anastomosis, the patch was tubed and closed anteriorly (Fig. 3.1). Animals were followed until they showed respiratory distress. After follow-up, the laryngotracheal complex was removed and incised posteriorly for macroscopical and histological (eosin-hematoxylin) evaluation. The internal site of the cartilage was photographed. The amount of bare cartilage and the amount of mucosal covered cartilage was determined by analyzing computer tracings. Data are expressed as % bare cartilage and % remucosalisation.

Fig. 3.1. Tube reconstruction-free cartilage graft.



A patch of ear cartilage measuring 1.6 cm (length) x 2.5 cm (axial circumference) is tubed and sutured into a tracheal defect after resection of a tracheal segment of 1.6 cm. The suture line of the cartilage tube is seen anteriorly (arrow). The black line indicates the section line of the histologic picture seen in Fig. 3.4.

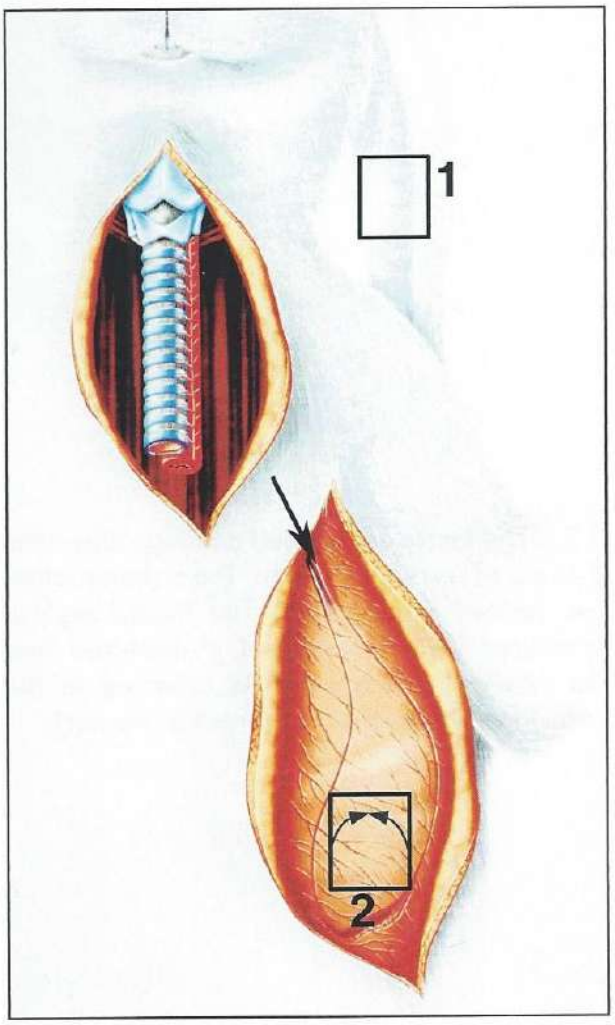
III.2.B Group 2. Cartilage tube wrapped with vascularized fascia (N=2)

A piece of autologous cartilage (25 x 16-mm) was brought towards the lateral thoracic area, sutured to the lateral thoracic fascial flap, and made into a tube (Fig.3.2.A). The vascular pedicle consists of the lateral thoracic vessels which emerge from the axillary vessels[1].
The skin was closed over the fascia enwrapped cartilage. The 2 animals within this group were followed for 1 month. After follow-up, the animals were anesthetized and the arterial vascular pedicle of the fascia flap was injected with blue silicone dye. Therefore, the lateral thoracic region was opened, and the left thoracic artery of the fascia flap was flushed through the cannula with 10 mL of heparinized, normal saline solution (10IU heparin/mL) followed by 5 mL of blue silicone dye (Microfil®, Canton Bio-Medical Products, Inc., Boulder, Co). After injection, animals were painlessly killed with an overdose of Nembutal and the fascial enwrapped tube was incised axially for macroscopical and histological evaluation.

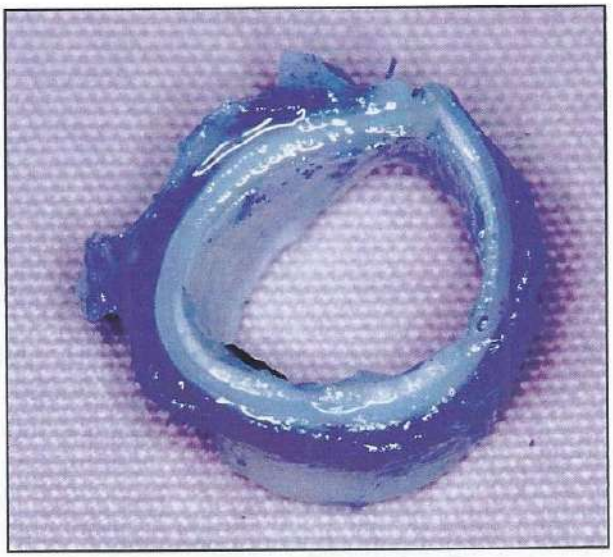
III.2.C Group 3. Cartilage graft wrapped with vascularized fascia and used as segmental tracheal replacement (N=5)

In this group, vascular connections between the autologous cartilage and the fascial flap were established before tracheal replacement. Therefore, a two-stage approach was required. The cartilage graft was sutured to the lateral thoracic fascia and made into a tube in an identical way as in group 2 animals (Fig. 3.2.A). After a 2 week period, the left thoracic area was reopened. The cartilage tube was transformed into a patch by reopening of the longitudinal suture line and was transferred to the neck region with the underlying fascial flap (Fig. 3.2.C). A 1.6 cm segment of cervical trachea was removed and the revascularized segment of cartilage was sutured into the tracheal defect (Fig. 3.3). The skin incisions were closed.
Respiration was observed daily. A CT scan was taken 1 week postoperatively to evaluate the airway lumen. Animals were euthanized when they showed respiratory distress. After the follow-up period, animals were killed painlessly with an overdose of Nembutal and the laryngotracheal complex was excised. Group 3 animals underwent blue silicone dye (blue Microfil®) injection of the lateral thoracic artery prior to tracheal harvest to better visualise the revascularization process. This material formed a cast of the vascular architecture of the fascia flap and the established vascular connections with the cartilage tube.
The tracheal reconstructions were removed, opened posteriorly, and photographed. The amount of bare cartilage and the amount of mucosal covered cartilage was determined by analyzing computer tracings. Data are expressed as % bare cartilage and % remucosalisation. The cartilage tubes were incised longitudinally for macroscopical and histological (eosin-hematoxylin) evaluation.

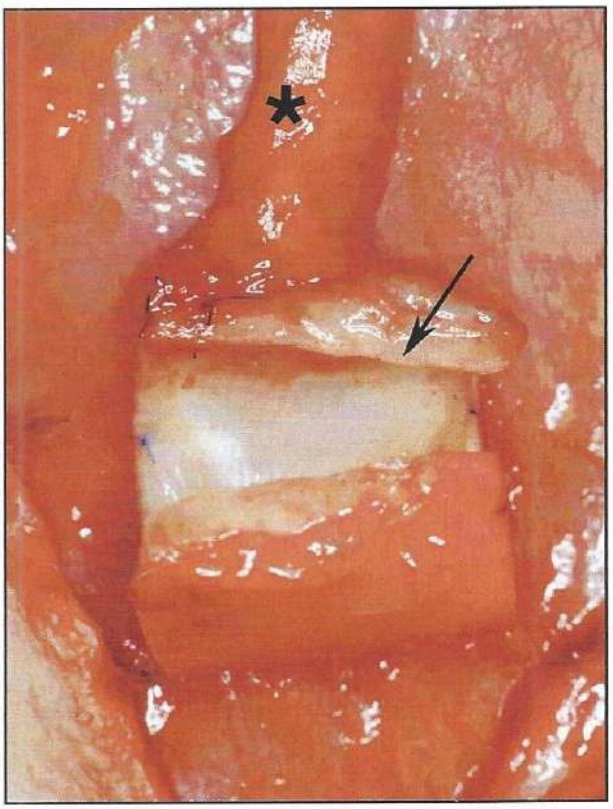
Fig. 3.2. Cartilage tube vascularized by lateral thoracic fascia.



3.2.A.Schematic presentation. A segment of ear cartilage (1.6 x 2.5 cm) (1) is transferred to the lateral thoracic fascia. The fascia is sutured to 1 side of the cartilage patch (2). The cartilage patch is made into a tube (small arrows) with a length of 1.6 cm. Arrow indicates arterial pedicle of fascia flap which is injected with blue Microfil® after follow-up.



3.2.B.Axially incised cartilage tube after 2 weeks of revascularization. The support and vitality of the cartilage was fully preserved. The cartilage tube was well attached to the blue microfil injected fascia flap.



3.2.C.The fascia enwrapped cartilage tube after 2 weeks of revascularization. The anterior suture line (arrow) is reopened. The fascial pedicle (indicated with an asterisk) is dissected and the cartilage patch which is attached to the vascularized fascia is transferred to the neck.

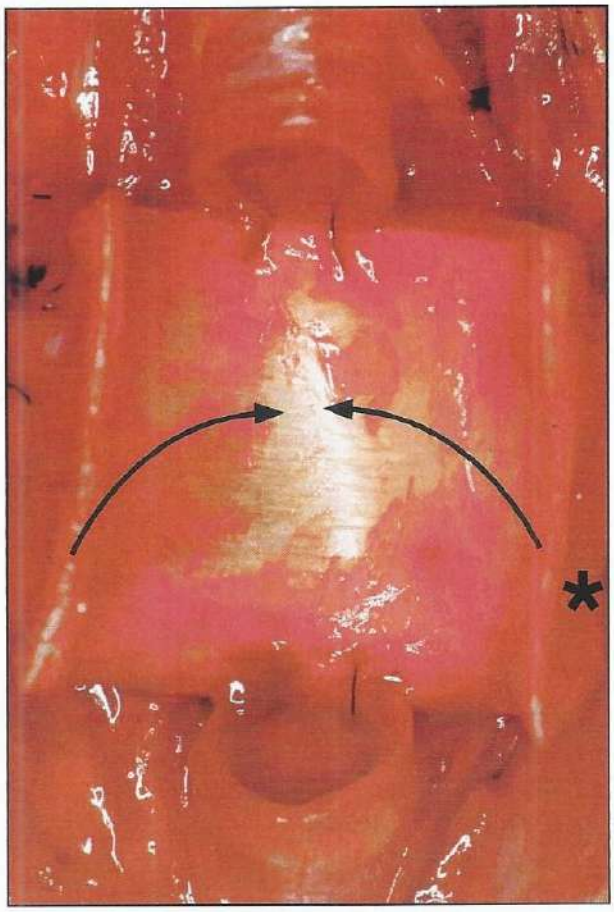


Fig. 3.3. Segmental tracheal replacement with cartilage tube wrapped with fascia.
The cartilage tube with its attached fascial envelope is transferred to a tracheal defect. The cartilage patch is sutured into the 1.6 cm tracheal defect. The patch is sutured into the defect from posterior to anterior. The longitudinal suture line (arrows) is situated anteriorly. The fascia flap around the cartilage tube is indicated with an asterisk.

III.3 Results

III.3.A Group 1

Group 1 animals showed respiratory distress after 22.4 days (mean); (SD=8.7) because of cartilage necrosis with loss of airway support. The surface area of the cartilage tube showed a mean of 9377 mm2 (SD: 660 mm2), whereas the surface area of the bare cartilage after follow-up showed a mean of 7671 mm2 (SD: 595 mm2) (Fig. 3.6). This represents 18.7 % (mean); (SD=10.8) of remucosalization of the cartilage tube. Macroscopical and histological examination showed that the major amount of the cartilage tube underwent necrosis with loss of airway support. Only the areas of cartilage near the anastomosis with the native trachea preserved their viability. The internal side of the viable segments of cartilage was covered with granulation tissue and respiratory epithelium. The measured remucosalization was limited to a distance between 0 and 2.7 mm from the anastomosis. The thickness of the regenerated mucosa was 4 times the thickness of the native tracheal mucosa (Fig. 3.4).

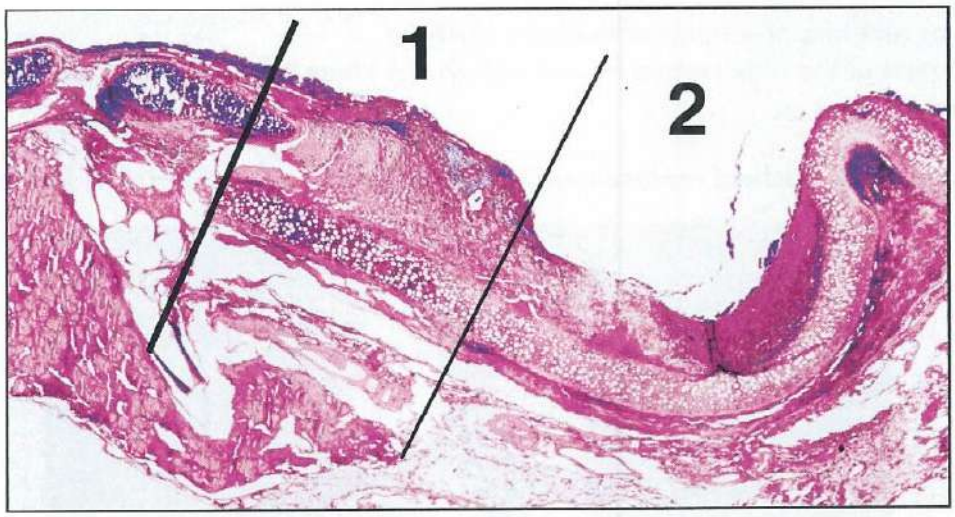


Fig. 3.4. Histology free cartilage graft.
The suture line between trachea and cartilage tube is indicated with a black line. The cartilage plate near the anastomosis (1) shows a normal morphology. This part of cartilage:

-is covered with a thick layer of granulation tissue,
-preserved its viability and
-is lined with respiratory epithelium. Note that most of the newly formed epithelial cells (leading edge) are flattened and cuboidal.

The cartilage in the middle of the tube (2):

-shows cartilage necrosis with eosinophilic ground substance,
-shows loss of cartilage support and
-is covered with pus (H&E, original magnification x10).

III.3.B Group 2

Group 2 animals preserved their full cartilage viability after being wrapped in vascularized fascia. The cartilage tubes preserved their supportive characteristics and they were attached to the blue, microfil injected fascia flap (Fig. 3.2.B). Histological evaluation showed normal cartilage surrounded by the fascia flap. The fascia flap succeeded in keeping the cartilage tube viable in the lateral thoracic position during a 1 month follow-up period.

III.3.C Group 3

Group 3 animals showed respiratory distress after 22.6 days (mean); (SD=4.1) because of cartilage necrosis with loss of airway support. The surface area of the cartilage tube showed a mean of 9427 mm2 (SD: 801 mm2), whereas the surface area of the bare cartilage after follow-up showed a mean of 7735 mm2 (SD: 956 mm2) (Fig. 3.6). This represents 18.1 % (mean); (SD=3.3) of remucosalization of the cartilage tube. A CT scan taken after 1 week showed the cartilage tube inside the airway with a well preserved tracheal lumen (Fig.3.5.A). Macroscopical examination showed that the cartilage tube was remucosalized at the anastomotic sites over a maximal distance of 3 mm (Fig. 3.5.B). The blood vessels of the regenerated mucosa were colored by blue microfil. This blue coloration came from collateral circulation of the fascia at the anastomotic areas because direct growth of fascial blood vessels through the cartilage tube is not possible. The middle segment of the tube consisted of bare cartilage. The bare cartilage lost its support and prolapsed into the airway lumen (Fig. 3.5.C).

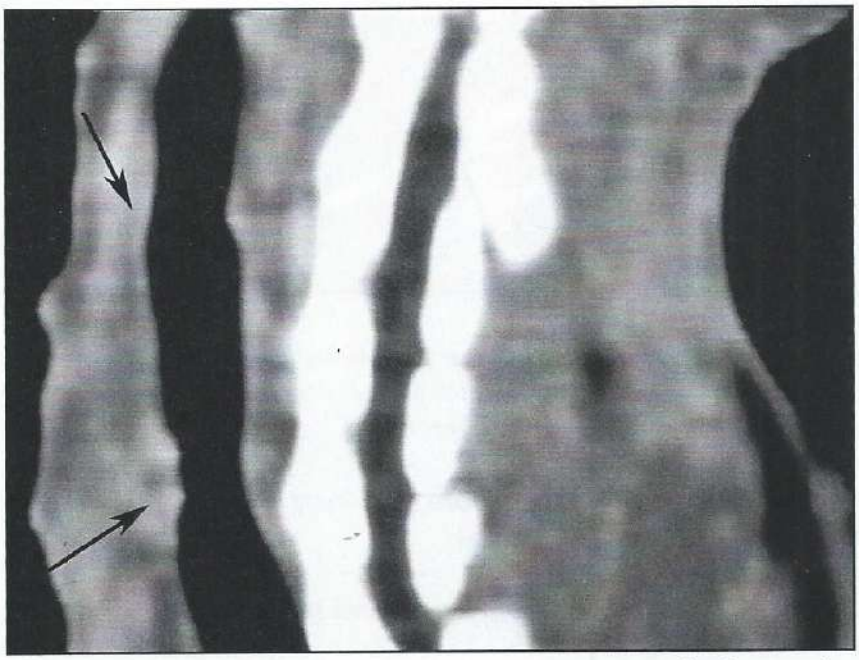
Histological examination showed (Fig. 3.5.D, 3.5.E):

-good viability of the remucosalized cartilage.

-necrosis and loss of support of the bare cartilage.

-A thickness of the regenerated mucosa which is 4 times the thickness of the native tracheal mucosa.

Fig. 3.5. Segmental tracheal replacement with cartilage tube wrapped with fascia



3.5.A. CT scan-sagittal section through tracheal reconstruction 1 week after tracheal replacement. The cartilage tube is situated between arrows. A sufficient airway lumen is visible 1 week after reconstruction. Animals were followed until signs of respiratory distress.

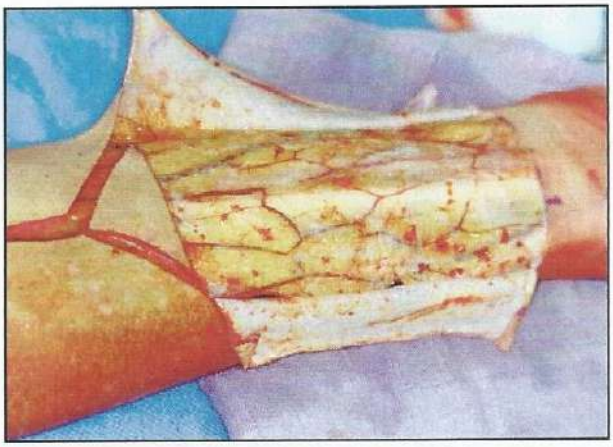


Fig. 1.11. A flap consisting of fascia and subcutaneous tissue may be taken at the volar side of the forearm after incision and dissection of the overlying skin.

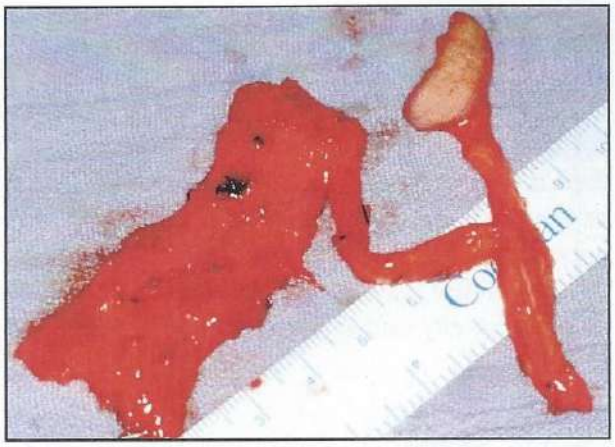


Fig. 1.12. The dissected fascial flap is axially perfused by the radial artery and vein. The flap can be transferred to the neck by microvascular anastomosis of the vascular pedicle.

The experimental and clinical flap have similar characteristics: they are thin and pliable, have a big flap surface area, and are axially perfused by a long and reliable vascular pedicle.The experimental and clinical flap differ in their way of transfer towards the neck: the radial forearm flap needs microvascular transplantation whereas the lateral thoracic fascia flap can be brought into the neck by rotation of the (lateral thoracic) vascular pedicle.

2. Mucosal lining

The mucosal lining of the reconstructive tissue may consist of respiratory epithelium or of squamous epithelium. A respiratory epithelial lining is found in tracheal transplants. However, this type of epithelium is not available outside the airway and hence not available when treating difficult tracheal stenosis cases. A mucosal lining consisting of squamous epithelium is found in the buccal area. Buccal mucosa can be used as a full-thickness graft. The vascularized fascial flap may be used as a 'transferable bed' to bring the mucosa viably inside the laryngotracheal defect.

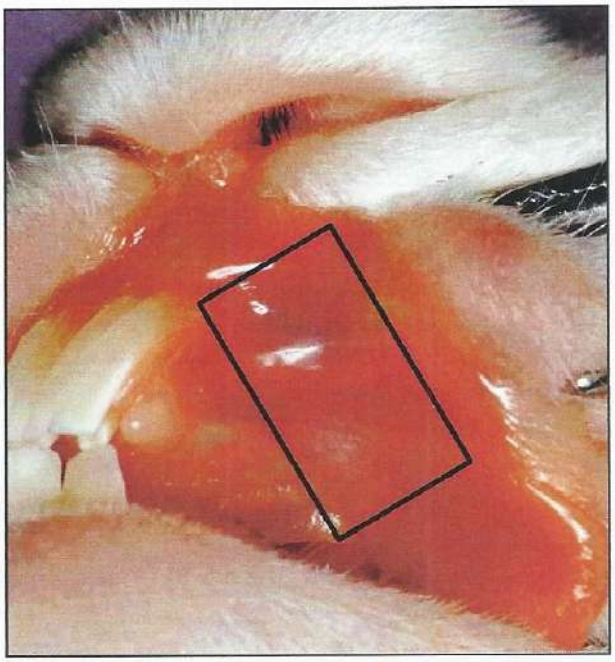


Fig. 1.13. Donor site of full-thickness buccal mucosa graft in rabbits.

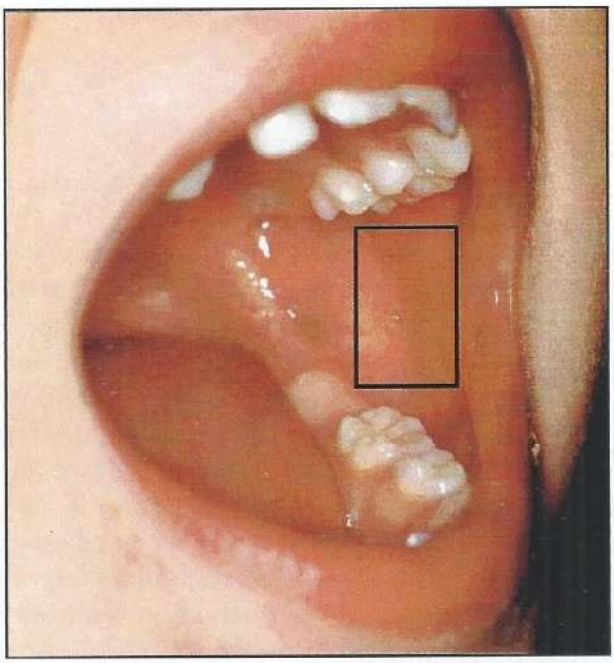


Fig. 1.14. Donor site of full-thickness buccal mucosa graft in humans.

3. Cartilage support

The cartilage support of the reconstructive tissue may consist of tracheal cartilage or of ear cartilage. Tracheal cartilage is found in tracheal transplants and will give the optimal support for the airway lumen. Cartilage support may also be provided by an elastic piece of cartilage that is sutured between the margins of an airway defect that has a smaller size than the size of the cartilage segment. Major amounts of elastic cartilage are available in the outer ear of a rabbit (Fig. 1.15). Only small amounts of elastic cartilage are available in humans (Fig. 1.16). Fibrocartilage is available in much larger quantities in the human rib but this type of cartilage has a low elasticity and is not suitable to realize an additional anterior airway lumen expansion.

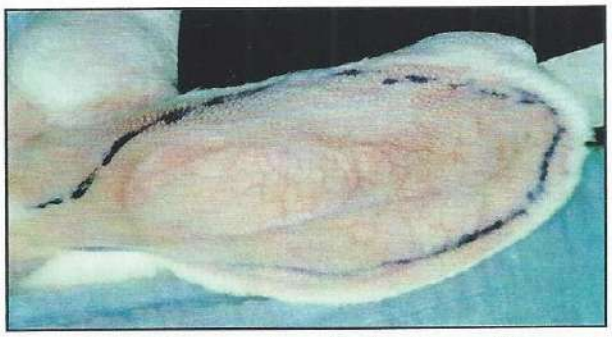


Fig. 1.15. Donor site of elastic cartilage in rabbits.

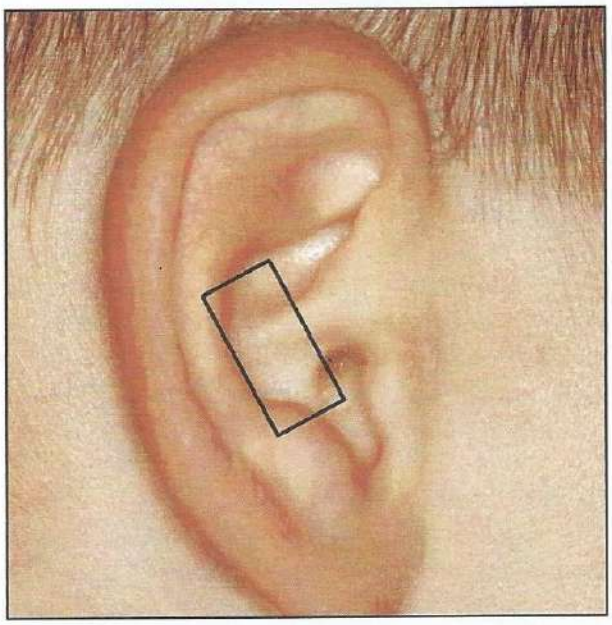


Fig. 1.16. Donor site of elastic cartilage in humans. In humans, an auricular graft with dimensions approaching 2 x 5 cm may be harvested without creating a deformity of the external ear.

I.4 Aim of this study

1. Study the healing of the different tissue components for tracheal repair. (mucosa, vascularization, cartilage)
2. Improving the tissues which are currently used in tracheal reconstruction.
   'Optimal tracheal repair tissue' does not exist outside the trachea so that all the currently used reconstructive options are lacking one or more of the basic tissue requirements. We wanted to study the importance of the mucosal lining and the cartilage support in airway reconstruction and to artificially combine the individual tissues (vascularization, mucosal lining, and support) with the goal of improving tracheal reconstruction.

In the second chapter we studied the healing of full-thickness mucosal defects using a new in vivo model. The effects of secondary healing were examined with this question in mind: is a mucosal lining really necessary when using tracheal reconstructive tissues?
In the third chapter we studied the healing of tubes of autologous cartilage with this question in mind: can a segment of trachea be replaced by a tube of autologous cartilage?
In the fourth chapter the individual repair tissues (cartilage, vascularized soft tissue, and mucosa) were evaluated after repair of an anterior airway defect. The different individual tissues were then combined and a composite tissue was developed.

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Chapter II. An investigation of airway wound healing using a novel in vivo model.

Abstract

Objective.
To study the amount of wound contraction and reepithelialisation occurring in the healing process of full-thickness mucosal defects treated with and without mitomycin.

Design.
A new wound healing model was developed in which the tracheal mucosa was exteriorized without interfering with the blood supply or with the cartilage support of the trachea. This was done by:

1. Orthotopic tracheal revascularization in vascularized fascia.
2. Isolation of revascularized segment after 14 days.
3. Posterior longitudinal incision of revascularized segment.
4. Exteriorization of tracheal mucosa with formation of anterior full-thickness mucosal defect.
5. Closure of posterior tracheal incision and reimplantation in the airway.

This model was used to study airway wound healing in 3 groups of animals

1. Controls (revascularization;exteriorization;reimplantation) (N=6)
2. Full thickness mucosal defect: patch defect (N=5), circumferential defect (N=3)
3. Full thickness mucosal defect after topical mitomycin application: patch defect (N=5), circumferential defect (N=3)

The animals were followed for periods varying from 2 to 4 weeks or until signs of respiratory distress. The surface areas of the wounds before and after follow-up were measured. Wound healing was studied histologically on axial and longitudinal sections.

Results.
Group 1. All the animals survived for 1 month. No significant difference existed between surface area of isolated trachea and of reimplanted trachea after follow-up.

Group 2. Five animals (patch defects) survived for 1 month. Full thickness mucosal defects healed by reepithelialisation and by a surface area reduction of 58.9 % (SD=10.5). The animals with the circumferential defects showed dyspnea after an average follow-up of 14 days due to excessive granulation tissue formation.

Group 3. Mitomycin reproducibly inhibited wound closure, yielding wounds that on average closed 56 % less than controls by day 14 ($p<0.001$). Histologic comparisons showed that mitomycin blocks angiogenesis during wound healing.

Conclusions.
A wound healing model based on tracheal revascularization, isolation, and reimplantation was developed in rabbits. This model allowed us to study the healing of full-thickness mucosal defects inside the airway.

II.1 Introduction

In comparison with the myriad of studies investigating various aspects of cutaneous wound healing, there is a real dearth of basic science research investigating mucosal wound healing of the airway. Currently, there is no effective way to study intraluminal events of reepithelialisation and wound contraction after airway injury. The amount of wound contraction and reepithelialisation for full-thickness mucosal airway defects is unknown. This information would be of interest to better understand the etiology of airway stenosis and to give insight into the principles leading to successful airway reconstruction. The difference in knowledge between mucosal and skin defects is because of the inaccessibility of the airway tissue for direct observation. In vivo wound healing studies of the larynx and trachea are based on endoscopic models[1, 2]. The problem with wounds that are made endoscopically is the difficulty in standardisation of the depth and dimensions of the wound.

A solution to this problem may be found in studying in vivo airway wound healing by using an invasive, open approach. After opening of the airway lumen, airway wounding can be performed in a controlled manner. The problem with an open approach however is the interference with the airway's blood supply after anterior incision of the airway wall. An in vivo experimental model that interferes with the airway's vascularization is not acceptable because of the importance of an intact blood supply for normal tissue healing. Another problem after anterior incision of the airway wall is the interference with the cartilaginous support which might also influence the healing process of mucosal wounds.

We developed an invasive in vivo model without interfering with the airway's blood supply and without interfering with the airway's cartilaginous support. The model consists of a complete isolation of tracheal segments after previous tracheal revascularization. With this model, the internal side of the trachea could be exteriorized and the trachea could be reimplanted after wounding without interfering with the blood supply or with the cartilaginous support. This model allowed us to study the healing of full-thickness mucosal defects and to study the influence of mitomycin application on mucosal wound healing. Recent publications have shown that mitomycin prevent scarring and fibrosis after tracheal wounding[3, 4]. In these studies, the effect of mitomycin application was evaluated in a model of subglottic stenosis. We wanted to evaluate the effect of mitomycin on normal mucosal wound healing.

II.2 Material and methods

II.2.A Experimental model-control animals

Six New-Zealand white rabbits (3 kg) were used to control the reliability of the experimental model. One animal was used to document the amount of revascularization after 2 weeks. Five animals were used to study the feasibility of tracheal reimplantation after exteriorization of the tracheal mucosa.

Stage 1. Vascular induction through staged tracheal transfer (Fig. 2.1.A)

A staged vascular induction technique was used. The concept is that a fascial flap can become a 'vascular carrier' and can be induced to provide an alternate blood supply through revascularization of a tracheal segment after a relatively short staging period in the orthotopic position.
In the first stage, the rabbits were premedicated with 0.3 ml/kg of Hypnorm® (10 mg fluanisone/0.2 mg fentanyl per ml) intramuscularly and anesthesia was maintained with halothane through mask ventilation. The left thoracic skin was incised and the left lateral thoracic fascia flap was isolated on the lateral thoracic vessels. The vascular anatomy and dissection technique of this fascial flap were described previously[5]. The cervical trachea was reached through a midline neck incision. The trachea was dissected from the underlying esophagus and cleared from most of the connective tissue without incising the trachea. The trachea was still perfused in situ but was stripped of its connective tissue envelope containing part of the blood supply. The ischemic cervical trachea was wrapped over 2 cm by the transposed lateral thoracic fascial flap. The lateral thoracic and cervical skin were closed with interrupted sutures.

Stage 2. Tracheal isolation, exteriorization of luminal site, tracheal reimplantation

After 14 days, the trachea on its fascial vascular carrier becomes transferable by vascular induction. In the second stage, the neck was reopened and the cervical trachea with a length 2 cm was dissected with its new, thin fascial envelope.
One animal was used to document the amount of revascularization after 14 days. This animal was sacrificed with an overdose of pentobarbital. The laryngotracheal complex was isolated from the airway tract on the vascularized fascia flap and was opened posteriorly. The left axillary region was opened, and the left lateral thoracic artery was cannulated with a 14-gauge catheter. The vasculature was flushed through the cannula with 10 ml of heparinized normal saline solution (10 IU of heparin per milliliter) followed by 5 ml of blue silicone dye (Microfil®; Canton Bio-Medical Products Inc., Boulder, Colo) (Fig. 2.1.B). Following these injection, the cervical trachea could be inspected macroscopically and the amount of revascularization could be evaluated.

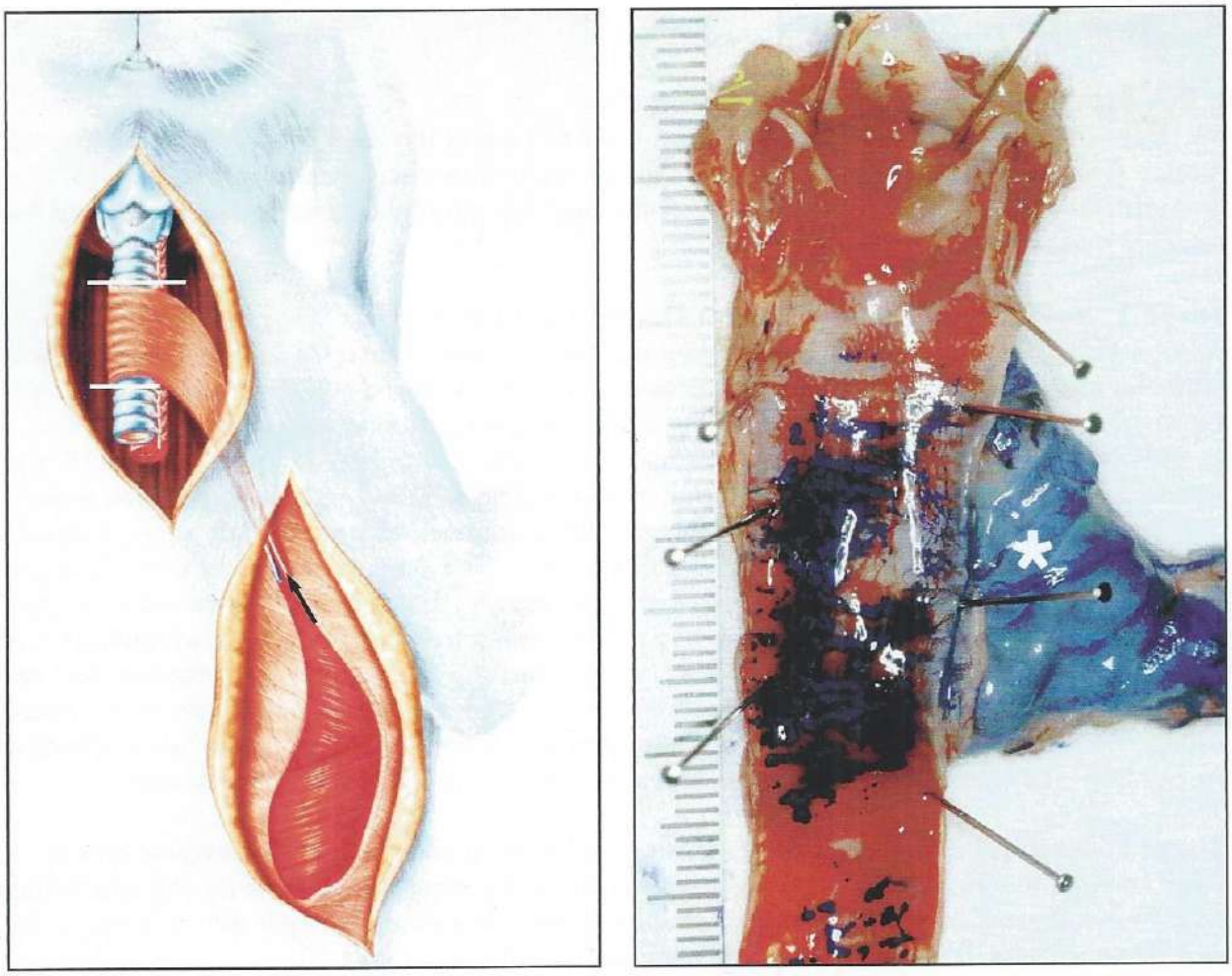


Fig. 2.1. Revascularization of the rabbit trachea

2.1.A. The left lateral thoracic fascial flap is dissected and transferred to the neck region. A 2 cm segment of cervical trachea is wrapped by the fascia flap.
After 2 weeks of fascial wrapping, the lateral thoracic artery was injected with blue Microfil® (arrow) in 1 rabbit. In the other animals, the fascial enwrapped segment of trachea was isolated from the airway (white lines).

2.1.B. Amount of revascularization after 14 days (control animal 1).
The laryngotracheal complex is visible after posterior, longitudinal incision and blue Microfil® injection of the vascular pedicle. The blue colored areas of the fascial enwrapped trachea are perfused by the fascial flap (asterisk).

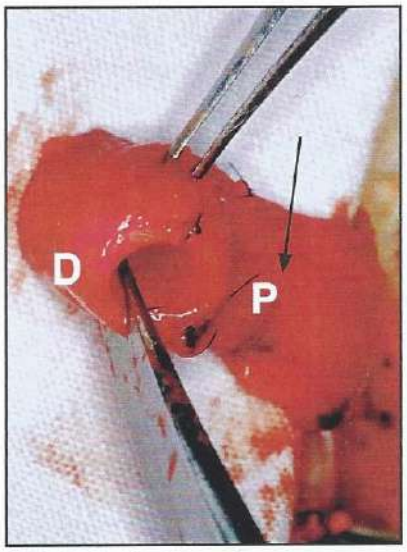
In the other 5 control animals, the validity of our new wound healing model was evaluated by comparing the mucosal surface areas of the isolated and the reimplanted tracheal transplant. In these animals, the fascia enwrapped tracheal segment was removed from the airway tract with preservation of the vascular pedicle of the fascia flap (Fig. 2.1.A).
After isolation, the segment was opened posteriorly by longitudinal incision of the distal site of the fascia flap and by longitudinal incision of the membranous trachea (Fig. 2.2.A).
After posterior incision, the tracheal patch was pinned onto cardboard in a stretched position with exposure of the mucosal lining (Fig. 2.2.B).
After taking a photograph with a ruler in place, the tracheal patch was made into a tube by closure of the membranous trachea and the incised fascial flap (Prolene 6-0). The tracheal tube was reintroduced into its original position in the airway defect. The animals breathed spontaneously during the entire operation. The cervical skin was closed with interrupted stitches.

Antibiotics were administered for four postoperative days in both operation stages. Rabbits were housed in separate quarters in an environment controlled facility and were fed a standard rabbit diet.

Stage 3. Control of tracheal segment

The fascia enwrapped segment was controlled 4 weeks after reimplantation. The rabbits (N=5) were anesthetized, the left axillary region was opened, and the left lateral thoracic artery was cannulated with a 14-gauge catheter. The vasculature was flushed through the cannula with 10 ml of heparinized normal saline solution followed by 5 ml of blue Microfil®. Following these injection, the larynx and cervical trachea were removed and opened dorsally to allow for a macroscopic inspection of the reimplanted tracheal segment. The rabbits were killed with an overdose of pentobarbital. The tracheas were rephotographed with a ruler in place.

Fig. 2.2. Experimental model-control animals.



2.2.A. Isolation of revascularized tube. A 2 cm segment of cervical trachea is isolated on the fascial vascular pedicle (arrow) and incised posteriorly. D=distal site of fascia flap; P=proximal site of fascia flap.

2.2.B. Isolated tracheal patch. The tracheal patch is pinned onto cardboard with exteriorization of the respiratory mucosa. Note the normal appearance of the mucosal blood vessels.

2.2.C. Macroscopy of reimplanted trachea after 1 month.
The vascular pedicle of the fascia flap was injected with blue microfil 2 weeks after reimplantation of the exteriorized patch. At that time, the laryngotracheal complex was removed and incised posteriorly with visualisation of the initial isolated patch (completely colored by blue Microfil®).

II.2.B Full thickness mucosal defects

B.1 Anterior patch mucosal defect (N=5) (Fig. 2.3)

The first stage was identical to the control animals except for the length of the revascularized trachea. A revascularized segment of trachea with a length of 1.5 cm was used to study the healing of full-thickness mucosal wounds. During the second stage, the revascularized trachea was isolated in an identical way as in control animals. An anterior mucosal wound was made during the second operation after exposure of the airway lumen. An anterior defect with a width of 0.6 cm was made using a bistouri for mucosal incision and microscissors for mucosal dissection (Fig. 2.3.A). Hemostasis of the exposed cartilage was achieved by even compression with sterile gauze. Closure and replacement of the trachea was done in an identical way as for the control animals. Animals were followed for 4 weeks or until they developed respiratory distress. After follow-up, the animals were anesthetized and their vascular pedicle was injected with blue Microfil®. The cervical trachea was removed, opened posteriorly, and photographed. To measure wound areas, photographic slides taken immediately following wounding and 4 weeks later were projected. The included ruler equalized their sizes, so that all images were of the same magnification. Wound areas were determined by analyzing computerized tracings. Data were expressed as "Wound Area" in mm^2. The wounded trachea was incised axially for macroscopical and histological (eosin-hematoxylin) evaluation.

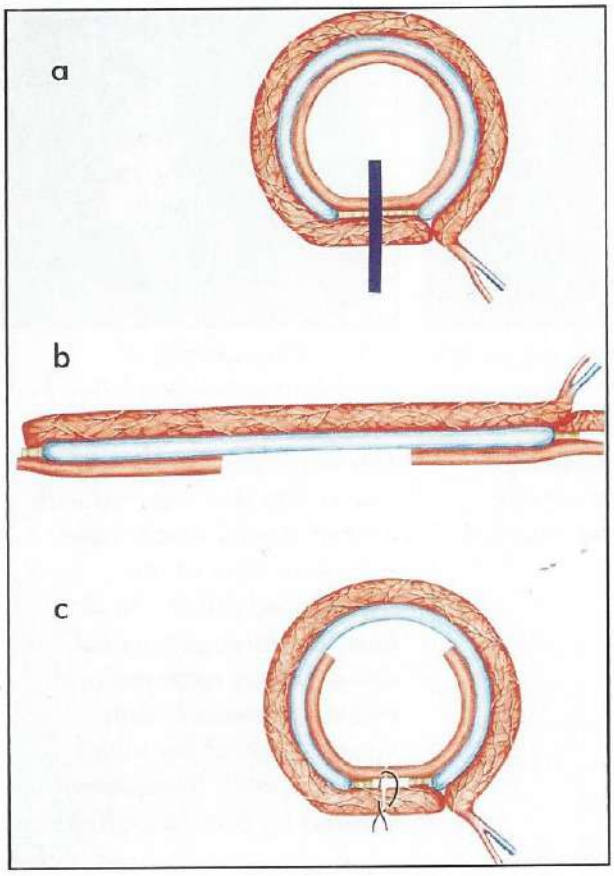


Fig. 2.3. Schematic presentation of anterior mucosal defect.

a. The isolated tracheal segment wrapped with fascia is incised posteriorly.

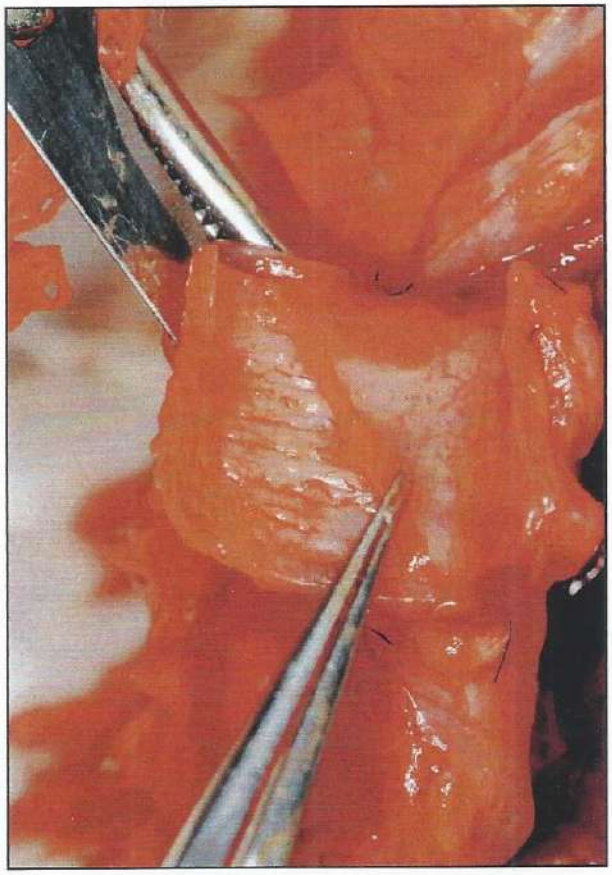
b. An anterior mucosal patch of .6 cm is removed from the exteriorized mucosal lining.

c. The tracheal patch is transformed into a tube by suturing of the incised fascial flap.

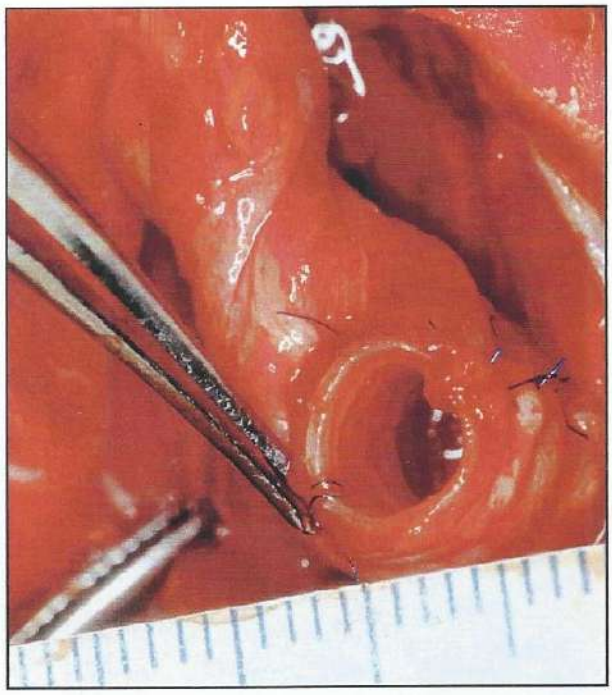
B.2 Circumferential defect (N=3)

The same surgical steps as for the anterior patch mucosal defect were made. The mucosal lining of the isolated patch was completely removed during the second operation stage (Fig. 2.4.A). After wounding, the tracheal patch was transformed into a tube (Fig. 2.4.B). Animals were followed until signs of respiratory distress became apparent. After follow up, the animals were euthanized and the tracheas were incised longitudinally for histological (eosin-hematoxylin) evaluation.

Fig.2.4. Circumferential mucosal defect.



2.4.A. Isolated tracheal patch. The mucosal lining is completely removed after revascularization and after isolation of the tracheal segment.



2.4.B. Isolated trachea tube. The patch consists of cartilage rings and intercartilaginous ligaments and is perfused by the fascial flap. The patch can be transformed into a tube by posterior closure. This denuded tube can be reimplanted into the airway tract.

II.2.C Full thickness mucosal defect after mitomycin application

C.1 Anterior patch mucosal defect after mitomycin application

During the second operation, 2 full thickness mucosal squares (0.6 cm x 0.6 cm) were excised (Fig. 2.5). Hemostasis was achieved by even compression with sterile gauze. The upper mucosal defect was treated with a single topical application of 0.5 ml (0.2 mg/ml) mitomycin whereas the lower mucosal defect was treated with 0.5 ml of saline. Drug delivery was via a soaked cotton pledget directly applied to the operative site for a duration of 5 minutes. The damaged tracheal patch was closed and replaced inside the tracheal defects. Five animals were followed during 2 weeks for morphometrical and histological evaluation. To measure wound areas, photographic slides taken immediately following wounding and 2 weeks later were projected. Wound areas were determined by analyzing computer tracings. Data were expressed as "Wound Area" in mm^2. The wounded trachea was incised longitudinally for macroscopical and histological (eosin-hematoxylin) evaluation.

Two extra animals were followed during 1 month for histological evaluation. The vascular pedicle (lateral thoracic artery) of the fascia flap was injected with blue Microfil® before tracheal harvest in all animals.

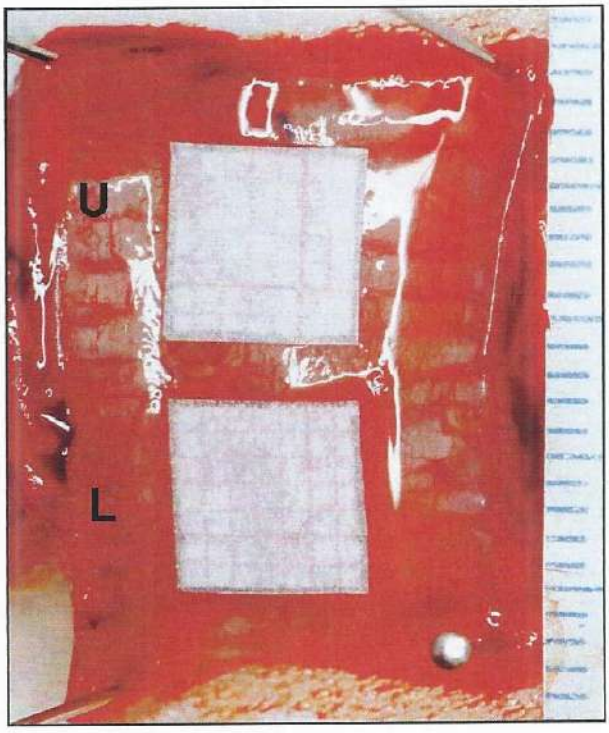


Fig. 2.5. Wound healing of anterior mucosal defect after mitomycin application.
Macroscopy of tracheal wound. Two full-thickness mucosal defects (0.6 cm x 0.6 cm) are formed after revascularization and after isolation of the tracheal segment. The upper mucosal defect (U) is treated with mitomycin whereas the lower mucosal defect (L) is treated with saline.

C.2 Circumferential mucosal defect after mitomycin application (N=3)

In this group, the completely denuded tracheal segments (Fig. 2.4.A) were treated with 1 ml of mitomycin (0.2 mg/ml). Animals were followed until signs of respiratory distress became apparent. After follow-up, the tracheas were incised longitudinally for histological (eosin-hematoxylin) evaluation. Differences between treatment groups were analyzed using the Student's t-test or a one-way analysis of variance. Statistical significance was defined as $p<0.05$.

All procedures were carried out using facilities and protocols approved by the University of Leuven Institutional Animal Care and Use Committee.

II.3 Results

II.3.A Experimental model-control animals (N=6)

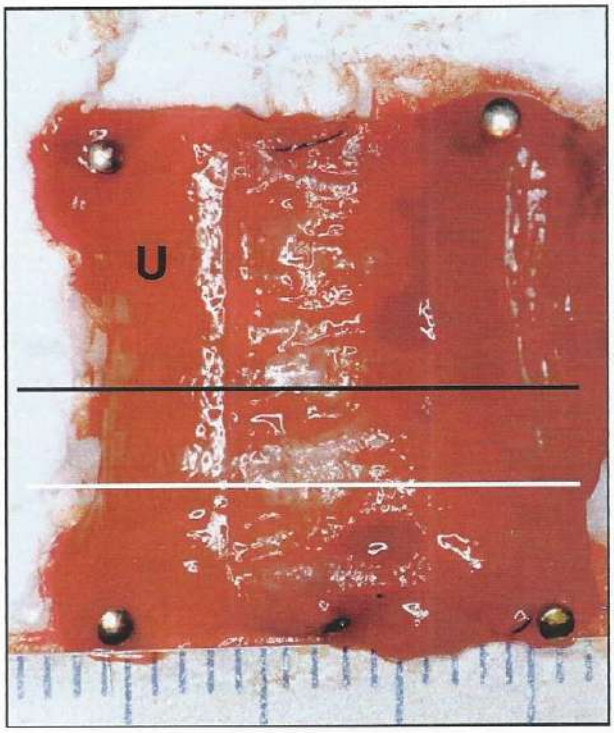
1. Documentation of the revascularization process (N=1). The mucosal lining of the fascia enwrapped segment of trachea was intensely colored with blue silicone after injection of the lateral thoracic artery. The blue coloration represents the amount of revascularization after 14 days of fascial wrapping and after patch formation (Fig. 2.1.B).
2. Exteriorization of respiratory mucosa (N=5). After follow-up, the surface area of the previously isolated segment could be identified because of the blue coloration of the mucosal lining (Fig. 2.2.C). No significant difference was found between the surface area of the patch during isolation (mean value 11500 mm^2; SD=541 mm^2) and the patch after follow-up (mean value 11744 mm^2; SD=555 mm^2).

II.3.B Full thickness mucosal defects

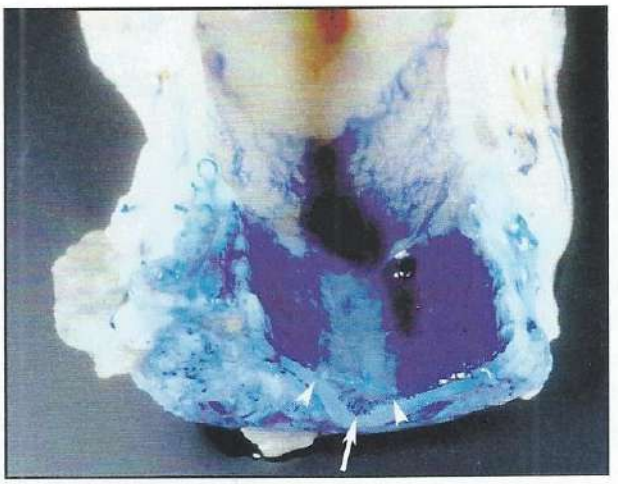
B.1. Anterior patch mucosal defect (N=5)

The surface area of the isolated trachea showed a mean of 8736 mm^2 (SD: 557 mm^2), whereas the surface area after healing showed a mean of 6908 mm^2 (SD: 595 mm^2) (Table 2.1). The surface area of the defect exhibited a mean of 4392 mm^2 (SD: 418 mm^2), whereas the surface area after healing was reduced to 1779 mm^2 (SD: 354 mm^2). This represents a mean of 58.9 % (SD=10.5) wound contraction (Fig. 2.7). Macroscopical and histological examination showed that this major amount of surface area reduction could be attributed to formation of granulation tissue and to wound contraction forces acting in the axial axis of the wound (Fig. 2.6). The thickest layer of granulation tissue was seen in the middle of the scar.

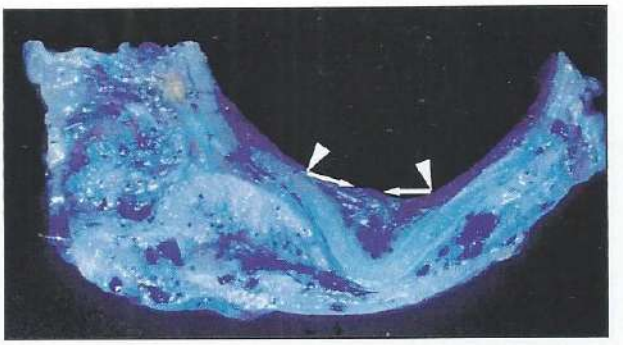
Fig. 2.6. Wound healing of anterior mucosal defect.



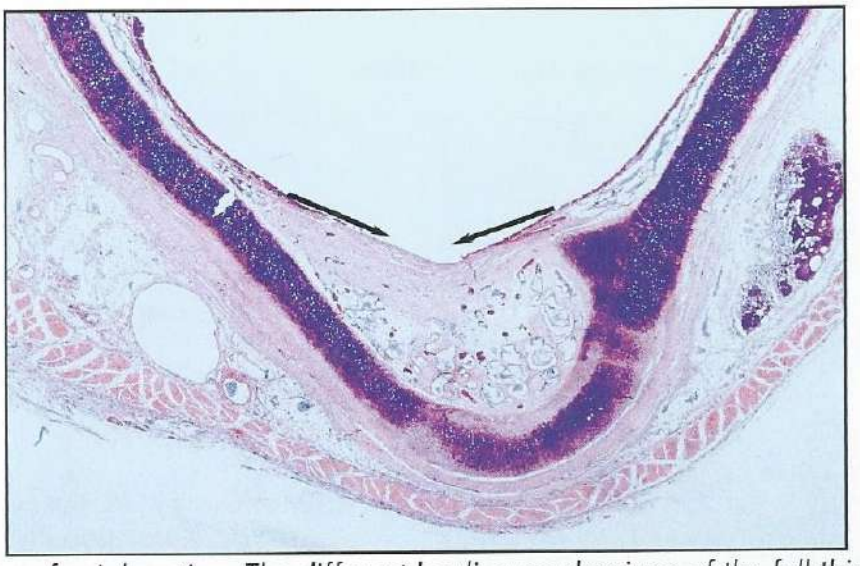
2.6.A. Macroscopy of tracheal wound. The anterior full-thickness defect (0.6 cm x 1.5 cm), sharply demarcated from the intact mucosa, is visible. After follow-up and after measuring of the surface areas, the transplant was incised in the midline (black line). The upper half (U) of the posteriorly incised transplant is visible in Fig. 2.6.B whereas an axial section of the lower half (white line) is visible in Fig. 2.6.C and Fig. 2.6.D.



2.6.B. Macroscopy of reimplanted trachea after 1 month. The upper half of the tracheal transplant (opened posteriorly) and the lower end of the larynx is visible. The intact mucosal lining (intensely colored with blue microfil) and the anterior scar (between arrowheads) is visible. The submucosal tissue is thickest in the center of the scar (arrow).



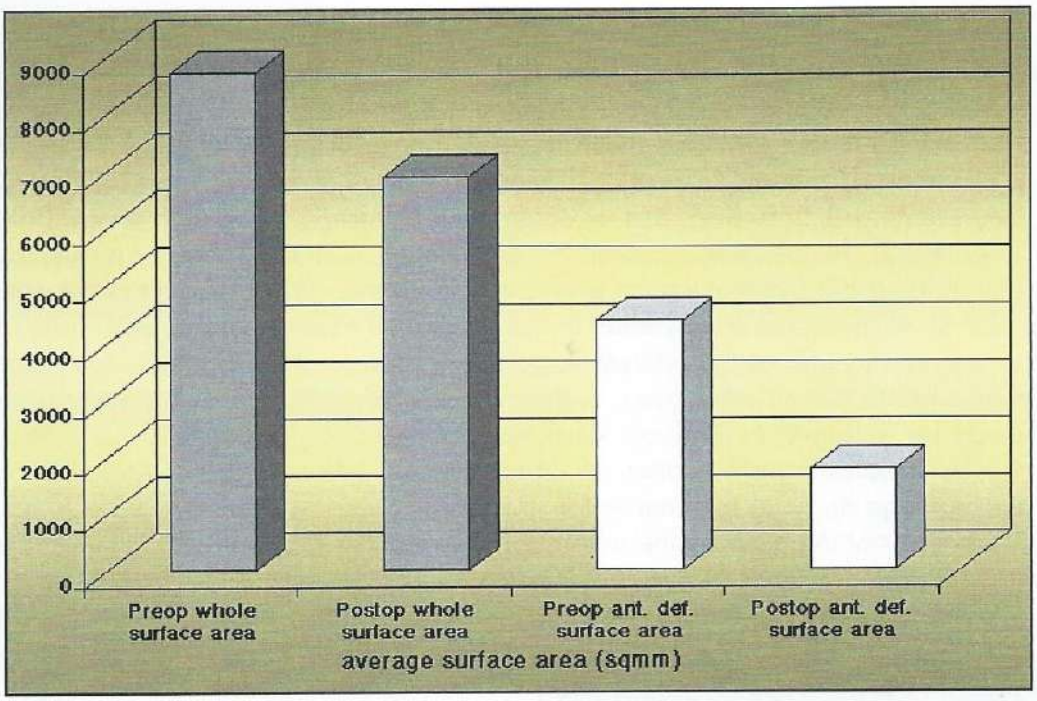
2.6.C. Macroscopy on axial section. The anterior mucosal defect after healing is visible between arrowheads. The surface area of the wound is reduced by thickening of the lamina propria and by wound contraction forces (arrows) acting on the cartilage support of the wounded area. The original shape of the cartilage ring has changed due to wound contraction forces.



2.6.D. Histology of axial section. The different healing mechanisms of the full thickness mucosal wound are visible (H&E, original magnification x4):

- Proliferation of granulation tissue formation is blocked after relining with epithelial cells. The thickest layer of granulation tissue is seen in the middle of the scar.
- The reepithelialization process is still incomplete in the center of the wound after 1 month. The newly formed epithelial cells (leading edge) are flattened and cuboidal.
- Wound contracton forces (arrows) are generated in the granulating wound with displacement of the cartilage towards the center of the scar.

Fig. 2.7. Wound contraction of anterior mucosal defect.



B.2 Circumferential defect (N=3)

The 3 animals with the circumferential tracheal defects survived for 12, 14, and 17 days. The animals showed respiratory distress because of excessive granulation tissue formation with obstruction of the airway lumen. Histologic evaluation showed excessive granulation tissue formation at the sites of the intercartilaginous ligaments (Fig. 2.8). No reepithelialisation from the anastomotic regions was detected.

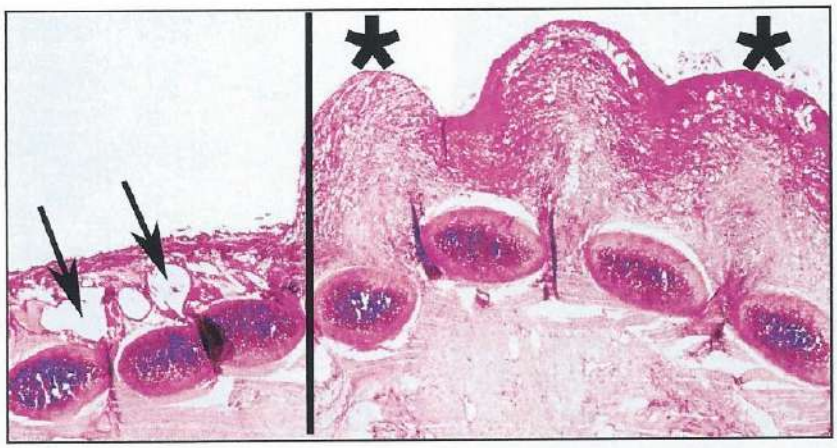
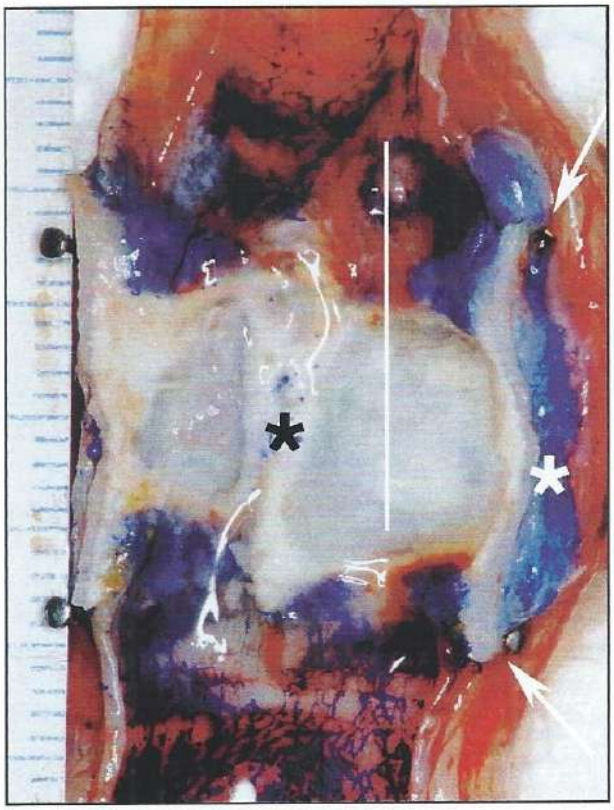
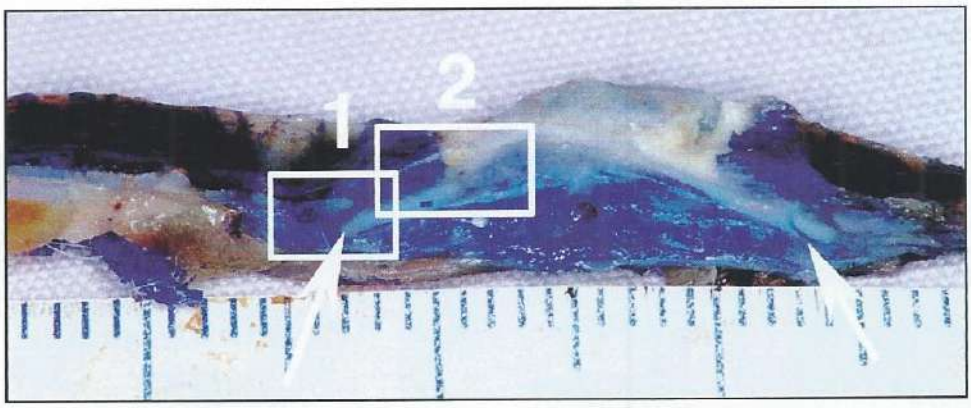


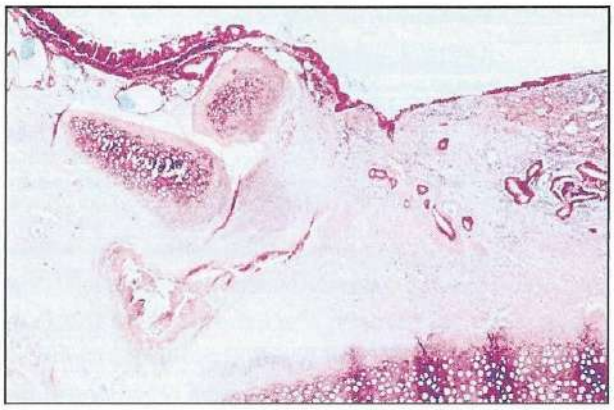
Fig. 2.8. Circumferential mucosal defect: histology on longitudinal section.
Denuded tracheal transplant on longitudinal section after 14 days of reimplantation. The place of anastomosis between intact trachea and denuded trachea is visible (black line).
The intact trachea shows a normal mucosal architecture with a lamina propria showing blue silicone dye injected blood vessels (arrows). The cartilage rings of the denuded trachea are intact and lined with a thick layer of granulation tissue. This granulation tissue is formed by angiogenic induction from the surrounding fascia flap. Vascularized tissue is growing through the intercartilaginous ligaments towards the airway lumen. The granulation tissue is not lined with epithelial cells (asterisks) and is thicker at the sites of the intercartilaginous ligaments (H&E, original magnification x10).



3.5.B. Macroscopy after follow-up. The artery of the fascia flap was injected with blue silicone dye before removal of the trachea. The trachea and the cartilage tube were opened posteriorly and pinned onto cardboard. The cartilage tube is situated between arrows. The fascia flap around the cartilage tube is indicated with a white asterisk. The anterior suture line of the cartilage tube is indicated with a black asterisk. An area of about 3 mm of the cartilage tube is lined with a blue colored mucosal lining. The middle 1 cm of the tube consists of bare cartilage. White line indicates place of longitudinal incision for macroscopical (Fig. 3.5.C) and histological (Fig. 3.5.D, E) examination.



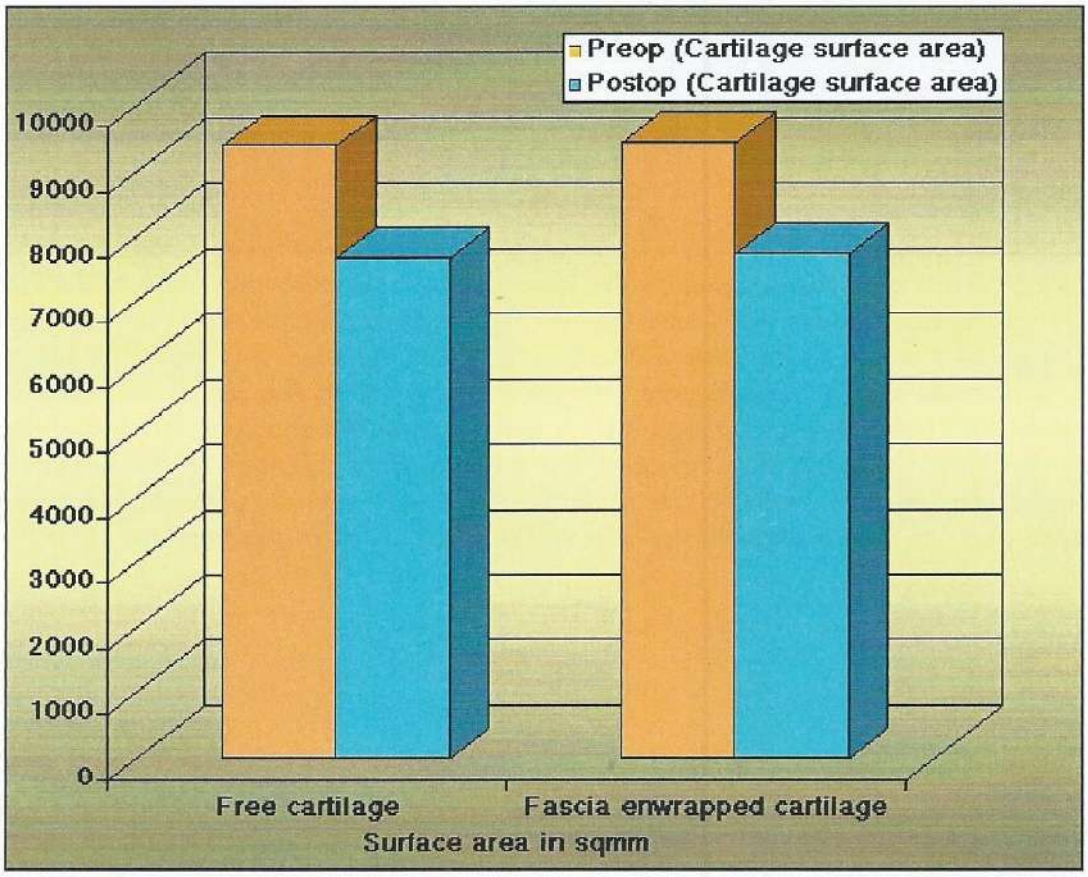
3.5.C. Macroscopy after follow-up and after longitudinal incision. Arrows indicate the lower and upper anastomosis of the cartilage tube. The cartilage tube is covered with a blue colored lining over a length of 3 mm at both anastomotic sites. Note that the internal lining of the ear cartilage at the anastomotic sites is much thicker than the thin respiratory mucosa of the native trachea. The regenerated lining of the cartilage tube is coming from the anastomotic sites of the native trachea. The blue coloration of the mucosal lining comes from collateral fascial blood supply at the anastomotic sites. The middle 1 cm of cartilage consists of bare cartilage. The cartilage in the middle part has lost its support and is prolapsing into the airway lumen.



3.5.D. Histology of boxed area 1. The lamina propria of the native trachea consists of a well vascularized (blue microfil injected blood vessels) connective tissue layer lined with ciliated epithelium. The lamina propria over the ear cartilage consists of a thick layer of granulation tissue lined with squamoid epithelium. The regenerated lamina propria consists of dense connective tissue with small blood vessels whereas the mucosal lining of the trachea consists of loose connective tissue with larger capillaries (Hematoxylin-eosin original magnification x 4).

3.5.E. Histology of boxed area 2. The anastomotic site of the cartilage tube is remucosalized and consists of viable cartilage. The middle part of the cartilage tube consists of bare cartilage with signs of necrosis (eosinophilic ground substance) and loss of support. F=microfil injected fascia flap (Hematoxylin-eosin original magnification x 10).

Fig. 3.6. Surface area of bare cartilage before and after follow-up.



III.4 Discussion

The question whether a tube of autologous cartilage could be used for segmental tracheal replacement has remained unanswered because research on segmental tracheal replacement is not available. The answer also has clinical importance because cartilaginous engineered tubes may be provided in the near future by tissue engineering techniques[4]. How cartilage tubes would heal inside an airway defect may partially be answered by information available from healing studies of tracheal reconstruction with patches of cartilage. Both clinical and animal studies have established that autologous costal and auricular cartilage grafts survive when placed in an anterior airway defect[9,10]. Patches of autologous cartilage undergo secondary healing with reepithelialization and revascularization induced from the margins of the defect. Most studies reported a full reepithelialisation and revascularization of the cartilage grafts. However, the majority of these studies were done with relatively small grafts (width of graft less than 5 mm)[9, 11,12]. Because the revascularization and reepithelialisation processes originate from the margins of the defect, healing of cartilage grafts will be dependent on the size of the graft. It can be anticipated that the central part of the graft may undergo necrosis if larger patches of cartilage are used. Our model allowed us to study the healing of large cartilaginous patches in the shape of a tube.

Essential for survival of the free cartilage tube was the coverage of the graft by granulation tissue. This occurred only in a small area around the anastomosis. The major amount of the cartilage graft remained uncovered, which resulted in necrosis and loss of the cartilage support. The limiting factor in the healing process of the cartilage tube was the ingrowth of granulation tissue. The remucosalized areas were characterized by viable cartilage and by a thick layer of granulation tissue with on top the regenerated epithelium. The regenerated lamina propria differed from the lamina propria of the intact trachea in thickness (regenerated lamina propria 4 times thicker than lamina propria of normal trachea) and its mode of vascularization (dense connective tissue with small blood vessels versus loose connective tissue with larger capillaries in the lamina propria of the normal trachea). A cartilage graft will not allow vessel ingrowth so that the only way to allow for angiogenesis and to induce granulation tissue formation was through the anastomosis with the native trachea.

A tube consisting of free auricular cartilage was not able to maintain a segmental tracheal replacement. The main reason for this was necrosis and collapse of the majority of airway exposed cartilage. In a second experiment, the blood supply around the cartilage tube was augmented in an attempt to protect the middle segment of the cartilage tube from undergoing necrosis. A vascularized fascia flap wrapped around the cartilage tube was able to preserve the structural and morphological integrity of the tube in a heterotopical position. We hoped that the fascia enwrapped cartilage could also preserve its viability within the orthotopical position awaiting complete remucosalization. Although improved vascularization around the tube was obtained, the healing pattern of the cartilage tubes with the surrounding vascularized fascia was nearly identical as the healing pattern of the free cartilage graft. Only a small area around the anastomosis (maximal 3 mm) became remucosalized and preserved its viability. The improved vascularization around the tube had no impact on angiogenesis acting at the anastomotic areas and had no influence on the necrosis of the middle part of the cartilage graft.

In our model, airway exposure of bare cartilage during a period longer than 2-3 weeks resulted in necrosis regardless of the amount of vascularization around the cartilage. The hostile environment of the airway destroyed the cartilaginous tubes until they were covered with vascularized connective tissue. Only the remucosalized cartilage areas preserved their viability and support.

The cartilage tube lined with perichondrium and surrounded by vascularized fascia has all the reported tissue requirements for optimal tracheal replacement[5]: it is vascularized, biocompatible, and non-immunogenic; it has intrinsic skeletal support to prevent airway collapse without the need for stenting, and it allows for reepithelialization. In this study it was shown that these tissue

characteristics are not sufficient for successful tracheal replacement. An important additional requirement is the availability of a mucosal lining because secondary healing is only seen around the anastomosis.
It can be concluded that efforts on cartilage tissue engineering need to be accompanied by efforts in obtaining a respiratory lining inside the tube. A viable cartilage tube with a viable epithelial lining will be necessary in order to obtain successful segmental tracheal replacement.

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Chapter IV. Prefabrication of composite tissue: the way towards improved tracheal reconstruction.

Abstract

Tracheal repair tissues were evaluated experimentally in order to provide an evidence-based choice for decision making in clinical tracheal reconstruction.
Tracheal reconstructive tissue was characterized as providing for vascularization, support and/or lining. A tissue equivalent was developed in the rabbit for each of the individual tissues. The individual tissues consisted of non-epithelialized soft tissue (vascularized fascia), epithelialized tissue (vascularized fascia grafted with buccal mucosa), and supportive tissue (ear cartilage). The 3 reconstructive tissues were evaluated in 30 rabbits after repair of an anterior laryngotracheal defect using morphometric and histological analysis.
After a 1 month follow-up period, defects repaired with non-epithelialized soft tissue showed a healing by secondary intention and a wound that was contracted to 44 % of the initial surface area of the defect. Mucosal lined soft tissue flaps and cartilage grafts showed a less than 10% wound contraction. Compared to cartilage grafts, mucosal lined soft tissue (vascularized fascia grafted with buccal mucosa) seemed preferable for clinical use because it showed a healing by primary intention.
A mucosal lined radial forearm fascia flap was used successfully in cases of restenosis after tracheal resection. A deficiency of the mucosal lined soft tissue was the absence of supportive tissue. In cases of extensive stenosis it might be useful to obtain additional expansion of the airway lumen by creating a convexity at the site of reconstruction.
In a second set of experiments we attempted to improve the mucosal lined soft tissue concept by adding elastic cartilage. A composite tissue consisting of vascularized fascia, buccal mucosa, and auricular cartilage was developed. Heterotopical prefabrication of the composite tissue was essential for survival of the cartilaginous component. Additional airway lumen expansion could be obtained after heterotopical flap prefabrication.
After experimental evaluation, the concept of the prefabricated composite tissue was successfully applied in a clinical case of long segment stenosis.
Experimental and clinical experience suggests that the combination of buccal mucosa and fascia form an optimized tissue combination for tracheal reconstruction. This combination can be improved by adding strips of autologous ear cartilage.

IV.1 Introduction

The best external surgical approach for tracheal stenosis is segmental resection with end-to-end anastomosis[1]. Augmentation of the tracheal lumen by inserting local, regional, or distant tissue is necessary when tracheal resection is not possible as for example in long segment stenoses (more than half of the tracheal length) or in cases of restenosis after tracheal resection. A stenosis of more than half the tracheal length is rather exceptional. However, because the indications for tracheal resections are growing, it can be anticipated that restenosis at the anastomotic site will become a more frequently encountered problem[2, 3, 4]. Restenosis poses a therapeutic problem because further resection is usually not possible and mostly, reconstructive tissue has to be used to augment the problematic airway segment.

Tracheal reconstruction by using repair tissue is a second choice solution because the optimal repair tissue is not available clinically. Optimal tracheal repair tissue should resemble the native tracheal tissue as close as possible and be composed of a cartilaginous support, an internal lining consisting of respiratory mucosa and a reliable blood supply. This optimal repair tissue cannot be found outside the airway. As a consequence, all the currently used reconstructive options are missing one or more basic requirements.

The most frequently used reconstructive tissues for tracheal lumen augmentation consist of cartilage grafts[5], and muscle flaps that are used as a carrier for skin[6], periosteum[7], or bone[8]. Results obtained with these reconstructive tissues are not constant because they all lack 1 or more requirements for optimal tracheal repair. Moreover, the choice between the different reconstructive options is difficult because no valuable comparison is available. Inherent to the rare pathology, all the reconstructive possibilities were applied in series with small amounts of patients, without clear definition of the repaired defects and with the amount of decannulated cases as only outcome variable.

A possibility to make an evidence based choice between the different reconstructive options may lie in a well-controlled experimental comparison model.

We therefore characterized the reconstructions by their constructing tissue components consisting of vascularization, support, and lining. The basic tissue requirements were translated into specific rabbit's tissues, which could be used for experimental comparison after repair of airway defects.

Important in tracheal reconstruction is to obtain a primary wound healing with a minimal amount of wound contraction and to obtain an airway lumen sufficient for normal respiration. We attempted to compare the different individual and composite repair tissues in their capacity to repair an anterior airway defect. From the experimental results obtained with this model, an optimized reconstructive concept was developed and successfully used in clinical cases of tracheal stenosis.

IV.2 Experimental evaluation of individual repair tissue

IV.2.A Material and Methods

The capacity of the different reconstructive tissues in preserving the surface area of an anterior laryngotracheal airway defect was evaluated. We used rabbit ear cartilage as supportive tissue and oral mucosa as internal lining. A vascularized fascia flap (lateral thoracic fascia) was used as non-epithelialized soft tissue and as a vascular carrrier for the mucosal graft (Fig. 4.1). The vascularized fascia flap was preferred as vascularized tissue because it provides for the thinnest axially perfused flap that is currently available.

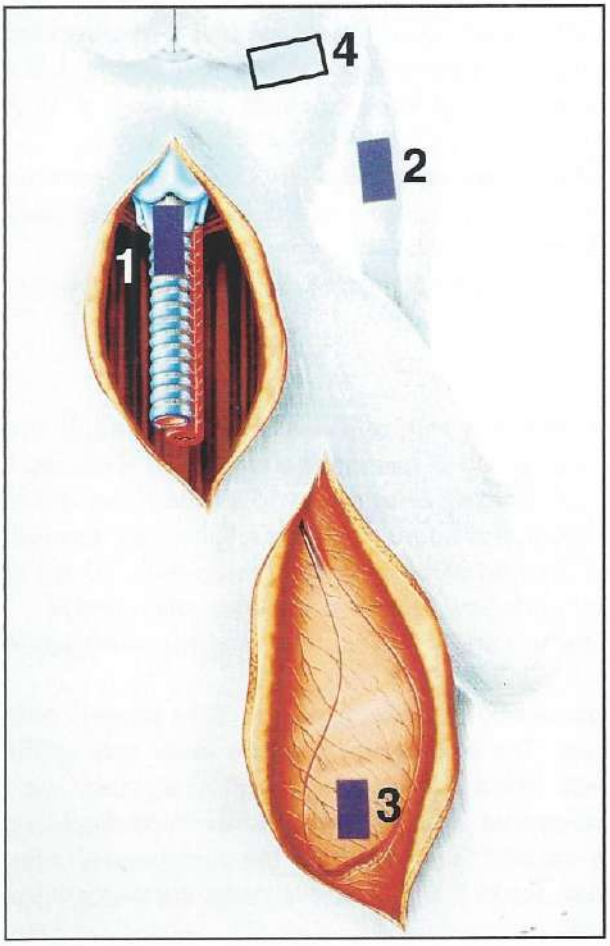


Fig.4.1. Reconstruction of anterior laryngo-tracheal defects.
A defect of 1.5 cm (length) by 0.8 cm (width) is created at the level of the cricoid and the upper trachea (1). This defect is repaired with a piece (0.8 x 1.5 cm) of auricular cartilage (2) or with a segment of the lateral thoracic fascia flap (3). The fascia flap can be used as non-epithelialized tissue or as epithelium lined fascia after applying a full-thickness buccal mucosal graft (4). An area of 1.5 x 0.8 cm -with or without full-thickness mucosa- is sutured into the anterior laryngotracheal defect.

A.1 Repair tissues

1.Fascia. (Vascularization+) Group 1-N=10

An area of 1.5 by 0.8 cm of the rabbit's lateral thoracic fascia[9] was used to close the airway defect (Fig. 4.1). The lateral thoracic fascia flap is located in the lateral thoracic area and axially perfused by the lateral thoracic artery and vein. The flap is transferable to the neck region by subcutaneous transposition on the vascular pedicle.

2.Cartilage. (Support+) Group 2-N=10

A cartilage plate (1.5 by 0.8 cm) with preserved perichondrium at each side was taken at the root of the outer ear.

3. Fascia + buccal mucosa. (Vascularization+, lining+) Group 3-N=10
Two full thickness mucosal grafts (0.75 cm by 0.8 cm) were taken at the left and right buccal area. The lateral thoracic fascial flap was dissected and the 2 mucosal grafts were sutured to the distal end of the fascial flap to obtain a mucosal covered area of 1.5 by 0.8 cm. The mucosa lined fascia was isolated on its vascular pedicle, rotated to the neck, and sutured into the anterior airway defect.

A.2 Airway defect

The animals were premedicated with 0.25 mg/kg of Imalgene® and 0.15 mg/kg of DomitorR® intramuscularly and anesthesia was maintained with halothane through mask ventilation. The cervical trachea was reached through a midline neck incision and the upper end was dissected over 2 cm from the surrounding tissues. An anterior laryngotracheal defect (measuring 1.5 x 0.8 cm) was created at the level of the cricoid and at the first three tracheal rings (Fig. 4.1). A photograph was taken of the anterior defect.
For the different reconstructions, a patch with a similar size as the defect was sutured into the defect with polypropylene (Prolene) 5-0. The skin incisions were closed and the rabbits were allowed to breathe without stent or tracheotomy. They were followed during 4 weeks.
Thirty mature New Zealand White rabbits, with a mean age of 6 months and weighing between 3 and 3.5 kg were used.

A.3 Evaluation

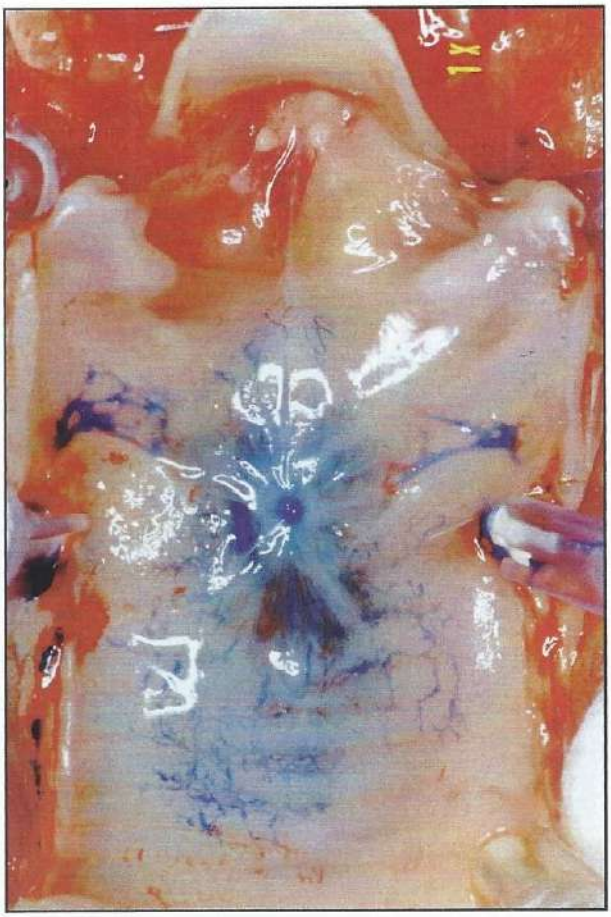
After the follow-up period, the animals were killed painlessly with an overdose of Nembutal and the laryngotracheal complex was excised. Animals in which a fascial flap was used (Groups 1 and 3) underwent silicone dye injection of the lateral thoracic artery prior to tracheal harvest to better visualize the revascularization process. Therefore, the lateral thoracic region was opened, and the left thoracic artery of the fascia flap was flushed through the cannula with 10 ml of heparinized, normal saline solution (10IU heparin/ml) followed by 5 ml of blue silicone dye.
This material formed a cast of the vascular architecture of the fascia flap and the established vascular connections with the tracheal patch.
All harvested tracheas (N=30) were incised longitudinally at their posterior side pinned onto cardboard, and photographed with a ruler in place. The photographs of the inner side of the reconstruction and the photographs of the defects (taken during the initial operation) were scanned on a color scanner and digitized into a computer. These color pictures were displayed on screen for tracing and calculation. The surface area of the defect and the surface area after reconstruction were outlined with a computer mouse. From these measurements the percentage reduction in surface area (SAR) could be calculated.
$SAR=SA_i-SA_f/SA_i$ where SA_f and SA_i are the final and initial surface area of the reconstruction, respectively.
For the overall comparison of the percentages of the reconstructed area for the 3 reconstructions, a Kruskal Wallis test was used. Statistical significance for the test was set at $p<0.05$.
The different reconstructions were evaluated macroscopically and histologically (axial sections). The tissues were embedded in paraffin and 4μm-thick axial sections were cut and stained with hematoxylin-eosin (H&E stain).

IV.2.B Results

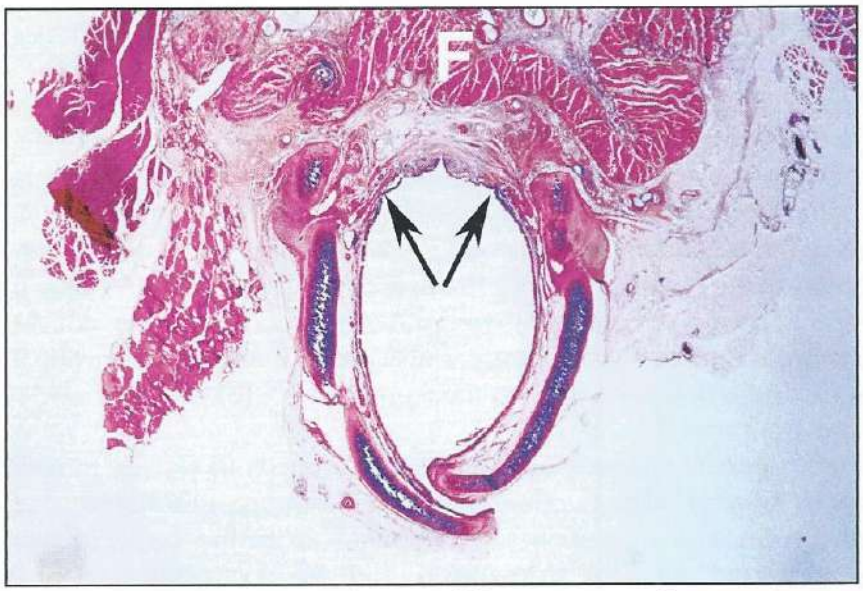
In order to have a group of 30 animals followed during a 4 week period, we had to operate on 35 animals. Five animals died during follow-up for reasons not related to their reconstruction. All the reconstructed areas of group 1 and group 3 animals were vascularized by the fascia flap and showed a blue coloration after blue Microfil® injection (Fig. 4.2.1.a, Fig. 4.2.3.a).
The surface area of the reconstruction was well preserved for group 2 and group 3 animals. The mean size reduction of the reconstructed surface area was 5.2 % (SE= 3.1%) in group 2 animals and 8.1% (SE=2.9%) after reconstruction with mucosal lined fascia. No statistical difference in wound contraction was found between group 2 and group 3 animals. In group 1, the mean size reduction of the reconstructed surface area measured 44.5% (SE=10.2%) and was significantly higher than in groups 2 and 3.
Histologically, the non-epithelialized reconstructions (Group1, 2) proved to be lined with respiratory epithelium by the time of evaluation. In group 1, remucosalisation started at the margins of the graft with progressive migration of the epithelium to the center of the graft over the 1-month follow-up period. After the follow-up period, the contracted surface area in group 1 was completely covered with respiratory epithelium (Fig. 4.2.1).
The cartilage grafts (group 2) underwent revascularization and reepithelialisation from the margins of the defect. Regenerated submucosal blood vessels and respiratory epithelium were less developed in the middle of the graft (Fig. 4.2.2).
The mucosal lined reconstructions (group 3) showed a primary healing with complete take of the oral mucosa on the fascial vascular carrier (Fig. 4.2.3).

Fig. 4.2 Morphology of patch reconstructions

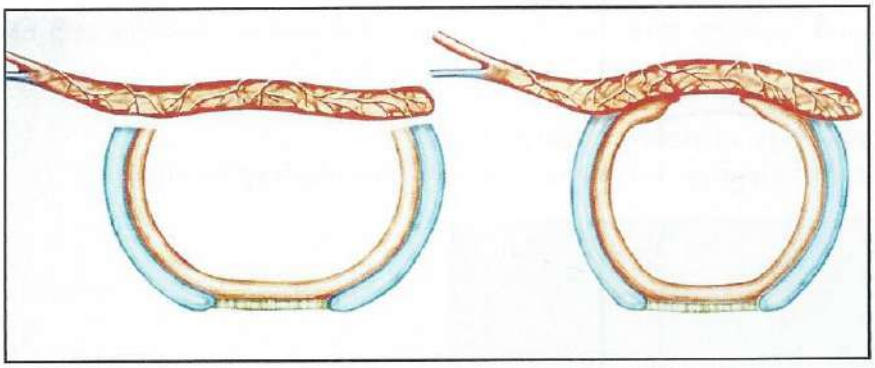
Fig. 4.2.1. Morphology after reconstruction with vascularized fascia.



4.2.1.a. Macroscopy fascia. Internal view after posterior longitudinal incision. The fascia is injected by blue Microfil®. Signs of wound contraction, which are most pronounced in the center of the patch, are visible.

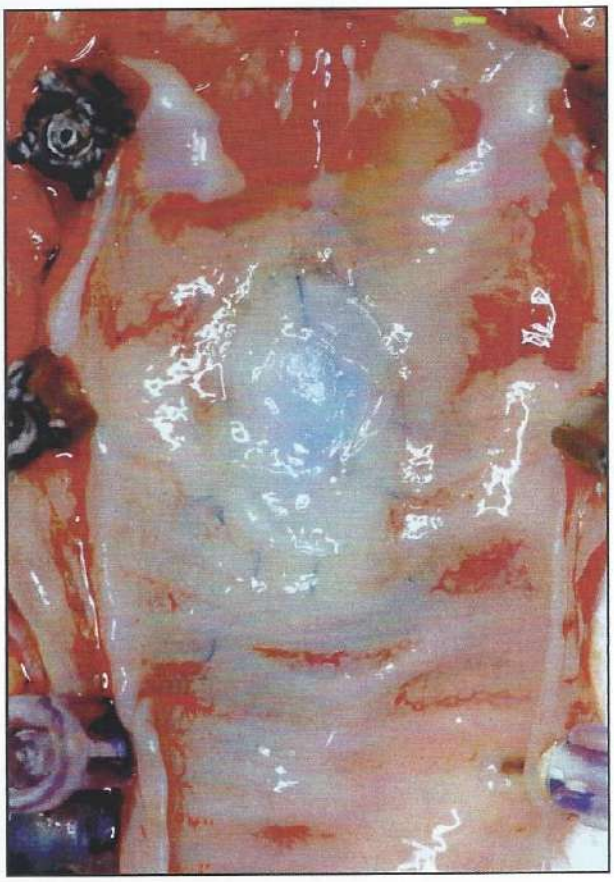


4.2.1.b. Histology fascia. The contracted area of fascia is seen between arrows. Regenerated respiratory epithelium covers the contracted area. F= fascia flap (H&E original x 5).

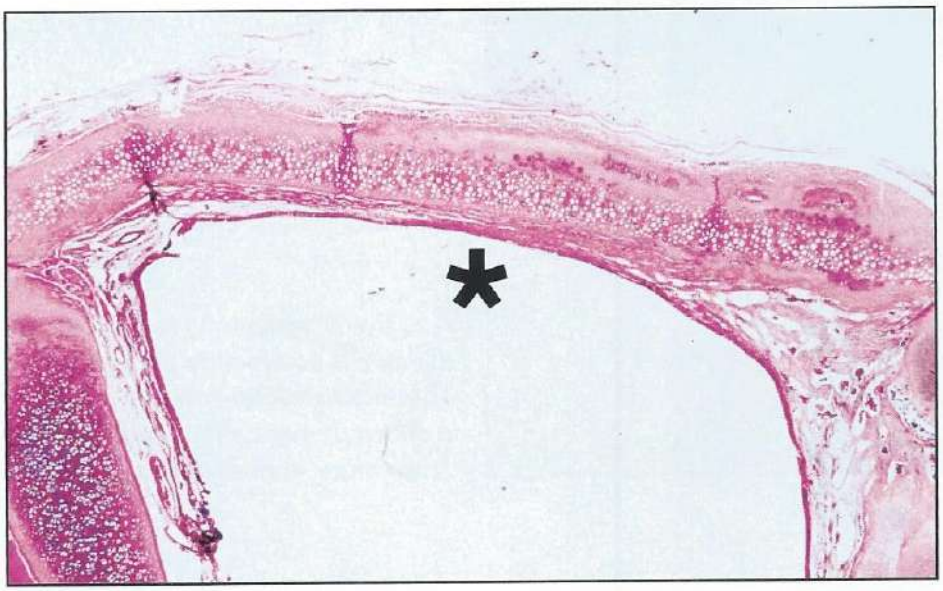


4.2.1.c..Schematic presentation of fascial healing. Healing of the fascia is characterized by reepithelialization from the defect margins and by wound contraction of the reconstructed defect area.

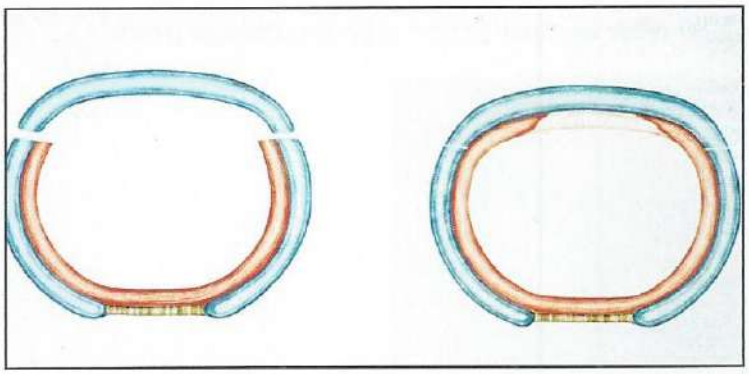
Fig. 4.2.2. Morphology after reconstruction with a cartilage graft.



4.2.2.a. Macroscopy cartilage. The Prolene sutures indicate the margins of the cartilage graft. The cartilage grafts are remucosalized from the margins of the defect. In this case, the central part of the graft was not completely reepithelialized.

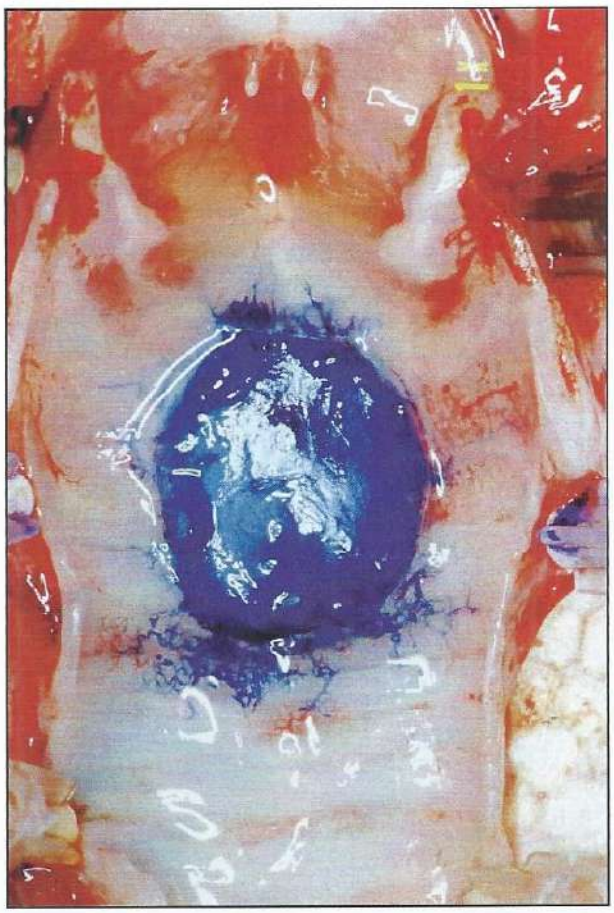


4.2.2.b. Histology cartilage. The cartilage graft is revascularized and reepithelialized from the margins of the defect. The regenerated submucosal blood vessels and respiratory epithelium are more developed at the margins of the cartilage graft than in the middle (asterisk) of the graft (H&E original x 5).

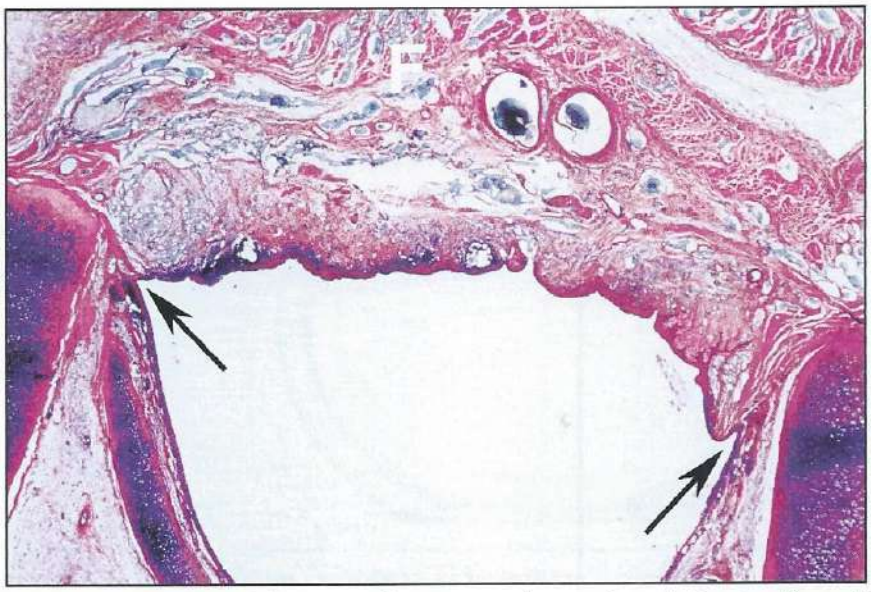


4.2.2.c. *Schematic presentation of cartilage healing. Revascularization and reepithelialization from the margins of the defect characterize healing of the cartilage graft.*

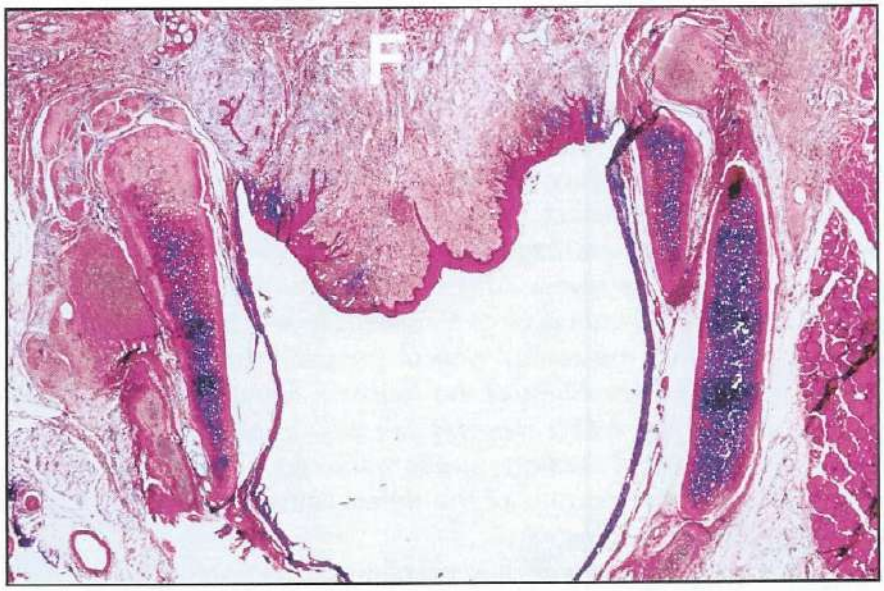
Fig. 4.2.3. Morphology after reconstruction with mucosa lined fascia.



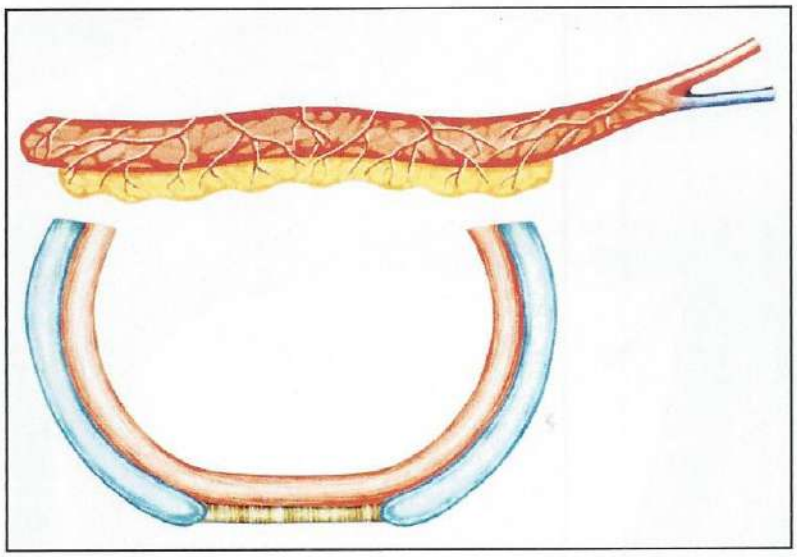
4.2.3.a. *Macroscopy mucosa lined fascia. Oral mucosa is completely colored by blue Microfil®. The reconstruction shows primary healing with a sharp demarcation line between the oral and respiratory epithelial lining.*



4.2.3.b. Histology mucosa lined fascia. The intensely perfused fascia flap (blue Microfil® injected) is lined with squamous epithelium. A sharp transition area exists between oral and respiratory mucosa (arrows). A flat reconstruction between the margins of the defect will be provided when the size of the mucosal graft is similar to the size of the defect. F= fascia flap (H&E original x 5).



4.2.3.c.Histology mucosa lined fascia. Prolapse into the lumen will result if a bigger patch is sutured into the defect. F= fascia flap (H&E original x 5).



4.2.3.d. Schematic presentation of healing of buccal mucosa. Primary healing with preservation of reconstructed surface area.

IV.2.C Discussion

Soft tissue without epithelial lining will heal by secondary intention when used inside the airway. This tissue undergoes reepithelialization in combination with a wound contraction process and with a significant reduction of the reconstructed surface area. Examples of clinically used tissues within this group are periosteum[7] and muscle[10]. Because of the wound contraction process, soft tissues without epithelial lining are not a first choice when augmentation of a stenotic airway is attempted.

Cartilage patches undergo secondary healing with reepithelialization and revascularization induced from the margins of the defect. Both clinical and animal studies have established that autologous costal and auricular cartilage grafts survive when placed in an anterior airway defect[11, 12]. However, because the revascularization and reepithelialisation processes originate from the margins of the defect, healing of cartilage grafts will be dependent on the size of the graft. It can be anticipated that the central part of the graft may undergo necrosis if a bigger patch of cartilage is used. The condition of the mucosal lining at the defect margins and the degree of vascularization of the defect margins will play important roles in cartilage healing. As a consequence, the healing of cartilage grafts within an airway stenosis may be more difficult because of scarring at the margins of the defect with problematic revascularization and reepithelialisation from these margins.

The vascularized fascia lined with buccal mucosa allows for a healing by primary intention. The vascular supply and the epithelial lining of the graft make the patch independent from the defect margins for its healing. No revascularization and no reepithelialization are necessary during healing and this may be advantageous in the clinical situation when a scarred wound bed is encountered. Wound healing with mucosa lined fascia is comparable to the wound healing seen when using skin lined flaps. A mucosal lining is however preferable for airway lining in order to prevent the crusting and desquamation seen when using skin grafts. A disadvantage of the mucosal lined fascia is the absence of supportive tissue. Essential when using a mucosal lined fascia flap into an airway defect is that the mucosal patch and the defect have a similar size. Prolapse into the airway will result if a mucosal patch, which is larger than the defect, is included (Fig. 4.2.3.c).

The mucosa lined fascia was used in 2 clinical cases of anastomotic stricture after segmental airway resection.

IV.3 Mucosa lined fascia. Case report

An 18-year-old boy was admitted to our department with severe laryngotracheal stenosis. The stenosis occurred after the patient was involved in a car accident 4 months earlier. At that time, the patient had been intubated for 3 weeks. After extubation he underwent an endoscopy and placement of a tracheostomy tube because of increasing respiratory distress. He was diagnosed as having subglottic stenosis. The stenosis was initially treated by dilatation and laser resection. He was referred to us in October 1997 presenting with a subglottic stenosis with complete obstruction of the lumen (Cotton grade 4), starting 1-cm subglottically. On CT scan, the stenotic segment was estimated as having a length of 3 cm. In November 1997 he underwent a resection of the cricoid cartilage anteriorly together with a resection of 4 cm of the proximal trachea including the tracheotomy. The mediastinal trachea was mobilized and a suprahyoid release was performed. The mediastinal trachea was sutured to the cricoid cartilage posteriorly and to the thyroid cartilage anteriorly. Although the upper and lower airway segments were mobilized maximally, the anastomosis (Dexon 2-0) was performed under some tension. The patient was extubated 1 day postoperatively and head flexion was maintained for 1 week. Five weeks postoperatively, the patient underwent a replacement of the tracheotomy because of increasing respiratory distress. On laryngoscopy a restenosis at the anastomosis was seen with a 90% reduction of the airway lumen. Two attempts to remove the fibrotic tissue with the CO2 laser were not successful. In January 1999 it was decided to do an augmentation tracheoplasty with a mucosal lined fascia flap. The laryngotracheal complex was incised longitudinally starting at the caudal end of the anterior commissure and ending in the tracheostomy incision. The restenosis was located at the caudal cricoid level and had a length of 2 cm. The laryngotracheal complex was incised over 5 cm to allow for a sufficient expansion of the stenotic area. An elliptical anterior defect with a length of 5 cm and with a width of 1.8 cm was obtained (Fig.4.3.a). The tracheotomy tube was removed and the patient was intubated orally.

The radial forearm fascia flap was used as vascular carrier for the mucosal lining because it provides fascial and fasciocutaneous tissue, vascularized by a long and reliable vascular pedicle consisting of the radial artery and vein.

A patch of fascia and subcutaneous tissue, measuring 8 by 5 cm, was exposed after a midline longitudinal incision of the radial forearm skin. A small strip of skin (measuring 1 by 5 cm) was included distally as a fasciocutaneous segment serving as a monitor for the viability of the flap postoperatively (Fig.4.3.b, c, d).

Two full-thickness mucosal grafts were harvested at the left and right buccal areas. The buccal mucosal defects were closed primarily. The mucosal grafts were trimmed and cleaned from underlying submucosal fat. The margins of the mucosal graft were secured to the fascia using interrupted sutures (Vicryl 6.0). A mucosa covered area of 1.8 x 5-cm was obtained.

The grafts were applied before dissection of the flap because the stability of the undissected fascia allowed for easy suturing. The longitudinal axis of the mucosal graft formed the tranverse axis of the fascial flap (Fig.4.3.b). After applying the mucosal grafts, the fascial flap was dissected on its vascular pedicle. The skin at the donor defect was closed primarily.

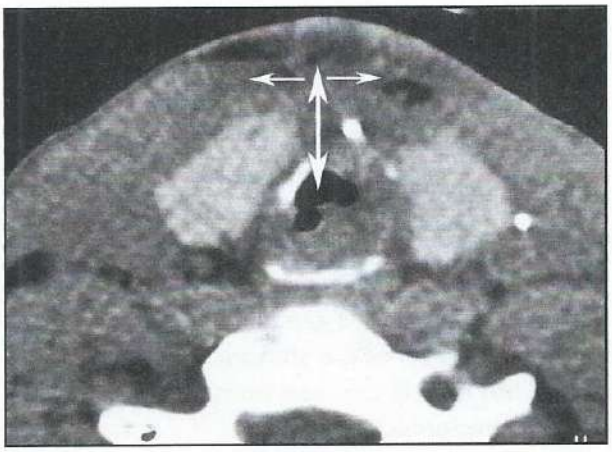
The mucosal covered area of the fascia flap was sutured into the anterior airway defect with Dexon 3.0. The distal end of the fascia flap was rotated over 180° and the fasciocutaneous island was brought in the neck incision to serve as a monitor. The monitoring skin paddle was used as a visible control of the viability of the flap in the postoperative period.

After insetting of the mucosa lined patch, the fascia flap was revascularized by an end-to-end anastomosis between superior thyroid and radial artery and by an end-to-side anastomosis between internal jugular vein and radial vein. The patient was extubated without problems 5 days postoperatively. A sufficient airway lumen with the mucosal lined fascia in the anterior defect was seen on a postoperative CT scan (Fig. 4.3.e).

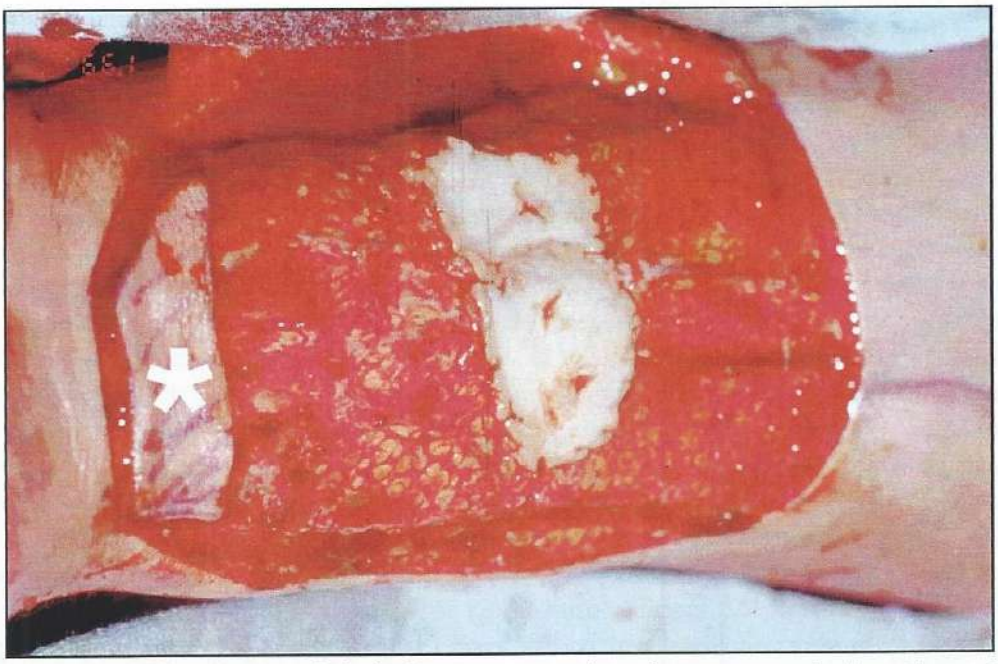
One other patient with a restenosis after segmental resection was reconstructed successfully in an identical way.

The 2 cases showed that a mucosa lined radial forearm fascial flap could be used successfully to close the airway defect after longitudinal incision and expansion of a stenotic area. The vascularized fascia allowed for easy healing in a scarred operation field and the mucosal grafts allowed for primary healing without wound contraction.
A disadvantage of the mucosa lined fascia is the absence of a cartilage component. Because support is lacking, a flat reconstruction between the margins of the anterior defect is the best result that can be obtained. Further expansion of the airway lumen by creating an anterior convexity is not possible and this may be a drawback when a more extensive stenosis needs reconstruction. In a second set of experiments, we wanted to investigate if we could improve this reconstructive concept by adding cartilage support to the mucosal lined fascia flap.

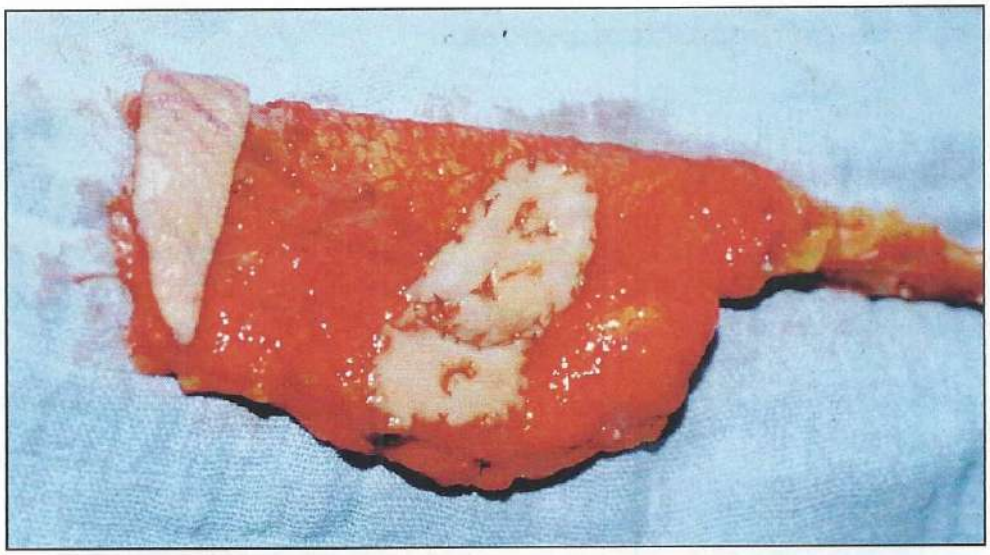
Fig. 4.3. Mucosa lined fascia-Case report.



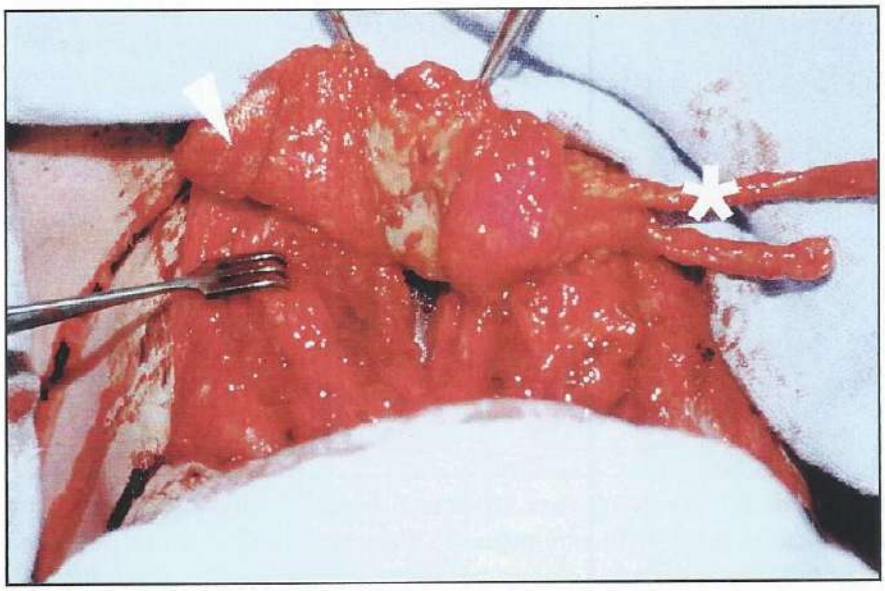
4.3.a..Preoperative CT scan. Axial CT scan through the anastomosis (area of caudal cricoid) after previous tracheal resection. The airway was incised anteriorly (double arrow) over a length of 5 cm and the stenotic area was expanded (arrows) with creation of an anterior defect.



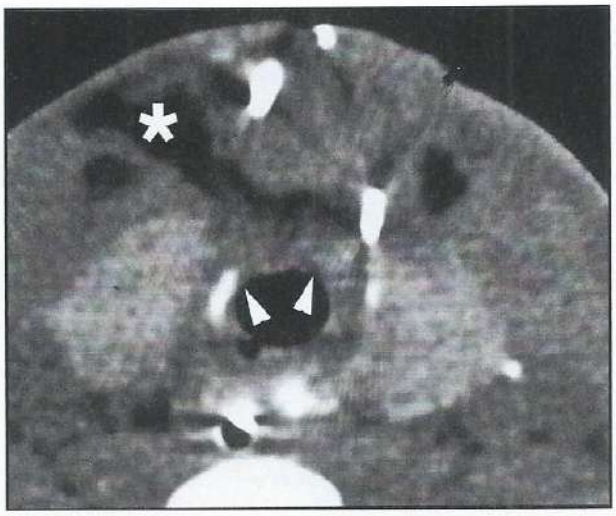
4.3.b. .Mucosa lined fascia. Two full thickness mucosal grafts were applied on the antebrachial fascia flap after elevation of the overlying skin (Dexon 6.0). Several sutures were placed in the middle of the mucosal graft to anchor it to the bed and to eliminate dead space. A mucosal covered area of 1.5 x 5 cm was obtained. The longitudinal axis of the area covered with mucosa was orientated in the transverse direction of the forearm. The monitoring skin flap is visible at the distal flap site (asterisk).



4.3.c. Mucosa lined fascia after flap dissection and isolation on the radial artery and vein.



4.3.d. The mucosa lined fascia is sutured into the longitudinally incised tracheal defect from inferior to superior. The vascular pedicle is indicated with an asterisk; the monitor flap is indicated with an arrowhead.



4.3.e. Postoperative CT. The mucosa lined fascia is sutured in the anterior airway defect. The full-thickness mucosa is visible between the arrowheads. The fascia flap is indicated by an asterisk. A flat reconstruction between the defect margins was obtained and this reconstruction was sufficient for normal respiration.

IV.4 Composite reconstruction model

IV.4.A Introduction

Cartilage support is essential when a more extended anterior lumen expansion is warranted. This support can be provided when an elastic piece of cartilage is sutured between the margins of an airway defect with a smaller size than the size of the cartilage segment. Elastic ear cartilage is available in major quantities in rabbits but only in small quantities in humans (Fig. 4.4). Fibrocartilage is available in much larger quantities in the human rib but this type of cartilage has a low elasticity and is not suitable to realize an additional anterior airway lumen expansion.

Theoretically, 3 different designs are possible when adding elastic cartilaginous support to a mucosa lined fascia flap.

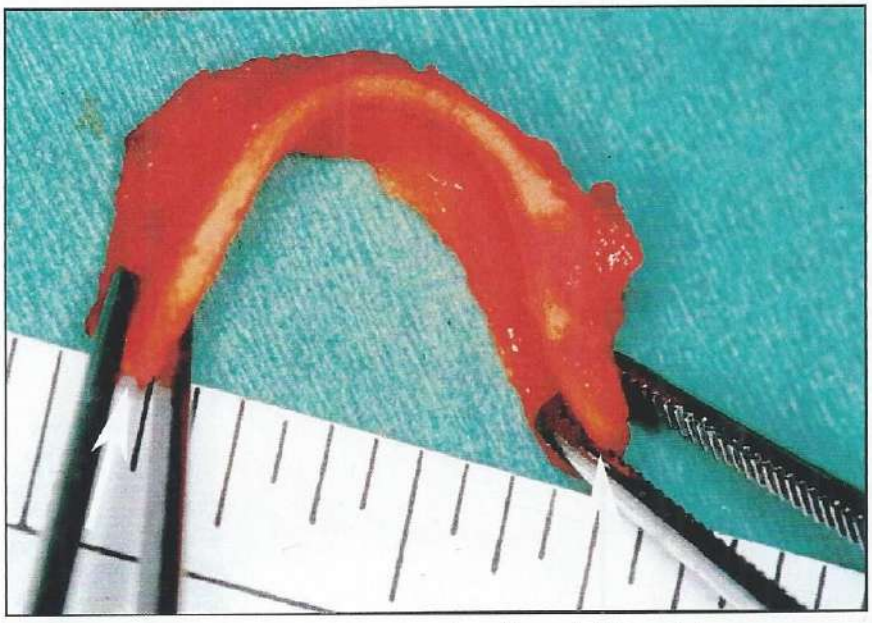
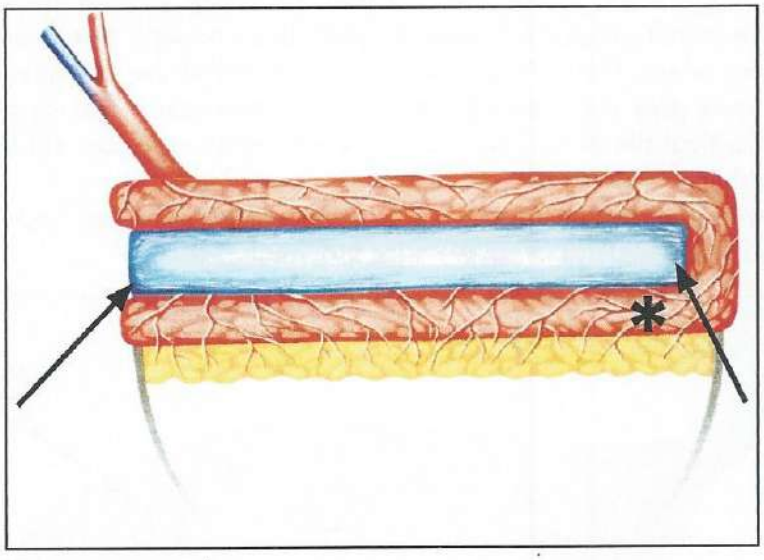


Fig.4.4. Cartilage support in airway reconstruction for additional lumen augmentation.
In humans, an auricular graft with dimensions approaching 2 x 5 cm may be harvested. An anterior convexity may be created if elastic cartilage with a length larger than the width of the defect is sutured to the margins of that defect (arrows).
Scale in Inches.

Composite tissue concept I

The cartilage graft is placed between an inner and outer fascial layer. The full thickness buccal mucosa graft is applied on the outer fascial layer after 180° rotation of the fascia flap (Fig.4.5.a). In this concept, both the cartilaginous and the mucosal component are well perfused by the fascial flap. The problem with this concept however is the low degree of support provided by the cartilage component. In order to obtain a convexity at the site of reconstruction, it is necessary to suture the margins of the elastic cartilage graft to the margins of a smaller airway defect. With the composite tissue concept I, the margins of the mucosal graft are sutured to the margins of the defect and as a consequence, the cartilage graft is located outside the defect without providing support to the reconstruction[13] (Fig. 4.5.a).

Fig. 4.5. Composite tissue reconstruction-theoretical possibilities I and II.



4.5.a. Composite tissue concept I.
The composite tissue is formed by a 180° rotation of the vascularized fascia. In this way, the cartilage is circumferentially covered and protected within a vascular bed. The full-thickness mucosal graft can be revascularized by capillary outgrowth from the inner layer of the vascularized fascia flap. A disadvantage of this concept is the low degree of support provided by the cartilage component. In order to obtain anterior expansion of the airway lumen, the margins of the cartilage graft (arrows) need to be sutured to the margins of the defect. In this concept, the cartilage margin, which is wrapped with fascia can not be sutured to the margin of the defect without interfering with the blood supply to the inner fascial layer (asterisk).

Composite tissue concept II

In this concept the mucosal graft is applied directly to the cartilage graft (Fig. 4.5.b). This theoretical concept provides cartilage support but the mucosal graft can not survive on the cartilage because the cartilage component will not allow for ingrowth of blood vessels.

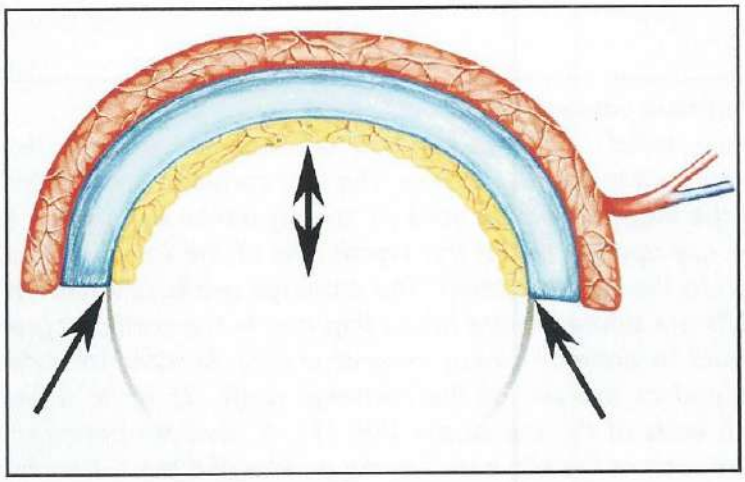


Fig. 4.5.b. Composite tissue concept II.
The cartilage component is sutured to the vascularized fascia and directly attached to the mucosal graft. In this concept, the margins of the cartilage graft (arrows) may be sutured to the margins of the defect with anterior expansion of the airway lumen (double arrow). The mucosal component will however undergo necrosis because cartilage will not allow vessel ingrowth from the fascia to the mucosal graft.

Composite tissue concept III

In this concept, cartilage strips are applied on the fascia flap so that the mucosal grafts are located between the cartilaginous framework. With this concept, the airway lumen can be expanded at the sites where the cartilage strips are sutured to the margins of the defect (Fig. 4.6). This concept may give some additional airway lumen expansion by providing cartilage support at the most critical site of the stenosis. The composite tissue concept III may theoretically improve the mucosal lined fascia flap.

The healing and amount of support provided by this theoretically most appealing concept was studied in the experimental setting of an anterior airway defect.

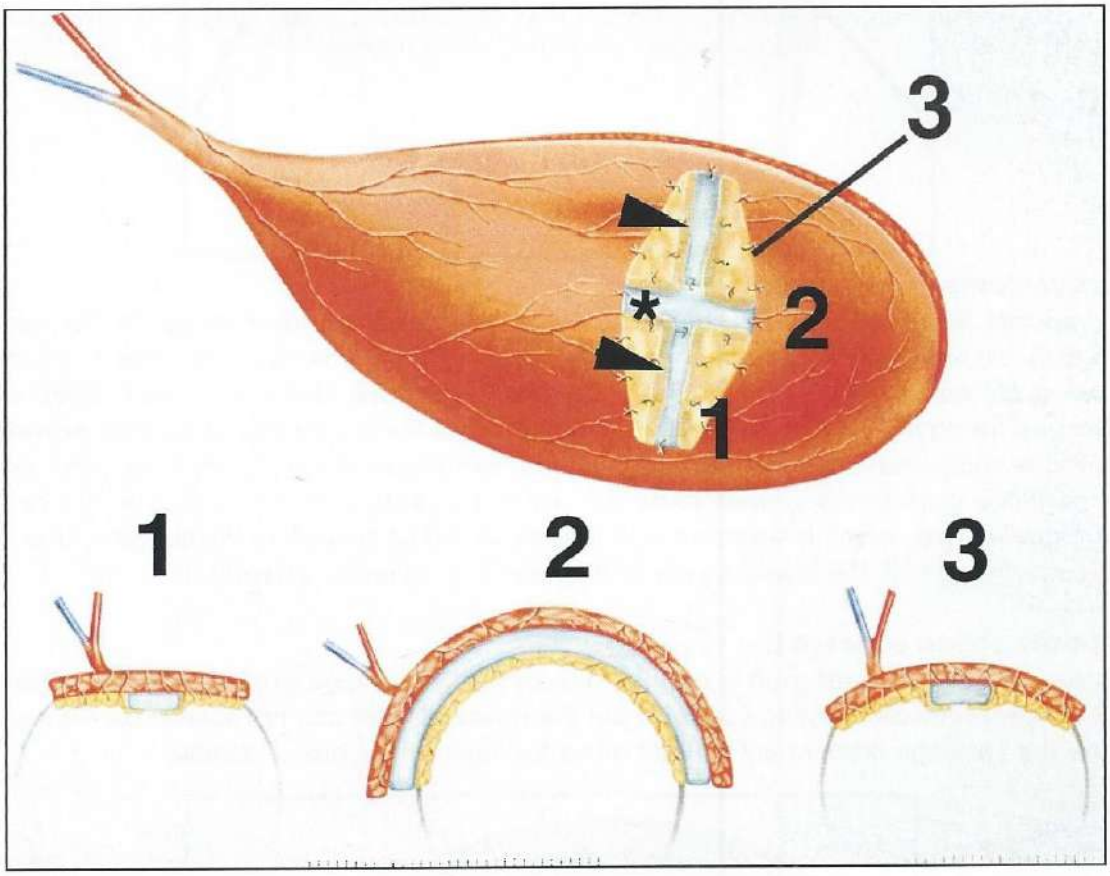


Fig. 4.6. Composite tissue concept III.
Cartilage grafts are sutured to the fascia flap between mucosal grafts. In this concept, 3 cartilage grafts are sutured to the fascial flap. The first cartilage graft (asterisk) is sutured to the longitudinal axis of the flap and will be used for airway lumen expansion. The 2 other cartilage grafts (arrowheads) are applied in the transverse axis of the fascia flap and are giving some longitudinal strength to the reconstruction. The cartilage grafts are sutured to each other. Four buccal mucosal grafts are sutured to the fascia flap and to the cartilage grafts to form a grafted area that will be used in anterior airway reconstruction. An anterior extension of the airway lumen will be obtained at the site of the cartilage graft (2) while a flat reconstruction will be obtained at both ends of the composite flap (1). A situation between the convex and flat reconstruction will be seen at the site between the middle and the extremity of the flap (3).
In this concept, the cartilage component may undergo secondary healing from 2 sites: from the oral mucosal grafts and from the respiratory epithelium at the margins of the defect.

IV.4.B Animal study

B.1 stage reconstruction

Healing of the cartilage component of the composite reconstruction was evaluated after repair of an anterior laryngotracheal defect in three rabbits. The composite tissue was formed at the lateral thoracic area. Three cartilages and 4 mucosal grafts were sutured to the fascia (Dexon 5-0) following the pattern shown in Figure 4.6. A grafted area of 1.5 x 2 cm was obtained.
The composite tissue was transplanted to an anterior laryngotracheal defect measuring 0.8 (width) x 2 (length) cm in 3 animals, involving the anterior cricoid and the first 4-5 tracheal rings. The rabbits were followed until the first signs of respiratory distress became apparent. At the time of dyspnea, animals were killed after injecting the vascular pedicle of the fascial flap with blue Microfil®. The reconstructions were assessed macroscopically and histologically.

Results

The three animals had a normal respiration during the first postoperative days. After an average follow-up time of 5 days, the animals showed signs of progressive respiratory distress. Postmortem evaluation of the grafts showed accumulation of secretions over the cartilage grafts. The cartilage grafts showed macroscopical (loss of support) and histological (eosinophilic ground substance, loss of cartilaginous tissue) signs of necrosis. The oral mucosal grafts showed ingrowth on the fascial flap with early signs of revascularization.
From the early postoperative follow-up in all 3 animals we learned that the initial support provided by the composite tissue concept III was lost due to necrosis of the uncovered, airway exposed, cartilage.

B.2 Preformed composite reconstruction

The principle of flap prefabrication was used to improve survival of the cartilage grafts. In this concept, transfer of the composite flap was delayed until the cartilage grafts were sufficiently healed and covered with buccal mucosa.
The composite tissue was formed at the lateral thoracic area. Three cartilages and 4 mucosal grafts were sutured to the fascia following the pattern shown in Figure 4.7. A grafted area of 1.5 x 2 cm was obtained. This grafted area was wrapped around a Gore-tex® tube to allow for healing of the cartilage grafts in a convex shape. The grafted area was inspected weekly under general anesthesia.
After 1 week of flap prefabrication, the mucosal grafts of the composite reconstruction showed a complete take and the bare cartilages were visible between the mucosal grafts. After 2 weeks, the peripheral parts of the cartilage grafts showed revascularization and remucosalization through angiogenesis and mucosal overgrowth from the surrounding buccal mucosal grafts (Fig. 4.7).
After 4 weeks the cartilage grafts were almost completely healed and covered by buccal mucosa in all 3 animals. After 1 month of flap prefabrication the cartilage grafts were sufficiently healed to allow for transplantation to the defect. After secondary healing of the cartilage grafts, the preformed composite tissue was dissected and transplanted to an anterior laryngotracheal defect measuring 0.8 (width) x 2 (length) cm in 3 animals, involving the anterior cricoid and the first 4-5 tracheal rings. One month after laryngotracheal reconstruction, the laryngotracheal complex was harvested after injection of the vascular pedicle of the fascia flap with blue Microfil®. The reconstructions were evaluated macroscopically and histologically (H&E staining) on axial sections.

Results

The cartilage grafts were fully healed and completely covered with mucosa in all three animals. The preformed composite reconstructions succeeded in expanding the reconstructed airway lumen (length of cartilage strip 0.7 cm longer than width of the anterior airway defect) (Fig. 4.8.1, 4.8.2).

The concept of composite tissue prefabrication allowed for a secondary mucosal healing of the cartilage grafts before being exposed to the airway lumen.

The principle of prefabrication of the composite tissue was subsequently used in a difficult clinical case of long segment stenosis.

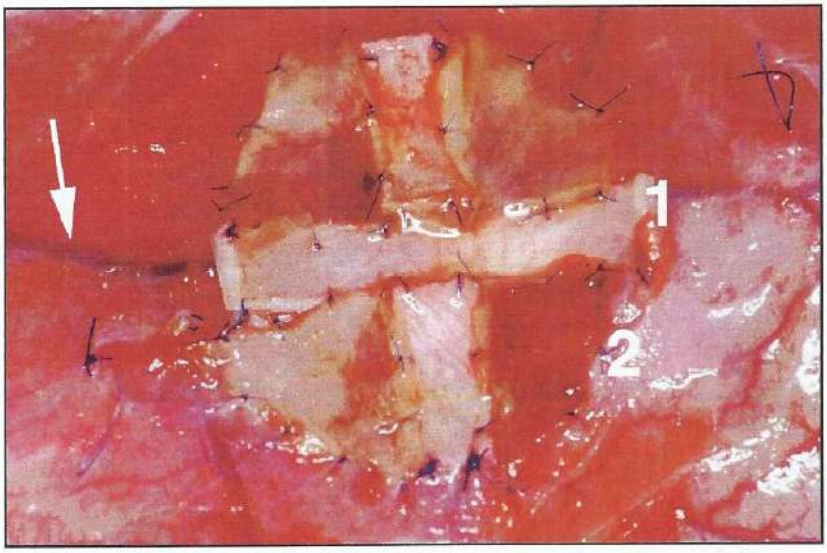
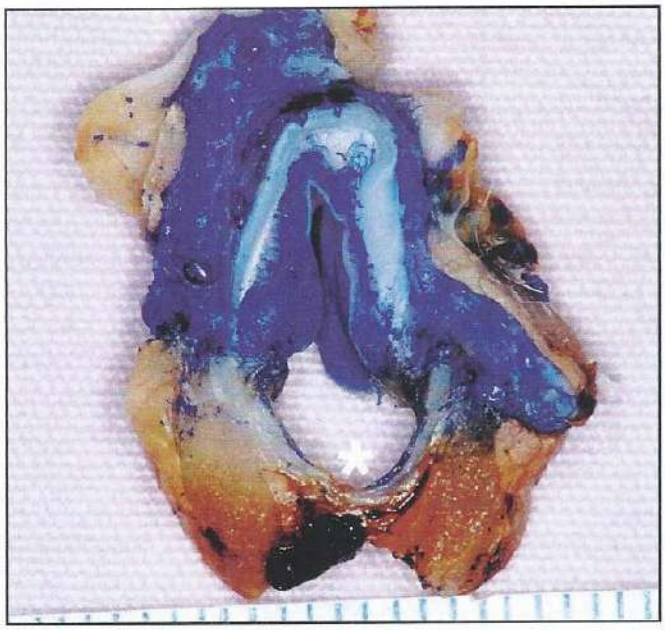
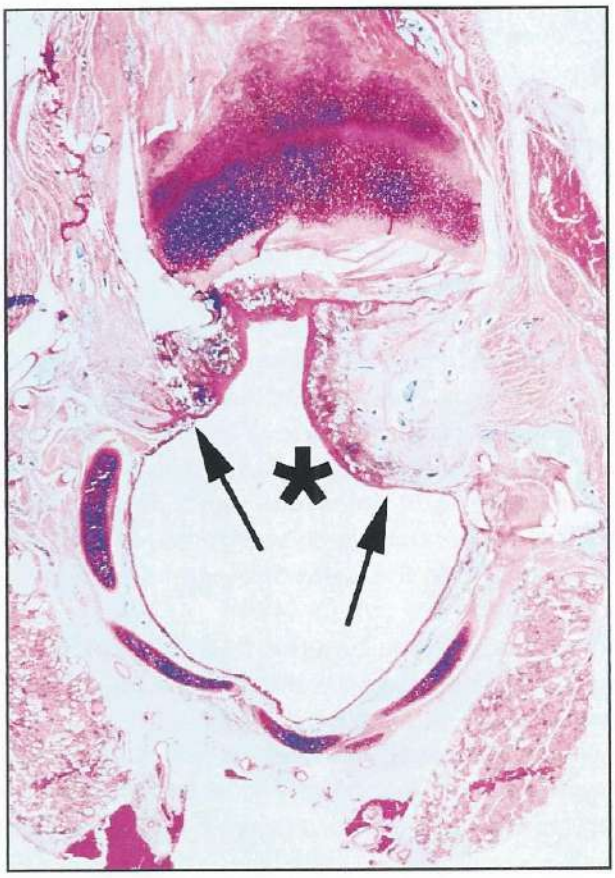


Fig. 4.7. Composite tissue design-Experimental study.
Situation 2 weeks after grafting of the fascia with mucosa and cartilage. The full-thickness mucosal grafts show complete survival. Submucosal vessel ingrowth is visible. The oral mucosa begins to grow over the edges of the cartilage grafts. Arrow points to lateral thoracic blood vessels running in the axial axis of the flap. After follow-up, the lateral thoracic artery may be injected with blue Microfil® so that the blood vessels which are from fascia origin can be identified (see figure 4.8).
1 indicates level of axial section of figure 4.8.1.
2 indicates level of axial section of figure 4.8.2.

Fig. 4.8. Preformed composite tissue-Experimental study.



4.8.1. Macroscopy of axial section at the level of the cartilage graft applied in the transverse axis of the reconstruction.
The cartilage graft is sutured between the margins of the anterior defect and expands the airway lumen anteriorly. A part of this anterior lumen extension is lost due to secondary healing with buccal mucosa. The mucosal lining is completely vascularized by the fascia flap (blue Microfil® injected). The regenerated buccal mucosa is much thicker than the respiratory mucosa which is visible in the remaining posterior trachea (asterisk).

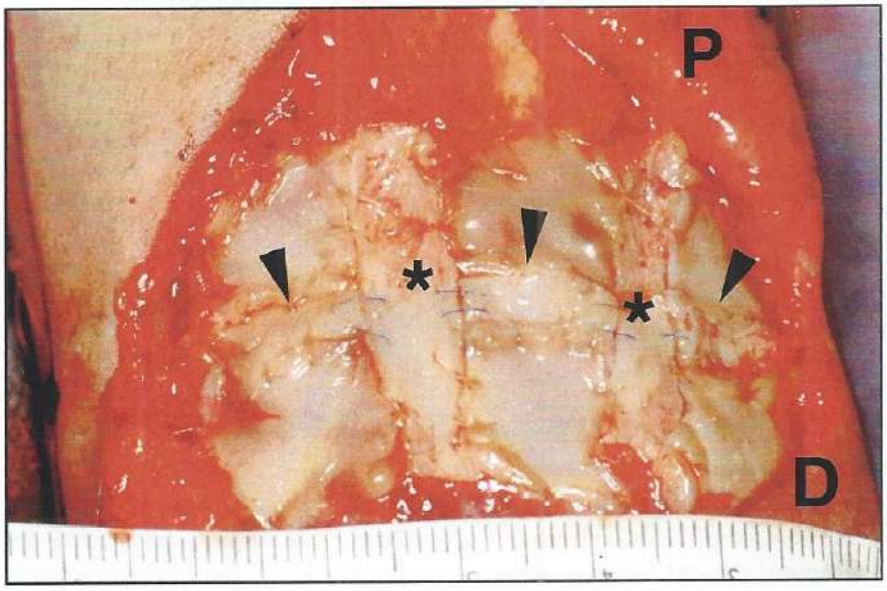


4.8.2. Histology of axial section at the level of the mucosal graft.
The cartilage strip, which is applied in the longitudinal axis of the reconstruction, is covered with buccal mucosa. The mucosal lined fascia is prolapsing into the lumen at the sites without cartilaginous support (asterisk).
The preformed flap, which was sutured into the anterior airway defect, is fully covered with buccal mucosa. A sharp demarcation between oral and respiratory epithelium is visible (arrows). Neochondrification is visible on extraluminal surface of cartilage graft (H.&E. original magnification x5).

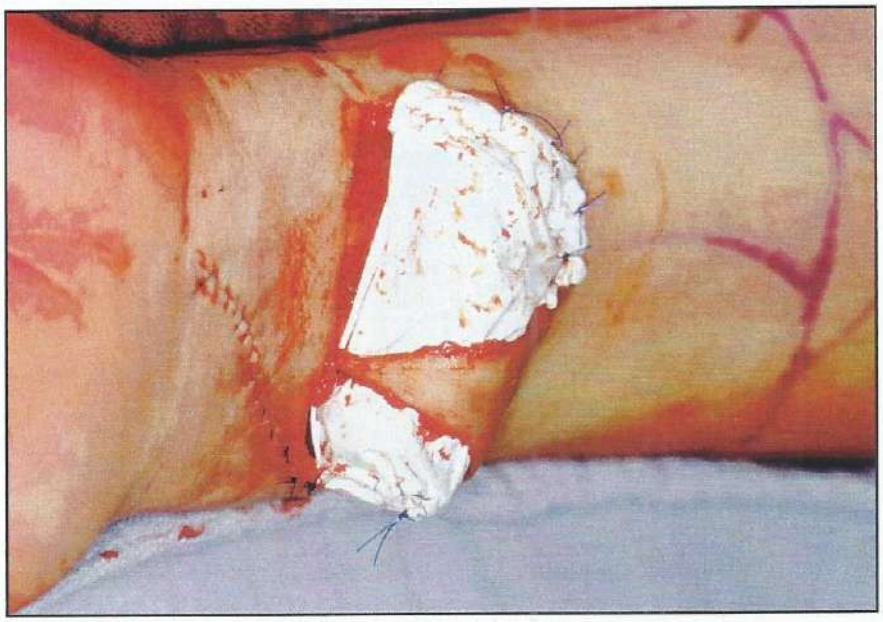
IV.5 Clinical application-case report: preformed composite tissue

A 38-year old male presenting with progressive stridor was urgently intubated. He was known as having laryngotracheal hypoplasia with episodes of dyspnea related to exercise. The present condition of extreme respiratory distress was triggered by an upper airway infection. Due to the relatively small laryngeal inlet, a pediatric tube was used for intubation. Attempts to extubate the patient proved to be impossible such that a tracheostomy was placed after 1 week.
After tracheostomy, the larynx and trachea were evaluated clinically and radiologically. On laryngoscopy, the glottic airway lumen was small with a short anterior-posterior dimension. Vocal fold mobility was preserved without signs of arytenoid ankylosis. A small airway lumen was found from the glottic level until the level of the tracheostomy. The length of the laryngotracheal stenosis measured 5.5 cm. The laryngeal cartilages had an abnormal form and shape and they were intensely calcified (Fig. 4.10.1). The mediastinal and thoracic trachea had a normal lumen (Fig. 4.10.4). In order to remove the tracheotomy, a tracheoplasty over a length of 5.5 cm was necessary. This intervention was also necessary in order to regain the ability to speak. The patient could not speak with the cannula because of a complete obstruction of the airway lumen above the tracheotomy site. Segmental airway resection or a slide tracheoplasty seemed impossible in this case because of involvement of the glottic level.
In a first operation stage, full thickness mucosal grafts from the right and left buccal area as well as auricular cartilage taken from both ears were grafted to the left radial forearm fascia. Two auricular cartilages measuring 2 by 4 cm were obtained. Two cartilage grafts of 1 x 4 cm were sutured (Dexon 4-0) to the longitudinal axis of the fascia to allow for anterior expansion of the reconstructed airway lumen (Fig. 4.9.1). Three cartilage grafts were sutured to the tranverse axis of the flap. The cartilage grafts were sutured to each other with Prolene 4-0. Six full thickness mucosal grafts were applied between the cartilaginous framework. A fascial area of 5.5 cm x 4 cm, grafted with mucosa and cartilage, was obtained after suturing the mucosal grafts to the fascia (Dexon 4-0). The grafted fascial area was sutured around a Gore-tex® tube to allow for secondary healing of the cartilage grafts in a convex shape. The forearm skin could not be closed over the preformed tissue and a Gore-tex® patch with paraffin gauze dressing was used to cover the tissue (Fig. 4.9.2). The preformed tissue was inspected after 10 days under general anesthesia. The Gore-tex® was then removed and the composite tissue was inspected. The sutures anchoring the grafts to the fascia were removed except for the Prolene sutures between the different cartilage strips.
A progressive growth of blood vessels and buccal mucosa over the cartilage grafts could be seen.
It was decided to transplant the preformed tissue 20 days after the initial operation. At that time, the cartilage strips were not completely reepithelialized but they were sufficiently healed at the margins with the mucosal grafts to allow for transplantation (Fig. 4.9.3). The forearm fascia was dissected on its vascular pedicle (Fig.4.9.4, 4.9.5). The neck was opened and the larynx and trachea were incised longitudinally from the level of the vocal folds to the level of the mediastinal trachea over a length of 5.5 cm. The anterior airway defect was expanded. The margins of the incised trachea were sutured to the strap muscles in order to maintain the expanded position of the incised anterior airway defect (Fig. 4.10.3). The tracheotomy tube was removed and the patient was intubated orally. The composite flap was sutured in the anterior airway defect using Dexon 3-0.
The flap was revascularized by suturing of the radial vessels to the superior thyroid artery (end-to-end) and to the internal jugular vein (end-to-side). A fasciocutaneous portion was included at the proximal site of the flap and sutured in the neck incision to serve as a monitor.
The patient was extubated without problems 5 days postoperatively. CT evaluation 10 days postoperatively showed a laryngotracheal airway lumen that was expanded with a factor of 3.The cartilage support was preserved and the reconstructed airway had a convex shape anteriorly (Fig. 4.10).

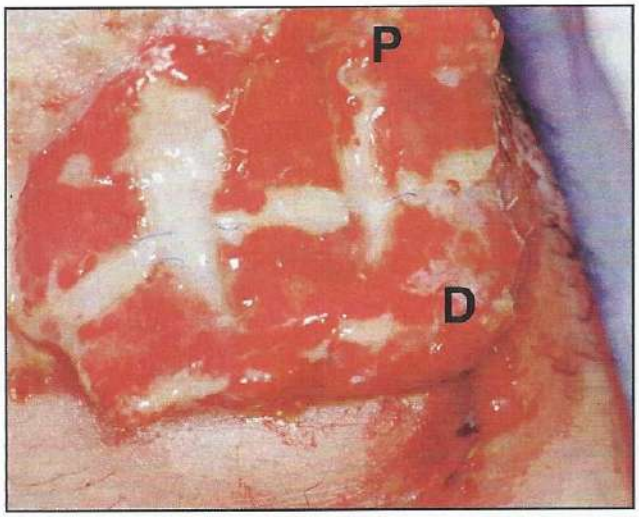
Fig. 4.9. Case report preformed composite tissue.



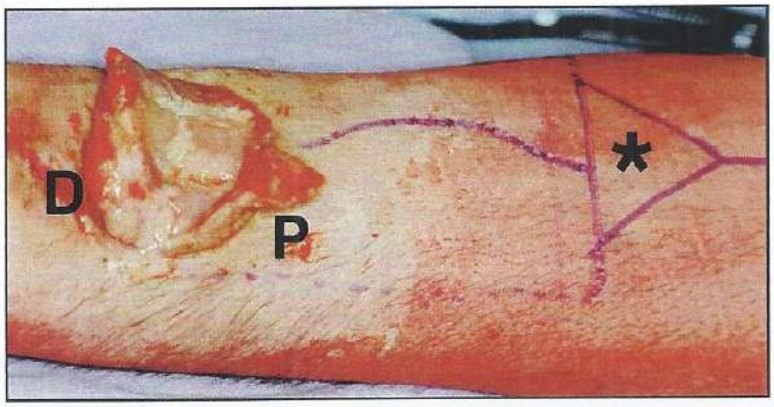
4.9.1. Preformed composite tissue.
The distal antebrachial fascia was grafted with a cartilaginous framework. Two strips of auricular cartilage (asterisk) measuring 1 by 4 cm were applied in the longitudinal direction of the forearm to provide for anterior lumen expansion of the reconstruction. Three shorter segments (arrowheads) were applied in the transverse direction of the forearm in order to give the reconstruction some rigidity in the longitudinal axis of the reconstruction. The cartilage grafts were sutured to the fascia (Dexon 4-0) and to each other (Prolene 4-0). Six mucosal grafts (1.2 x 1.2 cm) were sutured between the cartilaginous framework using Dexon 6- 0. The patch had a length of 5.5 cm (longitudinal axis of reconstruction-transverse axis of forearm) and a width of 4 cm (transverse axis of reconstruction-longitudinal axis of forearm). The distal site of the preformed composite tissue was wrapped around a tube to hold the cartilage grafts in a convex shape during healing. D=distal site of composite tissue; P=proximal site of composite tissue.



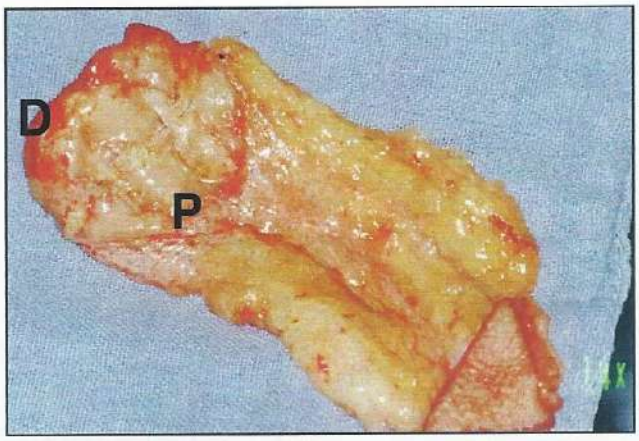
4.9.2. Preformed composite tissue-situation during waiting period.
The composite tissue flap is wrapped around a tube. The skin defect cannot be closed and the whole reconstruction is protected with a polytef (Gore-tex®) membrane.



4.9.3. Preformed composite tissue after 20 days.
The cartilage grafts are covered by oral mucosa at their margins. Note the convex shape of the composite tissue in the longitudinal axis of the forearm. The sutures anchoring the grafts to the fascia flap are removed except for the prolene sutures between the cartilage grafts.
D=distal site of composite tissue; P=proximal site of composite tissue.



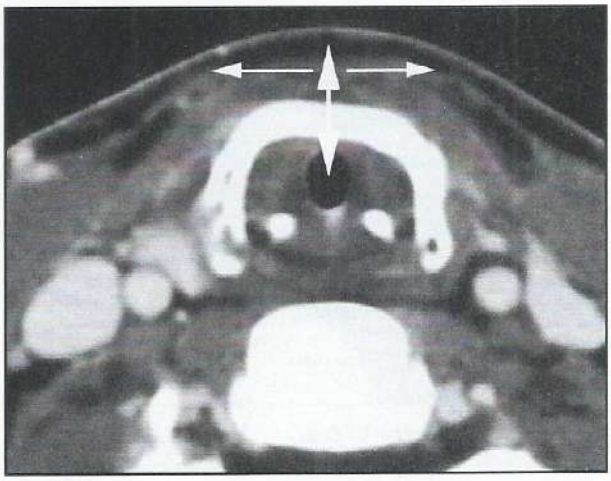
4.9.4Preformed composite tissue after 20 days at the time of flap dissection.
Incision of the forearm skin and outlining of the monitoring skin paddle (asterisk) is shown.
D=distal site of composite tissue; P=proximal site of composite tissue.



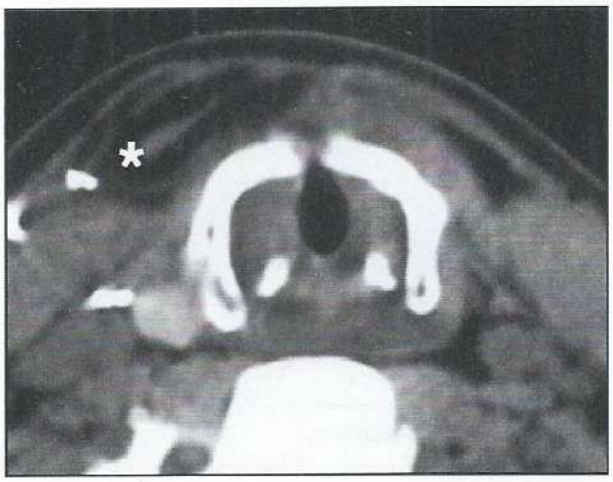
4.9.5. Preformed composite tissue after flap dissection.
The distal part of the flap contains the composite tissue. The proximal part of the fascial flap contains the monitoring skin paddle. The intervening tissue consists of vascularized fascia.
D=distal site of composite tissue; P=proximal site of composite tissue.

Fig. 4.10. Case report preformed composite tissue-Pre- and postoperative CT scan.

4.10.1.CT scan at glottic level.

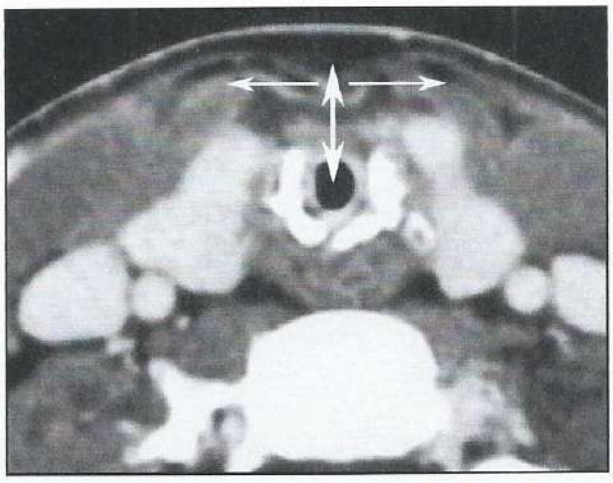


4.10.1a. Preoperative.
The thyroid cartilage is U-shaped with a short anterior- posterior length of the glottic airway lumen. The anterior commissure was incised (double arrow) and the anterior defect was slightly expanded (arrows).

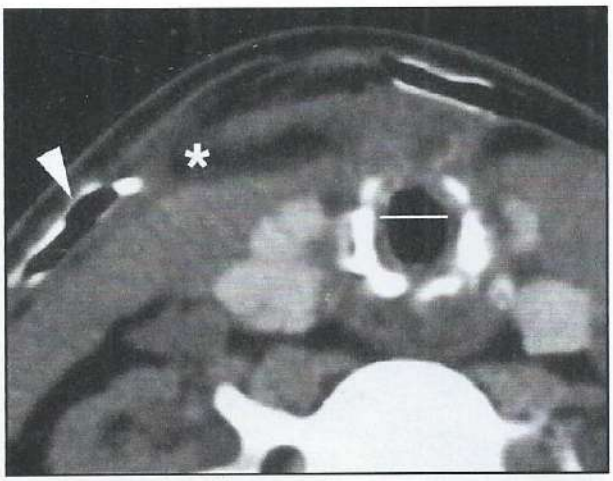


4.10.1b.Postoperative.
The upper end of the composite tissue is inserted in the anterior thyroid defect. This results in a lengthening of the anterior-posterior diameter. The fascia flap is indicated by an asterisk.

4.10.2.CT scan at cricoid level.

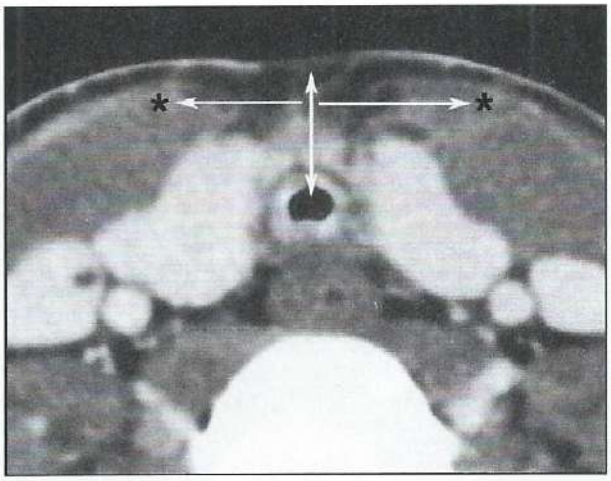


4.10.2a. Preoperative.
Small airway lumen. The airway is incised anteriorly (double arrow) and the anterior defect is expanded (arrows).

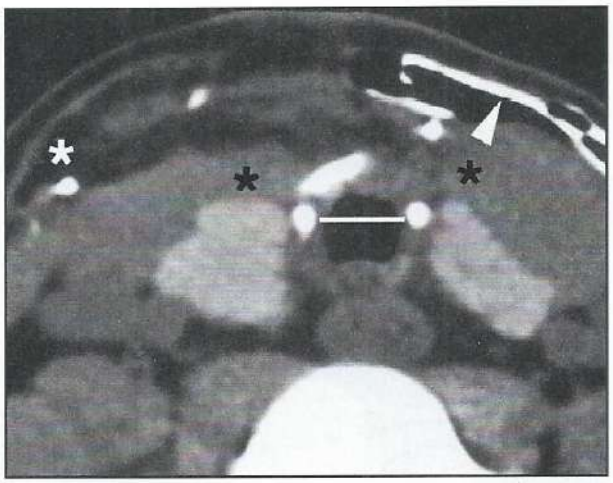


4.10.2b. Postoperative.
The patch is placed in the expanded anterior cricoid defect. Compared to a flat reconstruction between the defect margins (white line), the prefabricated composite reconstruction gives an additional anterior extension to the reconstructed airway lumen. The cartilage graft is partially ossified. The surface area of the airway lumen is augmented with a factor 3. Fascia flap is indicated with asterisk. Arrowhead points to postoperative drain.

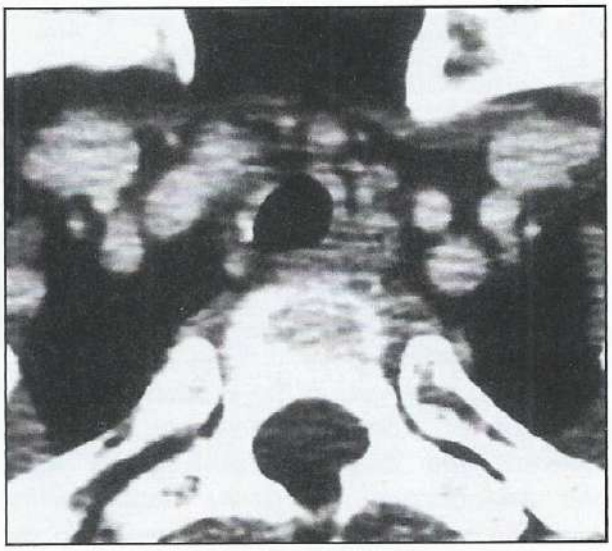
4.10.3. CT scan tracheal level.



4.10.3a. Preoperative.
Small airway lumen. The airway is incised anteriorly (double arrow) and the anterior defect is expanded (arrows). Some sutures (Prolene 4.0) were placed between the margins of the incised trachea and the strap muscles (asterisks) to hold the anterior defect in an expanded position.



4.10.3b. Postoperative.
The patch is placed in the expanded anterior tracheal defect. Note that the strap muscles are displaced to the margins of the anterior tracheal defect (black asterisks).
Compared to a flat reconstruction between the defect margins (white line), the prefabricated composite reconstruction gives an additional anterior extension to the airway lumen. The cartilage graft is partially ossified. The surface area of the airway lumen is expanded with a factor 3. White asterisk indicates fascia flap. Arrowhead points to postoperative drain.



4.10.3c. CT scan mediastinal trachea.
At the level of the upper mediastinum, the airway lumen has a normal calibre.

IV.6 General discussion

Cartilage grafts are frequently used in laryngotracheal reconstruction[5]. They are the optimal tissue to place between the divided halves of the cricoid cartilage to resolve a posterior laryngeal stenosis. Their use has become less popular in the anterior larynx and trachea because better results may be obtained with resection and end-to-end anastomosis[1,2]. A disadvantage when using free cartilage is that the revascularization and reepithelialization processes originate from the margins of the defect. The marginal revascularization and reepithelialization may be sufficient for small grafts in a well-vascularized wound bed but will be jeopardized in cases of scarring or when dealing with bigger strips of cartilage.

Mucosal lined fascia has the advantage of a primary healing and of a good intrinsic vascularity. This tissue may be used to resolve restenosis after segmental resection. It can be used in a 1-stage procedure with good survival of the full-thickness mucosal grafts. A prerequisite for mucosal survival is that the margins and the central portion of the grafts are carefully sutured to the vascularized fascia.

Composite tissue consisting of mucosal lining and strips of cartilage is the most adequate tissue that is currently available for airway wall reconstruction. This tissue can be used to repair critical airway segments. Flap prefabrication needs to be done to allow for survival of the cartilage component. Overgrowth of the bare cartilage by the mucosal lining –which is essential for cartilage survival– is a slow process. It took 1 month to obtain a complete coverage of the cartilage grafts (0.3 cm width) in the rabbit model. In the clinical case, relining of the cartilage grafts (1 cm width) was still incomplete after 20 days. Because of the slow remucosalization, the cartilage strips may undergo necrosis when they are immediately exposed to the airway lumen during a 1-stage procedure. After flap prefabrication, the cartilage grafts are sufficiently healed so that the cartilage support remains preserved.

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Summary

Segmental tracheal resection with primary anastomosis is not possible in cases of tracheal restenosis after previous tracheal resection and in cases of long segment stenosis. Currently, repair of segmental tracheal defects by tissue interposition is not possible. Tracheal allografts may be used experimentally but the need for immunosuppressive treatment prevents the use of allografts in patients. Restenosis and long segment tracheal stenosis need to be treated by anterior longitudinal incision of the stenosis and tissue repair of the anterior defect. Unfortunately there are no truly reliable repair tissues currently available in the clinical setting. Experiences from our experiments utilizing patches of revascularized tracheal allografts for anterior airway defects, we hypothesized that the reconstructive possibilities in the trachea can be improved, if a repair tissue with the combined characteristics of mucosal lining, cartilage support and vascularity would be made available.

The aims of this thesis are twofold. First, to study the healing of the different tissue components for tracheal repair using the rabbit as animal model and second, to improve the tissues which are currently used in tracheal reconstruction.

Chapter I gives an overview of the problem and the current repair possibilities. The most appropriate individual tissues that can provide for vascularisation, epithelial lining and support were defined in the rabbit model and in the clinical setting.

In Chapter II, the healing of full thickness mucosal defects using a new in vivo model was described. It was shown that granulation tissue formation, wound contraction and reepithelialisation characterize healing of full thickness mucosal airway defects. The slow reepithelialisation process is the main reason for overgrowth of granulation tissue and loss of airway lumen. Granulation tissue formation and wound contraction are inhibited after topical application of mitomycin. The main drawback of mitomycin application, however, is that the airway-exposed cartilage undergoes necrosis. If clinically available, tubes consisting of vascularized tracheal rings without mucosal lining would not lead to successful airway repair because excessive granulation tissue formation will lead to stenosis of the tube. A future direction in improved secondary healing of airway defects may lie in growth factors that promote reepithelialisation.

Chapter III addresses the question: can a segment of trachea be replaced by a tube of autologous cartilage? Morphometric and histologic analysis of rabbits' tracheal segments reconstructed with free and revascularized autologous cartilage, indicate that even if clinically available, tubes of autologous cartilage would not lead to successful airway repair. The problem with bare cartilage is that only a small area (near the anastomosis with the native trachea) becomes revascularized and remucosalized. The middle area always shows bare, airway exposed cartilage which will undergo necrosis. The limiting factor in the healing of autologous cartilage is the slow revascularization process. As such, a future direction in improved secondary healing of cartilage grafts may lie in growth factors that promote revascularization.

In chapter IV, after evaluating the different individual repair tissues and their combinations, it was shown that the mucosa lined fascia flap is the optimal tissue combination that can be used in a single stage procedure. Airway support can be improved when cartilage is added to the mucosal fascia flap. Prefabrication of the flap is then necessary to allow for survival of the cartilage component. With a prefabricated composite flap, it is possible to close an airway defect with limited anterior expansion of the reconstructed lumen. Segmental airway repair however is not possible with this composite tissue. A future direction in segmental tracheal repair may lie in inducing tolerance after tracheal allotransplantation so that immunosuppression is no longer necessary.

Samenvatting:

Resectie van tracheale segmenten met primaire anastomosering is niet mogelijk in geval van recidief stenose na voorafgaande trachearesectie en evenmin bij trachea stenosering over een lang traject. Allotransplantatie van de trachea is de enige mogelijkheid om circulaire tracheasegmenten te vervangen. Tracheale allotransplantatie kan experimenteel worden toegepast, maar de noodzaak tot immunosuppressieve behandeling van de receptor maakt klinisch gebruik ervan niet aangewezen. Restenosering van de trachea en stenosering over een lang traject moeten worden behandeld door middel van een longitudinale incisie van het deel en weefselherstel van het dusdanig gevormde voorste defect. Ook voor niet circulaire tracheadefecten zijn momenteel geen goede herstelweefsels voorhanden. Onze hypothese was dat autologe herstelweefsels aan de drie basisvereisten (slijmvliesbekleding, kraakbenige steun en bloedvoorziening) moeten voldoen om tot een optimaal tracheaherstel te komen.

Het doel van de studie was het belang van de drie weefselkarakteristieken individueel te onderzoeken bij wondheling en na herstel van de trachea.

In het eerste hoofdstuk worden de problemen die bestaan bij trachea reconstructie belicht.
De individuele weefsels die kunnen zorgen voor slijmvliesbekleding, kraakbenige steun en bloedvoorziening werden gedefinieerd zowel experimenteel (konijn), als klinisch.

In hoofdstuk 2 wordt de heling bestudeerd na aanbrengen van full-thickness slijmvliesdefecten. Hiervoor werd een nieuw in vivo wondhelingsmodel ontwikkeld. Het nieuwe model is gebaseerd op een uitwendig brengen van de slijmvliesbekleding van de trachea zonder te interfereren met de kraakbenige steun en de bloedvoorziening van de trachea. Heling van full-thickness slijmvliesdefecten werd gekenmerkt door granulatieweefselvorming, wondcontractie en een trage herbedekking met respiratoire epitheelcellen. Het traag verlopend epithelialisatieproces is de belangrijkste oorzaak voor de overmatige vorming van granulatieweefsel. Full-thickness slijmvliesdefecten geven aanleiding tot vernauwen en eventueel stenoseren van de luchtweg. De vorming van granulatieweefsel wordt tegengewerkt na lokale applicatie van Mitomycine op het wondbed. Mitomycine heeft namelijk een inhiberende werking op het proces van angiogenese. Het belangrijkste nadeel van mitomycine gebruik in dit model was het afsterven van het onbedekt blijvend kraakbeen. Een goed bevloeide luchtpijp zonder slijmvliesbekleding zou -indien beschikbaar- niet leiden tot succesvol tracheaherstel. Verder onderzoek kan zich toespitsen op groeifactoren die het re-epithelialisatieproces bevorderen.

In het derde hoofdstuk werd nagekeken hoe een buis bestaande uit autoloog kraakbeen heelt wanneer gebruikt als herstelweefsel voor een luchtpijpsegment. De proefdieren vertoonden dyspnœ gemiddeld 22 dagen na herstel van de trachea met vrije en gerevascularizeerde kraakbeentransplantaten.
Morfometrische en histologische analyses toonden een herbedekking van het kraakbeentransplantaat ter hoogte van beide anastomosen met intact slijmvlies over maximaal 3 mm. Het middendeel van het kraakbeentransplantaat vertoonde necrose.Verder onderzoek in verband met kraakbeenheling kan zich toespitsen op groeifactoren die de revascularisatie bevorderen.

De met mondslijmvlies bedekte fascia lap kombineert de weefselkenmerken slijmvlies en bloedvoorziening en is momenteel een van de meest optimale reconstructies die in de kliniek kan toegepast worden voor voorste tracheadefecten. De hersteltechniek toont een primaire heling en kan in één operatietijt doorgevoerd worden. Dit herstelweefsel kan nog worden geoptimalizeerd door toevoegen van elastisch kraakbeen. Om de vitaliteit van de kraakbenige komponent binnen dit samengestelde weefsel te verzekeren is prefabricatie van het weefsel vereist.

Het samengesteld weefsel wordt in het voorste luchtwegdefect geplaatst verschillende weken nadat de weefselkomponenten zijn samengebracht en nadat de kraakbeenkomponent bedekt is met slijmvlies. De geprefabriceerde weefselcompositie kan een luchtwegdefect herstellen met uitbouw van het luchtweglumen vooraan. Dit weefsel kan echter geen circulair tracheasegment herstellen. Circulair tracheaherstel is alleen mogelijk met allotransplanten. Verder onderzoek kan gebeuren met het doel immunologische tolerantie te bewerkstellingen bij toepassing van allotransplanten.

Curriculum vitae

The author of this thesis was born on the 24th of February, 1966 in Canlubang, Laguna, Philippines. There he attended primary school (Santa Cecilia Catholic School) and secondary school (Rizal Institute, Don Bosco) graduating as class salutatorian in 1982. He then studied at the University of the Philippines at Los Banos where he obtained his Bachelor of Science in Biology/Cell Biology (cum laude) in 1986. Driven by his childhood dream to wear a doctor's white coat, he went further to read medicine at the University of the Philippines, Manila. After his medical internship at the Philippine General Hospital in 1991, he took and passed the Philippine Medical Licensure exams. Shortly thereafter, he devoted his time to the upkeep of these three noble organs; the ear, the nose and the throat as a resident in training at the department of otorhinolaryngology head and neck surgery of the University of the Philippines-Philippine General Hospital. In 1995, he was appointed as chief resident of the department.
The following year, he received his board certification as an Ear, Nose, Throat specialist after passing the diplomate examinations of the Philippine Board of Otolaryngology Head and Neck Surgery. In the summer of 1997, he flew to Amsterdam as a clinical fellow in head and neck oncological surgery at the Academisch Ziekenhuis Vrije Universiteit under Prof. Dr. Gordon B. Snow. Lured by the possibility of getting a PhD and working in the field of laryngotracheal reconstruction, he crossed the border and landed in Leuven, Belgium. There he stayed at the department of otolaryngology-hns, Katholieke Universiteit Leuven as a fellow in laryngotracheal reconstruction and transplantation under Prof. Dr. Pierre Delaere. While in Leuven, he also finished his Master of Science thesis entitled, Tracheal patch autotransplantation:is a two-stage procedure really necessary? Late last year he started as a fellow in head and neck surgery at the Academisch Ziekenhuis Rotterdam (Dijkzigt).

List of Publications

Hardillo JAU, Vanclooster C, Delaere P. An investigation of airway wound healing using a novel in vivo model. (submitted)

Hardillo JAU, Delaere P. Tubes of autologous cartilage used as a segmental tracheal replacement: How do they heal? (submitted)

Delaere P, Hardillo JAU, Vandenhof B, Hermans R. Prefabrication of composite tissue: The way towards improved tracheal reconstruction. (accepted for publication, Annals of Otolaryngology)

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