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Review

Tracheal Tissue Engineering: Advances and Challenges

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Abstract

Traumatic tracheal injuries and congenital defects can be life threatening. Regenerating the trachea through tissue engineered scaffolds has emerged as an innovative alternative to traditional therapies. At present time, challenges in tracheal regeneration preclude clinical adoption, such as revascularization and promotion of favorable paracrine and immune signaling responses. This review summarizes current advances in tracheal regeneration and highlights key biological and engineering barriers to address to achieve functional tracheal regeneration.

Keywords: tracheal tissue enginneering; tracheal regeneration; advancements; barriers

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1. Introduction

Traumatic tracheal injuries and congenital airway defects in adult and pediatric populations are difficult to manage [1]. Most tracheal injuries are iatrogenic, although they may also result from blunt or penetrating trauma [2]. The true incidence is likely underestimated because many patients succumb before reaching definitive care [2,3]. Other tracheal abnormalities are caused by congenital defects, including severe laryngotracheal clefts (type IV). These cases are rare and fatal without intervention. The incidence of laryngotracheal clefts is between 1:10,000 and 1:20,000 live births [4]. In addition, extensive tracheal defects that arise following oncological resection present a significant challenge, as current surgical techniques cannot reliably reconstruct defects exceeding 40-50% of the tracheal length in adults and 30% in children [5]. These limitations highlight the urgent need for regenerative strategies using tissue engineered scaffolds capable of restoring functional tracheal tissue.

In 20% of cases, when surgical repair for tracheal defects is possible, it is complicated by granulation tissue formation, tracheal stenosis, anastomotic separation and leak, tracheoesophageal fistula (TEF), or tracheoinnominate fistula formation [6]. Additionally, almost 4.1% of these patients develop stenosis at the anastomotic site, requiring multiple additional procedures such as balloon dilation and reoperation [7]. Long-term comorbidities include aberrant airway anatomy, recurrent TEF, impaired mucociliary clearance leading to recurrent pulmonary infections, and tracheomalacia [8,9]. These complications significantly impair quality of life, causing psychological stress for patients and families while also increasing healthcare costs. This underscores the need for replacement strategies that can reduce complications, improve outcomes, and promote physiological tissue healing and regeneration. Early clinical attempts at tracheal replacement highlight significant challenges, including graft ischemia and failure, infection, and lack of epithelialization, thereby emphasizing the need for improved regenerative approaches [8]. This review will focus on advances in tracheal tissue engineering and the persistent challenges that need to be overcome to form functional tracheal tissue.

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2. Tracheal Embryogenesis, Anatomy and Regeneration: A Brief Overview

Understanding the embryology and anatomy of the trachea is essential to help guide optimal tracheal engineering techniques. The trachea is a crucial conduit that allows for the movement of air into the lungs, while protecting it from environmental hazards, and regulating humidity and temperature [10]. During embryogenesis, the trachea and esophagus form from a common foregut tube during the first trimester [11]. The trachea then separates from the esophagus to become its own structure [11]. The ventral foregut endoderm forms a diverticulum, or outpouching, that develops into the trachea [12]. The dorsal aspect of the foregut mostly contains mesenchymal cells which have been seen to play a role in the separation of the trachea and esophagus [12].

Anatomically, the trachea begins inferior to the larynx and bifurcates into the right and left mainstem bronchi within the thoracic cavity [10,13]. It is composed of 16-20 hyaline cartilage rings on the anterior and lateral walls with smooth muscle posteriorly, giving the trachea its characteristic U-Shape [10]. Fibroelastic connective tissue unites the tracheal rings and forms the layer in-between each ring that is continuous with the perichondrium [14]. Surrounding the trachea is a loose fibroconnective areolar tissue, which contains a rich network of blood vessels and nerves (**Figure 1**) [14].

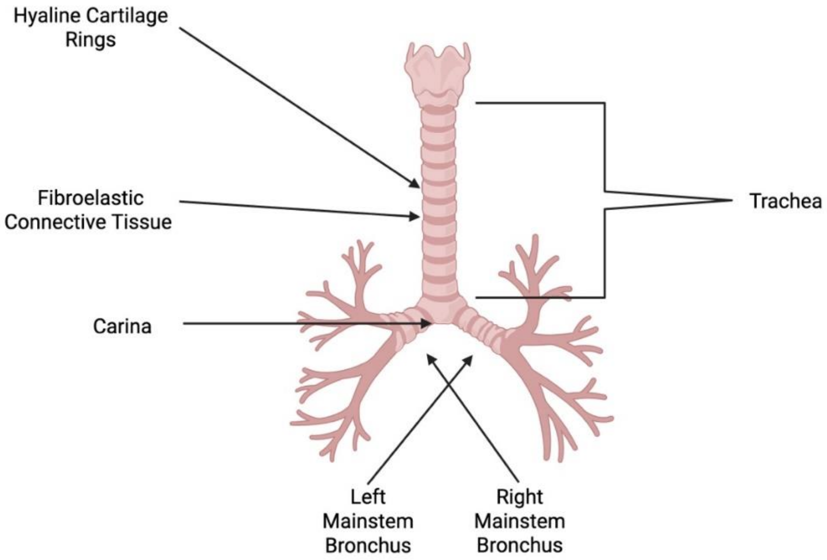

Figure 1. Tracheal Anatomy with key anatomical structures highlighted. Created in BioRender. Kosciuszek, N. (2026) https://BioRender.com/s2xo1f8.

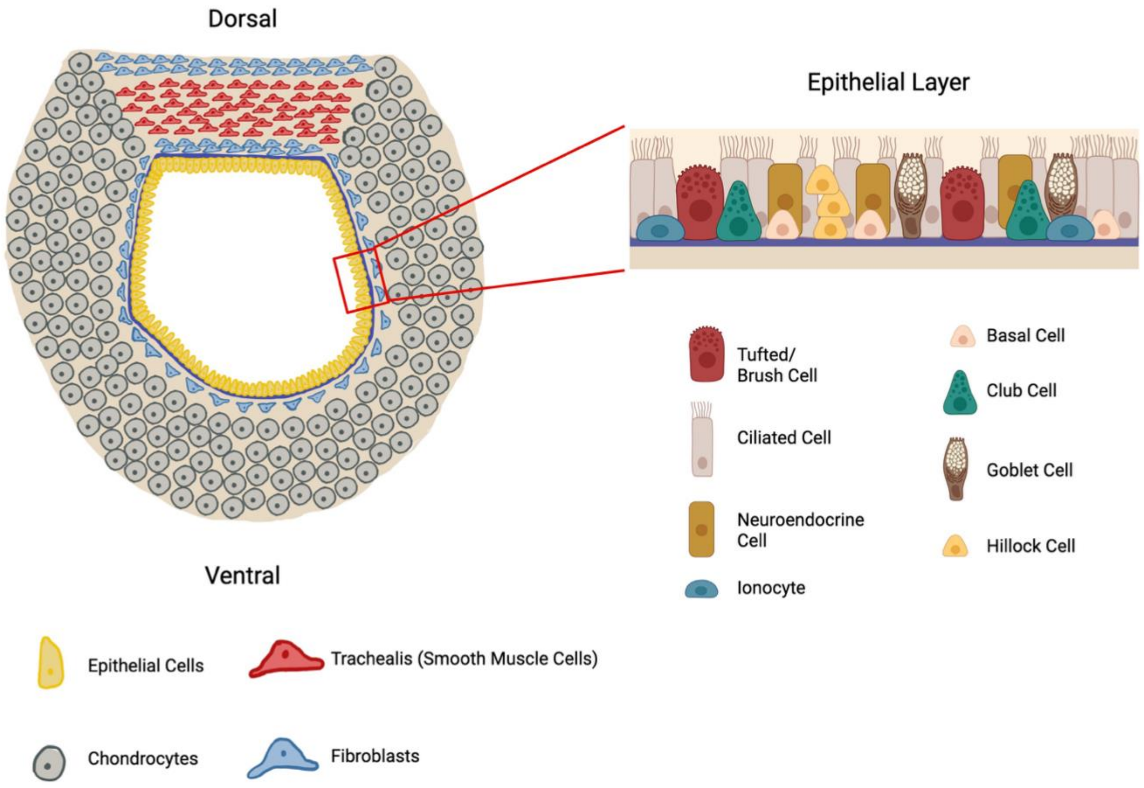

Figure 2. Cross section depiction of trachea highlighting the intricate layers of chondrocytes, fibroblasts, smooth muscle cells, and endothelial cells. Zoomed in panel represents a close up to the cells that are present in the endothelial layer. Created in BioRender. Kosciuszek, N. (2026) https://BioRender.com/7g7t7sd.

The inner lining of the trachea, specifically the epithelial layer, plays an important role in mucus production, immunity, and mucociliary clearance [10]. The epithelial layer is composed of numerous cells, specifically ciliated, basal, goblet, club, neuroendocrine, tuft, and hillock cells [15]. Surrounding the epithelial layer is a basement membrane, followed by smooth muscle (posteriorly), fibroblasts, and cartilage composed of chondrocytes (anteriorly) (**Figure 2**) [15]. These cellular components and their precise organization highlight the complex anatomy and infrastructure of the trachea, underscoring the challenges of regenerating a new trachea and the difficulty in designing scaffolds that replicate native tracheal structure and function. In addition, the trachea receives its blood supply from a large network of arteries originating from surrounding structures, illustrating the complexity of the trachea and the challenge of developing a robust vascular network to supply an engineered scaffold. [10].

In addition to its anatomic complexity, the trachea must withstand significant mechanical forces including cyclic compression and expansion, airflow shear stress, coughing pressures, and cervical motion in a variety of directions. The trachea maintains an intricate level of firmness while upholding its ability to be pliable. One group of researchers determined the degree of stiffness of tracheal cartilage depends on age with a stiffer trachea noted in older patients (20.5 pascals) compared to younger patients (12.2 pascals) [16]. Any engineered scaffold must maintain structural integrity and withstand external forces while preserving airway patency under these dynamic conditions.

When considering tracheal regeneration for diverse conditions ranging from congenital to acquired etiologies, it is important to understand the anatomical and functional differences in children and adults [17]. In children, the trachea is shorter, narrower, and posteriorly angled compared to adults [17]. In addition, a child’s trachea has more tracheal rings compared to adults (newborns:10 rings, adolescents: 8 rings, and adults: 6 or less from the sternal notch) [17]. The growth pattern of the trachea during development is highly debated with speculations ranging from linear growth patterns versus a polynomial growth pattern [17–19]. What is evident is that the trachea is not a "one-size fits all" organ, which further complicates the process required for regenerative engineering. For pediatric populations, scaffold development needs to account for increased growth over time so that engineered tissue will develop with the individual throughout adulthood, mitigating the need for additional surgical intervention.

3. Main Barriers to Regeneration

Successful tracheal regeneration requires overcoming several major challenges including restoration of epithelial function, vascularization of engineered constructs, immune compatibility, and mechanical stability (**Figure 3 and Table 1**). Each of these barriers must be addressed simultaneously and systematically to achieve durable airway repair and integration.

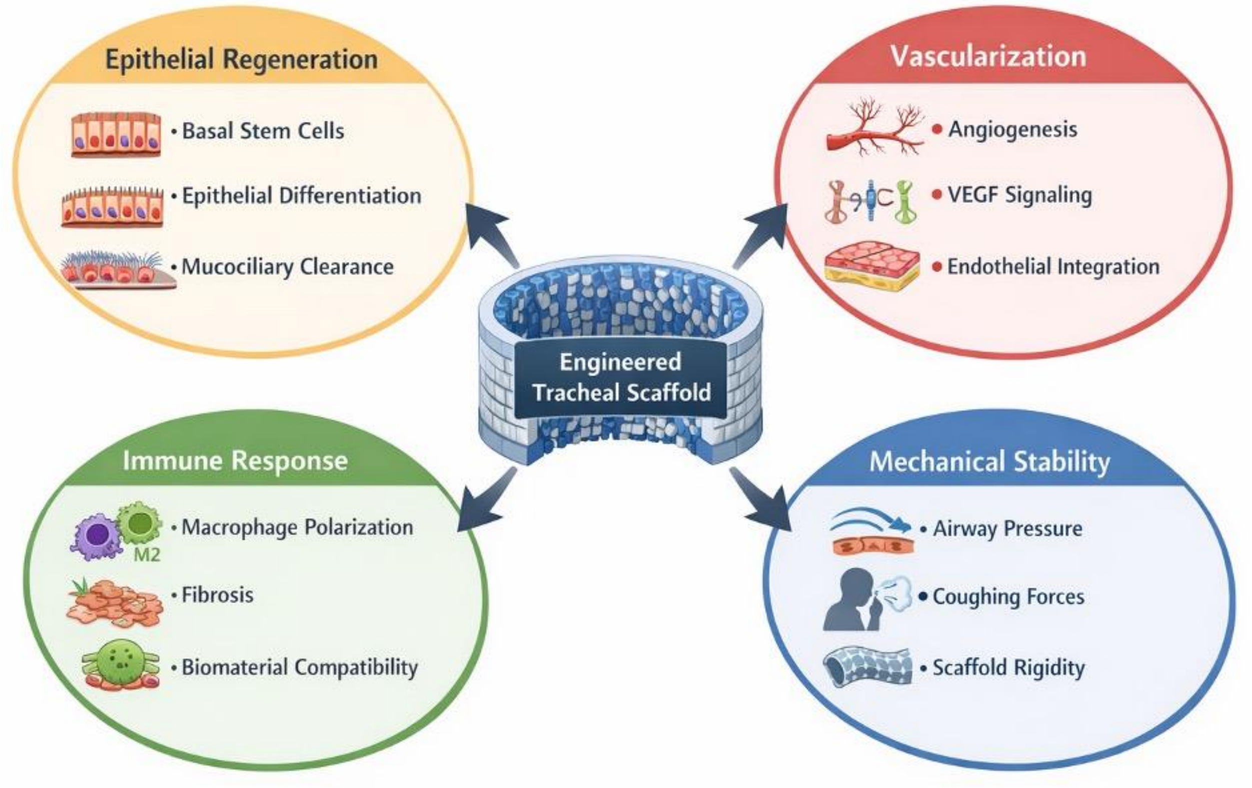

Figure 3. Overview of the key biological and engineering challenges that must be addressed to achieve successful tracheal tissue regeneration, including epithelial regeneration, vascularization, immune compatibility, and mechanical stability. Figure created with assistance of ChatGPT for design.

Table 1. Major biological and engineering challenges associated with tracheal regeneration.

Challenge	Biological / Engineering Issue	Current Strategies
Vascularization	Poor blood supply leads to scaffold ischemia and graft failure	Growth factor delivery (VEGF), pre-vascularized scaffolds, in vivo bioreactors [20]
Mechanical Stability	Airway must withstand airflow pressure, coughing, and neck motion	Synthetic polymers, reinforced scaffolds [21]
Immune Response	Foreign body reactions and fibrosis can cause scaffold rejection	Immunomodulatory biomaterials, cytokine delivery [22]

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Epithelial Regeneration	Functional airway epithelium required for mucociliary clearance	Stem cell seeding, epithelial progenitor cells [23]
Tracheal Growth	Airway growth requires adaptable scaffolds	Biodegradable and growth-permitting biomaterials [22]

4. Leveraging Innate Repair Mechanisms in the Trachea

The trachea has innate repair mechanisms that can be leveraged for tissue engineering purposes. The lateral tracheal vascular pedicle is a robust network that can provide nutrients during times of stress and injury. Preservation of this network would be critical for any engineered construct [14].

In general, the tracheal epithelium has a low turnover rate; however, when there is damage, quiescent progenitor cells, such as the hillock and basal stem cells, activate and rapidly undergo clonal expansion [24,25]. The hillock cells protect the basal stem cells during injury and inflammation, allowing the basal stem cells to proliferate and differentiate [25]. These basal cells can generate all six types of normal pseudostratified epithelial cells (i.e., mucociliary and secretory cells), which could be helpful for promoting appropriate tissue function and regeneration [25,26]. This regenerative potential makes basal cells particularly attractive tools for scaffold-based tissue engineering strategies. **Table 2** describes the pseudostratified epithelial cells and their function. For example, these cells could be isolated from a patient's airway, cultured and expanded in vitro, and used to seed a tissue engineered trachea. Butler et al. was able to culture human respiratory epithelial cells from endobronchial biopsies and demonstrated preserved mucociliary function [27]. This highlights the possibility of restoring mucociliary function when designing scaffolds.

Table 2. List of Pseudostratified Epithelial Cells Derived from Hillock and Basal Progenitor Cells.

Cell Name	**Cell Function**
Ciliated (Brush)	Mucociliary clearance – the cilia (hair-like projections) that beat in a wave-like movement to move mucus and debris out of the airway [28]
Goblet	Production of mucus that traps debris such as dust and pathogens from further entering the respiratory tract [29]
Basal	Multipotent stem cell – responsible for regenerating and repairing the epithelium by differentiating into ciliated and goblet cells [30]
Club (Clara)	Secretes proteins that reduce inflammation (uteroglobin) and regulate immune properties while having the capability to de-differentiate when basal cells are injured [31]
Pulmonary Neuroendocrine (PNECS)	Sensory and paracrine signaling that regulate airway tone, mucociliary clearance, and hyperresponsiveness to pathogens and airway irritants [32]

Tuft	Regulates the mucociliary clearance in response to chemical irritants that enter the respiratory tract [33]

Additionally, when the tracheal epithelium is compromised, the innate immune system works to repair the epithelium leveraging signaling pathways such as Wnt/β-catenin, Notch, and TGF-β to promote repair, growth, and healing [34]. As highlighted in **Table 2**, basal cells are a part of the tracheal epithelium that undergo differentiation to fit the tracheal environment [35]. These basal cells can be observed to proliferate and differentiate into goblet cells via the Notch pathway during times of stress and damage [34].

5. Cell Types Used in Tracheal Tissue Engineering and Regeneration

As previously mentioned, mesenchymal stem cells (MSCs) play an important role in the development of the trachea. Additionally, these cells are easy to harvest from various tissues (bone marrow, adipose, placenta, and umbilical cord), have anti-inflammatory properties, immune modulating capability, and multi-potency that can create a pro-healing environment [36]. Given this, many researchers utilize MSCs in their scaffold design for tracheal regeneration [37]. Shin et al. cultured MSCs on a porcine cartilage-based tracheal scaffold that was then implanted into defects in the trachea of rabbits and promoted the development of intact respiratory epithelium and patent luminal contour at the site of transplantation [38]. Other researchers cultured MSCs on a silk fibroin-hydroxyapatite scaffold and found that the scaffold was biocompatible and promoted MSC adhesion, growth, and proliferation, therefore showing promise as a possible scaffold for tracheal regeneration [39].

In addition to MSCs, researchers have utilized induced pluripotent stem cells (iPSCs) derived from fetal or adult somatic cells, which can be virally reprogrammed and differentiated using specific growth factors to the cell type of interest [40]. These cells are beneficial as they have unlimited proliferative capacity, can be patient-derived for autologous administration and help mitigate the ethical concerns of using embryonic stem cells (reference). One group incorporated iPSCs into a collagen sponge scaffold that was implanted into rats with tracheal defects [23]. Six of the eleven rats developed cartilage-like tissue within the tracheal defect, while the other five did not [23]. Another study utilized iPSCs to generate tracheal epithelium by differentiating iPSCs into ciliated epithelial cells over 30 days at air liquid interface culture and then incorporated these cells into a collagen scaffold [41]. This scaffold was then implanted into rats with a surgically created tracheal defect [41]. After seven days, functional ciliated epithelial cells were noted on the luminal side of the regenerated tissue [41].

Lastly, researchers have been incorporating primary cell lines such as chondrocytes into scaffolds to repair tracheal defects. Nomoto et al created a polypropylene tracheal prosthesis where autologous chondrocytes were harvested from rabbits' costal cartilage [42]. The bioengineered trachea seeded with autologous chondrocytes was then implanted into the tracheas of rabbits [42]. Fourteen weeks following implantation, regenerated cartilaginous tissue in the bioengineered trachea with maintenance of tracheal shape and structure was observed [42].

These examples of scaffolds demonstrate the benefit of using stem cells and primary cells to encourage appropriate tracheal tissue regeneration in tissue engineered constructs. Based on preliminary pre-clinical studies, MSCs provide immunomodulatory and anti-inflammatory benefits, whereas iPSCs offer greater differentiation potential but raise concerns regarding tumorigenicity and clinical safety. Next steps will be to determine which cell types or combination of cell types should be used to regenerate a functional trachea. Additionally, understanding the regenerative signaling pathways that regulate epithelial repair, cellular response, and tissue remodeling will be essential to model appropriate tracheal function and regeneration. As previously mentioned, pathways such as Wnt/β-catenin, Notch, and TGF-β play central roles in airway epithelial differentiation and stem cell activation. Therefore, understanding the pathways that influence tracheal cell behavior and identity will be critical for directing functional tracheal regeneration. **Table 3** summarizes commonly studied cell types used in tracheal tissue engineering along with their advantages and limitations.

Table 3. Cell types used in tracheal tissue engineering, along with their advantages and limitations.

Cell Type	Advantages	Limitations	Representative Study
Mesenchymal Stem Cells (MSCs)	Immunomodulatory, anti-inflammatory, multipotent, easy to isolate	Limited differentiation toward airway epithelium in some models	Shin et al. [38]
Induced Pluripotent Stem Cells (iPSCs)	High differentiation potential, can generate airway epithelial cells	Tumorigenicity concerns and complex differentiation protocols	Ikeda et al. [41]
Autologous Chondrocytes	Promote cartilage regeneration and structural support	Limited proliferation capacity and donor tissue requirements	Nomoto et al. [42]
Airway Basal Stem Cells	Native airway progenitors capable of regenerating epithelium	Difficult isolation and expansion	Lin et al. [25]
Endothelial Cells	Promote vascularization of scaffolds	Require supportive microenvironment for stability	Khalid et al. [43]

6. Biomaterials Used in Designing Scaffolds for Tracheal Tissue Engineering and Regeneration

Scaffolds are a three-dimensional template that provides a structural, mechanical, and biological construct that supports cell infiltration, growth, and proliferation. Scaffolds composed of various biomaterials have demonstrated promise in trachea regeneration and tissue engineering. However, the challenge with scaffolds is designing one with the optimal material that is biocompatible with humans, supports cell proliferation, is non-immunogenic, and has mechanical properties that support tracheal functions [21,44].

Materials used for bioengineering the trachea can be classified as natural, synthetic, or hybrid. Natural biomaterials, such as alginate, chitosan, collagen, fibrin, gelatin, hyaluronic acid, and soy protein [21,45–51], support cell adhesion, proliferation, and tissue regeneration in vitro and in vivo due to their similarity to native extracellular matrix [21]. For example, collagen–hyaluronic acid scaffolds have been shown to promote vascular development within engineered tracheal tissue [21,45]. When seeded with human umbilical vein endothelial cells and MSCs, these scaffolds demonstrated enhanced vascularization in a chick chorioallantoic membrane model [21,43]. While this study did not implant these scaffolds in vivo, it illustrates of the ability of collagen and hyaluronic acid to promote vascularization. These results are particularly important because insufficient vascularization remains one of the primary limitations in tracheal regeneration [21,52]. Natural biomaterials have limited mechanical strength, however, making them vulnerable to deformation under the mechanical stresses experienced within the airway [21]. One way to circumvent this limitation is through the use of stronger synthetic materials.

Synthetic materials enable the engineered tracheal device to withstand the physical stresses required to retain proper function. In addition, it is easier to standardize the formulation and synthesis of these materials, making their production scalable and reproducible [21]. Examples of synthetic materials include polylactic acid (PLA), polycaprolactone (PCL), polycarbonate (PC), thermoplastic polyurethane (TPU), polyglycolic acid (PGA), poly(lactic-co-glycolic) acid (PLGA), polyphosphoesters (PPE), polyethylene terephthalate (PET), and high-density polyethylene (HDPE) [21,53,54]. Materials such as PLA, PCL, aliphatic PC are biodegradable, whereas aromatic PC and TPU are not [21,22,53,54]. Modifying different chemical groups and properties of these materials allow for tunable biodegradation rates, which is advantageous for controlling tissue regeneration and healing. While there are significant advantages for the use of synthetic materials to encourage tracheal tissue regeneration, one drawback is limited biocompatibility which can cause an unwanted immune response and possible rejection [21].

In an effort to optimize the strengths of natural and synthetic materials, researchers are developing hybrid scaffolds. One study described a hybrid scaffold composed of PLGA knitted mesh with a collagen sponge impregnated with a gelatin hydrogel containing growth factor, basic fibroblast growth factor (bFGF), and implanted this into a rabbit tracheal defect model [55]. Six months following implantation, the bFGF hydrogel impregnated scaffold promoted regeneration of cartilage with growth and epithelization in-between the hosts' cartilage stumps [55]. Reportedly, the scaffold maintained its rigid strength, preventing tracheal collapse during healing [55]. While hybrid scaffolds provide improved mechanical stability and biocompatibility, long-term epithelial regeneration and integration remain inconsistent across experimental models. As previously mentioned, the challenge in developing a hybrid scaffold with synthetic and natural materials is incorporating materials that work together while minimizing host reaction. **Table 4** summarizes commonly used biomaterials in tracheal tissue engineering along with their advantages, limitations, and representative studies.

Table 4. Summary of Biomaterials Used in Tracheal Tissue Engineering and Their Advantages and Limitations.

Material	Type	Strengths	Limitations	Representative Study
Alginate	Natural	Biocompatible, supports cell adhesion and hydrogel formation	Weak mechanical strength, rapid degradation	Luo et al. [49]
Chitosan	Natural	Antimicrobial properties, promotes cell attachment	Limited mechanical stability	Nematollahi et al. [47]
Collagen	Natural	Mimics extracellular matrix, excellent cell compatibility	Rapid degradation and poor structural strength	Xu et al. [46]
Fibrin	Natural	Supports cell infiltration and angiogenesis	Weak mechanical properties, fast degradation	Dai et al. [48]
Gelatin	Natural	Promotes cell adhesion and proliferation	Low mechanical stability	Fares et al. [51]

Hyaluronic Acid	Natural	Supports cartilage regeneration and ECM signaling	Limited structural strength	Xu et al. [46]
Soy Protein	Natural	Biodegradable and supportive for cell growth	Limited studies in airway applications	Naik et al. [21]
HDPE	Synthetic	High mechanical strength and durability	Poor biodegradability and limited bioactivity	Naik et al. [21]
PLA	Synthetic	Biodegradable, tunable degradation rate	Can produce acidic degradation byproducts	DeStefano et al. [22]
PCL	Synthetic	Excellent mechanical strength, slow degradation	Hydrophobic surface limits cell attachment	Gandha et al. [53]
PC	Synthetic	Strong structural support	Limited biodegradability	Artham et al. [54]
PET	Synthetic	High durability and stability	Limited biocompatibility in regenerative applications	Naik et al. [21]
PGA	Synthetic	Biodegradable with good mechanical strength	Rapid degradation may compromise structural support	Naik et al. [21]
PLGA	Synthetic	Tunable degradation and widely used in tissue engineering	Degradation may produce acidic environment	Tatekawa et al. [55]
PPE	Synthetic	Tunable degradation and polymer properties	Limited long-term airway studies	Naik et al. [21]
TPU	Synthetic	Elastic and mechanically durable	Poor bioactivity for cell attachment	Naik et al. [21]

Abbreviations: HDPE - high-density polyethylene; PLA - polylactic acid; PCL – polycaprolactone; PC – polycarbonate; PET - polyethylene terephthalate; PGA - polyglycolic acid; PLGA - poly(lactic-co-glycolic) acid; PPE – polyphosphoesters; TPU - thermoplastic polyurethane

7. Specific Challenges with Engineering Tracheal Tissue

Scaffolds lack biochemical cues, such as of the presence of growth factors, cytokines, and extracellular matrix proteins, that are required for scaffold regeneration [20]. To overcome this, one group investigated the incorporation of extracellular matrix (ECM) to enable regeneration. ECM has been shown to play a crucial role in cell proliferation, adhesion, and viability [56]. In addition, the utilization of ECM with in vivo models has been shown to promote epithelization and prevent chronic inflammatory reactions that leads to secondary stenosis following tracheal repair [57–59]. One group took synthetic tracheal scaffolds and soaked them in lyophilized porcine ECM (pECM) or human ECM (hECM) and then seeded the scaffold with lung epithelial cells from a human lung [20].

The scaffolds coated with pECM or hECM had a greater proportion of living cells and increased cell density at 24 hours and 7 days [20]. In addition, cell proliferation was enhanced by 24 hours in the scaffolds that were coated with pECM and hECM compared to standard cell well plates and uncoated scaffolds [20]. The presence of extracellular matrix proteins helped with cell differentiation and growth by providing important ligands and proteins that are typically present in native tissue. Therefore, this study highlights a developing area of tissue engineering research where the optimization of scaffold conditions promotes favorable environments for cells to grow.

Tracheal epithelial regeneration remains challenging as many factors, such as extracellular proteins, signaling molecules and inflammation, play a role in differentiation of the basal cells. These cells are sensitive to stresses and quickly adapt. One strategy for promoting epithelial regeneration involves modulation of the oxidative stress environment. Chen et al. evaluated hydrogels composed of chitin, tannic acid, aspartic acid, and L-arginine (LA@PA-TA@C) for the repair of damaged tracheal epithelium. After four weeks, the regenerated epithelium closely resembled native airway tissue, with the presence of ciliated, goblet, and basal cells. The authors attributed these outcomes partly to the antioxidant properties of tannic acid, which reduced oxidative stress and cellular apoptosis [34]. By limiting oxidative injury, basal cells were able to survive, proliferate, and differentiate into functional epithelial cells. This study illustrates how scaffold composition can influence cellular signaling pathways and promote epithelial regeneration.

Immune response also plays a critical role in the success of implanted tracheal scaffolds as inflammatory reactions can impair tissue integration and regeneration [60]. One of the most important regulators of this response is the macrophage [60,61]. Macrophages exhibit distinct functional phenotypes that influence scaffold outcomes. Pro-inflammatory M1 macrophages promote cytokine release, fibrosis, and graft failure, whereas pro-regenerative/anti-inflammatory M2 macrophages support tissue remodeling and scaffold integration. Excessive M1 macrophage activation can lead to extracellular matrix deposition and tracheal narrowing, ultimately compromising airway patency. For this reason, researchers are actively exploring strategies to modulate macrophage polarization toward the M2 phenotype [60,61]. For example, delivering immunomodulary molecules, such as interleukin-4 (IL-4) or interleukin-13 (IL-13), to promote M2 macrophage polarization that will elicit a pro-regenerative environment, allowing for tissue repair and scaffold integration within the native host environment [62]. Reduced systemic side effects and long-term scaffold viability were observed when IL-4 and IL-13 were slowly released from nanoparticles embedded within a scaffold [62]. This demonstrates a potential strategy for circumventing M1 macrophage polarization and reducing fibrosis, stenosis, and scaffold rejection. In addition to macrophage polarization, the host response to implanted scaffolds involves foreign body reactions, cytokine signaling cascades, and fibrotic remodeling. Excessive fibrosis can lead to airway narrowing and impaired scaffold integration, highlighting the importance of designing biomaterials that minimize chronic inflammation.

The type of material used in the scaffold plays an integral role in the degree of immune response it generates. It has been noted that decellularized scaffolds may contain residual immunogenic material leading to fibrosis [61]. On the other hand, fully synthetic scaffolds have low bioactivity and mount less of an immune response [61]. However, this can lead to persistent and chronic inflammation that ultimately causes rejection of the scaffold [61]. Therefore, designing a scaffold with limited immunogenicity remains a challenge.

Lastly, designing a scaffold that has a durable blood supply for the graft and cells is critical and, as previously stated, remains one of the most significant barriers in tracheal tissue engineering. Without rapid angiogenesis, implanted scaffolds are prone to ischemia, impaired epithelial regeneration, and eventual graft failure. Currently, pre-vascularized scaffolds, growth delivery systems, and in-vivo bioreactor techniques have been examined [20]. Growth factor delivery systems that deliver factors, such as vascular endothelial growth factors, have been shown to enhance endothelial cell proliferation and promote angiogenesis in tracheal scaffolds [20]. Although promising, one setback of growth factor delivery systems is the need to have controlled release of factors, otherwise aberrant vascular growth occurs [20]. Pre-vascularized scaffolds allow for early integration of the scaffold and can reduce ischemia of the scaffold and cells, but these scaffolds can be difficult to create [20]. Finally, the use of in-vivo bioreactors allows researchers to implant an engineered scaffold into a vascular rich region of the body, such as the omentum, to promote natural development of blood vessels prior to implanting into the trachea [16]. This approach enhances endothelialization and scaffold maturation [20], thereby improving long-term tissue viability with a reduction in ischemic complications [20]. While promising, this novel and relatively new approach require multiple surgical procedures that can be time consuming and costly [20].

8. The Future of Tracheal Regeneration

Future engineered tracheal scaffolds will benefit from a multilayered design leveraging the strength of both synthetic and natural biomaterials, incorporation of key regenerative cells and extracellular matrix. The use of 3D bioprinting technology has immense potential given its ability to personalize the design, create multiple layers, and incorporate cells as well as extracellular matrix within the construct. Using this technology, different scaffold layers could be bioprinted. For example, one layer could incorporate natural biomaterials seeded with epithelial and endothelial cells optimized for epithelialization and vascular regeneration, while another layer could support cartilage formation and mechanical stability with chondrocyte seeded synthetic material. Such multifunctional constructs would allow researchers to address several barriers to tracheal regeneration within a single engineered scaffold.

9. Conclusion

This review highlights recent advances in tracheal tissue engineering while emphasizing the major challenges that remain. Although substantial progress has been made in identifying suitable biomaterials, cell sources, and immune-modulating strategies, major challenges remain in vascularization, mechanical stability, and enabling epithelial regeneration while minimizing immune-mediated fibrosis and rejection. Addressing these challenges will require multidisciplinary approaches that integrate biomaterial engineering, stem cell biology, and regenerative biochemical signaling modulation in order to develop a clinically viable method of restoring functional tracheal tissue.

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Abbreviations

The following abbreviations are used in this manuscript:

TEF	Tracheoesophageal Fistula
VEGF	Vascular Endothelial Growth Factors
TGF-β	Transforming growth factor-beta
MSC	Mesenchymal Stem Cell

iPSCs	Induced pluripotent stem cells
PLA	Polylactic Acid
PCL	Polycaprolactone
PC	Polycarbonate
TPU	Thermoplastic polyurethane
PGA	Polyglycolic acid
PLGA	Poly(lactic-co-glycolic) acid
PPE	Polyphosphoesters
PET	Polyethylene terephthalate
HDPE	High-density polyethylene
bFGF	Basic fibroblast growth factor
ECM	Extracellular matrix
hECM	Human extracellular matrix
pECM	Porcine extracellular matrix
IL-4	Interleukin-4
IL-13	Interleukin-13
3D	Three-dimension

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