Review Article

Tracheal replacement

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Abstract: Non-malignant and malignant obstruction of the tracheal airway causes significant morbidity and mortality. With increased use of artificial airways, benign and iatrogenic complications are increasing. A tracheal stenosis that is less than 5 cm in length can be resected with end-to-end anastomosis. Longer tracheal lesions can be treated in a palliative way by placement of a stent to secure airway lumen patency. The management of tracheal defects is an evolving field. Tracheal transplantation and tracheal regeneration may bring major treatment advances to cases with long-segment tracheal involvement. This review examines the current possibilities and future prospects in the area of tracheal transplantation and regeneration.

Keywords: Trachea; transplant; revascularization; regeneration

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Immediate repair of long-segmental defects

Prosthetic tracheal repair

In recent years, most synthetic materials used for tracheal replacement have been tested in experimental animal research. From these studies, it became clear that definitive prosthetic replacement of the airway wall is not possible (1). To date, nearly all surgical prostheses that have been successful were observed in potentially sterile mesenchymal tissues. No example of successful prosthetic repair can be cited in the respiratory or gastrointestinal tract. The internal site of the airway tract belongs to the outside world, and bacterial contamination at the interface between the airway and prosthesis prevent its in-growth (*Figure 1*). The complications of wound breakdown at the anastomoses can be temporarily delayed by wrapping the prosthesis in vascularized tissue, mostly transposed omentum.

Palliative treatment of long-segmental defects

Long-segmental tracheal defects, which result after removal of malignant tumors are extremely rare. The only possibility for immediate reconstruction of these defects is to reduce the length of the defect by inserting a silicone stent, which is sutured to the upper and lower margins of the defect. A free fasciocutaneous skin tube (lateral thigh flap, radial forearm flap) can be used to wrap the silicone stent as a temporary closure (*Figure 2*) (2).

Tracheal allotransplantation

Introduction

The trachea is one of the few organs that are exceptionally difficult to transplant because of the technical difficulty to restore the blood supply to the graft. The blood supply of the 12 cm-long trachea depends in its entirety on small blood vessels branching out into numerous even smaller vessels, each of them subsequently penetrating the trachea in between the cartilage rings to provide blood supply to segments of the mucosal lining. If a part of the trachea is removed from the airway, all blood supply is interrupted. The removed

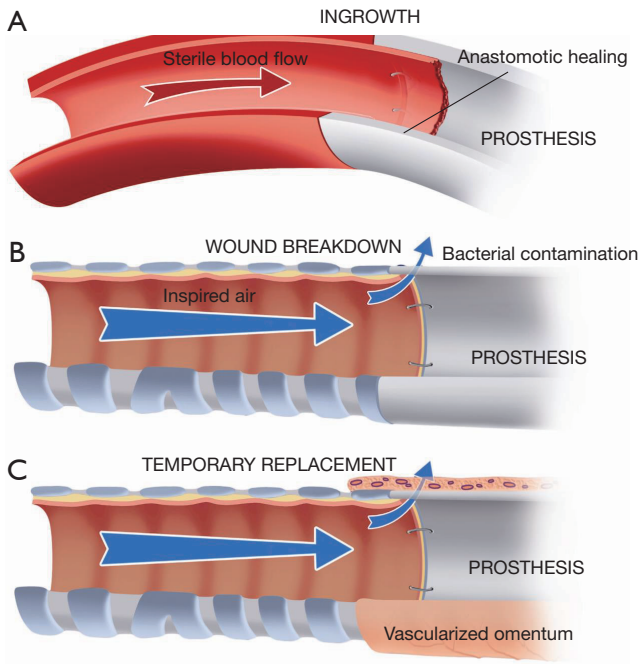

Figure 1 Prosthetic replacement: airway versus vascular conduits. (A) Blood vessel prosthesis. Endothelialization of the luminal surface of vascular grafts occurs only 1 to 2 cm into the graft from the anastomotic site. These endothelial cells are derived from adjacent, native endothelium and they enable the anastomosis to heal; (B) airway prosthesis. In the respiratory tract, the flow of inspired air will lead to bacterial contamination and wound breakdown at the anastomosis. The respiratory epithelium will not grow over the prosthesis-airway anastomosis; (C) airway prosthesis wrapped in vascularized tissue. A prosthesis may act as a temporary airway stent when it is wrapped by well-vascularized tissue (e.g., omentum). The vascularized tissue around the prosthesis may temporarily avoid the complications of wound breakdown at the anastomotic sites.

part of the trachea cannot survive, even if it were to be placed back into the airway straightaway (*Figure 3*). Our group has a 20 year-long research record in the field of tracheal revascularization and holds a leading position in the development of tracheal transplantation by means of vascularized segmental units.

A tracheal transplant may be necessary to repair surgical defects of the laryngotracheal airway tract that are unsuitable for segmental resection and autologous tissue repair. With the exception of some anecdotal, poorly documented cases performed without blood supply restoration (3) or immunosuppressive medication (4), no clinical tracheal allotransplants have been transplanted orthotopically as an isolated composite tissue graft. In tracheal allotransplantation, it is important to deal with both immunosuppression and indirect revascularization in a heterotopic position. The first documented preserved viability of a heterotopically revascularized allotransplant was published by Klepetko *et al.* in 2004 (5). The graft was revascularized in the omentum of a patient who underwent lung transplantation from the same donor. Ultimately, the trachea transplant was not used, but its viability was documented for at least 60 days.

The first documented revascularized tracheal allotransplant to be reported was published in 2010 (6).

Our approach to tracheal heterotopic revascularization, orthotopic transplantation, and withdrawal of immunosuppressive medication is based on a series of six cases (*Figure 4*) (7). For tracheal allotransplantation, we consider a "good match" to mean that the donor is of the same blood group as the patient.

Surgical technique

Revascularization of the trachea is the first step towards successful tracheal transplantation. The typical arterial and venous blood supply, consisting of several small tracheo-esophageal branches, does not enable direct tracheal transplantation. Currently, the only reliable way to achieve tracheal revascularization is to wrap the isolated trachea with a well-vascularized soft tissue flap perfused by a vascular pedicle, which then allows for transfer of the revascularized trachea to an airway defect. The forearm fascia flap pedicled on the radial artery and vein has proven to be reliable for tracheal revascularization (7). It is important to have complete immobility between the trachea and the surrounding recipient's vascular bed to obtain a fast revascularization of the blood vessels of the tracheal adventitia (*Figure 5*).

Revascularization has to be achieved by the outgrowth of capillary buds from the fascia flap (recipient blood vessels) uniting with those on the adventitia (donor blood vessels) of the tracheal segment. Inosculation is the establishment of direct vascular anastomoses between the vascularized soft tissue flap and the adventitia of the trachea.

Compared to a free skin graft, there are two additional barriers to revascularization for a tracheal allograft. The cartilage rings and intercartilaginous ligaments may interfere with the revascularization of the mucosal lining of the cartilaginous trachea. Cartilaginous tissue does not allow for the ingrowth of blood vessels. Revascularization

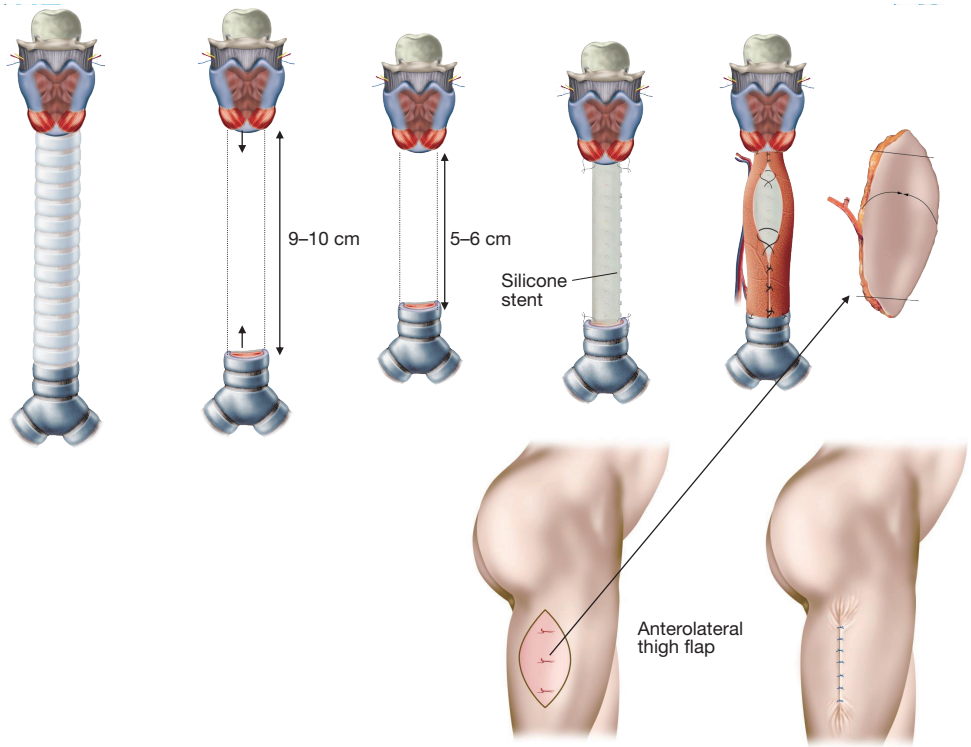

Figure 2 Palliative treatment of long-segment tracheal defects.

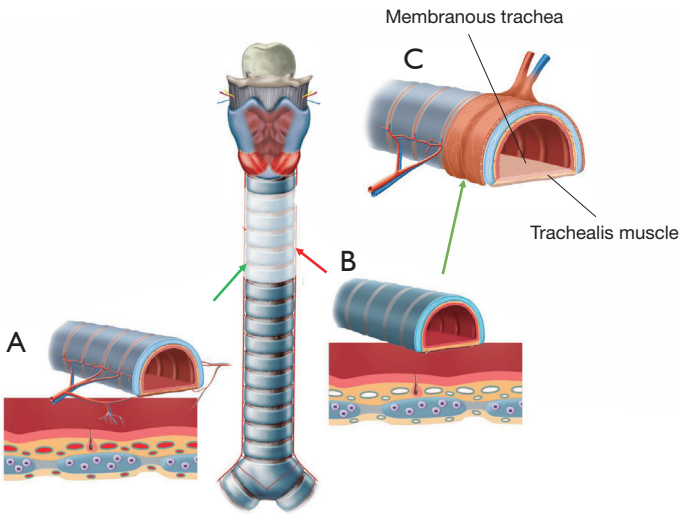

Figure 3 Vascularized and de-vascularized trachea. (A) In the healthy native trachea, the blood supply is ensured by a network of small blood vessels penetrating the trachea between the cartilage rings. Successful grafting (green-colored arrow) of a segment of the trachea requires the segment to have an intact and independent blood supply; (B) prelevation of a tracheal segment inevitably leads to interruption of its blood supply. Successful transplantation (red-colored arrow) requires restoration of an adequate blood supply, as in A. This is extremely difficult; (C) de-vascularized tracheal segments can become revascularized in a heterotopical position. The cartilaginous trachea can undergo progressive revascularization when wrapped with well-vascularized tissue. In humans, revascularization of the membranous trachea will be difficult because the trachealis muscle forms a barrier for mucosal revascularization. We learned that heterotopic tracheal revascularization occurs in a safer way after excision of the membranous trachea.

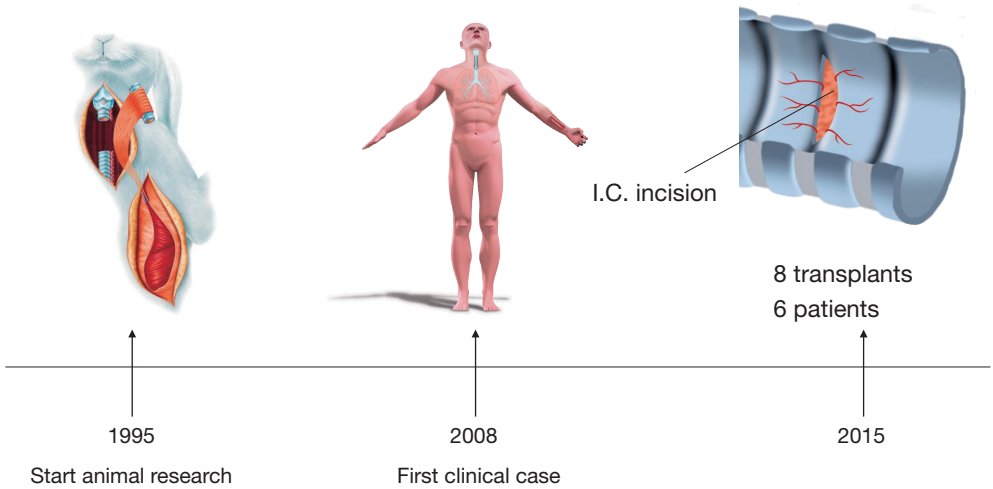

Figure 4 Overview of our experience in tracheal allotransplantation. Eight transplants were used in six patients. Two of the initial transplants were lost after withdrawal of immunosuppressive therapy. Important is to make partial incisions of the intercartilaginous (I.C.) ligaments at the time of forearm implantation to preserve the viability of the transplant after cessation of immunosuppressive drugs.

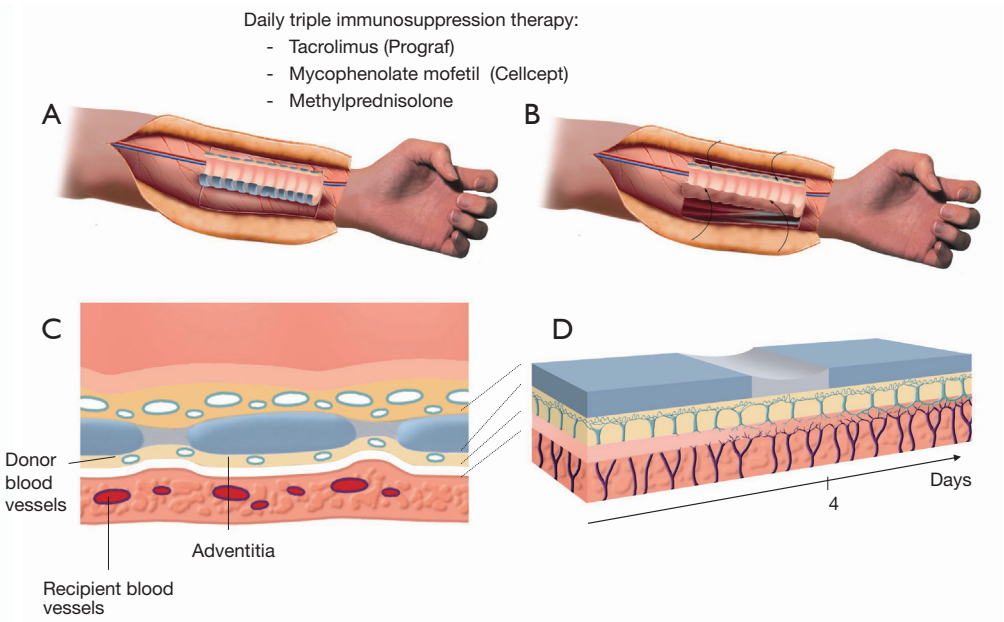

Figure 5 Orthotopic tracheal revascularization. The approach to heterotopic revascularization is shown. The forearm skin is incised and dissected away from the underlying fascia and subcutaneous tissue. After removal of the membranous part (A), the trachea is wrapped with the radial forearm fascia (B) and the forearm skin flaps are sutured to the incised trachea. Revascularization can be achieved by the outgrowth of capillary buds from the native vascularized tissue to unite with capillaries in the adventitia of the trachea (C). This link-up should be well advanced by the third day (D).

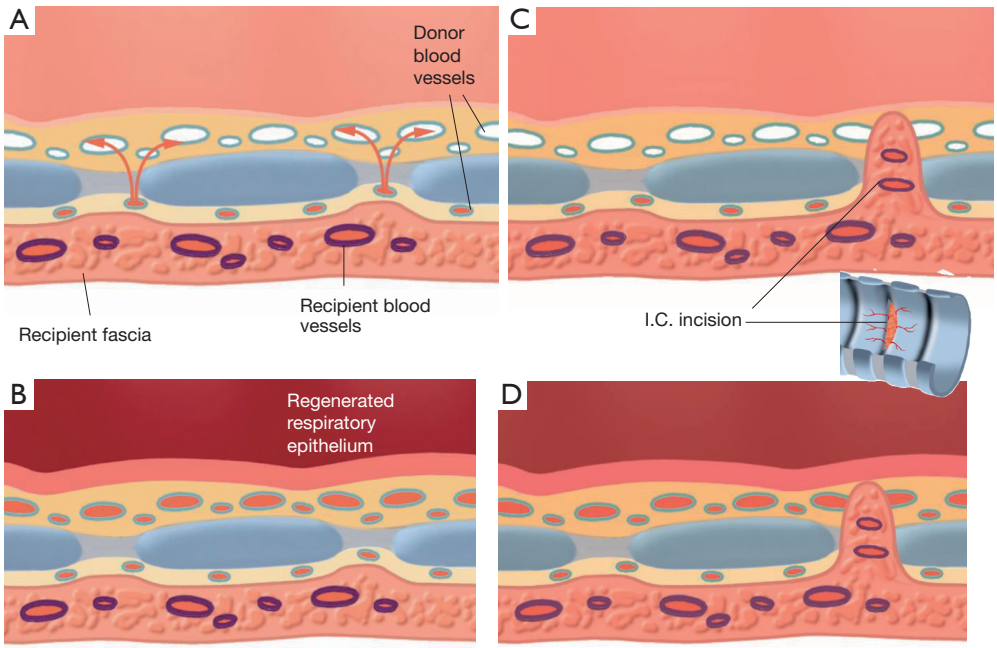

Figure 6 Tracheal revascularization and mucosal regeneration. (A) The cartilaginous trachea is revascularized (red arrows) by the surrounding tissues through the intercartilaginous ligament; (B) regeneration of the donor respiratory epithelium occurs simultaneously with the revascularization process; (C,D) partial incision of the intercartilaginous ligament (inset) will bring the recipient blood vessels closer to the donor submucosal capillaries, which will result in advancing of the revascularization process.

of the mucosal layer of an avascular tracheal segment occurs through the intercartilaginous ligaments (*Figure 6*). Full revascularization and mucosal regeneration of the cartilaginous trachea can be achieved within 2–4 months of the trachea being implanted in the forearm. Incision of the intercartilaginous ligaments will foster the revascularization process by bringing the recipient blood vessels closer to the submucosal capillaries.

A tracheal allotransplant is a composite tissue transplant that may be used to restore the airway, with the goal of improving quality of life. The benefits garnered by tracheal allotransplantation have to be balanced against the morbidity of long-term immunosuppression therapy. Immunosuppressive medication should be withdrawn before immunosuppressant-related complications occur. The cartilage tissue seems to escape immunologic rejection owing to the absence of blood vessels, and because the chondrocytes are protected within a matrix (6,8,9). In our initial patient series of tracheal transplantations, it became clear that the intercartilaginous ligaments formed an obstruction for the ingrowth of native blood vessels (*Figure 7*). The placement of intercartilaginous incisions at the time of forearm implantation was an important adaptation. The incisions of the intercartilaginous ligaments facilitated revascularization, enabling the ingrowth of recipient vessels into the submucosal space of the transplant. When incisions through the intercartilaginous ligaments were made at regular intervals, full revascularization and mucosal regeneration of the cartilaginous allotransplant could be obtained in a shorter time period. Moreover, regularly spaced intercartilaginous incisions provide avenues for angiogenic recipient vessels to breach the ligamentous barrier and thus grow into the submucosal space of the transplant tissue after withdrawal of immunosuppressants.

Clinical examples

Of the six patients treated so far, five patients were treated for a long-segment stenosis and one patient was transplanted to resolve a long-segment laryngotracheal involvement by a chondrosarcoma. Our approach to a long-segement stenosis is shown in *Figure 8*.

Tracheal allotransplantation was used in the treatment of a patient with an extended laryngotracheal chondrosarcoma. The patient involved was a 63-year-old man. The tumor developed over a period of more than 10 years. His airway

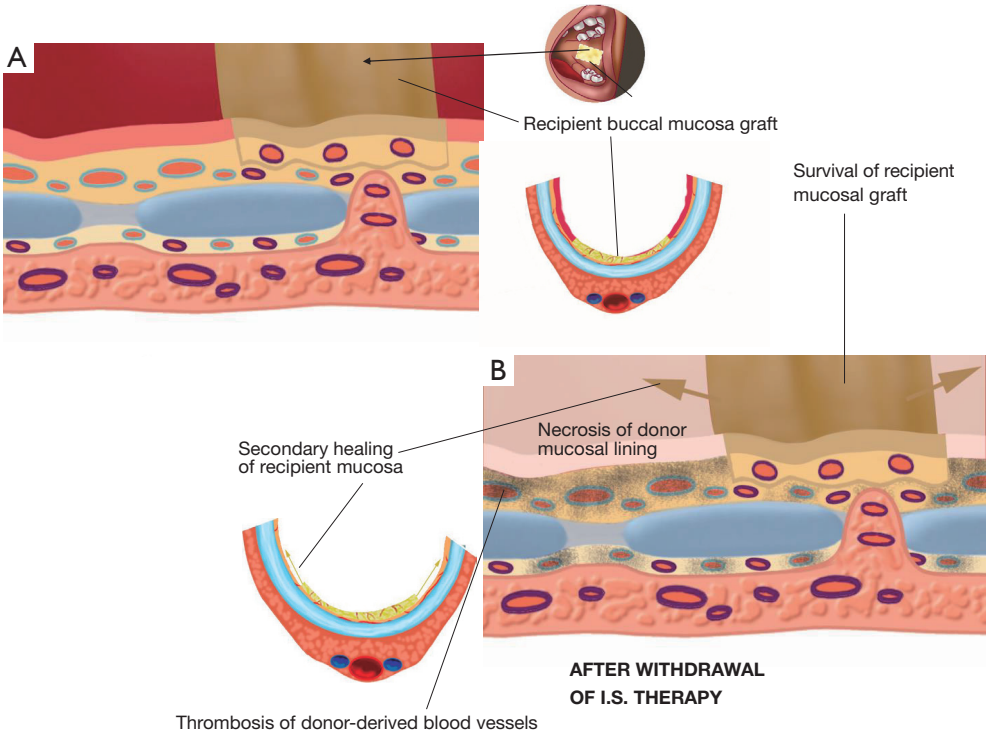

Figure 7 Importance of intercartilaginous incisions and of recipient mucosa. (A) After tracheal allograft revascularization and mucosal regeneration, recipient buccal mucosa can be introduced into the midportion of the allotransplant. A mucosal defect is created in the central part of the transplant and the midportion is grafted with a full-thickness mucosal graft from the recipient's buccal area; (B) after withdrawal of immunosuppressive drugs: immunologically-induced lymphocytes attack the microcirculation. Inflammatory vascular infiltrates will lead to thrombosis of donor-derived blood vessels and to necrosis of the mucosal layer. The intercartilaginous ligaments were observed to be acting as a barrier to the ingrowth of recipient blood vessels. The intercartilaginous incisions will allow for ingrowth of recipient blood vessels into the submucosal space of the transplant. These newly formed recipient blood vessels will allow the recipient mucosal lining in the midportion of the transplant to survive immunosuppressant withdrawal. The surviving recipient mucosal graft will allow for secondary healing of the areas of donor epithelial lining that underwent necrosis (yellow arrows).

could be preserved by the placement of a silicone stent. Due to the stagnation of secretions, he required periodical bronchoscopic cleaning of the stent. Since the last time, he had developed several acute episodes of stent blockages, which made definitive treatment necessary. Four months after implantation of a suitable allograft in the left forearm, the tumor was resected through an anterior cervical incision with a sternotomy extension (*Figure 9*). The potential for tumor progression while under immunosuppression for a low-grade malignancy was considered to be low and was confirmed by CT scan at the time of orthotopic transplantation, which demonstrated a nearly unchanged tumor bulk. Immunosuppressive medication was gradually phased out between 15 and 18 months after orthotopic transplantation. The transplant's morphology remained intact after withdrawal of immunosuppressive therapy. It seems that the mucosal repopulation of the transplant after cessation of immunosuppressants can occur with minimal loss of airway lumen (*Figure 10*).

A circumferential airway repair may be necessary after resection of malignant tumors. Tracheal allotransplantation at the time of tumor resection will be possible only for low-grade malignancies and not for other malignant tumors, because of the risk of tumor progression in the 3-month period of pretransplant immunosuppression. A circumferential defect left by tumor resection can be reconstructed temporarily with a stent wrapped in vascularized tissue. This type of reconstruction must be considered temporary due to inevitable stent-related complications. Tracheal allotransplantation may be considered in those patients with a temporary repair who

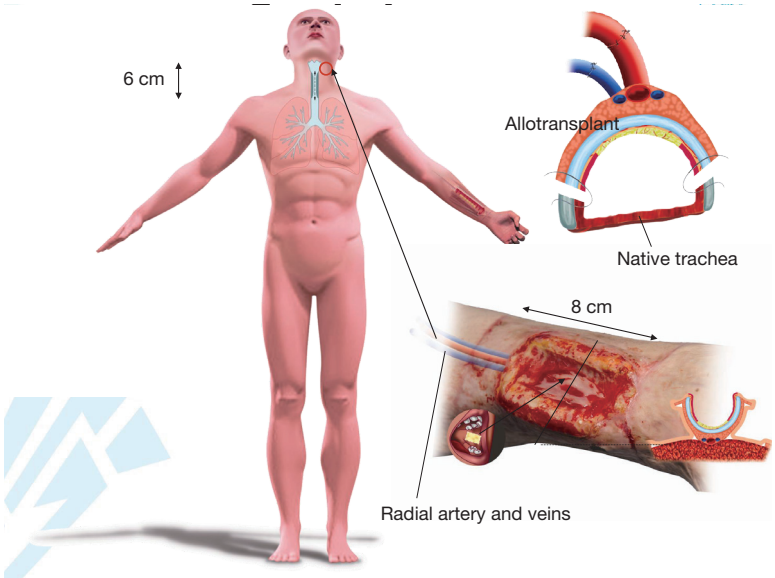

Figure 8 Allotransplantation of a long-segment tracheal stenosis. Orthotopic transplantation of a tracheal transplant to resolve a long-segment (6 cm) airway stenosis is illustrated. An eight cm long tracheal allotransplant is implanted at the forearm. During the first weeks the luminal site of the transplant is protected by the application of fibrin glue. After revascularization, a buccal mucosa graft from the recipient can be applied to the midportion of the transplant to allow for a safe withdrawal of immunosuppressive drugs. The long-segment tracheal stenosis is incised longitudinally (double arrow). After full revascularization and mucosal regeneration have been achieved, the tracheal allotransplant is transplanted from the forearm to the airway defect on a radial vascular pedicle. The radial blood vessels are sutured to the neck vessels to facilitate revascularization. The cartilaginous trachea is sutured into the airway defect to restore the concavity of the airway lumen. Withdrawal of immunosuppressive therapy can start 1 year after orthotopic transplantation.

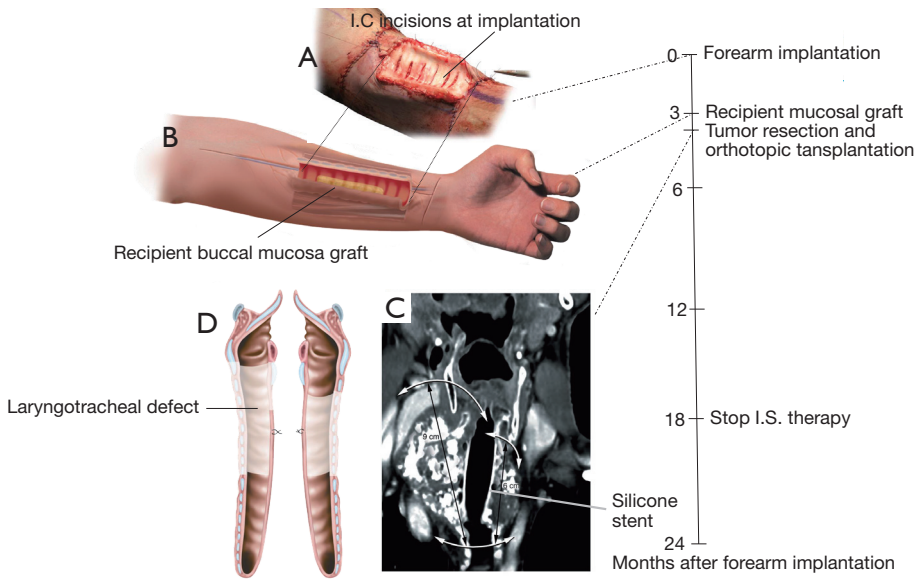

Figure 9 Patient with low-grade chondrosarcoma. Tracheal allotransplant at time of forearm implantation with I.C. incision (A) and after full revascularization with a recipient buccal mucosa graft at it's midportion (B). Tumor involvement visible on a coronal CT scan image (C). The airway lumen is bridged by a silicone stent. The degree of resection is indicated with white, two-headed arrows. The lengths of the tracheal resection were 9 cm (right) and 6 cm (left) (scale =1 cm). After 4 months, the tumor could be resected and the tracheal allotransplant was used to repair the laryngotracheal defect (D).

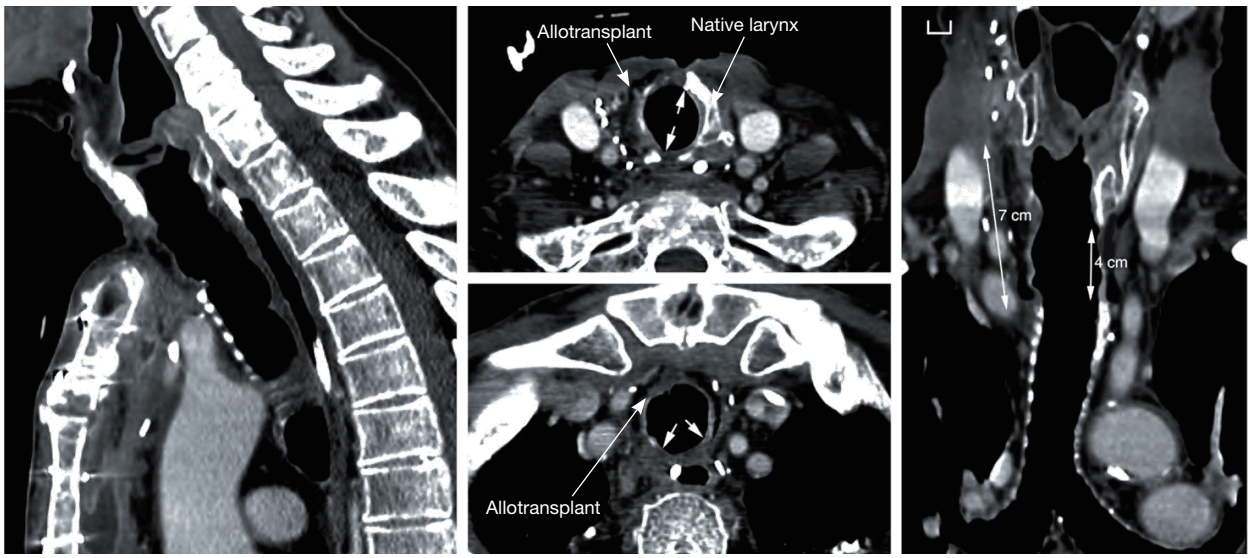

Figure 10 CT scan after orthotopic transplantation and after withdrawal of immunosuppressive drugs. A CT scan 2 years after orthotopic transplantation and 6 months after cessation of all immunosuppressive therapy is shown. Note the absence of cartilage calcification in the allotransplant (scale =1 cm). (A) Sagittal reformatted CT scan; (B) axial CT scan at laryngeal level; (C) axial CT scan at level of cervical trachea; (D) coronal reformatted CT scan.

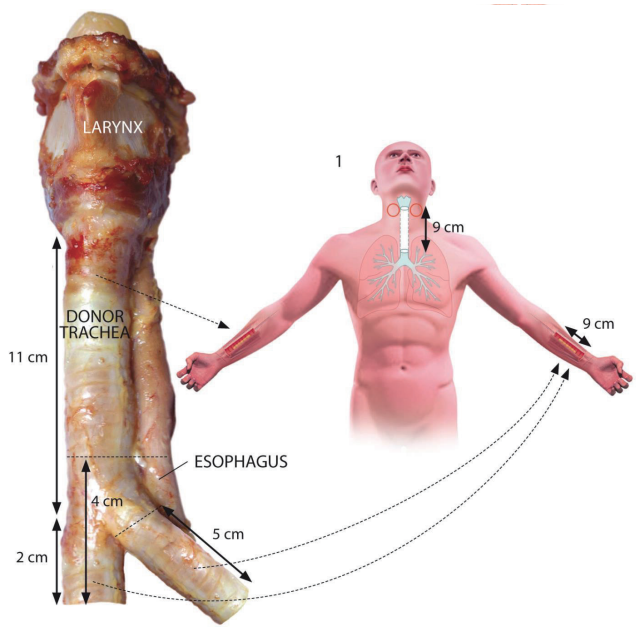

Figure 11 Implantation of two tracheal allografts for circumferential airway repair. The full length of the trachea and main bronchi can be used for allotransplantation. Two cartilaginous tracheal segments with a length of 9 cm may be implanted at two forearm sites. By suturing the two allotransplants together, a tube may be created for circumferential airway repair.

remain tumor-free.

The best protocol for circumferential allotransplantation may lie in a bilateral transplantation of the cartilaginous trachea (*Figures 11*,*12*).

Tracheal regeneration

Regeneration versus secondary healing

The relative contribution of tissue regeneration versus scarring in the healing of the airway mucosal lining depends on the extent of injury inflicted. A superficial epithelial wound can heal by way of regeneration of the surface epithelium (*Figure 13*) (10). Indeed tissues with a high proliferative capacity, such as airway tract epithelia, renew themselves continuously and, after injury, can regenerate above the basal membrane as long as the stem cells in these tissues have not been destroyed.

If a tissue injury is severe and involves damage of both epithelial cells and the submucosal layer, healing cannot be accomplished by regeneration alone. Under these conditions, the main healing process is repair by deposition of collagen, causing the formation of a scar. Future therapies should aim to promote regeneration and reduce scar tissue formation when dealing with full-

thickness mucosal tracheal defects. Exploration of the potential use of stem cells for true regenerative healing is ongoing. The present challenge for regenerative medicine is to overcome the barriers to regeneration of the mucosal and epithelial lining in full-thickness epithelial defects. However, regeneration of full-thickness mucosal defects is not yet possible.

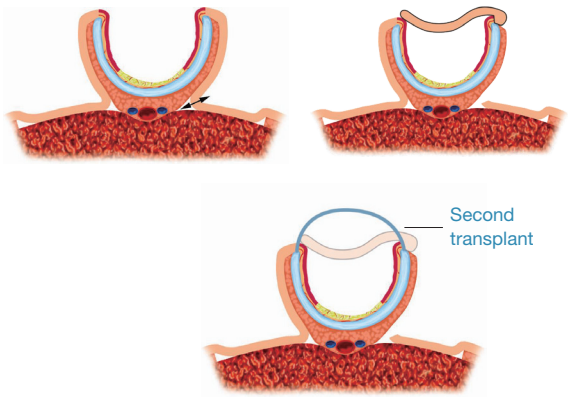

Figure 12 Circumferential airway repair. The first transplant is used to restore the posterior and lateral walls of the airway. A part of the forearm skin can be included as a temporary reconstruction of the anterior wall. In a second operation, the second transplant can be used to replace the forearm skin and to further augment the airway lumen.

The unrealistic prospect of tracheal regeneration

Since 2008 the trachea has been termed the first human organ that can be man-made with stem cells (11). Meanwhile an engineered trachea has been implanted in several patients. This achievement has received a lot of attention in medical journals as well as in the press. Indeed, the engineered windpipe was seen to be the first step towards other forms of organ regeneration. Classic organ transplantations with their typical side effects due to anti-rejection medication could then be replaced by growing organs from the body's own cells. However, the optimism surrounding organ regeneration has proved to be completely unfounded. In fact, the engineered trachea is an example of blatant scientific deception.

The engineered trachea was represented as a regenerated trachea after applying bone marrow cells to a de-cellularized (12) or synthetic scaffold (*Figure 14*) (13). There is no scientific foundation whatsoever to assume why stem cells would support airway tissue regeneration in this setting. In addition, even if a trachea-like organ would be generated, it would irrefutably fail after implantation if adequate blood supply had not been restored. As expected, the implantation of de-cellularized and synthetic scaffolds resulted in extremely high morbidity and mortality rates (14). At this point in time, this form of airway regeneration should be regarded as hypothetical and scientifically unfounded (15,16).

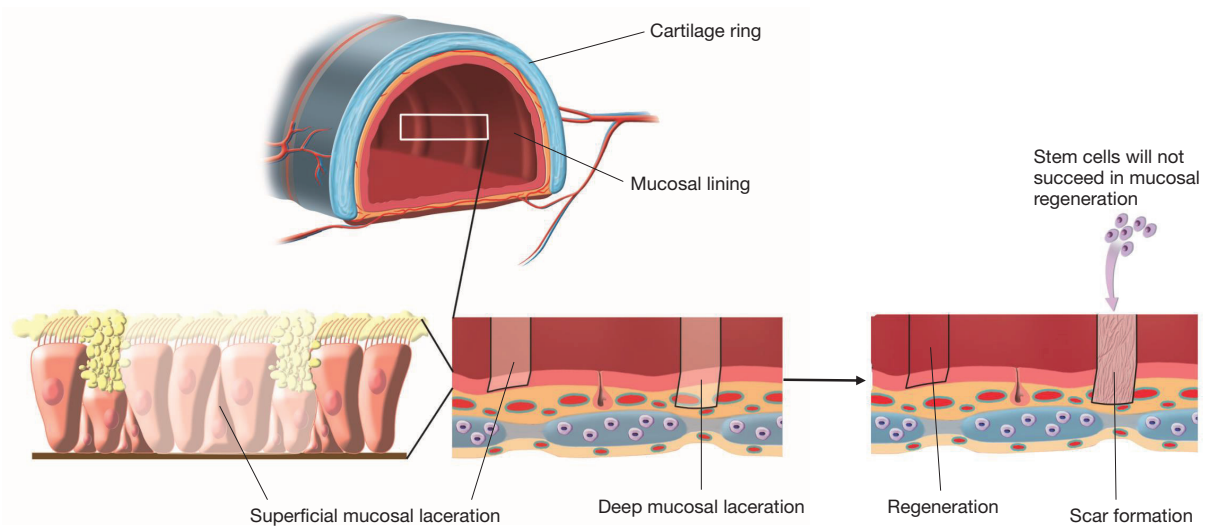

Figure 13 Regeneration of airway tissue. The basement membrane of the mucosal layer supports a pseudostratified epithelium, the surface layer of which is columnar and ciliated, with deeper layers of oval or rounded basal cells. A superficial epithelial wound can heal through regeneration of the surface epithelium. Tissues with high proliferative capacity renew themselves continuously and can regenerate after injury above the basal membrane through proliferation and differentiation of basal cells.

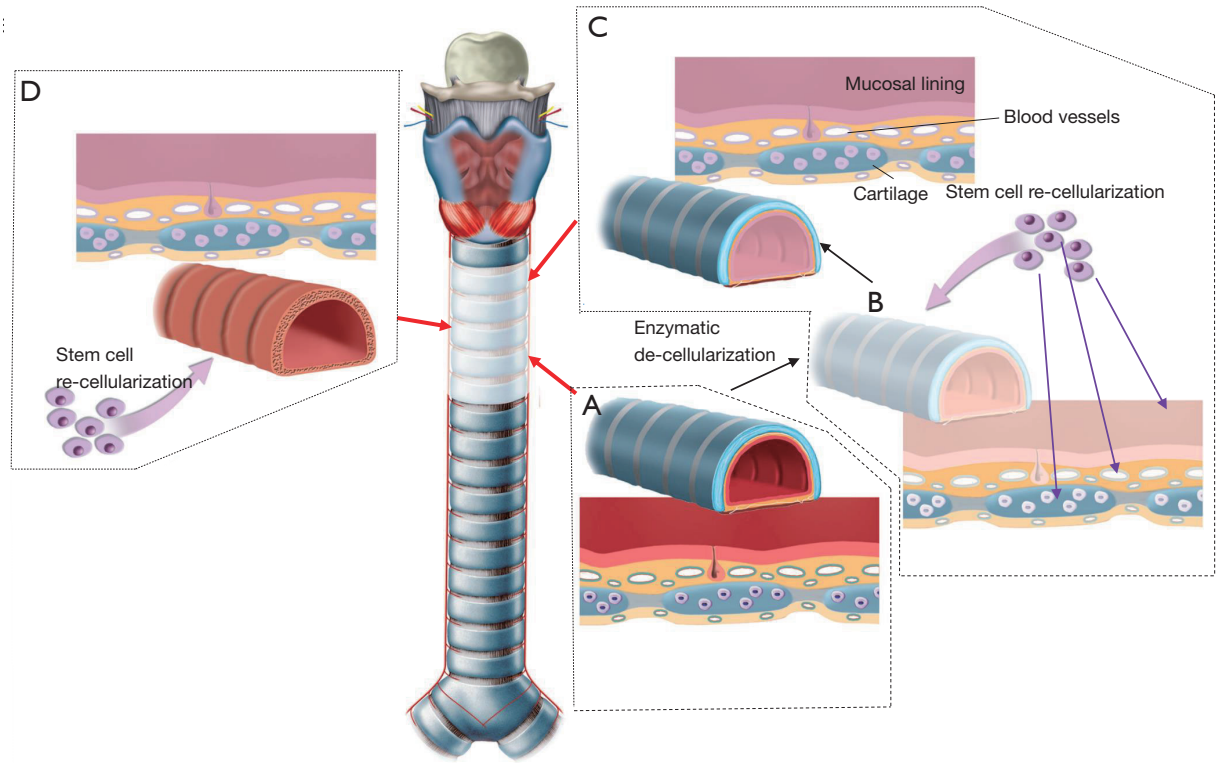

Figure 14 How the engineered trachea was represented. (A) De-vascularized native trachea; (B) as a first step towards a presumed 'stem-cell engineered regenerated trachea', a detergent is used to destroy all viable cells, leaving a scaffold of connective tissue; (C) a 'stem-cell engineered regenerated trachea': it is hypothesized that stem cells penetrate the connective tissue and subsequently regenerate cartilage, blood vessels and respiratory mucosa. This presumed regenerated trachea is implanted without restoration of any blood supply (red-colored arrow); (D) it is hypothesized that stem cell-mediated re-cellularization of a synthetic scaffold may also lead to a fully regenerated trachea that can be transplanted inside the airway (red-colored arrow).

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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