Tracheal replacement for primary tracheal cancer

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Purpose of review
To summarize the so far applied clinical methods of tracheal replacement, comparing pros and cons of conventional and tissue-engineered approaches.

Recent findings
Several strategies have been most recently described to replace the trachea-like aortic homografts, allotransplantation, and tissue engineering. Allotransplantation requires lifelong immunosuppression and this may be ethically questioned being not a lifesaving procedure. Tissue-engineered tracheal transplantation has been clinically applied using biological or bioartificial tubular or bifurcated scaffolds reseeded with mesenchymal stromal cells, and bioactive molecules boosting regeneration and promoting neovascularization.

Summary
Tracheal tissue engineering may be a promising alternative to conventional allotransplantation in adults and children. Different methods have been developed and are currently under active clinical investigation, and await long-term results.

Keywords
biological and artificial scaffold, donor organs, tissue engineering, trachea transplantation

INTRODUCTION

Primary tracheal cancers are rare tumors that can originate from different cellular components of the trachea such as the respiratory epithelium, salivary glands, and mesenchymal structures. Squamous cell carcinoma, more frequent in men, and adenoid cystic carcinoma, evenly distributed between sexes, represent the majority of them and account for 0.1–0.4% of all malignant diseases. Unfortunately, most are diagnosed only when more than 75% of the internal airway lumen is obstructed so that the primary diagnosis is often made late in the natural history and course of the disease, leading to advanced tumor progression and inoperability [1].

Curative treatment is only provided by surgical resection. Clinical experience has shown that a safe end-to-end anastomosis can be achieved if the affected segment of the trachea does not extend over half of the entire length in adults or one-third in children. If those critical values are surpassed, surgical removal within safe margins of the airway involved by the tumor cannot be performed, leaving therefore only palliative measures such as stenting, tumor debulking, or radiotherapy to delay tumor progression [2]. There are few experimental options described in the literature, but none of them reached standard clinical practice yet [3,4▪,5].

The trachea appears as a simple conduit for passing air from the upper airway into the lungs to enable gas exchange, but serves more important functions. The respiratory epithelium with its mucociliary apparatus cleans the inhaled air from unwanted organisms. Due to the exposure to both external and internal mechanical forces, the trachea must present a certain longitudinal flexibility and other important peculiarities that permit neck movements and lateral rigidity to withstand positive and negative pressures during breathing and other actions (Table 1).

Therefore, there are specific requirements for a tracheal substitute that have to be fulfilled: it should be flexible and rigid, biocompatible, non-immunogenic, airtight [2], with an internal lining of

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KEY POINTS

- The basic principles of tissue engineering are as follows: natural or synthetic scaffold, cells, bioreactor, and bioactive molecules/factors.
- Tissue engineering provides promising alternatives to conventional transplantation and can avoid the need of donor organs and immunosuppression.
- Synthetic scaffolds could be custom-made and rapidly produced; however, novel strategies are necessary to protect material from contamination and necrosis.
- New guidelines and paradigms for transplantation medicine must be formulated in order to address the ongoing questions and ethical concerns regarding the handling of tissue-engineered tissues/organs.

cells to prevent infection [6] and sufficient vascularization to avoid graft necrosis. Furthermore, pediatric grafts should parallel the somatic growth once implanted. Unfortunately, the trachea lacks a well outlined vascular supply but is provided by a delicate network of vessels, which originate from the right inferior thyroid and bronchial arteries [1]. This represents a major challenge to achieving sufficient vascular support, especially during allogenic replacement.

Allotransplantation of a trachea

The first ever case describing a tracheal allogenic transplant in humans was published in 1979 by Rose *et al.* [7]. A cadaveric tracheal graft was transplanted first heterotopically into the sternocleidomastoid muscle to provide revascularization (for 3 weeks) and then orthotopically repositioned with the resulting vascularized muscular section. In 1993, Levashov *et al.* [8] described a second case by doing a single-stage transplantation using omentopexy to provide indirect vascularization and immunosuppression. The presented cases seemed promising by that time, despite the absence of any evidence or proof of allograft functionality and viability. Ten years later, Klepetko *et al.* [9] demonstrated successful heterotopic revascularization in the omentum of an allogenic tracheal graft with maintained structural and functional integrity. The importance of a combined arterial and venous revascularization for the survival of long segmental allogenic tracheal grafts was first described by Macchiarini *et al.* [10] in a pig model. Tintinago *et al.* [11] confirmed the findings later in a clinical case of an 18-year-old male patient.

Unfortunately, allogenic strategies depend on lifelong immunosuppression that leads to side-effects and also puts patients with malignancies at a higher risk [2]. In contrast to early reports, the epithelial cells and chondrocytes of the trachea have an immunocompetent function [12], and trigger acute or chronic rejection. Methods such as cryopreservation, irradiation, or detergent enzymatic treatment have been utilized to reduce this immunocompetence of the graft but with adverse effects [13–17]. These methods can result in a mechanical and structural impairment with subsequent alteration of cell engraftment and mechanical strength. Aside from some documented clinical transplantations of cryopreserved allografts, there are 131 worldwide cases described using chemical fixated allografts both in children and adults [18]. Despite this large number of allotransplantations, only one patient suffered from a malignancy (adenoid cystic carcinoma) and postoperative outcome was poor. Moreover, this method relies on the posterior wall of the recipient to reconstruct the trachea. This makes its application for circumferential extended malignancies inappropriate. In addition to that, the method requires the placement of a stent and longlasting preoperative graft processing (~70 days). Another method to replace the trachea has been reported by Wurtz *et al.* [19,20]. The authors described the use of an aortic homograft combined with a permanent intraluminal stent to support the structural integrity. The long-term follow-up was promising from the oncological point of view. However, no cartilage-ring formation could be seen which resulted in permanent stenting to give the graft structural support [20,21].

All methods described so far required either continuous stenting, which is associated to chronic infections and graft contamination, donor-organ and immunosuppressive therapy to avoid rejection, or complex graft production. To overcome those hurdles, an alternative for conventional tracheal transplantation may be tissue engineering.

Table 1. Biomechanical characteristics of bioartificial scaffolds required to mimic human neck movements

Requested testing	End limits
Flexion-extension bending	Flexion to 70° and extension to 60° with an expected strain limit of 40%
Axial tension/compression	Strain limits, 40% for tension and 20% for compression
Right/left lateral bending	±48° with an expected strain limit of 40%
Right/left axial rotation @ 0° flexion	±75°
Right/left axial rotation @ 0° max flexion	±70°

TISSUE ENGINEERING

Tissue engineering demonstrated its feasibility in experimental but also in the clinical setting. Aside from the trachea, early clinical data are available for the bladder, blood vessels, cartilage, skin, and heart valves. Tissue engineering can be applied to reconstruct damaged tissues and/or restore organs' function. Basically four different components are combined in tissue engineering: cells (either autologous or allogenic); a biological or synthetic-based scaffold; a bioreactor (*in vitro* or *in vivo*); and lastly bioactive molecules/factors to enhance and support the endogenous regenerative capacity. Below we will provide an in-depth understanding of how the different components integrate toward tissue engineering.

Cells

A variety of cell types may potentially be used for tracheal tissue engineering. Currently, some have only been in the experimental phase such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). However, adult stem cells such as amniotic fluid stem cells, fat-derived or bone marrow-derived mesenchymal stem cells (MSCs), and terminally differentiated cells (such as epithelial, chondrocytes, endothelial, and muscle cells) have already been clinically applied. The easy availability of these adult stromal cells and differentiated cell types can provide an in-vitro setting to investigate the mobilization of endogenous progenitor cells to enrich the in-vivo repopulation of the transplanted graft. In a clinical setting, mononuclear cells which include the mesenchymal subfraction [4▪] and epithelial and/or chondrocytes [3,5,22] are of particular interest because they have low ethical implications, are easily available and, most importantly, are of autologous origin, which eliminates the need for lifelong immune-suppressants (Table 2).

Scaffold

The ideal scaffold should be nonimmunogenic, nontoxic, noncarcinogenic, and be able to allow cell adhesion and proliferation. As the trachea also has more specific characteristics such as air-tight and liquid-tight seals as well as adequate structural support (longitudinal flexibility and lateral rigidity) to preserve airway patency and allow rapid epithelialization, it is important that these scaffolds also maintain all anatomical and functional properties of the trachea. The optimal scaffold architecture should also mimic the target tissue microenvironment and maintain tissue-specific mechanical characteristics. There is an assortment of scaffolds which can be based on natural matrix, decellularized [22,23], cryopreserved organs/tissues, collagen structures [24] or are made from synthetic

Table 2. Types of cells that could potentially be used during tissue-engineered approaches

Cell type	Cell source	Advantages/disadvantages	Clinical experience
Pluripotent stem cells			
Embryonic stem cells (ESCs)	Embryonic tissue	Pros: pluripotency	Only preclinical studies
		Cons: allogenic, ethical concerns, possible teratoma formation	
Induced pluripotent stem cells (iPSC)	Autologous tissue biopsy, reprogramming required	Pros: pluripotency, autologous	Only preclinical studies
		Cons: possible teratoma formation	
Adult stem cells			
Amniotic fluid/placenta/umbilical cord blood-derived cells	Aborted fetal tissue	Pros: multipotency, easy isolation, low risk of teratoma formation, high expansion capacity	Mostly preclinical studies
		Cons: tumorgenicity ?	
Mesenchymal stem cells	Bone marrow aspiration, peripheral blood, adipose tissue	Pros: multipotency, low-to-moderate risk of dedifferentiation, high-expansion capacity	Trachea, pulmonary valve, acute graft-versus-host disease
		Cons: immunogenicity ?	
Differentiated cells			
	Tissue biopsy, peripheral blood	Pros: no or low risk of teratoma	Trachea, bladder, cartilage
		Cons: unipotency, immunogenicity (allogenic), limited expansion capacity	

biodegradable or nondegradable materials (e.g., polyester urethane, polypropylene mesh, polyethylene glycol-based hydrogel, polyhydroxyacids, poly-ε-caprolactone, etc.).

There are various methodologies that are available to engineer a nonimmunogenic scaffold by removing cellular components and major histocompatibility complexes (MHC I/II), such as chemical agents [enzymatic, ionic, nonionic, alkaline, acidic, zwitterionic, chelating, etc.], physical, and mechanical processes (such as agitation, perfusion, etc.). This is essential because it reduces the chances of immunoreaction. Attention must also be paid to the scaffold's mechanical integrity and biocompatibility when removing immunogenic particles [25▪]. In particular, the extracellular matrix (ECM) is of vital importance in order to enable the successful repopulation of cells on the scaffold, neo-angiogenesis, and the development of the biotechnological interface.

Currently, using a combination of both natural (e.g., collagen) and synthetic (polymers) substances in developing the scaffold is another possible option for future transplantations [26], which can provide novel avenues to this field of tissue engineering [27]. Cell sheet (scaffold-free) technology is also another clinical alternative solution. The reduction in inflammatory responses and improved integrity were demonstrated by this promising application but the impaired mechanical properties made this method unfeasible for long gap reconstruction.

Bioreactor

Bringing cells and scaffold together will need an environment that mimics the native characteristics of the target tissue. Cell engraftment, proliferation, and differentiation can significantly differ in various surroundings. The bioreactors that are utilized are proposed to mimic the natural environment of the airways. Besides Bader and Macchiarini [28] and Jungebluth *et al.* [4▪] describing a single-staged orthotopic approach, Delaere *et al.* [5] recently proposed a multistaged heterotopic in-vivo engineering approach.

Bioactive molecules

The great progress in the understanding of cells homing and signaling can change the field tremendously. Molecules, growth factors, or proteins can have different impacts on cell migration, differentiation, and guidance of cellular in-growth. These bioactive molecules may be administered in various ways, such as local or systemic injections or intraprocessing delivery. A clinically applied concept for the trachea has been described by Bader and Macchiarini [28] and further investigated by Jungebluth *et al.* [4▪] using erythropoietin to reduce inflammatory responses and cell apoptosis, granulocyte colony stimulating factor to mobilize endothelial progenitors, and mesenchymal stem cells and different growth factors to induce cell differentiation on the scaffold. This area should further be investigated because the recent clinical data have provided some evidence of an improved clinical outcome.

CLINICAL APPLICATION OF TISSUE-ENGINEERED TRACHEAL GRAFTS

To date, four different tissue-engineered approaches have been transferred into the clinic to replace and/or reconstruct various tracheal defects and diseases. As the indications differ a lot from each other and there is a lack of long-term outcome data, it is difficult to determine the optimal technique. In 2002, Omori *et al.* [29] transplanted an approximately 50 mm long nonseeded Marlex mesh tube covered by a collagen sponge. Within 2 months, an epithelialization was observed and an improvement of this re-epithelialization was detected after 20 months. However, the replaced length of trachea was not critical and, thus, real clinical benefit is rather unknown.

Walles *et al.* [22] decellularized a porcine jejunum patch with an intact vascular system and then reseeded it with different resident cells. In 2003, the first patient was treated with this method. Since then, the concept has been further developed, even though the basic principles of jejunum decellularization were maintained. However, only small (not-segmental) defects have been treated so far. A potential disadvantage for this technique is the relatively longlasting processing time and low mechanical resilience capacity, and the impossibility to circumferentially replace tracheal segments. Macchiarini *et al.* [3] used in 2008 a decellularized donor trachea with maintained biomechanical characteristics of the native trachea re-seeded with the patient's own epithelial cells and stem cells derived chondrocytes. Since then, nine other patients underwent a tracheal transplantation using a similar approach. Neo-vascularization was induced and supported by mobilizing the omentum and wrapping the graft in situ after implantation. The disadvantage of this method is the long decellularization protocol and the constant need of a donor organ. However, Elliott *et al.* [30▪] reported recently the 2 years of follow-up of a successful transplantation in a child using the aforementioned method. Delaere *et al.* [5] used a method

Table 3. Strategies for a tracheal replacement

Method	Method	Pros and cons	Application field
Allotransplantation	Donor organ		
	Fresh organ	Cons: immunosuppression needed	Few reports in humans
	Cryopreserved, irradiated	Pros: no immunosuppression	Few reports in humans
		Cons: initial stenting	
	Chemical fixation	Pros:	100 adults
		no immunosuppression	31 children
		Cons:	only one cancer patient
		relies on local reconstructive tissue	
		long processing	
	Fresh aortic allograft	Pros: no immunosuppression	Initial clinical application in six human cases
		Cons: requires permanent stenting	
Composites	In-vivo allograft epithelialization	Pros:	
		no long-term immunosuppression	Initial clinical cases
		Cons:	
		relies on a donor trachea	
		initial immunosuppression	
	Polypropylene mesh covered with collagen sponge	Pros:	Initial clinical application (patch)
		no immunosuppression	
		Cons:	
		no long segments	
Tissue-engineered transplantation	Biological scaffold		
	Decellularized human trachea	Pros:	Initial clinical application (circumferential entire or partial trachea to treat both malignant and benign diseases)
		no immunosuppression	
		biodegradable	
		maintained ECM	
		biocompatibility	
		cell homing	
		pro-angiogenicity	
		Cons:	
		donor-dependent	
		long processing	
	Decellularized porcine jejunum	Cons:	One clinical case of malignancy (patch)
		mechanical strength	
		shortage of donor	
		processing time	
		immunogenicity?	
	Synthetic scaffold		
	POSS covalently bonded to PCU	Pros:	One clinical case (circumferential entire trachea + bifurcation to treat malignancy)
		customized	
		rapid and cheap production	
		Cons:	
		lack of pro-angiogenic factors	

PCU, poly-[carbonate-urea] urethane; POSS, polyhedral oligomericsilsesquioxane.

equally described by Rose *et al.* in 1979 [7]. With this concept, they used a combination of immunosuppressant-dependent allogenic transplantation and heterotopic in-vivo re-population of the donor organ as a tissue engineered component. The drawback of this method is the very longlasting and complicated engineering period, need of multiple operations, and of immunosuppressive medications in the early phase. The first synthetic-based Y-shaped scaffold re-seeded with the patient's stem cells has been transplanted into a patient in 2011. Jungebluth *et al.* [4▪] utilized a computed tomography scan of the patient to reconstruct the removed tracheal section with most accuracy. To provide neo-vascularization to the synthetic graft, different growth factors had been applied into the graft's wall and systemically to the patient. Besides, the omentopexy was also performed as previously described.

CONCLUSION

Despite the relatively simple architecture of the trachea, its replacement has been challenging over the past century and none of the so far described methods can claim to be superior over the others in all respects. The difficulties are related to the anatomical position of the trachea, its continuous exposure to the external environment, and direct connection to the distal airways and lungs. However, one can agree on some basic principles (Table 3).

The conventional tracheal transplantation has some obvious disadvantages. The donor and the recipient must match their MHC profile to be eligible but even so these patients require lifelong immunosuppressive medication, which is associated with severe negative side-effects and high medical costs. Local regulations and ethical guidelines can always influence and delay the process as the method is donor-dependent. This type of tracheal allotransplantation does not appear to be the most promising method for future treatment in tracheal cancer patients.

Tissue engineering is an alternative treatment and has demonstrated its feasibility in the clinical scenario. Different methodologies are available and long-term follow-up will ascertain the ideal strategy. Hence, the intermediate method of using an allogenic donor trachea that is re-seeded in a heterotopical in-vivo model shows quite promising results so far but is very longlasting and, at least in the beginning, associated with immunosuppressive medication. Using decellularized donor tracheas is also rather time consuming and attention must be paid to not destroy the ECM during the tissue processing. The method requires likewise a donor but immunosuppressive medication can be avoided and preliminary results seem promising.

Synthetic meshes combined with collagen sponges are a fast and easy method to be reproduced. The method relies on the endogenous epithelialization of the implanted graft but experimental studies demonstrated that long segment tracheal replacement does not function with unseeded grafts. Synthetic-based scaffolds re-seeded *in vitro* with autologous cells prior to implantation have several advantages. There is no need of organ donor or immunosuppressive medication postoperatively. The scaffold can be custom-made when it comes to the replacement of the entire trachea with the two main bronchi (Y-shaped). However, strategies must be developed to support neo-angiogenesis and in-situ cell re-population. The application of bioactive molecules during the processing and postoperatively accelerates endogenous regenerative capacity, cell homing, wound healing, and reduced inflammatory responses.

It would also be interesting to include gender and individual specific responses on different constructs to improve this novel approach. Finally, the current guidelines and paradigms for transplantation medicine must be re-evaluated in order to address the ongoing questions and ethical concerns regarding the handling of tissue-engineered tissues/organs.

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Conflicts of interest

All authors have no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 181).

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This study describes the 2-year follow-up of a 12-year-old boy who was born with long-segment congenital tracheal stenosis and treated with a stem-cell-based tissue-engineered tracheal transplant. The utilized scaffold was a decellularized donor trachea processed with the enzymatic solution method in accordance with Macchiarini's method from 2008.