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REVIEW

Translating tissue-engineered tracheal replacement from bench to bedside

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Abstract There are a variety of airway diseases with different clinical settings, which may extend from a surgical approach to total organ replacement. Tissue engineering involves modifying cells or tissues in order to repair, regenerate, or replace tissue in the body and seems to be a promising approach for airway replacement. The successful implantation of stem-cell-based tissue-engineered trachea in a young woman with end-stage post-tuberculosis left main bronchus collapse serves as a prototype for the airway tissue-engineered-based approach. The trachea indeed could represent a perfect model system to investigate the translational aspects of tissue engineering, largely due to its low-oxygen needs. This review highlights the anatomy of the airways, the various disease conditions that cause damage to the airways, elaborates on the essential components of the tissue-engineering approach, and discusses the success of the revolutionary trachea transplantation approach.

Keywords Decellularization · In situ tissue engineering · Mesenchymal stem cells · Regenerative medicine · Tissue engineering · Trachea · Transplantation

Introduction

Structure and function of airway

The conducting airway is divided into the upper airway (nose, oral cavity, pharynx, larynx) and the lower airway (tracheobronchial tree: trachea, bronchi, bronchioles, alveolar ducts, alveolar sacs). The tracheobronchial tree can be structurally subdivided into cartilaginous airways (also called conducting zone) and non-cartilaginous airways (respiratory zone) [1]. The inhaled air, moving through the larynx, reaches the trachea, which is a fibromuscular tube supported by approximately 15–20 C-shaped cartilaginous rings (which give rigidity and prevent collapse during inspiration). The adult trachea is about 10–13 cm in length and 1.5–2.5 cm in diameter, whereas the infant trachea is 4 and 0.36 cm, respectively [2]. There are approximately two cartilaginous rings per cm of tracheal length and the first ring is generally broader than the rest. The tracheal cartilaginous rings are highly elastic and become inflexible with age or trauma. Inward airflow from the trachea branches off to the two bronchi (one to the right lung and one to the left lung); the bronchi contain C-shaped cartilage rings [2]. The airways systematically branch over an average of 23 generations of dichotomous branching and the main stem bronchi represent the tracheobronchial tree's first generation. Deeper in the lungs, each bronchus divides into secondary and tertiary bronchi, which continue to branch into smaller airways called bronchioles. There is no cartilage in the bronchioles, and this lack of airway support often plays a major role in respiratory disease. The bronchioles end in air sacs called the alveoli. Alveoli are bunched together into 15–20 clusters to form alveolar sacs. On the surface of each alveolus, there is a network of small pulmonary capillaries where at alveolar-capillary membrane level blood oxygenation takes place.

Tissue organization of airway

The airway is mainly composed of three major components: (1) a mucosal lining of epithelial and connective

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tissue (pseudostratified ciliated cells, basal cells, goblet cells, submucosal glands, Clara cells, neuroendocrine cells, brush cells); (2) a supporting connective tissue layer mostly cartilaginous; and (3) a layer of smooth-muscle bundles (interstitial cells, mast cells). Moreover, in non-cartilaginous airway, there are about 40 different highly specialized cell types both structurally and functionally.

Along with differentiated specific cells, the presence of airway stem cells has been recently demonstrated. Indeed, deriving from mesenchyme, it is speculated that airway connective tissue may also contain undifferentiated stem cells [3, 4]. Currently, much research is focused on isolating airway stem cells, and it has been suggested that more than one kind of stem cell is required to support the upkeep and repair of the airway. In the adult lung, cells at the broncho-alveolar junction, expressing specific markers (such as SPC and CC10), have been proposed to represent lung epithelial progenitor cells and are able to contribute to both the alveolar (distal) and bronchiolar (proximal) lineages [5]. However, a significant challenge for this type of work is the overall rarity of stem cells, and research studies will be necessary to identify and characterize airway stem cells.

Damage to the airway

There are more than 40 conditions that affect the airway and impact a person's ability to breathe. According to the British Lung Foundation, respiratory disease is globally the second biggest killer after cardiovascular diseases. The airway can be damaged due to stenosis, infection, cancer, congenital anomalies, trauma, and foreign-body aspiration (a detailed list is provided in Table 1). Along with these pathologies, tracheal stenosis, a condition where the windpipe becomes narrower, affects 4–13% of adults (in the USA alone) and occurs in 1–8% of neonates. It can be divided based on: the location (supraglottic stenosis-tracheal narrowing above the larynx, or subglottic stenosis-tracheal narrowing below the vocal cords) or on stenosis conditions (congenital—from birth, or acquired—developed later in life). Congenital tracheal stenosis is a life-threatening, rare disorder with an estimated incidence of 0.6–2.9%. The clear etiology is still not clear, but it is hypothesized that the most severe form of stenosis occurs during the fourth week of gestation (affecting respiratory development) and the less severe stenosis would occur between the eighth and tenth weeks of gestation (impaired development of cartilages and their supporting tissue). Acquired tracheal stenosis can indeed develop from a number of causes: (1) as a result of treatments such as endotracheal intubation, tracheotomy, radiotherapy, tracheal resection, reconstruction, dilation, tracheobronchial airway stent, or past surgery; (2) external injury; (3) cancer; or (4) autoimmune conditions (like polychondritis, sarcoidosis, papillomatosis, amyloidosis, and Wegener's granulomatosis); (5) infections such as bacterial—tuberculosis and fungal infections. There are some conditions, such as idiopathic subglottic stenosis, where no known cause has been identified. Prematurely born children may also often have breathing/swallowing problems mainly due to blockage of the larynx (voice box), trachea, or throat.

Clinical management of airway damage

Since the 1950s, tracheal resection and end-to-end anastomosis has been the "gold standard" clinical approach for the treatment of most of airway diseases, such as tracheal stenosis, tracheomalacia, tumors, (the major accomplishments are highlighted in Table 2).

However, when the disease length extends more than 50% of the total (in adults) or one-third (in small children) tracheal length, safe reconstruction would not leave enough length of the native airway for primary reconstruction and safe reconstruction results impossible [6]. In these cases, the use of tracheal stents, t-tubes, or cannulas are the most useful approaches for airway surgery. However, existing prosthetic replacements are imperfect, and have resulted in life-threatening and uncorrectable complications, such as risk of inflammatory response, complications due to limited durability of the prosthesis, increased susceptibility to infection, and need for re-operations [7]. One of the choices of treatment with the most potential for end-stage disease is organ transplantation, however, the two main concerns in this field are rejection and shortage of organs. The possible solution to the problem of rejection is gene therapy; while xenotransplantation can circumvent the problem of the shortage of organs. However, in spite of recent advances like cloning of mammals [8], the xenotransplantation is still not an attractive treatment option [9]. In the last few years, a new field of reconstructive surgery has emerged and seems to be a promising approach for improving the quality of life by replacing missing functions through rebuilding body structures: the regenerative medicine approach. In Table 3, the major breakthroughs in the field of trachea transplantation are reported. According to the authors' own experiences, the reported transplantations have a clinically positive outcome: the transplanted tissue-engineered airways were airtight and functionally and mechanically completely integrated into the adjacent airway, with no signs of chronic inflammation, granulation tissue, infection, or erosion. After only 4 months from the transplantation (and until now), the patients can perform regular physical activity, their quality of life has returned to normal, and their lung-function parameters are all within the normal range for age and sex, which suggests that the regenerative approach could be the only technique of the

Table 1 List of causes and conditions of airway damage

Condition	Description	Reference
Congenital		
Tracheal agenesis	Trachea may be completely absent	[74]
Tracheal atresia	Trachea is partially in place but considerably under formed	[75]
Tracheal web	Thin layer of tissue draped across the tracheal lumen	[76]
Short- and long-segment tracheal stenosis, cork-screw trachea	Rings of cartilage varying in length, location, and severity	[77, 78]
Tracheomalacia	Trachea is floppy due to weakness of the tracheal walls	[79]
Vascular anomalies	Abnormally formed blood vessels leading to tracheo-esophageal compression	[80]
Tracheoesophageal fistulas	Abnormal connection (fistula) between the esophagus and the trachea	[81]
Tracheal cysts	Evaginations of embryologic tracheal buds	[82]
Trachiectasis	Abnormal tracheal bifurcation above the level of the carina	[83]
Laryngotrachesophageal cleft	Defect of the posterior larynx and trachea and the anterior wall of the esophagus	[84]
Cancer		
Adeno cystic lung carcinoma	Primary malignant tumor, non-small cell lung cancer usually begins in the periphery of the lungs	[85]
Squamous cell lung carcinoma	Primary malignant tumor, usually starts in the bronchial tubes in the central part of the lungs	[86]
Infection		
Tuberculosis	Caused by *Mycobacterium tuberculosis*	[87]
Lemierre's syndrome	Nasopharyngitis due to *Fusobacterium necrophorum*	[88]
Deep cervical space infections	Mixed infections caused by aerobic and anaerobic bacteria	[89]
Croup	Acute viral respiratory illness	[90]
Bacterial tracheitis	Most often caused by *Staphylococcus aureus*	[91]
Rhinoscleroma	Caused by *Klebsiella rhinoscleromatis*	[92]
Tracheal stenosis		
Postintubation	Clinical problem caused by regional ischemic necrosis of the airway	[93]
Post-tracheotomy	Stenosis following tracheostomy	[94]
Trauma		
Facial trauma	Vehicle accidents	[95]
Laryngotracheal, inhalation injuries, endotracheal tube	Injuries due to thermal and chemical substance and postextubation	[96]
Foreign-body aspiration		
Penetration syndrome	Foreign bodies migrate through trachea and lodge in bronchi, e.g., food products	[97]
Autoimmune and associated diseases		
Wegener's granulomatosis	Antineutrophilcytoplasmic antibody (ANCA)-associated vasculitis	[98]
Sarcoidosis	Granulomatous infiltration and obstruction of the upper airways	[99]
Pulmonary amyloidosis	Proteinaceous deposits on tracheal walls	[100]
Relapsing polychondritis	Recurrent episodes of inflammation of cartilaginous tissues	[101]
Tracheopathiaosteoplastica	Benign cartilaginous and osseous metaplasia of tracheobronchial tree	[102]

Table 2 Major accomplishments in tracheal surgical procedures

1.	1951: First case of tracheal resection and primary anastomosis [103]
2.	1984: Tracheoplasty using autologous pericardial patch [104]
3.	1989: First description of slide-tracheoplasty in two patients [105]
4.	1994: Reported good results with the slide tracheoplasty in four patients [106]
5.	1996: Use of cadaveric human tracheal homograft [107]

Table 3 Timeline of major accomplishments in trachea transplantation

1. 1979: First tracheal allotransplantation in man [108]
2. 2002: Grillo reviewed and discussed the need for tracheal replacement, distinct from resection with primary anastomosis, and the many efforts over the past century to accomplish this goal experimentally and clinically [6]
3. 2003: Tracheotomy surgery that combined donor trachea cartilage with tissue from his patient. This innovation minimized the potential for rejection and, significantly allowed the patient to communicate normally post-surgery [109]
4. 2004: First human transplantation of a bioengineered airway tissue: first clinical application of a tissue-engineered airway patch obtained seeding autologous muscle cells and fibroblasts on a decellularized porcine jejunal segment [110]
5. 2008: First tissue-engineered airway transplant: a patient affected by end-stage bronchomalacia has been transplanted with the first tissue-engineered airway, obtained using a 6-cm decellularized cadaveric tracheal segment and the recipient's own stem cells to replace the recipient's lower trachea and left bronchus [11]
6. 2010: First vascularized tracheal allotransplantation: the tracheal allograft was wrapped in the recipient's forearm fascia, after revascularization and mucosal reestablishment, the tracheal allograft was moved to its correct anatomical position with an intact blood supply [111]
7. 2010: First windpipe transplant in a child: a 10-year-old boy with a rare condition called long-segment congenital tracheal stenosis has been transplanted with a complete tissue-engineered trachea, autologous bone marrow stem cells were collected, and applied to the graft in situ in the body, to rebuild the cellular component of the trachea [73]

many attempts at tracheal replacement that seems to offer any real promise.

Regenerative medicine, an emerging novel approach in modern medicine, is based on the "re-growing" of living tissue in the laboratory using the patients' own cells (healthy cells extracted from the donor site or stem cells) and on the stimulating the body's own natural healing process by activating the body's inherent ability to repair and regenerate. It has emerged as a potential alternative to tissue or organ transplantation. Innovative regenerative medicine therapies, that aim to heal or reconstruct diseased tissue and support the regeneration of diseased or injured organs, are now available and there are about 70 companies involved in the business of implantable tissue-engineered products; with few already commercially available [10].

The recent advances and developments in tracheal tissue engineering [11, 12] and stem cells [13–15] could very well make the regenerative medicine a reality for tracheal replacement.

Regenerative medicine

Regenerative medicine approach is based on a combination of three elements: living cells, a matrix (scaffold) to support the living cells, and cell-signaling systems to stimulate the cells and their surrounding environment in order to repair, maintain, replace, or enhance tissue/organ function as well as to develop new tissue. Regenerative medicine has recently been accepted as a useful clinical discipline to ensure and enhance the quality of life in patients undergoing organ reconstruction and tissue bioengineering and has already provided functional human organ replacements elsewhere [16, 17]. The interest of tracheal replacement research has therefore turned to guided tissue-engineered regeneration, the only technique that seems to offer any real promise. This has, indeed, been recently demonstrated by the successful clinical application of the first tissue-engineered tracheal construct (Fig. 1) [11]. The approach engineers a functional tracheal graft based on three key components: (1) autologous cells harvesting, in vitro culture, and mesenchymal stem cells differentiation, (2) a human donor trachea decellularization via a detergent-enzymatic method which allows to maintain extracellular matrix structures, and (3) a bioreactor, which permits to reseed the autologous cells on the appropriate (external and internal) matrix decellularized surfaces (Fig. 2). Herein, these three components will be reviewed in relation to reported literature data.

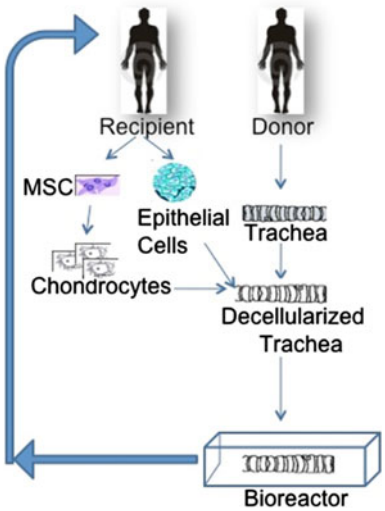

Fig. 1 Flowchart showing the pipeline of translational-clinical research for airway diseases

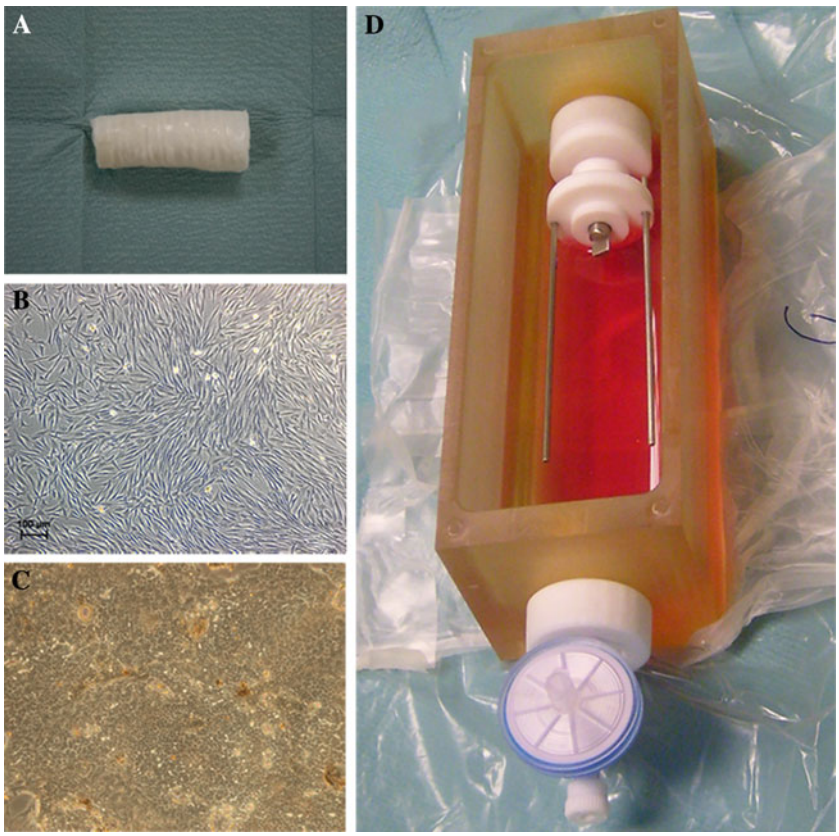

Fig. 2 Key components used to generate the tissue-engineered airway: **a** human donor decellularized trachea, **b** mesenchymal stem cell-derived chondrocytes, **c** epithelial cells, and **d** novel double-chamber bioreactor

Cells

The source and choice of cells are essential issues for replacement of damaged tissue. It has been clearly established that a patient's own cells (autologous cells) are the ideal choice: indeed, both immune rejection problems and regulatory issues can be avoided.

Tracheal tissue is composed of epithelial cells, chondrocytes, collagen matrix, and blood vessels. Epithelial cells can be harvested from nasal or tracheal mucosa, chondrocytes from auricular, costal, nasal septum, or bone joints cartilage, while endothelial and smooth-muscle cells from arteries or veins. The two main challenges concerning the "cellular" part of the tracheal tissue-engineering approach are: (1) the complete epithelialization of tracheal lumen (respiratory epithelial cells), and (2) the development of hyaline cartilage (chondrocytes) with suitable mechanical properties. Several groups have pursued the identification of cell lines and culture conditions that support high levels of cell differentiation and maintain cellular vitality and functionality [18]. However, currently no consensus exists about a well-defined tissue-engineering technique that maximizes cell growth, adhesion, and structural integrity.

Regarding epithelial cells, in vitro cell culture conditions played a main role for the obtainment of an in vivo fully developed respiratory epithelium [19–22]. It has been demonstrated that primary epithelial cells are not always easy to culture and tend to be differentiated rapidly with increased passage number [21, 23]. Recently, Pfenninger et al. [24] suggested that (1) a basal lamina-equivalent of collagen fibers, (2) extracellular factors secreted by fibroblasts, and (3) the creation of an air–liquid interface system are the three key factors necessary for inducing in vitro epithelial cell differentiation. However, these conditions need a fibroblast feeder layer, and are quite complex [24]. Adopting the Rees protocol [25], using specific serum-free medium, bovine pituitary factor, and epidermal growth factor, stable and differentiated cultures of bronchial epithelial cells at the fourth passage have been obtained and have been successfully used to obtain the re-epithelialization of the decellularized tracheal graft [11, 26]. However, some uncertainty about precisely which epithelial sources are best for particular tissue-engineering challenges still remains. Epithelial cells can be harvested from the tracheal, nasal, or bronchial mucosa. In our clinical study, nasal epithelial cells had a proliferation rate that was too fast, and apoptosis occurred in earlier passages than bronchial cells. As a consequence, only bronchial cells were used for graft development [11]. Moreover, in a case of patients with malignant tracheal tumors, both tracheal and bronchial epithelial cells can not be used to reseed tracheal grafts. More detailed studies are necessary to define which respiratory epithelial cell source could provide a more

efficient and effective source with which reepithelialize airway grafts.

Regarding chondrocytes, different chondrogenic sources and different procedures have been evaluated and cartilaginous tissues with different properties have been till now obtained [18]. However, the invasiveness of biopsy, the loco-regional and/or general anesthesia, donor site pain, and limited tissue availability render essential the finding of alternative chondrocyte sources.

In order to obtain a universal cellular source, stem cells, with their capacity for unlimited self-renewal and the production of more committed progenitor cells, have been evaluated. At least three different populations in the trachea and/or upper airway (basal cells [27], keratin-rich cells [28], and secretory cells [29]) that may be stem or progenitor cells have been identified, however, the methods to isolate them and knowledge on the key stem cell properties are still limited. Recently, it has been demonstrated that mesenchymal stem cells (MSCs) can differentiate not only into bone, connective tissue cells, and smooth muscle fibers [30–33] but also into chondrocytes [34], which make them interesting candidates for tissue-engineered tracheal cartilaginous regeneration. Amniotic MSCs, seeded on a polymeric scaffold and implanted in the uterus, allowed to obtain an engineered three-dimensional cartilage suitable for perinatal tracheal regeneration [35]. Along with the possibility to consistently in vitro expand mesenchymal progenitor cells without affecting their differentiation capacity, bone marrow MSCs have drawn research interest because: (1) the use of local anesthesia for bone marrow aspirates averts the risks associated with general anesthesia in critically injured patients, and (2) the possibility to obtain large numbers of each cell types from MSCs cells spars the patients from multiple procedures [36, 37]. However, it has been demonstrated that maintaining differentiated chondrocytes in monolayer culture induces a shift of their biosynthetic profile to a fibroblast-like phenotype [38], with a probably consequent in vivo formation of fibrocartilage instead of hyaline cartilage in the implant [39]. The use of growth factors in MSC expansion medium, prior differentiation, has been shown to have a significant effect on the ability of human MSCs to undergo chondrogenesis [36, 37, 40, 41]. Bone marrow MSCs, cultured in the presence of basic fibroblast growth factor [42] and induced to differentiate, in a controlled way, using transforming growth factor β [43], dexamethasone, insulin, and recombinant parathyroid hormone-related protein (to prevent terminal hypertrophic differentiation of MSC derived chondrocytes and to reduce the risk of calcification of implanted cartilage) [44], allowed us to obtain vital, functional, and phenotypically stable chondrocytes with which re-cellularized the cartilaginous side of decellularized tracheal graft [11, 26] (Fig. 3).

Scaffold

A scaffold should be a structure capable of supporting three-dimensional tissue regeneration, and, as a consequence, composition, biocompatibility, biodegradability, adequate surface properties, three-dimensional architecture, and mechanical properties of the scaffold greatly influence tissue regeneration. Several synthetic degradable

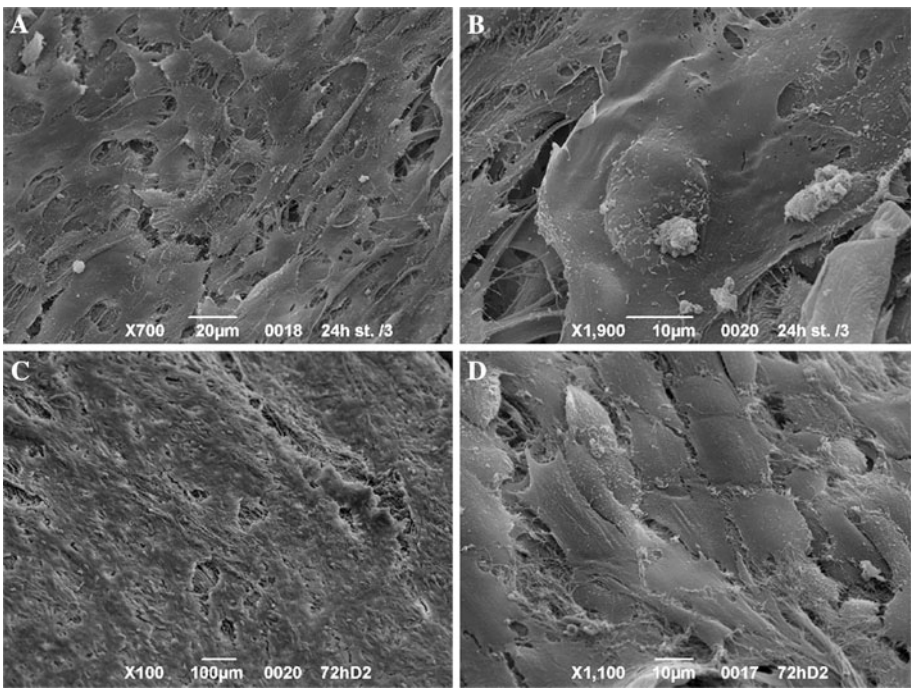

Fig. 3 Scanning electron microscopy (SEM) images, at different amplifications, showing decellularized matrices seeded with chondrocytes after 24-h (**a**, **b**) and 72-h (**c**, **d**) dynamic culture periods

polymers (polyglycol acid, polylactic acid, alginate gel) or biomaterials (collagen, chitosan, hyaluronic derivates) have been evaluated for their ability to support airway reconstruction. However, these constructs are far from clinical applications because their functionality, biomechanics, and viability, correlated to an adequate and continuous vascularization, are not ascertained [6, 26, 45]. Moreover, most of the synthetic or natural matrices seemed unable to resist collapse and, in many occasions, developed early stenosis [46].

Recent studies have demonstrated that synthetic or naturally derived scaffolds, such as collagen-enforced poly-lactic-glycolic acid non-woven mesh [47], multiple-layer scaffold, consisting of a collagen sheet on the inner side (to enhance the immigration of epithelial cells), a polyglycolic acid nonwoven mesh in the middle (to support chondrogenesis) and a copolymer (L-lactide/ε-caprolactone) coarse mesh on the outside (to impart elasticity) [48] or ring-shaped type II collagen sponge reinforced by a graft of poly(ε-caprolactone) [49], developed a tracheal substitute with mechanic properties and gross morphology similar to that of the native tracheal cartilage. Naturally derived scaffolds, such as fibrin/hyaluronic acid composites, have been demonstrated to provide tracheal luminal epithelial regeneration and a favorable environment for chondrocytes to maintain their phenotype and synthesize cartilage ECM [50]. However, either polymeric or natural scaffolds may trigger in vivo inflammation reactions, with consequent graft failure to sufficiently support a permanent shape and size in engineered tissue. Researchers have then evaluated alternative tissue-engineered approaches using scaffold-free cartilage grafts composed of chondrocyte sheets that can be used for the reconstruction of cylindrical tracheal cartilage [51–53]. However, it took more than 2 months in vivo implantation for the constructs to become stiff as the native trachea and it resulted difficult to control the shape of the in vivo cartilage formation [51, 52].

Extracellular matrix (ECM)-derived prosthetic materials, maintaining the natural ECM composition, without releasing toxic biodegradable products or inducing inflammation, have been demonstrated to play an active part in the regulation of cell behavior, affecting cell proliferation, migration, differentiation, and, as a consequence, tissue regeneration [54, 55]. Based on this consideration, recent researches have focused their attention on decellularized matrices as potential scaffolds for the obtainment of a functional, tissue-engineered trachea. The main goal of the decellularization approach is to remove most or all of the cellular and nuclear material from the tissue/organ (making substitutes nonimmunogenic), without or only minimally altering the composition, biological activity, and mechanical integrity of the remaining ECM. Different decellularization protocols have been evaluated to obtain tracheal grafts, and most were highly efficient in removing cellular materials but cause disruption of glycosaminglycans and substantially reduced laminin and fibronectin ECM content, compromising the ability of the scaffold to provide mechanical support during the remodeling process [56–58]. Using a detergent-enzymatic method (DEM) (Fig. 4) [59], animal tracheal matrices characterized by ultrastructure and physical properties unaltered respect native tissue and able not to elicit any rejection response when used as allografts or xenografts have been obtained [60–62], suggesting the possible and promising use of these scaffolds as suitable support for tissue-engineered tracheal constructs. Using the DEM approach, a complete decellularization of human tracheal matrices was obtain (Fig. 5), preserving all ECM structural components (luminal basement membrane include), necessary for cell attachment and repopulation, and native morphological and biomechanical properties [11, 63]. Decellularized human tracheal matrices supported cell adhesion and proliferation, representing an immediate ideal environment for cells and allowing the obtainment of a cellular, functional, tissue-engineered airway [11].

An important issue to consider with a transplanted organ is the challenge of vascularization. Concerning tracheal

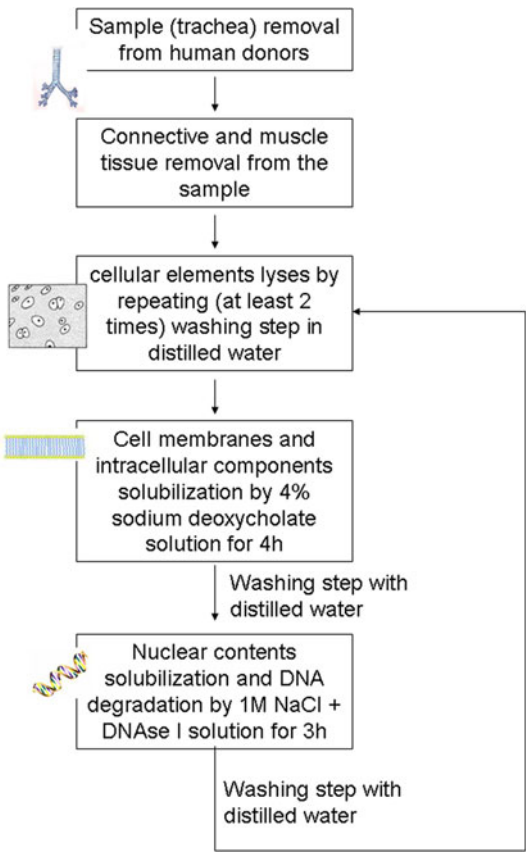

Fig. 4 DEM approach used for tracheal decellularization

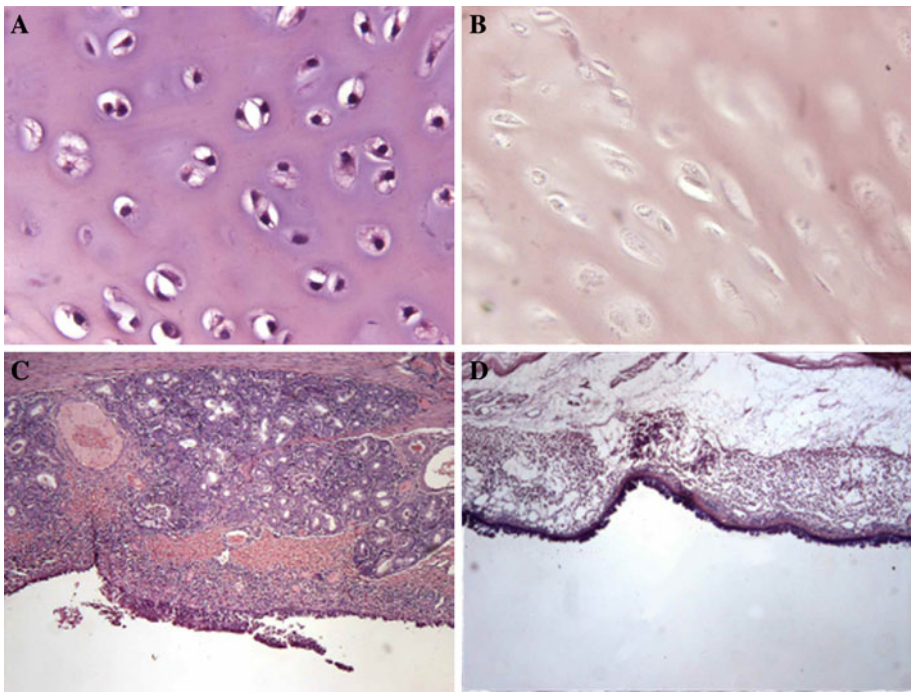

Fig. 5 Hematoxylin and eosin (H&E) staining of transversal sections of native (**a**, **c**) and decellularized (**b**, **d**) trachea. As the figures show, a complete decellularization of both cartilagineous (**a**, **b**, 400×) and epithelial (**c**, **d**, 200×) tracheal side was obtained, preserving all ECM structural components

tissue engineering, a graft which promote rapid functional angiogenesis results to be essential to the restoration of tracheal function, being ischemia (due to poor vascularization) one of the primary risk factors for airway transplantation. In our experience with human bioengineered trachea, after four postoperative days, laser-Doppler analysis showed a rich microvascular bed, and at 1 month post-implantation, a complete revascularization of the graft was confirmed by the mucosal bleeding elicited by biopsy [11]. We therefore consider it possible that the decellularization procedure did not remove all the factors with an impact or an influence on angiogenesis, such as basic fibroblast growth factor (bFGF) and transforming growth factor $\beta 1$ (TGF $\beta 1$), and that decellularized human tracheal matrices, containing growth and angiogenic factors, may serve as a good storage depot during tissue regeneration, and could play a key role in timely in vivo construct revascularization.

Bioreactors

Bioreactors can be used to aid the in vitro development of tissue-engineered tissue by providing biochemical and physical regulatory signals to cells and encouraging them to grow, proliferate, undergo differentiation, and/or to produce extracellular matrix prior to in vivo implantation. A number of bioreactors are currently available and many are under development like the "breathing" dynamic cell–air interface bioreactor for controlled differentiation of stem cells [64].

Regarding tracheal bioreactor-based tissue-engineering technology, it has been demonstrated that intra-scaffold continuous medium flow (using rotational bioreactors) induced the development of a more functional cartilage tissue respect to the static one [65, 66]. Lin et al. [67] demonstrated that the use of an air–liquid bioreactor provided essential mechanical stimuli necessary to promote chondrocyte proliferation and matrix secretion. In another study, using a custom-designed bioreactor, a rabbit neotrachea was fabricated in vivo [68], however, the bioreactor had only a limited success, because the design was suitable only for short tracheal segment grafts (1–2 cm long) and no epithelial cells seeding has been achieved.

The double-chamber bioreactor used to properly repersonalize the world's first stem-cell-based tracheal graft was based mainly on three key requirements: (1) the provision of different culture conditions on either side of the organ wall for the culture of two different cell types, (2) the need to maintain construct oxygenation (mass transport of gases and nutrients) despite the thickness and the length (more than 4 cm) of the implant wall, and (3) the need of autoclavability and the ease of handling [11, 63, 69]. The commercial version of this bioreactor was launched by Harvard Bioscience Inc. and named it the ORGANIZER Series Model 100 "In Breath" bioreactor.

The first human tissue-engineered trachea replacement was highly encouraging, and demonstrated that a cellular, tissue-engineered airway with mechanical properties allowing normal functioning and significant improvement of the airway appearance and free from the risks of rejection can be produced. However, a long-lasting engineering period, high costs, potential risks of cell differentiation instability, and contamination represent the scientific,

clinical, and commercial bottlenecks standing in the way of full integration of this regenerative medicine technology into routine clinical care. It then results clear the need to develop a simple, reproducible, and routine technique that is commercially effective and socially acceptable for a large population of patients.

In situ tissue engineering

The in situ tissue-engineering approach is based on tissue formation directly in the patient's body: a scaffold, placed at the site of a tissue defect, plays the role of a biological incubator to allow host cells to migrate into it and form the regenerated tissue, meanwhile as the patient's body plays the role of a natural bioreactor [70]. Recent studies have demonstrated that the intraoperative or bedside application on scaffold of autologous bone marrow cells or of MSCs, in presence of specific environmental signals, allowed the regeneration of a functional trachea [71, 72]. Developing strategies to facilitate targeting of transplanted MSCs as well as endogenous MSCs to injured tissues could then be a clinically more feasible approach to assist the regeneration of tracheal tissue. Following adequate stimulation, stem cells and progenitor cells can indeed be mobilized out of the bone marrow to circulate into the peripheral blood. The molecular mechanisms that control the mobilization of specific stem cell subsets from the bone marrow are currently being intensely investigated. However, it is believed that boosting the mobilization of these stem cells, using, e.g., specific factors, will provide novel therapeutic approaches for tissue regeneration. In other words, if a co-stimulation occurs in the presence of an appropriate scaffold, the cells are triggered to achieve a strong and high-quality remodeling activity that is better, faster, and more tightly controlled than in any in vitro activity [12]. In a very recent breakthrough trachea transplantation (March 2010) [73], this concept has been successfully utilized in a real-life situation: a decellularized trachea, in situ seeded with autologous bone marrow MSCs (externally) and islands of respiratory cells (internally), has been transplanted in a 12-year-old boy, along with a cocktail of specific boosting/regenerative factors designed to prompt stem cells to grow and differentiate into new tissue once inside the body (erythropoietin as boosting factor, granulocytes colony stimulating factor as recruitment factor and beta-transforming growth factor as commitment factor). Since it was a total tracheal replacement (7 cm), by far the longest section of trachea ever transplanted, a custom-made bioabsorbable stent made of polydioxanone (PDS) has been placed in the inner surface in order to give strength to the new trachea. The patient was weaned off ventilation a few days later, and was immediately breathing very well. During April and May, the patient underwent weekly endoscopies to remove thick secretions. After the stent dissolved, which took about 2 months, the trachea was able to support itself with no signs of collapse or tracheomalacia. The epithelium is now returning, the trachea is healthy, widely patent, supported, well vascularized, and there have been no signs of necrosis.

Conclusions

The trachea is a complex, highly organized structure, and at the same time a perfect model system to study and develop the translational aspects of tissue engineering. The tracheal bioreactor-based tissue-engineering technology consisting of autologous bone marrow-derived chondrocytes and epithelial cells grown on a decellularized donor trachea using a double-chamber rotating bioreactor is a very powerful and successful technology for trachea transplantation. This approach, however, resulted to have bottlenecks standing in the way of full integration into routine clinical care. The in situ tissue-engineering concept, a spin-off from the core technology, could be a very efficient, simple, and a truly natural and globally regulatory issues abiding strategy to perform bedside tracheal engineering replacements. These technologies have not only opened the door to the development of cell-based regenerative therapy in patients with early-stage airway disease, but have also provided the required impetus to the field of stem cell-based regenerative medicine.

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