Published in final edited form as:

Ann Otol Rhinol Laryngol. 2016 November ; 125(11): 873–885. doi:10.1177/0003489416656646.

Clinical Translation of Tissue Engineered Trachea Grafts

Tendy Chiang, MD^{1,2,3}, Victoria Pepper, MD^{1,4}, Cameron Best, BA¹, Ekene Onwuka, MD^{1,5}, and Christopher K Breuer, MD^{1,4}

¹Tissue Engineering and Surgical Research, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA

²Department of Pediatric Otolaryngology, Nationwide Children's Hospital, Columbus, Ohio, USA

³Department of Otolaryngology–Head & Neck Surgery, The Ohio State University College of Medicine, Columbus, Ohio, USA

⁴Department of Pediatric Surgery, Nationwide Children's Hospital, Columbus, Ohio, USA

⁵Department of Surgery, The Ohio State University, Wexner Medical Center, Columbus, Ohio, USA

Abstract

Objective—To provide a state-of-the-art review discussing recent achievements in tissue engineered tracheal reconstruction.

Data Sources and Review Methods—A structured PubMed search of the current literature up to and including October 2015. Representative articles that discuss the translation of tissue engineered tracheal grafts (TETG) were reviewed.

Conclusions—The integration of a biologically compatible support with autologous cells has resulted in successful regeneration of respiratory epithelium, cartilage, and vascularization with graft patency, although the optimal construct composition has yet to be defined. Segmental TETG constructs are more commonly complicated by stenosis and delayed epithelialization when compared to patch tracheoplasty.

Implications for Practice—The recent history of human TETG recipients represents revolutionary proof of principle studies in regenerative medicine. Application of TETG remains limited to a compassionate use basis; however, defining the mechanisms of cartilage formation, epithelialization, and refinement of in vivo regeneration will advance the translation of TETG from the bench to the bedside.

Keywords

tissue engineering; tracheal replacement; regenerative medicine; airway reconstruction

Reprints and permissions: sagepub.com/journalsPermissions.nav

Corresponding Author: Tendy Chiang, Department of Pediatric Otolaryngology, Nationwide Children's Hospital, 555 S 18th Street, Suite 2A, Columbus, OH 43205-2664, USA. Tendy.Chiang@nationwidechildrens.org.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Introduction

Long-segment tracheal defects can arise congenitally or secondarily as a result of trauma, infection, or malignancy (Figure 1). Successful management of long-segment tracheal defects, regarded as more than 50% of the trachea in adults or 30% in children, exceeds the limits of primary reconstruction. Beyond reconstructive strategies, the pursuit of tracheal substitutes has been directed toward development of a construct that maintains a consistent and predictable long-term conduit for respiration. Grillo¹ described approaches involving foreign materials, nonviable tissues, autogenous tissues, and transplantation for tracheal replacement. These techniques have had limited success due to chronic infection, granulation, vascular erosion, resection, and development of malacia.

Regenerative medicine and tissue engineering serve to integrate cells, a 3-dimensional scaffold, and manipulation of cell signaling to induce new autologous tissue or “neotissue” formation.² The goal of tissue engineering is not to manage or augment failed tissue but to replace it. The ideal tracheal replacement would be nonimmunogenic, biocompatible, durable, and possess growth potential, which can be all be achieved with tissue engineering.

In 2008, the first segmental replacement with a tissue engineered tracheal graft (TETG) was implanted in a patient with severe bronchial stenosis following treatment of tuberculosis.³ Since then, there have been 9 published reports of TETG implantation in humans with a total of 15 reported patients who have undergone tracheal replacement.^{3–9}

Despite promising research, many hurdles remain for a TETG to make the transition from bench to bedside. The aim of this review is to assess the feasibility for TETGs and discuss advances that are facilitating this transition. Specific interest is directed to segmental tracheal replacement and in vivo studies.

Methodology/Search Strategy

A structured search of the current literature was performed on PubMed. The date of the most recent search was October 16, 2015. Search terms included: *tissue engineered trachea*, *regenerative medicine*, *tracheal replacement*, and *tissue engineering and trachea*. Relevant lists of studied papers were searched to identify further relevant papers. References of papers examined were then evaluated for additional relevant papers. No exclusions were made on the basis of study type or language. Emphasis was placed on publications dated in the past 5 years.

The search identified 384 potentially relevant articles, and upon further review of abstracts, 112 were deemed relevant. This consisted of 72 original reports, 29 reviews, and 11 case reports. In addition, a search for these terms was also performed within the lay press. A total of 85 studies are cited in this review.

Discussion

Successful tissue engineering relies on 3 components; namely, the scaffold, the cell source, and the humoral and mechanical signaling factors that orchestrate neotissue formation

(Figure 2). These factors can be modulated to suit the organ that is being replicated, and the design approaches to integrate these 3 components as well as transition them into the patient may be vastly different depending on the desired organ. Options range from ectopic cell expansion to direct implantation “off-the-shelf” without cell seeding. Each option has benefits and disadvantages in terms of time, risk of rejection, risk of contamination, and complications (Figure 3), and it is the current goal to determine what techniques are most optimal for generation of the TETG with regard to safety and efficacy. In the context of a TETG, there are inherent challenges with airway reconstruction and neotissue formation that are not encountered in other constructs. First, the cartilage framework of the trachea receives its nutrients via passive diffusion, and the avascular nature of hyaline cartilage is challenged by poor regenerative ability. Preservation of scaffold integrity needs to be balanced with the rate of cartilage regeneration. Second, the airway is not a sterile environment. A TETG is immediately exposed to contamination, which can compromise neotissue development. Third, orthotopic graft failure or compromise can result in significant morbidity in the form of airway obstruction, respiratory distress, and death. Thus, the scaffold must be designed to stimulate epithelial growth for clearance of secretions while also providing the structural integrity to prevent airway collapse. Both preclinical and clinical results demonstrate the complexity of this task.

Scaffolds

To achieve the structural integrity necessary for the airway, many different scaffolds have been explored, but they can generally be classified as decellularized tracheal constructs, biosynthetic, or scaffold-free constructs.

Decellularized Tracheal Scaffolds—The development of decellularized tracheal scaffold came in response to initial outcomes using cadaveric human tracheal allografts. Cadaveric allografts were initially described in 1980, where harvested specimens were preserved in formalin, Thimerosal, or acetone.¹⁰ These grafts did not support cellular ingrowth and resulted in the development of stenosis and malacia.¹¹ Alternatively, decellularization works through removal of cell and immunogenic material from the extracellular matrix (ECM), preserving the mechanical and bioinductive profile of the graft.^{12–14} Since mechanical properties of the tracheal ECM are primarily related to collagen, glycosaminoglycans, and elastin, decellularized tracheas have been demonstrated to have similar biomechanical profiles to native trachea.¹³ While the bioinductive properties are not completely understood, the microstructure maintained in decellularized scaffold also appears to play an important role in neotissue formation through chemoattraction, cell support, and signaling.¹³ This is demonstrated by not only the chondrocyte repopulation and re-epithelialization but by the presence of muscle bundles, serous glands, and nerve fibers that have been observed in tissue engineered decellularized tracheal grafts.^{15,16}

Decellularization protocols vary in length, ranging from 3 days to 12 weeks, and use a variety of methods and solutions to disrupt cell membranes and denature proteins.^{3,17–21,22} Variation in protocols has been shown to result in significant differences in ECM composition and structural integrity.²³ While most decellularized tracheal matrices demonstrate support for epithelial growth, some studies suggest that the rate of chondrocyte

repopulation does not adequately bridge the gap between ECM degradation and cartilage repopulation.^{12,21} Animal studies suggest that TETGs demonstrate 2 phases of degradation: early degradation of mucosal connective tissue followed by delayed degradation of cartilage ECM.²¹ Ideally, the rate of these phases should match the rate of neotissue formation; faster degradation will lend to instability and malacia of the TETG, and prolonged degradation of a scaffold can cause a foreign body inflammatory response, resulting in stenosis and scar formation.²⁴ In addition to achieving the appropriate rate of degradation and regeneration, scaffold supply is dependent on donor availability, which limits the broad utilization of this technique.

Biosynthetic Scaffolds—The inherent limitations in decellularized and cadaveric tracheas have led to the development of biosynthetic scaffolds, which are constructed from commercially available materials. These constituents are biologically inert, nonimmunogenic, and easily customizable. In addition, these synthetics can range from rapidly absorbable to permanent, allowing customization of design. The scaffolds are typically composed woven polymers, forming a porous environment that facilitates cell seeding and contributes to the biomechanical profile.

Scaffolds of Marlex mesh reinforced with polypropylene spiral coated with porcine-derived collagen have demonstrated patency and partial epithelialization for segmental defects in the animal model and have been used successfully for nonsegmental or patch repair in humans.^{25–30} However, reepithelialization was significantly delayed, noting persistent graft exposure 5 years after implantation.²⁵ The addition of seeded cells to this construct improved rates of epithelialization, with observed rates of epithelialization in the murine model by 2 weeks.^{26,28}

The first bioartificial nanofiber TETG was produced from polyhedral oligomericsilsesquioxane poly-(carbonate-urea) urethane (POSS-PCU), a nanocomposite polymer.⁹ The stiffness of the materials led to compliance mismatch, granulation tissue formation, and the development of a fistula at the distal anastomotic site that required numerous endoscopic interventions.³¹ Additionally, these grafts demonstrated poor vascularization and epithelialization as well as the potential for infection.

The complications encountered with POSS-PCU led to the pursuit of more physiologic constructs consisting of polyblends of electrospun materials. Polyethylene terephthalate (PET) and polyurethane (PU), which are both FDA-approved polymers, have both been explored in combination and in isolation.^{31–33} Modification of the PET/PU ratios can result in biomechanical characteristics similar to native trachea. The PET-only grafts demonstrated greater seeded cell retention, but both constructs demonstrated viability *in vivo*.³¹

Growth potential is a particular consideration for pediatric patients who may need TETG implantation. Resorbable constructs permit growth by conferring early structural support and then degrading as neotissue formation replaces the scaffold. Examples include: polyglycolic acid (PGA), polylactic-co-glycolic acid (PLGA), poly-(L-lactide-co-ε-caprolactone) (PLCL), and polyhedral oligomericsilsesquioxane poly-(ε-caprolactone) (POSS-PCL) and urea urethane.^{34–42} The PLCL/TGF-B1-loaded gelatin scaffolds with cultured chondrocytes

demonstrated similar biomechanical characteristics to native trachea after heterotopic placement in nude mice for 8 weeks.³⁶ An alternative graft composed of POSS-PCL, the biodegradable counterpart of POSS-PCU, was shown to maintain structural integrity for a minimum of 4 to 6 months with estimated degradation over 24 months.⁴³ This graft was later transitioned to the clinic and used in the first human bioartificial nanocomposite TETG implant.^{43,44}

Scaffold-Free/Biologic Constructs—While traditional tissue engineering relies on the presence of a scaffold, there are drawbacks, including immunogenicity, altered cell phenotype, and modified cell-cell interactions. Scaffold-free constructs bypass these issues by harnessing techniques that do not require cell seeding or an exogenous, 3-dimensional material.⁴⁵ Subsets of scaffold-less tissue engineering include self-organization and self-assembly techniques.

Self-organization techniques involve the formation of new tissue with the application of external forces.⁴⁵ These techniques include bioprinting and cell-sheet engineering. As an example, fabricated sheets of cartilage obtained from the auricular cartilage of New Zealand white rabbits has been used in combination to a muscle/silicone construct to create a neotrachea that was ectopically cultured and orthotopically transplanted 12 to 14 weeks after initial implantation.⁴⁶ Although they demonstrated mechanical stability without malacia, all rabbits expired from obstruction/stenosis between 1 and 39 days after surgery.

Self-assembly techniques result in spontaneous tissue formation in the absence of external forces: Cells seeded on a nonadherent surface develop neotissue by adhering to each other in order to minimize free energy. Self-assembly in TETG has been reported using human mesenchymal stem cell derived cartilaginous rings and cylinders generated through a custom ring-to-tube assembly system.⁴⁷ This scaffold-free TETG demonstrated the same or better biomechanical properties when compared to native rat trachea.

The variety of scaffold constructs that have been explored, as well as the wide variation in outcomes, demonstrates the need for further preclinical validation to find an optimal scaffold design. While most of these options have shown promising results in terms of epithelialization, similar results are lacking when evaluating cartilage regeneration or vascularization. In addition, there continues to be significant issues of mechanical failure or stenosis associated with each scaffold design (Table 1).

Cell Targets in TETG

The 2 predominant cellular targets in tracheal tissue engineering have been chondrocytes and ciliated pseudostratified columnar epithelium (respiratory epithelium). Chondrocytes form the structural support of the trachea, and pseudostratified columnar epithelium confers the functional characteristics of the native airway, including barrier for infection, secretion clearance, and humidification. In order to achieve these results, many cell types have been used alone or in combination including chondrocytes, epithelial cells, fibroblasts, adipose tissue, chondrocytes, mesenchymal amniocytes, and mesenchymal stem cells (bone-marrow mononuclear cells).^{17,20,26–28,34,38,48–58}

In decellularized constructs, it has been demonstrated that chondrocyte and epithelial cell proliferation are both needed for graft survival and patency.⁴⁹ Cell expansion can take place in vitro (cells are seeded onto a scaffold and expanded in a bioreactor prior to implantation), in vivo (cells are seeded at the time of implantation with the host serving as a bioreactor), or in situ (scaffold is implanted without donor stem cells and endogenous host cells are recruited for neotissue formation).²²

The clinical outcome of grafts has also been linked to the presence of living cells. While this factor has been dose-dependent in other constructs, there is currently minimal data within the tracheal literature regarding whether an increase in viable cells seeded improves outcomes. Quantification of cell seeding can be performed with manual or automated cell count, DNA assays, flow cytometry, and scanning electron microscopy. Real-time assessment of cell viability and seeding density using a colorimetric-based system has been used clinically to verify the integrity of a TETG prior to transplant.³³

Since differentiated cell targets include the culture of chondrocytes and respiratory epithelium, an obvious cell source is chondrocytes harvested from cartilages, achieved by taking pieces of trachea, nasal septum, rib, and auricular cartilage. These cells can then be isolated and expanded in vitro.^{40,42,46,59–62} While easy to harvest, the elastic auricular cartilage has failed to provide the integrity of hyaline cartilage needed for a tracheal construct.⁴⁶ Alternative sources including tracheal and nasal septal chondrocytes resulted in histologically similar cartilage to that of the native trachea but were not as strong as native trachea.⁶³ Differentiated targets for reepithelialization have reported success with tracheal epithelial cells, tracheal fibroblasts, gingival fibroblasts, buccal mucosa, and skin.^{3,8,26,28,29,49,64,65}

Stem cells have been used to facilitate the development of neotissue. Variants of mesenchymal stem cells (MSC) have been used, ranging from bone marrow, amniotic fluid, and adipose tissue for TETG.^{17,18,20,26,43,47,49,61,66,67} Bone marrow–derived and adipose–derived MSC have been most commonly used given their availability and accessibility. However, complete epithelialization has been observed in constructs using bone marrow–derived mesenchymal stem cells (BM-MSC), autologous epithelial cells, buccal mucosal graft, or tracheal fibroblasts.^{6,8,29,49} It is unclear if respiratory epithelialization represents survival of donor epithelium or regeneration by the recipient modulated by a paracrine effect from seeded cells. However, epithelial migration from the anastomotic sites has been demonstrated in some studies, suggesting recipient derived neotissue.²⁸ This restoration of respiratory epithelium is a slow process and can require up to 1 to 2 years for full repopulation in both preclinical and clinical models.^{4,6} Alternative studies in rabbits have demonstrated the differentiation of labeled adipose–derived MSC into ciliated epithelium, stromal cells, and cartilage.¹⁷ While these results are conflicting, it is possible that the cell fate may be a combination of both theories and that it may be dependent on the other factors creating the TETG, including the scaffold and bioengineering approach.

State of Translational Research in TETG

Table 1 provides a comprehensive review of preclinical in vivo tissue engineered tracheal graft original reports. Published experiments performed in murine (Mu), rabbit (Rb), porcine

(Po), canine (Ca), and ovine (Ov) models are grouped by design approaches, scaffold materials, and seeded cell sources. These categories are weighed against the most critical TETG outcome criteria: epithelialization, cartilage regeneration, vascularization, mechanical failure or the need for stent placement, and the development of stenosis. Study designs that involve segmental orthotopic implant are delineated. In this manner, a birds-eye view of the current state of preclinical TETG research is provided.

Complete epithelialization was observed in each animal model and is most frequently achieved via in vitro culture yet is feasible with direct implantation and in situ regeneration. Scaffold material choice does not seem to limit the formation of a functional epithelium, as our literature search identified reports in decellularized, biologic, and synthetic constructs alike. Similarly, seeded cell source does not appear to impact graft epithelialization and is clearly feasible in segmental TETGs. Cartilage regeneration, while not impossible via in situ and direct implantation techniques, seems to be the most feasible via ectopic and in vitro culture, with myriad reports in every animal model except for the murine species. Cartilage regeneration does not appear to be linked to any one particular scaffold material; however, the bulk of the progress in cartilage regeneration has been accomplished with synthetic scaffolds. In contrast to epithelialization, cartilage regeneration may be more dependent on seeded cell type, with differentiated chondrocytes presenting a clear advantage in the body of work reviewed. In our review, the most daunting metric to achieve has been construct vascularization, upon which the degree of epithelialization and cartilage regeneration may be closely linked. Ectopic and in vitro culture may best promote graft vascularization; however, direct implantation in canines also produced vascularized neotracheal tissue.^{21,30,68} The relationship between scaffold type and vascularization potential is not clear, but the most frequent reported material to meet this criterion was a synthetic scaffold. Interestingly, reports of graft mechanical failure or stenosis are also most frequently reported with synthetic scaffolds, and chondrocyte seeding is most associated with mechanical failure. The development of airway stenosis is a significant hurdle in the translation of the TETG to the clinic. Graft stenosis is the predominant morbidity in segmental orthotopic TETG implantation and presents the greatest hurdle to clinical translation, and all approaches to create segmental replacement are plagued by these complications to some degree. The optimal scaffold type(s) and cell seeding source(s) to prevent TETG stenosis are still unclear.

An analysis of these results identifies 2 important implications of the preclinical work to date. The first is that there is greater morbidity in studies using segmental orthotopic replacement when compared to partial tracheal reconstruction with an interposed graft (patch tracheoplasty), identifying a research priority for the safe and efficacious translation of full segment TETG to the clinic. The second is that the optimal method to generate a tissue engineered trachea that is completely epithelialized, regenerates cartilage, is vascularized and biomechanically sound, and resists stenosis will most likely require integration of the most successful methodologies. The next generation of preclinical animal studies evaluating various tissue engineered tracheal constructs may benefit from systematic and rigorous examination of each design variable. Additionally, an alternative perspective may suggest that fulfillment of all outcome criteria may not be necessary for a successful

TETG; for example, a nondegradable ringed construct may not require cartilage regeneration for long-term viability.

Current Clinical Experience With Tissue Engineered Tracheal Grafts

In recent years, there have been several reports of TETG for the clinical management of long-segment airway defects, spanning both the scientific literature and lay press. All published outcomes are presented in Table 2. The current clinical application for TETG demonstrates clinical success but is evaluated by heterogeneous metrics. Bronchoscopic findings, need for dilation and stenting, pulmonary function testing, laser Doppler, serologic testing, computed tomography, quality-of-life questionnaires, histology, and immunohistochemistry have all been examined.^{3,4,6-9,16}

A series of 7 reported patients, including 1 performed in 2005 in a 78-year-old male with thyroid cancer, have demonstrated the successful use of TETG technologies undergoing patch tracheoplasties. This series of patients used a Marlex mesh covered with collagen fibers and seeded with venous blood. Results have demonstrated patency out to a 34-month follow-up and complete epithelialization.⁶⁸⁻⁷⁰ More recently, there has been a similar effort at patch tracheoplasty using decellularized porcine jejunum, which has shown complete incorporation to the surrounding tissues on bronchoscopy.⁷¹

The first reported segmental TETG implantation was performed in a 30-year-old female who presented with tuberculosis infection of her trachea and proximal bronchi resulting in severe stenosis and malacia.³ A donor trachea underwent a 6-week decellularization process and was seeded with bronchial mucosa and BM-MSC. Proximal graft stenosis developed 6 months after surgery, requiring 16 bronchoscopies in 5 years with the placement of stents during 14 of these interventions. Bronchoscopic biopsies demonstrated evidence of recellularization at 1 year postimplantation, with return of respiratory epithelium at 2 years.⁴

In 2014, a 76-year-old male received a decellularized TETG seeded with expanded BM-MSC to replace a 4.5 cm segment of posttraumatic subglottic and tracheal stenosis.¹⁶ Unfortunately, his hospitalization was complicated by several episodes of respiratory distress requiring 6 bronchoscopies, candida infection, and eventual death on postoperative day 23 from a myocardial infarction. Autopsy demonstrated a patent TETG with neovascularization, squamous epithelium, and muscle bundles in the submucosa with serous glands and nerve fibers, suggesting graft-enabled migration of host cells.

The first pediatric TETG recipient was reported in 2012 on a patient with congenital tracheal stenosis and pulmonary sling.⁶ Initial management at birth with autologous patch tracheoplasty and stenting resulted in stent erosion into the aorta at 3 years of age, resulting in tracheal homograft placement and subsequent replacement due to infection. Homograft failure with vascular erosion occurred at 10 years of age, resulting in TETG implantation at 12 years of age. A donor trachea underwent a 3-day decellularization and was seeded intraoperatively with BM-MSC and small patches of harvested tracheal mucosa. The omentum was interposed to improve perfusion to the graft site.⁶ He required 25 procedures postoperatively for secretion clearance, granulation removal, and placement of 2 bioabsorbable stents.⁷ Normal ciliated respiratory epithelium was observed by 42 months.

To avoid the need for a donor trachea and a long decellularization process, the first reported bioartificial nanocomposite TETG was implanted in 2011.^{9,72} A 36-year-old male diagnosed with tracheal mucoepidermoid carcinoma involving the carina initially was managed with surgery and radiation therapy. He was diagnosed with recurrence at the right main stem bronchus 2 years later and was offered resection with tracheal replacement with TETG. A bioartificial nonresorbable nanofiber scaffold was constructed from POSS-PCU was seeded with BM-MSc in a bioreactor for 36 hours prior to implantation and reseeded with BM-MSc immediately before implantation, which was performed with interposed omentum. Bronchoscopy with biopsies 2 months postoperatively demonstrated granulation and signs of epithelialization, and the patient was reported to be asymptomatic 5 months after transplantation.

Of interest is a series of studies combining the techniques of both tracheal transplantation and tissue engineering. Initial studies in a rabbit model using heterotopic implantation followed by orthotopic transplantation of the tracheal allograft demonstrated the potential of this combination.⁷³ These experiments were followed by the implantation of a fresh cadaveric allograft into the forearm of a 55-year-old female who had sustained long-segment posttraumatic tracheal stenosis.⁸ The patient underwent immunosuppression for 229 days, with orthotopic transplantation after 4 months in the form of a microvascular free flap based off of the radial artery and 2 radial veins. Viable cartilage lined with squamous epithelium composed of chimeric neotissue was identified at the time of transplant. Donor-derived epithelium disappeared after withdrawal of immunosuppression without significant impact on the cartilaginous framework. It is speculated that the donor cartilage was not immunogenic due to the lack of direct vascular exposure to the graft. Computed tomography obtained 1 year after transplant were similar to those obtained 1 week after transplantation.

The largest series of TETG implantations in humans is referenced in a review article that describes 9 patients who underwent decellularized TETG and 8 patients who underwent replacement with a bioartificial nanocomposite TETG.⁵ Five of the patients who underwent decellularized TETG were reported as alive, 4 of which are stent-dependent. All patients who underwent replacement with decellularized TETG for malignant etiologies died of systemic tumor recurrence, and 1 patient who underwent replacement for congenital tracheal stenosis died of gastrointestinal bleeding. Of the 8 patients who underwent bioartificial nanocomposite TETG implant, 6 patients are reported as alive, and 2 patients are reported as dead from unrelated reasons.

In addition to the cases documented within the medical literature, there have been several highly publicized cases within the media. This includes the case of a 20-year-old male with tracheal cancer who underwent tracheal replacement with a biosynthetic scaffold and subsequently died of unknown causes.^{74–76} Additionally, a 2-year-old female with tracheal agenesis underwent a tracheal replacement with a biosynthetic graft but subsequently died from complications unrelated to the graft.^{77,78} While these accomplishments within the clinical world show a vast array of outcomes, they also show both promise and the need to continue to critically evaluate outcomes.

Implications for Practice

The recent history of human TETG recipients represents revolutionary proof of principle studies in regenerative medicine, but it also highlights challenges in the field. The approaches to tracheal replacement remain diverse, ranging from decellularized to bioartificial scaffolding, undifferentiated to differentiated cells, and in vitro expansion to immediate implantation using the body as a bioreactor. While each strategy has its benefits and drawbacks, no one approach has proven to be superior.

Currently, clinical use of TETG for large segment tracheal defects remains reserved for compassionate use only. A position statement from the International Society for Cellular Therapy in 2014 stated that while significant progress is being made by many international groups, this discovery is largely taking place in parallel and in isolation, with little to no collaboration among basic/translational communities with clinical and commercial efforts.⁷⁹ The summit of experts resulted in a collection of key questions and general recommendations to advance the field. Emphasis needs to be placed on comprehensive reporting of clinical outcomes, mechanisms of neotissue formation, and collaboration between regulatory agencies and industry partners.

To advance the field of airway tissue engineering, molecular and cellular mechanisms mediating neotissue formation in TETG need to be defined. While a TETG patch tracheoplasty model has been demonstrated in the murine model, only recently has the feasibility of orthotopic segmental tracheal transplant in the murine model been reported using syngeneic transplant and implantation of decellularized grafts.^{26,28,80,81} Additional hurdles for the transition of the TETG from the bench to the bedside involve the facilities and skill sets required for in vitro cell culture and Good manufacturing practice (GMP) laboratory facilities. Evolution of in vivo tissue engineering using the recipient as a bioreactor, thereby avoiding the need for in vitro expansion and development of closed, disposable cell seeding system for operator independent cell isolation and seeding, would improve the accessibility of TETG and the feasibility for widespread use.^{50,82}

Conclusions

Current clinical and translational studies have yet to identify the most effective strategy for tracheal replacement. Further studies to identify the mechanisms of epithelialization and cartilage repopulation are necessary. Trials comparing varying scaffold and cell seeding techniques with the application of uniform, comprehensive characterization as well as protocolization of interventions will help homogenize data for improved outcome metrics.

Acknowledgments

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Grillo, HC. Surgery of the Trachea and Bronchi. Lewiston, NY: BC Decker; 2004.

2. Langer R, Vacanti JP. Tissue engineering. *Science*. 1993; 260:920–926. [PubMed: 8493529]
3. Macchiarini P, Jungebluth P, Go T, et al. Clinical transplantation of a tissue-engineered airway. *Lancet*. 2008; 372:2023–2030. [PubMed: 19022496]
4. Gonfiotti A, Jaus MO, Barale D, et al. The first tissue-engineered airway transplantation: 5-year follow-up results. *Lancet*. 2014; 383:238–244. [PubMed: 24161821]
5. Jungebluth P, Macchiarini P. Airway transplantation. *Thorac Surg Clin*. 2014; 24:97–106. [PubMed: 24295665]
6. Elliott MJ, De Coppi P, Spegiorin S, et al. Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year follow-up study. *Lancet*. 2012; 380:994–1000. [PubMed: 22841419]
7. Hamilton NJ, Kanani M, Roebuck DJ, et al. Tissue-engineered tracheal replacement in a child: a 4-year follow-up study. *Am J Transplant*. 2015; 15(10):2750–2757. [PubMed: 26037782]
8. Delaere P, Vranckx J, Verleden G, De Leyn P, Van Raemdonck D. Tracheal allotransplantation after withdrawal of immunosuppressive therapy. *N Engl J Med*. 2010; 362:138–145. [PubMed: 20071703]
9. Jungebluth P, Alici E, Baiguera S, et al. Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study. *Lancet*. 2011; 378:1997–2004. [PubMed: 22119609]
10. Herberhold C, Franz B, Breipohl W. Chemical preserved human trachea as prosthesis in covering tracheal defects—first experiences (author's transl). *Laryngol Rhinol Otol (Stuttg)*. 1980; 59:453–457. [PubMed: 7464363]
11. Tanaka H, Maeda K, Okita Y. Transplantation of the cryopreserved tracheal allograft in growing rabbits. *J Pediatr Surg*. 2003; 38:1707–1711. [PubMed: 14666448]
12. Zang M, Zhang Q, Chang EI, Mathur AB, Yu P. Decellularized tracheal matrix scaffold for tracheal tissue engineering: in vivo host response. *Plast Reconstr Surg*. 2013; 132:549e–559e.
13. Badylak SF. The extracellular matrix as a biologic scaffold material. *Biomaterials*. 2007; 28:3587–3593. [PubMed: 17524477]
14. Sun F, Pan S, Shi HC, et al. Structural integrity, immunogenicity and biomechanical evaluation of rabbit decellularized tracheal matrix. *J Biomed Mater Res A*. 2015; 103:1509–1519. [PubMed: 25044712]
15. Conconi MT, De Coppi P, Di Liddo R, et al. Tracheal matrices, obtained by a detergent-enzymatic method, support in vitro the adhesion of chondrocytes and tracheal epithelial cells. *Transpl Int*. 2005; 18:727–734. [PubMed: 15910302]
16. Berg M, Ejnell H, Kovacs A, et al. Replacement of a tracheal stenosis with a tissue-engineered human trachea using autologous stem cells: a case report. *Tissue Eng Part A*. 2014; 20:389–397. [PubMed: 24004248]
17. Batioglu-Karaaltin A, Karaaltin MV, Ovali E, et al. In vivo tissue-engineered allogenic trachea transplantation in rabbits: a preliminary report. *Stem Cell Rev*. 2015; 11:347–356. [PubMed: 25351181]
18. Wood MW, Murphy SV, Feng X, Wright SC Jr. Tracheal reconstruction in a canine model. *Otolaryngol Head Neck Surg*. 2014; 150:428–433. [PubMed: 24367052]
19. Weymann A, Patil NP, Sabashnikov A, et al. Perfusion-decellularization of porcine lung and trachea for respiratory bioengineering. *Artif Organs*. 2015; 39(12):1024–1032. [PubMed: 25894696]
20. Gray FL, Turner CG, Ahmed A, Calvert CE, Zurakowski D, Fauza DO. Prenatal tracheal reconstruction with a hybrid amniotic mesenchymal stem cells-engineered construct derived from decellularized airway. *J Pediatr Surg*. 2012; 47:1072–1079. [PubMed: 22703772]
21. Remlinger NT, Czajka CA, Juhas ME, et al. Hydrated xenogeneic decellularized tracheal matrix as a scaffold for tracheal reconstruction. *Biomaterials*. 2010; 31:3520–3526. [PubMed: 20144481]
22. Fishman JM, Lowdell M, Birchall MA. Stem cell-based organ replacements—airway and lung tissue engineering. *Semin Pediatr Surg*. 2014; 23:119–126. [PubMed: 24994525]
23. Haykal S, Soleas JP, Salna M, Hofer SO, Waddell TK. Evaluation of the structural integrity and extracellular matrix components of tracheal allografts following cyclical decellularization techniques: comparison of three protocols. *Tissue Eng Part C Methods*. 2012; 18:614–623. [PubMed: 22332979]

24. Badylak SF, Valentin JE, Ravindra AK, McCabe GP, Stewart-Akers AM. Macrophage phenotype as a determinant of biologic scaffold remodeling. *Tissue Eng Part A*. 2008; 14:1835–1842. [PubMed: 18950271]
25. Nakamura T, Teramachi M, Sekine T, et al. Artificial trachea and long term follow-up in carinal reconstruction in dogs. *Int J Artif Organs*. 2000; 23:718–724. [PubMed: 11075903]
26. Kobayashi K, Suzuki T, Nomoto Y, et al. A tissue-engineered trachea derived from a framed collagen scaffold, gingival fibroblasts and adipose-derived stem cells. *Biomaterials*. 2010; 31:4855–4863. [PubMed: 20347137]
27. Nomoto M, Nomoto Y, Tada Y, et al. Bioengineered trachea using autologous chondrocytes for regeneration of tracheal cartilage in a rabbit model. *Laryngoscope*. 2013; 123:2195–2201. [PubMed: 23821554]
28. Nomoto Y, Kobayashi K, Tada Y, Wada I, Nakamura T, Omori K. Effect of fibroblasts on epithelial regeneration on the surface of a bioengineered trachea. *Ann Otol Rhinol Laryngol*. 2008; 117:59–64. [PubMed: 18254373]
29. Okano W, Nomoto Y, Wada I, et al. Bioengineered trachea with fibroblasts in a rabbit model. *Ann Otol Rhinol Laryngol*. 2009; 118:796–804. [PubMed: 19999366]
30. Yamashita M, Kanemaru S, Hirano S, et al. Tracheal regeneration after partial resection: a tissue engineering approach. *Laryngoscope*. 2007; 117:497–502. [PubMed: 17334311]
31. Ajallouei F, Lim ML, Lemon G, et al. Biomechanical and biocompatibility characteristics of electrospun polymeric tracheal scaffolds. *Biomaterials*. 2014; 35:5307–5315. [PubMed: 24703872]
32. Lee JH, Park HS, Oh SH, et al. Triple-layered polyurethane prosthesis with wrinkles for repairing partial tracheal defects. *Laryngoscope*. 2014; 124:2757–2763. [PubMed: 24986797]
33. Jungebluth P, Haag JC, Lim ML, et al. Verification of cell viability in bioengineered tissues and organs before clinical transplantation. *Biomaterials*. 2013; 34:4057–4067. [PubMed: 23473965]
34. Luo X, Liu Y, Zhang Z, et al. Long-term functional reconstruction of segmental tracheal defect by pedicled tissue-engineered trachea in rabbits. *Biomaterials*. 2013; 34:3336–3344. [PubMed: 23380355]
35. Tsukada H, Gangadharan S, Garland R, Herth F, DeCamp M, Ernst A. Tracheal replacement with a bioabsorbable scaffold in sheep. *Ann Thorac Surg*. 2010; 90:1793–1797. [PubMed: 21095312]
36. Park JH, Hong JM, Ju YM, et al. A novel tissue-engineered trachea with a mechanical behavior similar to native trachea. *Biomaterials*. 2015; 62:106–115. [PubMed: 26041482]
37. de Mel A, Yap T, Cittadella G, et al. A potential platform for developing 3D tubular scaffolds for paediatric organ development. *J Mater Sci Mater Med*. 2015; 26:141. [PubMed: 25737129]
38. Shin YS, Choi JW, Park JK, et al. Tissue-engineered tracheal reconstruction using mesenchymal stem cells seeded on a porcine cartilage powder scaffold. *Ann Biomed Eng*. 2015; 43:1003–1013. [PubMed: 25253469]
39. Britt JC, Park SS. Autogenous tissue-engineered cartilage: evaluation as an implant material. *Arch Otolaryngol Head Neck Surg*. 1998; 124:671–677. [PubMed: 9639478]
40. Lin CH, Hsu SH, Huang CE, Cheng WT, Su JM. A scaffold-bioreactor system for a tissue-engineered trachea. *Biomaterials*. 2009; 30:4117–4126. [PubMed: 19447489]
41. Grimmer JF, Gunnlaugsson CB, Alsberg E, et al. Tracheal reconstruction using tissue-engineered cartilage. *Arch Otolaryngol Head Neck Surg*. 2004; 130:1191–1196. [PubMed: 15492167]
42. Tsao CK, Ko CY, Yang SR, et al. An ectopic approach for engineering a vascularized tracheal substitute. *Biomaterials*. 2014; 35:1163–1175. [PubMed: 24239301]
43. Teoh GZ, Crowley C, Birchall MA, Seifalian AM. Development of resorbable nanocomposite tracheal and bronchial scaffolds for paediatric applications. *Br J Surg*. 2015; 102:e140–e150. [PubMed: 25627127]
44. Crowley C, Birchall M, Seifalian AM. Trachea transplantation: from laboratory to patient. *J Tissue Eng Regen Med*. 2015; 9:357–367. [PubMed: 26052583]
45. Athanasiou KA, Eswaramoorthy R, Hadidi P, Hu JC. Self-organization and the self-assembling process in tissue engineering. *Annu Rev Biomed Eng*. 2013; 15:115–136. [PubMed: 23701238]
46. Weidenbecher M, Tucker HM, Gilpin DA, Dennis JE. Tissue-engineered trachea for airway reconstruction. *Laryngoscope*. 2009; 119:2118–2123. [PubMed: 19806650]

47. Dikina AD, Strobel HA, Lai BP, Rolle MW, Alsberg E. Engineered cartilaginous tubes for tracheal tissue replacement via self-assembly and fusion of human mesenchymal stem cell constructs. *Biomaterials*. 2015; 52:452–462. [PubMed: 25818451]
48. Kanzaki M, Yamato M, Hatakeyama H, et al. Tissue engineered epithelial cell sheets for the creation of a bioartificial trachea. *Tissue Engineering*. 2006; 12:1275–1283. [PubMed: 16771640]
49. Go T, Jungebluth P, Baiguero S, et al. Both epithelial cells and mesenchymal stem cell-derived chondrocytes contribute to the survival of tissue-engineered airway transplants in pigs. *J Thorac Cardiovasc Surg*. 2010; 139:437–443. [PubMed: 19995663]
50. Jungebluth P, Bader A, Baiguera S, et al. The concept of in vivo airway tissue engineering. *Biomaterials*. 2012; 33:4319–4326. [PubMed: 22440048]
51. Mohd Heikal MY, Aminuddin BS, Jeevanan J, Chen HC, Sharifah SH, Ruszymah BH. Autologous implantation of bilayered tissue-engineered respiratory epithelium for tracheal mucosal regeneration in a sheep model. *Cells Tissues Organs*. 2010; 192:292–302. [PubMed: 20616535]
52. Tatekawa Y, Kawazoe N, Chen G, Shirasaki Y, Komuro H, Kaneko M. Tracheal defect repair using a PLGA-collagen hybrid scaffold reinforced by a copolymer stent with bFGF-impregnated gelatin hydrogel. *Pediatr Surg Int*. 2010; 26:575–580. [PubMed: 20425118]
53. Komura M, Komura H, Otani Y, et al. Tracheoplasty with cartilage-engineered esophagus environments. *J Pediatr Surg*. 2015; 50:1093–1098. [PubMed: 25783340]
54. Hong HJ, Chang JW, Park JK, et al. Tracheal reconstruction using chondrocytes seeded on a poly(L-lactic-co-glycolic acid)-fibrin/hyaluronan. *J Biomed Mater Res A*. 2014; 102:4142–4150. [PubMed: 24443290]
55. Kamil SH, Eavey RD, Vacanti MP, Vacanti CA, Hartnick CJ. Tissue-engineered cartilage as a graft source for laryngo-tracheal reconstruction. *Arch Otolaryngol Head Neck Surg*. 2004; 130:1048–1051. [PubMed: 15381591]
56. Fuchs JR, Hannouche D, Terada S, Vacanti JP, Fauza DO. Fetal tracheal augmentation with cartilage engineered from bone marrow-derived mesenchymal progenitor cells. *J Pediatr Surg*. 2003; 38:984–987. [PubMed: 12778408]
57. Kunisaki SM, Freedman DA, Fauza DO. Fetal tracheal reconstruction with cartilaginous grafts engineered from mesenchymal amniocytes. *J Pediatr Surg*. 2006; 41:675–682. [PubMed: 16567175]
58. Chang JW, Park SA, Park JK, et al. Tissue-engineered tracheal reconstruction using three-dimensionally printed artificial tracheal graft: preliminary report. *Artif Organs*. 2014; 38:E95–E105. [PubMed: 24750044]
59. Gilpin DA, Weidenbecher MS, Dennis JE. Scaffold-free tissue-engineered cartilage implants for laryngotracheal reconstruction. *Laryngoscope*. 2010; 120:612–617. [PubMed: 20058322]
60. Kim DY, Pyun J, Choi JW, et al. Tissue-engineered allograft tracheal cartilage using fibrin/hyaluronan composite gel and its in vivo implantation. *Laryngoscope*. 2010; 120:30–38. [PubMed: 19877186]
61. Hashemibeni B, Goharian V, Esfandiari E, et al. An animal model study for repair of tracheal defects with autologous stem cells and differentiated chondrocytes from adipose-derived stem cells. *J Pediatr Surg*. 2012; 47:1997–2003. [PubMed: 23163989]
62. Kojima K, Bonassar LJ, Roy AK, Vacanti CA, Cortiella J. Autologous tissue-engineered trachea with sheep nasal chondrocytes. *J Thorac Cardiovasc Surg*. 2002; 123:1177–1184. [PubMed: 12063466]
63. Kojima K, Bonassar LJ, Ignatz RA, Syed K, Cortiella J, Vacanti CA. Comparison of tracheal and nasal chondrocytes for tissue engineering of the trachea. *Ann Thorac Surg*. 2003; 76:1884–1888. [PubMed: 14667605]
64. Hamilton N, Bullock AJ, Macneil S, Janes SM, Birchall M. Tissue engineering airway mucosa: a systematic review. *Laryngoscope*. 2014; 124:961–968. [PubMed: 24129819]
65. Kim J, Suh SW, Shin JY, Kim JH, Choi YS, Kim H. Replacement of a tracheal defect with a tissue-engineered prosthesis: early results from animal experiments. *J Thorac Cardiovasc Surg*. 2004; 128:124–129. [PubMed: 15224031]

66. Haykal S, Salna M, Zhou Y, et al. Double-chamber rotating bioreactor for dynamic perfusion cell seeding of large-segment tracheal allografts: comparison to conventional static methods. *Tissue Eng Part C Methods*. 2014; 20:681–692. [PubMed: 24392662]
67. Clark ES, Best C, Onwuka E, et al. Effect of cell seeding on neotissue formation in a tissue engineered trachea. *J Pediatr Surg*. 2016; 51(1):49–55. [PubMed: 26552897]
68. Omori K, Nakamura T, Kanemaru S, Magruffov A, Yamashita M, Shimizu Y. In situ tissue engineering of the cricoid and trachea in a canine model. *Ann Otol Rhinol Laryngol*. 2008; 117:609–613. [PubMed: 18771079]
69. Omori K, Nakamura T, Kanemaru S, et al. Regenerative medicine of the trachea: the first human case. *Ann Otol Rhinol Laryngol*. 2005; 114:429–433. [PubMed: 16042099]
70. Kanemaru S, Hirano S, Umeda H, et al. A tissue-engineering approach for stenosis of the trachea and/or cricoid. *Acta Otolaryngol Suppl*. 2010:79–83. [PubMed: 20879824]
71. Steinke M, Dally I, Friedel G, Walles H, Walles T. Host-integration of a tissue-engineered airway patch: two-year follow-up in a single patient. *Tissue Eng Part A*. 2015; 21:573–579. [PubMed: 25316325]
72. Roberts, M. [Accessed June 23, 2016] Surgeons carry out first synthetic windpipe transplant. <http://www.bbc.com/news/health-14047670>. Published July 7, 2011
73. Delaere P, Liu Z, Sciot R, Welvaart W. The role of immunosuppression in the long-term survival of tracheal allografts. *Arch Otolaryngol Head Neck Surg*. 1996; 122:1201–1208. [PubMed: 8906055]
74. Fountain, H. [Accessed June 23, 2016] Christopher Lyles, got synthetic trachea, dies at 30. http://www.nytimes.com/2012/03/07/health/research/christopher-lyles-got-synthetic-trachea-dies-at-30.html?_r=0. Published March 7, 2012
75. Fountain, H. [Accessed June 23, 2016] Synthetic windpipe is used to replace cancerous one. <http://www.nytimes.com/2012/01/13/health/research/surgeons-transplant-synthetic-trachea-in-baltimore-man.html>. Published January 12, 2012
76. Hasan, L. [Accessed June 23, 2016] First US patient gets stem cell trachea transplant. <http://abcnews.go.com/Health/Wellness/us-patient-artificial-trachea-transplant/story?id=15354809>. Published January 13, 2012
77. Moisse, K. [Accessed June 23, 2016] Girl dies after groundbreaking trachea transplant. <http://abcnews.go.com/Health/girl-dies-groundbreaking-trachea-transplant/story?id=19604605>. Published July 8, 2013
78. Lapook J. Two-year-old gets windpipe made from her own stem cells. Two-year-old gets windpipe made from her own stem cells. [Accessed June 23, 2016] Published July 25, 2013.
79. Weiss, DJ., Elliott, M., Jang, Q., Poole, B., Birchall, M. International Society of Cell Therapy Pulmonary Scientific C. Tracheal bioengineering: the next steps. *Cytherapy; Proceeds of an International Society of Cell Therapy Pulmonary Cellular Therapy Signature Series Workshop*; Paris, France. April 22, 2014; 2014. p. 1601-1613.
80. Kuten JC, McGovern D, Hobson CM, et al. Decellularized tracheal extracellular matrix supports epithelial migration, differentiation, and function. *Tissue Eng Part A*. 2015; 21:75–84. [PubMed: 24980864]
81. Tani A, Tada Y, Takezawa T, et al. Regenerative process of tracheal epithelium using a collagen vitrigel sponge scaffold. *Laryngoscope*. 2013; 123:1469–1473. [PubMed: 23625522]
82. Kurobe H, Maxfield MW, Naito Y, et al. Comparison of a closed system to a standard open technique for preparing tissue-engineered vascular grafts. *Tissue Eng Part C Methods*. 2015; 21:88–93. [PubMed: 24866863]
83. Mohd Heikal MY, Aminuddin BS, Jeevana J, et al. Autologous implantation of bilayered tissue-engineered respiratory epithelium for tracheal mucosal regeneration in a sheep model. *Cells Tissues Organs*. 2010; 192(5):292–302. [PubMed: 20616535]
84. Kojima K, Bonassar LJ, Roy AK, et al. A composite tissue engineered trachea using sheep nasal chondrocyte and epithelial cells. *The FASEB Journal*. 2003; 17:823–828. [PubMed: 12724341]
85. Asnaghi MA, Jungebluth P, Raimond MT, et al. A double chamber rotating bioreactor for the development of tissue-engineered hollow organs: from concept to clinical trial. *Biomaterials*. 2009; 30(29):5260–5269. [PubMed: 19647867]

Figure 1.
Summary of congenital and acquired conditions requiring long-segment tracheal reconstruction.

Figure 2.

The classic tissue engineering paradigm includes a scaffold, seeded cells, and humoral signaling. Combinations of these 3 design components give rise to design approaches to create the optimal tissue engineered tracheal graft.

Figure 3. Approaches to the creation of a tissue engineered tracheal graft are grouped into 4 categories: ectopic culture, in vitro culture, in situ regeneration, and direct implantation. Advantages and disadvantages of each are highlighted to demonstrate current challenges facing the field. Photographs reproduced with permission.^{35,84,85}

Table 1

Preclinical Experience With Tissue Engineered Tracheal Grafts.^a

Approach	Epithelialization	Cartilage Regeneration	Vascularization	Mechanical Failure/Stent Required?	Stenosis?
Ectopic culture	Rb ^{34,48,53}	Rb ^{34,42,46,53}	Rb ^{34,42,46}	Rb ³⁴	Rb ⁴⁶
	Po ⁵⁵	Po ⁵⁵		Ov ⁶²	Ov ⁶²
In vitro culture	Rb ^{17,34,38,48,52,54,58}	Rb ^{17,34,38,40,42,46,54,58,59}	Rb ^{17,34,40,42,46,54,58}	Rb ^{34,59}	Rb ^{40,46}
	Po ⁴⁹	Ca ⁶¹			Po ⁶⁶
	Ov ^{20,56,57,83}	Ov ^{56,57}			Ov ²⁰
	Mu ^{26,28}	Rb ^{27,58}	Rb ²⁷		Ov ⁶⁷
In situ regeneration	Rb ^{27,58}	Po ⁵⁰	Ov ⁶⁷		
	Po ⁵⁰				
	Ov ⁶⁷				
	Mu ⁸⁰	Ca ^{30,68}	Rb ³²	Ca ¹⁸	Mu ⁸⁰
Direct implantation	Rb ³²		Ca ^{21,30,68}	Ov ³⁵	Rb ³²
	Ca ^{21,30,68}				
	Ov ³⁵				
	Mu ⁸⁰				
Scaffold material	Rb ¹⁷	Po ⁵⁰	Rb ¹⁷	Ca ¹⁸	Mu ⁸⁰
	Po ^{21,49,50}		Ca ²¹		Po ⁶⁶
	Ov ²⁰				Ov ²⁰
	Mu ⁸¹	Rb ^{38,53}	Mu ⁸¹		
Biologic	Rb ^{38,53,60}	Ca ⁶¹			
	Mu ²⁸	Rb ^{27,34,40,42,54,58}	Rb ^{27,32,34,40,42,54}	Rb ^{34,41,42}	Rb ^{32,40,41}
	Rb ^{27,32,34,41,48,54}	Po ⁵⁵	Ca ^{30,68}	Ov ^{35,62}	Ov ^{62,67}
	Po ⁵⁵	Ca ^{30,68}	Ov ⁶⁷		
Synthetic	Ca ^{30,68}	Ov ^{56,57,62}			
	Ov ^{35,56,57,67,83}				
	Mu ⁸¹	Ca ^{30,68}	Mu ⁸¹	Ov ³⁵	Rb ³²
Cell seeding					

	Outcome Measure				
	Epithelialization	Cartilage Regeneration	Vascularization	Mechanical Failure/Stent Required?	Stenosis?
Epithelial cells	Rb ³²		Rb ³²		
	Ca ^{31,30,68}		Ca ^{21,30,68}		
	Ov ³⁵				Po ⁶⁶
	Mu ²⁸	Po ⁵⁰			
	Rb ⁴⁸				
Adipose SC	Po ^{49,50}				
	Ov ⁸³				
	Mu ²⁶	Rb ¹⁷	Rb ¹⁷	Ca ¹⁸	
Chondrocytes	Rb ¹⁷	Ca ⁶¹			
	Rb ^{27,34,52-54}	Rb ^{27,34,40,42,46,53,54,59}	Rb ^{27,34,40,42,46,54}	Rb ^{34,41,59}	Rb ^{40,41,46}
	Po ^{49,55}	Po ⁵⁵		Ov ⁶²	Ov ⁶²
	Ov ⁵⁶	Ov ^{56,62}			
Mesenchymal amniocytes	Ov ^{20,57}	Ov ⁵⁷			Ov ^{20,57}
BM-MSc	Rb ^{38,58}	Rb ^{38,42,58}	Rb ⁴²		Po ⁶⁶
	Po ⁵⁰	Po ⁵⁰	Ov ⁶⁷		Ov ⁶⁷
	Ov ^{56,67}	Ov ⁵⁶			

^aReports of preclinical animal experiments weighing design approach, scaffold material selection, and cell seeding source against tissue engineered tracheal graft outcome metrics including epithelialization, cartilage regeneration, vascularization, mechanical failure, and stenosis. Reference numbers of studies involving segmental tissue engineered trachea graft constructs are underlined. While much progress has been made in the laboratory, the ideal combination of design variables to create an optimal TETG has yet to be translated to the clinic, and much work is required to understand the cellular and molecular mechanisms of neotrachea development.

BM-MSc, bone marrow–derived mesenchymal stem cells; Ca, canine; Mu, murine; Ov, ovine; Po, porcine; Rb, rabbit; SC, stem cells.

Table 2

Human Applications of Tissue Engineered Trachea Grafts.^a

Authors	Patient	Indication	Scaffold		Cell Source	Modulator	Outcome	Follow-Up	Reference
2005 Omori, et al	78-year-old female	Thyroid cancer	Marlex mesh patch with spiral rings covered by collagen sponge		Venous blood	None	Epithelialization on bronchoscopy (7 m), patent airway	34 mos	68,69
2008 Macchiarini et al	30-year-old female	Post-tuberculosis chronic tracheitis with bronchomalacia	Decellularized cadaveric trachea		Bronchial epithelial cells, chondrocytes, BM-MNC	FGF, TGF-β3, rPTHrP, Dex, Insulin	Epithelialization on bronch (1 m), critical stenosis (12 m) requiring stenting × 14	5 y	3,4
2008 Omori, et al	77-year-old male	Subglottic stenosis s/p tracheostomy	Marlex mesh patch with spiral rings covered by collagen sponge		Venous blood	none	Epithelialization (2 m)	2 mos	68
	71-year-old male	Recurrent thyroid cancer	Marlex mesh patch with spiral rings covered by collagen sponge		Venous blood	None	Small leak treated with drainage, epithelialization (22 m)	22 mos	68
	59-year-old female	Recurrent thyroid cancer with invasion into trachea	Marlex mesh patch with spiral rings covered by collagen sponge		Venous blood	None	Complete epithelialization (11 m)	12 mos	68
2010 Kanemaru et al	71-year-old female	Tracheal stenosis s/p intubation (trauma)	Marlex mesh patch with spiral rings covered by collagen sponge		Venous blood	FGF	Patent airway	6 mos	70
	39-year-old female	Tracheal stenosis s/p intubation (asthma)	Marlex mesh patch with spiral rings covered by collagen sponge		Venous blood	FGF	Patent airway	6 mos	70

	Authors	Patient	Indication	Scaffold		Cell Source	Modulator	Outcome	Follow-Up	Reference
		45-year-old male	Tracheal stenosis s/p intubation (burn)	Marlex mesh patch with spiral rings covered by collagen sponge		Venous blood	FGF	Patent airway	6 mos	70
2010	Delaere et al	55-year-old female	Tracheal stenosis falling tracheostomy	Tracheal allograft into forearm, free flap		None (buccal mucosa used as patch)	None	Patent airway (by CT)	1 y	73
2011	Jungebluth et al	36-year-old male	Tracheal and bronchial cancer	POSS-PCU		Peripheral mononuclear cells	TGF-B3, G-CSF, hrEPO	Fungal overgrowth, granulation on bronch (2 m), asymptomatic (5 m)	5 mos	9, 72
2011	Unpublished (Macchiarini)	30-year-old male	Tracheal cancer	Unpublished		Unpublished	Unpublished	Deceased	Unpublished	74–76
2012	Elliott et al	12-year-old male	Congenital tracheal stenosis	Decellularized cadaveric trachea		BM-MNC	hrEPO, G-CSF, TGF-B3	Malacia (6 wks) requiring stenting, granulation, and retained secretions	42 mos	6, 7
2013	Unpublished (Macchiarini)	2-year-old female	Tracheal agenesis	Unpublished		Unpublished	Unpublished	Deceased	Unpublished	77, 78
2014	Berg et al	76-year-old male	Tracheal stenosis s/p trauma and tracheostomy	Decellularized cadaveric trachea		Inferior turbinate chondrocytes, BM-MNC	None	Fungal overgrowth, deceased of MI, patent graft at autopsy	23 d	16

Authors	Patient	Indication	Scaffold	Cell Source	Modulator	Outcome	Follow-Up	Reference
2015 Steinke et al	26-year-old male	Long-segment tracheoesophageal defect secondary to caustic ingestion	Decellularized porcine jejunum (used as patch)	Microvascular endothelial and skeletal muscle cells	None	Complete incorporation into surrounding tissue (2.5 y)	2.5 y	71

Abbreviations: BM-MNC, bone marrow-derived mononuclear cells; FGF, fibroblast growth factor; TGF- β 3, transforming growth factor beta 3; rPTHrP, recombinant parathyroid hormone related peptide; Dex, dexamethasone; POSS-PCU, polyhedral oligomeric silsesquioxane poly-[carbonate-urea] urethane; hrEPO, human recombinant erythropoietin; MI, myocardial infarction

^aAll published reports of clinically applied TETGs from both the academic literature and the lay press emphasize the need for thorough documentation and analysis of TETG outcomes and highlight the challenges in engineering full segment tracheal grafts. Initial clinical experience with the TETG should inform the next generation of preclinical investigations to optimize and validate the use of TETG.