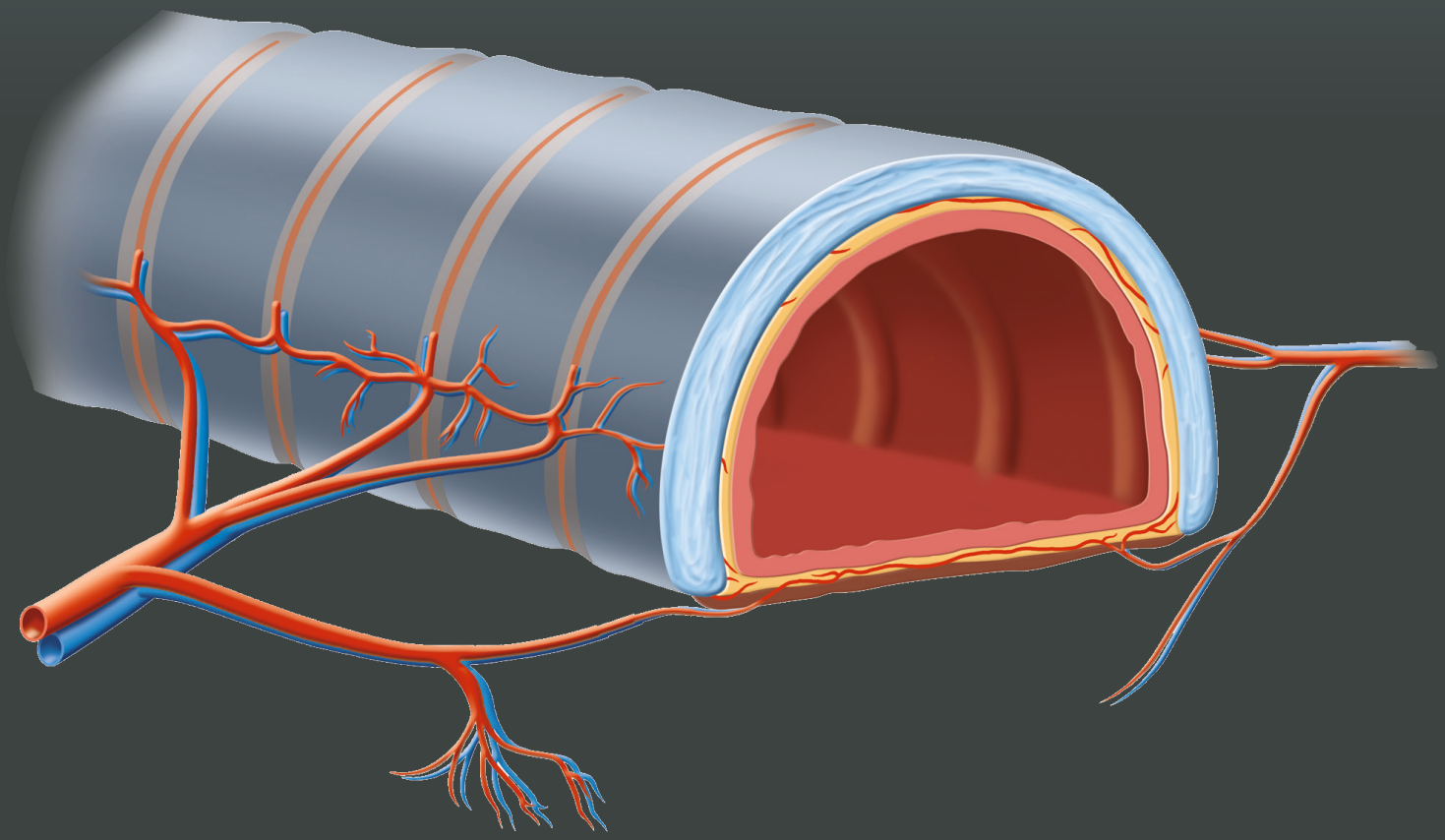
Tracheal Transplantation

Current possibilities

Pierre Delaere



Leuven University Press

TRACHEAL TRANSPLANTATION

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Cover illustration: Morphology of the trachea. The morphological structure of the trachea with cartilage rings, the respiratory ciliated epithelium, and the very rich blood supply that runs in between the cartilage rings in order to supply the mucosa. The posterior part of the trachea is the membranous trachea, where the cartilage tissue is replaced by smooth muscle fibers.

Contents

Preface

This ebook presents insights and advances that have been realized over the past 30 years from both experimental and clinical laryngotracheal airway research.

Our own research, conducted between 1990 and 2001, has focused on experimental revascularization and transplantation of tracheas in rabbit models. These studies have produced two important clinical applications: tracheal autotransplantation for reconstruction of extended hemilaryngectomy defects after tumor removal, and patch tracheal allotransplantation to resolve long-segment stenoses and restenoses after segmental resections.

The main lesson we gained over this extended period of research is that the morphology of the laryngotracheal airway is extremely complex. At first sight, the trachea may appear to be a simple tube for air transport. However, the cartilaginous framework of this airway, combined with its ultrathin mucosal lining and rich, but difficult to handle, blood supply, makes it to one of the most challenging tuberous organs to repair and transplant.

This ebook is intended to highlight both the intricacies of the laryngotracheal airway and the reconstructive approaches that can potentially restore airway function, particularly in relation to laryngotracheal stenoses and defects.

1. Anatomy and wound healing of the cricotracheal airway

1.1. Anatomy of the cricotracheal airway

This chapter highlights relevant anatomical features specific to surgical procedures for the cricotracheal airway.

At the laryngeal level, the subglottis is the narrowest part of the airway, and most stenoses occur at this level. The cricoid cartilage provides the only circumferential framework for the airway (Fig. 1.1), and therefore, it maintains the competency of the airway. Accordingly, if surgical resection or trauma compromises the circumferential support that this cartilage provides, an airway can be adversely affected. Hence, the cricoid cartilage is a crucial structure in any ablative or reconstructive procedure.

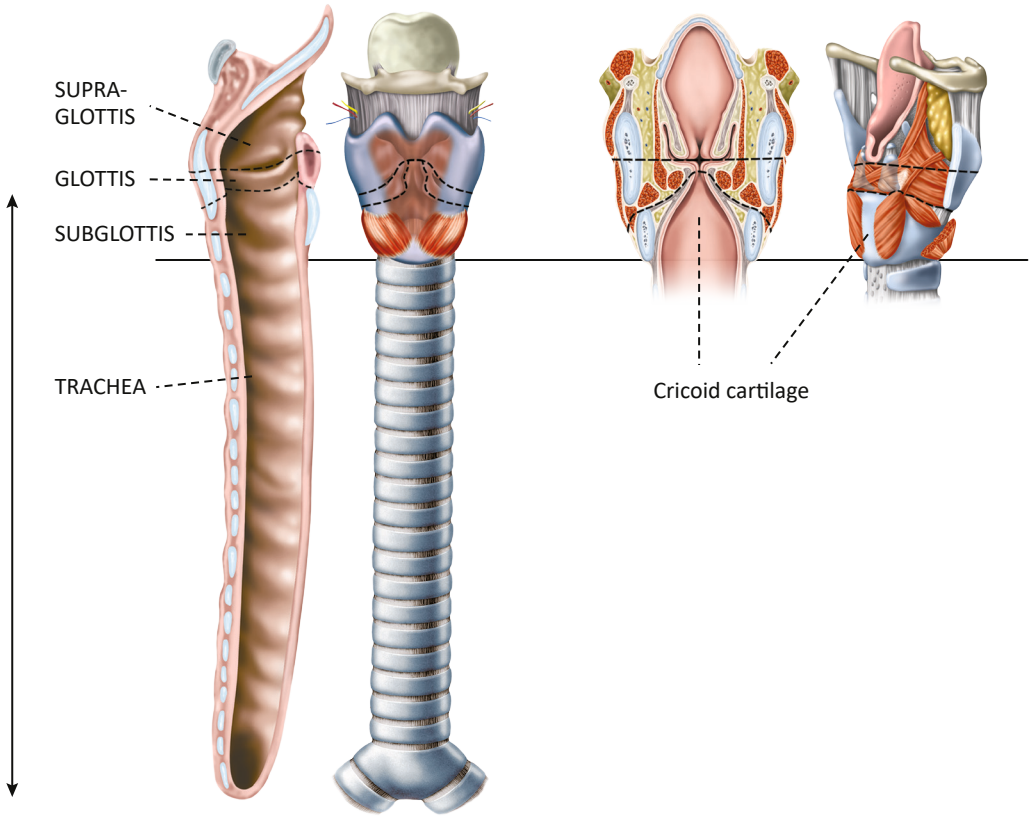

Figure 1.1. Topographical anatomy of the trachea.
The cricotracheal airway (indicated with the extended double arrow line at the far left) is the most vulnerable region for stenosis.

The average length of the adult human trachea is 11.8 cm (range, 10–13 cm) from the infracricoid level to the top of the carinal spur. Typically, there are 18 to 22 cartilaginous rings within this length, with approximately two rings per centimeter. In an adult male, the internal diameter of the trachea measures approximately 2.3 cm laterally and 1.8 cm anteroposteriorly. These measurements can vary in proportion to the size of an individual, and they are smaller in women. In addition, the rings of the adult trachea are normally C-shaped, with the posterior membranous wall connecting the arms of the

"C" in an essentially straight line. This line usually measures less than one-third the circumference of the trachea. The proportion of cross-sectional cartilaginous length to the length of the membranous wall also does not change from infancy through developmental growth [1].

A surgeon usually visualizes the trachea in the thyroidectomy position, with the neck extended. The structure of the trachea is one-half cervical and one-half thoracic. However, when the neck is flexed, the trachea becomes almost entirely mediastinal because the cricoid cartilage drops to the level of the thoracic inlet. In elderly individuals, the latter position can become more permanent secondary to cervical kyphosis. When viewed laterally in an upright individual, the trachea courses backward and downward at an angle from a nearly subcutaneous position at the infracricoid level to rest against the esophagus and vertebral column at the carina. Anteriorly, the thyroid isthmus passes over the trachea near the second tracheal ring. The lateral lobes of the thyroid are closely applied to the trachea, and a common blood supply is obtained from the branches of the inferior thyroid artery. In the groove that exists between the trachea and the esophagus there are recurrent laryngeal nerves which extend from beneath the arch of the aorta on the left side. As a result, these laryngeal nerves have a longer distance in proximity to the trachea on the left side than on the right where the nerves are able to loop around the subclavian artery and then approach the groove. These nerves enter the larynx between the cricoid and thyroid cartilages, just anterior to the inferior cornu of the thyroid cartilage (Fig. 1.2).

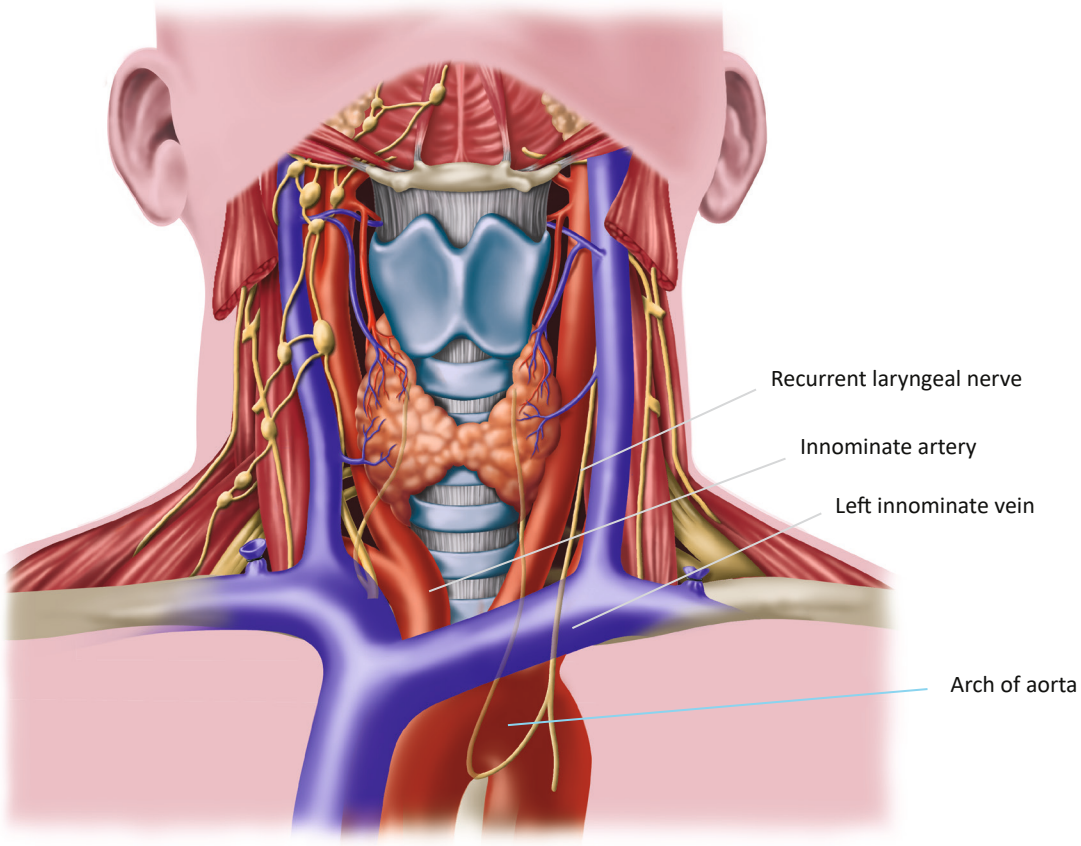

Figure 1.2. Topographical anatomy of the trachea - frontal view.

The anterior pretracheal plane can be easily accessed via a cervical approach. The innominate vein lies anteriorly, away from the trachea. The innominate artery, however, crosses over the midtrachea obliquely from its point of origin from the aortic arch to the right side of the neck. In children, the innominate artery is higher and is encountered in the lower part of the neck. In some adults, the artery is unusually high and it crosses the trachea at the base of the neck when slight cervical extension is present. At the level of the carina, the left main bronchus passes beneath the aortic arch, while the right main bronchus lies beneath the azygos vein. The pulmonary artery lies just in front of the carina. On either side of the trachea is fibrofatty tissue which contains lymph node chains. A large packet of nodes also lies just beneath the carina. The course of the trachea from an anterior cervical position to a posterior mediastinal position brings it into proximity of major vascular structures. As a result, it is difficult to access the entire trachea via a single incision (Fig. 1.3). Accordingly, Grillo [2] has emphasized that when surgical procedures are being planned for tracheal lesions, these anatomic relationships must be precisely defined in relation to the extent and nature of the lesions.

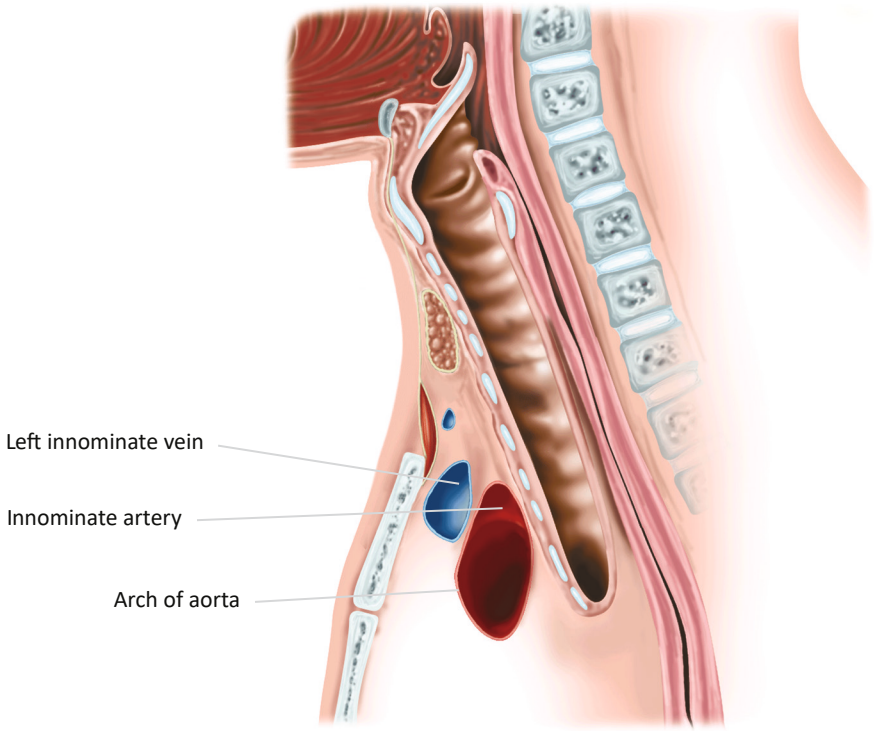

Figure 1.3. Topographical anatomy of the trachea - sagittal view.
The average length of the adult trachea is 10–13 cm from the lower border of the cricoid cartilage to the tip of the carinal spur.

Cartilaginous rings provide lateral rigidity to the human trachea. They extend over approximately two-thirds the circumference of the trachea and the posterior wall is membranous. The trachea is lined with respiratory mucosa which tightly adheres to the inner surface of the cartilage. The epithelium of the trachea is columnar and ciliated. Cilia on the tracheal epithelium sweep particles trapped by mucus upward in a synchronized wave-like fashion. In habitual smokers and those affected by chronic throat irritations, the cilia may be damaged or destroyed. As a result, these individuals may have an increased risk of squamous metaplasia. If metaplasia does develop, secretions can be cleared successfully with coughing. This observation, in combination with the feasibility of cutaneous reconstructions and intraluminal stents, makes it clear that ciliated epithelium, although highly desirable, is not essential for tracheal reconstructions.

During coughing and spasms, considerable contraction of the muscular membranous wall can occur, with the tips of the cartilage drawn inward. With age, calcification of tracheal rings is generally observed. Local trauma or operation can also lead to calcification of cartilaginous rings. Meanwhile, under normal conditions, the trachea slides easily within a layer of fibrofatty areolar tissue from the neck to the mediastinum.

The trachea is able to function as a conduit for ventilation, it can clear secretions, and it warms, humidifies, and cleans air for the respiratory zone. Coughing and intrinsic defense mechanisms also keep the airway free of foreign material.

Regarding the blood supply for the human trachea, it is segmental and largely shared with the esophagus. The blood supply is principally derived from multiple branches of the inferior thyroid artery above, and from bronchial arteries below. Moreover, while the arteries approach the trachea laterally, there are fine branches that pass anteriorly and posteriorly to the trachea (Fig. 1.4). Miura and Grillo [3] demonstrated that the inferior thyroid artery nourishes the upper trachea, usually through a pattern of three principal branches with fine subdivisions and extremely fine collateral vessels. However, many variations have been observed, as noted by Salassa and colleagues [4]. In general, bronchial vessels nourish the lower trachea, carina, and mainstem bronchi. Sometimes the internal mammary artery contributes. For surgery, excessive circumferential dissection with division of the lateral pedicles can easily devascularize the trachea.

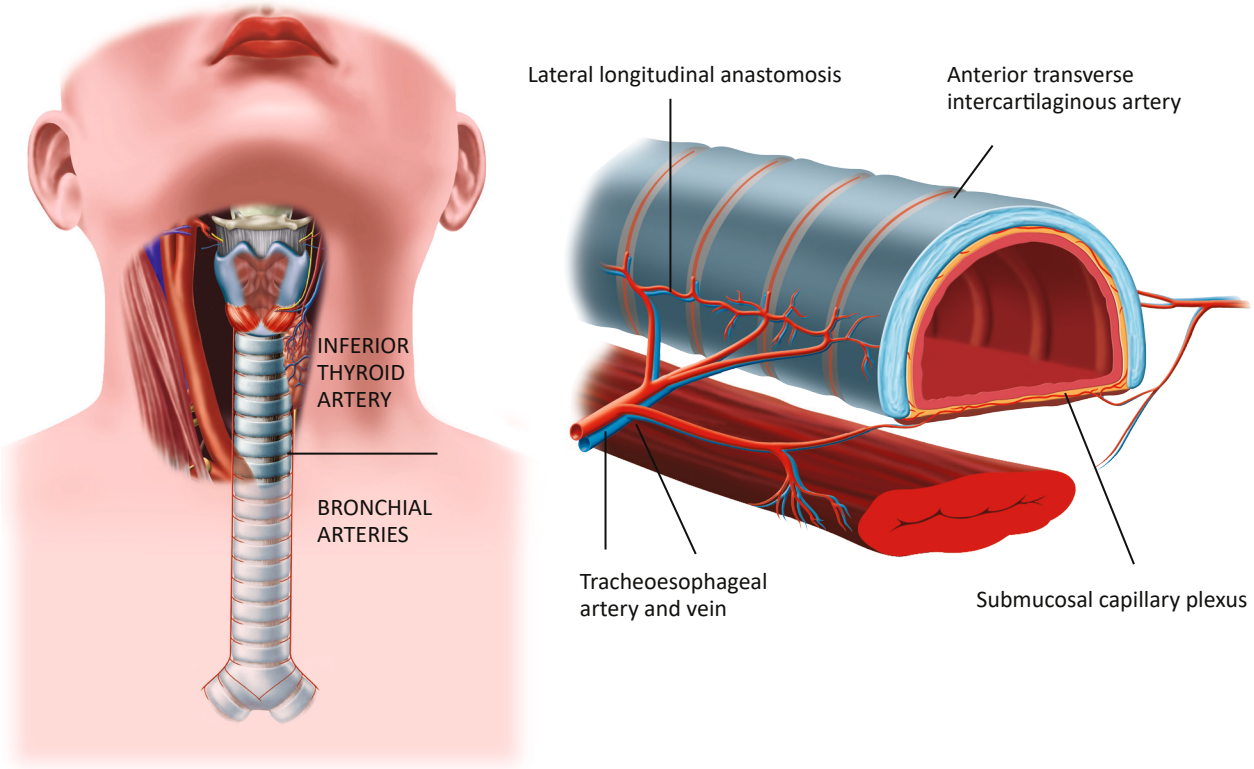

Figure 1.4. Schematic view of the available blood supply for the trachea.
The rich vascular network beneath the endotracheal mucosa originates from transverse intercartilaginous arteries that derive from a lateral longitudinal anastomosis.

1.2. **Wound healing of the cricotracheal airway**

Healing that occurs after disruption of the mucosal lining of the cricotracheal airway represents a basic biological process and it is needed to protect against bacterial invasion. The relative contribution of tissue regeneration versus scarring during healing of the mucosal lining depends on the extent of injury that has been inflicted. For a superficial epithelial wound, healing involves regeneration of the surface epithelium (Fig. 1.5) [5]. Indeed, tissues with a high proliferative capacity, such as airway tract epithelia, renew themselves continuously. Following an injury, airway tract epithelia can regenerate above the basal membrane as long as the stem cell population in these tissues is maintained. The repair and regeneration process involved is mediated via several mechanisms, including basal cell spreading and migration, followed by proliferation and differentiation of epithelial cells.

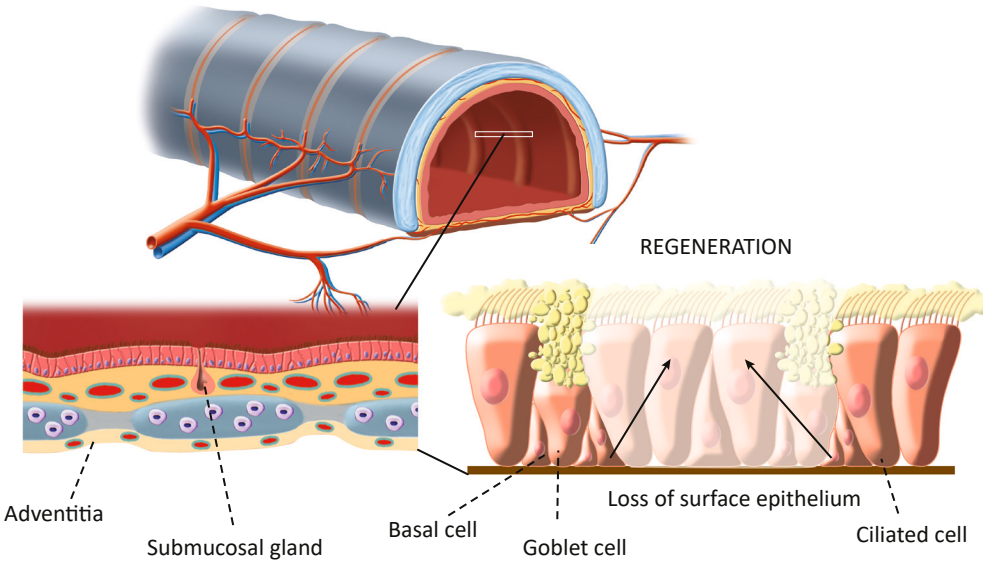

Figure 1.5. Regeneration of respiratory epithelium. The basement membrane of the mucosal layer supports a pseudostratified epithelium. The surface layer is columnar and ciliated, while deeper layers include oval or rounded basal cells. Goblet cells are interspersed in the epithelium and they secrete mucus. This mucus lubricates the tracheal surface and traps foreign particulate matter such as dust and bacteria. Goblet cells constitute approximately 20–30% of the cells in the more proximal trachea, and then they decrease in number distally. The submucosal layer is composed of a loose mesh of connective tissue that contains large blood vessels, nerves, and mucous glands. The ducts of the glands pierce the overlying layers and open at the surface. The outermost tunic, the adventitia, is composed of loose connective tissue that contains small blood vessels that supply the trachea.

A superficial epithelial wound is healed with regeneration of the surface epithelium. Tissues with a high proliferative capacity renew themselves continuously and can regenerate after injury above the basal membrane with proliferation and differentiation of basal cells.

When a tissue injury is severe and both epithelial cells and the submucosal layer are damaged, healing cannot be accomplished by regeneration alone. The healing process for these injuries requires a deposition of collagen to allow a scar to form. The healing pattern of a circumferential full-thickness mucosal defect is illustrated in Figure 1.6.

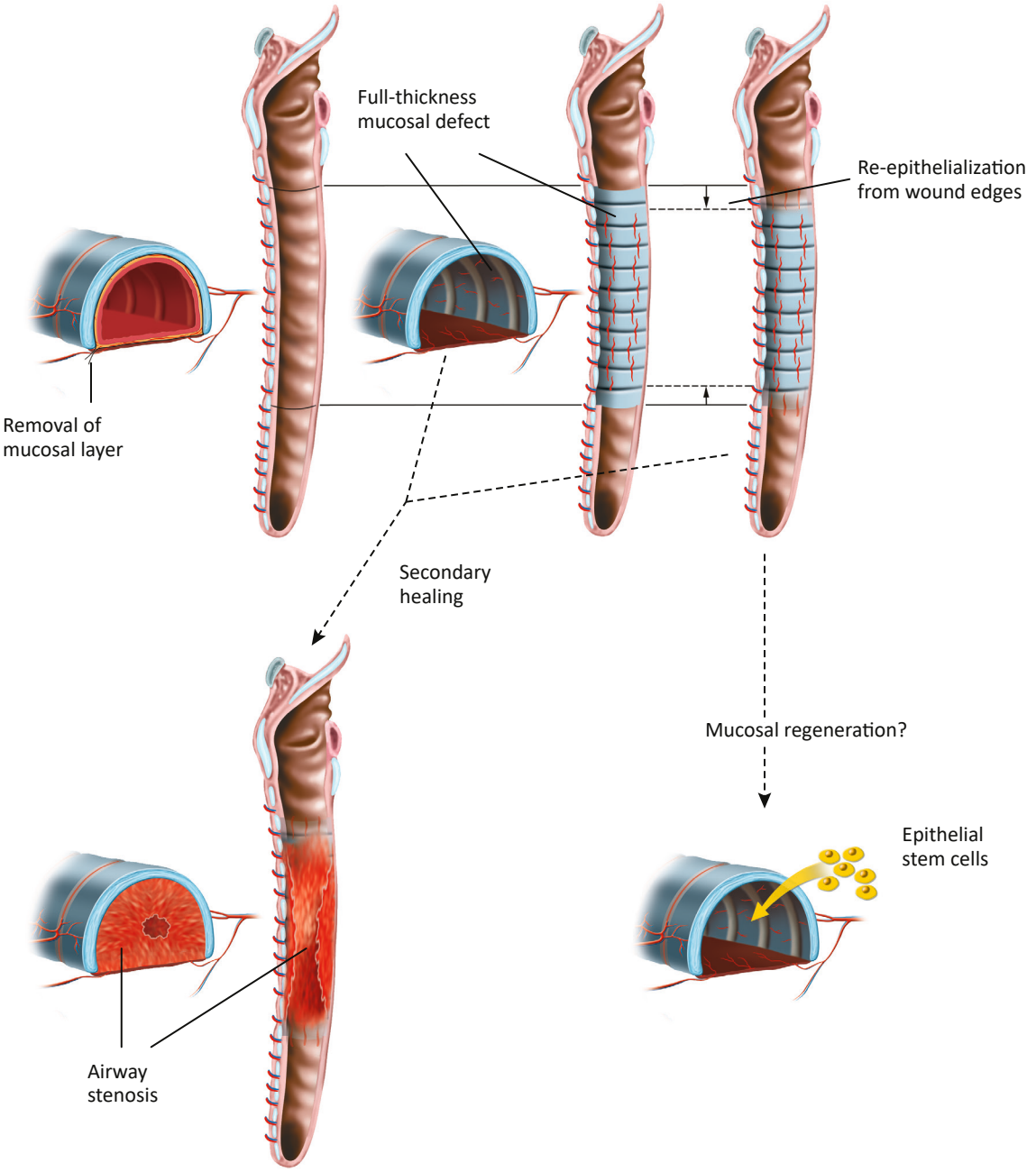

Figure 1.6. Wound healing of a circumferential full-thickness tracheal mucosal defect. Healing of full-thickness mucosal wounds involves the formation of granulation tissue, wound contraction, and re-epithelialization from the wound edges (indicated with short black arrows). Airway stenosis can develop from circumferential wounds in the middle of a denuded segment. Healing of anastomotic sites can involve the growth of several millimeters of respiratory epithelium into the wound margins. The remaining tissue between those margins that remains uncovered will eventually be covered by granulation tissue. To date, it is not possible to induce regeneration of the mucosal lining of a trachea segment by applying epithelial stem cells (colored yellow).

An important factor in the etiology of laryngotracheal stenosis is the extent of damage to the mucosal lining.

The cuff pressure of an endotracheal tube should be adequate, yet not impair mucosal blood flow [6]. It has been shown that continuous lateral wall cuff pressures > 30 cm H_2O compromise blood flow, while cuff pressures > 50 cm H_2O completely obstruct tracheal wall blood flow [7]. However, compliance of the tracheal wall is also a factor. During the early phase of post-intubation airway stenosis, mucosal ulceration and perichondritis develop, and these conditions are followed by formation of exophytic granulation tissue. As healing progresses, granulation tissue is gradually replaced with mature fibrotic tissue and the wound contracts, thereby giving rise to a classical mature airway scar (Fig. 1.7).

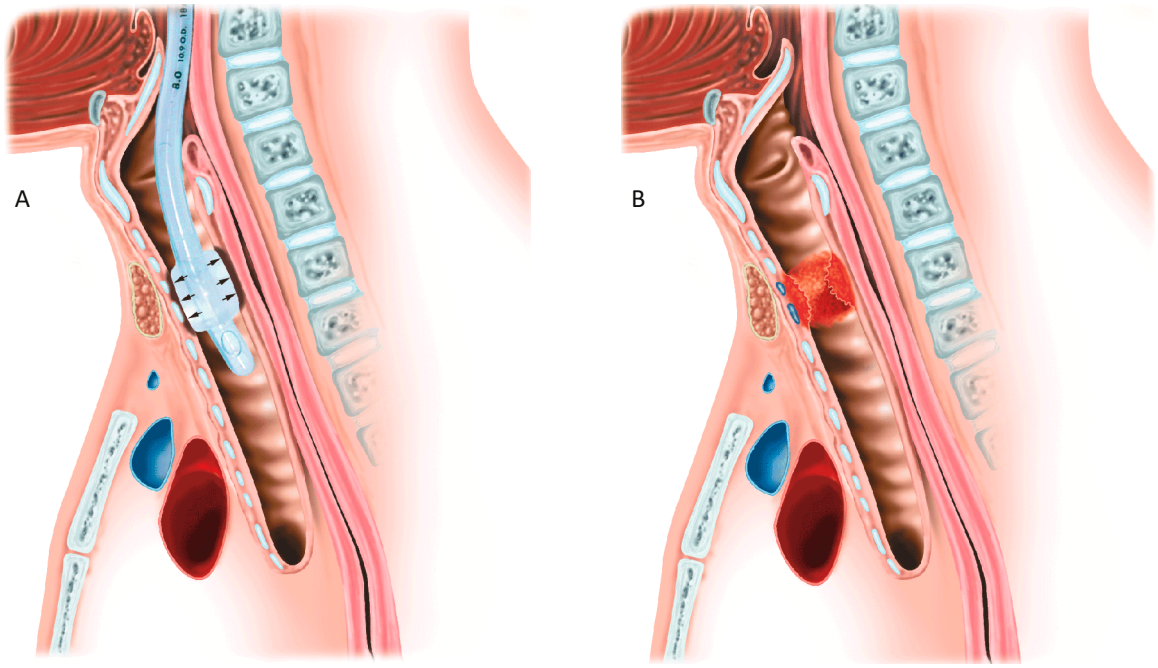

Figure 1.7. Wound healing of a mucosal defect due to pressure necrosis induced by an overinflated cuff.
A. Cuff pressures greater than 30 cm H_2O impede mucosal capillary blood flow and this can lead to mucosal ischemia. B. During cuffed intubation, when the pressure on the tracheal mucosa is greater than the mean capillary perfusion pressure of the mucosa, mucosal necrosis with secondary healing can develop.

The evolution of mucosal damage towards stenosis is not well understood. An important question regarding the healing process of mucosal airway defects is to what extent a wound will contract, and to what extent a wound will re-epithelialize.

One way to avoid wound contraction is to localize wounds in an area of skin that is adherent to underlying structures. For example, the rabbit ear model exhibits minimal wound contraction because the dermis on the inner surface of the rabbit ear is tightly adherent to the underlying cartilage. Correspondingly, full-thickness wounds in this area have exhibited greater than 90% healing as a result of granulation tissue production and epithelial cell migration from the periphery of the wound.

Moreover, this model has consistently produced scars that are histologically similar to hypertrophic scars in humans. When there is a delay in wound re-epithelialization as a result of limited wound contraction, excessive production of granulation tissue occurs. This is subsequently followed by development of a hypertrophic scar after re-epithelialization is complete [8]. A similar wound healing mechanism has been observed inside the trachea since the mucosa is tightly adherent to underlying cartilage rings.

Future therapies for full-thickness mucosal tracheal defects should aim to promote regeneration and reduce scar tissue formation. Accordingly, studies to examine the potential for stem cells to induce regenerative healing represent an active area of research. In addition, barriers to regeneration of the mucosal and epithelial linings of full-thickness epithelial defects remain an ongoing challenge, with the regeneration of full-thickness mucosal defects currently considered impossible.

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2. Tracheal reconstruction and its limitations

Non-malignant and malignant obstructions of the tracheal airway can cause significant morbidity and mortality. A common cause of tracheal stenosis includes damage from endotracheal and tracheostomy tubes. With increased use of artificial airways, the incidence of iatrogenic complications are increasing. While minor tracheal lesions may be treated endoscopically, more extensive lesions usually require more invasive procedures to maintain the airway lumen. A tracheal stenosis that is less than 5 cm in length can be resected with end-to-end anastomosis.

2.1. Tracheal resection

For tracheal stenosis, resection of the trachea and primary anastomosis can be performed, and this is an accepted and preferred procedure. Moreover, excellent results have been reported for this procedure in many large series [1, 2]. The length of trachea that can be safely resected widely varies according to patient age, posture, body habitus, extent of disease, and history of prior tracheal surgery. However, resections of less than 4 cm of trachea are almost always tolerated, unless incomplete mobilization or fixed cervical kyphosis induces anastomotic tension. Furthermore, while there is no universally applicable limit for the amount of trachea that can be resected, resections of more than half the tracheal length have rarely succeeded. In addition, the airway must be under full control at all times during reconstructive surgery of the trachea to prevent hypoxia (Fig. 2.1).

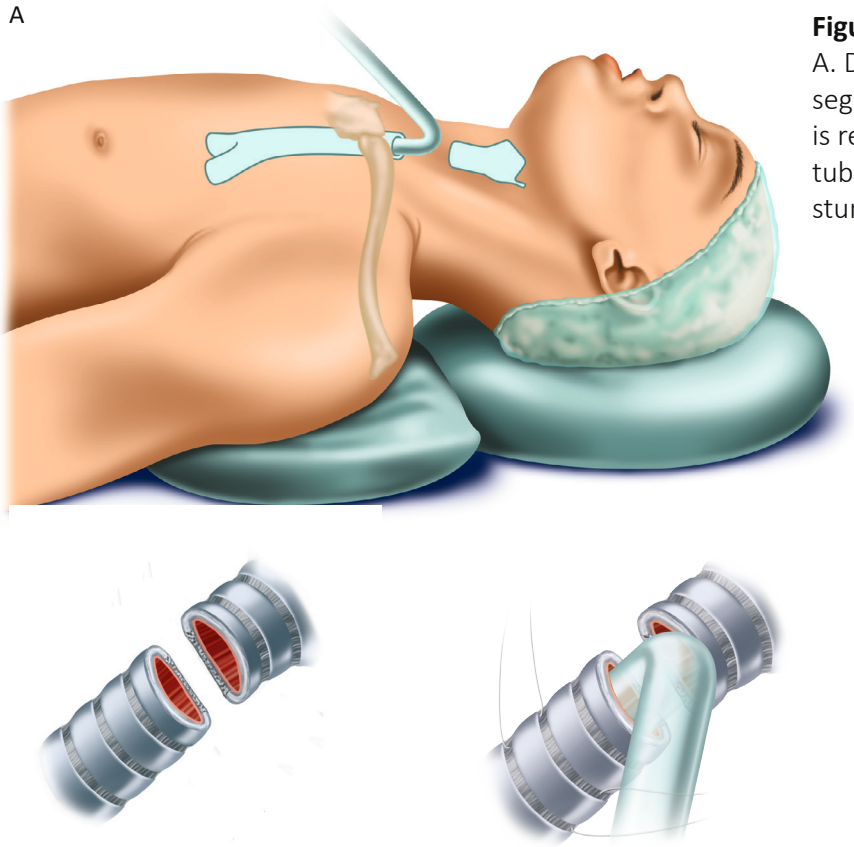

Figure 2.1. Tracheal resection. A. During resection of an involved segment, the orotracheal tube is replaced with an endotracheal tube through the distal tracheal stump.

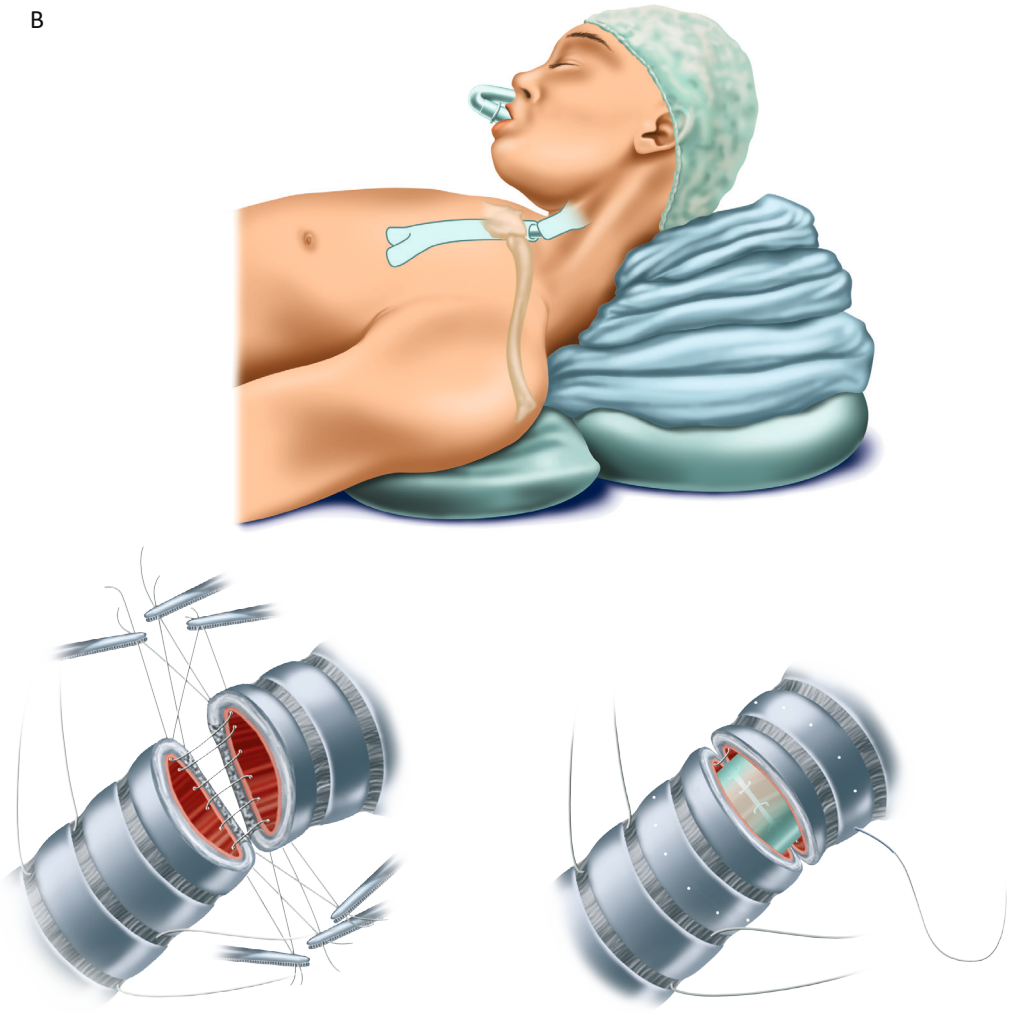

Figure 2.1. Tracheal resection (continued).
B. For a posterior anastomosis, inverted interrupted 3-0 vicryl stitches are initially used, with knots tied on the outside. Lateral and anterior anastomoses are subsequently performed with interrupted 3-0 vicryl stitches. Upon completion of the operation, the original endotracheal tube is advanced into the distal airway and the anastomotic sutures are tied. Cervical flexion decreases tension on the suture line approximating the ends of the tracheal anastomoses.

Surgical treatment for tracheal stenosis is generally a reliable and successful procedure when performed by an experienced surgeon. Grillo et al. have reported the largest series to date, and a low rate of anastomotic complications (9%) and a 95% success rate were described [3]. Other experienced groups have reported a similar success rate with mortality rates less than 3% [2, 4]. These series have also identified a few risk factors for complications, and these include: a resection length > 4 cm, diabetes, previous radiotherapy, and an associated laryngotracheal reconstruction. In addition, re-stenosis is a challenging complication and it can occur when an anastomosis is performed under too much tension.

2.2. **Cricotracheal resection**

When benign stenosis of the upper trachea also involves the subglottic larynx, one-stage reconstruction is possible [5]. Cricotracheal resection with anastomosis of the trachea to the thyroid cartilage is the treatment of choice for (idiopathic) subglottic stenosis with normal vocal fold mobility. Briefly, for this reconstruction, the anterior subglottic larynx is resected, and the posterior cricoid lamina is bared, yet preserved, in order to protect the recurrent laryngeal nerves (Fig. 2.2). Next, the distal tailored trachea is advanced to replace the anterior subglottic laryngeal wall and to resurface the posterior cricoid plate with the membranous tracheal wall. Overall, this procedure has had favorable outcomes [6, 7]. Moreover, if the repair is precise, stenting of the anastomosis is not necessary. However, stenting is necessary when a cartilage graft is positioned between the divided halves of the cricoid plate in combination with posterior glottic stenosis (Fig. 2.3).

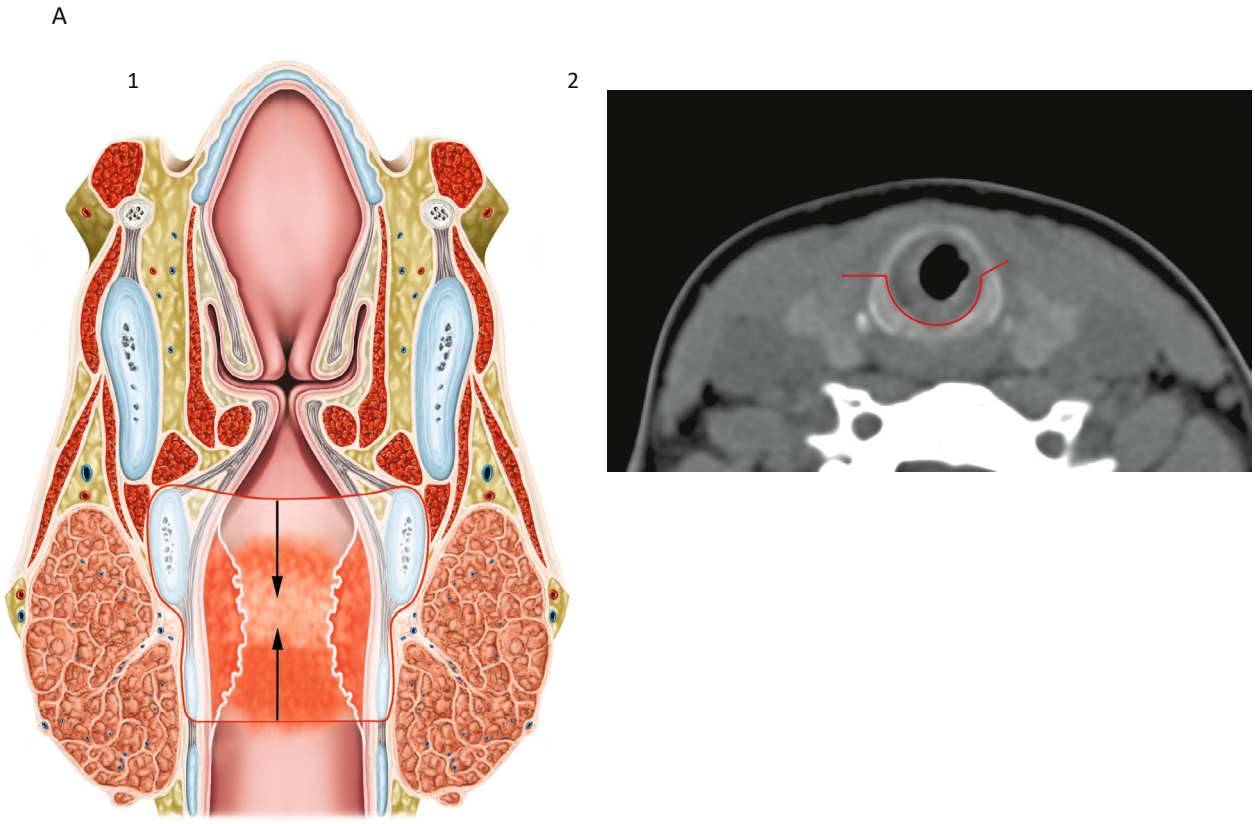

Figure 2.2. Cricotracheal resection.
A. Coronal sectional view (1) and axial CT scan (2) of a cricotracheal resection where the extent of resection for idiopathic subglottic stenosis is indicated (red lines). The arrows indicate the direction of the anastomosis performed between the trachea and the remaining cricoid and thyroid cartilages.

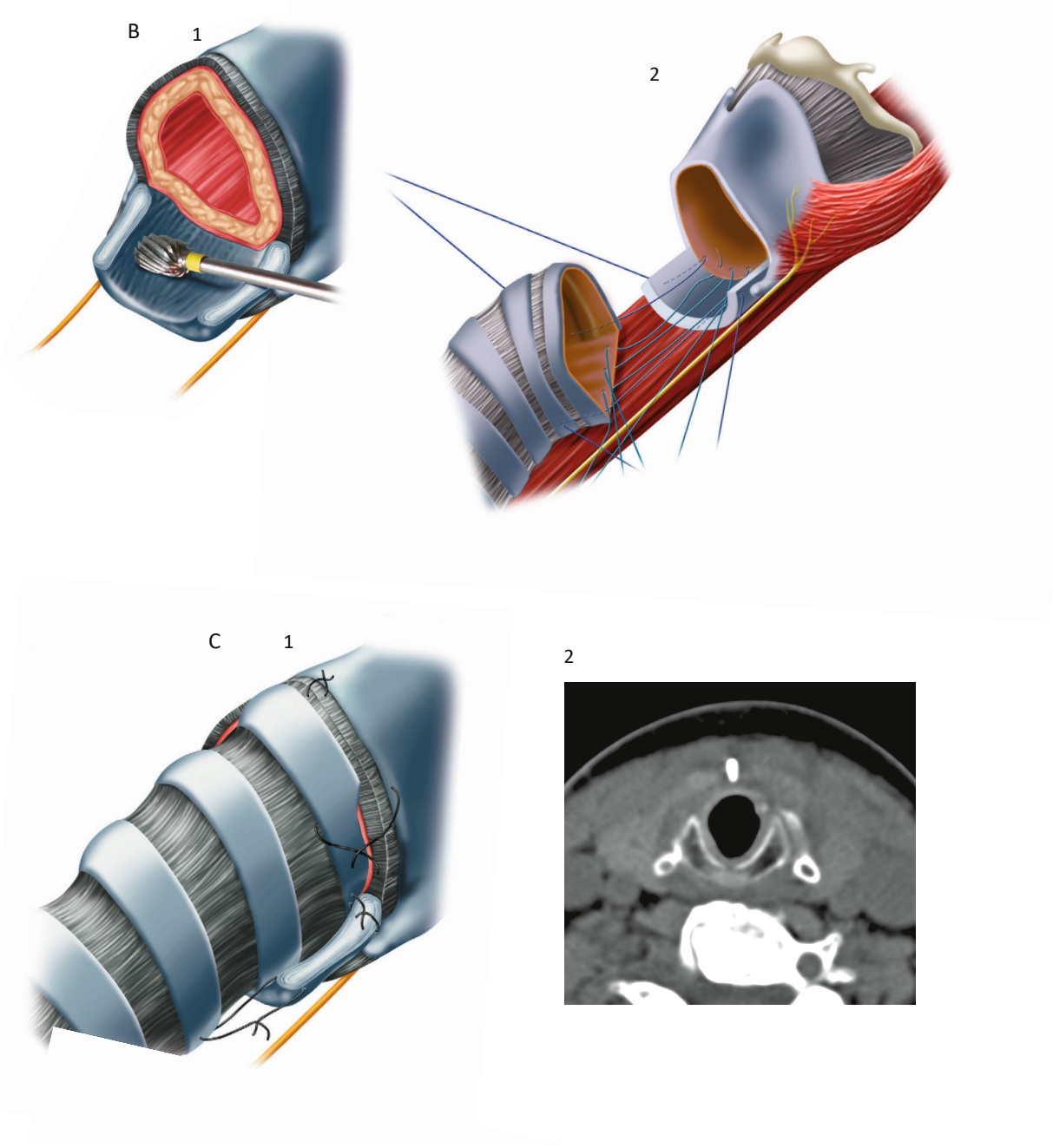

Figure 2.2. Cricotracheal resection (continued).
B. A lateroanterior view of a cricotracheal anastomosis. Widening of the cricoid plate was achieved with a diamond burr (1) and suture placement is shown for the posterior cricotracheal anastomosis (2).
C. A lateroanterior view of a cricotracheal (lateral and posterior) and thyrotracheal (anterior) anastomosis (1). An axial CT scan is shown of the completed anastomosis (2).

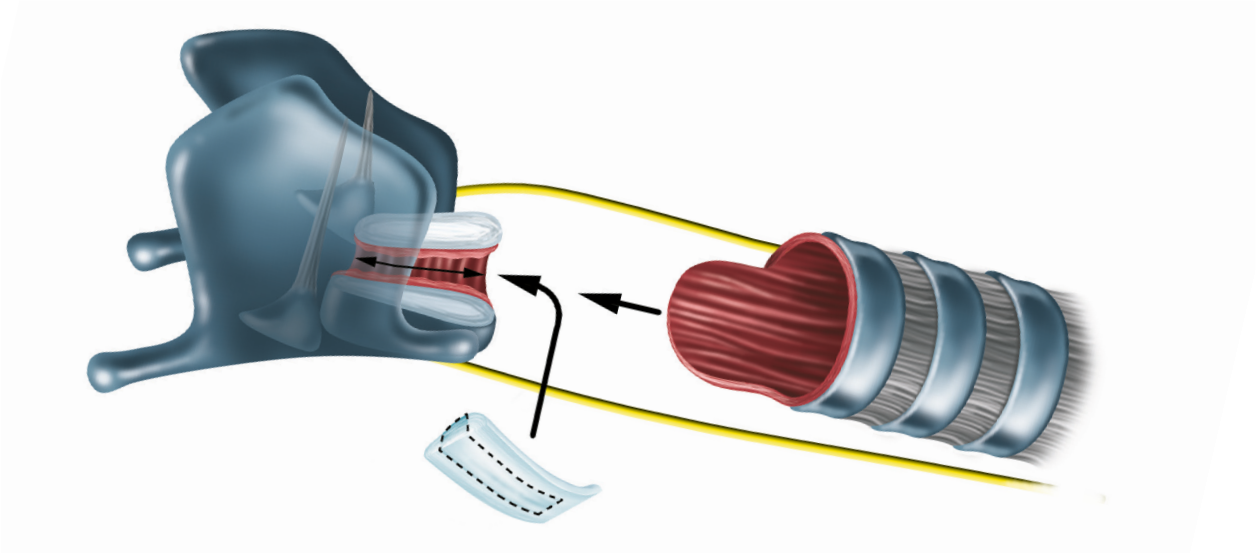

Figure 2.3. Cricotracheal resection with a posterior costal cartilage graft applied to a subglottic and posterior glottis stenosis.
A pedicled flap of membranous trachea can be obtained by removing two or more distal rings of a tracheal stump. Then, a posterior midline incision of the cricoid plate is made (two-headed arrow) as part of a temporary thyrotomy, and a costal cartilage graft is interposed (curved arrow) between the two parts of the posterior cricoid. After the trachea is advanced upward (arrow), its membranous portion is sutured to the mucosa of the posterior commissure of the larynx. A laryngeal stent is usually in place for 3–4 weeks to provide support and aid healing.

2.3. **Slide tracheoplasty**

Short-segment congenital tracheal stenosis is effectively managed with resection and reconstruction. However for juveniles, the length of the resection must be limited [8]. Furthermore, vicryl sutures 5-0 are employed for the anastomosis.

Long congenital stenosis is usually caused by complete tracheal rings and it can be managed with a slide tracheoplasty [9, 10]. Briefly, the long stenotic segment is transected at its midpoint, while the upper and lower halves of the stenosis are divided vertically posteriorly and anteriorly, respectively. After the corners are trimmed, the two segments are slid together and an anastomosis is performed (Fig. 2.4). As a result, the tracheal circumference is doubled, the cross-sectional area is quadrupled, acceptable tension is achieved, and the blood supply remains adequate. The advantages of this direct method versus the application of patches of cartilage or pericardium are: (a) the trachea is reconstructed with vascularized tracheal tissue, thereby minimizing granulation tissue or necrosis; (b) the lumen is immediately epithelialized; (c) prompt extubation is usually achieved postoperatively; and (d) a need for cardiopulmonary bypass is eliminated, except when a pulmonary artery sling or other cardiac anomalies are present. Moreover, Wright and associates [8] have demonstrated that long-term growth of the trachea occurs when this repair approach is used. However, it is important to note that a slide tracheoplasty is not useful for acquired, long-segment stenoses.

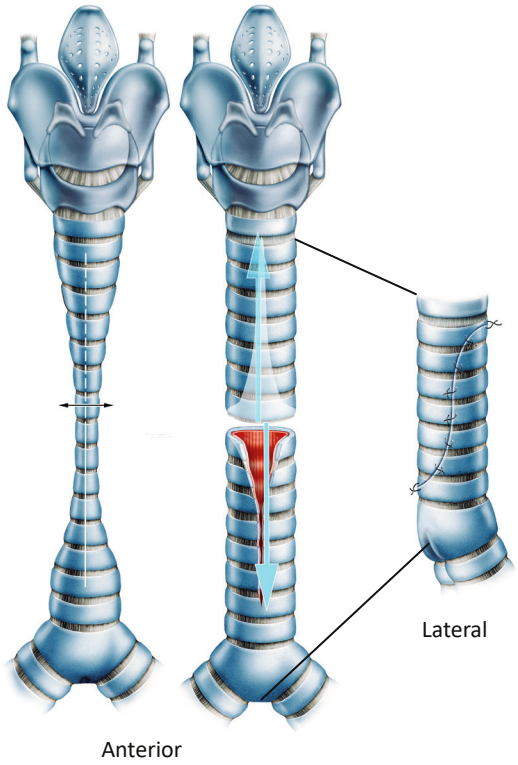

Figure 2.4. Slide tracheoplasty.
To perform a slide tracheoplasty, the trachea is first transsected at the midpoint of the stenotic segment (indicated with a double arrow). Next, the posterior surface of the upper stenotic segment (indicated with a dotted white line) and the anterior surface of the lower segment (indicated with a white line) are vertically incised. The two ends are then slid together and anastomosed.

2.4. **Limitations**

Segmental resection with end-to-end anastomosis is the preferred treatment for a stenosis that encompasses less than 50% of the tracheal length. When the length of a resected trachea is greater than 50% of the tracheal length in adults, or greater than 30% of the tracheal length in children, release of the surrounding anatomical structures will be insufficient to allow for a tracheal resection with direct end-to-end anastomosis.

Another challenging situation is an anastomotic stricture after segmental resection. These strictures are usually related to excessive tension at the suture line and they occur in approximately 10 % of patients undergoing resection [11]. While a silicone stent (i.e., a T-tube [12]) can provide temporary airway support (Fig. 2.5), tracheal reconstruction or transplantation will be necessary as a definitive treatment for both restenoses after segmental resection and long-segment stenoses.

Primary tracheal neoplasms (including adenoid cystic carcinomas and squamous cell carcinomas) may require the resection of trachea segments that are longer than 5–6 cm, and this requires reconstruction with a tracheal substitute (Fig. 2.5D).

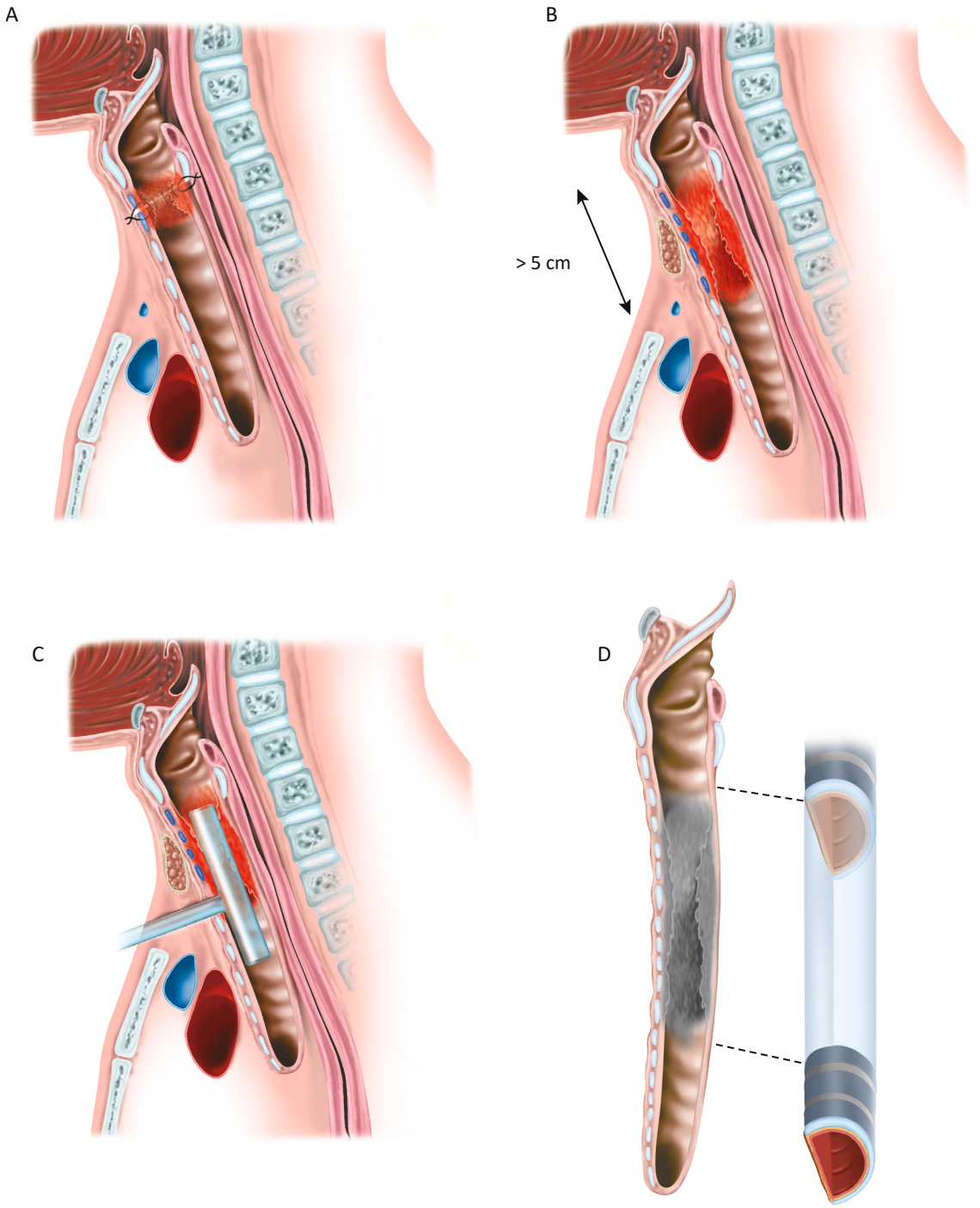

Figure 2.5. Limitations of current surgical techniques.
A. Restenosis at an anastomosis after segmental resection.
B. Long-segment stenosis involving a tracheal length longer than 5 cm.
C. T-tube placement through the dilated stenosis.
D. Tracheal neoplasms that require long-segment resection and tubular reconstruction.

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3. Obstacles for circumferential tracheal replacement

3.1. Prosthetic replacement

To date, all surgical prosthesis that have been successful (i.e., vascular conduits) have been performed in potentially sterile mesenchymal tissues. In contrast, research with prosthetic devices placed inside the airway have been unsuccessful since the 1960s [1]. This is partially due to the potential for synthetic materials to increase the risk of granulation, infection, and erosion of adjacent organs [2-4]. The latter can lead to fistulas with surrounding vessels, such as the aorta or the brachiocephalic artery, and this increases the risk of life-threatening hemoptysis. In addition, synthetic materials can provoke inflammatory granulomas that obstruct the lumen (Fig. 3.1).

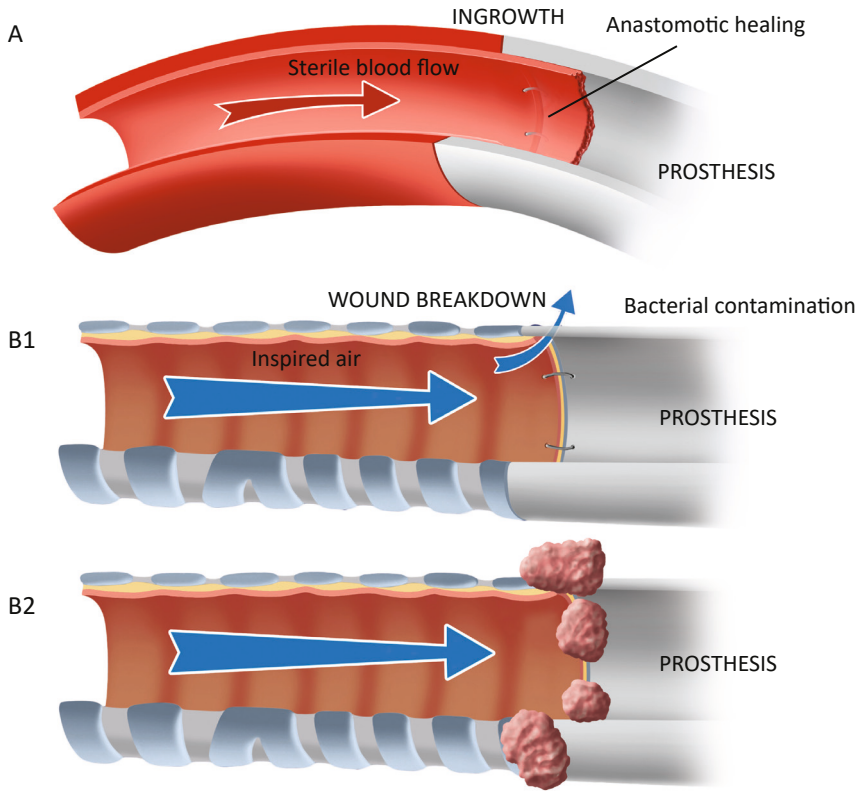


Figure 3.1. Prosthetic replacement of tubular conduits.
A. Blood vessel prosthesis. Endothelialization of the luminal surface of a vascular graft only extends 1-cm into the graft from the anastomotic site. The endothelial cells derive from adjacent, native arterial endothelium and they facilitate healing of the anastomosis.
B. Airway prosthesis. In the respiratory tract, the flow of inspired air can lead to bacterial contamination and wound breakdown at anastomoses. In addition, respiratory epithelium will not grow over a prosthesis-airway anastomosis (1). As a result, infection and formation of granulation tissue can occur at an anastomosis (2).

A prosthesis may act as a temporary airway stent when it is encompassed by well-vascularized tissue (i.e., omentum). However, this vascularized tissue will only temporarily prevent complications such as wound breakdown, infection, and formation of granulation tissue at anastomotic sites (Fig. 3.2).

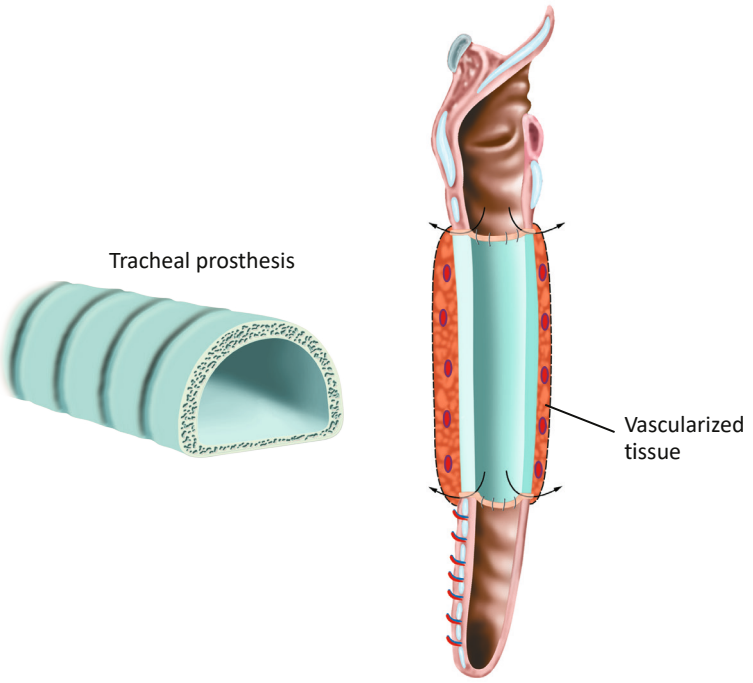


Figure 3.2. An airway prosthesis encompassed by vascularized tissue. Arrows indicate wound breakdown, infection, and formation of granulation tissue at an anastomosis between a prosthesis and native trachea tissue.

3.2. **Autologous tissues**

Fabre and colleagues have proposed a single-stage operation to construct a tube from a forearm fasciocutaneous flap that is vascularized by radial vessels and reinforced by rib cartilage. The rib cartilages are interposed transversally in subcutaneous tissue (Fig. 3.3) [5] and a neotracheal conduit is constructed from a large, rectangular fasciocutaneous flap. Before the fasciocutaneous rectangle is transformed into a tube by suturing the main lengths together, several costal cartilage segments are obtained from the patient's rib cage to be inserted between the skin and the fascia within the subcutaneous tissue to ensure transverse rigidity of the tube. Typically, 6 or 7 cartilaginous segments (each 5 mm wide, 9 cm long) are elevated from the most caudal ribs and then are slid into place, with care taken not to injure the skin vascular supply from the perforators. Structural rigidity of the neotracheal tube is achieved by suturing the ends of each costal cartilage between each other. Since 2004, 12 patients have undergone this operation [5].

The main limitation of this technique is the cutaneous lining consisting of keratinized, stratified squamous epithelium. This will lead not only to an absence of mucociliary clearance but also to crusting and desquamation. Neotracheas require aggressive management of bronchial secretions, and a temporary tracheostomy may be necessary. Consequently, this reconstruction should not be indicated for patients who lack excellent diaphragmatic and respiratory function which provide effective coughing. Another limitation of this technique is the risk of cartilage fracture in elderly patients affected by extensive rib cartilage calcifications.

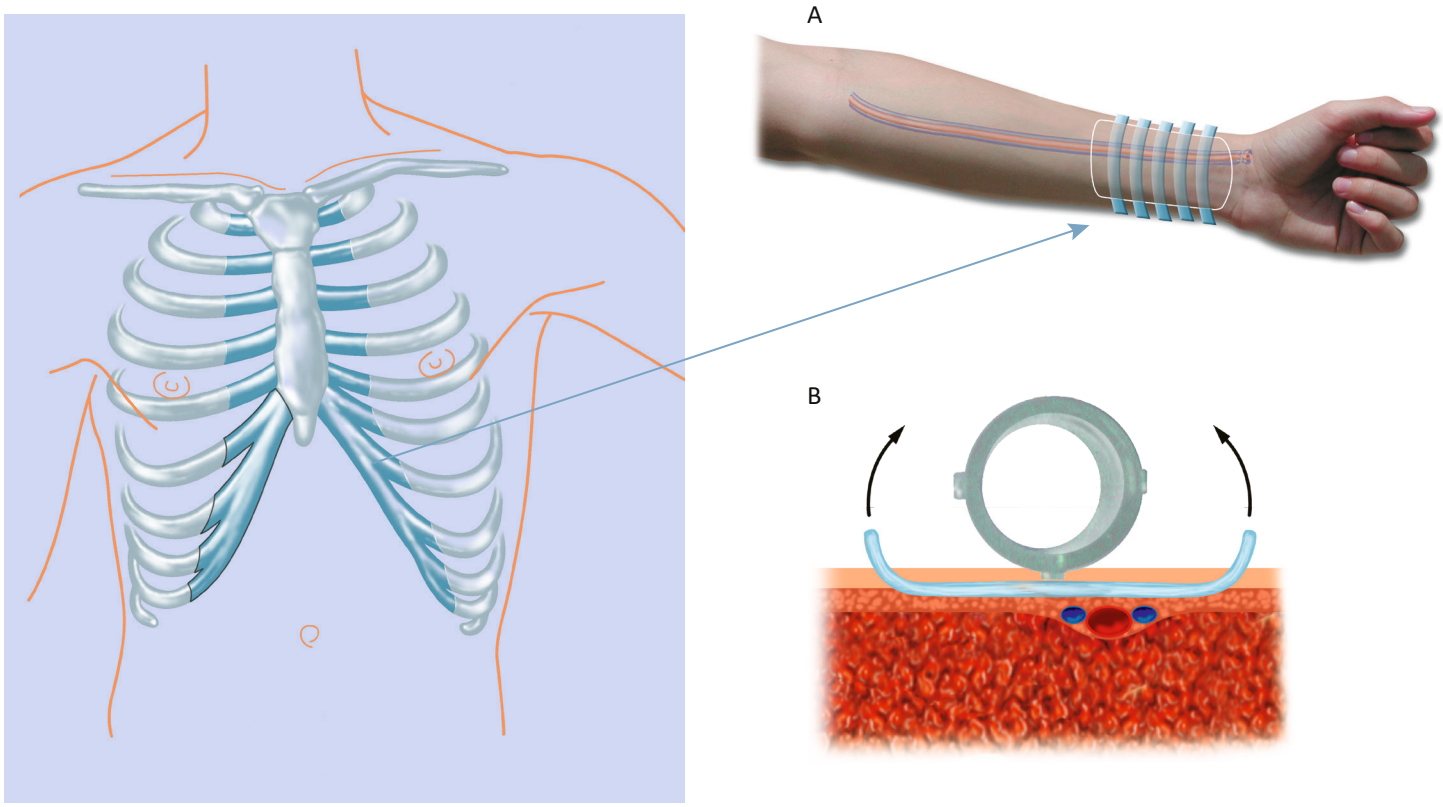


Figure 3.3. Surgical procedure involving autologous tissue.
A. A forearm free flap after cartilage rib insertion.
B. Suturing of the cartilages around a transitory silicone stent (indicated with arrows).

In 2014, a 4-year follow-up report was published regarding a child who had undergone a total autologous tracheal replacement [6]. A new trachea was manufactured after a 12-cm segment of constricted trachea was removed from the patient. The new trachea consisted of a myocutaneous latissimus dorsi-free flap into which four chondrocartilage slings were inserted subdermally every 2 cm (Fig. 3.4). The flap was formed into a tubular structure around a Y-shaped silicone tracheal prosthesis and a tracheostomy was performed at the junction between the native trachea and the new trachea. At postoperative day 9, the silicone stent was removed. Daily aspiration of particulate matter from the tracheobronchial tree was performed for six weeks to prevent bronchial plugging and pneumonia due to skin desquamation and lack of endoluminal clearance. The tracheostomy was maintained for more than two years.

Improvements concerning aspects of the cutaneous lining of the reconstructed airway and the cartilage rings (i.e., graft rigidity) in this technique remain to be addressed [6].

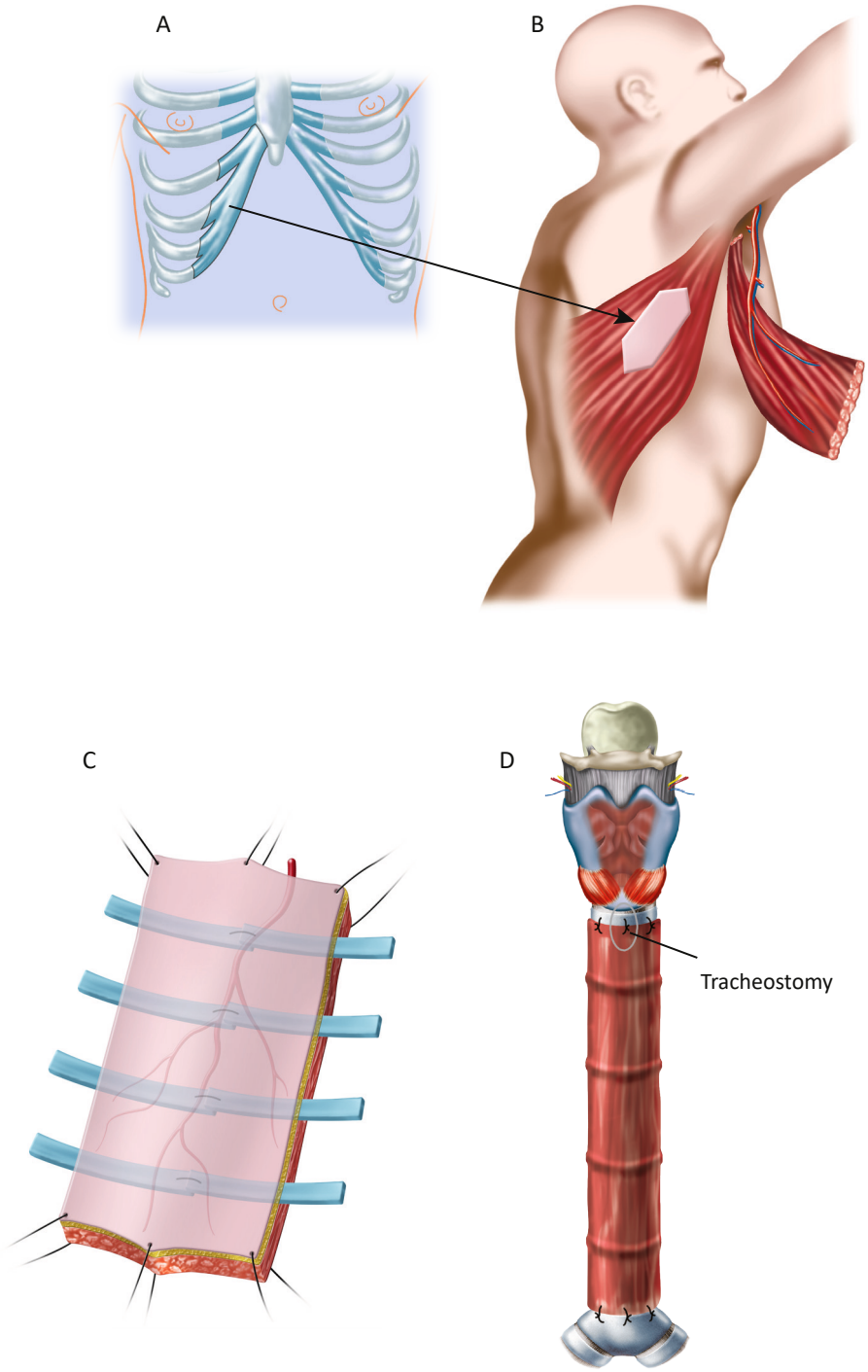


Figure 3.4. Surgical procedure involving autologous tissue (continued).
Harvesting of the components necessary to create a new trachea is shown. Briefly, hyaline cartilage is harvested from the 7th, 8th, and 9th chondrocartilage (A) and is subsequently cut into four cartilage slings measuring 80 × 5 × 2 mm. A musculocutaneous latissimus dorsi flap (12 × 8 cm) with skin paddle is also harvested (B). The four costal grafts are inserted subdermally to be protected by skin on the outside and by muscle on the inside (C). A reinforced latissimus dorsi flap is sutured and shaped into a tubular structure around a silicone tracheal prosthesis to form a new tracheal tube. The graft is sutured superiorly to the first tracheal ring and inferiorly immediately above the carina (D). A tracheostomy is performed above the proximal end of the new trachea.

Circumferential reconstruction of long tracheal segments with autologous tissue is a challenging procedure with a complicated postoperative course. From a technical point of view, carving of rib cartilage strips to form cartilage rings is a particularly challenging aspect of this procedure. To date, only a surgical team in Paris has successfully performed this procedure. It remains to be seen if other groups will succeed in reproducing this technique.

3.3. **Tracheal regeneration**

The term, “tissue engineering”, implies that replacement of tissues and organs can be achieved with isolation and culturing of cells outside the body. These cells are later seeded into a biocompatible scaffold prior to implantation.

3.3.1. **Whole organ regeneration**

Organ transplantation currently represents the gold standard treatment for all diseases leading to irreversible organ failure. However, despite efforts to increase the supply of suitable organs for transplantation, a significant gap still exists between the number of organ donors and the number of recipients. As a result, an organ shortage exists. Tissue engineering and regenerative medicine efforts share the same ultimate goal: to create functional tissues or whole organs for use as ‘replacement parts’ for the human body. Successful achievement of this goal would represent a groundbreaking advance in clinical transplantation.

Experimental attempts at organ regeneration have been reported for human organs such as kidney [7], lung [8, 9], heart [10], liver [11], and small intestine [12]. More recently, a new technology for whole organ regeneration has been described which employs organ decellularization to create a three-dimensional extracellular matrix that preserves the native architecture of a tissue, including the vasculature. Restoration of blood supply to a regenerated organ is an essential aspect of this regeneration concept (Fig. 3.5). However, possible clinical applications for any of these regeneration attempts are currently precluded due to the challenges associated with the production of clinically functional organs. Given the complex morphology of any organ, the ability to successfully demonstrate this regeneration concept would represent a “miraculous” achievement.

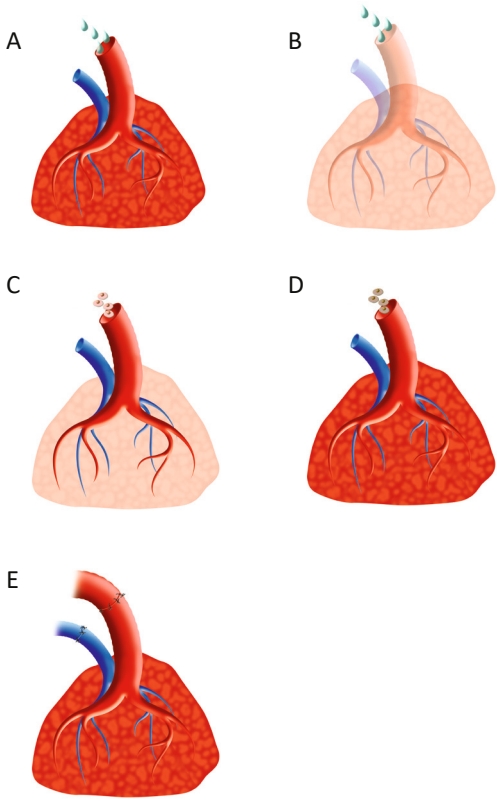


Figure 3.5. Whole organ engineering according to a decellularization-recellularization protocol.
Tissue decellularization is achieved by flushing an organ with detergent solutions through its native vascular system. This removes native cell components while preserving the extracellular matrix (A,B). The decellularized scaffold can then support engraftment of specialized parenchymal cells to form a functional organ (C,D).

The ultimate goal of this protocol is to maintain macrovascular and microvascular trees so that an organ can be re-incorporated into a recipient’s vascular system. However, recellularization and bioengineering of functional organs remain to be accomplished (E).

3.3.2. **Tracheal regeneration with a biological scaffold**

Bioengineering has a controversial history in tracheal replacement. The first reports of tracheal regeneration described the use of cryopreserved aortic allografts as biological matrices without stem cells for extensive airway reconstruction [13]. Based on animal experiments, it was expected that *in vivo* regeneration of the epithelium and cartilage would occur after implantation. There are several patients who have been treated with an aortic allograft wrapped in tissue with good blood perfusion. Most often, abdominal fat (omentum) has been wrapped around an aortic graft, including its intact blood supply [13, 14]. Regeneration of cartilage rings and respiratory epithelium was reported in a recently published series involving 13 patients who had segments of their trachea or bronchus replaced with cryopreserved aortic allografts wrapped with a local muscle flap [15]. Tsukada and colleagues described a tracheal replacement procedure that was performed with a silicone-stented, fresh aortic allograft in animals [16]. However, the authors concluded that this substitute is unsuitable for primary tracheal replacement because it undergoes shortening of the grafted area. For example, shortening of up to 87.5% was observed within one year of the replacement procedure [16] (Fig. 3.7).

In 2008, Macchiarini et al. [17] presented the first case of a 30-year-old woman with severe bronchomalacia who had her left bronchus replaced with an enzymatically decellularized human donor trachea, which was colonized in a bioreactor by epithelial cells and mesenchymal stem-cell-derived chondrocytes originating from the patient [17, 18]. Because the graft included cells cultured from the recipient, no anti-donor antibodies or immunosuppressive drugs were administered [17] (Fig. 3.6).

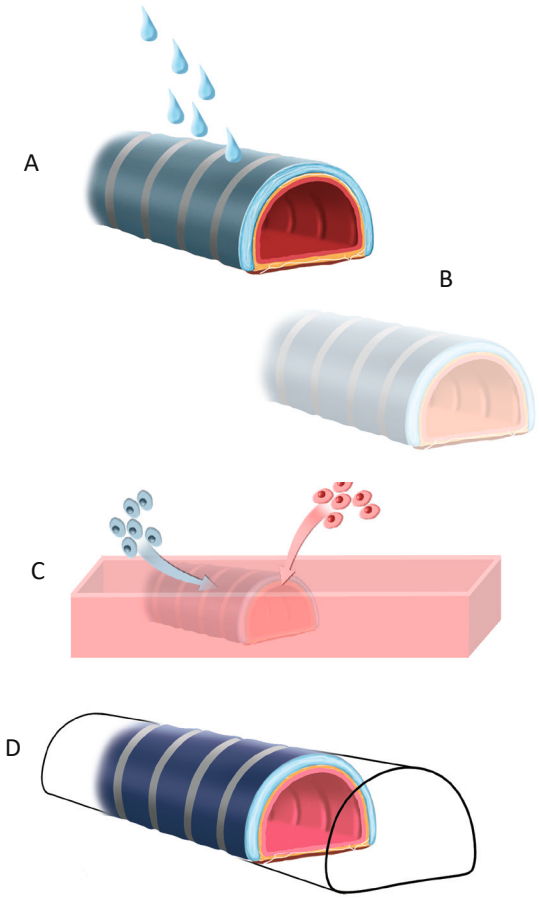


Figure 3.6. Tracheal engineering according to a decellularization-recellularization protocol.
A scaffold was created by removing the cells and antigens from a human donated trachea (A, B). The trachea was then colonized by epithelial cells and mesenchymal stem cell-derived chondrocytes which were cultured from cells obtained from the recipient (C). After a few days in a bioreactor, the "engineered" and avascular trachea was successfully transplanted to repair a diseased part of the patient's own bronchus (D).

Two principal cell types were needed for recellularization of the upper airway scaffold: chondrocytes and epithelial cells. Epithelial cells were harvested in the form of nasal epithelia, while chondrocytes were harvested from bone marrow-derived hematopoietic progenitor cells [19]. In the procedure described by Macchiarini et al. [17], the tracheal graft and the cells of the patient were brought together in a bioreactor. The mucosa cells were placed inside the tracheal graft, while the bone marrow cells were placed on the outside of the graft. The cells were then allowed to colonize over a few days in the bioreactor.

Following a successful surgery to insert the graft, the recipient had a functional airway which exhibited a normal appearance and mechanical properties. For four months the patient's quality of life improved. However, after four months, the patient's airway narrowed again and collapsed, requiring clinicians to insert a stent to keep her airway open. The stent was removed six months later, although several more stents were inserted and removed in subsequent years. Eventually, a left pneumectomy was performed in 2016 due to graft failure. The patient also experienced chronic lung infections and colonization, as well as numerous bronchoscopic procedures. However, despite this case receiving significant attention in medical journals [17] and in the press [20], its use of an engineered trachea appears to represent an example of scientific deception [21, 22].

In 2011, a revolutionary report was published regarding the first tissue engineered trachea to be implanted in a child. A 12-year-old Irish boy underwent the surgery in London with more or less the same team of doctors that performed the first case in 2008. Notable differences between these cases are that a bioreactor and mucosal cells from the nose were not used in the latter procedure. Instead, the donor's trachea was enzymatically treated to destroy all of the donor's cells, and then it was covered with the recipient's own bone marrow cells and immediately implanted in the airway to undergo '*in vivo* regeneration' [23, 24]. This achievement was extensively reported in international media and was described as the first successful stem-cell based regeneration of an organ in a child [25].

Extremely astonishing among the tracheal regeneration approaches that have been described are that regeneration and transplantation have reportedly occurred without restoration of blood supply. Originally, the most significant technical breakthrough in tissue reconstruction occurred in 1902 when Alexis Carrel reported on a method of blood vessel anastomosis. For developing this method he was awarded the Nobel Prize in 1912. In subsequent years, the application of vascular surgery for blood supply restoration has resulted in revolutionary advances in tissue transplantation. In contrast, the recent achievements obtained with tissue-engineered tracheal transplants do not include any experimental or clinical data to indicate the status of blood supply restoration. Thus, despite the description of these proposed tracheal regeneration concepts as "successful", they are theoretically impossible.

In all of the published cases which have described a biological scaffold [14, 17, 18, 23], the patients were functioning with a synthetic stent to support the reconstructed airway lumen (Fig. 3.7). Hence, the published results correspond with the outcomes expected according to our current knowledge of airway repair. Moreover, the biological scaffolds were wrapped in vascularized omentum so that inevitable stent-related complications would be delayed.

For the decellularization-recellularization approach, no physical explanation has been provided as to why stem cells would penetrate the connective tissue of the scaffold and regenerate cartilage, epithelial, and endothelial components. Moreover, even if this very unlikely phenomenon occurs, it would not influence the ultimate outcome which is that the native trachea will undergo resorption after re-implantation. Tissue-engineered tracheas which are used with a stent wrapped with vascularized tissue may function for longer periods of time, provided that stent-related complications, such as formation of granulation tissue at the top or bottom edges of the stent, are treated.

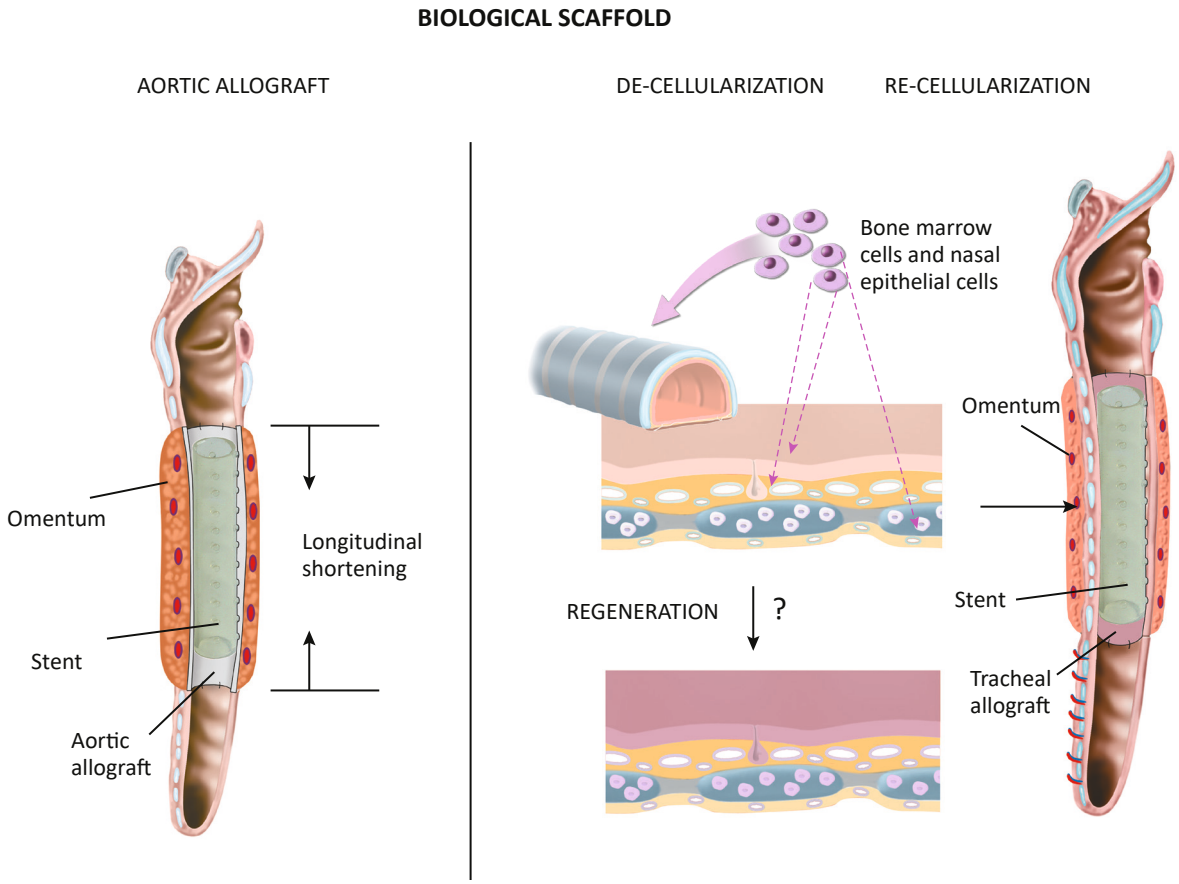


Figure 3.7. "Successful" tracheal tissue engineering.
The left and right panels show an aortic allograft and the decellularization-recellularization procedure applied to a biological scaffold that was used in a 'successful' tracheal regeneration approach. Both of the patients involved were stent-dependent. Longitudinal shortening of the defect due to wound contraction occurred with the aortic allograft and this may have been misinterpreted as tracheal regeneration [16]. Moreover, regarding the decellularization-recellularization step, there is no physical explanation why stem cells would penetrate the connective tissue of the scaffold and regenerate the cartilage, epithelial, and endothelial components.

3.3.3. **Tracheal regeneration with a synthetic scaffold**

Another aspect to consider regarding the recently described tracheal regeneration approaches is the use of synthetic scaffolds. In 2011, the trachea was heralded in medical professional literature [26] and in popular media [27] as the first synthetic organ to be engineered by using a synthetic scaffold and bone marrow cells. This realization was recognized as the beginning of a new era of stem cell-engineered organ regeneration with synthetic scaffolds. A synthetic scaffold was developed at University College London and the surgery to implant it was performed at the Karolinska Institute in Sweden. Evidence-based research predicts that a synthetic airway will fail after weeks or months when the sutures between vital airway tissue and the synthetic material detach (Fig. 3.8). The surgeons at the Karolinska Institute wrongfully reported that the procedure was a success within the initial symptom-free window (i.e., the first four months post-surgery) [26].

Unfortunately, and as expected, serious complications were observed after this initial four-month window and these resulted in the death of the patient during the subsequent two years of follow up [28]. For patients who receive a synthetic trachea, their rates of morbidity and mortality are very high.

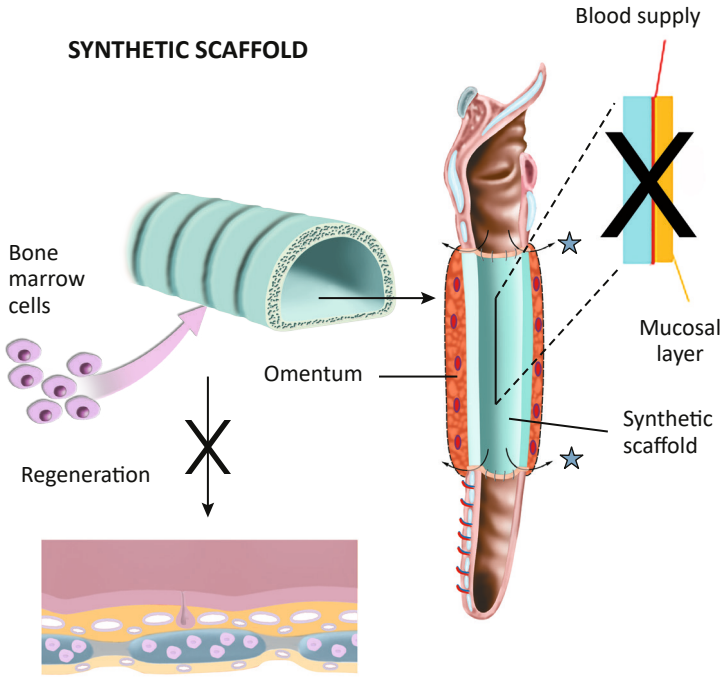


Figure 3.8. Misreporting of tracheal tissue engineering as successful.
The researchers who reported performing a 'successful synthetic trachea transplantation' relied on three hypotheses. One, that stem cells would penetrate the synthetic material used to regenerate cartilage, blood vessels, and respiratory mucosa; two, that healing and sealing of the anastomosis between the vital trachea and scaffold would be observed; and three, the presumed regenerated trachea would be implanted without any further restoration of blood supply. However, after the initial symptom-free window, the sutures between the native trachea and the synthetic scaffold detached (indicated with asterisks), thereby resulting in major complications and death of the patient. To date, growth of a thin layer of blood vessels over a synthetic scaffold has not been observed (crossed detail), and this is needed to support ingrowth of a mucosal graft.

In the future, misleading tracheal regeneration claims can be avoided by ensuring that clear visualization (i.e., a CT scan) of the trachea is provided in the absence of a stent (Fig. 3.9).

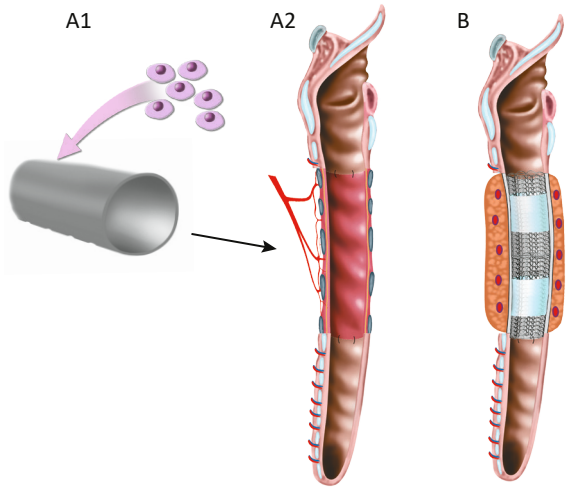


Figure 3.9. Avoiding misleading tracheal regeneration claims.
A. Tracheal regeneration performed with use of a biological or synthetic scaffold and stem cells (1). Direct visualization of the regenerated tracheal construct without a stent is needed to support a claim of tracheal regeneration (2).
B. To date, the 'evidence' provided for tracheal regeneration has included visualization of an omental-wrapped scaffold with an intraluminal stent in place to temporarily preserve the airway lumen.

3.4. **Tracheal allotransplantation**

3.4.1. **Direct revascularization**

The main obstacle to performing a direct tracheal transplantation is that the segmental blood supply of the trachea originates from several tracheoesophageal branches which are too small to undergo a microvascular transfer (Fig. 3.10) [29]. However, the upper 4 cm of a trachea has been transplanted as part of a laryngeal transplant on the superior thyroid arteries (Fig. 3.11).

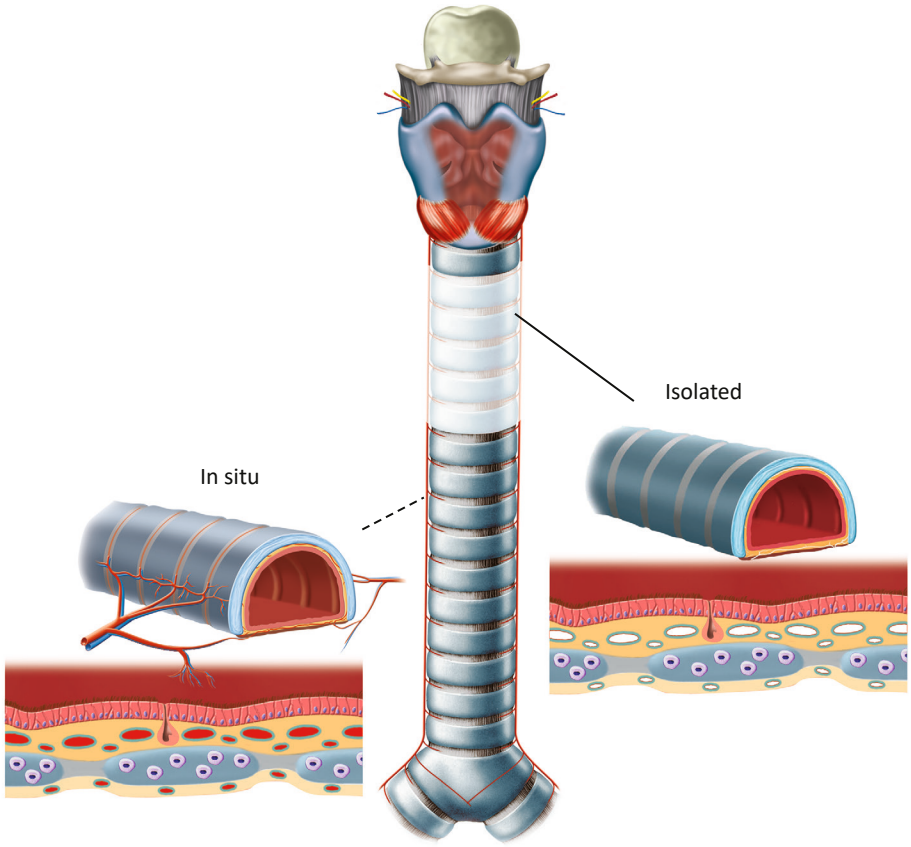


Figure 3.10. Providing a blood supply for an allotransplanted trachea is a key obstacle.
An *in situ* tracheal segment and its associated segmental blood supply are shown (left). Arterial and venous supplies to the trachea do not provide direct revascularization due to their small diameter. An avascular tracheal segment after isolation is also shown (right).

Transplantation of a fresh laryngeal allograft was performed to replace a stenotic larynx following a motorcycle accident. The allograft included a 5-ring segment of trachea, thyroid, parathyroids, a portion of the attached pharyngeal wall, both superior laryngeal nerves, and both recurrent nerves. Arterial, venous, and neural anastomoses were performed, and perfusion was established during the early stages of the procedure. Despite one episode of rejection, health and function of the transplant were observed to be good after 40 months [30].

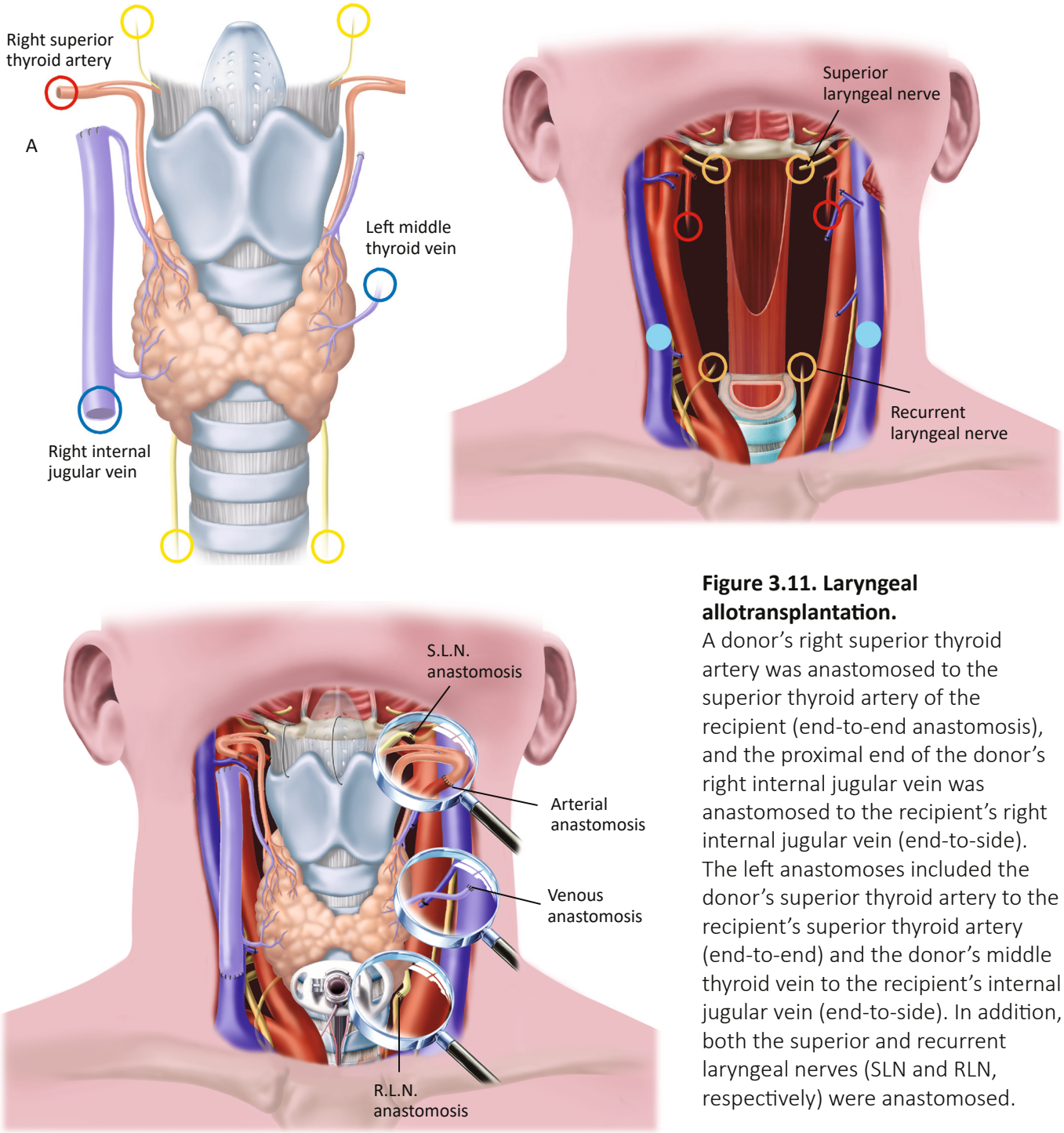


Figure 3.11. Laryngeal allotransplantation.
A donor's right superior thyroid artery was anastomosed to the superior thyroid artery of the recipient (end-to-end anastomosis), and the proximal end of the donor's right internal jugular vein was anastomosed to the recipient's right internal jugular vein (end-to-side). The left anastomoses included the donor's superior thyroid artery to the recipient's superior thyroid artery (end-to-end) and the donor's middle thyroid vein to the recipient's internal jugular vein (end-to-side). In addition, both the superior and recurrent laryngeal nerves (SLN and RLN, respectively) were anastomosed.

The upper part of the trachea, similar to the thyroid gland, receives its arterial blood supply from the branches of the superior thyroid artery. Therefore, the upper part of a trachea was directly transplanted with the corresponding larynx and thyroid gland. Unfortunately, direct revascularization represents not a clinically relevant transplantation technique for isolated tracheal segments due to the complexity and invasiveness of the procedure.

3.4.2. **Indirect revascularization**

3.4.2.1. History of tracheal allotransplantation

The first clinical tracheal transplantation performed was reported in 1979 [31]. A donor trachea was implanted heterotopically under the sternocleidomastoid muscle of a recipient and then was transferred into an orthotopic position three weeks later. No immunosuppression was required and short-term integration with surrounding tissue and reepithelialization were achieved:

Lancet 1979;1:433:

"SiR,—We would like to present a preliminary report of the first allogeneic tracheal transplantation in man. Detailed studies in immunologically well-defined individuals showed that tracheal transplants, in contrast to other organ transplants, induce only a weak graft-rejection reaction even in the presence of major histoincompatibility. This may reflect weak organ-specific antigenicity. We concluded that the mechanisms leading to necrosis of tracheal transplants had been misinterpreted as allograft rejection whereas they were more likely to have been ischaemic and infectious in nature. In a series of further experiments we found that tracheas heterotopically implanted into the muscles of the neck remained free of ischaemic and infectious complications. They became revascularised and all tissue components remained intact. This is the background to our attempt at heterotopic/orthotopic tracheal transplantation in man. The recipient was a 21-year-old male with an extensive tracheal stenosis affecting eight segments as a result of reanimation procedures. The donor (blood group compatible), also a 21-year-old male, died of severe brain trauma resulting from an accident. The ischemic time of the resected ten-segment tracheal piece was 160 min. After closure of both oral and aboral lumens of the tracheal segment by mucous flaps from the posterior wall of the transplant it was implanted heterotopically into the sternocleidomastoid muscle of the recipient. It was accepted without complications. 3 weeks later, the transplant was mobilised and orthotopically implanted isoperistaltically, accompanied by a vascularised muscular section from the sternocleidomastoid. Tracheostomy or endothetic support was regarded as unnecessarv. The tracheal allograft has become integrated and it has functioned perfectly for 9 weeks without any evidence of rejection, ischaemia, or infection. The patient has been discharged. We believe that combined hetero-orthotopic transplantation of tracheal allografts constitutes a valuable approach to the treatment of the human trachea. "

Major shortcomings of this report are the statements that the trachea induces only weak antigenicity, while the mucosal lining and capillaries from the donor are highly antigenetic [32]. In addition, immunosuppressive therapy was not administered and the report did not document the viability of the allograft or the long-term outcome.

A second case was reported in 1993, *Eur J Cardiothorac Surg* 1993;7:383-386 [33]:

"The authors observed a 24-year-old female with idiopathic fibrosing mediastinitis affecting the thoracic segment of the trachea and producing marked stenosis. An attempt at one-stage allotransplantation of the trachea with omentopexy of the graft was made. In spite of immunosuppressive therapy (cyclosporine and azathioprine), signs of rejection appeared on the 10th postoperative day, which were arrested by antithymocyte globulin and loading doses of corticosteroids. Subsequently, the condition of the patient became stable. By the end of the 2nd month the graft appeared vital, its lumen being about 10 mm, the lines of anastomoses were epithelialized. Four months after operation signs of the graft stenosis appeared, possibly caused by progressive fibrosing mediastinitis. Because of this complication a silicon stent was used for the prophylaxis of further graft stenosis. This satisfactory result of tracheal

allotransplantation is thought to be related to adequate selection of the donor-recipient pair, modern immunosuppressive therapy and utilization of omentopexy for early graft revascularization."

A major shortcoming of this reported procedure is that orthotopic one stage revascularization and transplantation of the allotransplant was performed, and this method has proven to be unreliable as a revascularization technique [34, 35].

To achieve indirect revascularization by wrapping a trachea in vascularized recipient tissue, a tracheal allograft must be in a heterotopic position and the patient be maintained on an immunosuppressive therapy. These requirements were first reported by Klepetko et al. in 2004 [36] when they described revascularization of a trachea in the omentum of a patient who received a lung transplant from the same donor. Ultimately, the tracheal transplant was not used, but it remained viable up to 60 days. In this case, a 57-year-old male patient with terminal chronic pulmonary disease and a history of multiple episodes of respiratory failure had a recurring need for mechanical ventilation and tracheostomies. As a consequence of these interventions, tracheal stenosis had developed. The patient was offered a staged procedure which consisted of a bilateral lung transplantation and transfer of a donor's trachea into the recipient's abdomen to be wrapped in the greater omentum (Fig. 3.12). This approach provided the additional option that resection of a stenotic tracheal segment could be followed by reconstruction with a tracheal allotransplant after it was revascularized and transferred on the pedicled omentum.

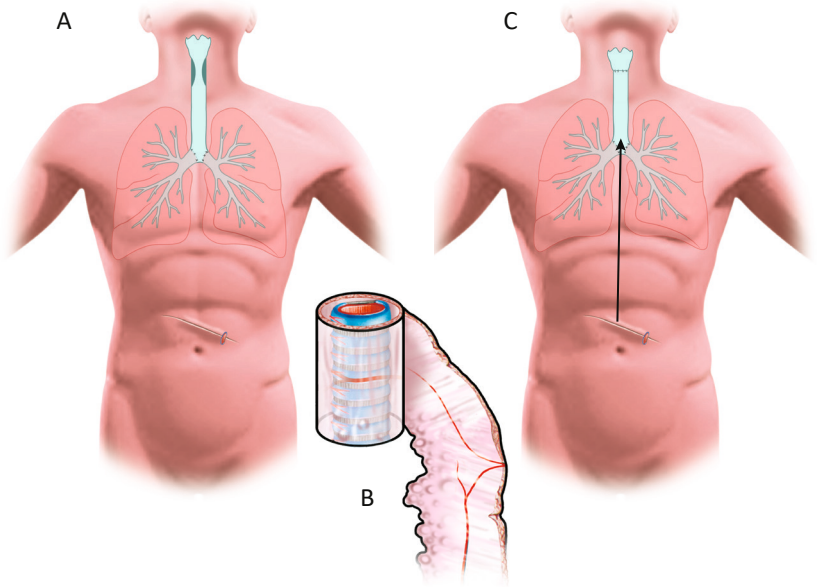


Figure 3.12. Heterotopic revascularization of the trachea in the greater omentum.
A patient who underwent bilateral lung transplantation received a donor trachea that was placed in the abdomen (A) and wrapped with the greater omentum (B). Use of a revascularized trachea was intended to resolve tracheal stenosis (arrow) (C).

Definitive repair of the tracheal stenosis was performed six months after a lung transplantation. The patient underwent a cricotracheal resection and reconstruction with direct end-to-end anastomosis achieved. When the tracheal allograft was harvested for investigation, a histological examination revealed that viable cartilage was covered by respiratory epithelium with excellent vascularization of the tracheal wall.

This case illustrates that indirect revascularization of the trachea may be possible if heterotopic wrapping of a trachea in omentum is performed [36].

3.4.2.2. Experimental tracheal allotransplantation

Based on the technique developed for heterotopic wrapping of a trachea in vascularized fascial flaps, experimental animal research on tracheal allotransplantation was started in 1993 [32, 37]. In rabbit models, revascularization of tracheal allotransplants was observed after heterotopical wrapping was performed with a lateral thoracic fascial flap (Movie 1). The recipient's blood supply to the fascial flap was found to induce revascularization and mucosal regeneration over a 14-day period. Moreover, the vascular induction process from the surrounding fascia was found to be mediated by intercartilaginous ligaments (Figs. 3.13, 3.14).

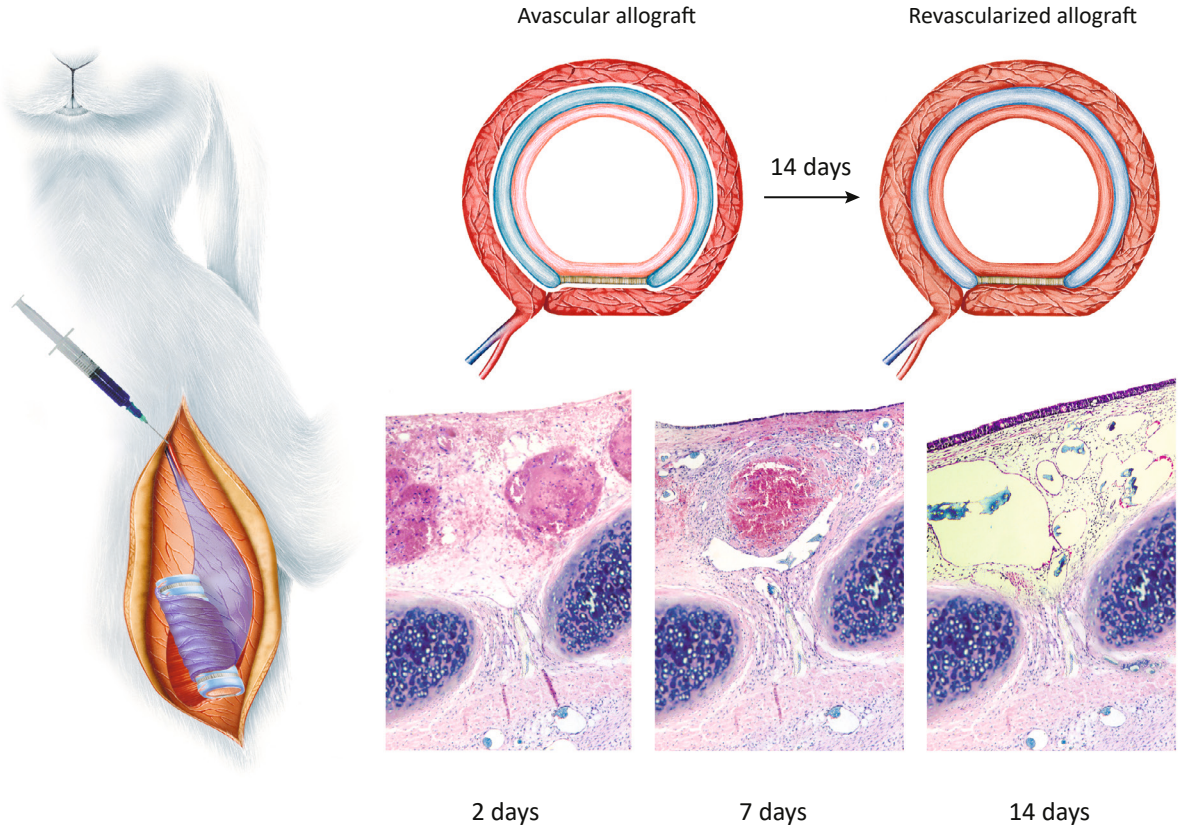


Figure 3.13. Tracheal allograft revascularization and mucosal regeneration.
Revascularization has been studied in rabbits administered cyclosporine (10 mg/kg/d) for immunosuppression. In these studies, a blue silicone dye was injected into the lateral thoracic artery at various timepoints. After 2 days, thrombosis of the submucosal blood vessels and disintegration of the respiratory epithelium were observed. After 7 days, early revascularization with recanalization of thrombosed submucosal blood vessels and regeneration of the surface epithelium were observed. After 14 days, full submucosal revascularization and regeneration of the respiratory epithelium were observed.

Experimental evidence indicates that basal epithelial cells survive the ischemic period following isolation of the allograft. In addition, epithelial regeneration from basal cells follows the revascularization process of submucosal blood vessels.

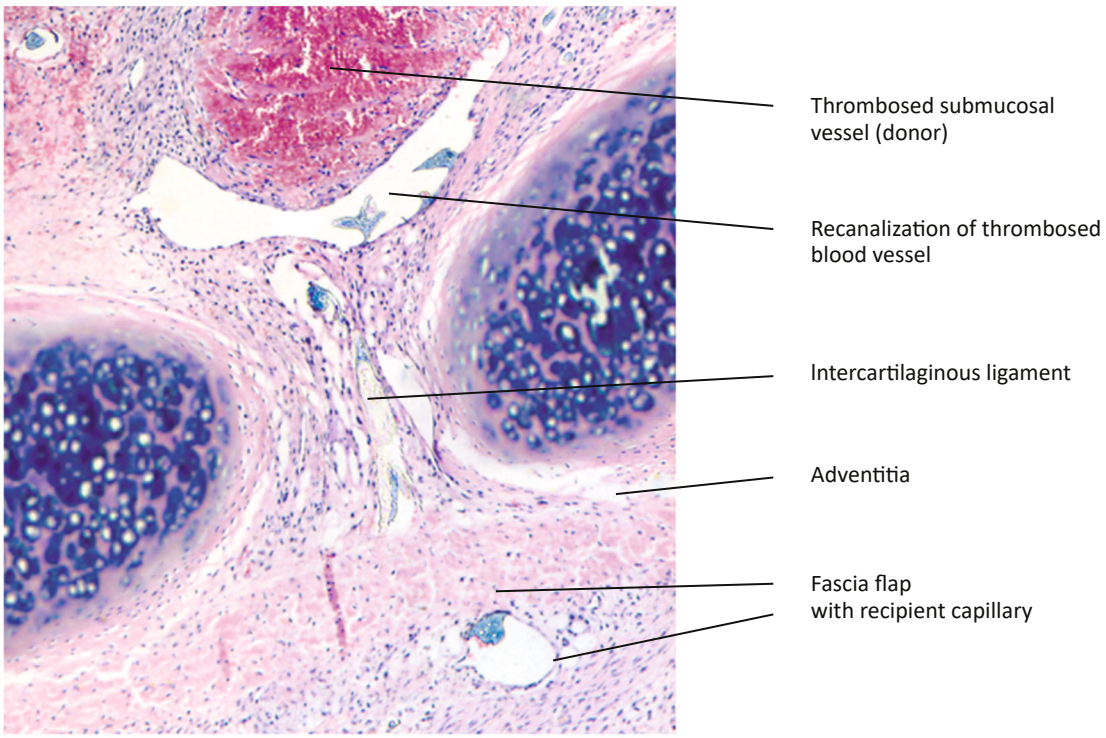


Figure 3.14. Tracheal allograft revascularization – histological details of intercartilaginous ligaments.
Revascularization that occurs from the fascia flap to the donor's submucosal space through the intercartilaginous ligament is shown. In this image, it is not clear if revascularization occurs due to outgrowth of recipient capillaries to the donor's submucosal space (angiogenesis), or due to recanalization of existing, yet thrombosed, capillaries of the donor (vascular induction).

Rabbit tracheal allografts that have been maintained under continuous immunosuppression with cyclosporine (10 mg/kg/d) have not exhibited rejection. For example, after a 14-day revascularization period in a heterotopic position, an allograft was transplanted orthotopically on a newly created vascular pedicle of a fascial flap (Figs. 3.15, 3.16). In parallel, a full-thickness skin graft of the donor animal was used as an external monitor for the rejection process (Fig. 3.17). Acute rejection occurred following an orthotopic tracheal transplantation when short-term immunosuppressive treatment was discontinued (Figs. 3.18-20).

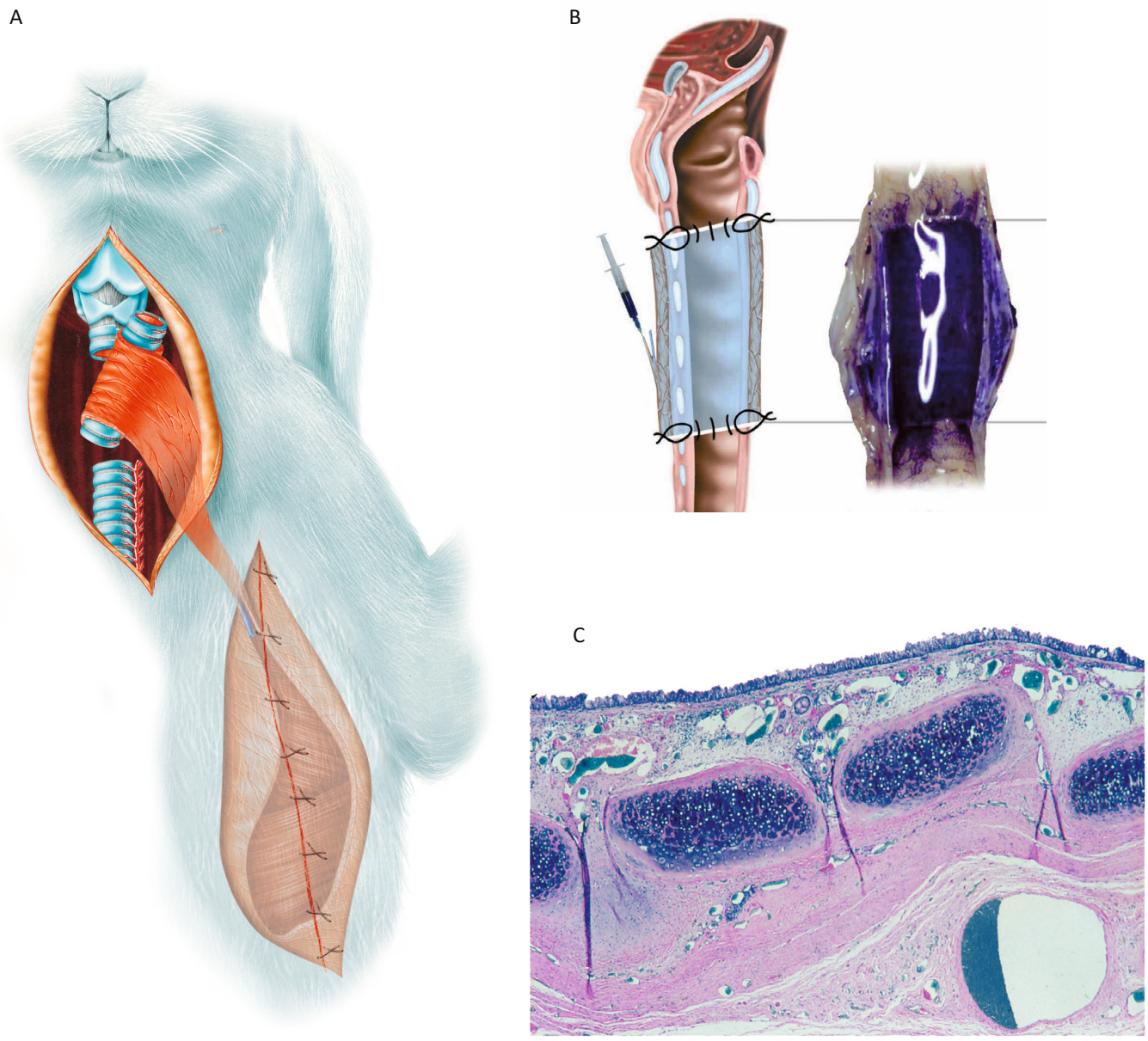
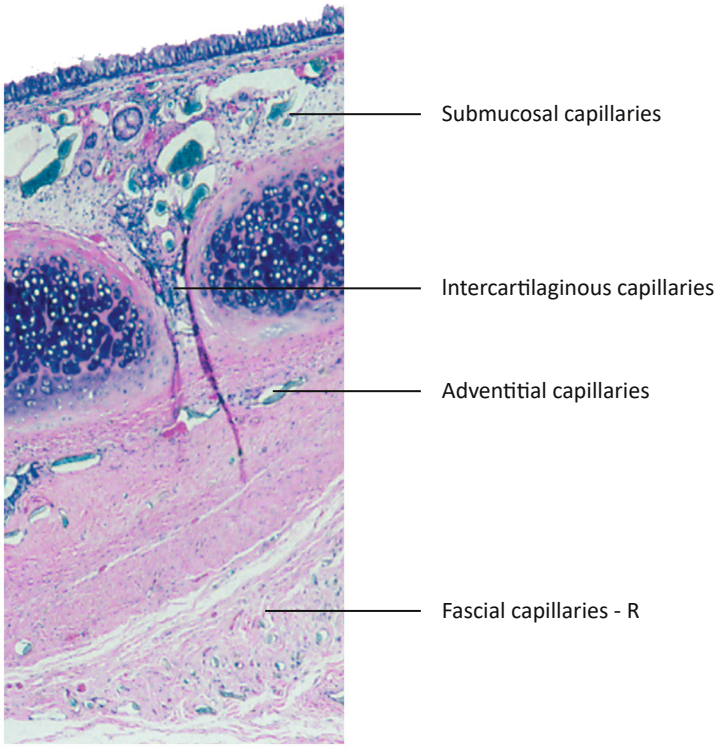
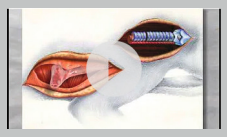


Figure 3.15. Tracheal allotransplantation in an orthotopic position.
A. A tracheal allotransplantation was performed on the lateral thoracic artery and vein. Transposition of the fascial flap to the neck was performed with preservation of the vascular pedicle.
B. Postmortem macroscopy of a tracheal allograft 4 weeks after an orthotopic transplantation was performed. Blue silicone dye was injected into the vascular pedicle and both the fascia and mucosal lining of the tracheal transplant were uniformly stained.
C. Histology of a tracheal transplant (H&E). The blood supply from the fascial flap reaches the mucosal lining via intercartilaginous ligaments. All blood vessels and capillaries were stained following an injection of blue silicone dye. An enlarged view of the outlined area is shown in Fig. 3.16.



Movie 1*



Experimental tracheal Transplantation.

* Due to the nature of the video clips, a YouTube account might be required in order to enable access.

Figure 3.16. Tracheal allotransplantation - histology at an intercartilaginous ligament.
The fascial capillaries observed in this image derive from the recipient. However, the revascularization studies that were performed did not clearly distinguish which capillaries in the adventitial, intercartilaginous, and submucosal spaces derive from the recipient (R) versus the donor.

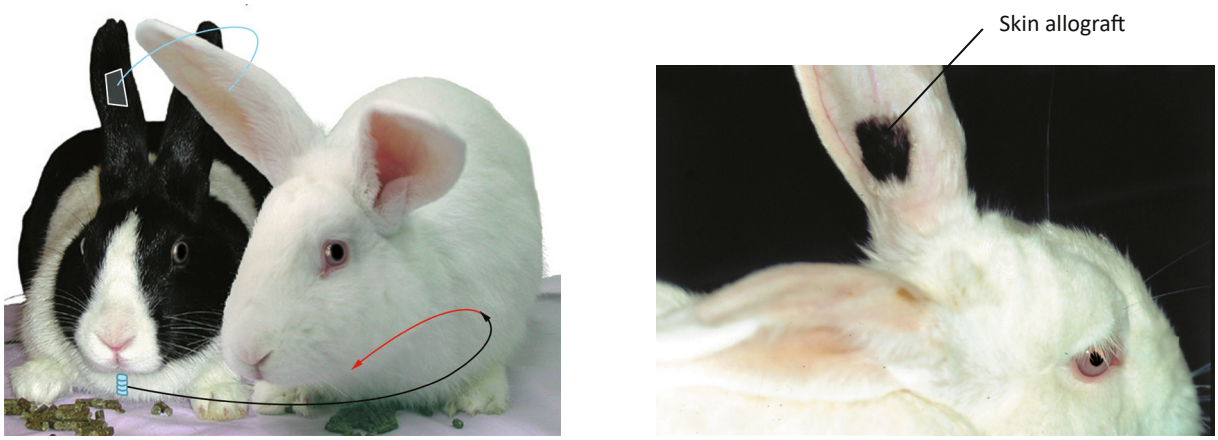


Figure 3.17. Tracheal allotransplantation performed between New Zealand white rabbits (recipients) and Dutch belted rabbits (donors).
A tracheal allograft was first implanted in the lateral thoracic fascia (black arrow) to undergo revascularization (1). Two weeks later, the allograft was orthotopically transplanted (red arrow-2). In parallel with the heterotopical implantation, a full-thickness skin patch of the donor was grafted on the external ear of the recipient to serve as an external monitor of the rejection process.

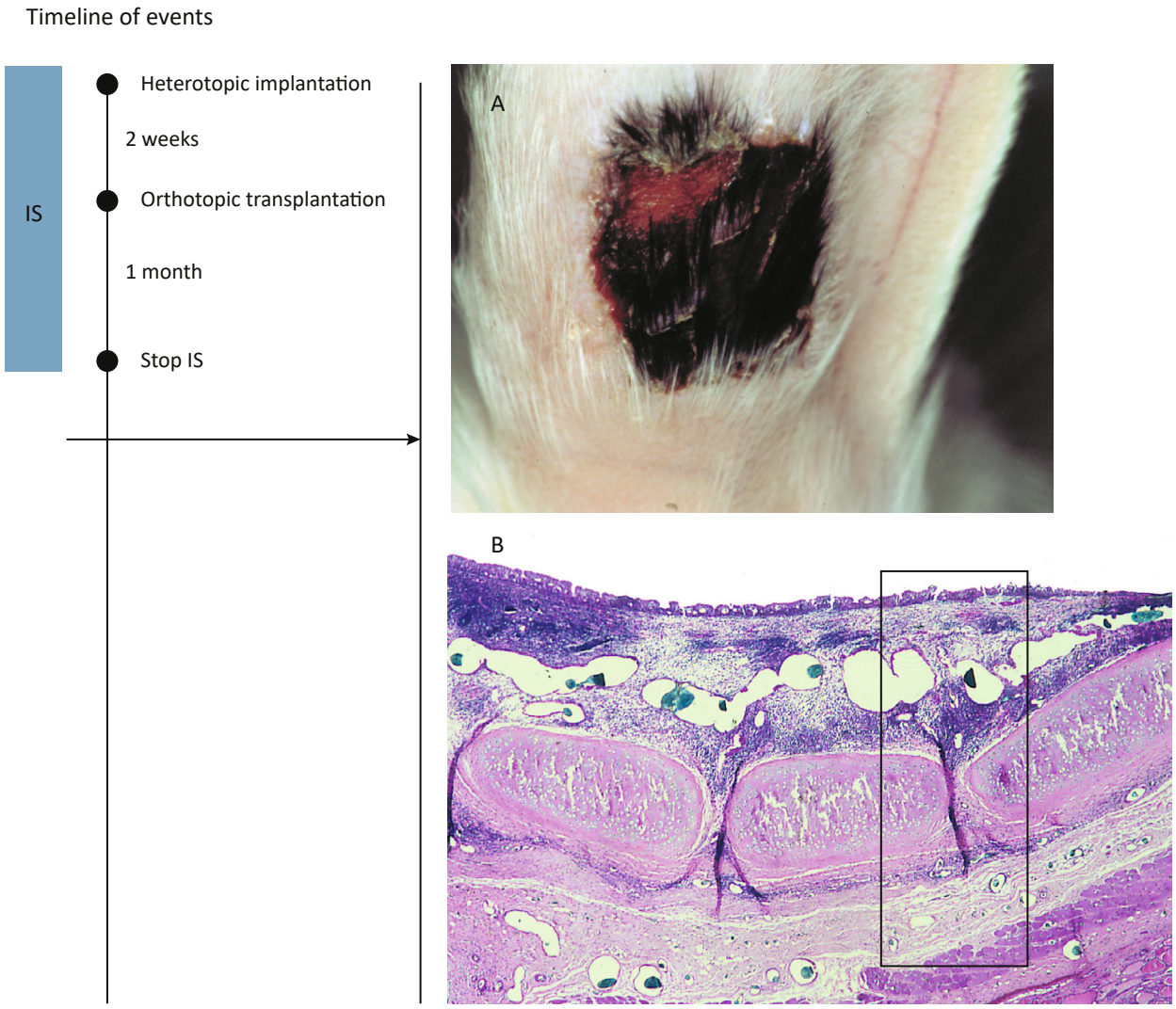


Figure 3.18. Time table for acute rejection of an tracheal allotransplant.
Shortly (1-2 weeks) after immunosuppressive medication (IS) was discontinued, both the skin graft (A) and the tracheal allotransplant (B) showed signs of immunologic rejection. (B) A histologic image of hematoxylin and eosin staining of the tracheal wall shows lymphocytic rejections occurring at both submucosal and adventitial sites of the transplant. An enlarged view of the outlined area is shown in Fig. 3.19.

Acute tracheal allograft rejection is characterized by immunologically-induced lymphocytes attacking the microcirculation of a transplant. Rejection leads to thrombosis of donor-derived blood vessels and necrosis of the mucosal layer (Fig. 3.18). Approximately 10 days after short-term immunosuppressive treatment was discontinued, respiratory distress occurred.

Rejection studies conducted with immunosuppressed rabbits have demonstrated that the trachea is subject to the same immunologic conditions as all other allogenic tissues. Furthermore, the most important component of tracheal rejection is cell-mediated and the targeted cell population is the allograft endothelium.

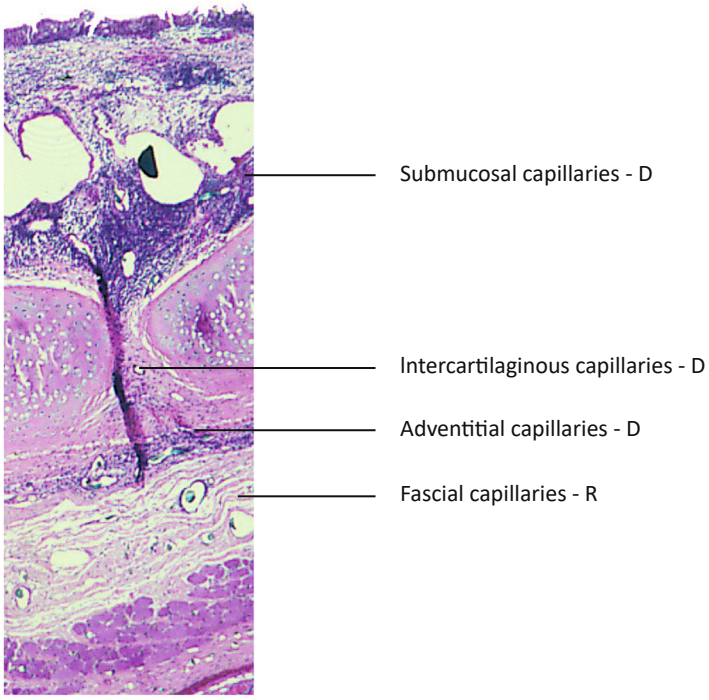


Figure 3.19. Histology of acute rejection of a tracheal allotransplant.
Mucosal, intercartilaginous, and adventitial blood vessels and capillaries were attacked by lymphocytes during acute rejection of a tracheal allotransplant. Meanwhile, fascial blood vessels and capillaries from the recipient (R) were unaffected. D – donor (H&E).

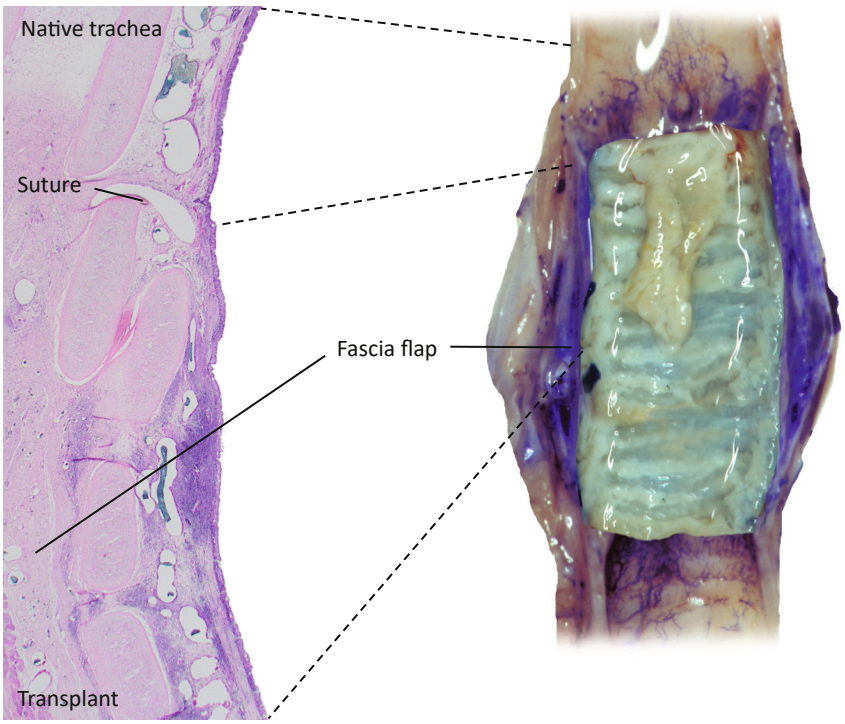


Figure 3.20. Morphology of acute transplant rejection.
Left: Histology (H&E) of a transplant 8 days after short-term immunosuppressive treatment was discontinued. An infiltration of mononuclear cells is observed near the donor endothelium in the submucosal, intercartilaginous, and adventitial spaces of the transplant. Meanwhile, blood vessels from the recipient in the native trachea and fascia remained unaffected.

Right: Macroscopic view of the transplant 14 days after immunosuppressive treatment was discontinued. Full mucosal necrosis is observed, while the fascia flap around the tracheal transplant is viable.

Acute rejection studies have clearly demonstrated that all of the capillaries in the submucosal, intercartilaginous, and adventitial spaces of a transplant derive from the donor. Hence, initial revascularization involves inosculation between fascial blood vessels of the recipient and adventitial blood vessels of the donor. Revascularization of the tracheal wall is induced from the surrounding fascia, yet it does not involve angiogenesis or the outgrowth of recipient blood vessels into donor tissues.

3.4.3. **Clinical tracheal allotransplantation**

Revascularization of the trachea is the first step towards a successful tracheal transplantation. Currently, the only reliable method for achieving tracheal revascularization is to wrap an isolated trachea with a well-vascularized soft tissue flap that is perfused by a vascular pedicle. This may allow transfer of the revascularized trachea to an airway defect. In Figure 3.21, we provide an overview of our initial clinical concept (2008) for heterotopic revascularization with a radial forearm flap [38].

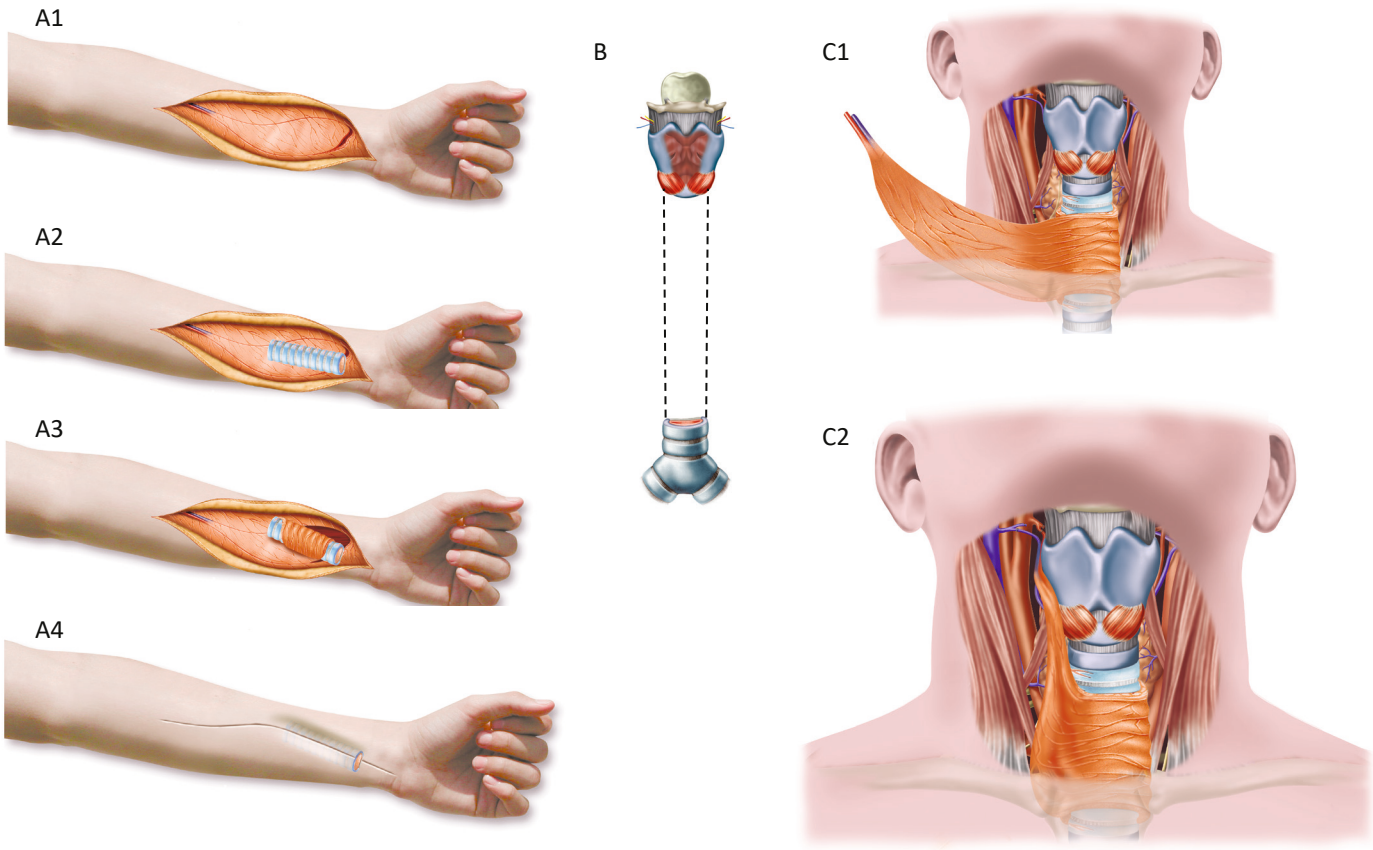


Figure 3.21. Initial concept of clinical tracheal allotransplantation after revascularization with radial forearm fascia.
A blood-group compatible trachea was immediately harvested from a heart-beating donor and directly implanted into the forearm of an immunosuppressed recipient (A1-A4). Our goal was to obtain full revascularization of the trachea in order to perform a tracheal allotransplant and reconstruct a circumferential tracheal defect (B). Orthotopic transplantation may be attempted by suturing the radial blood vessels to the blood vessels in the recipient's neck (C1, C2) if the heterotopic allograft revascularization has been successful.

A sternotomy approach during lung procurement allowed a tracheal segment to be taken from a blood group-matched (heart beating) donor. To resolve a long tracheal stenosis, an 8-cm-long donor trachea was placed with the appropriate orientation on subcutaneous tissue dissected from a recipient's left forearm. The allograft was circumferentially wrapped with fascia and subcutaneous tissue to achieve revascularization (Fig. 3.22). Immunosuppressive therapy with tacrolimus (to obtain a trough level of 12 to 15 µg per liter), azathioprine (100 mg per day), and corticosteroids (0.4 mg

per kilogram of body weight per day) was begun intravenously and then maintained orally thereafter (tacrolimus, 6 mg per day; azathioprine, 100 mg per day; and methylprednisolone, 4 mg per day).

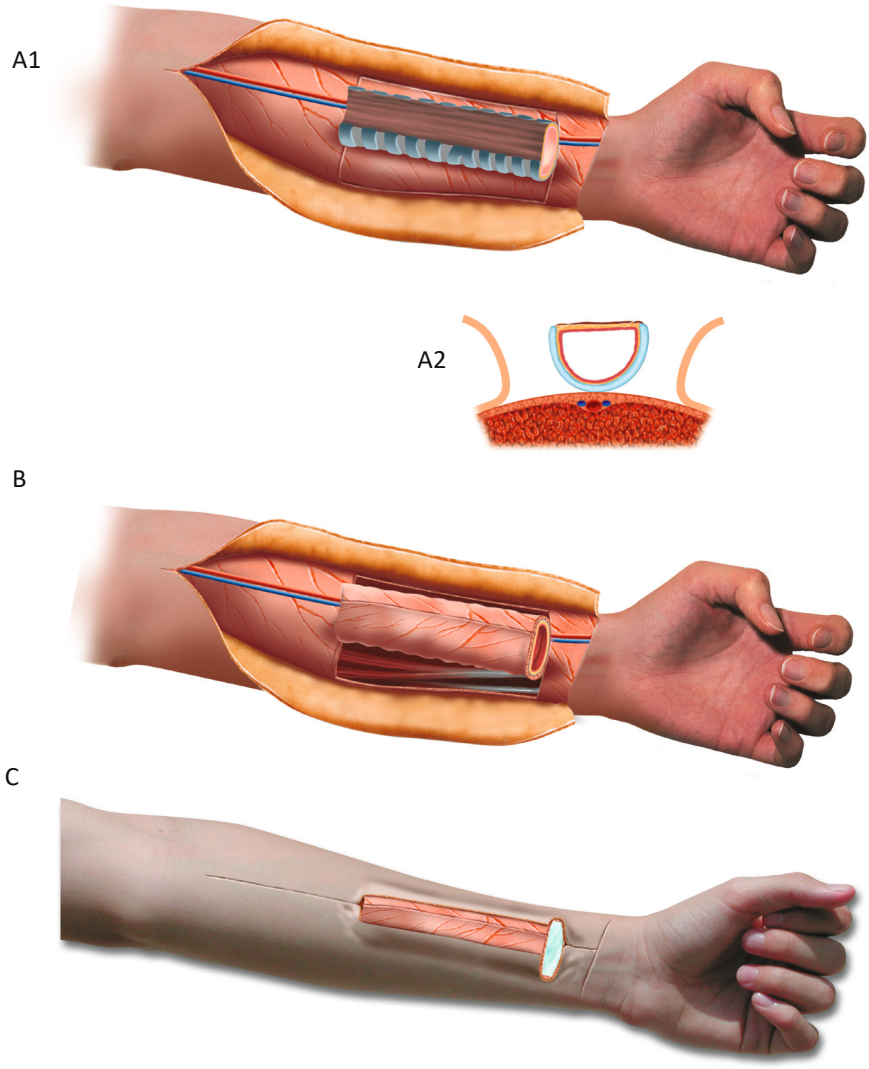


Figure 3.22. Implantation of an allograft in the forearm.
An 8-cm-long tracheal allograft is shown with its cartilaginous portion positioned over the subcutaneous tissue of a recipient's forearm (A1). A cross-section of the avascular allograft is also shown (A2). Subcutaneous tissue and fascia were dissected from the underlying muscles and wrapped around the tracheal allograft to cover the cartilaginous and membranous regions (B). Forearm skin was closed over a fascial-wrapped trachea (C).

Within the first few weeks following implantation, the cartilaginous trachea exhibited early signs of revascularization, although avascular necrosis was observed in the posterior membranous sheath. The necrotic posterior tracheal wall was removed after 2 weeks. Potential mechanisms mediating revascularization of a tracheal allograft are presented in Figure 3.23 [38].

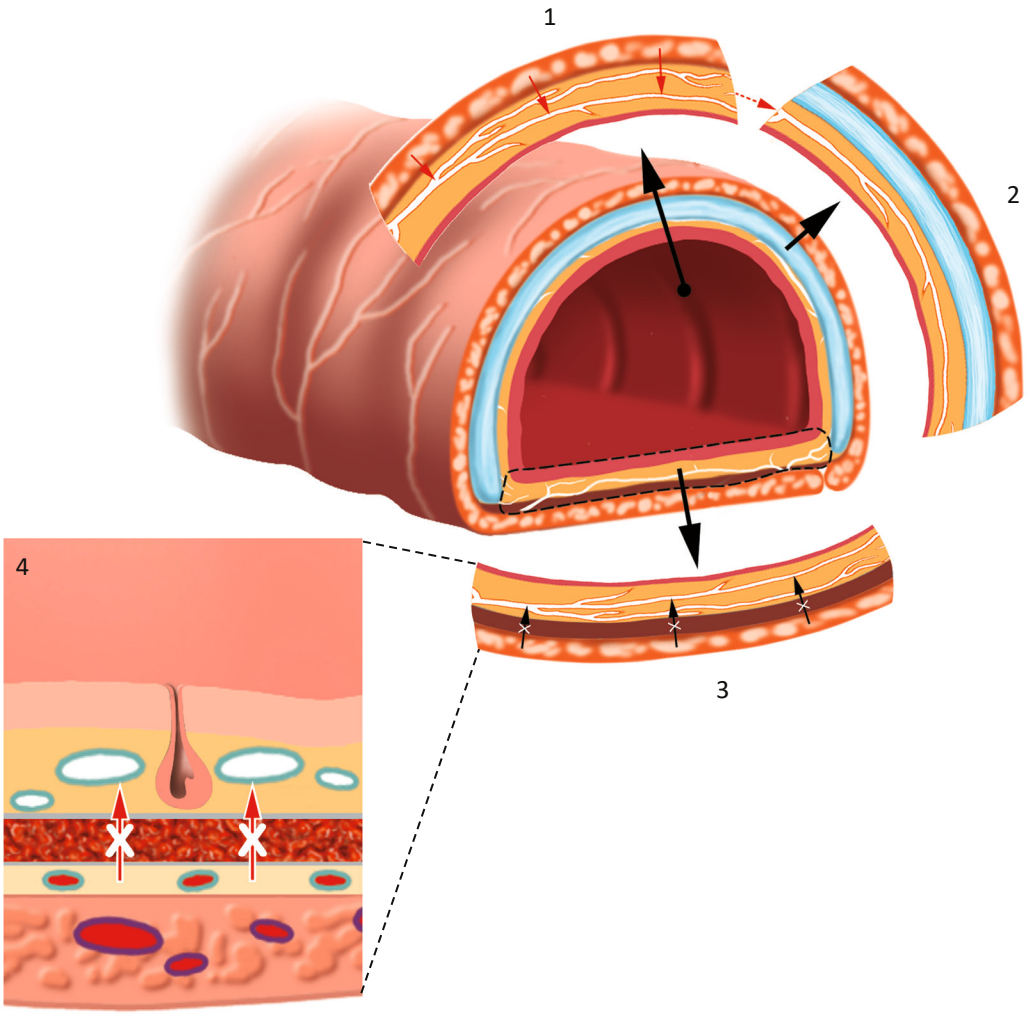


Figure 3.23. Revascularization after circumferential wrapping of a tracheal allograft with forearm fascia.
Cross-sectional views of a cartilaginous trachea at the levels of an intercartilaginous ligament (1), a cartilage ring (2), and the membranous trachea (3). The trachealis muscle prevented vascular induction of the submucosal capillaries and revascularization of the membranous trachea [indicated with arrows in 3 and 4 (enlarged view)]. As a result, necrosis developed. Revascularization of a cartilaginous trachea may be initiated from intercartilaginous spaces (indicated with red arrows). Cartilage is avascular and does not allow revascularization from the fascia. Submucosal capillaries at the cartilage rings also underwent revascularization from surrounding intercartilaginous spaces (indicated with a red dotted arrow).

Despite observations of circumferential revascularization of a trachea in rabbit models (Fig. 3.24), this approach is not optimal for tracheal revascularization in a clinical setting due to the tendency for a membranous trachea to undergo necrosis. However, a cartilaginous trachea can undergo slow and progressive revascularization (Figs. 3.25, 3.26).

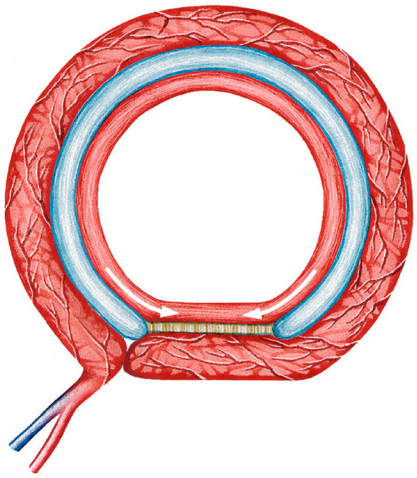


Figure 3.24. Revascularization after circumferential tracheal wrapping in a rabbit model.
In rabbits, the membranous trachea is only a small part of the circumference of the trachea. This smaller dimension facilitates revascularization from surrounding submucosal blood vessels of the cartilaginous trachea (indicated with arrows).

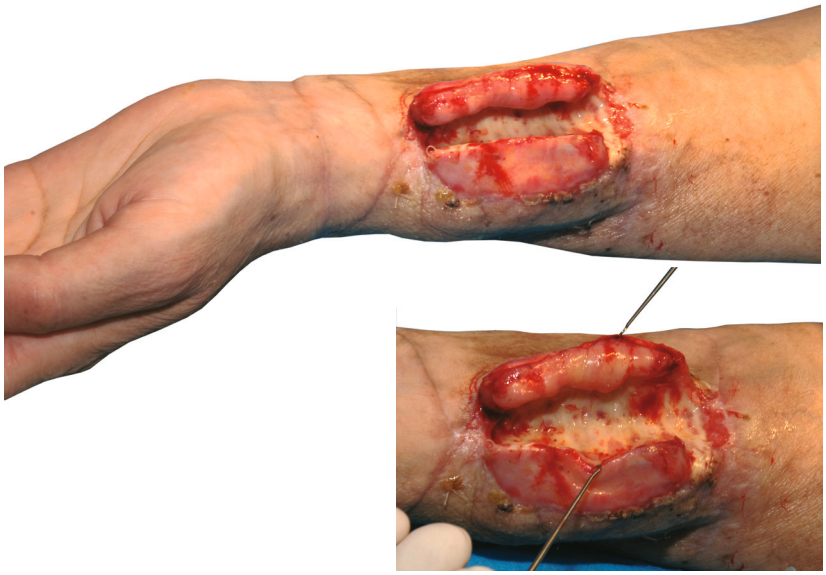


Figure 3.25. A tracheal allotransplant after removal of the membranous region.
Slow and progressive revascularization of a cartilaginous trachea was directly observed three weeks after the trachea was implanted in the forearm.

In Figure 3.26, the revascularization process of a cartilaginous trachea wrapped with recipient fascia is shown. For this process, complete immobility between the trachea and the surrounding recipient's vascular bed is important in order to achieve more rapid revascularization of the blood vessels of the tracheal adventitia (Fig. 3.26). Revascularization will occur when capillary buds exhibit outgrowth from the fascia flap (i.e., the recipient's blood vessels) and contact the blood vessels of the adventitia (i.e., the donor's blood vessels) of the tracheal segment. Inosculation represents the establishment of direct vascular anastomoses between the vascularized fascia flap and the adventitia of the trachea.

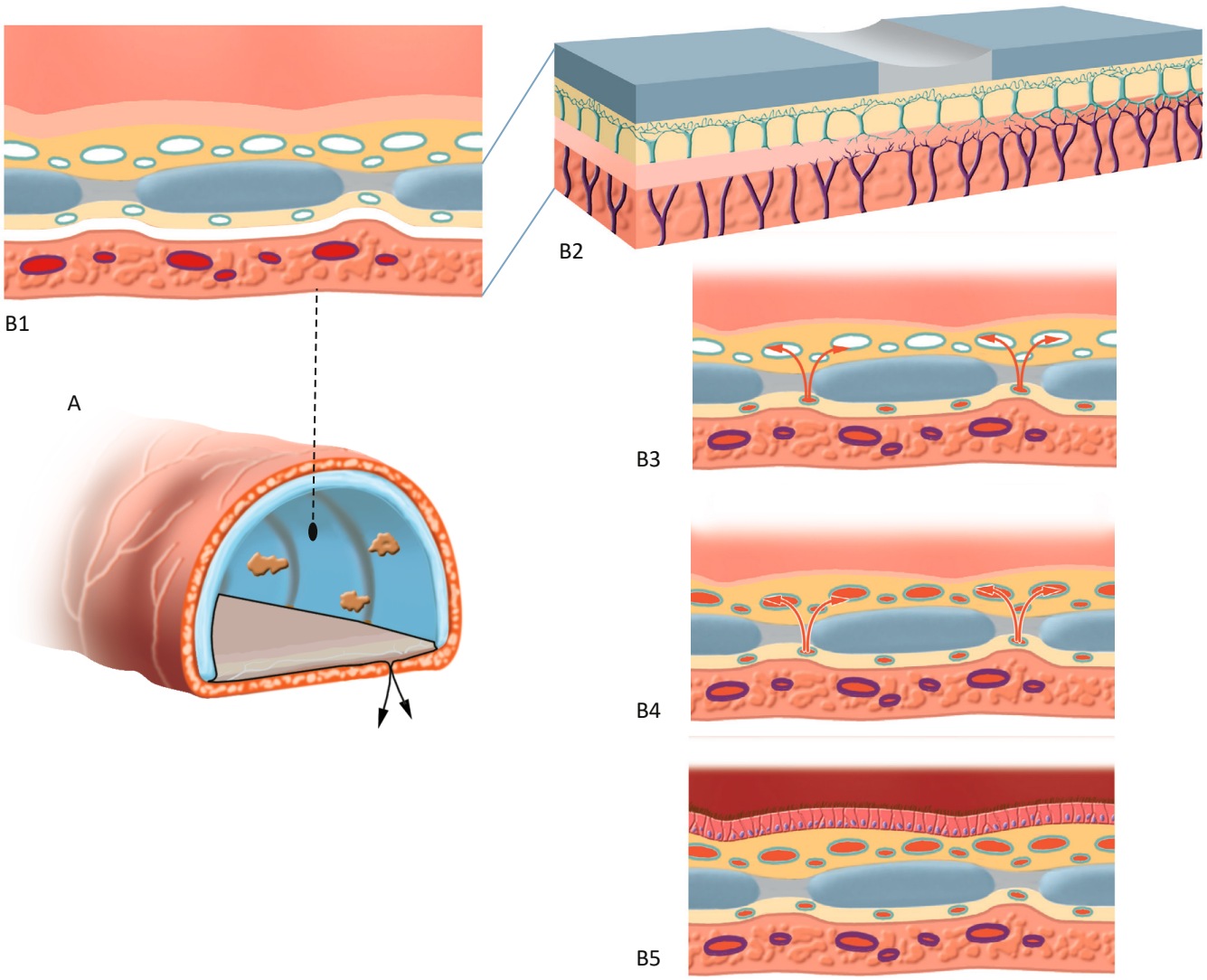


Figure 3.26. Trachea revascularization and mucosal regeneration.
A. A cross-sectional view at the level of a cartilage ring shows a cartilaginous trachea with small areas of early revascularization. In addition, the necrotic membranous trachea has been removed (indicated with a double arrow). Cross-sectional views are also provided immediately after implantation (B1), during revascularization of the adventitial capillaries (B2), after revascularization of the adventitial space and during vascular induction of the submucosal space (B3), and after revascularization of the submucosal blood vessels (B4). Capillaries colored blue derive from the donor while capillaries colored purple derive from the recipient. Regeneration of donor respiratory epithelium also occurs simultaneously during the revascularization process (B5).

Initially, the fascia adheres to the trachea via fibrin. Revascularization is subsequently achieved when the outgrowth of capillary buds from the native vascularized tissue is able to reach the capillaries in the adventitia of the trachea. Concomitantly, the fibrin present is infiltrated by fibroblasts, and this gradually transforms the initial tenuous adhesion of a fibrin clot into a definitive attachment made up of fibrous tissue.

Compared to a free skin graft, there are two additional barriers to revascularization of a cartilaginous tracheal allograft. The cartilage rings and intercartilaginous ligaments may interfere with revascularization of the cartilaginous trachea. In particular, the cartilage rings form a complete barrier. Revascularization of the mucosal layer of an avascular tracheal segment proceeds through intercartilaginous ligaments. Full revascularization and mucosal regeneration of the cartilaginous trachea can be achieved within three months of implantation of a trachea in the forearm.

3.5. **Conclusion**

Currently, there is no safe and dependable method for tracheal replacement. In addition, there are no reliable reconstruction methods for the repair of circumferential tracheal defects (Fig. 3.27). While cartilage reinforced free flaps have been used successfully, the complexity of this procedure and the associated postoperative care make it difficult to reproduce.

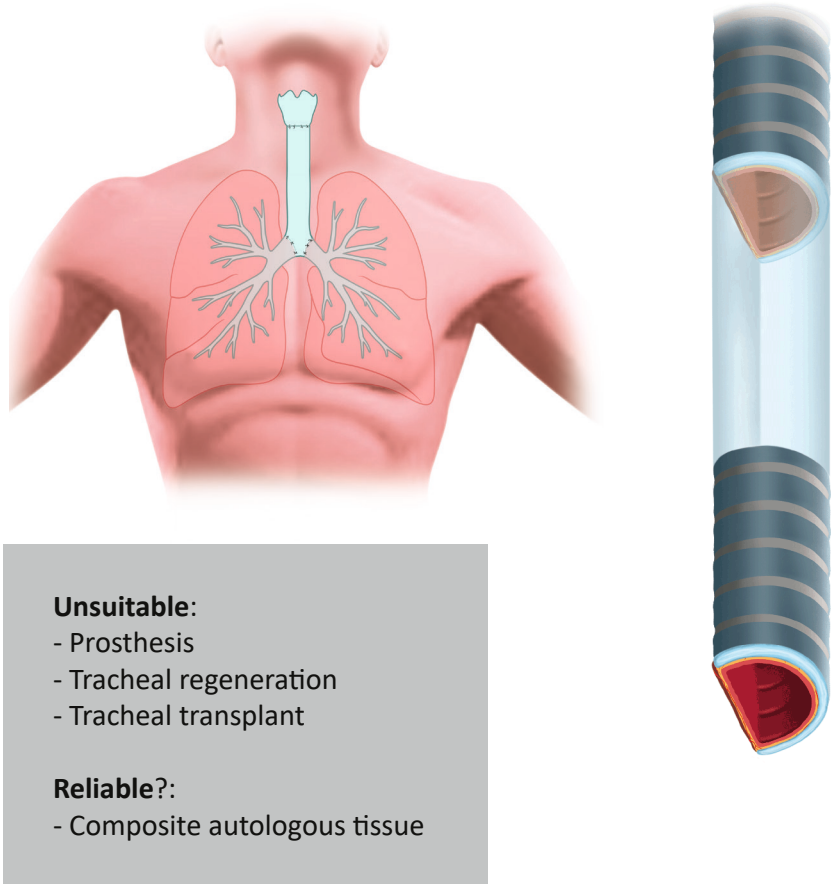


Figure 3.27. Possible clinical applications of circumferential tracheal replacement.
Segmental defects may result after resection of a primary tracheal neoplasm, resection of a long-segment stenosis, and resection of a re-stenosis after segmental resection.

Most cases involving long segment stenosis and re-stenosis after segmental resection can accommodate a longitudinal incision of the stenosis and preservation of a posterior tracheal remnant. In these cases, patch reconstruction with restoration of the concavity of the airway lumen may represent

an optimal solution. Thus, the possibility of performing a tracheal transplant for patch reconstruction has been explored (Fig. 3.28).

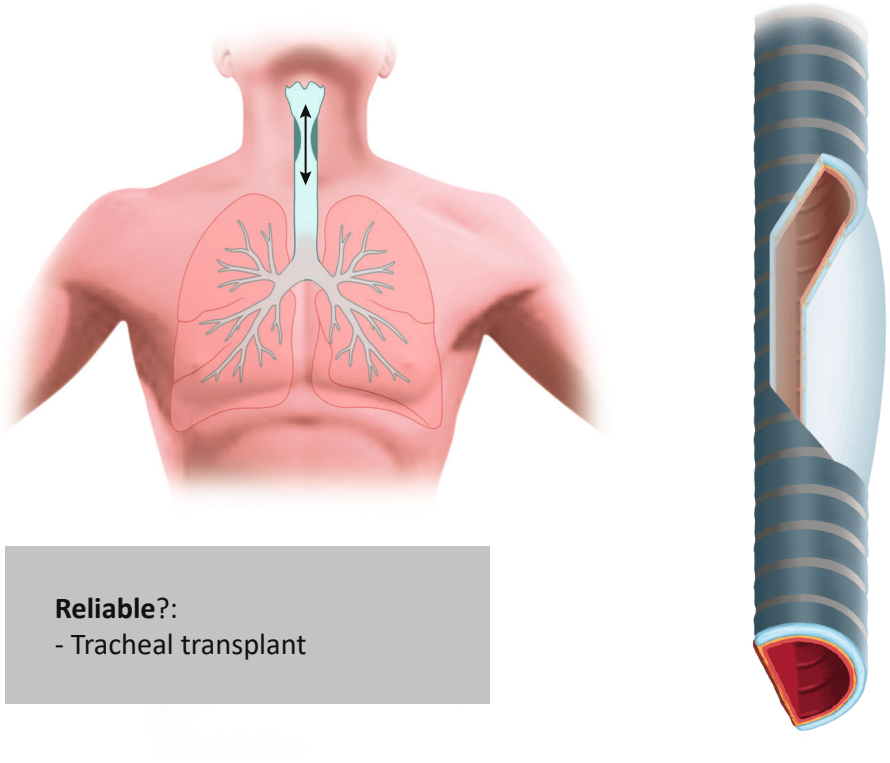


Figure 3.28. Possible clinical applications of patch tracheal replacement.
A patch reconstruction may be necessary after longitudinal incision (double arrow) of a long-segment stenosis and a re-stenosis after segmental resection.

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4. **Optimal reconstructive tissue for patch laryngotracheal repair**

An airway defect may be circumferential after resection of a malignant tracheal tumor. However, the majority of laryngotracheal airway mutilations involve patch defects that result from a longitudinally incised long-segment stenosis or re-stenosis after segmental resection. In these cases, some native airway tissue may remain.

The optimal tissue for repair of a patch laryngotracheal airway defect is a vascularized segment of the cartilaginous trachea which is lined with respiratory mucosa. Preservation of a reliable blood supply to this segment is important, and it is the most difficult factor to address. Currently, the microvascular techniques available cannot directly restore blood supply to a trachea. However, blood supply to a trachea can be restored indirectly by wrapping the trachea in a fascia flap that is well perfused from a vascular pedicle. This allows a revascularized tracheal segment to be transplanted. Another important factor is the respiratory mucosal lining. This ultrathin lining protects the cartilaginous framework of a trachea from undergoing necrosis and this lining will minimally interfere with the luminal surface area of a reconstructed airway.

Both a vascular blood supply and the mucosal lining of a trachea will help preserve the unique characteristics of the tracheal cartilaginous framework during restoration of the cricotracheal airway. The unique characteristics of the cartilaginous framework are attributed to the axial elasticity of tracheal cartilage rings and the longitudinal elasticity provided by intercartilaginous ligaments (Figs. 4.1, 4.2).

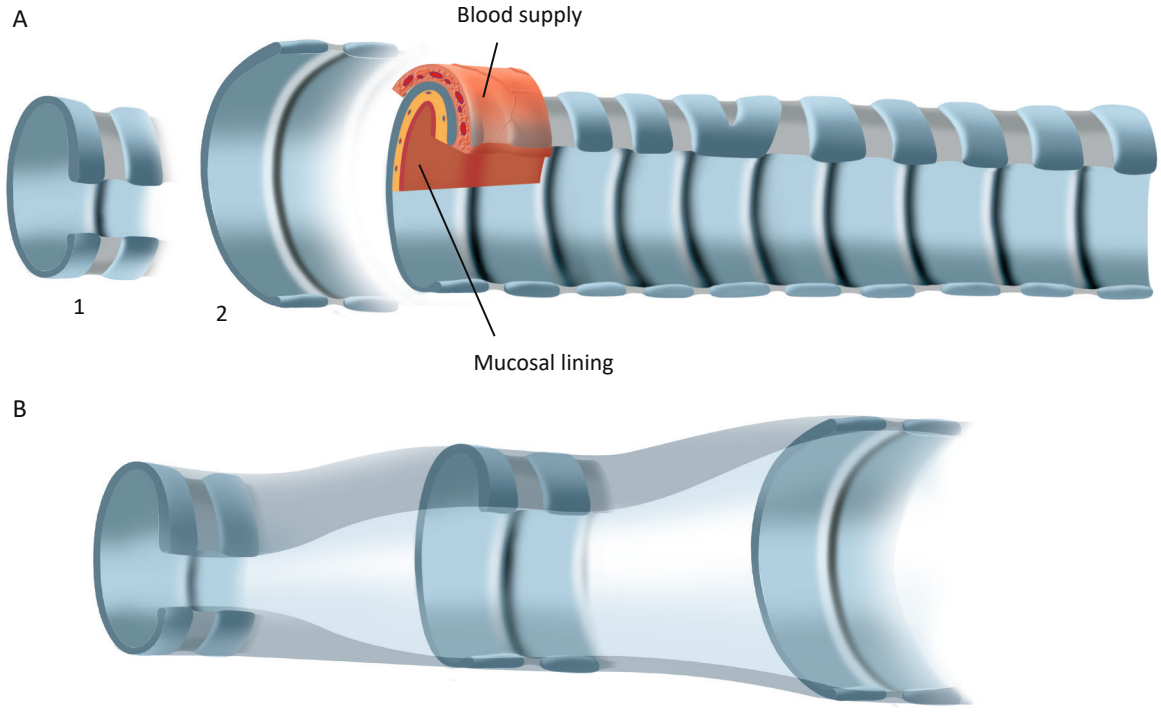


Figure 4.1. The optimal reconstructive tissue for cricotracheal airway repair and cartilage support.
A. Axial elasticity of a cartilaginous trachea is shown with greater (1) or lesser (2) degrees of bending applied to a cartilage ring.
B. Longitudinal elasticity of a cartilaginous trachea. The degree of bending can differ between the two ends of a patch.

The elasticity of cartilage rings in the axial plane can result in more or less convexity of a tracheal patch according to the width of a defect. In addition, longitudinal elasticity of a cartilage patch can lead to differences in convexity at the ends of a patch. The latter is an important consideration in cases where defect reconstructions have different requirements at both sites. For example, extended laryngeal defects can have different requirements at glottic versus subglottic sites (see also chapter 5).

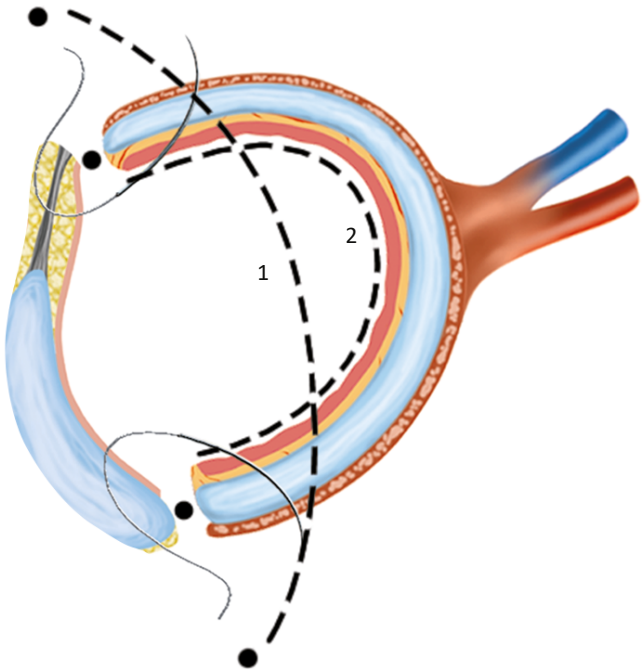


Figure 4.2. Axial elasticity of a revascularized patch of cartilaginous trachea.
The extent of external airway support provided by the reconstructive tissue is indicated with dotted lines. The same tracheal patch will provide less (1) or more (2) convexity, depending on the width of the defect.

Histological (Fig. 4.3) and ultrastructural (Figs. 4.4, 4.5) features of hyaline cartilage may clarify the mechanisms mediating the unique elastic characteristics of the cartilaginous framework of the trachea.

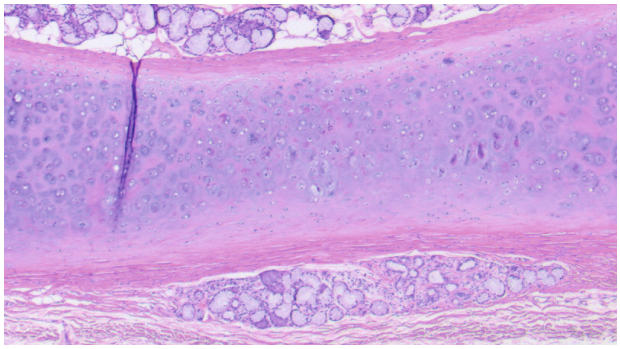


Figure 4.3. Histology (H&E) of hyaline tracheal cartilage.
Tracheal hyaline cartilage is enclosed by a layer of dense connective tissue, the perichondrium, which is essential for cartilage growth. This connective tissue investment is rich in fibroblasts, undifferentiated mesenchymal cells, blood vessels, and nerves. Chondrocytes are flattened near the perichondrium and more round in deeper regions. Collagen fibers, water, and ground substance constitute the matrix. Basophilia of this cartilage is due to the presence of glycosaminoglcans (GAGs), such as chondroitin sulfate, in ground substance. Damaged hyaline cartilage is unable to be repaired because in the adults the chondrocytes cannot undergo mitosis.

Chondrocytes in hyaline cartilage are highly specialized cells which synthesize and maintain all of the components of the extracellular matrix. As a result, the extracellular matrix has a unique and highly ordered molecular organization. A total of 60–70% of the wet weight of hyaline cartilage derives from water, and both water and inorganic salts provide the resilience and lubricating capabilities of this cartilage. The remaining constituents include structural macromolecules such as collagens, proteoglycans (PGs), and noncollagenous proteins. Type II collagen accounts for 90–95% of the collagen present in hyaline cartilage, and it forms a fibrillar meshwork that mainly provides tensile strength and shape. PGs that are present in the matrix are negatively charged. Consequently, these PGs attract large numbers of positively charged water ions. PGs are also composed of a core protein with complex carbohydrates (i.e., GAGs, glycosaminoglycans) that radiate from this core, much like bristles radiate from a brush. GAGs consist of repeating, negatively-charged disaccharide units of various lengths that may be sulfated or not. The former, mainly chondroitin sulfate, dermatan sulfate, and keratan sulfate, attach noncovalently to hyaluronic acid, a non-sulfated GAG, to form large PG aggregates known as aggrecans.

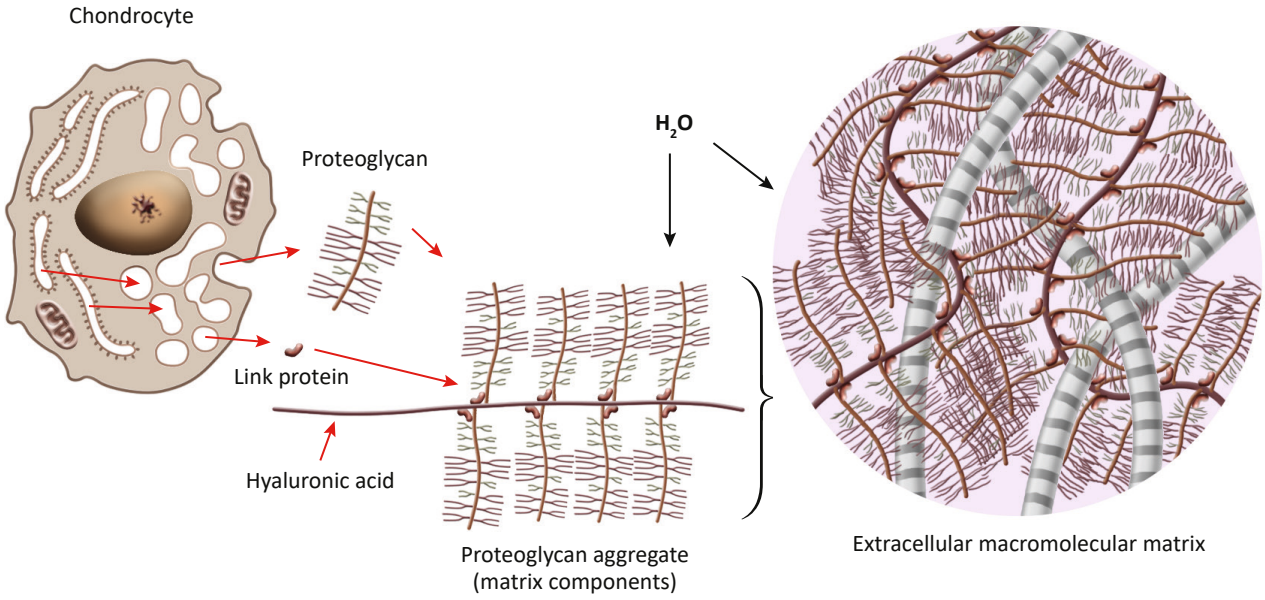


Figure 4.4. Elasticity of hyaline cartilage.
Chondrocytes synthetizes and secretes both type II collagen and proteoglycans (PGs), and these interact to form a hydrated macromolecular matrix of cartilage.

Interactions between aggrecan molecules, water molecules, and the collagen fibril network form the basis for the resistance of hyaline cartilage to compression and its resilience.

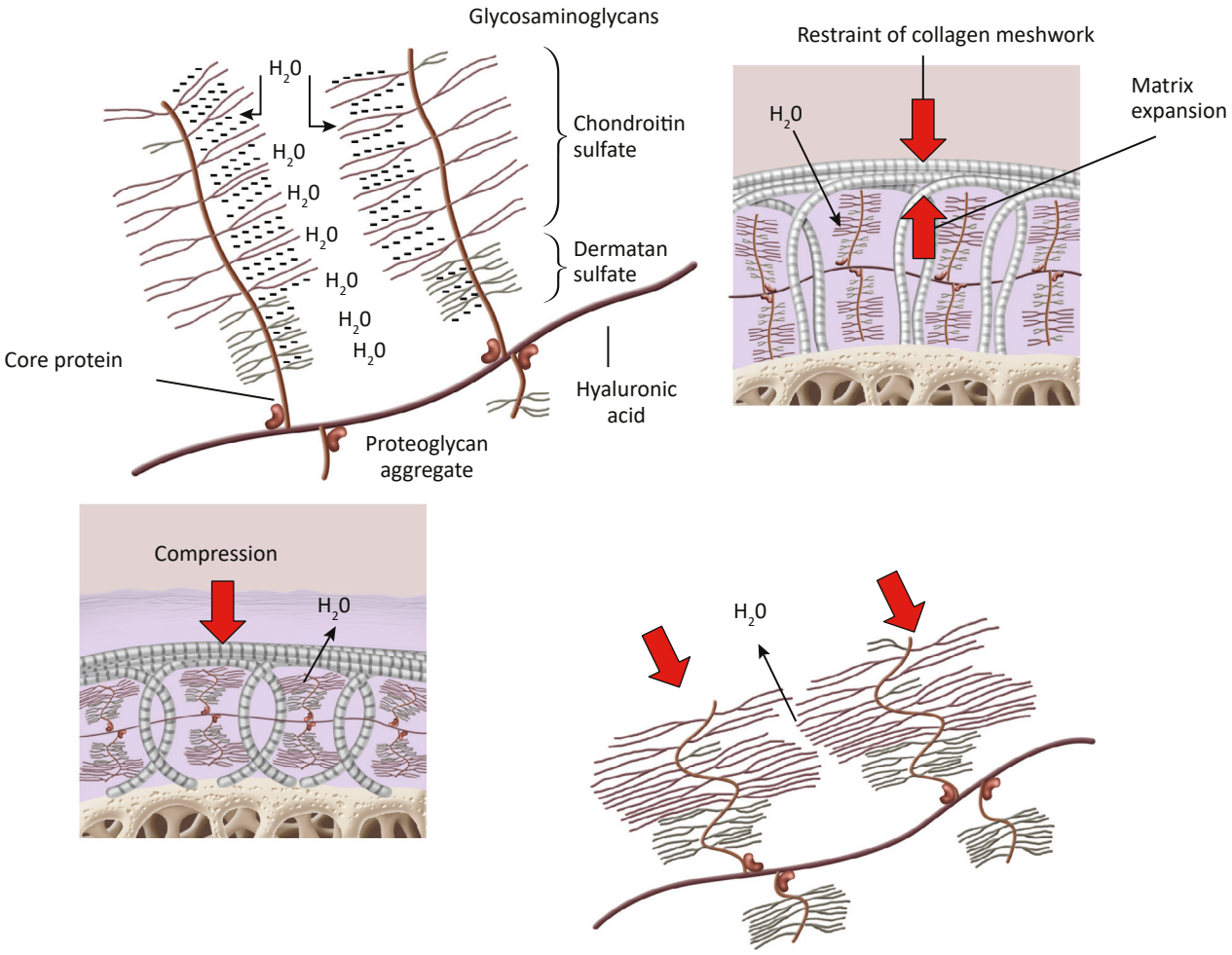


Figure 4.5. Elasticity of hyaline cartilage (continued).
Negatively charged GAG side chains repel one another and attract water, thereby leading to an increase in matrix volume. However, this expansion is limited by the collagen meshwork in hyaline cartilage. Conversely, matrix compression pushes GAG side chains together to release water molecules and decrease matrix volume. Decompression allows re-expansion of molecules and matrix volume.

The most obvious airway defect that can be reconstructed with a vascularized cartilaginous tracheal patch is an extended hemilaryngectomy defect (Fig. 4.6). This defect includes only half of the original cricoid cartilage due to resection of a lateralized T2b-T3 glottic cancer or a lateralized cricoid chondrosarcoma. In this situation, a 4-cm segment of cartilaginous trachea can be used as reconstructive tissue if this segment can be successfully transplanted with a preserved blood supply [1].

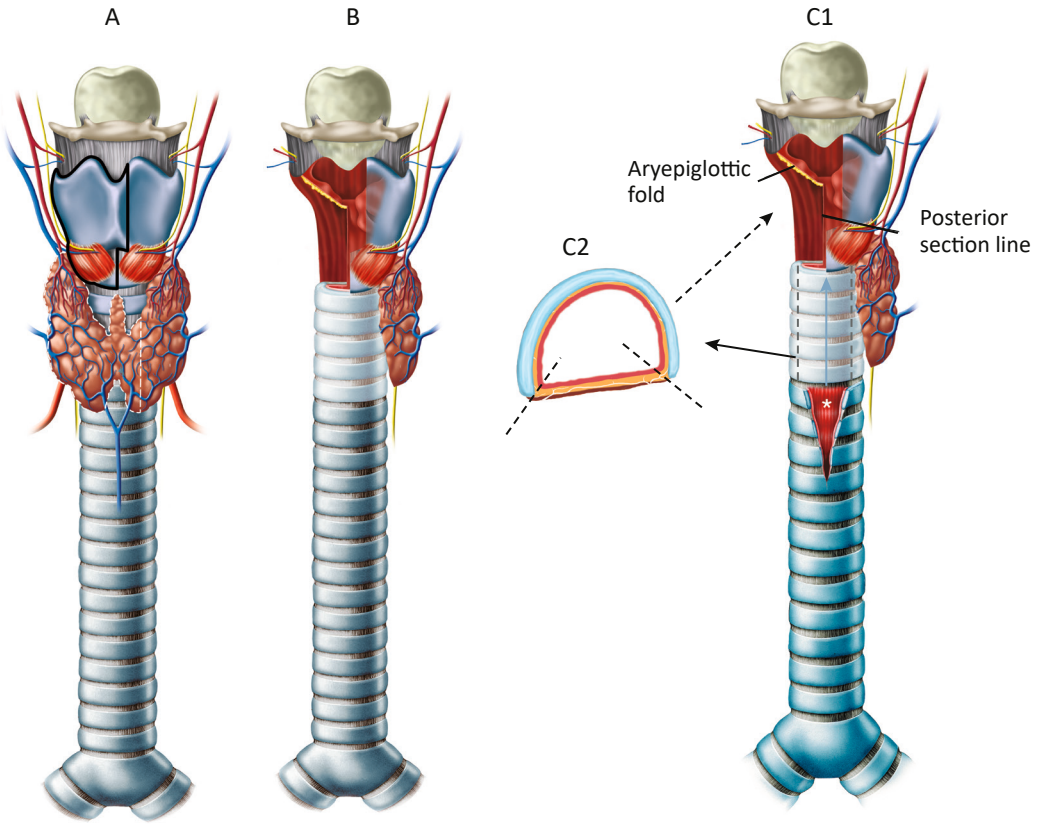


Figure 4.6. Extended hemilaryngectomy defect.
A. A laryngeal defect due to removal of a lateralized tumor with subglottic extensions is outlined. B. An extended hemilaryngectomy defect is shown that includes half of the cricoid cartilage. The upper 4 cm of the cervical trachea could be used as 'optimal reconstructive tissue' for its repair. C. A total of 4 cm of the cartilaginous cervical trachea is isolated (C1, indicated with a black arrow) to be used as a patch to restore the airway at the glottic and subglottic levels (indicated with a dashed arrow). The remaining trachea can be pulled upwards (indicated with a blue arrow) towards the reconstructed larynx. A temporary tracheostomy (indicated with an asterisk) is maintained. A tracheal patch can be placed inside the laryngeal defect if its blood supply is preserved (Fig. 4.7).

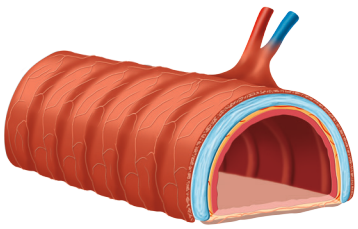


Figure 4.7. A vascularized cartilaginous tracheal patch.
For transfer of a segment of trachea to a laryngeal defect, it is necessary to provide a blood supply for the segment with a flap perfused by a vascular pedicle which allows a microvascular transfer.

References

1. Delaere PR, Hermans R. Tracheal autotransplantation as a new and reliable technique for the functional treatment of advanced laryngeal cancer. Laryngoscope 2003;113:1244-1251.

5. Orthotopic revascularization and tracheal autotransplantation

5.1. Introduction

In 1998, we started testing the concept of tracheal autotransplantation in clinical practice for the treatment of one-sided tumors of the larynx. Since then, tracheal autotransplantation has been developed as a technique to reconstruct a functional larynx in patients undergoing extended hemilaryngectomy for selected laryngeal tumors (Fig. 5.1).

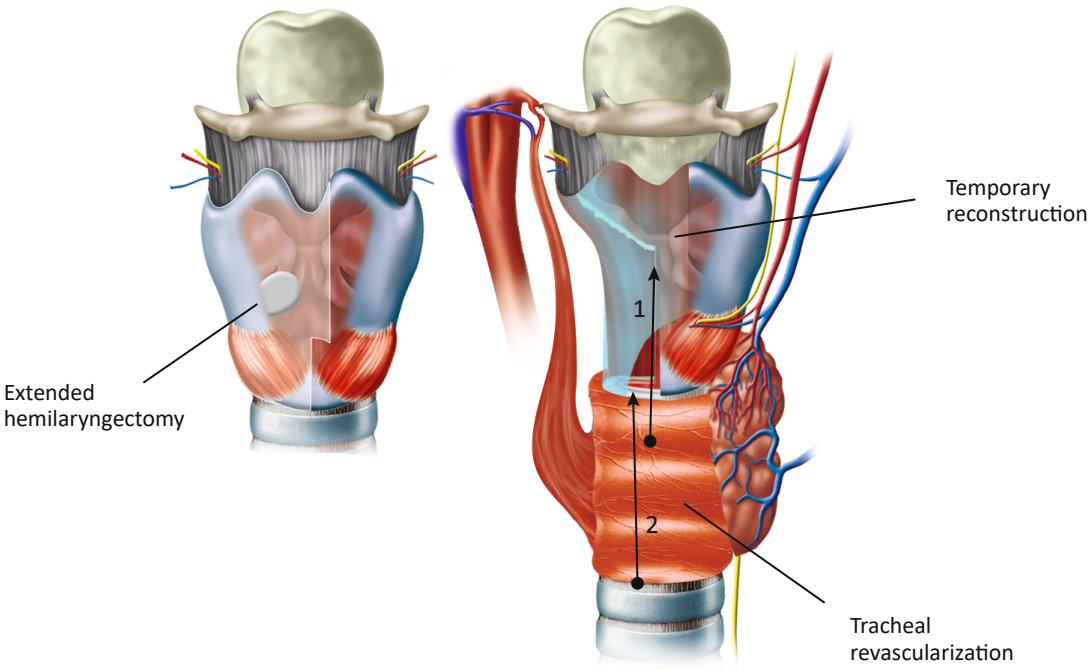


Figure 5.1.Principle of tracheal autotransplantation.
Left: Illustration of an extended laryngectomy defect due to a lateralized tumor.
Right: Overview of the tracheal autotransplantation procedure. Briefly, the tracheal segment is transplanted into an extended hemilaryngectomy defect in two operations. In the first operation, tumor resection and orthotopic revascularization are performed. Tracheal revascularization is achieved by wrapping the trachea with forearm fascia. The radial blood vessels are sutured to blood vessels in the neck. Tracheal autotransplantation (1) is subsequently performed two months later. Tracheal continuity is restored with upward mobilization of the mediastinal trachea (2).

In patients with advanced unilateral chondrosarcomas of the cricoid and or unilateral glottic squamous cell carcinoma (SCC) with subglottic extension (Fig. 5.2), involvement of the cricoid necessitates a more aggressive surgical resection than can be achieved with classical partial laryngectomy procedures. Previously, such patients had no other choice than to undergo a total laryngectomy. However, the development of a tracheal autotransplantation method allows a hemilaryngectomy to

achieve complete tumor resection, while an autotransplant aims to preserve laryngeal speech, prevent a permanent tracheostomy, and maintain swallowing function.

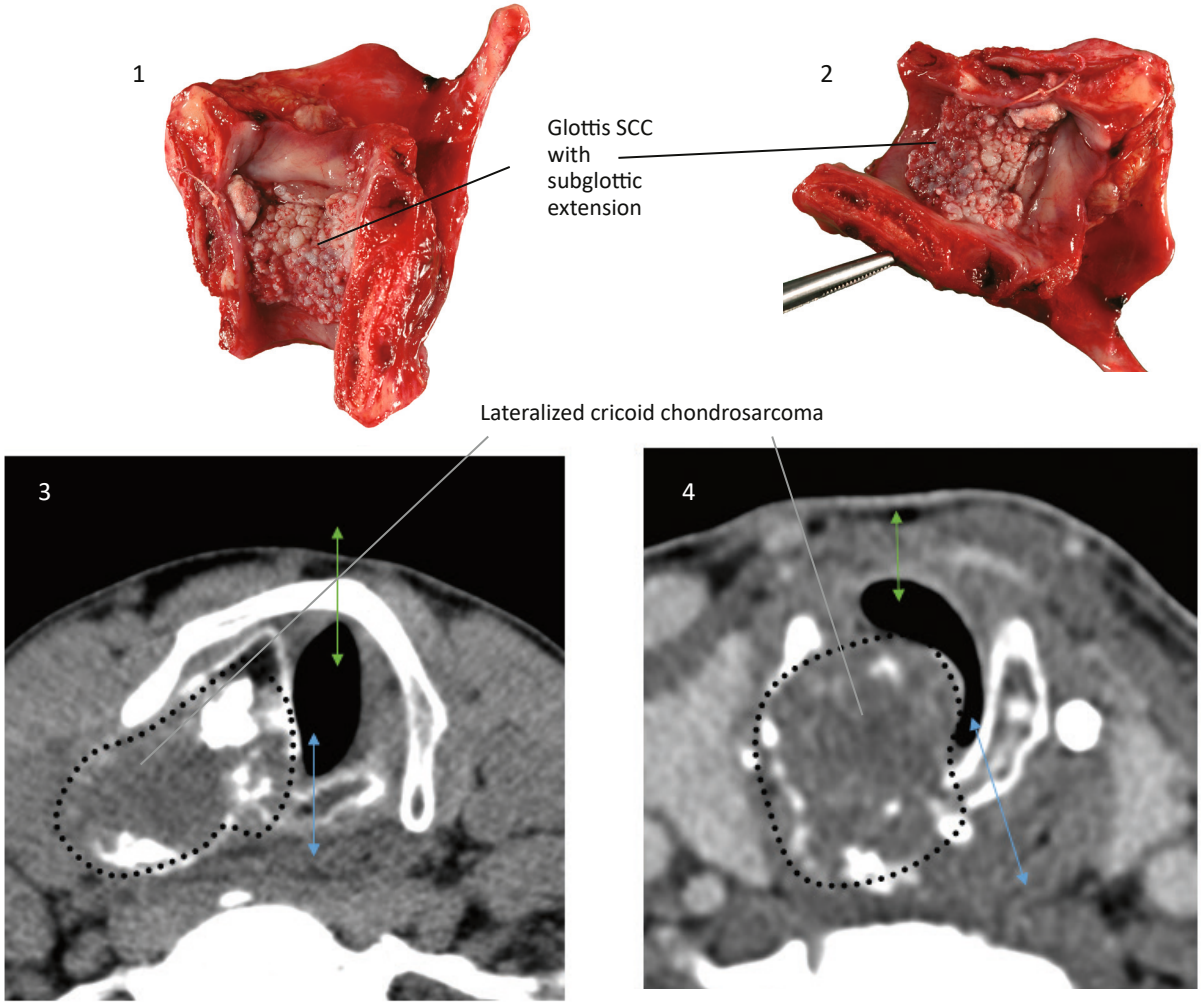


Figure 5.2. Suitable tumors for tracheal autotransplantation.
Upper panel (1, 2): A resected specimen of a glottic SCC with subglottic extension. On the tumor side, the full height of the cricoid cartilage requires resection. If the tumor reaches the midline anteriorly, the anterior commissure is resected.
Lower panel: An axial CT image of a cricoid chondrosarcoma at the glottic (3) and subglottic (4) levels. The dotted outlines indicate tumor extension. Anteriorly, an incision is made at the midline (indicated with green double arrow). A lateralized cricoid chondrosarcoma can be resected with close margins. Here, the tumor reaches the posterior midline and a posterior incision is made 1 cm over the midline on the contralateral site (indicated with blue double arrows).

Over the last few decades, there has been considerable interest in larynx preservation strategies for the treatment of laryngeal SCC. Correspondingly chemotherapy, radiotherapy, chemoradiotherapy, and transoral laser microsurgery have been increasingly used [1]. However, these organ-sparing strategies have significant limitations. For example, transoral laser microsurgery is primarily indicated

for the treatment of early stage carcinoma of the glottis (without compromised vocal fold mobility and without subglottic extension). However, for resection of more advanced stage glottic SCC, and especially for salvage resection of radiorecurrent glottis SCC, transoral laser microsurgery yields inferior local control rates [2]. Similarly, for the treatment of cricoid chondrosarcomas, the use of radiotherapy and chemotherapy has not proven successful. For such patients, tracheal autotransplantation offers a valuable alternative to total laryngectomy.

The first human tracheal autotransplantation was performed in 1996 following extensive preclinical testing (see 5.6, Our initial tracheal autotransplantation approach). Based on this promising early work, we designed an optimized reconstructive autotransplant approach which involves a two-step procedure. Initially, a segment of the cervical trachea is orthotopically provided with an independent vascular supply. Then, the trachea is used as an autograft to reconstruct a surgical hemilaryngectomy defect [3-7]. After implementing this adapted technique at our center in 2003, we have now treated a series of more than 30 patients with advanced cricoid cartilage chondrosarcoma or glottic SCC [7].

5.2. **Tumor resection and restoration of sphincteric function**

Suitable tumors for resection include glottic SCC stage T2-T3N0 and lateralized cricoid chondrosarcomas, both of which cannot be treated with an endoscopic approach. The procedure starts with a lateral neck dissection (levels II,III, and IV) and includes an ipsilateral thyroidectomy (Figs. 5.3 - 5.6) (Movie 2, 3).

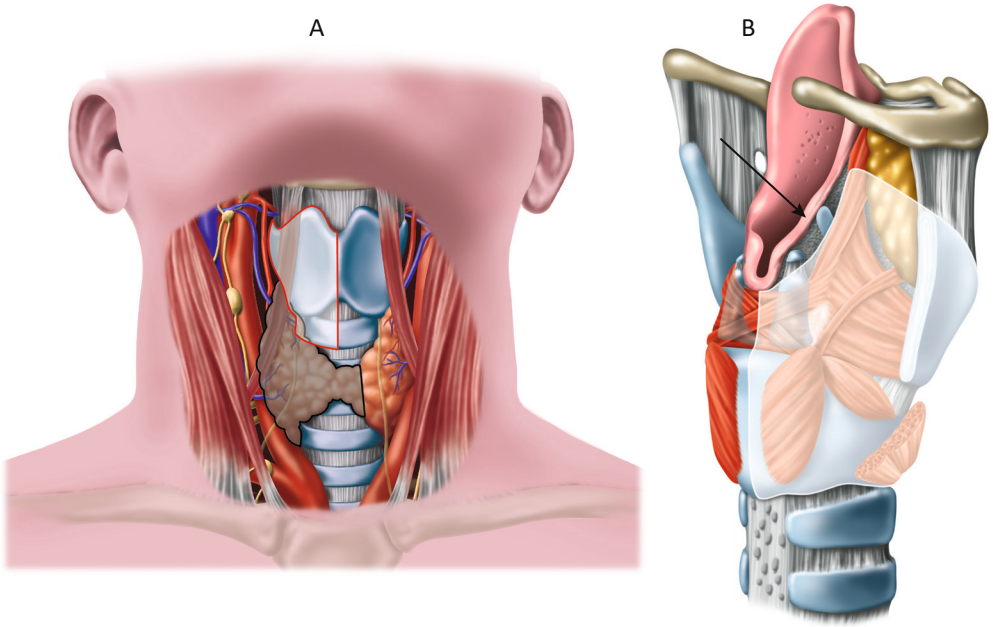


Figure 5.3. An illustrated overview of tumor resection.
A. Frontal view. The amount of larynx included in the resection is indicated with a red line. One cm of the contralateral thyroid cartilage may also be included in the resection. Briefly, an anterolateral neck dissection will be performed, followed by ipsilateral removal of the thyroid gland, the recurrent nerve, and the tracheoesophageal lymph nodes (indicatad with a black line).
B. Lateral view. The aryepglottic fold (labeled with a black arrow) will be preserved.

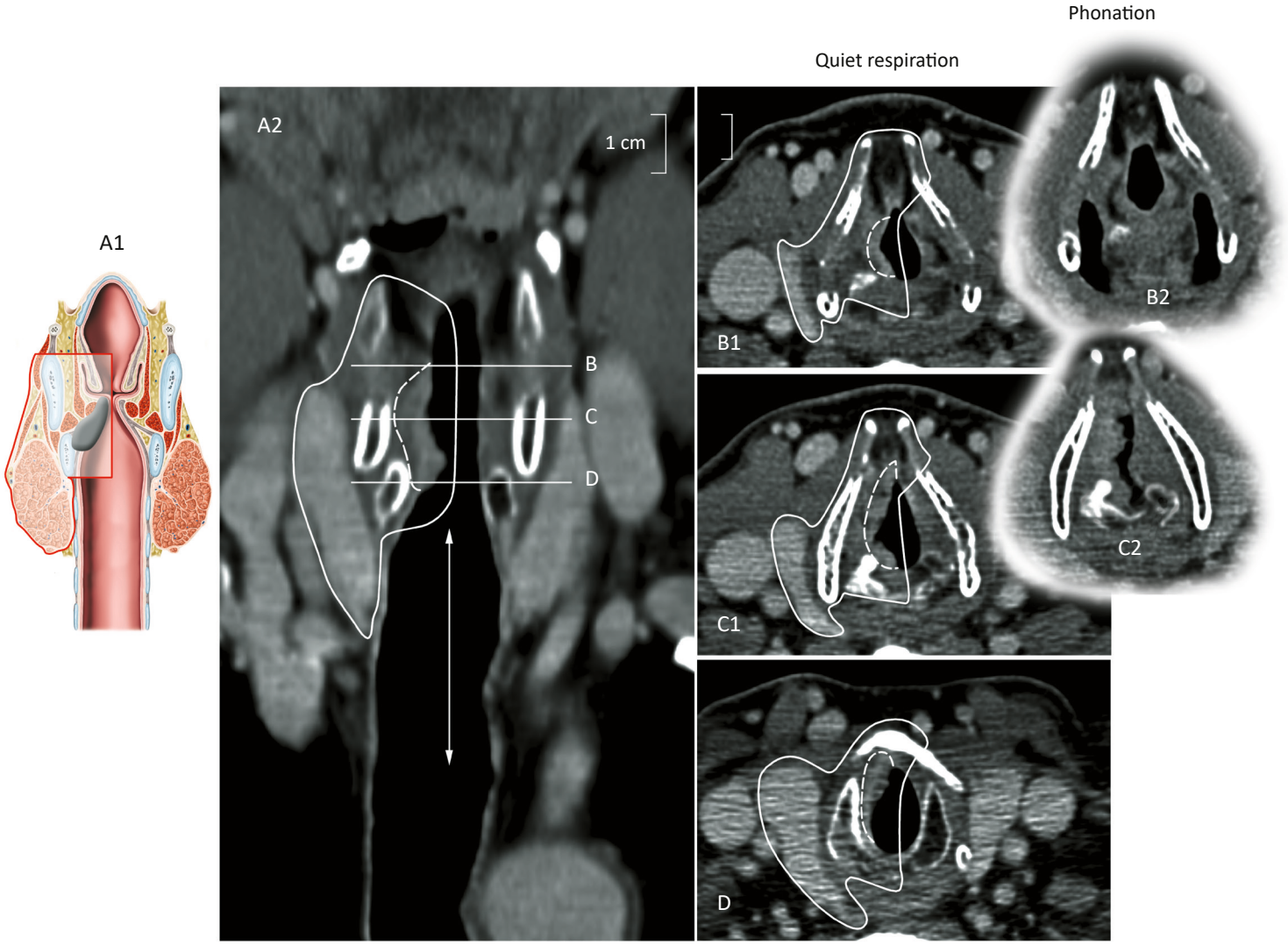


Figure 5.4. CT views of a right T3 glottic SCC with subglottic extension and its resection. A. A coronal view illustration of a right T3 glottic SCC with subglottic extension. The area outlined in red (A1) and white (A2) represents the extent of tumor resection. The dotted white line represents the tumor to be resected. The double arrow indicates the extent of cervical trachea to be used for reconstruction. B. Axial CT scan at the supraglottic level during quiet respiration (1) and during phonation (2). The airway lumen size during quiet respiration is identical to the airway lumen size during phonation. C. Axial CT scan at the glottic level during quiet respiration (1) and during phonation (2). The airway lumen size during quiet respiration is more than double the size of the airway lumen during phonation. D. Axial CT scan at the subglottic level. The area resected to remove a right T3 vocal fold cancer is outlined in white.

On the preoperative CT scan, laryngeal closure is observed at the glottic level during prolonged phonation of "i" on a CT scan performed during quiet respiration and phonation (Fig. 5.4).

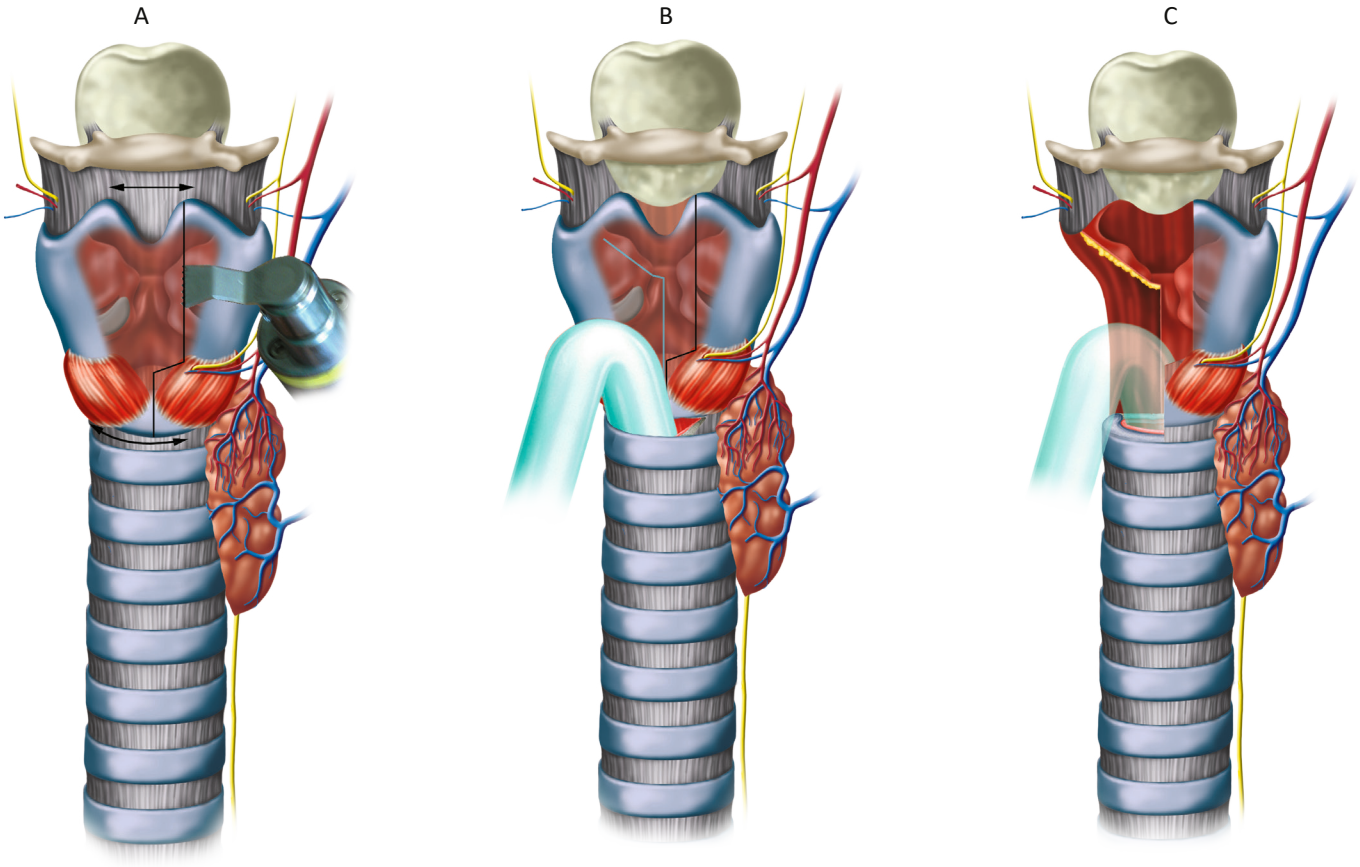


Figure 5.5. An illustrated overview of the steps to resect a right glottis SCC with subglottic extension.
A. An incision is made in the thyrohyoid and cricothyroid ligaments (indicated with two double arrows). If the SCC reaches the anterior commissure, the anterior commissure may be included in the resection as well (indicated with a black line in A and B).
B. An incision is made at the midline of the larynx at the posterior commissure (indicated with a blue line). An intubation tube is placed in an incision made in the cricotracheal ligament.
C. After tumor resection, the upper margin of the aryepiglottic fold at the tumor site is preserved (shown in yellow).

Tracheal autotransplantation allows for conservation laryngectomy for unilateral glottic cancer with vocal fold fixation and infraglottic tumor extension reaching the upper border of the cricoid cartilage, two major contraindications for all 'classic' conservation procedures. An extended hemilaryngectomy may be utilized for unilateral T2-T3 glottic cancer with posterior subglottic extension greater than 5 mm yet without extension into the ventricle (Fig. 5.6). Furthermore, the resection can be extended towards the anterior third of the contralateral vocal fold for tumors which reach the anterior commissure. The aryepiglottic fold, which can safely be preserved, subsequently plays a an important role in restoration of sphincteric laryngeal function. It also allows a posterior midline reconstruction to be performed at the glottic and supraglottic levels. Sphincteric function after resection is then restored by bringing the epiglottic remnant down to the level of the vocal fold and by closing the gap between the aryepiglottic fold and the epiglottis at the side of resection (Figs. 5.7, 5.8, 5.9) (Movie 4).

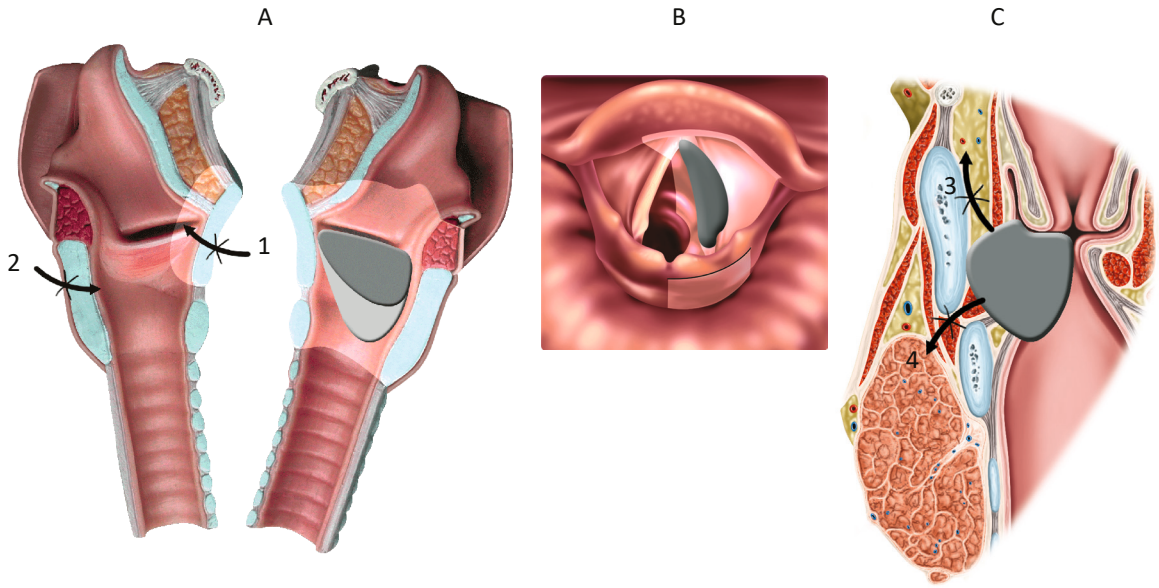


Figure 5.6. Maximal tumor extension of a glottic SCC that allows for an extended hemilaryngectomy to be performed.
In all three views, tumor extension is shown in grey. A. Midsagittal incisional view. The tumor should not cross the anterior (1) or posterior (2) commissure. B. Endoscopic view of the tumor extension with excision margins (resection area is bleached). C. Coronal view. The supraglottis (3) should be free of any tumor and the tumor may not invade the cricothyroid ligament (4).

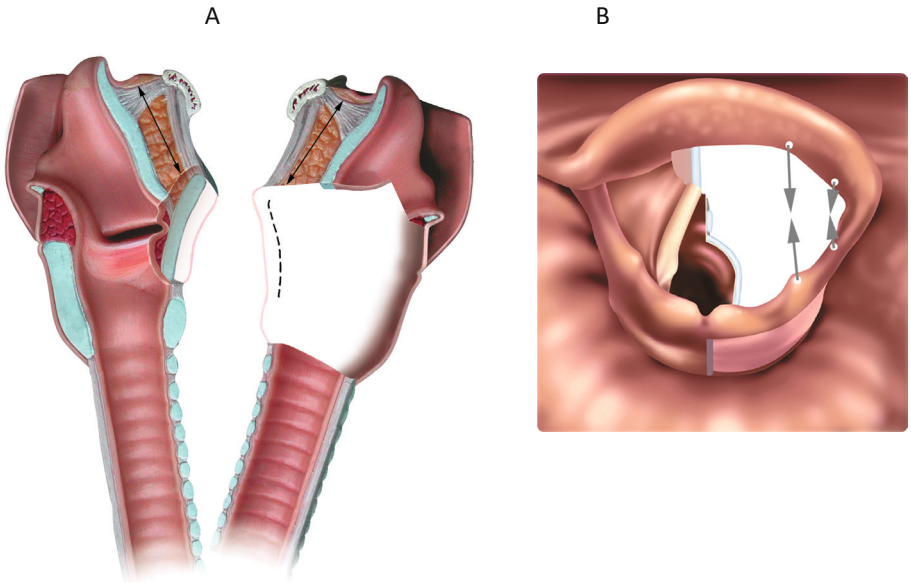


Figure 5.7. Defect after extended hemilaryngectomy.
A. Midline sagittal incisional view. The hyoepiglottic attachments will be released (indicated with black double arrows). B. Endoscopic view. The anterior commissure is included if the tumor reaches the anterior site of the vocal fold. Two or three Vicryl 3.0 sutures (represented by grey arrows) are placed between the aryepiglottic fold and the epiglottis at the side of resection.

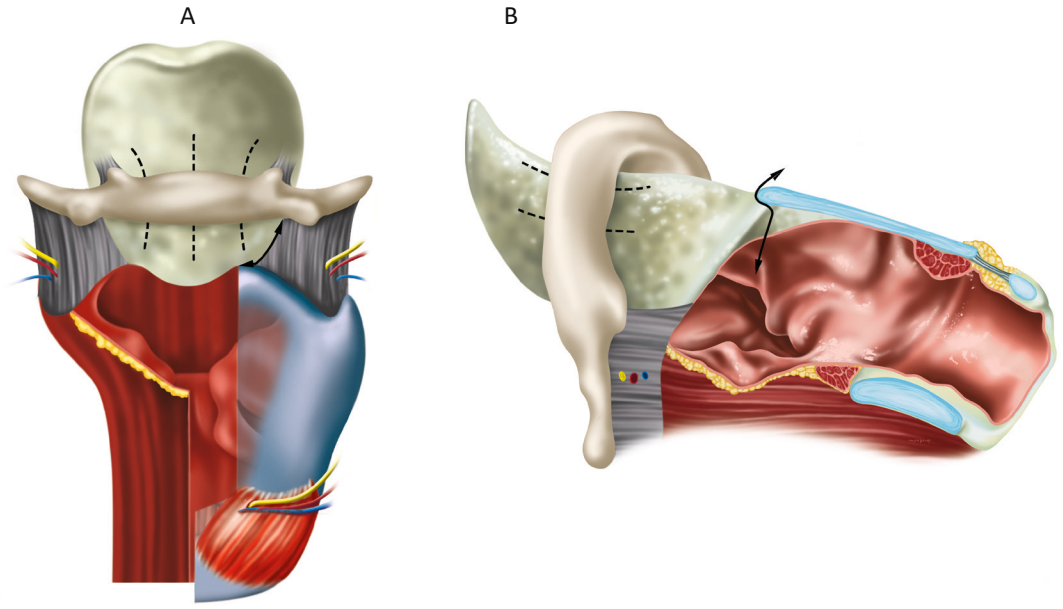


Figure 5.8. Defect after extended hemilaryngectomy (continued).
A. Frontal view. B. Lateral view. The hyoepiglottic attachments will be released (indicated with dashed lines) and the remaining thyroepiglottic attachment will be partially incised (indicated with double arrow).

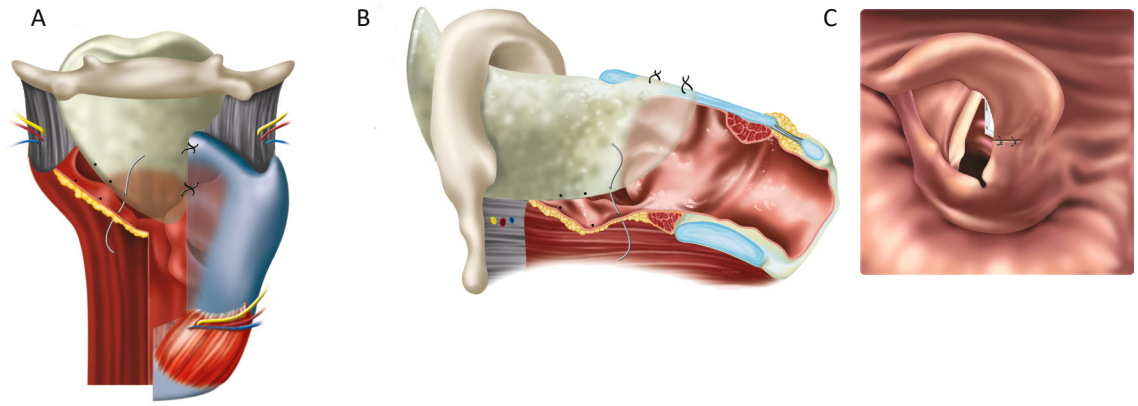
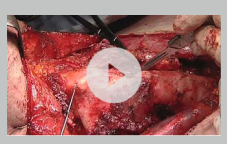


Figure 5.9. Restoration of sphincteric function.
A. Frontal view; B. Lateral view; C. Endoscopic view. The epiglottic petiole is sutured to the thyroid cartilage at the level of the vocal fold. Two or three Vicryl 3.0 sutures are placed between the aryepiglottic fold and the epiglottis at the side of resection (A,B). After the sutures are in place, the aryepiglottic fold is brought proximal to the midline (C).

Movie 2 and 3*



Tumor resection of a SCC and chondrosarcoma.

Movie 4

Midline reconstruction of the aryepiglottic fold.

* Due to the nature of the video clips, a YouTube account might be required in order to enable access.

5.3. **Tracheal revascularization and temporary laryngeal reconstruction**

After an extended hemilaryngectomy is performed, the vascular situation of the cervical trachea is rather similar to that of a cervical trachea after total thyroidectomy (Fig. 5.10). However, after the former procedure, one thyroid lobe remains.

After tumor resection, the cervical trachea is wrapped with subcutaneous tissue and fascia from the radial forearm. Meanwhile, the hemilaryngeal defect will be repaired temporarily with a radial forearm skin paddle. A tracheostomy will be necessary for respiration (Figs. 5.11-5.16).

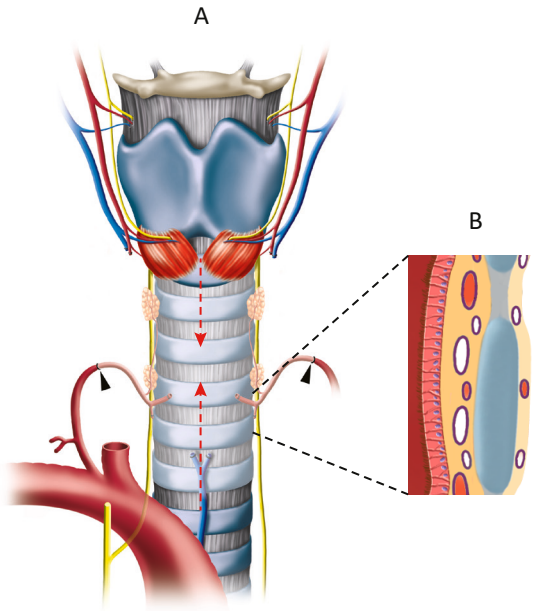


Figure 5.10. Blood supply to a cervical trachea after total thyroidectomy.
A.The blood supply of the cervical trachea derives from branches of the inferior thyroid artery. These branches are interrupted (indicated with arrowheads) during a total thyroidectomy. Collateral mucosal blood supply from above and below (indicated with dashed arrows) prevents the cervical trachea from undergoing avascular necrosis. Ischemia without risk of avascular necrosis is optimal for inducing a new blood supply after an ischemic trachea is wrapped in a well vascularized soft tissue flap. B. A cross-sectional view through the tracheal wall illustrates the ischemic condition that develops after the extrinsic blood supply is interrupted.

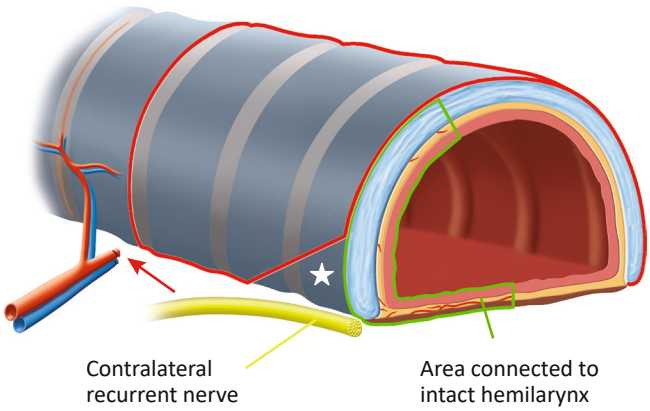


Figure 5.11. The portion of the cervical trachea that is wrapped with fascia.
A superolateral view of the trachea. The upper 4 cm of the cervical trachea is dissected from the surrounding thyroid gland to be wrapped with fascia (outlined with a red line). The segmental tracheal blood supply is then interrupted after the soft tissue connections are removed (indicated with a red arrow). A triangular area (1.0 x 1.5 cm) that is adjacent to the intact recurrent nerve remains in place and is not wrapped with fascia (labeled with a white star).

A

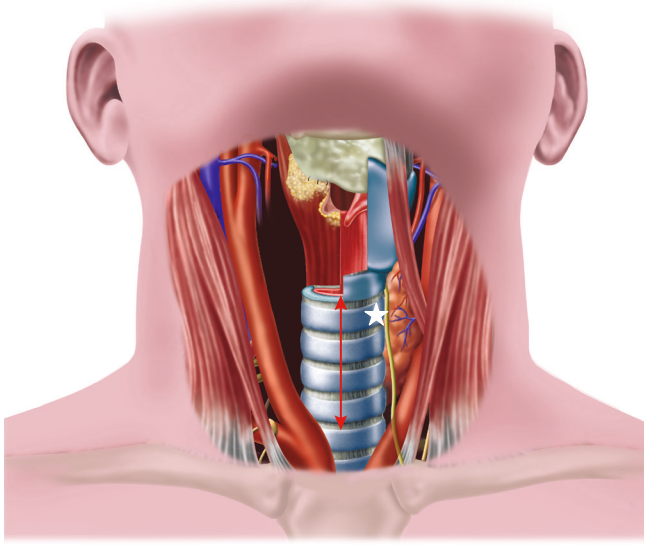
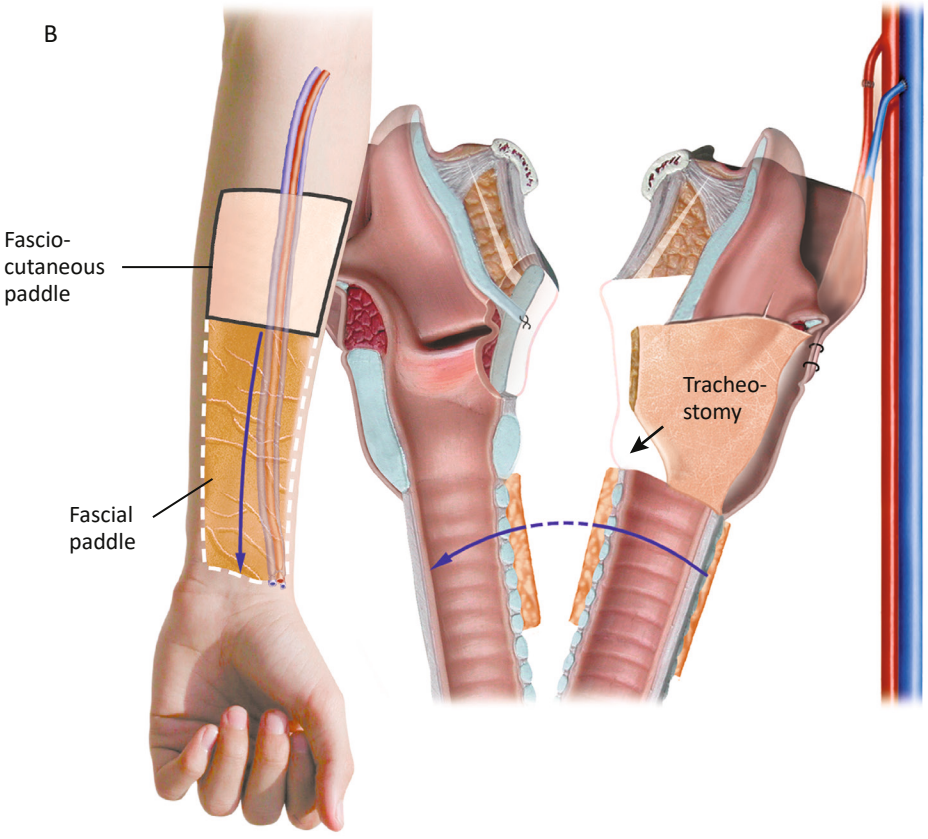


Figure 5.12. Tracheal revascularization and temporary reconstruction.
A. Frontal view. The upper 4 cm of the cervical trachea (indicated with a red double arrow) will be wrapped with fascia, except for a triangular area close to the recurrent nerve (labeled with a white star).
B. Midsagittal incisional view. The radial forearm flap includes a fascio-cutaneous paddle and a fascial paddle. The latter provides revascularization of the cervical trachea (indicated with a blue arrow), while the skin paddle serves as a temporary closure for the hemilaryngectomy defect. The radial blood vessels are sutured to blood vessels in the neck.
C. Endoscopic view after the skin has been sutured to the laryngeal defect.

B



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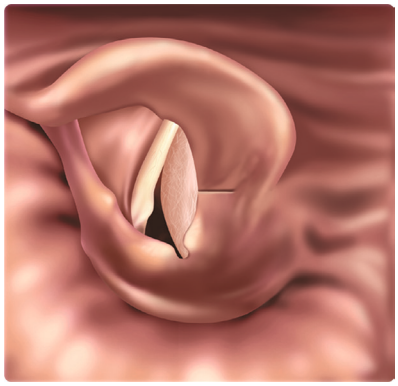


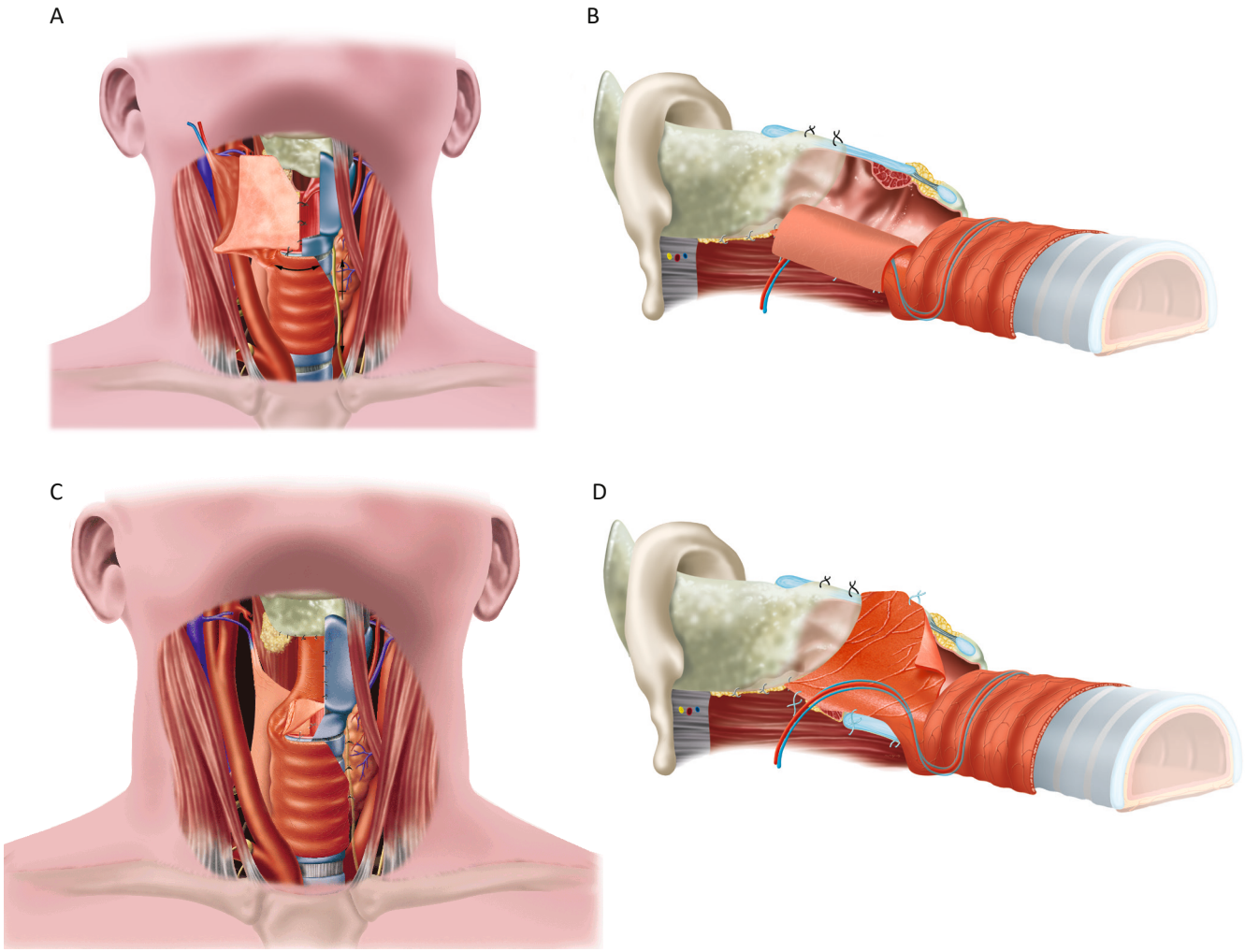
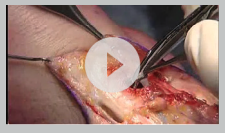


Figure 5.13. Tracheal revascularization and temporary reconstruction.
Frontal (A) and lateral (B) views of forearm fascia wrapped around a 4-cm segment of the cervical trachea. The upper surface of the forearm fascia lies against the tracheal wall. The lateral site of the skin flap is sutured to the posterior laryngeal section margin of the cricoid cartilage from inferior to superior.

Frontal (C) and lateral (D) views of a temporary reconstruction with the skin island of the radial forearm flap placed into the laryngeal defect. A 'tracheostomy' is positioned at the caudal part of the reconstructed larynx and the radial blood vessels are sutured to blood vessels in the neck. The superior thyroid artery (in an end-to-end anastomosis) and the internal jugular vein (in an end-to-side anastomosis) are mostly used as recipient vessels.



Movie 5
Radial forearm flap.

Movie 6
Tracheal revascularization.

For wrapping of a trachea in vascularized fascia, it is necessary to isolate the trachea from its surrounding tissue connections (Movie 5, 6). As a result, the extrinsic blood supply provided by small tracheoesophageal vascular branches is disrupted. However, the intrinsic mucosal blood supply remains preserved (Fig. 5.14).

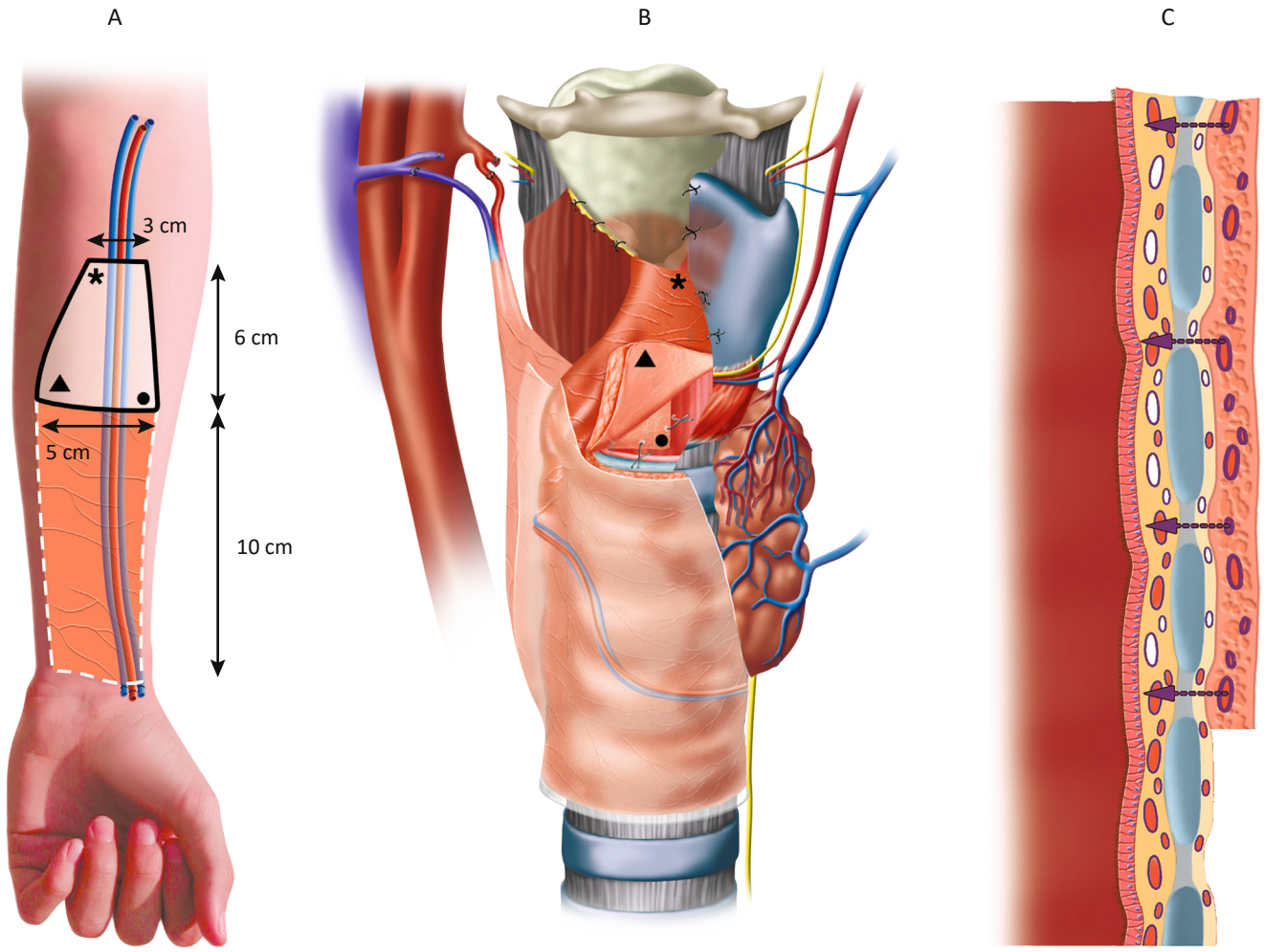


Figure 5.14. Overview of orthotopic tracheal revacularization.
A.The fascial paddle of the radial forearm flap measures 5 x 10 cm while the skin paddle measures 5 x 6 x 3 cm. The first lateral side of the skin flap is sutured to the posterior laryngeal section margin from inferior (site labeled with a black dot) to superior. B. The mobilized tracheal segment of the trachea (which includes 4 cm of the cervical trachea) is wrapped with the fascial paddle, except for a triangular area at the site of entrance of the left recurrent nerve into the larynx. The second lateral side of the skin paddle is sutured to the anterior laryngeal section line from superior (labeled with an asterisk) to inferior (labeled with a triangle). The lower edge of the latter is not sutured into the laryngeal defect and it serves as a visible monitor for the radial forearm flap. An opening that is left at the caudal larynx that serves as a site for a tracheostomy between the first and second operation. A Gore-tex® membrane (Preclude Pericardial Membrane, 0.1 mm, W.L. Gore and Associates, Inc., Flagstaff, AZ, USA) is applied over the fascial-enwrapped trachea to prevent adhesions. C. Vascular connections will be formed between the fascia and the trachea (indicated with dashed arrows). The driving force for tracheal revascularization from the fascia flap is the ischemia of the mobilized trachea.

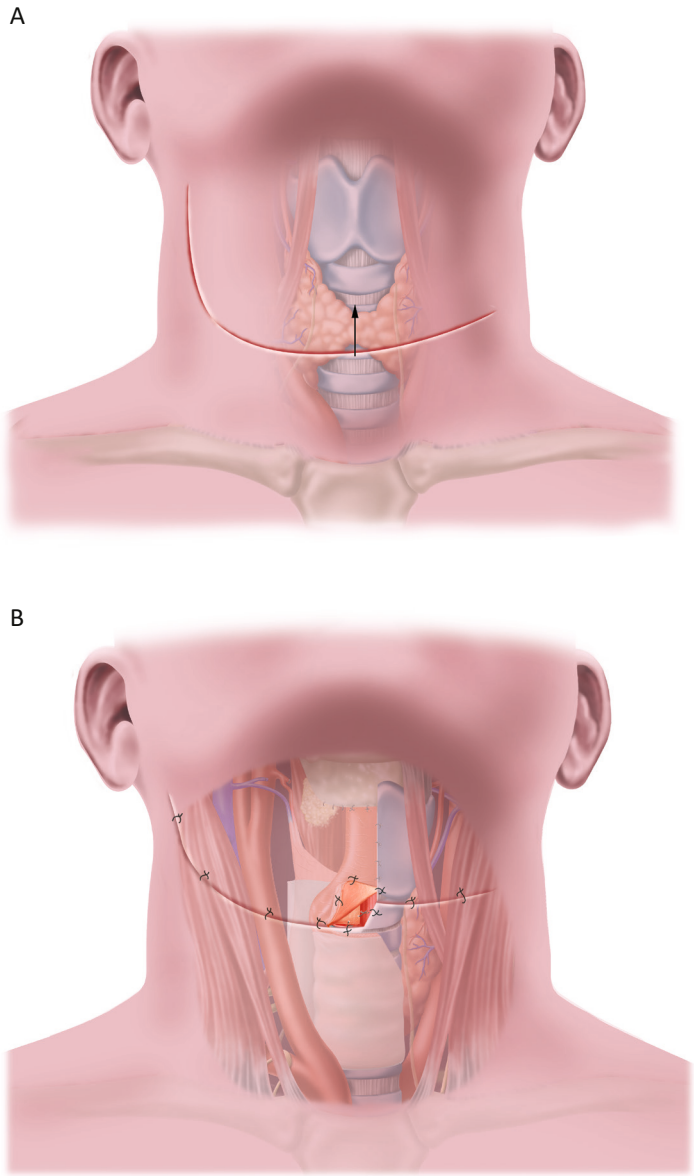
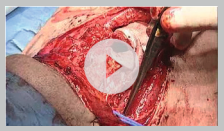


Figure 5.15. Initial incision (A) and final suturing (B) in the first operation of tracheal autotransplantation.
A. An incision is made 2-3 cm above the sternal notch which extends to the mastoid on the tumor side. After the first operation, the lower neck skin flap is sutured to the first tracheal ring (indicated with an arrow) without tension. B. To close the neck, the upper neck skin flap is attached to the superior side of the 'tracheostomy'. The lower edge of the radial skin serves as an external monitor for the viability of the radial forearm flap.

After this intervention, the patient can close the glottis chink during speech and swallowing. However, a tracheostomy is needed for respiration (Fig. 5.15) (Movie 7). Some aspiration of saliva and liquids will be observed. It is advisable to place a percutaneous gastrostomy to allow safe feeding until the tracheostomy is closed.



Movie 7
Temporary reconstruction and function after the first operation.

An overview of tracheal revascularization and temporary larynx reconstruction is provided in the CT scans shown in Figure 5.16.

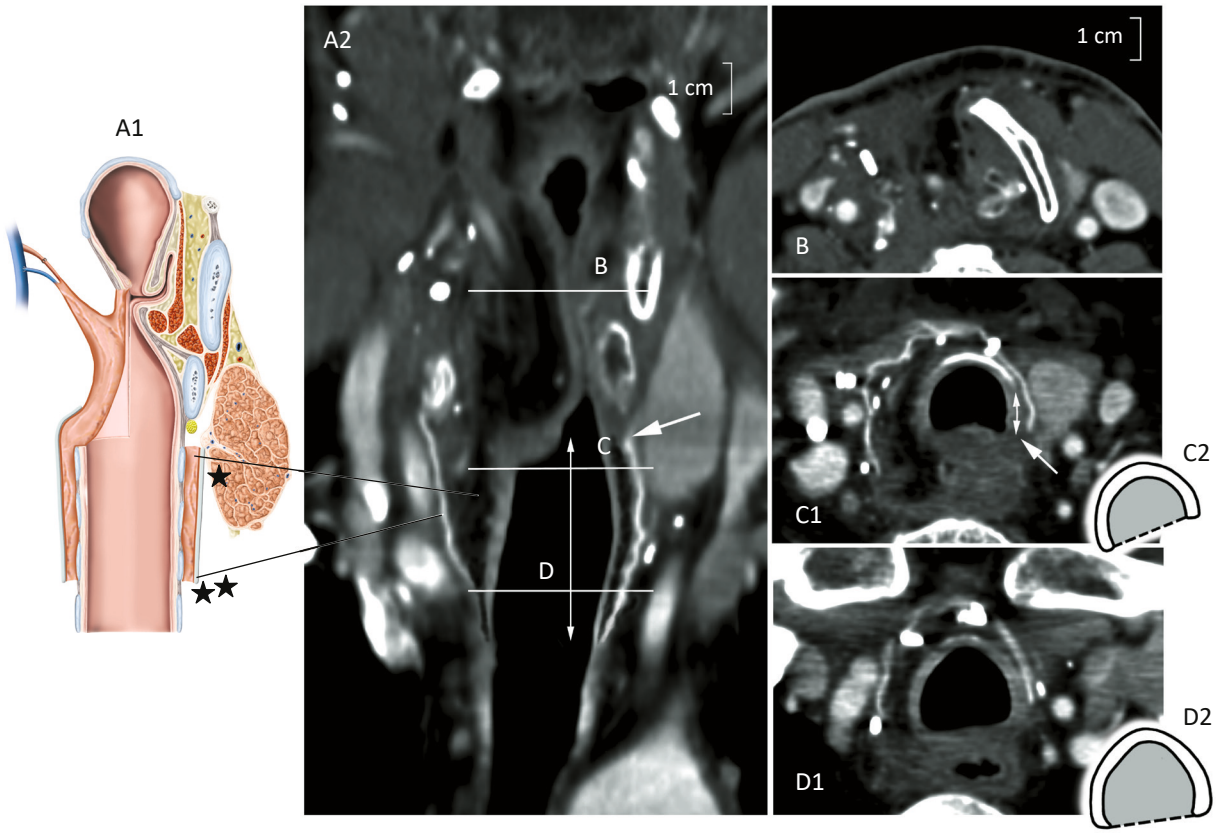


Figure 5.16. Overview after the first operation of tracheal autotransplantation.
A. A coronal view illustration of the procedure performed in the first operation (A1, A2). A2. Coronal CT scan. The length of cervical trachea to be used for larynx reconstruction is indicated by the white double arrow. The arrow points to the entrance of the recurrent nerve into the larynx (A2,C1). B. Axial CT scan at the glottic level. Complete obliteration of the laryngeal lumen is observed. C. Axial CT scan at the upper tracheal level (1). The accompanying schematic shows the extent of the cartilaginous trachea included in the autotransplant (2). The white double arrow shows a 1-cm segment of the cervical trachea near the recurrent nerve which is not included in the autotransplant. D. Axial CT scan at the lower tracheal level (1). The accompanying schematic shows the extent of the cartilaginous trachea which will be included in the autotransplant (2).
*: radial forearm fascia
**: Gore-Tex® membrane

5.4. **Tracheal autotransplantation**

Four to six weeks after the first intervention, vascular connections will have been established between radial forearm fascia and the cervical trachea, thereby allowing the revascularized trachea to be resected (Figs. 5.17, 5.18). The vascular connections are actually sufficient after two weeks (see 5.6., Our initial tracheal autotransplantation approach). However, for organizational reasons, 4–6 weeks is the interval that is optimal for the two-step procedure.

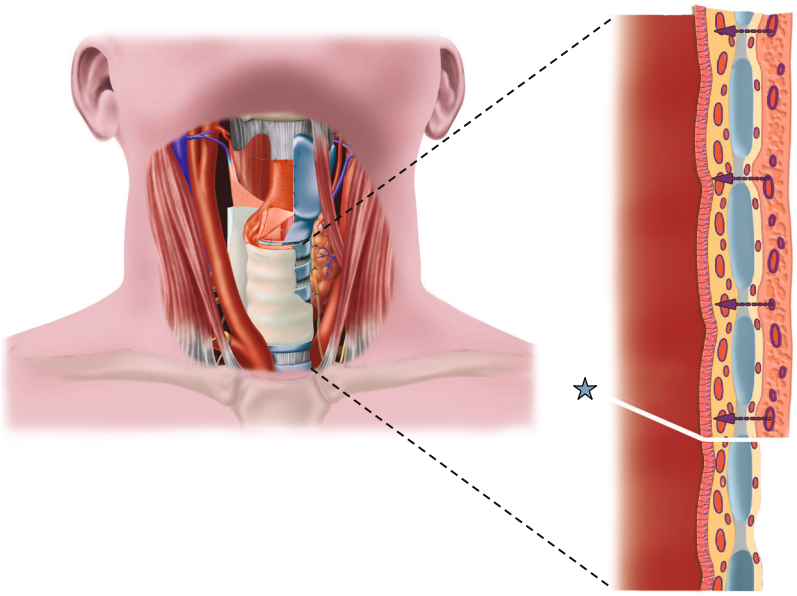


Figure 5.17. Revascularized trachea. After 4-6 weeks, the cervical trachea is fully vascularized by its surrounding fascia (arrows). Fascial revascularization allows the fascia-wrapped tracheal segment to be resected. This leads to interruption of the upper and lower intrinsic mucosal blood supply (indicated with blue star).

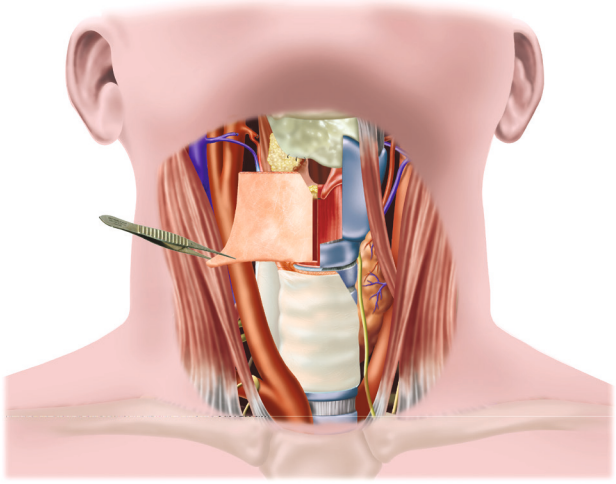


Figure 5.18. Removal of the fasciocutaneous paddle from the laryngeal defect. The anterior, superior, and posterior suture lines between the radial forearm skin flap and the laryngeal remnant are opened and the skin paddle is completely separated from the laryngeal remnant.

During the second operation, the skin paddle is removed from the laryngeal defect and de-epithelialized (Movie 8). The cervical trachea is then autotransplanted with an intact blood supply to the hemilaryngeal defect, followed by mobilization and suturing of the mediastinal trachea to the reconstructed larynx. A tracheostomy is also maintained between the reconstructed larynx and the mediastinal trachea (Figs. 5.19–5.23). A Gore-Tex® membrane prevents adhesions between the fascia and the surrounding tissues. This membrane also facilitates isolation of the tracheal autotransplant (Fig. 5.19).

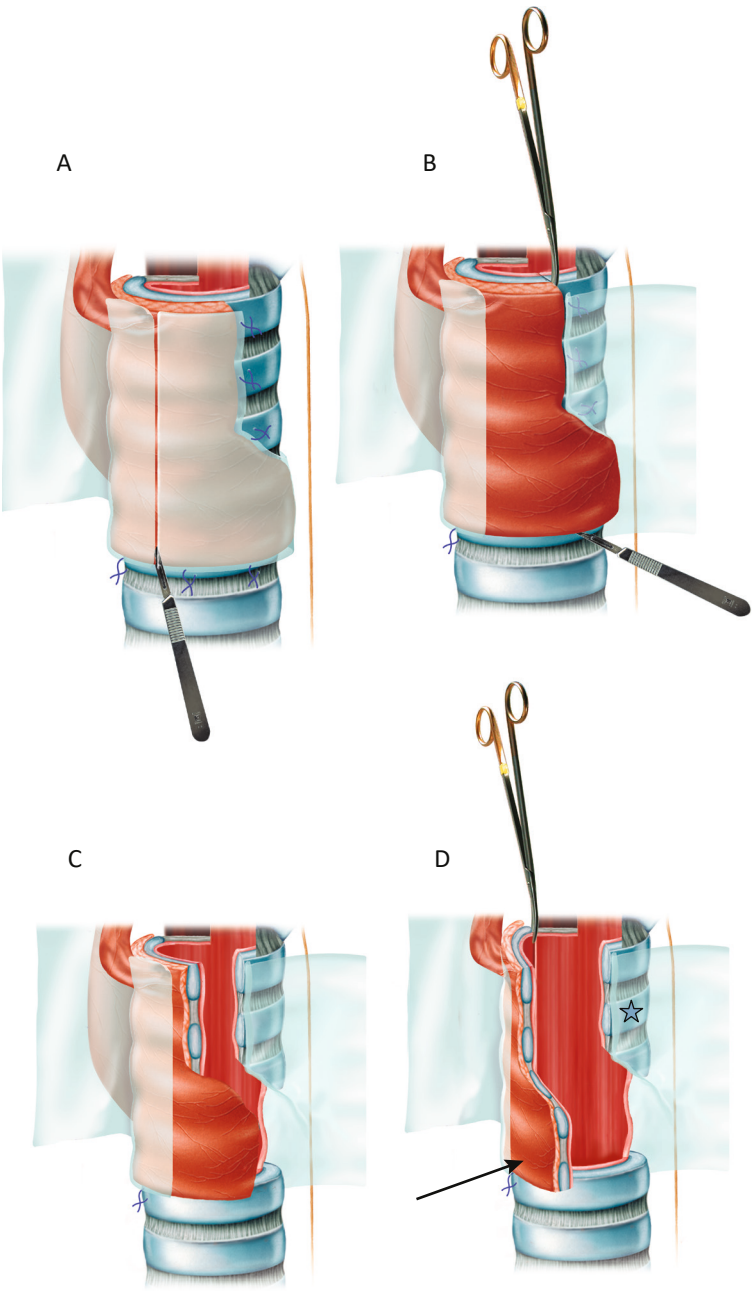
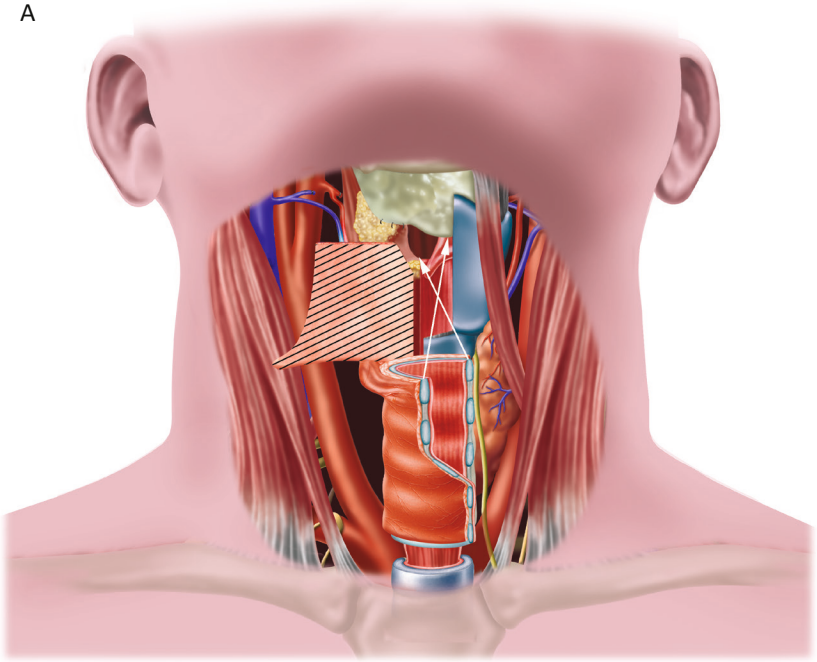


Figure 5.19. Isolation of a tracheal transplant with its new blood supply.
A. An incision is made at the midline of the Gore-Tex® membrane. B. The distal part of the membrane is turned to the site of the preserved hemilarynx to protect the recurrent nerve during tracheal isolation. The trachea can then be safely incised in the sleeve between the distal end of the membrane and the distal end of the fascia flap. Another tracheal incision is made caudal to the fascia-wrapped trachea. C. The tracheal patch is rotated to the site of the hemilaryngectomy. D. After rotation, an incision is made in the trachea between the cartilaginous ring and the membranous trachea. The portion of the Gore-Tex® membrane that wraps the neck vessels facilitates upward mobilization of the revascularized trachea. A triangular area adjacent to the recurrent nerve will remain in place (labeled with a blue star). The full width of the cartilaginous trachea (indicated with an arrow) will be isolated at the lower site (Movie 9).

A



B

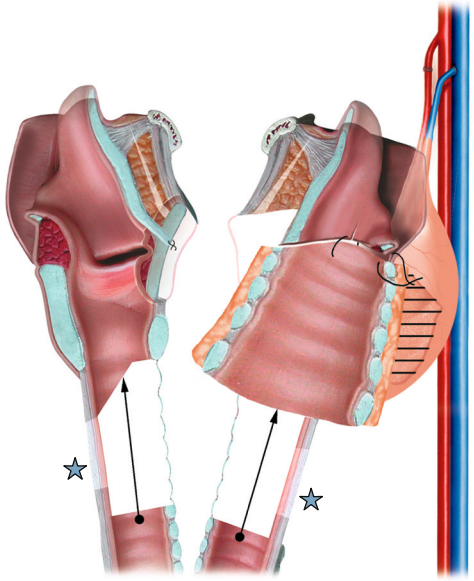
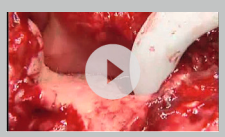


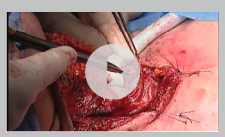
Figure 5.20. Tracheal autotransplantation.
A. Frontal view. Isolation of the revascularized trachea. The radial forearm skin flap is de-epithelialized (indicated with diagonal shaded area) before the isolated tracheal patch is transferred to the laryngeal defect (indicated with two white arrows).
B. Midline sagittal incisional view. The revascularized trachea is transplanted to the laryngeal defect. The skin paddle is removed from the defect and de-epithelialized (shaded area). The mediastinal trachea is then sutured to the reconstructed larynx (indicated with arrows). A portion of the membranous trachea (labeled with a blue star) will be resected to allow for anastomosis of the tracheal stump to the reconstructed larynx. Transfer of the tracheal patch is performed without manipulation of the vascular pedicle (Movie 10).

Movie 8



Removal of the skin flap from the defect.

Movie 9



Tracheal isolation.

Movie 10

Tracheal autotransplantation.

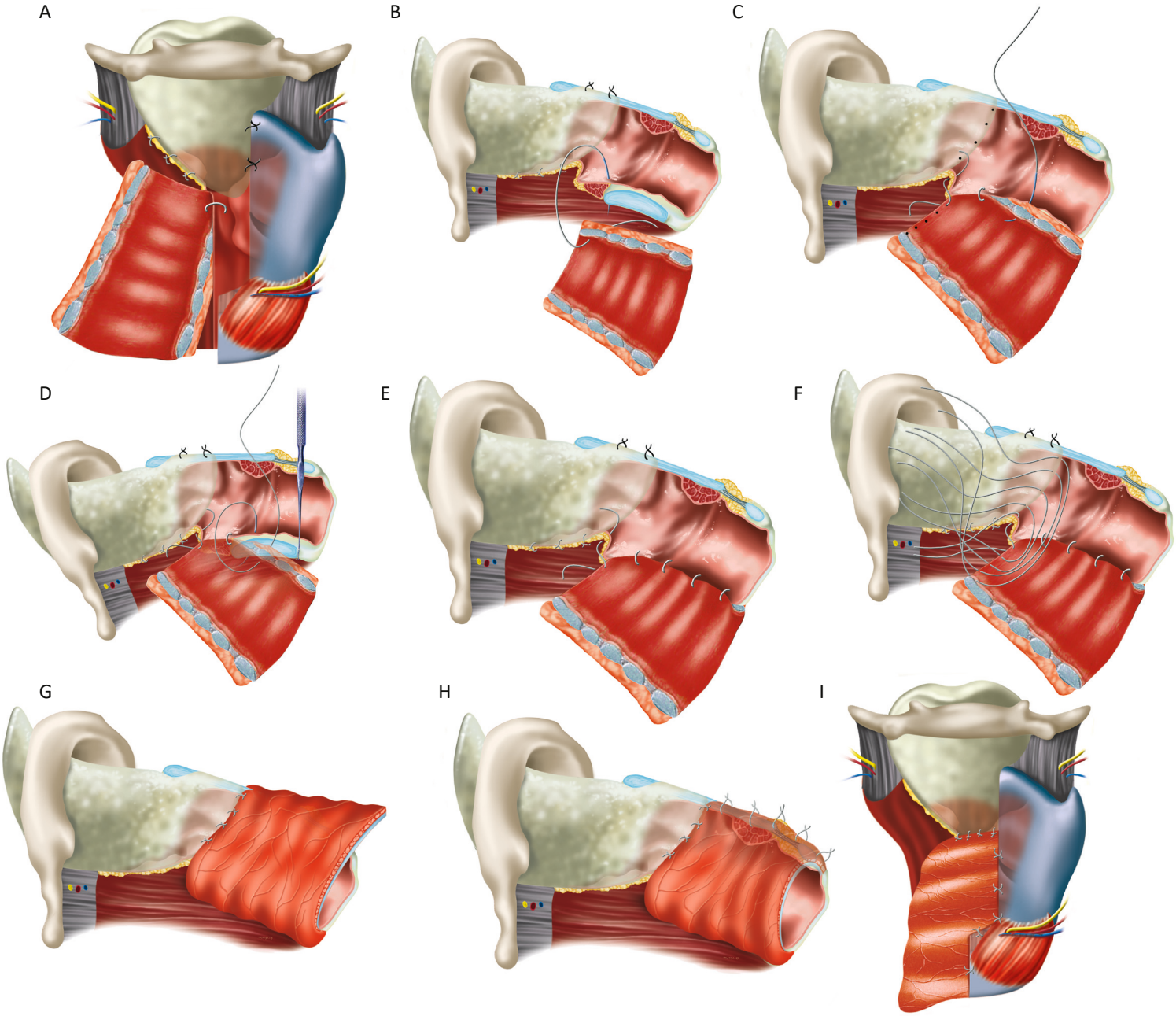


Figure 5.21. Overview of the sutures needed to inset the tracheal transplant.
A, I: Frontal views; B-H: Lateral views. A-E. Posterior sutures are placed from superior to inferior. F. Sutures needed for superior anastomosis. G-I. Anterior sutures are placed form superior to inferior.

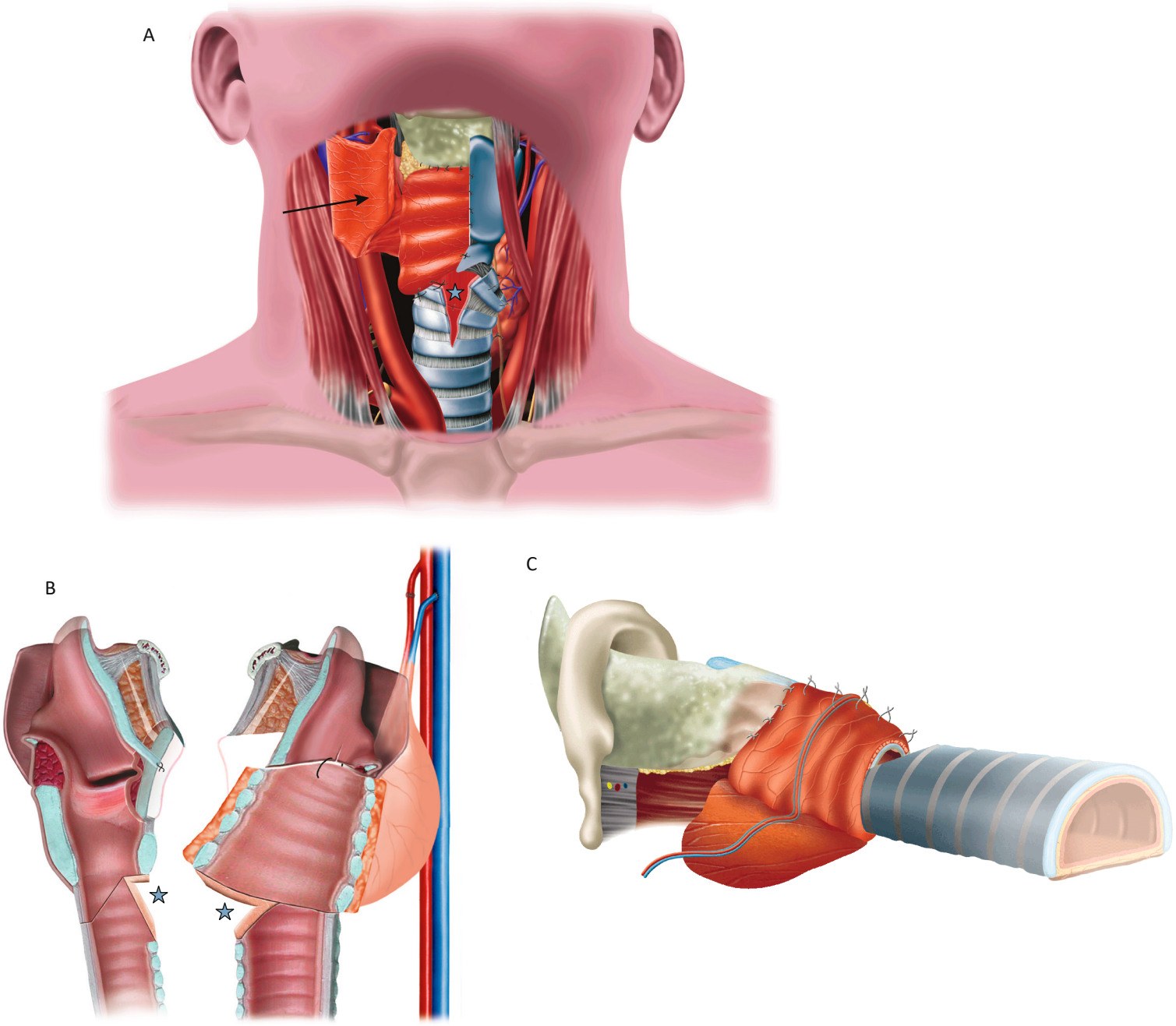


Figure 5.22. After tracheal autotransplantation.
A. Frontal view of a tracheal autotransplant with the de-epithelialized fasciocutaneous paddle indicated with an arrow. Two rings of the mediastinal trachea are incised anteriorly to form a tracheostomy (labeled with a blue star). B. Midline sagittal incisional view of the neck skin flaps sutured to the tracheostome (indicated with blue stars). C. Lateral view after tracheal autotransplantation and anastomosis of the tracheal stump to the reconstructed larynx.

After reconstruction, the airway continuity will be re-established by moving the tracheal stump up to the lower end of the reconstructed larynx. After tracheal autotransplantation, the continuity between the lower cervical trachea and the reconstructed larynx is still preserved. A small rim of the membranous trachea is removed (Fig. 5.23.A) to allow for easy end-to-end anastomosis. A blunt finger dissection is then performed to release the tracheal wall down into the mediastinum. The stump of the lower cervical trachea is anastomosed posteriorly and laterally towards the cricoid cartilage and to the hemilaryngeal patch (Fig. 5.23). An end-to-end anastomosis is performed without tension.

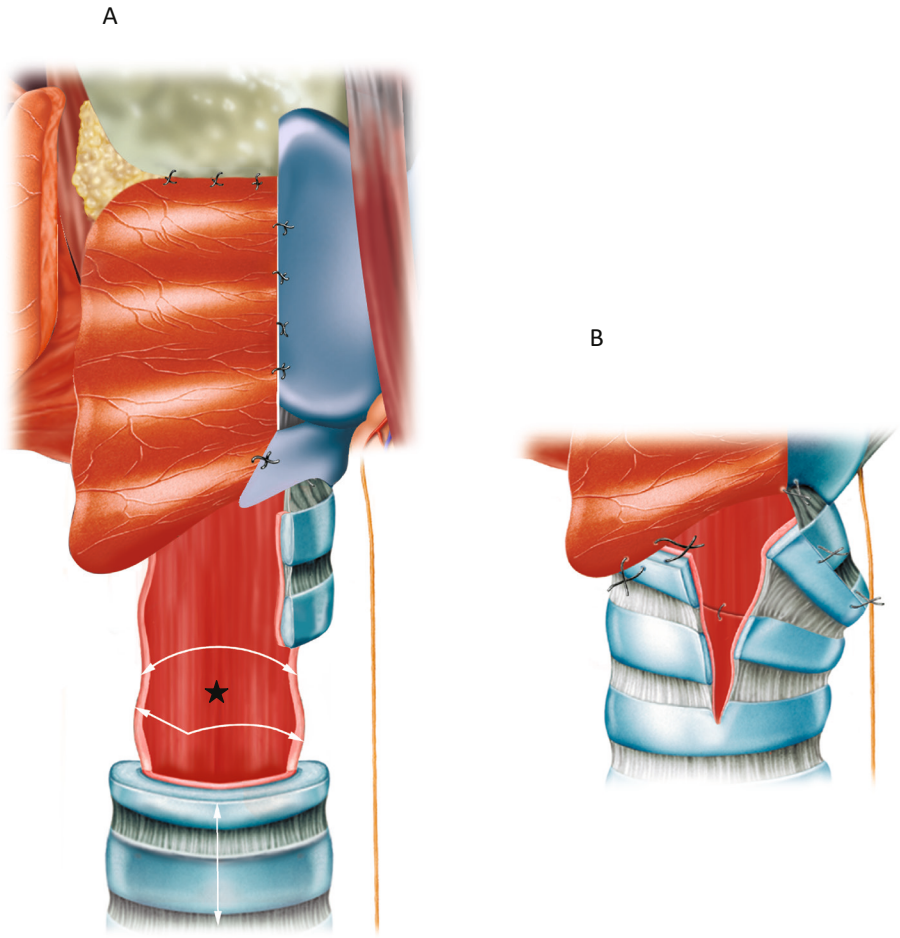
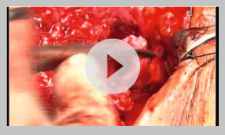


Figure 5.23. Suturing of the mediastinal trachea to the reconstructed larynx.
A. A small portion of the membranous trachea is resected (labeled with a black star) to facilitate an end-to-end anastomosis between the reconstructed larynx and the lower cervical trachea. The upper two rings of the tracheal stump are incised anteriorly (indicated with a two-headed arrow). B. After suturing the tracheal stump to the reconstructed larynx, the remaining tracheostomy is positioned anteriorly (Movie 11).



Movie 11
Mediastinal trachea sutured to a reconstructed larynx.

5.5. **Closure of tracheostomy**

The tracheostomy can be closed after all laryngeal functions have fully recovered. This usually occurs 4-8 weeks after tracheal autotransplantation (Figs. 5.24-5.27) (Movie 12).

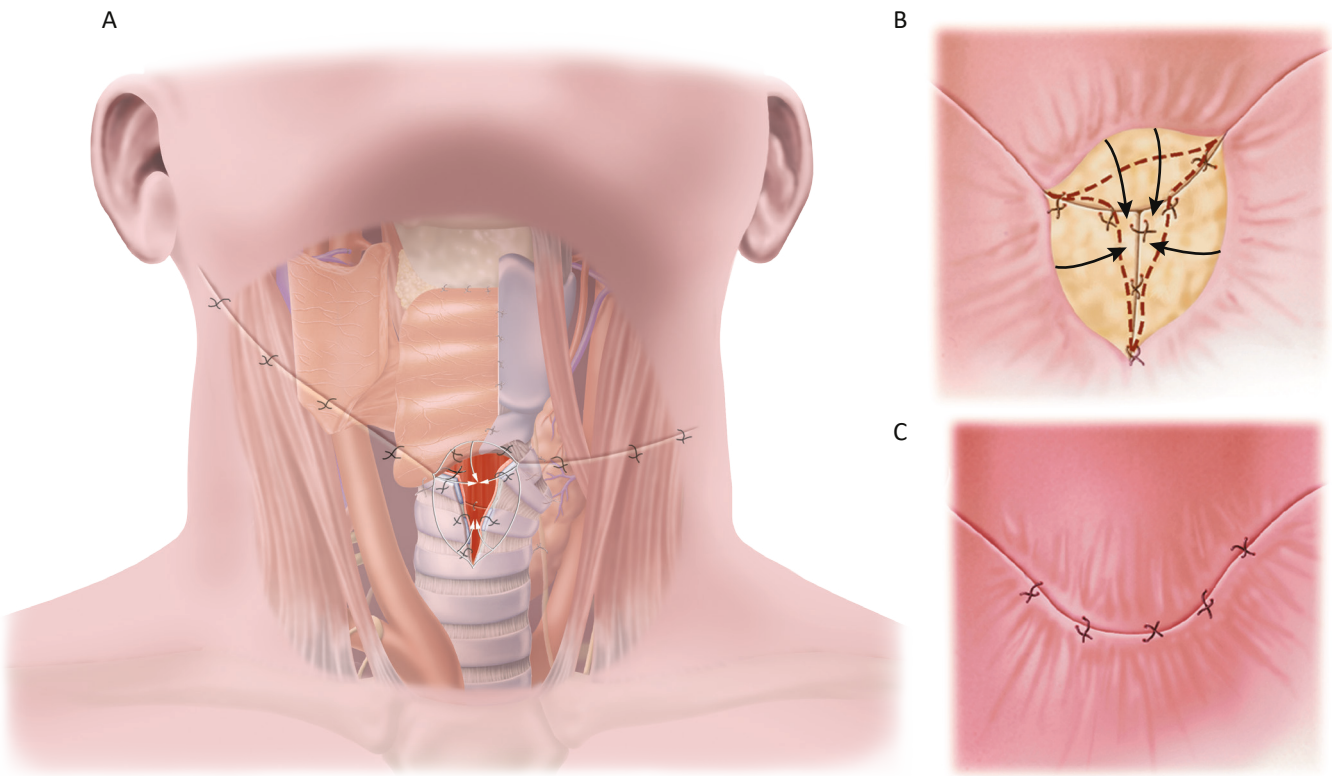
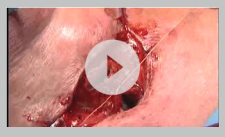


Figure 5.24. Tracheostomy closure.
A. The neck skin around the tracheostomy is incised and inverted (indicated with arrows). B. Following inversion of the skin flaps, red dotted lines indicate the circumference of the tracheostome before closure. Arrows show the second layer closure which is performed with the upper and lower neck skin flaps. C. Both the upper and lower neck skin flaps are undermined and then closed as a second layer.

Movie 12

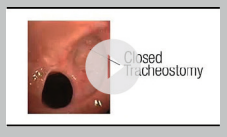


Closure of the tracheostomy.

Movie 13

Function after chondrosarcoma resection.

Movie 14



Function after tracheostomy closure.

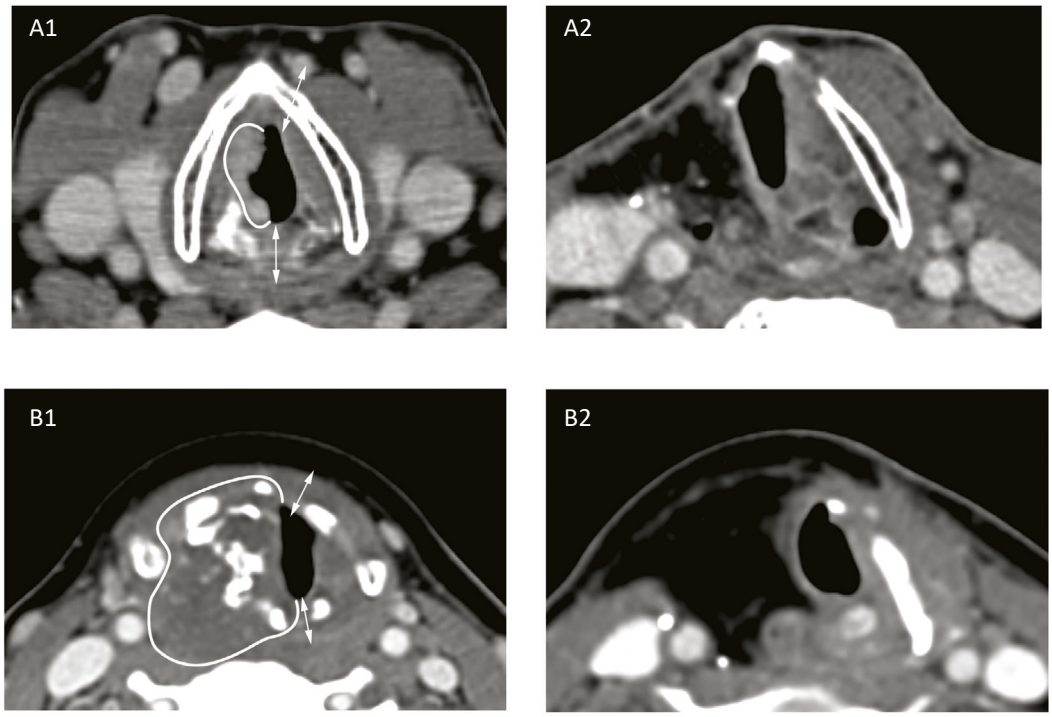


Figure 5.25. Laryngeal morphology on CT scans at the glottic level before and after tracheal autotransplantation.
A. Axial CT scan at the glottic level. A carcinoma of the right vocal fold (outlined with a white line) and its resection margins (indicated with double arrows) are shown before (A1) and after (A2) tracheal autotransplantation. B. Axial CT scan at the glottic level. Chondrosarcoma of the right hemilarynx (outlined with a white line) and its resection margins (indicated with double arrows) are shown before (B1) and after (B2) tracheal autotransplantation.

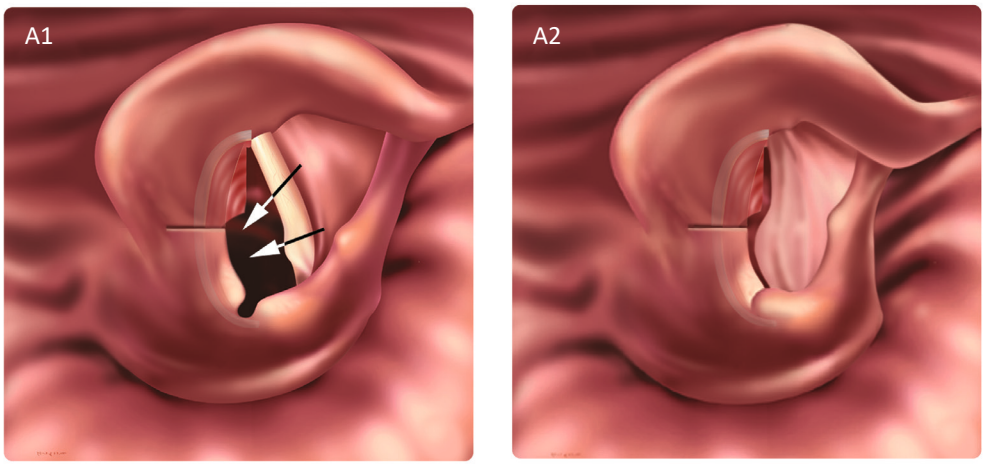


Figure 5.26. Laryngeal morphology at the glottic level after tracheal autotransplantation.
Endoscopic view after tracheal autotransplantation during inspiration and abduction (A1) and during phonation and adduction (A2). Closure (indicated with arrows) occurs between the intact false fold and the aryepiglottic fold at the site of resection (Movie 13, 14).

A CT scan provides an overview after tracheal transplantation (Fig. 5.27). Phonation occurs at the supraglottic level.

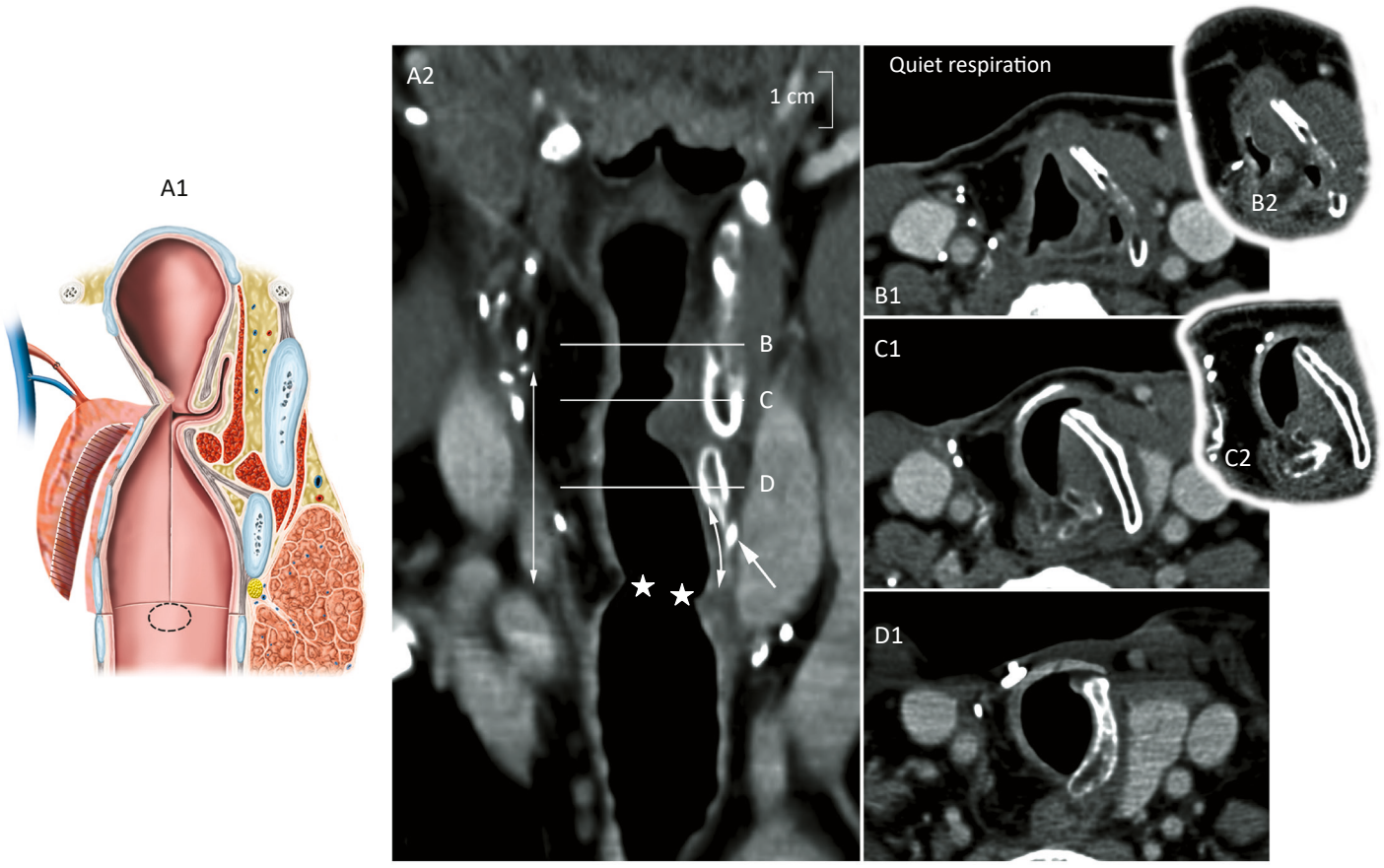


Figure 5.27. Overview after the second operation of tracheal autotransplantation.
A. A coronal view illustration of the procedure performed in the second operation. The dotted circle indicates the site of tracheostomy (A1). A2. Coronal reformatted CT scan. The length of cervical trachea to be used for larynx reconstruction is indicated with a white double arrow. The short double arrow labels a 1 cm segment of the cervical trachea that is near the recurrent nerve and is not included in the autotransplant. The arrow indicates the entry point of the recurrent nerve into the larynx. Anastomosis of the mediastinal trachea to the reconstructed larynx is indicated with white stars. B. Axial CT scan at the supraglottic level during quiet respiration (1) and during phonation (2). The airway lumen is closed during phonation. C. Axial CT scan at the glottic level during quiet respiration (1) and during phonation (2). The glottic gap is not closed during phonation. D. Axial CT scan at the subglottic level (1).

5.6. **Our initial tracheal autotransplantation approach**

Initially, the tracheal autotransplantation concept was developed as a two-stage procedure. In the first operation, the cervical trachea was wrapped with a fascia flap from the radial forearm and revascularized on blood vessels from the neck (Fig. 5.28). The second operation was performed two weeks later and it includes tumor resection and tracheal autotransplantation (Fig. 5.29).

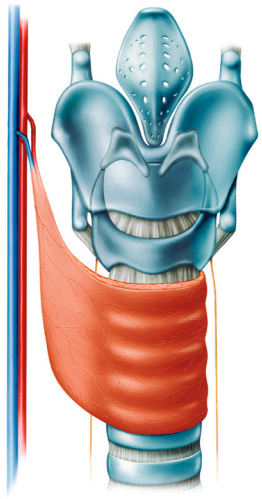


Figure 5.28. Initial concept – first operation.
A 4-cm segment of the cervical trachea is wrapped with radial forearm fascia. The radial vessels are sutured on blood vessels in the neck. The larynx is untouched.

An advantage of this initial approach was that a tracheostomy was avoided between the first and second interventions. However, there were also significant disadvantages associated with this initial approach. First, tumor resection during the second stage is oncologically unsafe for laryngeal carcinomas. Second, with a bulky tumor present, extubation of the patient could lead to dyspnea. Based on these major drawbacks, we modified the concept such that tumor resection would be performed during the first intervention along with temporary reconstruction of the hemilaryngeal defect

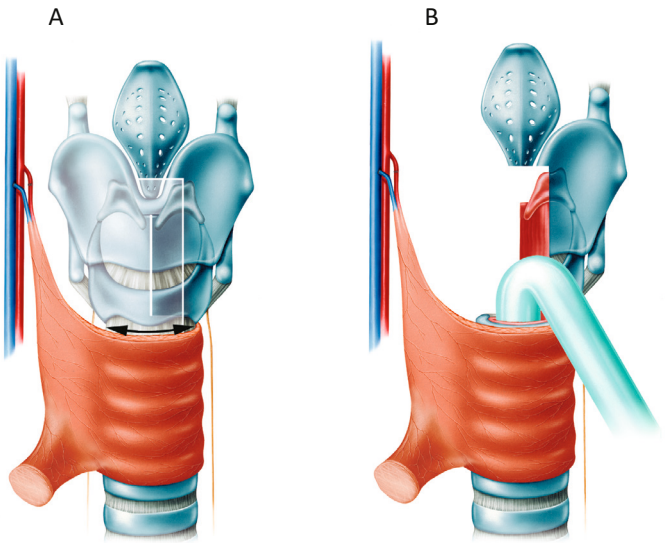


Figure 5.29. Initial concept – second operation.
A. The region of the tumor undergoing resection is outlined in white and shaded. An incision is made in the cricotracheal ligament along the black double arrow.
B. Following tumor resection, an intubation tube is placed into the proximal trachea.

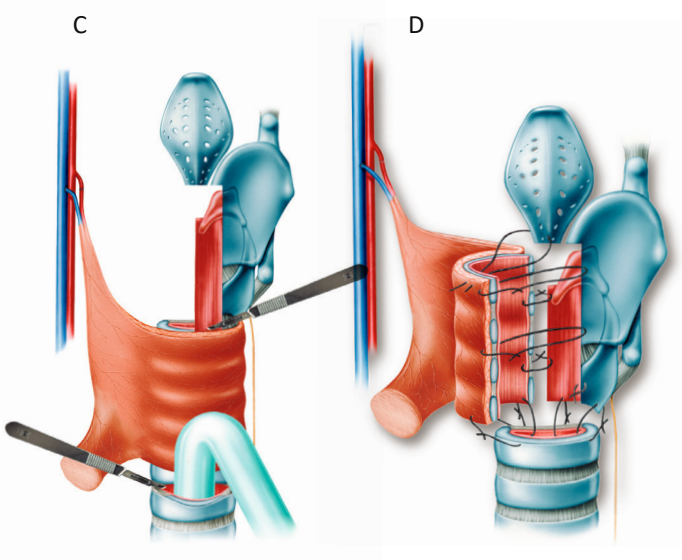


Figure 5.29. Initial concept – second operation (continued).
C. The tracheal autotransplant is isolated with the intubation tube placed below the fascia-wrapped trachea. D. The tracheal autotransplant is placed into the laryngeal defect. The mediastinal trachea is then sutured to the reconstructed larynx.

5.7. **Conclusion**

In order to achieve optimal oncological and functional outcomes following tracheal autotransplantation, a careful patient selection is of the utmost importance. Since the tracheal autotransplantation technique necessitates preservation of one cricoarytenoid unit, T2 or T3 laryngeal SCC (e.g., unilateral T2 with impaired vocal fold mobility or T3 with subglottic extension and/or arytenoid cartilage fixation) are considered possible candidates, provided that the tumor does not extend over the anterior commissure, which makes resection with safe margins while preserving a sufficient portion of the contralateral vocal fold impossible. This is a primordial requirement for both primary as salvage cases. For advanced chondrosarcoma cases, limited extension over the posterior commissure is not a contraindication for the autotransplant technique because wide resection margins are not required.

In addition to tumor location and extension, functional status and patient age are two additional important factors which need to be taken into account when considering tracheal autotransplantation. Ideally, good candidates for this technique have a high or medium performance status (e.g., World Health Organization (WHO) performance status classification categories 0, 1 or 2), they have no restrictive or obstructive lung disease (except if due to a laryngeal tumor) and they have quit smoking prior to surgery. Meanwhile, in years past, patient age has not been considered an important selection criteria. However, in our experience, we have observed worse functional results in patients older than 65 years who have undergone tracheal autotransplantation.

A drawback of the tracheal autotransplantation technique is its complexity and the need for a two-stage approach. However, the latter condition provides an additional safety since it allows for section margins to be reevaluated before a definitive reconstruction is performed, thereby possibly ameliorating oncological outcome. Moreover, despite its complexity, it is important to understand that this technique offers a functional alternative to a total laryngectomy for patients with advanced cricoid chondrosarcoma and also for selected patients with glottic SCC, both in the primary and salvage settings.

Thus, tracheal autotransplantation has proven to be a functional alternative to total laryngectomy in selected cases of unilateral T2 or T3 glottic SCC and in cases of advanced chondrosarcoma of the cricoid cartilage. In addition, this technique has provided excellent functional results in regard to respiration,

vocal recovery, and swallowing, without compromising oncologic outcome, especially in patients ≤ 65 years of age and for patients with chondrosarcoma [7].

Therefore, tracheal autotransplantation represents an optimal patch reconstruction for airway defects as a well vascularized technique that provides optimal cartilage support and maintenance of the respiratory mucosal lining.

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6. Heterotopic revascularization and allotransplantation of the cartilaginous trachea

6.1. Revascularization and rejection of the cartilaginous trachea

An allotransplant of the cartilaginous trachea may be useful to repair patch defects in the laryngotracheal airway tract which are unsuitable for segmental resection and autologous tissue repair. In 2008, we started performing patch tracheal allotransplants for these indications. Between 2008 and 2019, we performed ten patch tracheal allotransplantations in seven patients [1-3]. In this patient series, optimization of the revascularization strategy for this technique was explored. In addition, the possibility of reducing and eventually discontinuing immunosuppressive therapy was examined. However, three allotransplants were lost due to immunological rejection when immunosuppressive therapy was discontinued.

For this method, the membranous trachea is removed and the allograft is subsequently wrapped in forearm fascia and subcutaneous tissue for revascularization (Figs. 6.1–6.4). Revascularization and mucosal regeneration evolve slowly over a period of 2–3 months and small areas of necrosis can develop at the margins of the transplant (Fig. 6.4).

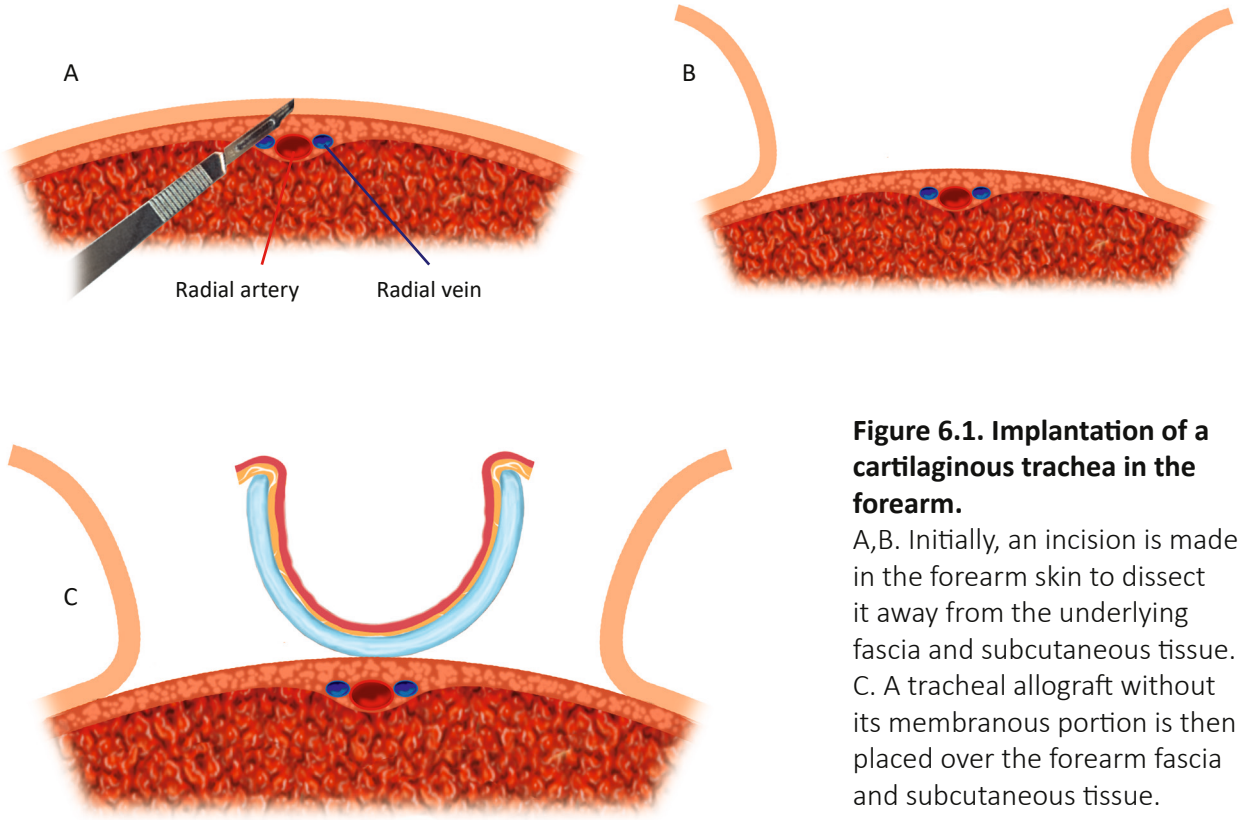


Figure 6.1. Implantation of a cartilaginous trachea in the forearm.
A,B. Initially, an incision is made in the forearm skin to dissect it away from the underlying fascia and subcutaneous tissue. C. A tracheal allograft without its membranous portion is then placed over the forearm fascia and subcutaneous tissue.

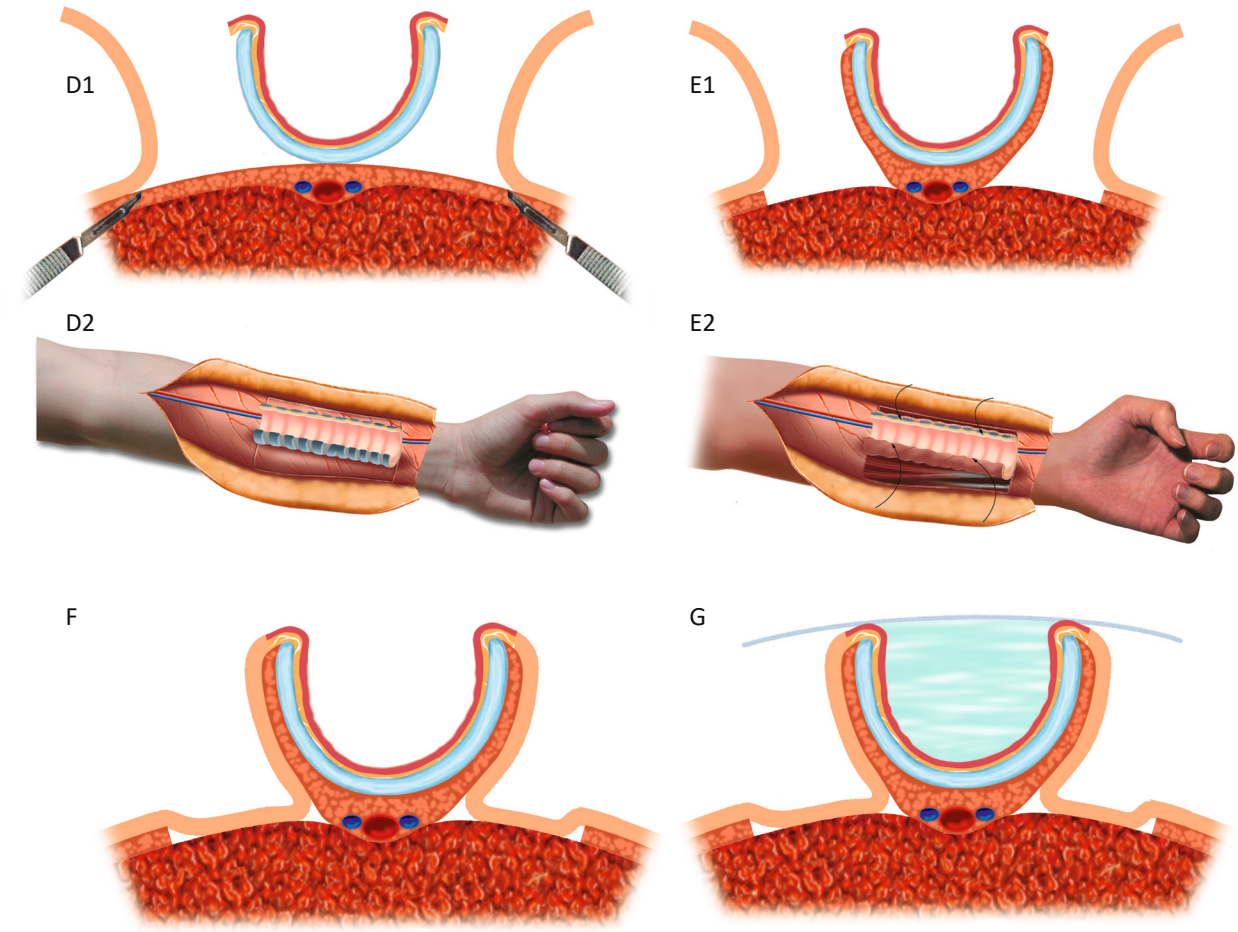


Figure 6.2. Implantation of a cartilaginous trachea in the forearm (continued).
Incisions are made in the fascia and subcutaneous tissue of the forearm (D1, D2) to dissect them from the underlying muscles. The attachments of the vascular pedicle are not disturbed (E1, E2). The cartilaginous trachea is then wrapped with radial forearm fascia (E1, E2) and the forearm skin flaps are sutured to the incised trachea (arrows in E2, F). G. The luminal site of the transplant is protected by an application of fibrin glue and the transplant is covered with a protective Gore-Tex® membrane sheet.

Figure 6.3. A cartilaginous trachea undergoing revascularization in a patient.
The forearm of a patient is shown with a Gore-Tex® soft tissue patch protecting a tracheal transplant as it undergoes revascularization.

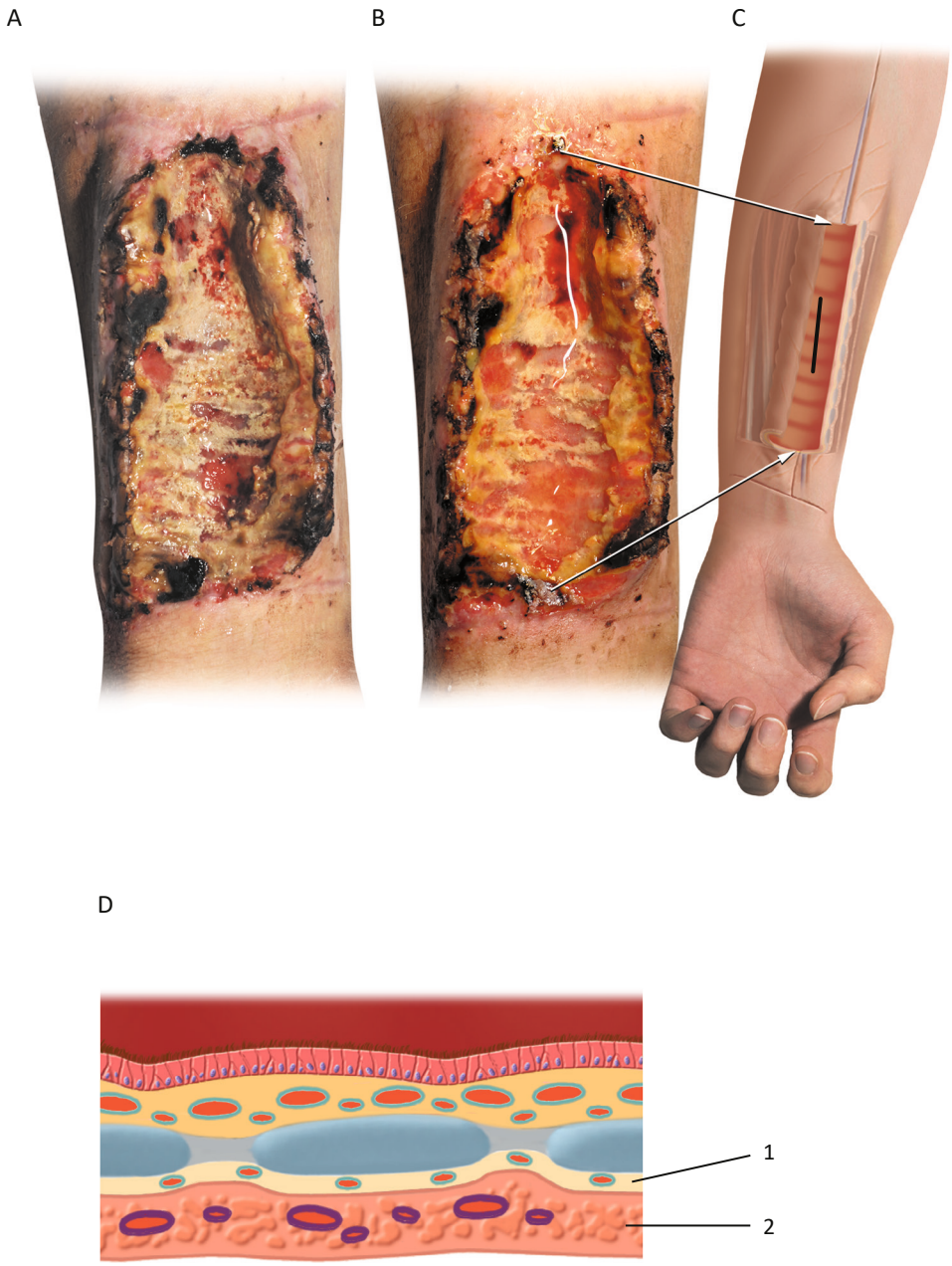


Figure 6.4. Images of heterotopic revascularization of a cartilaginous trachea in a patient. Revascularization of a tracheal allograft 1 month (A) and 2 months (B) after implantation. Revascularization and mucosal regeneration evolve slowly over 2–3 months. Small areas of necrosis are observed at the margins of the allograft. C. An illustration of a revascularized tracheal patch in the forearm is provided to represent what is imaged in panels A and B. D. A cross sectional view (the black line in C indicates where the incision is made) representing revascularization of the tracheal mucosal lining. Vascular connections form between the adventitia (1) and the surrounding fascia and subcutaneous tissue flap (2). Capillaries colored in blue derive from the donor, while capillaries colored in purple derive from the recipient.

A tracheal transplant will be ready for orthotopic transplantation three months after its implantation in forearm tissue. The most common indications for allotransplantation of a tracheal patch are airway stenoses with lengths greater than 5 cm and difficult re-stenoses after segmental resection. The small posterior remnant of the airway wall, which remains after an incision is made in the stenosis, serves as the recipient site of the allograft. The allotransplant is then sutured into the defect and its radial blood vessels are sutured to blood vessels in the neck to facilitate revascularization (Figs. 6.5, 6.6).

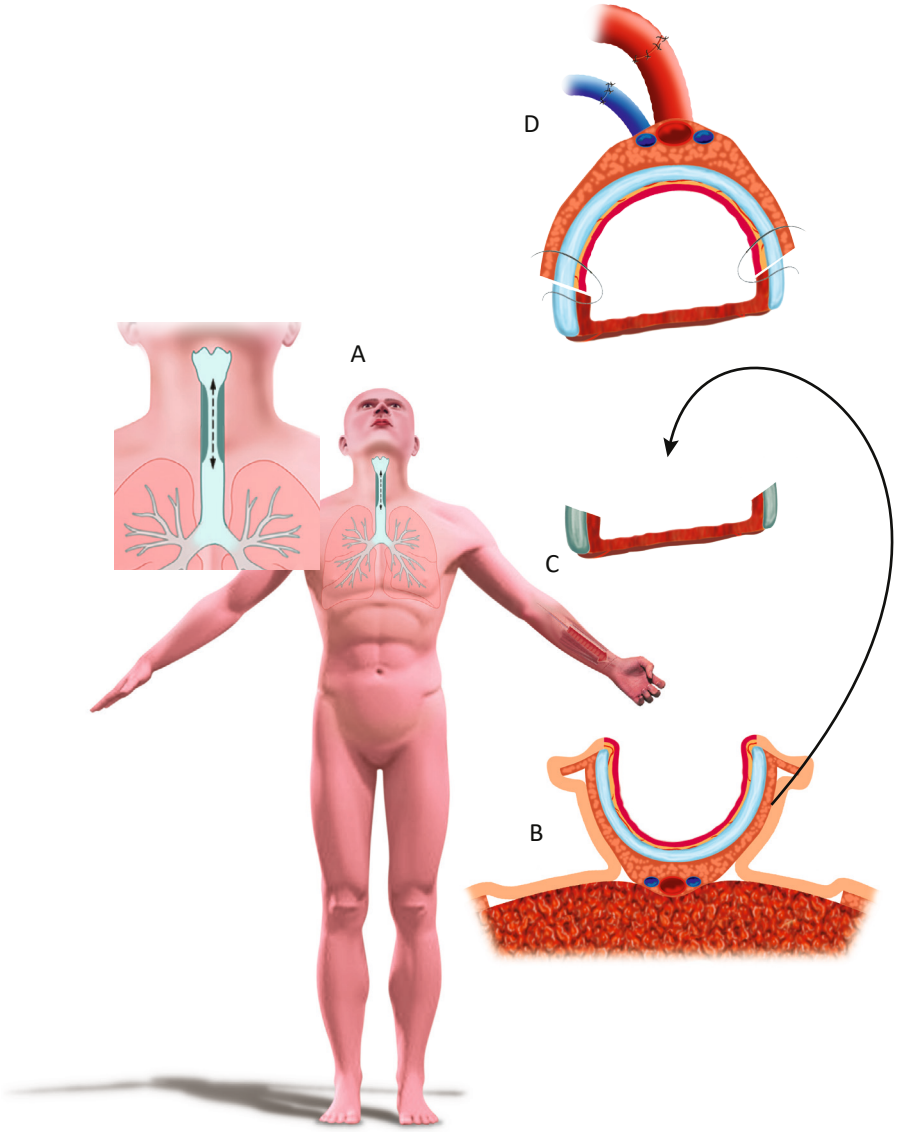


Figure 6.5. Orthotopic transplantation.
Orthotopic transplantation of a tracheal transplant to resolve long-segment airway stenosis. The long-segment tracheal stenosis is incised longitudinally [indicated with a dashed double arrow in (A) and expanded in (C)]. After full revascularization and mucosal regeneration have been achieved, the tracheal allotransplant is transplanted from the forearm (B) to the airway defect (C) on the radial vascular pedicle. D. The cartilaginous trachea is then sutured to the airway defect to restore concavity of the airway lumen. The radial blood vessels are connected to blood vessels in the neck (Movie 15).

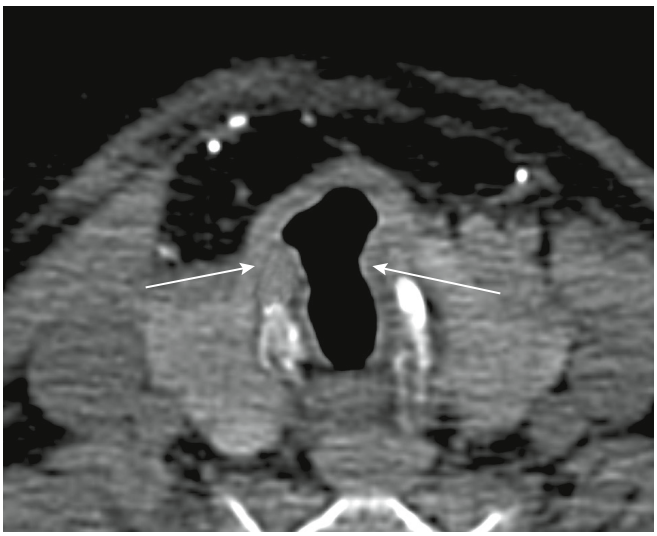
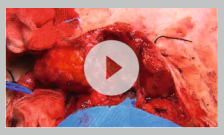


Figure 6.6. CT scan after tracheal autotransplantation.
Restoration of airway wall convexity is achieved with a patch tracheal allotransplant (between arrows).



Movie 15*
Orthotopic transplantation of a cartilaginous trachea.

* Due to the nature of the video clips, a YouTube account might be required in order to enable access.

A tracheal allotransplant is a composite tissue which can be used to restore the airway and improve a patient's quality of life. However, the benefits of a tracheal allotransplantation need to be balanced against the morbidity associated with long-term immunosuppression therapy. Therefore, attempts to withdraw immunosuppressive medications should be made before immunosuppressant-related complications occur. Cartilage tissue appears to escape immunologic rejection due to an absence of blood vessels in this tissue, and because its chondrocyte cells are protected within a matrix [1,3,4]. Thus, the mucosal lining is the primary site of rejection in an unprotected graft [5,6]. After immunosuppression therapy has been maintained for a year, it may be possible to gradually reduce and discontinue the administration of immunosuppressive drugs. However, this will increase the possibility that the donor-derived mucosal lining and mucosal capillaries of a graft will undergo necrosis. In contrast, the recipient's blood vessels will escape rejection and may potentially preserve the viability of an allotransplant. In our initial patient series of tracheal transplantations, it was observed that intercartilaginous ligaments obstructed the ingrowth of native, recipient blood vessels (Fig. 6.7). Furthermore, the development of full-thickness mucosal necrosis ultimately led to necrosis of the cartilage component and stenosis of the reconstructed airway lumen [2]. Therefore, measures that facilitate repopulation of the mucosal lining with endothelial and epithelial cells derived from the recipient will be important for preserving full transplant viability after immunosuppressive therapy is withdrawn. Such measures include the introduction of incisions into intercartilaginous ligaments and recipient mucosal grafts.

Both clinical (Fig. 6.7) and experimental (Fig. 6.8) rejections clearly illustrate that differences exist between vascular induction during revascularization of tracheal allografts and true angiogenesis. Moreover, these observations are consistent with the fact that vascular induction and angiogenesis with outgrowth of blood vessels are representing two different processes. For example, vascular induction of donor mucosal capillaries by recipient blood vessels around a transplanted tissue may occur through intercartilaginous ligaments, whereas true angiogenesis is only possible in areas where the recipient's blood vessels directly contact the submucosal layer of the graft.

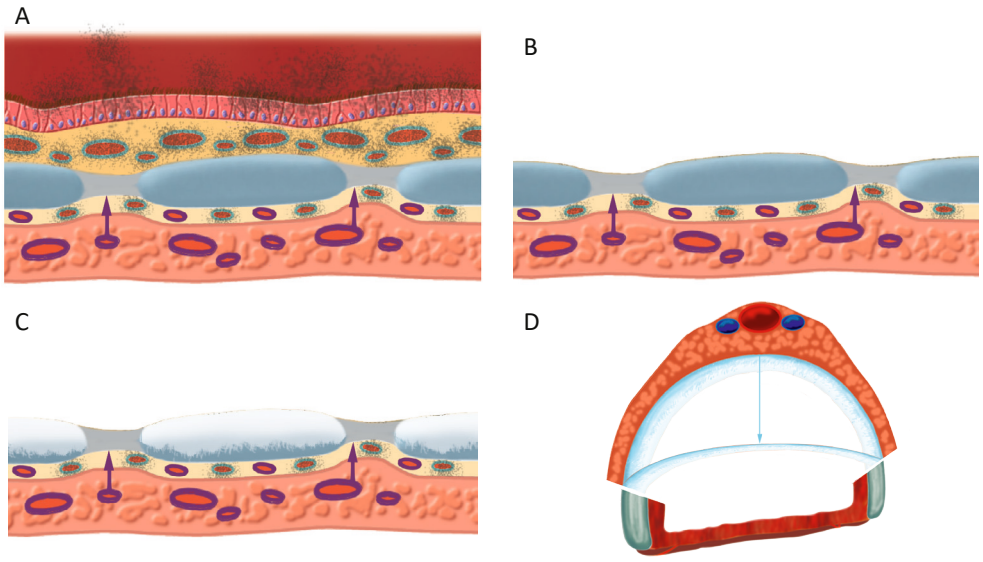


Figure 6.7. Discontinuing the administration of immunosuppressive drugs.
When immunosuppressive therapy is discontinued, donor-derived endothelial cells and epithelial cells undergo rejection. A. Immunologically-induced lymphocytes (represented by small black dots) attack the microcirculation and epithelial cells of a transplant. B. Inflammatory vascular infiltrates induce thrombosis of donor-derived blood vessels and necrosis of the mucosal layer. Resulting tissue ischemia during chronic rejection may then become a trigger for induction of angiogenesis and the growth of recipient blood vessels into the allotransplant (indicated with arrows in A). However, in the first patients who underwent transplantation, it was observed that their intercartilaginous ligaments acted as a barrier to the ingrowth of recipient blood vessels (indicated with arrows in B and C). This resulted in necrosis of the mucosal lining and eventually a loss of cartilage viability (indicated as grey/white shading in C) and collapse of the transplant (indicated with an arrow in D). Capillaries colored blue derive from the donor and are attacked by lymphocytes. Capillaries colored purple derive from the recipient and remain intact.

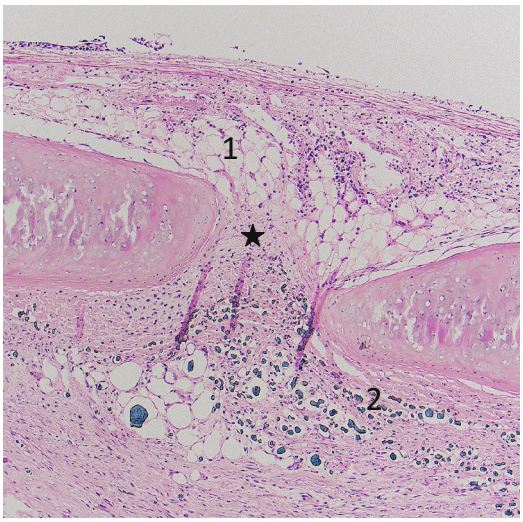


Figure 6.8. Histology of a tracheal allotransplant in a rabbit after immunologic rejection.
Hematoxylin and eosin staining shows end stage rejection in the mucosal layer and necrosis in the submucosa and epithelial lining (1). Capillaries of the fascia and the adventitial layer are filled with blue silicone dye (2) which was injected in the artery of the fascia flap. The fascia and adventitial layer provide capillaries from the recipient to preserve their viability. However, the intercartilaginous ligaments obstruct the ingrowth of blood vessels into the submucosal layer of the transplant (labeled with a black star).

6.2. **Intercartilaginous incision**

The most important adaption of the revascularization concept was to allow recipient blood vessels to undergo angiogenesis in the submucosal space of the tracheal allograft. This process was facilitated by making partial incisions in the intercartilaginous ligaments during the forearm implantation procedure. These incisions disrupt the barrier for angiogenetic outgrowth of recipient vessels. When incisions were made at regular intervals, revascularization and mucosal regeneration of the cartilaginous allotransplant was observed within a period of 2–3 months (Figs. 6.9, 6.10). While this period of time for revascularization was similar to that observed for transplants without incised ligaments, the placement of regularly spaced intercartilaginous incisions allowed the recipient vessels to undergo angiogenesis through the ligamentous barrier and grow into the submucosal space of the transplant tissue. The successful integration of recipient vessels into the transplant tissue enables the cartilage component of the transplant to survive after immunosuppressive therapy is discontinued (Fig. 6.10).

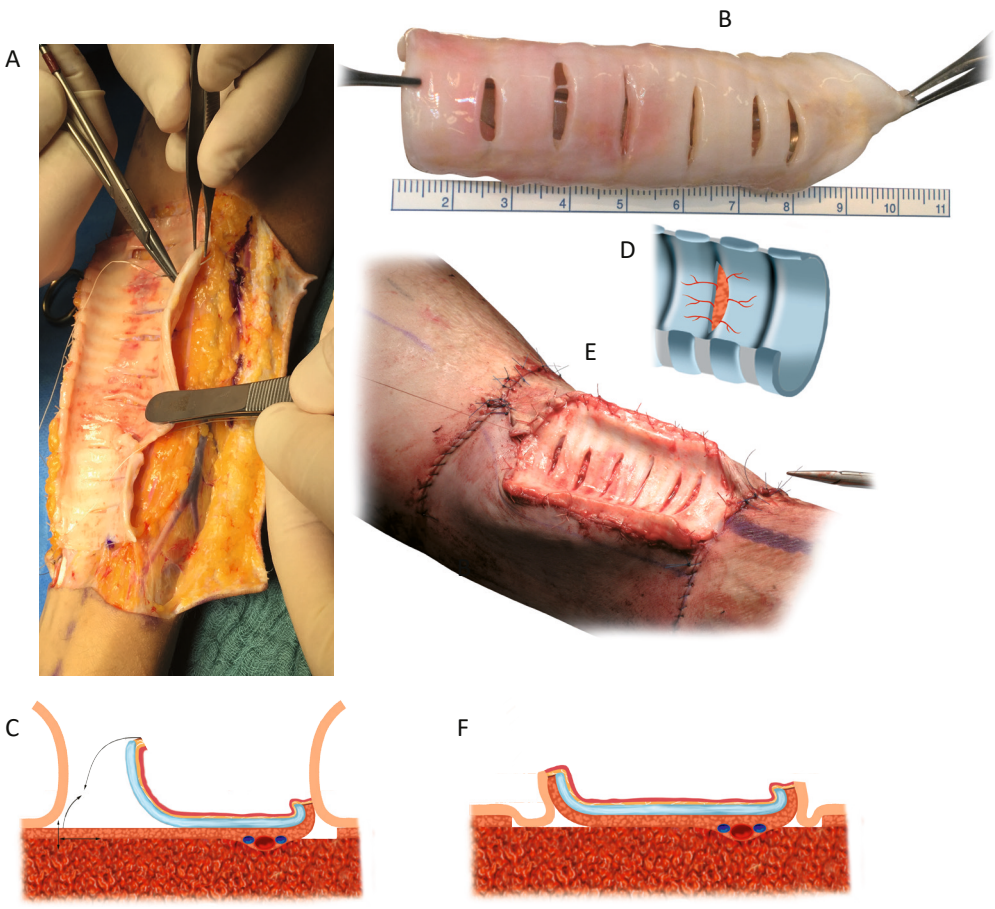


Figure 6.9. Intercartilaginous incisions are made during forearm implantation.
A. Intercartilaginous incisions are made in a tracheal allotransplant during its implantation into the forearm.
B. Partial incisions are made in the intercartilaginous ligaments in alternating anterior intercartilaginous spaces.
C. Fascial wrapping is performed with minimal dissection of the radial forearm fascia (indicated with arrows).
D. Disruption of the intercartilaginous ligaments facilitates growth of the recipient's blood vessels into the submucosal space of the transplant.
E,F. After implantation, the allograft loses part of its concave shape. However, its shape will be regained during orthotopic transplantation.

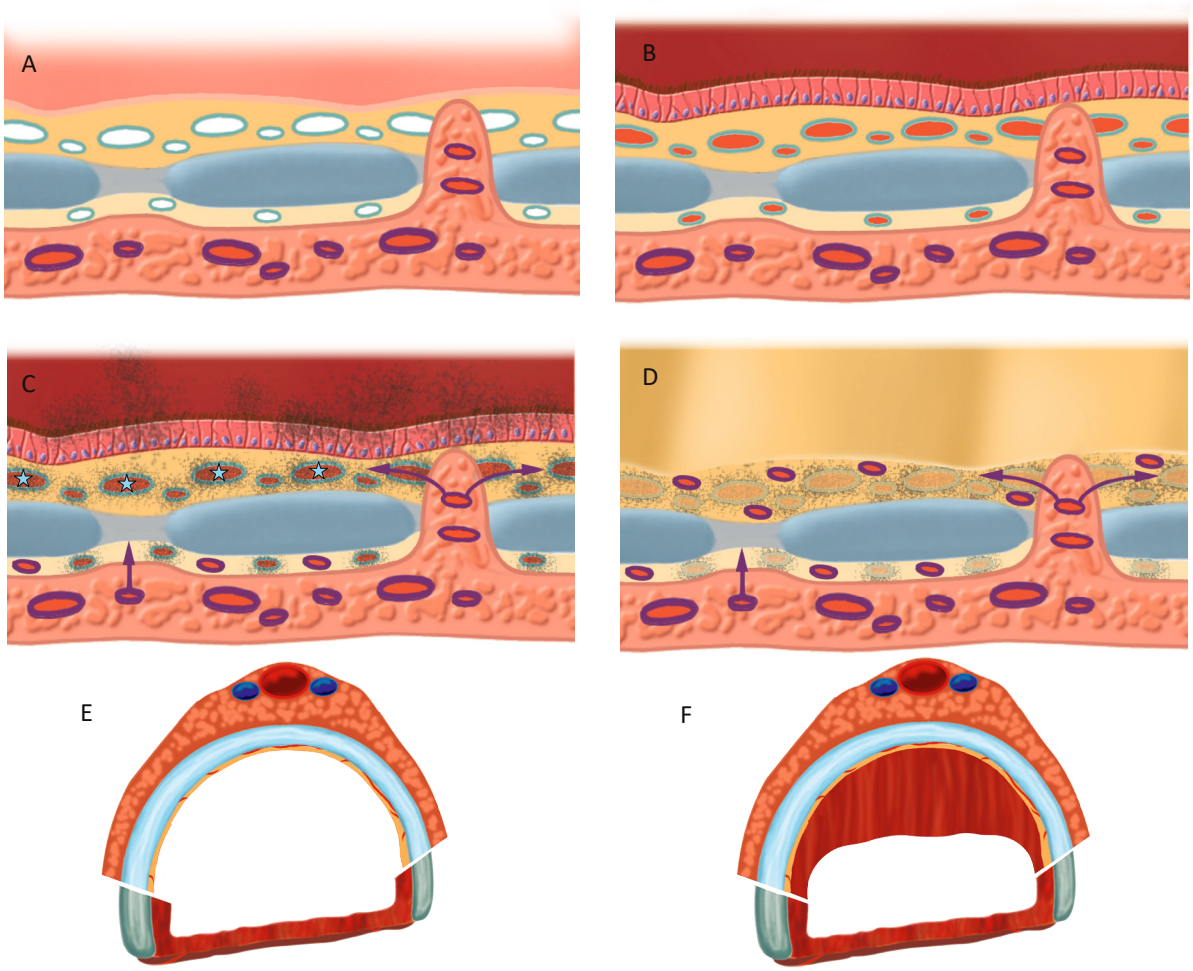


Figure 6.10. Intercartilaginous incisions and withdrawal of immunosuppressive therapy.
A,B. Revascularization of the submucosal layer is induced in the presence of a fascial flap. The incisions made in intercartilaginous ligaments have little influence on the length of the revascularization period since angiogenesis and the outgrowth of blood vessels is a slow process. Revascularization and mucosal regeneration have been observed to plateau after a 2–3 month period. The capillaries outlined in blue originate from the donor and the capillaries outlined in purple originate from the recipient.
C. During the administration of immunosuppressive drugs, the recipient's blood vessels will grow into the submucosal space of the transplant through incisions made in the intercartilaginous ligaments (indicated with purple arrows). However, when immunosuppressive drugs are no longer administered, both donor-derived blood vessels and epithelial cells are attacked by lymphocytes (represented by small black dots). Eventually, blood vessels from the donor will undergo thrombosis (indicated with asterisks) and the epithelium will become necrotic (D). Outgrowth of blood vessels occurs through incisions made in the ligaments of the recipient. This outgrowth ensures that vascularized tissue will be present at the luminal site of the allograft, thereby facilitating survival of the cartilage tissue (D,E). However, in the absence of a mucosal lining, a vascularized connective tissue layer will enable healing via secondary intention, and this will result in narrowing of the reconstructed airway lumen (F).

When incisions are made in intercartilaginous ligaments, cartilage allografts are resistant to necrosis and they remain viable when surrounded by well-vascularized recipient tissue. However, secondary healing after necrosis of the donor epithelium leads to loss of the reconstructed airway lumen that is provided by the transplant (Fig. 6.10).

6.3. **Buccal mucosal grafting**

After immunosuppressive medication is withdrawn and necrosis of the donor's mucosal lining occurs, secondary healing with loss of the reconstructed airway lumen can occur. To avoid these situations, recipient mucosa should be introduced into the mucosal lining of a revascularized tracheal transplant. Buccal mucosal grafts are the best option because respiratory mucosa is difficult to handle as a free graft. Full-thickness skin grafts are also less suitable because they are keratinized and thicker than buccal mucosa grafts.

Recipient mucosal grafts can be introduced after portions of a donor's respiratory mucosa are removed. Generally, a free mucosal graft that is a little bigger than the estimated defect is harvested (Fig. 6.11). A mucosa stretcher is used to retract and provide hemostasis to the donor bed providing improved access and ease of harvest for the surgeon. Local anesthetic is injected at the periphery of the marked outline and deep into the central area of the donor site to help elevate and hydro-dissect the oral mucosa from the underlying soft tissues. The graft is then incised and dissected off of the buccinator muscle, while avoiding Stensen's duct. The defect can then be closed with an absorbable suture, or it can be left open to undergo closure by secondary intention. To encourage revascularization of the graft, proper defatting and removal of all excess submucosal tissue, fat, and muscle should be performed [7,8].

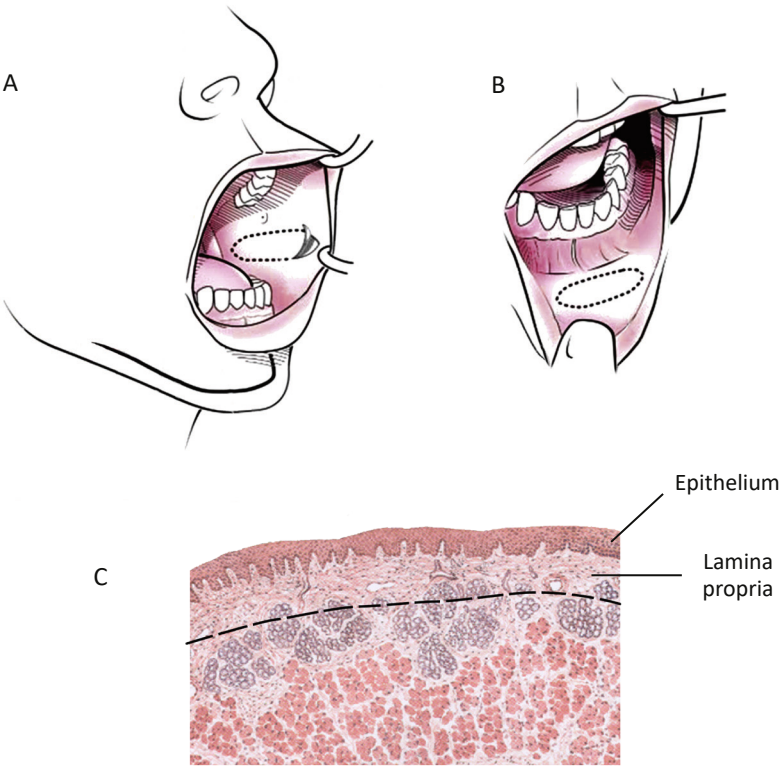


Figure 6.11. Free buccal mucosa graft. Possible sources of mucosal grafts are outlined in the inner side of the cheek (A) and in the lower lip (B). C. Anatomically, the oral mucosa consists of a thick nonkeratinized stratified squamous avascular epithelium and a vascular lamina propria (shown with H&E staining). Black dashed line indicates dissection plane for harvest of a mucosal graft.

After revascularization, the donor's respiratory epithelium is removed from the midportion of the transplant and denuded sites are grafted with recipient buccal mucosa (Fig. 6.12). Ciliated epithelium is considered to have an important role in mucosal transport. However, the ciliary function of this epithelium is not primordial. For example, smokers with squamous metaplasia of the mucosa do not experience significant problems, although the ability to cough up phlegm needs to be maintained.

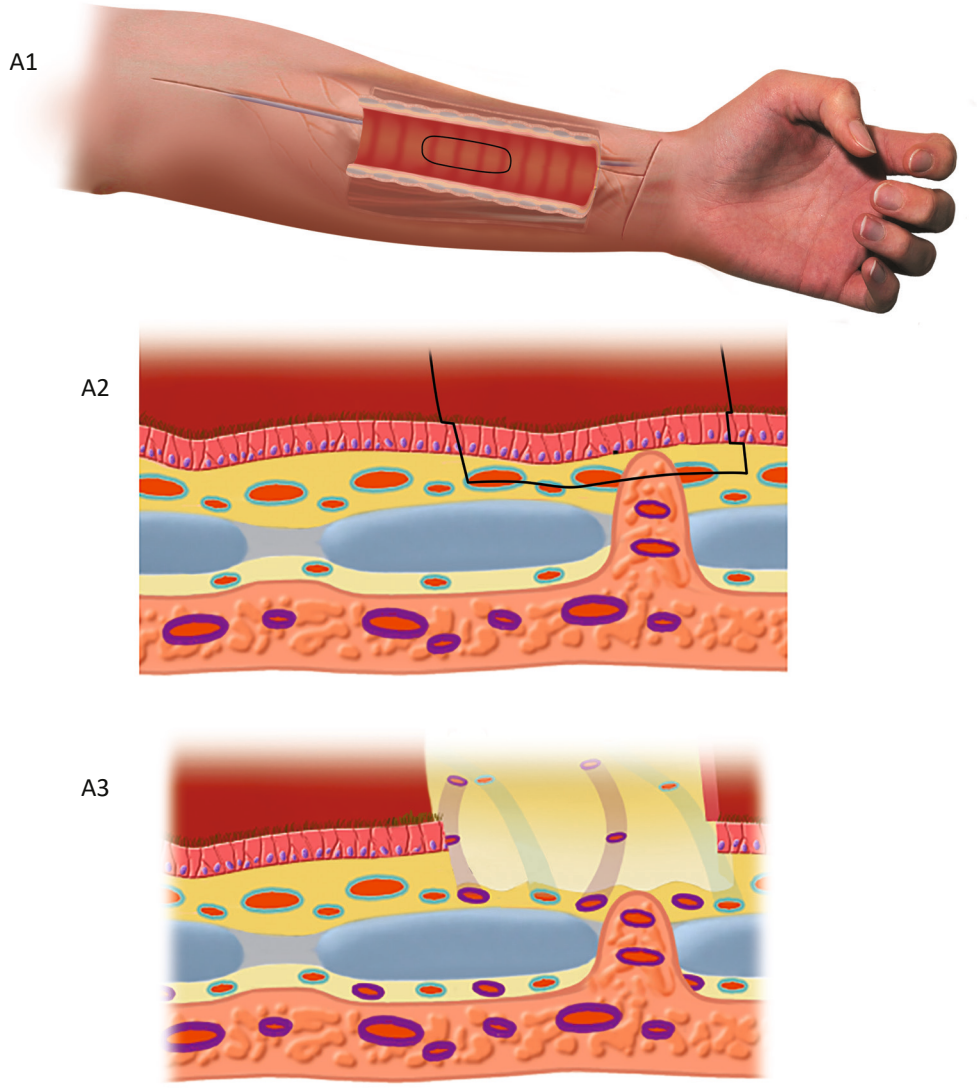


Figure 6.12. Grafting of a revascularized allotransplant with full-thickness buccal mucosa.
A1. Outlining of the segment of respiratory mucosa that will be resected at the midportion of the allotransplant.
A2. Axial section at midportion of the transplant. Outlining of the segment of respiratory mucosa that will be resected.
A3. Axial section at midportion of the transplant after removal of donor mucosa. Vascularized bed after resection of respiratory mucosa. The vessel wall of the donor capillaries are blue colored while the vessels wall of the recipient capillaries are purple colored. Recipient capillaries have grown into the submucosal space of the transplant.

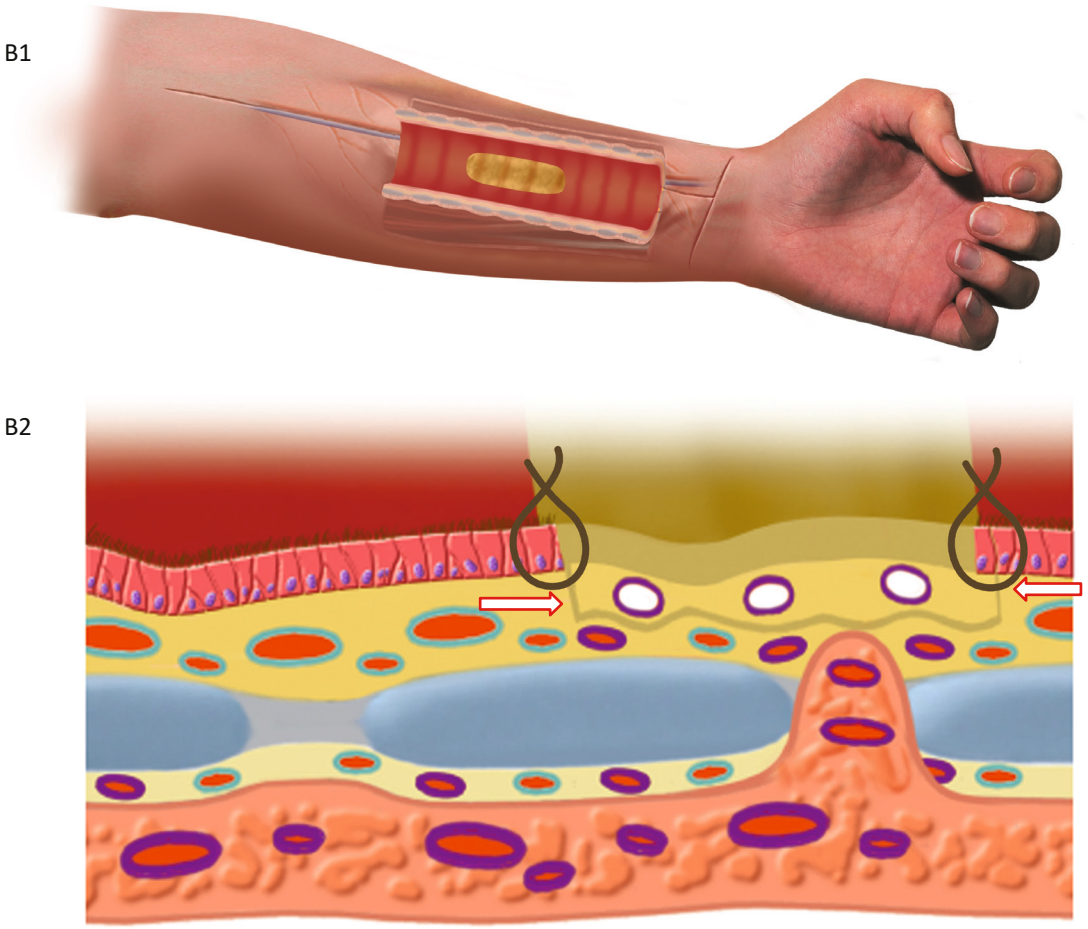


Figure 6.12. Grafting of a revascularized allotransplant with full-thickness buccal mucosa (continued).
B1. A buccal mucosal graft is sutured into the midportion of the transplant after a similar amount of donor mucosa is removed.
B2. A good graft inset is necessary to ensure immobilization of the graft on its bed and to prevent haematoma formation. The full thickness graft is sutured to the wound edges circumferentially with independent sutures. The blood supply of the neighbouring tissue (arrows) may provide an additional source to successfully revascularize the allograft.

A good graft inset is necessary to ensure immobilization of the graft on its bed and to prevent haematoma formation. Mucosal grafts rely on the underlying vascularity of the bed to maintain cellular respiration [9]. After ingrowth of the buccal mucosal graft, the allotransplant can be orthotopically transplanted to repair a difficult airway defect. Most of the indications for this procedure involve long-segment stenoses. One patient in our own series was treated for a low-grade chondrosarcoma with involvement of the trachea and the lower part of the right hemilarynx (Figs. 6.13-6.16).

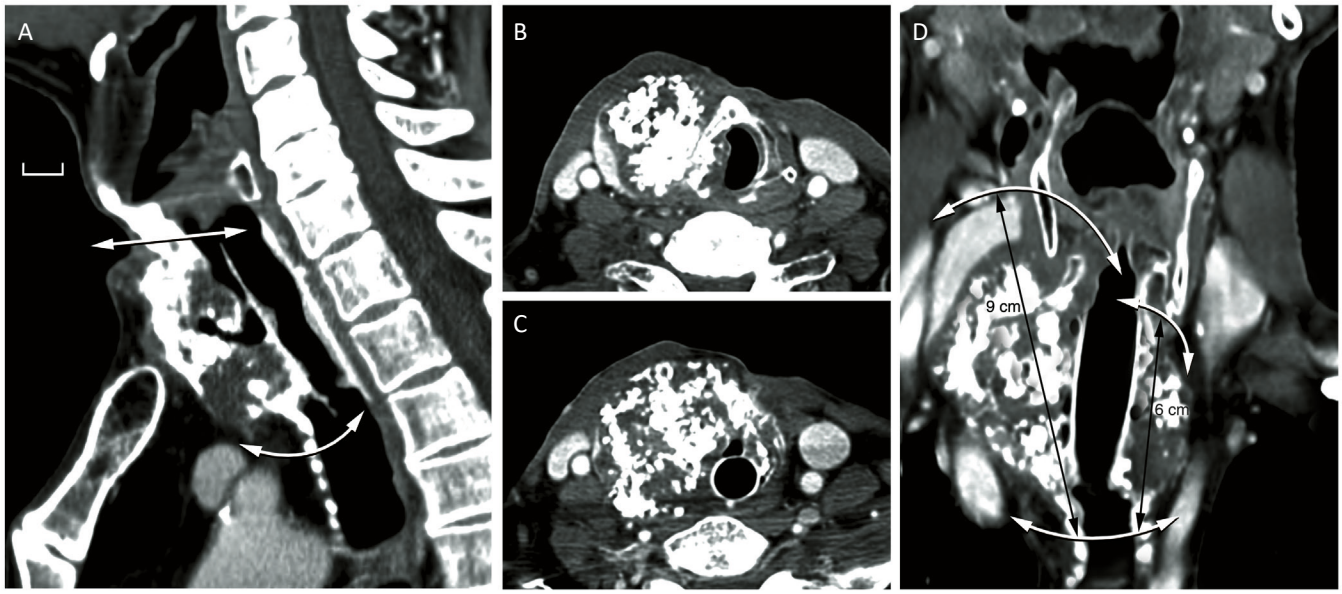


Figure 6.13. CT scan of tracheal chondrosarcoma before transplantation.
Tumor extent is shown in: (A) a sagittal reformatted CT scan, (B) an axial CT scan at the subglottic level, (C) an axial CT scan at the tracheal level, and (D) in a coronal reformatted CT scan. The airway lumen is bridged by a silicone stent that holds the airway open. The extent of resection is indicated with white, two-headed arrows. Lengths of laryngotracheal resection were 9 cm (right) and 6 cm (left) (scale: 1 cm). A tracheal segment of 6 cm was resected together with the lower part of the right hemilarynx.

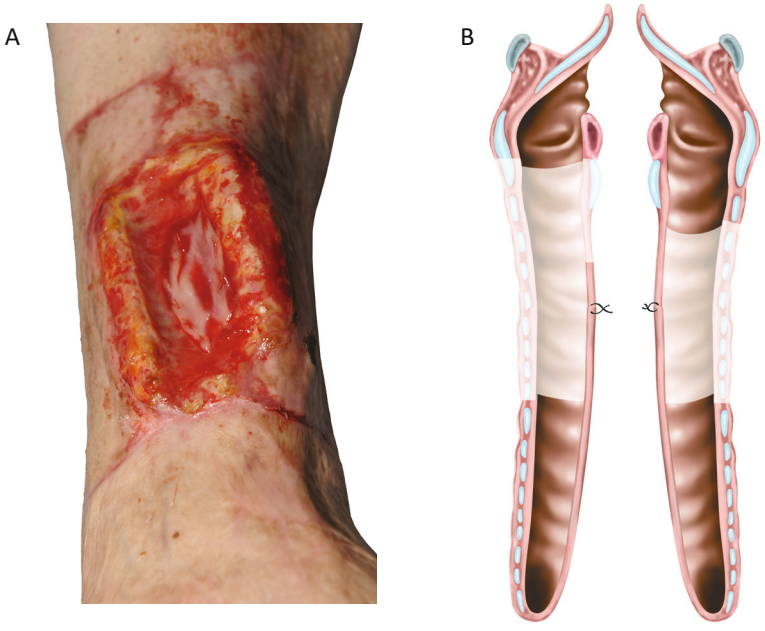


Figure 6.14. Situation after chondrosarcoma resection.
A. Orthotopic tracheal transplant prior to withdrawal of immunosuppressive drugs. A procedure developed to minimize secondary healing after discontinuing immunosuppressive therapy is shown. After full revascularization of the tracheal transplant is achieved in the forearm, a full-thickness buccal mucosa graft from the recipient's cheek is grafted to the midportion of the trachea transplant (after a similar extent of the donor respiratory mucosa is removed)
B. Midsagittal incisional view of the laryngotracheal defect.

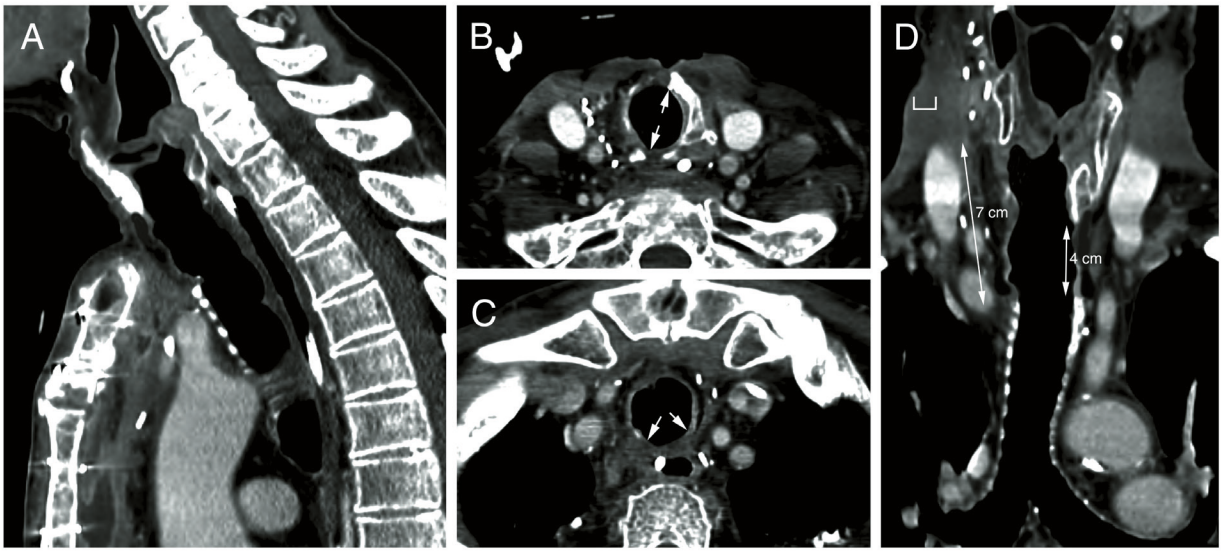


Figure 6.15. CT scan after patch tracheal transplantation.
CT scans obtained after orthotopic transplantation and prior to discontinuation of immunosuppressive drugs. Note the absence of cartilage calcification in the allotransplant (scale: 1 cm). A. A sagittal reformatted CT scan. B. An axial CT scan at the laryngeal level. The tracheal transplant is indicated by the two white arrows. C. An axial CT scan at the level of the cervical trachea. The tracheal transplant is indicated with two white arrows. D. A coronal reformatted CT scan. The tracheal transplant measures 7 cm on the right side and 4 cm on the left side.

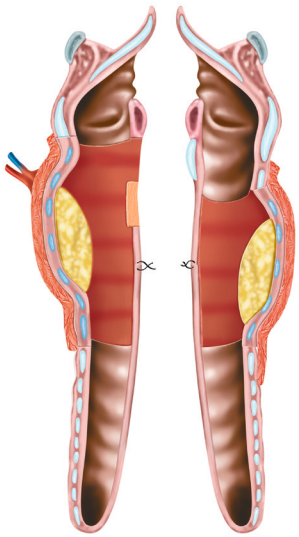


Figure 6.16. Orthotopic tracheal transplantation before discontinuing immunosuppressive treatment.
A midsagittal incisional view of a laryngotracheal defect that is reconstructed with a tracheal allotransplant. The buccal mucosa graft (colored yellow) is visible at the midportion of the transplant.

Withdrawal of immunosuppression treatments began four months after a heterotopic implantation was performed and was completed after 1 year. A time line that includes revascularization, orthotopic transplantation, and withdrawal of immunosuppressive drugs is shown in Figure 6.17.

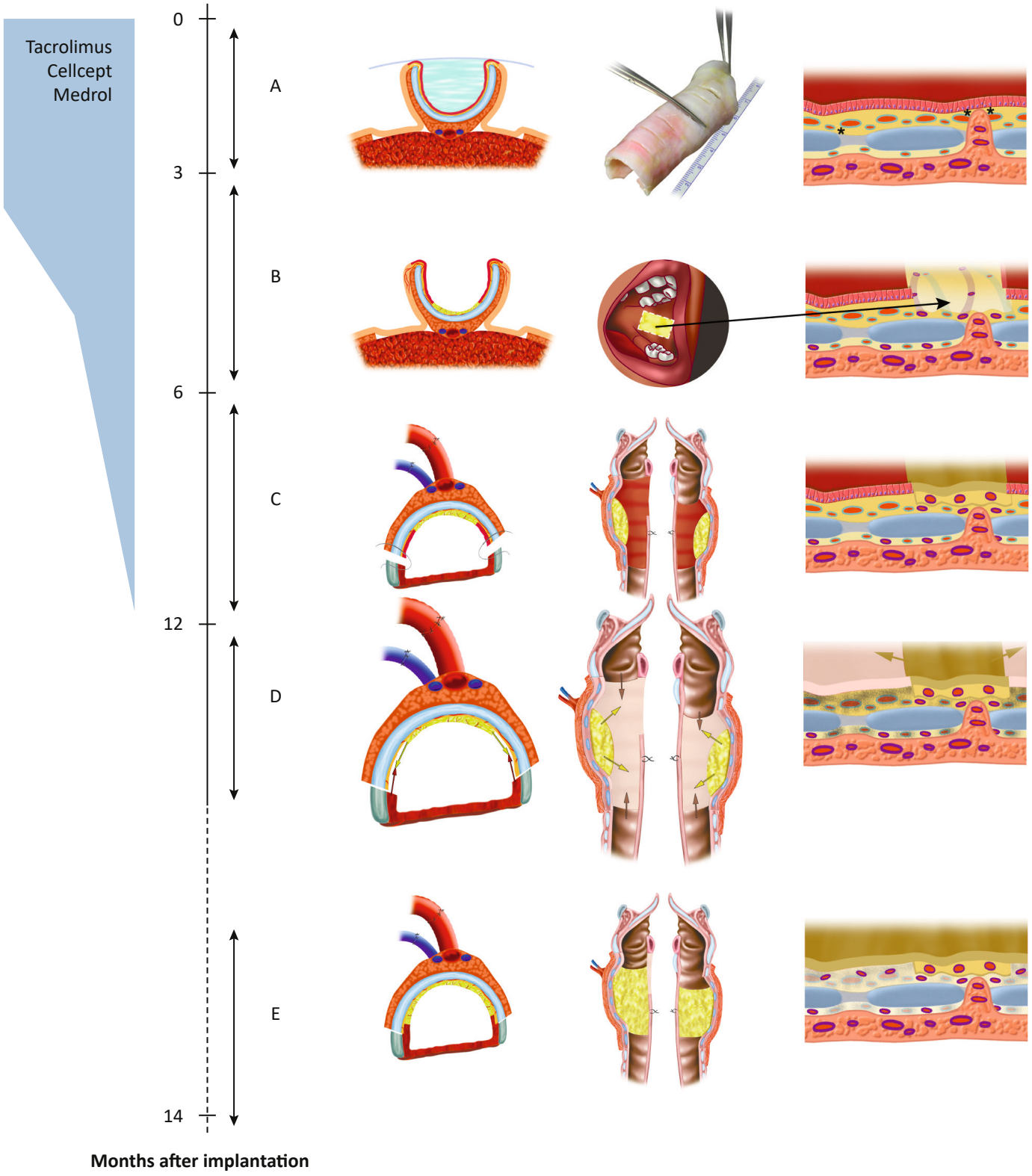
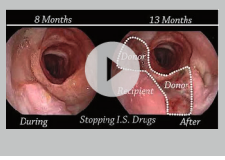

Tacrolimus
Cellcept
Medrol
0
3
6
12
14
A
B
C
D
E
Months after implantation

Figure 6.17. Time line for tracheal allotransplantation.
Timeline for clinical procedure (left), key surgical steps (middle), and postulated processes of revascularization, tissue rejection, and regeneration (right). Timeline (in months) shows the principal procedural steps (**A-E**) and timing of immunosuppression (Tacrolimus 9 mg/d, Cellcept 2 g/d, Medrol 24 mg/d). **A. Heterotopic allograft revascularization**. Freshly harvested tracheal allograft is wrapped with fascia and subcutaneous tissue on the radial side of the forearm. Fibrin glue (light blue) on the luminal side prevents the mucosa from drying. Before implantation, partial incisions are made in alternating anterior intercartilaginous spaces. Recipient blood vessels (purple lining) from forearm tissues mediate vascular induction of donor blood vessels (blue lining) in the adventitia, which, in turn, mediate vascular induction of donor blood vessels in the submucosa, either through intact intercartilaginous ligaments (*) or direct contact in areas where ligaments are incised (**). Revascularization allows the donor respiratory mucosa to regenerate, a process that is complete within 3 months. **(B) Implantation of recipient buccal mucosa.** Once the graft is fully revascularized, the central portion of the respiratory donor mucosa is removed and replaced with a graft from the recipient's mouth mucosa (yellow). Angiogenesis occurs with ingrowth of recipient blood vessels from the surrounding forearm tissues into the mucosal space of the transplant, through intercartilaginous ligament incisions. **(C) Orthotopic vascularized transplantation.** After ingrowth of the recipient mucosal graft, the revascularized tracheal graft can be transplanted orthotopically with its newly created vascular pedicle to repair the airway defect (illustrated in a sagittal laryngotracheal view). Immunosuppressive medication is gradually phased out and stopped at 1 year (blue bar). **(D) Allorejection of donor noncartilaginous tissues.** Withdrawal of immunosuppression provokes immunologic rejection of residual donor mucosal tissues, with inflammatory infiltrates, vascular thrombosis, and necrosis of the mucosal layer. The donor cartilage is immune-privileged and not susceptible to allorejection. Noncartilaginous tissues are replaced via outgrowth of the recipient buccal mucosa (yellow arrows) and recipient respiratory mucosa at the anastomotic sites (brown arrows). **(E) Replacement of noncartilaginous recipient-type tissues.** Situation after healing of rejected donor mucosal lining.

The intercartilaginous incisions facilitate repopulation of the submucosal layer by recipient capillaries after immune rejection. These newly formed blood vessels allow the mucosal lining from the recipient, which is inserted in the midportion of the transplant, to survive after withdrawal of immunosuppressive medication. Full-thickness mucosal grafts inhibit wound contraction at the midportion of the transplant. Meanwhile, the remaining donor mucosal sites undergo necrosis and secondary wound healing with ingrowth of recipient epithelium and with some loss of the reconstructed airway lumen (Figs. 6.18, 6.19; Movie 16). After the tracheal transplant has been populated with non-ciliated recipient epithelium, the loss of mucociliary clearance at the transplanted site is compensated for by clearance through coughing (Movie 16).



Movie 16
Healing of a tracheal allotransplant after withdrawal of immunosuppressive drugs.

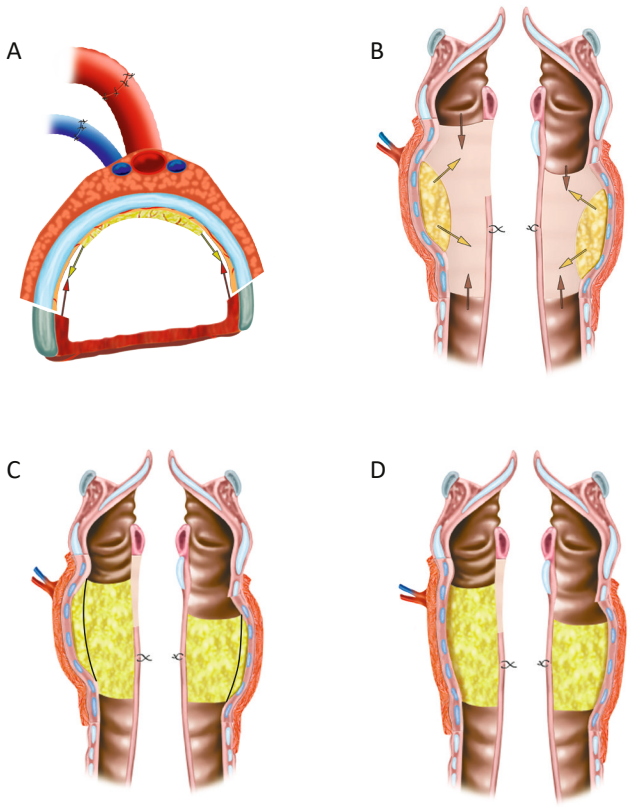


Figure 6.18. Mucosal healing of a tracheal transplant after withdrawal of immunosuppressive medication.
Axial (A) and sagittal midline incisional (B) views of a reconstructed airway after withdrawal of immunosuppressive medications. The revascularized allotransplant consists of both recipient and donor tissues. The donor's epithelial lining and endothelium will disappear after withdrawal of immunosuppressive medication. These areas will subsequently be repopulated by recipient vessels and recipient epithelium from a buccal mucosal graft (indicated with yellow arrows), and also by surrounding respiratory mucosa (indicated with brown arrows). C. This re-epithelialization will occur with secondary healing with some loss of the reconstructed airway lumen (indicated with black lines). D. Allotransplant after completion of secondary healing.

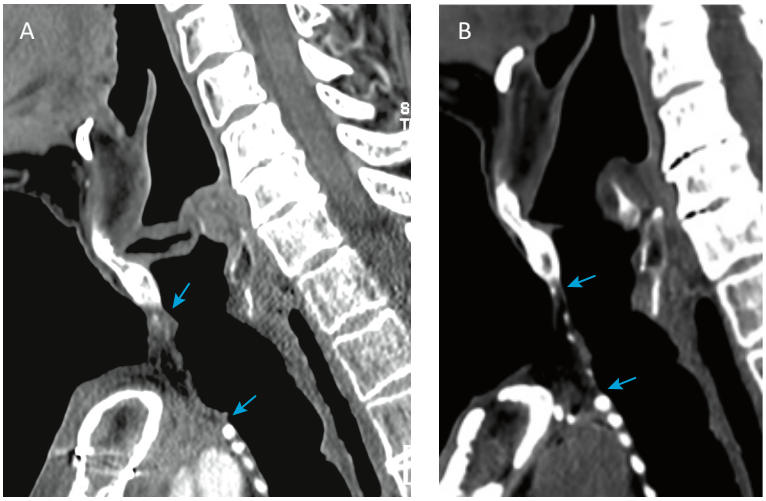


Figure 6.19. Mucosal healing of a tracheal transplant observed in CT scans after administration of immunosuppressive drugs is discontinued.
Sagittal reformatted CT scans obtained before (A), and one year after (B), immunosuppressive drugs are withdrawn from a patient who underwent a tracheal transplant. The dimensions of the transplant indicated between the arrows are reduced due to secondary healing after necrosis which developed in regions of the donor-derived mucosal lining.

6.4. **A more rapid revascularization process and reduced secondary healing**

A major drawback of the tracheal transplantation concept is the long period of time that is needed for donor mucosal revascularization and regeneration to occur. It takes approximately 2–3 months before full mucosal regeneration is established. During this period, the margins of the transplant are at risk of necrosis and the circumference of the cartilaginous trachea is reduced due to tissue loss. Interestingly, we observed in one of our patients that the revascularization phase could be shortened from 2–3 months to 2–3 weeks when the luminal surface of the transplant was covered with radial forearm fascia (Figs. 6.20–6.22).

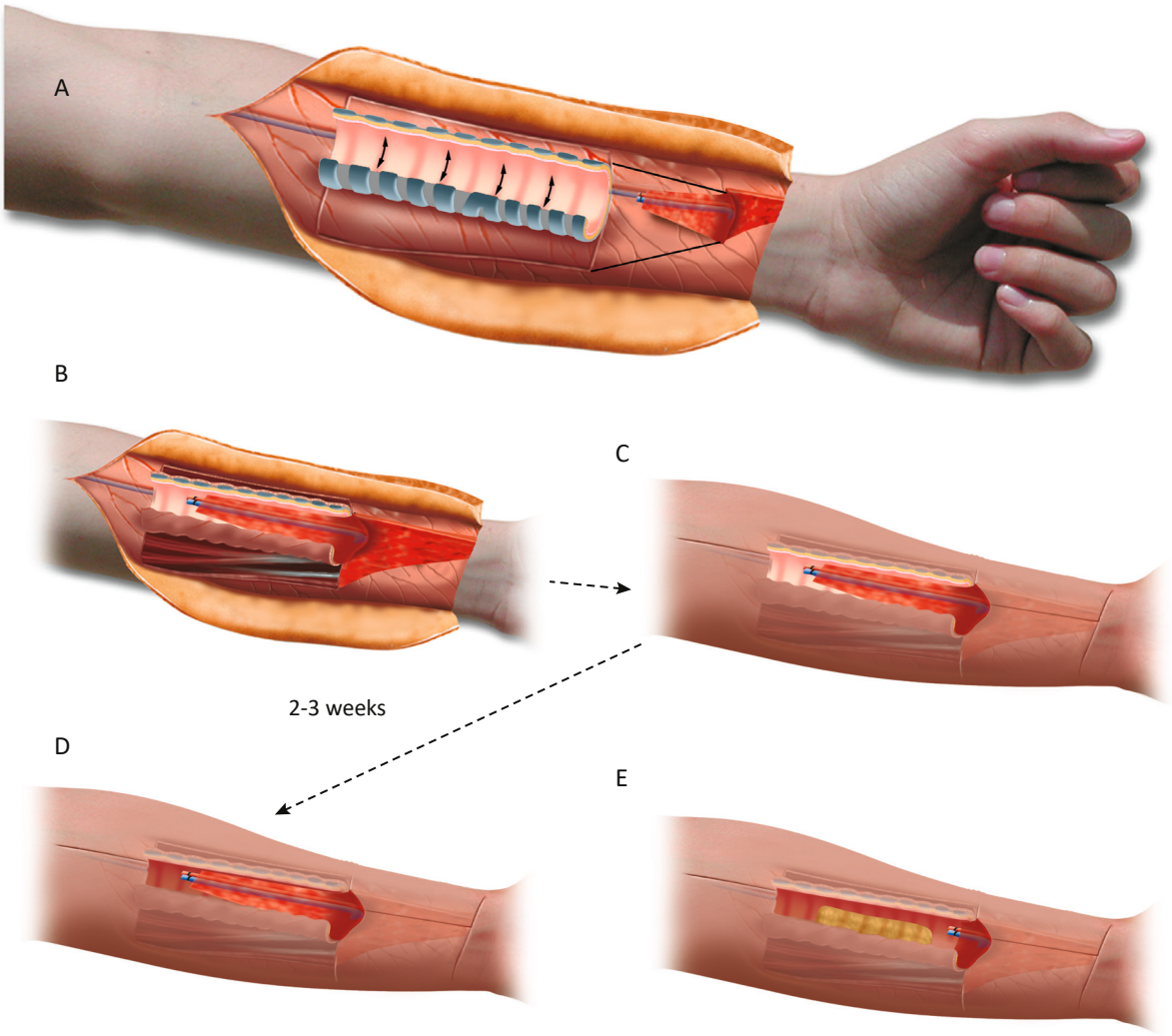


Figure 6.20. Optimal protocol for patch tracheal revascularization.
When an allotransplant is implanted in a more proximal position, a larger amount of distal fascia is available to cover the luminal site of the transplant. Therefore, the distal radial forearm fascia should be rotated inside the tracheal allograft. Double arrows indicate where intercartilaginous incisions should be made (A). By covering the luminal site of the transplant with well vascularized tissue mucosal revascularization and regeneration occur more rapidly (B-D). After revascularization, a buccal mucosal graft from the recipient is implanted in the central portion of the allograft. After ingrowth of the buccal mucosal graft, the inside rotated portion of the fascia flap is removed from the luminal site of the transplant (E).

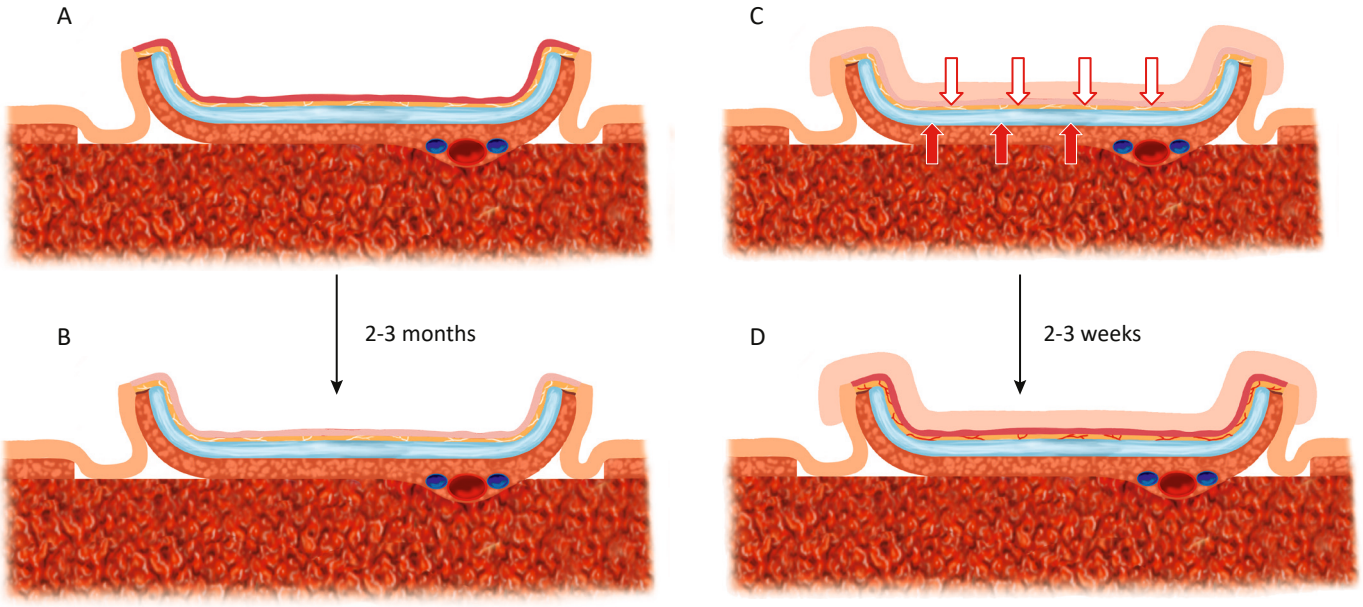


Figure 6.21. Optimal protocol for patch tracheal revascularization (continued).
A,B. Mucosal revascularization and epithelial regeneration occur over a period of 2-3 months when the luminal site is uncovered. C,D. However, the revascularization period can be reduced to 2-3 weeks when the luminal site is covered by well-vascularized tissue (represented by a pink tissue layer). In C, the mechanisms which may contribute to more rapid revascularization of the submucosal space include stimulation of the vascular induction process by surrounding fascia (indicated with red arrows) and a diffusion of nutritients from the luminal fascia to the epithelial cells (indicated with white arrows).

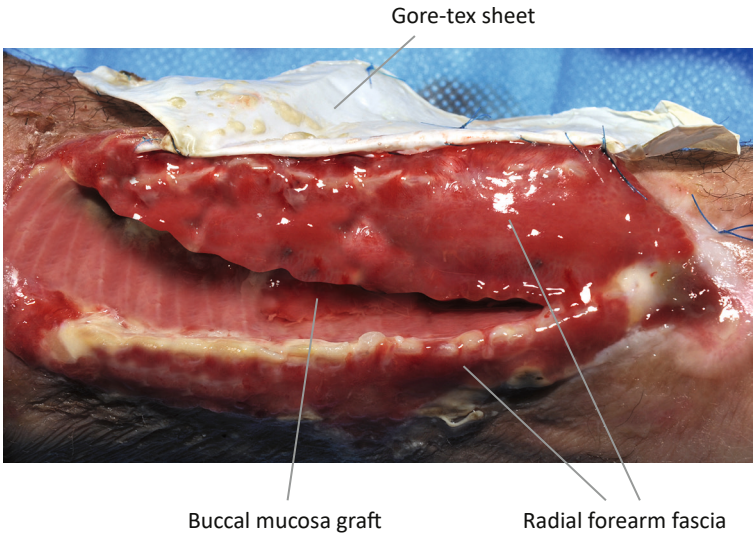


Figure 6.22. Optimal protocol for patch tracheal revascularization (continued).
Image of a tracheal transplant with distal radial forearm fascia covering the luminal site. A buccal mucosa graft is visible at the midportion of the graft.

To maximally preserve the lumen of a reconstructed airway after immunosuppressive drugs are withdrawn, it is advisable to replace a large portion of the transplant's central mucosal lining with recipient buccal mucosa (Fig. 6.23).

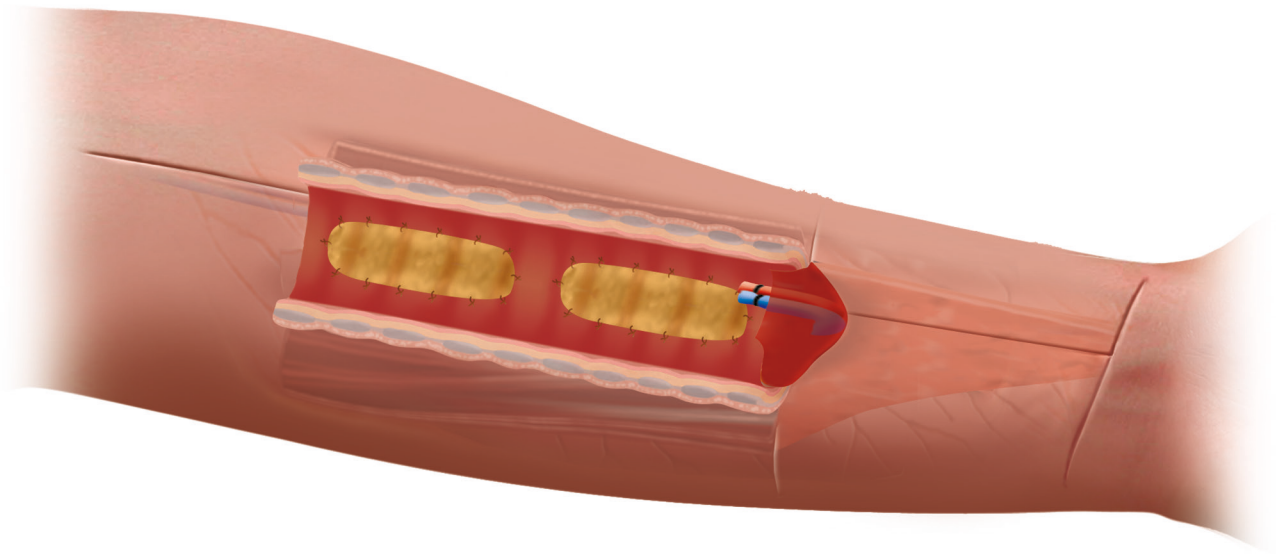


Figure 6.23. Minimization of secondary healing.
A large portion of the central region of a tracheal transplant is grafted with two buccal mucosa grafts to minimize secondary healing.

6.5. **Conclusion**

The optimal transplantation protocol that has been described involves intercartilaginous incisions and a large area of buccal mucosa grafting to provide a controlled process of progressive vascularization, rejection of allogeneic mucosa, and preservation of cartilage viability (which is otherwise immune-privileged). This vascularized transplantation technique with temporary immunosuppression generates a chimeric trachea, and the immune-privileged nature of the cartilage involved has a central role. This technique holds promise for patients needing extensive airway reconstruction because the resulting chimeric trachea graft does not require ongoing immunosuppression, and this is a highly desired, yet elusive goal, in the field of allotransplantation [10,11] (Fig. 6.24).

This technique was developed on the basis of animal research [5,6] and autotransplantation experience [12]. The technique was further refined in a series of seven patients (1-3). Based on our latter experience, we learned that vascular induction of the submucosal blood supply during graft revascularization is a distinct process from true angiogenesis. For example, vascular induction of donor mucosal capillaries by recipient blood vessels around a transplanted tissue can occur through intercartilaginous ligaments. In contrast, true angiogenesis involves the growth of recipient blood vessels into the submucosal space, and this is only possible in areas where recipient blood vessels are able to directly contact the submucosal layer of the graft. Therefore, the most important adaptation of the tracheal autotransplant procedure was to allow recipient blood vessels to grow into the submucosal space of a graft. This growth was facilitated by making partial incisions in the intercartilaginous ligaments. These incisions disrupt the ligament barrier to angiogenic outgrowth of recipient vessels and it enables growth of the recipient vessels into the submucosal space of the transplant.

Another important factor in optimizing the tracheal autotransplant procedure was the use of a recipient buccal mucosal graft in the central part of the transplant. Buccal mucosa was chosen because respiratory mucosa is difficult to handle as a free graft. After discontinuing immunosuppressive therapy, the buccal mucosa graft is perfused by an ingrowth of recipient blood vessels. When the donor respiratory epithelium is eventually degraded, the buccal mucosal graft serves as a replacement of the surrounding transplant's inner lining (Movie 17). At transplant sites that are lined with nonciliated squamous epithelium, loss of mucociliary clearance is compensated with coughing.

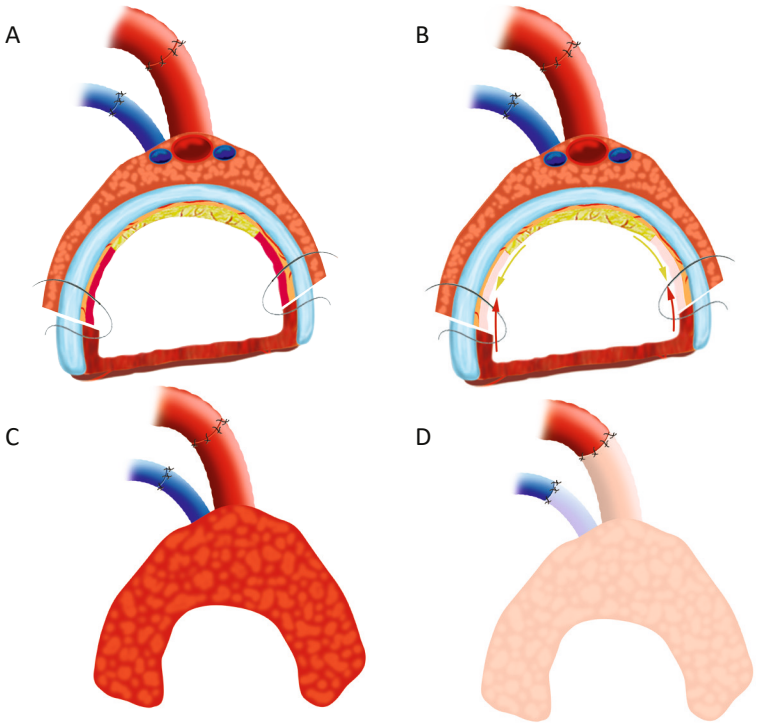


Figure 6.24. Tracheal allotransplant represents a unique procedure among composite tissue allotransplants.
A,B. A trachea allotransplant involves interactions between donor and recipient tissues. After withdrawal of immunosuppressive therapy, only the donor respiratory epithelium undergoes necrosis (bleached area in B).

C,D. In comparison, other composite tissue allotransplants consist of different tissues which are perfused by a vascular pedicle which is anastomosed to recipient vessels. When immunosuppressive therapy is withdrawn for these allotransplants, all donor tissues are susceptible to necrosis (bleached area in D).

By performing intercartilaginous incisions and using recipient buccal mucosa, immunosuppressive medication has been able to be safely and gradually reduced before being completely discontinued 9–12 months after forearm implantation. With cessation of immunosuppressive therapy, recipient epithelium allows secondary healing of necrotic areas to occur.

The trachea is transplanted as a composite tissue, and cartilage structure is a critical functional element of this graft. Cartilage is avascular, and thus, it relies on indirect nutrition from surrounding tissues. Cartilage is also immune-privileged with its chondrocyte cells protected within a matrix. The revascularization procedure we have described, in combination with carefully timed immunosuppression, takes advantage of these unique properties to preserve tracheal cartilage tissue and its structure. Meanwhile, noncartilaginous tissues are replaced with recipient tissues.

Finally, the revascularization period of a tracheal allotransplant has been reduced without tissue loss by covering the luminal site of the heterotopic trachea with the distal part of a radial forearm fascia flap. An optimal revascularization concept to be applied to future patients is shown in Figure 6.25.

Movie 17
Overview of a tracheal patch allotransplantation.

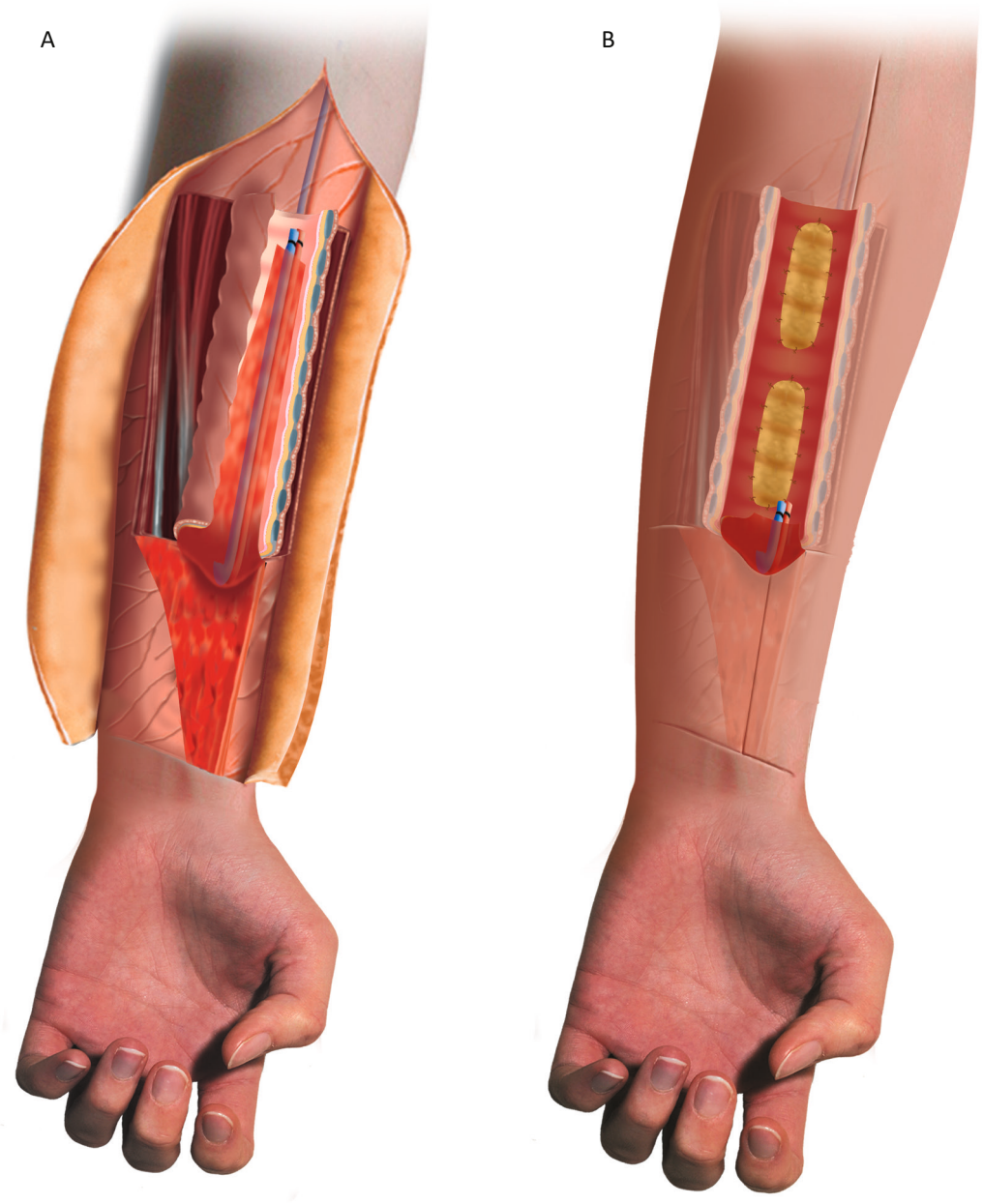


Figure 6.25. Optimal heterotopic revascularization concept.
A. A tracheal allotransplant with intercartilaginous incisions is fully covered at its luminal site with radial forearm fascia to facilitate rapid and complete revascularization.
B. The central part of the transplant is grafted with several buccal mucosal grafts. Only a small amount of donor respiratory mucosa remains at the margins.

6.6. **Future direction: Circumferential tracheal allotransplantation**

Due to the need for immunosuppressive drugs and a two-stage approach tracheal allotransplants are contraindicated for long-segment tracheal resections after removal of malignant tumors. For complete long-segment stenoses requiring tube reconstruction (which are rare), circumferential airway repair with a tubed allotransplant is needed.

It is possible that the latter could be developed with radial forearm skin serving as a new posterior membranous trachea (Fig. 6.26).

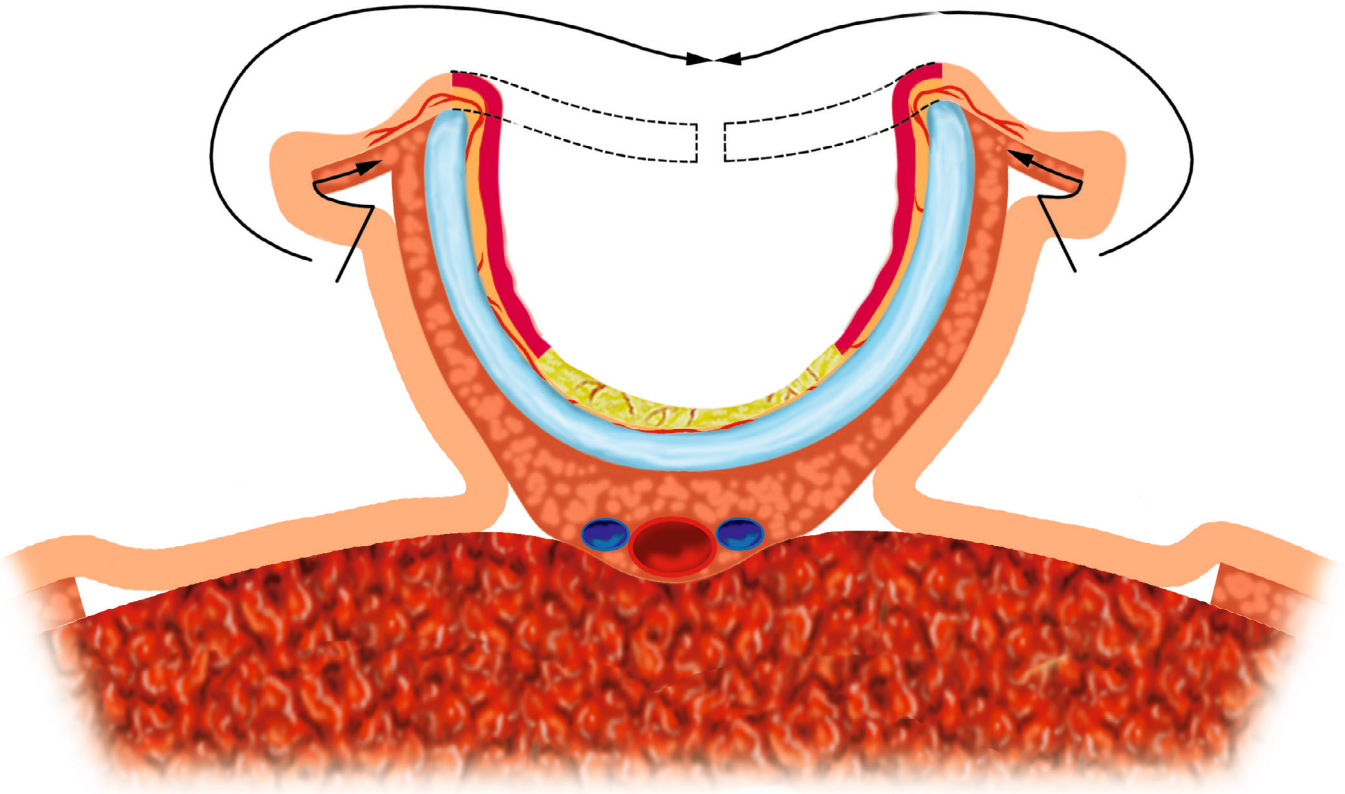


Figure 6.26. Tubed tracheal allotransplant for circumferential airway repair.
An axial sectional view shows the vascular connections that can develop between forearm skin and an allotransplant after implantation. These vascular connections allow an incision in the skin to form a new posterior wall. After the forearm skin grows into the margins of the allograft, it could be incised (indicated with short arrows) and used as the posterior wall of a tubed tracheal allotransplant (indicated with long arrows).

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