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TRACHEAL TISSUE ENGINEERING

Learning Objectives

After completing this chapter, students should be able to:

1. Describe the structure of the trachea, including vascularization and innervation.
2. Discuss congenital tracheal stenosis, including symptoms and classification schemes.
3. Describe the genetic regulation of tracheal development, including the role of various genes and changes in branching patterns.
4. Describe tracheal stenosis in cases of tracheal intubation and tracheostomy.
5. Discuss the use of balloon dilation, stents, and trachea resection and reanastomosis for the treatment of tracheal stenosis.
6. Describe design considerations for tracheal tissue engineering.
7. Describe the process of bioengineering 3D artificial tracheas.
8. Discuss tissue engineering models to support the fabrication of artificial tracheas.
9. Describe examples of artificial tracheas and clinical applications in adult and pediatric patients.

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CHAPTER OVERVIEW

In this chapter, we begin with a discussion of the structure and function of the trachea, including organization and distribution of various cell types. We also discuss the formation of tracheas during embryogenesis and the genetic signals that regulate tracheal development. We then discuss common tracheal disorders, including congenital tracheal stenosis and tracheal stenosis during tracheal intubation and tracheostomy. We then discuss some of the treatment modalities that are used in cases of tracheal disorders. We describe the use of balloon dilation and stents and the use of surgical reconstruction for the treatment of tracheal disorders. After providing this framework, we next move on to discuss the role of tissue engineering in the development of artificial tracheal tissue. We start with a discussion of design criteria for tracheal tissue engineering and then present a process flow chart identifying the steps required to fabricate 3D artificial tracheas. We then provide specific examples of artificial tracheas that have been developed in research laboratories, and we relate these examples to our design criteria and process flow chart. The field of tracheal tissue engineering has been one of the success stories in tissue engineering, with two examples of clinical applications for 3D artificial tracheas, one in adult patients and one in pediatric patients. We conclude our chapter by presenting this seminal work in the field of tracheal tissue engineering.

7.1 STRUCTURE AND FUNCTION OF THE TRACHEA

Introduction—The trachea, commonly referred to as the windpipe, is part of the respiratory system and serves as a conduit for air from the larynx to the bronchioles within the lungs (1–4). The trachea extends from the sixth cervical vertebra to the fifth thoracic vertebra at which point it bifurcates into the left and right bronchi, which feed into each of the two lungs. The trachea is a rigid cylindrical tube with a complex organization of epithelial cells, extracellular matrix, and cartilaginous support matrix. The length of the trachea in humans is 10–12 cm with a diameter in the range of 20–25 mm (5).

Ciliary Escalator—The organization of cells and the extracellular matrix in the trachea is very complex. The trachea consists of several cell types, including pseudostratified epithelial cells and goblet cells. The part of the trachea containing these two cell types is known as the ciliary escalator or the mucociliary escalator. The epithelial cells contain ciliary structures, which are hair-like protrusions from the surface of the cell. The goblet cells produce mucus, which consists of a protein known as mucin. The function of the mucus is to trap foreign bodies, including bacteria and other microbes, from the inhaled air and prevent the foreign bodies from entering into the respiratory system. Once these microbes are trapped, coordinated movement of the epithelial cells, including the ciliary escalator, functions to transport the foreign bodies to the pharynx where they can either be swallowed or expectorated. Thus, the ciliary escalator serves as a defense mechanism against

entry of foreign bodies to the respiratory system, with epithelial cells, ciliary structures, and mucus-producing goblet cells acting in tandem to achieve this function.

Lamina Propria—The next layer in the trachea is known as the lamina propria, which consists of many cell types, like macrophages and mast cells, and extracellular matrix components like elastin. The elastin provides flexibility to the trachea, thereby supporting the changes in lumen diameter during inhalation and exhalation of air. The macrophages remove any foreign matter that has entered the respiratory system by escaping the first level of defense provided by the mucus and the ciliary structures.

Submucosa—The next layer of tracheal tissue is known as the submucosa; it contains numerous mucous and serous glands. The mucous glands in the submucosal layer of the trachea produce mucus, which travels to the luminal surface of the tissue and combines with the mucus produced by the goblet cells. The serous glands contain serous cells, which are the primary defensive cells of the submucosal layer and secrete antimicrobials to target airborne pathogens.

Cartilaginous Rings—The next layer in the trachea is a thick layer of hyaline cartilage. The trachea consists of 16–20 rings of hyaline cartilage. The rings of the hyaline cartilage are designed to provide structural support and are known as tracheal rings. The hyaline cartilage in the tracheal rings allows the trachea to remain open in the absence of positive air pressure; in the absence of tracheal rings, the trachea would collapse during respiration, due to changes in air pressure and the force exerted by the surrounding tissue. These tracheal rings are macroscopic structures, are visible with the naked eye, and can also be felt by touching the neck. They measure about 1 mm in thickness and are separated by narrow distances, although they merge to form a continuous cartilaginous layer. The first and last tracheal rings have distinct characteristics, with the former being broader then the central tracheal rings and the latter being broad in the middle.

Adventitia—The outermost layer of the trachea is known as the adventitia; it consists of loose connective tissue which provides structural support and connects the trachea to the esophagus and other neighboring organs.

Smooth Muscle Tissue—The trachea also has smooth muscle tissue, which functions to regulate the diameter of the trachea based on oxygen demand. Smooth muscle relaxation results in an increase in tracheal diameter, thereby increasing airflow to the lungs to meet an increase in oxygen demand.

Vascularization—The vascularization structure of the trachea is very complex, as there is not a single major artery feeding the entire trachea structure (6,7). Rather, the vascular organization of the trachea has multiple major arteries feeding different parts of the tissue. The cervical portion of the trachea, which is the part closer to the larynx, receives its blood supply either from the inferior thyroid artery or from the subclavian artery. These major arteries branch into several smaller vessels known as the tracheoesophageal branches, which feed both the trachea and the esophagus. The blood supply to the thoracic trachea is fed by the bronchial arteries or the supreme intercostal artery. As in the case for the cervical trachea, these major arteries form tracheoesophageal branches that feed the trachea and the esophagus. The tracheoesophageal branches feeding the trachea are further divided into smaller

vessels known as the primary tracheal arteries. The primary tracheal arteries divide further to form intercartilaginous arteries, which feed directly to the tissue between the cartilage rings. The trachea also has an extensive capillary network feeding the mucosal and submucosal tissue. The cartilage rings do not have blood vessels directly feeding into the tissue, but instead depend on diffusion from the capillaries in the submucosal tissue.

Innervation—The trachea, and the bronchus which enters the lungs, are innervated by branches of the vagus nerve (8–11), which serves to regulate smooth muscle contraction and relaxation. Smooth muscle contraction and relaxation in turn regulate the airflow to the lungs. Smooth muscle relaxation occurs in response to stimulation by sympathetic division of the autonomic nervous system, resulting in an increase in airflow to the lungs. Smooth muscle contraction occurs in response to stimulation by the parasympathetic division of the autonomic nervous system, which in turn causes a decrease in diameter and reduced airflow to the lungs.

7.2 CONGENITAL TRACHEAL STENOSIS

Congenital tracheal stenosis (CTS) is a condition in which variable segments of the trachea are narrow, a condition which can prove to be fatal in neonates and infants (12–18). CTS can be caused by complete tracheal cartilage rings and thickening of the submucosal tissue, leading to narrowing in the internal diameter of the trachea. Under normal physiological conditions, the cartilage rings in the trachea are disk-shaped and have a specific configuration that allows airflow during respiratory function. However, in cases of CTS, the cartilage rings are circular in configuration and have a reduced diameter, thereby constricting the trachea and limiting airflow during respiratory function. Long segment CTS (LCTS) refers to cases of CTS in which greater than half the length of the trachea undergoes stenosis.

CTS is associated with other congenital disorders including those of the cardiovascular, pulmonary, and gastrointestinal system, and if left untreated, CTS can be fatal. Until about two decades ago, most cases of CTS were fatal due to obstruction of the respiratory tract. However, with recent advances in medical technology, the survival rate of patients with CTS has significantly improved. While variable between hospitals, the survival rate has been reported to be in the range of 78–92% (12).

CTS is considered to be a rare disease by the Office of Rare Diseases Research at the National Institute of Health, which defines a rare disease as any disease having a prevalence of less than 200,000 in the entire US population. While the prevalence of CTS is not very high in the US population, the complications that arise due to obstruction of the airflow track can be fatal; therefore, treatment and management of CTS has received significant attention in the recent literature.

Classification—There are three classifications of CTS based on anatomical characteristics of the cartilage rings. This system has given rise to type I, type II, and type III CTS (15). In type I CTS, the entire trachea is affected, and there is fairly uniform stenosis throughout the length of the trachea. The stenosis in

the airflow tract is uniform and is caused by deformations in all or almost all of the cartilage rings. In type II CTS, there is a tapering pattern associated with the trachea with an increase in stenosis along the length of the trachea. Terms like funneling and tapering have been used to describe this condition. In this condition, the upper parts of the trachea are not affected and the cartilage rings at the upper part of the trachea are normal. However, the cartilage rings closer to the ends of the trachea are smaller in size, leading to a tapering in the diameter of the airflow track. In type III CTS, there is no regular pattern for the tracheal cartilage rings, and only a few regions of the trachea are affected. In this case, only a small portion of the respiratory tract is affected.

Symptoms and Diagnosis—The symptoms of CTS can manifest within the first few weeks after birth or can take several months (15). Some symptoms include problems with breathing or wheezing, chest congestion, and pneumonia (15). Another symptom of CTS is stridor, which refers to noisy breathing resulting from turbulent airflow in the narrowed trachea. CTS can be diagnosed by imaging techniques like x-rays, CT scans, or MRIs and may also require microlaryngoscopy and bronchoscopy (MLB). MLB is a direct visualization technique in which an endoscope is placed within the larynx (microlaryngoscopy) and/or bronchi (bronchoscopy), through the mouth to visualize the airway. Photographs and videos allow direct visualization of the larynx, trachea, and the bronchi; image processing and analysis tools can be used to extract valuable information about airway stenosis from the endoscope.

CTS and Development of the Trachea—During the third to fourth week of development, the respiratory primordium dilates and undergoes bifurcation, leading to the initial stages of tracheal development (19). By week 8 of development, early rudiments of tracheal cartilage can be found; these develop to form mature cartilage rings by week 10, including the addition of smooth muscle tissue to the trachea. It is believed that there are two stages of abnormalities during tracheal development that can give rise to CTS (19). Any deficiencies in tracheal development during the 4-week time period will affect formation of the entire respiratory system and can lead to a severe form of CTS with associated disorders of the respiratory system (19). However, if abnormalities in tracheal development occur between the 8- and 10-week time period, it is believed that the process of cartilage formation will be affected, leading to deficiencies in the formation and organization of cartilage rings in the trachea.

7.3 GENETIC REGULATION OF TRACHEAL DEVELOPMENT

Tracheal development is a very complex process that is regulated by changes in the expression of specific genes at specific time points during the developmental path (20,21). The drosophila has been used extensively to dissect the genetic pathways that regulate tracheal formation. In the drosophila, the trachea is used to deliver oxygen to all tissues; it consists of a single monolayer of epithelial cells without the complex organizational structure of mammalian tracheas. The process of tracheal

development begins with the commitment of ectodermal stem cells to a tracheal lineage, followed by reorganization of these cells to form clusters or sacs around the peripheral end of the embryos. This is followed by migration of these cells to the central region of the embryo, followed by invagination to form tracheal placodes, which are the earliest semblance of a cylindrical structure with a luminal surface; these placodes can be viewed as a very early precursor to a trachea. The committed tracheal cells in the placodes then initiate a complex pattern of branching, starting with the formation of primary and secondary branches and ending with the formation of terminal branches. Tracheal development is regulated by changes in the expression of several genes, some of which include *trachealess, branchless, breathless, pantip-2, and blistered* while also involving the participation of the EGF and FGF signaling pathways (20,21).

Formation of Tracheal Placodes—Tracheal cells are formed by differentiation of ectodermal cells to generate clusters or sacs of cells, known as tracheal placodes, which are lined up on either side of the embryo. On average, there are 10 tracheal placodes that are formed on either side of the embryo, and each one contains about 80 cells. The differentiation of ectodermal cells to tracheal cells is regulated by the expression of the trachealess gene, which converts the planar ectodermal cells to sacs of tracheal cells (22–27). Mutations in the trachealess gene have inhibited the formation of tubes in drosophila and therefore have prevented the formation of the trachea (28). One very interesting fact about tracheal development in drosophila is that the total number of cells remains constant throughout the process. Although there are significant changes in cell distribution and organization, there is no accompanying cell proliferation. The formation of branching networks to support tracheal development is a result of cell migration and reorganization.

Invagination of Tracheal Placodes—Once sac formation is complete, the next step in the process is migration of tracheal placodes to the center of the embryo and invagination of the tracheal placodes, which refers to reorganization of the tracheal cells within the sacs to form a hollow structure. The cells within the tracheal placodes are still retained within this structure, although they organize to form a hollow chamber at the center. This process is due to migration and reorganization of the existing cells that have populated the tracheal placodes. It has been shown that epidermal growth factor signaling plays an important role in the invagination of tracheal cells in the placodes (29,30). It has been proposed that upregulation in the expression of rhomboid (rho) in the tracheal cells leads an increase in EGF signaling, which in turn affects invagination of tracheal placodes. It has also been shown that mutation in rho leads to defects in cell migration and hampers the invagination of tracheal placodes.

Formation of Primary Branches—After the formation of the tracheal placodes, the next step in the process is the formation of primary branches, which is followed by the formation of second and terminal branches. The primary branching pattern is initiated by the expression of branchless, which has been shown to serve as a critical determinant of primary branching patterns (31). The expression of branchless is turned on within clusters of cells that are about to initiate primary branches, and is turned off once the branching pattern has been completed. Branchless encodes a

protein that functions as a ligand for the breathless receptor, and subsequent signaling pathways provide instructive cues for the development of primary branching patterns. The role of branchless has further been demonstrated by mutations in the gene, which restrict the formation of primary branches from tracheal placodes. The protein encoded by branchless is homologous to fibroblast growth factors (FGFs), thereby suggesting that FGF signaling may be involved in regulating the formation of primary branches during tracheal development.

Formation of Secondary Branches—The next step in the process is extension of the primary branches to form secondary branches, which appear a few hours after the primary branches. The formation of the secondary branches is also regulated by the relative expression of several genes, with Pantip-2 being one such example (20). Pantip-2 has been shown to be expressed in many cells in the primary branches, which eventually lead to the formation of secondary branches. As the secondary branches form and continue to expand, the expression of Pantip-2 is restricted to the lead cells, which are located at the ends of the primary branches. The expression of Pantip-2 was shown to be progressively limited to fewer cells at the leading tip of the primary branches as the branching pattern continued.

Formation of Terminal Branches—The final step of tracheal development is the formation of terminal branches from the secondary branches, a process that is regulated by the expression of several genes, including a gene known as blistered (32). Blistered encodes a protein known as the Drosophila Serum Response Factor, the expression of which has been shown to be upregulated in all tracheal cells that undergo terminal branching. It has also been shown that the expression of drosophila serum response factor is regulated by the expression of branchless. As we have seen in the formation of primary tracheal branches, branchless encodes a protein that is homologous to FGF, leading to the activation of the FGF signaling pathway. Activation of the FGF signaling pathway is an earlier event in the formation of primary branches, which has been shown to lead to the activation of blistered and the formation of terminal branches.

7.4 POST INTUBATION AND POST TRACHEOSTOMY TRACHEAL STENOSIS

Introduction—In a previous section, we studied CTS and the complications that can lead to tracheal resection/reanastomosis or the need for complete tracheal replacement. Examples of some other conditions that may require similar intervention include post-intubation and post-tracheostomy tracheal stenosis (33–38). Post-intubation and post-tracheostomy tracheal stenosis do not have a very high incidence, estimated to be 4.9 cases per year for every one million of the general population (39). The incidence of these disorders has been reported to be in the range of 10–22% of all patients that need to be intubated. Further, only 1–2% of these patients suffer severe complications. Just as we saw in the previous case when we discussed CTS, the prevalence of cases in the United States is not very high; nonetheless, patients who have severe complications

require care in tertiary centers, and many times, these complications can lead to fatalities. Therefore, there is an imperative need to understand the progression of such disorders and develop effective tools for the management and treatment of these conditions, some of which we will study in subsequent sections of this chapter.

Endotracheal Intubation and Tracheostomy—Endotracheal intubation requires insertion of a flexible tube in the trachea (40–45). This tube is connected to a mechanical ventilator to support respiration. The endotracheal tube is inserted through the mouth, and once inserted, it is secured in place by inflation of a balloon cuff at the end of the tube. Mechanical ventilators are used routinely during surgical procedures as a way to regulate respiratory parameters. While endotracheal intubation is a safe procedure, the pressure inserted by the balloon cuffs on the trachea can lead to cell necrosis and cuff stenosis. In cases when there is trauma to the respiratory tract or large blockages that disrupt the airflow to the lungs, a tracheostomy can be performed by creating a direct interface between atmospheric air and the trachea. In this case, an incision is made in the neck region of the patient, which is followed by cutting through a small portion of the thyroid gland. This is followed by a small incision in the cartilaginous rings of the trachea, and a tracheostomy tube is then placed and secured in the trachea. This creates a direct pathway for atmospheric air to flow through the trachea and to the bronchi and the lungs. While the procedure is considered to be safe, complications like tracheal stenosis can occur with long-term tracheostomies.

Post-Intubation and Post-Tracheostomy Tracheal Stenosis—One of the potential complications of tracheal intubation and tracheostomy is stenosis, narrowing of the airflow track that leads to compromised respiratory function (39). In the case of tracheal intubation, stenosis can arise from the balloon cuff, leading to a condition known as cuff stenosis. The purpose of inserting an inflated balloon during tracheal intubation is to secure the tracheal tube within the trachea; increasing the pressure within the balloon cuff provides direct contact with the luminal surface of the trachea. If the pressure in the balloon cuff is increased beyond the pressure in the local blood vessels feeding into the submucosal tissue, the blood flow to the tissue will be significantly reduced. Cartilaginous rings of the trachea do not have an independent blood supply, but rely on diffusion of nutrients from the submucosa tissue. Any reduction in the blood supply to the submucosa tissue will adversely affect the cartilaginous rings and can lead to cell necrosis and loss of structural integrity of the tracheal tissue; this necrosis and loss of integrity in turn can lead to tracheal stenosis, which in case is referred to as cuff stenosis.

Post-tracheostomy tracheal stenosis is a result of tissue damage resulting from insertion of the tracheostomy tube. Granulation tissue, which is formed as a result of the wound healing process, can directly lead to stenosis by blocking the flow of air in the trachea. In addition, structural damage to the cartilaginous rings by direct physical contact with the tracheostomy tube can lead to loss of structural integrity of the trachea and lead to stenosis. Also, formation of granulation tissue from the wound healing process around the cartilaginous rings can further accelerate stenosis.

7.5 TREATMENT MODALITIES FOR TRACHEAL STENOSIS

As we discussed in the previous sections, tracheal stenosis can occur due to congenital disorders like CTS or can be a result of endotracheal intubation or tracheostomy. These conditions affect infants and adults, although the prevalence of congenital disorders is lower compared to complications arising from tracheal intubation or tracheostomy. In order to treat tracheal stenosis, several strategies have been developed, some of which include the use of stents, balloon dilation, tracheal resection, and reanastomosis.

Endoscopic Balloon Dilation for Tracheal Stenosis—This procedure involves insertion through the mouth of a balloon catheter within the trachea (46–50). Once positioned within the trachea, the balloon is gradually inflated to apply radial pressure to the luminal surface of the trachea. This increase in radial pressure acts to increase the internal diameter of the trachea and reduce the extent of stenosis. The pressure in the balloon is then reduced, causing it to deflate, and the catheter is then removed.

This procedure has been conducted extensively in the United States and other parts of the world; a recent report summarizing the findings from 209 patients has been prepared (51). The most significant findings was that endoscopic balloon dilation resulted in significant improvement of tracheal stenosis and was not associated with any major complications.

One example of a balloon dilation system that is currently available on the market is the CRE^{TM} Pulmonary Balloon Dilator by Boston Scientific. The catheter is available in different dimensions, with an internal diameter ranging from 8 mm to 18 mm at a pressure of 3 atmospheres (information from company website). The length of the catheter is fixed at 75 cm while the length of the balloon itself varies from 3.0 mm to 5.5 mm.

Airway Stents—A stent is a hollow, cylindrical prosthesis that maintains luminal patency and provides support (52–57). Stents can be inserted within the trachea to alleviate stenosis and reduce the extent of airway narrowing (58–65). The devices are implanted in the trachea and retained for an extended time period. This is unlike balloon dilation, in which a catheter is inserted into the trachea a single time and then removed from the patient (although the procedure may be repeated several times). According to a recent publication by Mehta, an ideal airway stent should have the following characteristics (54): 1) easy to insert and remove, 2) customized to fit the dimensions and shape of the stricture, 3) able to re-establish the airway and maintain luminal patency with minimum rate of migration, 4) made of an inert material that does not irritate the airway, precipitate infection, or promote granulation tissue formation, 5) able to exhibit similar clearance characteristics like the normal airway so that mobilization of secretions is not impaired, and 6) economically affordable.

Airway stents are considered to be a long-term solution for the treatment of tracheal stenosis because they can remain in place for months, with reports of stents remaining implanted for periods in excess of 50 months. While there are risks associated with all surgical interventions, implantation of stents in the trachea

is safe. In one study in which 42 patients were treated for tracheal stenosis, the patients first underwent balloon dilation, followed by implantation of silicone stents (66). In this study, 5% of the patients suffered from complications resulting from granuloma formation, which were successfully treated, and an additional 5% of the patients required repeat surgery for stent replacement, which was successfully conducted.

There are two categories of airway stents: metal stents and tube stents. One example of an airway tube stent is the Montgomery Safe-T-Tube™ series, which is offered by Boston Medical Products. The stent is available in 5 different styles: pediatric, standard, thoracic, extra-long, and tapered. The standard stents are made from implant grade silicone and come in different dimensions, ranging from 10 mm to 16 mm in external diameter (information from company website).

The second category of airway stents are metallic mesh stents, which can be coated with a plastic covering and may also be balloon-expandable or self-expanding. Balloon-expandable stents are implanted in a deflated form and, once inserted at the desired position, are secured by inflation of the balloon. Self-expanding metallic stents are made with shape memory alloys like nitinol, and are maintained in a specific configuration at a lower temperature. Once inserted inside the body, the increase in temperature causes the material to change configuration, thereby allowing it to fit into its functional position. Metal stents are easier to work with and can be easily placed within the trachea via flexible bronchoscopy under local anesthesia in an outpatient setting. One example of a metal stent for tracheal stenosis is the Ultraflex™ tracheobronchial stent, made by Boston Scientific. Ultraflex stents are made with nitinol, an alloy of titanium and nickel that comes in different dimensions, ranging in external diameter from 146 mm to 20 mm and ranging in length from 40 mm to 80 mm (information from company website).

Tracheal Resection and Reconstruction—Tracheal resection and reconstution is a surgical procedure which involves removal of a small portion of the trachea that has been affected by stenosis and reconstruction of the remaining tissue to form a complete, though shorter, trachea (67–74). Removal of up to 4–5 cm of the trachea can be conducted. Prior to conducting the surgery, it is important to recreate the geometry of the trachea and identify the region of stenosis; this identification can be achieved by CT scans of the neck and chest region. Direct laryngoscopy and bronchoscopy also need to be conducted in order to obtain an accurate assessment of the tracheal stenosis. Tracheal resection and reconstruction is an invasive surgical procedure that is only conducted after unsuccessful attempts using other strategies, like balloon dilation. For the duration of the surgical procedure, the patient needs to be placed on a mechanical ventilator due to the resection of the trachea.

The surgical procedure requires a low traverse cervical incision for tracheal stenosis in the upper portions, while stenosis in the lower parts of the tracheal may require an additional upper midline sternostomy. The strap muscles are retracted to expose the trachea, which is separated from the surrounding tissue and secured

in place. The part of the trachea that is affected is excised, and the open ends of the tissue are sutured together.

Careful consideration and planning goes into the surgery due to potential complications that can occur. Mechanical ventilation is needed for 18 to 24 hours after the surgical procedure has been completed. Steroids are required to reduce edema for several weeks after the surgery. Other potential complications include the formation of granulation tissue, infection, and injury to the laryngeal nerve.

A recent study looked at the success rate of 110 patients after tracheal resection and reanastomosis (75). In this study, the length of the trachea resected ranged from 2.0 cm to 6.5 cm, with a median length of 3.5 cm. The most frequent post-surgical complication reported was recurrent nerve paralysis, observed in 5.5% of the cases. The long-term results of these surgeries were evaluated 12 to 226 months after the surgery, and 93.5% of the patients reported satisfactory results. The mortality rate related to the surgical procedure was reported to be 2.7%.

7.6 DESIGN CONSIDERATIONS FOR TRACHEAL TISSUE ENGINEERING

The design considerations for tracheal tissue engineering can be easily stated as "*bioengineered tracheas should be similar in form and functional to mammalian tracheas*." This is the overarching theme in tracheal tissue engineering, and specific requirements have been defined to meet this objective. This is not only the case for tracheal tissue engineering; it also applies across tissue systems, as we will come across in the later chapters.

The specific design considerations for tracheal tissue engineering have been eloquently defined by Macchiarini and Grillo as (76,77): 1) biocompatibility, 2) liquid- and air-tight, 3) nonimmunogenic and minimal inflammatory response, 4) nontoxic and noncarcinogenic, 5) avoidance of collapse by reasonable strength, 6) support cell engraftment, 7) support neovascularization, 8) possibility of growth, 9) resistance to fibroblastic and bacterial invasion, 10) standardized easy and short fabrication, 11) customizable and low-cost, 12) easy surgical handling, 13) provide physiological environment similar to ECM, 14) minimal necessity of donors and accessibility, 15) result in predictably successful engraftment, 16) provide or support epithelial resurfacing, 17) avoid stenosis or late buckling, 18) avoid accumulations of secretions, 19) must not dislocate or erode over time, and 20) permanent constructions.

7.7 PROCESS OF BIOENGINEERING ARTIFICIAL TRACHEAS

In Chapter 1, we presented a general scheme to bioengineer 3D artificial tissue. In this section, we will discuss a modified process scheme that has been adopted to bioengineer artificial tracheas. The process scheme presented here is based on

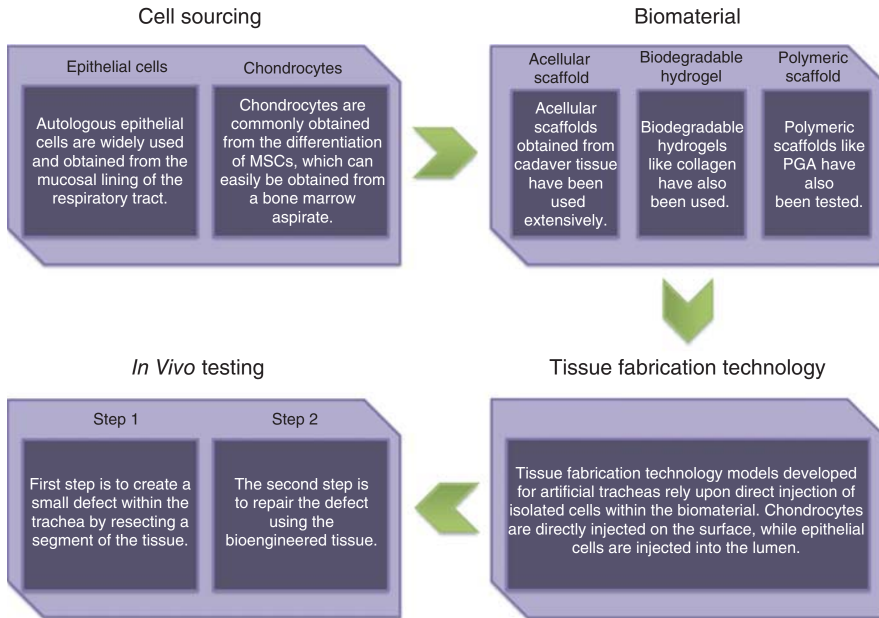


Figure 7.1 Process of Bioengineering Artificial Tracheas—Tubular scaffolds have been fabricated using many different biomaterials, including acellular grafts, biodegradable hydrogels, and polymeric scaffolds. Epithelial cells have been isolated from a tissue biopsy of the mucosal layer, while chondrocytes have routinely been obtained by differentiation of bone marrow MSCs. Tissue fabrication technologies for tracheal tissue engineering have commonly involved the use of direct injection strategies for incorporation of chondrocytes. Epithelial cells are injected into the lumen of the tissue graft. Once the tubular biomaterial has been cellularized, the artificial trachea is tested for *in vivo* efficacy using a small animal injury model, in which part of the trachea is resected and replaced with the artificial tissue.

recent publications that have presented varying strategies to bioengineer 3D trachea using different tissue engineering platforms (Figure 7.1).

As we discuss this process scheme, we will quickly see that attempts to bioengineer trachea have used different cell sources, many different biomaterials, and several tissue fabrication technologies. We will also see that bioreactor technology has not been extensively integrated in the process flow sheet for tracheal tissue engineering, as has been the case for several other tissue systems. The same is true for vascularization efforts, as there are not many examples of tissue-engineered tracheas with a blood vessel supply incorporated into the artificial tissue.

In this section, we provide a general scheme to bioengineer 3D artificial tracheas, and in the next section, we describe specific examples of tissue-engineered models for artificial tracheas. This is followed by two recent examples of tissue-engineered tracheas that have been used in clinical applications for the treatment of patients.

Cell Sourcing—It may be recalled that the trachea consists of several cell types, including epithelial cells on the luminal surface and chondrocytes within

the cartilaginous rings. Most efforts for bioengineering tracheas have focused on identifying suitable sources for these two cell types. Autologous epithelial cells have been widely used and are obtained from the mucosal lining of the respiratory tract. A tissue biopsy can be obtained using minimally invasive methods and the tissue biopsy can be digested to isolate epithelial cells. Chondrocytes have been commonly obtained from the differentiation of mesenchymal stem cells (MSCs), which can easily be obtained from a bone marrow aspirate. Once the MSCs are isolated and cultured *in vitro*, several protocols have been developed to effectively drive the differentiation of cultured MSCs toward a chondrocyte lineage. Autologous epithelial cells and MSC-derived chondrocytes have been a preferred source for tracheal cells, both for animal studies and for clinical studies.

Biomaterial Development—There has been considerable variation in the choice of biomaterials that have been used to bioengineer artificial tracheas. Acellular scaffolds obtained from cadaver tissue have been used extensively, including in clinical studies discussed later in this chapter. In addition, biodegradable hydrogels, like collagen, have also been used; and polymeric scaffolds like PGA have also been tested. Composite biomaterials with PCL and collagen have also been used for tracheal fabrication. Scaffold-free technologies have not been evaluated due to challenges in the formation of hollow structures that can support changes in fluid hemodynamics over long time periods.

Tissue Fabrication Technology—Tissue fabrication technology is perhaps one area that is lacking in the field of tracheal tissue engineering. Many of the models that have been developed for artificial tracheas have relied upon direct injection of isolated cells within the biomaterial. As we have discussed before, direct injection technology provides limited spatial resolution for the cells. There have not been any reports using cell/organ printing or solid freeform fabrication technologies to support the development of artificial tracheas.

Bioreactors—Bioreactors are another technology that have not been developed very effectively in the tracheal tissue engineering field. There have been few studies that report the use of bioreactors for the development of artificial tracheas. There was one study in which a bioreactor system was developed for scaffold cellularization, and the resulting tracheas were used clinically. In addition, there have been two studies that describe the use of bioreactors to support the fabrication of artificial trachea. In another study, a bioreactor was developed to provide physiological conditioning to an artificial trachea after fabrication of the tissue graft. In this case, an artificial trachea was fabricated by culturing chondrocytes on a composite biomaterial and cultured under static *in vitro* conditions for several days prior to bioreactor conditioning. The bioreactor was designed to provide continuous media flow to support the metabolic requirements of the artificial trachea. In this bioreactor system, the rotational speed of the bioreactor could be varied, thereby culturing the trachea under controlled shear stress regimes.

Vascularization—There have not been any reports of *in vitro* methods for vascularization of artificial tracheas. Instead, the strategy has been to utilize acellular grafts to bioengineer artificial tracheas, and once implanted, the acellular scaffolds supported neovascularization from host tissue. Indeed, this has been the case for

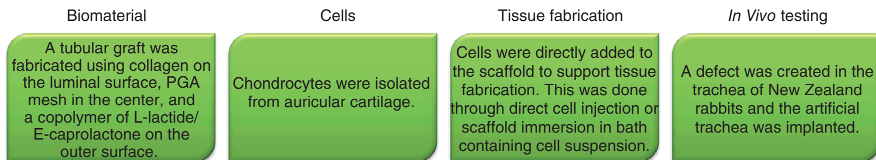
the two clinical studies, both of which have demonstrated neovascularization after tracheal implantation. However, the need for *in vitro* vascularization still persists and continues to be an area that requires attention.

In Vivo Testing—There have been several groups that have demonstrated the efficacy of bioengineered tracheas using small animal models. While there have been variations in the specific models used, they all aim to create a small defect within the trachea by resecting a segment of the tissue and then replacing this defect using the bioengineered tissue.

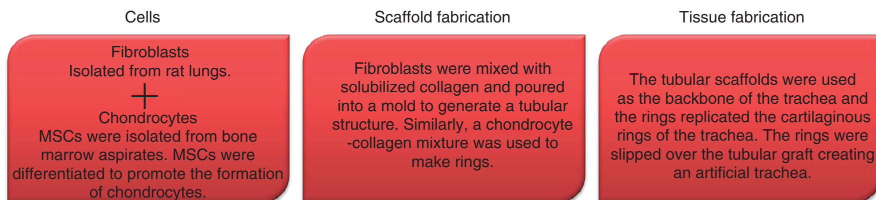
7.8 TISSUE ENGINEERING MODELS FOR ARTIFICIAL TRACHEAS

In this section, we will look at several models that have been developed to fabricate artificial trachea using tissue engineering technologies (Figure 7.2).

(a) Example 1 – trilayer artificial trachea



(b) Example 2 – artificial trachea based on collagen



(c) Example 3 – artificial trachea based on pga mesh

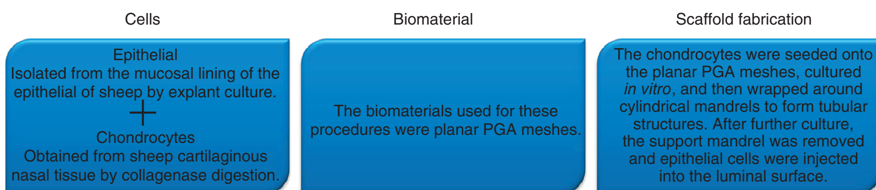


Figure 7.2 Models of Artificial Tracheas—(a) Tri-layer Artificial Trachea—A tri-layer tubular scaffold was fabricated and populated with autologous chondrocytes, which were isolated from auricular cartilage. Once an artificial trachea was bioengineered, it was tested *in vivo* to assess efficacy. **(b) Artificial Trachea Based on Collagen**—Fibroblasts were mixed with collagen and used to form tubular grafts. The same strategy was used to fabricate ring structures, which were coupled with the tubular grafts to form an artificial trachea. **(c) Artificial Trachea based on PGA Mesh**—A planar PGA mesh was populated with chondrocytes isolated from a nasal septum tissue biopsy. The planar mesh was rolled up to form a hollow tubular structure, and epithelial cells were injected within the lumen, resulting in the formation of an artificial trachea.

As we review these models, we see that different researchers have tested different biomaterials, several cell sources have been used, and researchers have adopted various tissue fabrication technologies. In addition, we will look at the use of bioreactor technology to fabricate tracheal tissue or to provide physiological conditioning and tissue development post-fabrication. As we study these models, we will have an opportunity to relate many tissue engineering principles, namely those presented in earlier chapters, for the fabrication of artificial tracheas.

Example 1 for Tissue-Engineered Tracheas—A tri-layer tracheal graft was fabricated using collagen, nonwoven PGA mesh, and a copolymer of (L-lactide/ϵ-caprolactone) (78). A tubular graft was fabricated using these three materials, with collagen on the luminal surface, PGA mesh in the center, and the copolymer of L-lactide/ϵ-caprolactone was used on the outer surface. The tri-layer tracheal graft was designed with three separate materials to provide different functions. The collagen layer was designed to support the attachment and functionality of epithelial cells, the central PGA mesh was designed to support chondrocyte viability, and the outermost copolymer layer was designed to provide elasticity to the trachea. Chondrocytes were isolated from auricular cartilage, which was obtained from New Zealand rabbits. Biopsies were obtained and digested in a collagenase solution, and the isolated cells were maintained and expanded under controlled cell culture conditions. In order to support tissue fabrication, the cells were directly added to the scaffold. As the details of the cellularization protocol were not provided, it can be assumed that the cells were added either by direct injection into the scaffold using a syringe, or by immersing the scaffold within a bath containing the cell suspension. Once cellularization was complete, the scaffold was maintained in a cell culture incubator for 24 hours prior to *in vivo* testing using an animal model. It should be noted that the artificial trachea was not populated with epithelial cells during *in vitro* tissue fabrication; instead, this model is based on the hypothesis that *in vivo* implantation of the artificial trachea will lead to epithelization using autologous cells from the host.

The effectiveness of the artificial trachea was tested using a small animal injury model. Using New Zealand rabbits for *in vivo* testing, a defect was created and the artificial trachea was sutured in place. Controlled release of transforming growth factor was used to support chondrogenesis. Three months post-evaluation, epithelization of the luminal surface was demonstrated in the artificial trachea, along with accumulation of cartilage tissue. This clearly demonstrated the feasibility of tissue engineering strategies to bioengineer artificial tracheas.

Example 2 for Tissue-Engineered Trachea—A very interesting approach to bioengineer artificial tracheas was recently published. In the previous example and in others we have come across in the book, the scaffold fabrication and cellularization have been independent, though sequential, events in the tissue fabrication process. In this example, these two were coupled (79). Cells were obtained from rats, with fibroblasts being isolated from the lungs and MSCs isolated from bone marrow aspirates. MSCs were subjected to a differentiation strategy to promote the formation of chondrocytes; differentiation was induced using cell culture media supplemented with β-glycerophosphate, ascorbic acid phosphate, and dexamethasone.

Collagen was used as the scaffolding material to support the fabrication of artificial trachea.

Cells, either fibroblasts or MSC-differentiated chondrocytes, were mixed with solubilized collagen, and this cell-gel mixture was poured into a mold to generate tubular or ring-shaped structures. Tubular structures were constructed with fibroblasts and were designed to provide the backbone of the trachea or the major structural component. The rings that were fabricated using chondrocytes were designed to replicate the cartilaginous rings of the trachea. Once fabricated, the rings were slipped over the tubular grafts; the tubular grafts replicated the backbone of the trachea while the rings replicated the cartilage rings. This process supported the fabrication of an artificial trachea that was close in form to mammalian trachea.

Once fabricated, the artificial trachea was implanted in rats after a tracheal defect, and was shown to survive for very short time periods not exceeding two days. While the tissue fabrication technology adapted in this model was very interesting, there was no consideration for the formation of an epithelial lining that may have contributed to the limited *in vivo* success of the artificial trachea. The use of fibroblasts was also interesting; it appears the fibroblasts were used to provide structural support in the formation of a hollow tubular structure, which could not be easily accomplished with the collagen itself. Nonetheless, the model was novel and interesting, and upon further development and optimization, it could lead to a viable strategy to bioengineer artificial tracheas.

Example 3 for Tissue-Engineered Tracheas—In another very interesting and novel approach to bioengineer artificial tracheas, epithelial cells and chondrocytes were obtained from the nasal septum of sheep (80). Epithelial cells were isolated from the mucosal lining of the epithelial by explant culture, which means that tissue biopsies were plated on a tissue culture plate, resulting in the outgrowth of epithelial cells from the tissue sample. Chondrocytes were obtained from the cartilaginous tissue, which was subjected to a collagenase digestion procedure. The chondrocytes were seeded onto planar PGA meshes, cultured *in vitro*, and then wrapped around cylindrical mandrels to form tubular structures. These cellularized constructs were implanted in a subcutaneous pocket in nude mice to support the formation and development of the tissue graft. The tissue graft was implanted with the cylindrical mandrel in place. Upon explantation, the support mandrel was removed from the tissue graft, and epithelial cells were injected into the luminal surface. The tissue graft was cultured *in vitro* after injection of the epithelial cells, which led to the fabrication of an artificial trachea. While the artificial trachea was not tested for *in vivo* functionality, the anatomical and functional properties of the artificial tissue were shown to be comparable to that of mammalian tracheas.

Concluding Remarks—In this section, we have looked at several examples of tissue engineering strategies that have been used to fabricate artificial tracheas. We have seen the use of different materials, different sources of cells, and very different tissue fabrication technologies. The reader can easily relate many of these strategies to various topics that have been covered in previous chapters, including cell sourcing, stem cell differentiation, biomaterial development, and tissue fabrication technologies. In addition, based on the information that has been provided

in earlier chapters, the reader can design custom strategies that can lead to the fabrication of artificial trachea. One limitation of the strategies that we have looked at is the inability to incorporate bioreactor technology in the fabrication of artificial tracheas, either for scaffold cellularization or for physiological conditioning. Indeed, this has been one of the limitations in the field of tracheal tissue engineering, as there are very few studies that describe the use of bioreactor technology to support the fabrication of artificial trachea.

7.9 TRACHEAL TISSUE ENGINEERING—AN EXAMPLE OF A CLINICAL STUDY

There have been two successful clinical studies relating to the transplantation of tissue-engineered tracheas, one of which is described in this section (Figure 7.3)

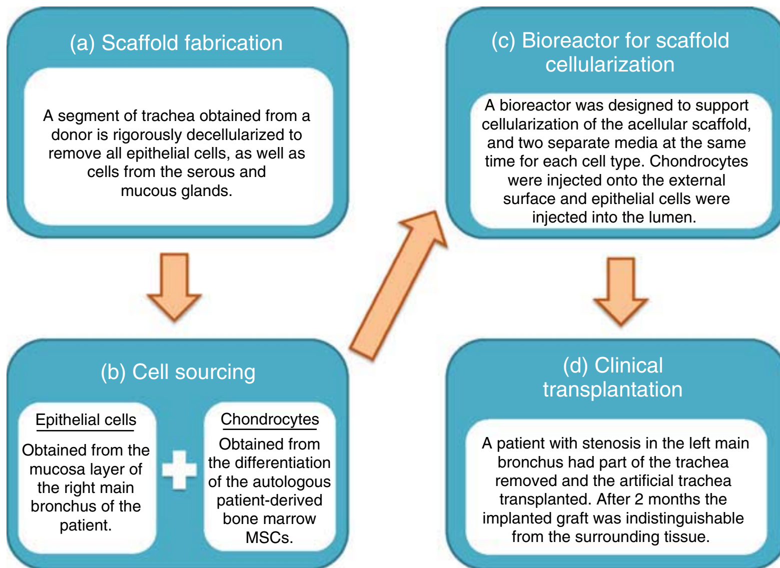


Figure 7.3 Fabrication of Artificial Trachea for Clinical Applications—(a) Scaffold Fabrication—Donor tracheal tissue was subjected to a decellularization protocol designed to remove the cells, leaving behind an intact extracellular matrix. **(b) Cell Sourcing**—Epithelial cells were isolated from a biopsy of the mucosal layer of the right main bronchus. Chondrocytes were obtained from differentiation of bone marrow MSCs. **(c) Bioreactor for Scaffold Cellularization**—Chondrocytes are added to the scaffold by direct injection of the cells onto the outer surface of the acellular scaffold using a microsyringe. Epithelial cells were added to the scaffold by delivery of the cells to the luminal surface of the acellular scaffold. **(d) Clinical Transplantation**—A segment of the left main bronchus was removed due to stenosis and replaced with the tissue-engineered trachea.

and a second one, which is described in the next section (81,82). Indeed, fabrication of artificial trachea has been one of the more successful areas of research in the field of tissue engineering. The clinical studies have been instrumental in validating the feasibility of tissue and organ engineering and have demonstrated many of the principles of tissue fabrication. The first of these clinical studies was reported in 2008 (82), while the second was reported in 2012 (81).

Scaffold Fabrication—The first successful human transplantation of tissue-engineered trachea was reported in a seminal publication in the year 2008 (81). An artificial trachea was fabricated using an acellular scaffold. A segment of trachea, which measured 7 cm in length, was obtained from a human donor and subjected to a very rigorous decellurization protocol. As we have seen before, decellularization strategies are designed to remove all cellular components from the tissue, leaving behind an intact extracellular matrix. The ECM has the right composition, distribution, and orientation of various proteins, making it suitable to support the tissue fabrication process. In this case, the decellularization process consisted of 25 cycles using sodium deoxycolate and deoxyribonuclease I. It was demonstrated that the decellularization protocol removed all epithelial cells, cells of the serous and mucous glands, and almost all of the chondrocytes associated with the cartilaginous rings. It was also demonstrated in this study that the decellularization process did not damage the properties of the ECM or change the mechanical properties in any significant manner.

Cell Sourcing—Patient-derived autologous cells were used to fabricate the artificial trachea, which completely removed the need for immunosuppression. In order to fabricate artificial trachea, epithelial cells were required to populate the luminal surface, while chondrocytes were required to populate regions of the trachea that give rise to the cartilaginous rings. In this study, the epithelial cells were obtained from the right primary bronchus, while the chondrocytes were obtained by differentiation of bone marrow mesenchymal stem cells. Both cell sources used in this study were autologous, which means they were obtained from the patient.

In order to source epithelial cells, a small tissue biopsy was obtained from the mucosal layer of the right main bronchus. The tissue biopsy was transported to the laboratory in phosphate-buffered saline containing penicillin and streptomycin. The tissue specimen was digested using trypsin, and the isolated epithelial cells were plated on a tissue culture surface and maintained in a cell culture incubator. The cells were cultured in a DMEM based media and supplemented with bovine pituitary extract and recombinant epidermal growth factors.

Chondrocytes were obtained by controlled differentiation of autologous patient-derived bone marrow mesenchymal stem cells (BMMSCs). BMMSCs were obtained from a bone marrow aspirate and were maintained in a DMEM based media supplemented with basic fibroblast growth factor. The cells were cultured in tissue culture flasks and subpassaged at 90% confluency. In order to differentiate BMMSCs to chondrocytes, the BMMSCs were cultured for 72 hours in complete media containing human transforming growth factor, recombinant parathyroid hormone-related peptide, dexamethasone, and insulin.

Bioreactors Design—A novel bioreactor was designed to support cellularization of the acellular scaffold with epithelial cells on the luminal surface and chondrocytes on the external surface. The bioreactor was housed within a polysulphone chamber, which provided anchoring points for the acellar scaffold. Chondrocytes were directly applied to the external surface of the acellular graft using a direct injection strategy with a microsyringe. Epithelial cells were injected into the lumen of the acellular scaffold through a separate access port engineered into the bioreactor. The concentration of chondrocytes and epithelial cells was adjusted to one million cells per milliliter for both cell types. The bioreactor was designed to accommodate two separate media formulations at the same time: one for the chondrocytes and one for the epithelial cells. The acellular graft was cultured in the bioreactor for 96 hours prior to implantation. Another novel feature of the bioreactor was the design of a component that rotated between a liquid phase and an air phase, which was a mechanism designed to enhance oxygenation, increase the supply of nutrients, and support waste removal.

Implantation of Tissue-Engineered Trachea—The artificial trachea was implanted in a patient; this is one of the few examples of successful clinical applications of tissue-engineered grafts. The artificial trachea was used to replace the left main bronchus in a patient. The patient was discharged from the hospital ten days after the surgery was performed and was monitored for up to 2 months post-surgery. Remarkably, the implanted graft was indistinguishable from the surrounding tissue and was vascularized by the host tissue and there were no signs of inflammatory cells 60 days after surgery. This is indeed a remarkable fate and one of the true accomplishments in the field of tissue engineering.

Concluding Remarks—The example presented here serves to demonstrate one of the most significant milestones in the field of tissue engineering: the clinical application of artificial tissue to improve a patient's quality of life. In addition, this example serves to demonstrate the role of biomaterials, cells, and bioreactors for the fabrication of artificial tissue; a clear validation of the building blocks of tissue engineering. Tissue engineering has been defined as tissue fabrication in Chapter 1 of this book; here, we see how this works in a clinical setting.

7.10 TRACHEAL TISSUE ENGINEERING—A SECOND EXAMPLE OF A CLINICAL STUDY

In this section, we discuss a second case of a tissue-engineered trachea being used for the treatment of a patient (82) (Figure 7.4). In this case, the patient was born with a birth defect known as congenital tracheal stenosis, which has been described earlier in this chapter. This patient was treated using several strategies, which included autologous patch tracheoplasty, implantation of balloon-expandable stainless steel stents, and transplantation of a tracheal homograft after complications resulting from the stent. At the age of 10, the patient suffered further complications and required additional surgical intervention. Therefore, in this case, a tissue-engineered trachea proved to be a viable treatment option for this patient.

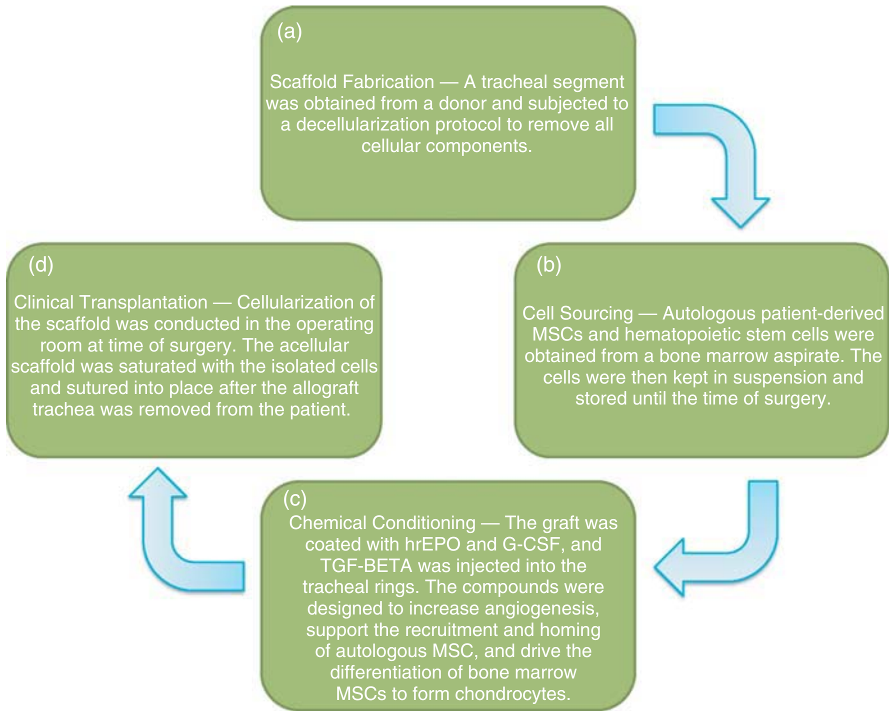


Figure 7.4 A Second Example: Fabrication of Artificial Tracheas for Clinical Applications—(a) Scaffold Fabrication—Donor tracheal tissue was subjected to a decellularization protocol designed to remove the cells, leaving behind an intact extracellular matrix. **(b) Cell Sourcing**—Bone marrow aspirate is used to source autologous MSCs and hematopoietic stem cells. **(c) Chemical Conditioning**—Several chemical compounds were used to drive the differentiation of MSCs to form chondrocytes, to support the recruitment of circulating MSCs, and to increase angiogenesis. **(d) Clinical Transplantation**—A segment of the trachea was removed due to stenosis and replaced with the tissue-engineered trachea.

Scaffold Fabrication—The strategy for scaffold fabrication was the same one used in the first example. A tracheal segment was obtained from a donor and subjected to a decellularization protocol to remove all cellular components, leaving behind an intact extracellular matrix.

Cell Sourcing—Autologous cells were used in this case; they consisted of a mixture of patient-derived mesenchymal stem cells and hematopoietic stem cells, both of which were obtained from a bone marrow aspirate. The isolated cells were not maintained and/or expanded in culture; on the contrary, the cells were retained in suspension and stored until the time of the surgery. This means that the number and proportion of cells at the time of isolation remained the same until the time of surgical implantation.

There are significant differences in the cell sourcing strategy used for the two clinical studies that we have presented. In the previous example, the isolated cells

were maintained and manipulated in culture prior to surgical transplantation, whereas in the current example, the cells were stored in suspension prior to use. In the previous example, the epithelial cells were isolated from a biopsy of the right main bronchus, and the chondrocytes were obtained from differentiation of bone marrow MSCs. This is in contrast to the current example, which used unmodified MSCs and hematopoietic stem cells obtained from a bone marrow aspirate.

Surgical Engraftment—Cellularization of the acellular scaffold was conducted in the operating room at the time of surgery. The acellular scaffold was saturated with the isolated cells, and the cellularized scaffold was sutured into place along the length of the trachea after removal of the allograft trachea that was previously implanted into the patient. The graft was coated with erythropoietin and granulocyte-colony stimulating factor, and transforming growth factor was injected into the tracheal rings. These compounds were designed to increase angiogenesis, support the recruitment and homing of autologous MSC, and drive the differentiation of bone marrow MSCs to form chondrocytes.

Comment—There are a few significant differences between the two clinical examples presented in this chapter. In the current example, the cells and the scaffold were coupled together at the time of surgical engraftment, as will be described later in this section; there was no scaffold cellularization prior to surgical implantation. Along the same line of thought, this also means that the strategy described in this study does not require or make use of bioreactors, either for cellularization and/or for physiological conditioning. This is consistent with the tissue engineering model that has been adapted in the study: coupling of cells and scaffold at the time of surgical engraftment. This model is significantly different from the previous one presented in this chapter in which cellularization of the scaffold was conducted prior to surgical implantation; in this case, bioreactors were used to support scaffold cellularization and tissue fabrication. These are two completely different strategies, each of which has proven to be successful in the clinical setting.

There were some short-term surgical complications resulting from implantation of the artificial trachea. However, once the patient stabilized, steady recovery was reported. Fifteen months after surgery, the implanted trachea showed complete epithelialization on the lumen surface, and the patient had normal lung function. Two years after surgical intervention, the patient was reported to be "*well, growing, and had not needed medical intervention for 6 months*."

Discussion—As we saw in the previous example, this case study serves to demonstrate successful implantation of a tissue-engineered trachea and is a remarkable demonstration of accomplishment in the field. There are clear differences between the two cases, particularly with regard to the specific tissue engineering technology adapted. In the first case study, an artificial trachea was developed *in vitro* and then transplanted; scaffold cellularization and maturation took place under controlled *in vitro* culture conditions and made use of bioreactor technology. In the second case, scaffold cellularization was conducted at the time of surgery, and the scaffold was immersed in the cell suspension and immediately transplanted; several compounds were added to the trachea to promote development and maturation during *in vivo* culture of the implanted tissue

graft. While different, both of these strategies proved to be successful as judged by the most important criteria, which is the ability to improve patient quality of life. Indeed, both of these studies are seminal in the field and clearly demonstrate the tremendous potential for the field of tissue engineering.

SUMMARY

Current State of the Art—In this chapter, we have looked at several examples for the development of 3D artificial tracheas. The models presented in this chapter served to illustrate many of the principles we have studied during the course of this book, including cells, biomaterials, bioreactors, and tissue fabrication technology. We have seen that different combinations of these fundamental principles in tissue engineering lead to very different results and very different models for 3D artificial tracheas. We have also seen that careful selection of design variables, like the source of cells, type of biomaterial, cellularization strategy, and culture conditions, including bioreactor conditioning, have a significant impact on the form and function of 3D artificial trachea. Perhaps most importantly, we have described the application of tissue engineering technology in a clinical setting. We studied examples of 3D artificial tracheas that have been transplanted in patients to significantly enhance the quality of life for these patients. These clinical studies are described as seminal publications, and serve to demonstrate and validate the potential impact of tissue engineering.

Thoughts for Future Research—There has been considerable progress made in the field of tracheal tissue engineering. However, the development of biomaterials to support fabrication of 3D artificial tracheas remains one area of research that has not fully matured. Mammalian tracheas are hollow structures that have a high demand on the mechanical properties of the extracellular matrix. In tissue engineering, acellular scaffolds have been used extensively to support fabrication of artificial tracheas; this is due to the high mechanical stability of acellular scaffolds. While acellular scaffolds have proven to be effective for fabrication of transplantable tissue, a generation of new biomaterials needs to be developed; materials which have mechanical properties that are comparable to acellular grafts are necessary. A generation of biomaterials needs to be synthesized to support the fabrication of 3D artificial tracheas with mechanical properties comparable to that of mammalian tracheas.

PRACTICE QUESTIONS

1. Describe the structure of the mammalian trachea. Include a description of the different cell types and the functions of these cells. Also describe the extracellular matrix components that constitute the mammalian trachea.
2. What is congenital tracheal stenosis (CTS)? Explain the classification scheme for CTS. What are some of the symptoms associated with CTS? How can CTS be diagnosed?

3. Describe the genetic regulation of tracheal development.
4. What are the causes of post-intubation and post-tracheostomy tracheal stenosis?
5. What are some treatment modalities for tracheal stenosis? Discuss the relative advantages and disadvantages of the treatment modalities that you describe.
6. In Chapter 4, we studied two strategies for tissue fabrication using scaffold-free technology: self-organization and cell sheet engineering. Start by providing a brief description of these two strategies to support tissue fabrication. Do you believe either one of these can be used to support the fabrication of artificial tracheas? If so, which one do you think is more suitable and why?
7. During our discussion of tracheal tissue engineering, there was an evident lack of interest in the development of bioreactor technology to support the formation and culture of artificial tracheas. If you were to develop bioreactors for tracheal tissue engineering, where would you use the bioreactors in the tissue fabrication process? Give one specific example, including a design for the bioreactor, that can be used to support the fabrication of artificial tracheas.
8. During our discussion on stem cells for tracheal tissue engineering, we studied a few examples using primary bone marrow MSCs to support the fabrication of artificial tracheas. Instead of using bone marrow MSCs, we can also use iPS cells to fabricate artificial tracheal tissue. Do you believe that iPS cells will be advantageous for development of artificial tracheal tissue? Explain your answer.
9. During our discussion in Chapter 2, we studied iPS cells and hES cells and their potential application during the tissue fabrication process. Which one of these two will you choose to fabricate artificial tracheas and why?
10. Acellullar scaffolds have been used extensively to support the fabrication of artificial tracheas. What are some advantages of acellular scaffolds? What are some of the disadvantages of acellular scaffolds for the fabrication of tracheal tissue?
11. In Chapter 4, we discussed cell and organ printing for the fabrication of artificial tracheas. Provide a brief description of cell and organ printing. Describe how you would use cell and organ printing to bioengineer artificial tracheas.
12. In Chapter 5, we provided a process flow chart that can be used to determine the best strategy to induce vascularization in artificial tissue and organs. Using this process flow chart, develop a strategy to engineer vascularization in artificial tracheal tissue.
13. In Chapter 5, we discussed three strategies to induce vascularization in artificial tissue and organs: biologically replicated, biologically mediated, and

biologically inspired. Start by providing a brief description of each of these three strategies. Which one will you use for the vascularization of artificial tracheas and why?

14. Develop a process flow chart for the fabrication of artificial tracheas. Include a discussion of the following items: cell sourcing, biomaterial selection, scaffold cellularization, vascularization, and bioreactor conditioning.

15. What are some of the critical scientific and technological challenges in the field of tracheal tissue engineering? What can you do to overcome these challenges?

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