Whole Organ and Tissue Reconstruction in Thoracic Regenerative Surgery

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Learning Objectives: Educational objectives. On completion of this article, you should be able to (1) identify the key components for bioengineering organs and tissues, (2) determine the associated risks and drawbacks in the field of tissue engineering, (3) identify ethical challenges and considerations in tissue engineering.

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This work was supported by European Project FP7-NMP-2011-SMALL-5 grant 280584-2 (Biomaterials for Tracheal Replacement in Age-related Cancer via a Humanly Engineered Airway), ALF Medicine grant LS1101-0042 (Stockholm County Council) (Transplantation of Bio-engineered Trachea in Humans), the Swedish Heart-Lung Foundation (trachea TE), Dr Dorka Stiftung (bioengineering of tracheal tissue), and the Government of the Russian Federation grant 11.G34.31.0065.

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Abstract

Development of novel prognostic, diagnostic, and treatment options will provide major benefits for millions of patients with acute or chronic respiratory dysfunction, cardiac-related disorders, esophageal problems, or other diseases in the thorax. Allogeneic organ transplant is currently available. However, it remains a trap because of its dependency on a very limited supply of donated organs, which may be needed for both initial and subsequent transplants. Furthermore, it requires lifelong treatment with immunosuppressants, which are associated with adverse effects. Despite early clinical applications of bioengineered organs and tissues, routine implementation is still far off. For this review, we searched the PubMed, MEDLINE, and Ovid databases for the following keywords for each tissue or organ: *tissue engineering, biological and synthetic scaffold/graft, acellular and decelluar(ized), reseeding, bioreactor, tissue replacement,* and *transplantation.*

We identified the current state-of-the-art practices in tissue engineering with a focus on advances during the past 5 years. We discuss advantages and disadvantages of biological and synthetic solutions and introduce novel strategies and technologies for the field. The ethical challenges of innovation in this area are also reviewed.

 ■ Mayo Clin Proc. 2013;88(10):1151-1166

According to the World Health Statistics 2012 report, released by the World Health Organization, there were 57 million deaths in 2008, of which 36 million (63%) were attributed to noncommunicable diseases (NCDs). The top 4 contributors to NCD-related deaths were cardiovascular disease (48%), cancer (21%), chronic respiratory disease (12%), and diabetes mellitus (3.5%).[1] The total number is projected to increase to 52 million by 2030. In addition, approximately 44% of NCD deaths were identified as premature deaths (defined as death at <70 years). The financial impact from this on the health care systems of the world is enormous (Table 1). In addition, costs from mortality, research, pain, and lost social contributions should be taken into account.[2] Hence, the numbers presented in Table 1 are likely underestimates of the actual values.

Most chest diseases are curable with standard medical or surgical treatments, but chronic diseases are usually either difficult to treat or nontreatable. Many chronic heart and lung diseases require allogeneic transplant and lifelong immunosuppressants, which is currently the only curative treatment available. Moreover, congenital or chronic diseases that affect structures such as the trachea, esophagus, or diaphragm cannot be cured and are usually managed with palliative measures. Allogeneic organ transplant for these disorders is currently not available.

Tissue engineering (TE) has emerged as the most promising therapeutic option to address the limits now confronting transplantation medicine and to widen the domains in which transplantation medicine can be applied. It is a young interdisciplinary field of research and clinical applications that focuses on the repair, replacement, or regeneration of cells, tissues, or organs. It requires several key components, including but not restricted to (1) scaffolds or matrices (biological or synthetic), (2) cells (autologous, allogeneic, or xenogeneic), (3) bioreactors, and (4) bioactive molecules (Figure 1). For this review, we searched the PubMed, MEDLINE, and Ovid databases for the following keywords for each tissue or organ: *tissue engineering, biological and synthetic scaffold/graft, acellular and decellular(ized), reseeding, bioreactor, tissue replacement,* and *transplantation.*

We specifically discuss the current developments of organ engineering and TE in the different areas of the respiratory, upper digestive, and cardiovascular systems with a note on related ethical issues.

SCAFFOLDS

Tissue engineering involves the design, evaluation, modification, and maintenance of living cells or tissues embedded in scaffolds. The scaffolds, which can be either biological or synthetic, are expected to mimic the target tissue architecture. They must also have the appropriate physical, chemical, and mechanical properties to enable cell penetration and tissue formation in 3 dimensions, when seeded or reseeded with host cells. For biological scaffolds, it is essential to find an optimal target-specific decellularization method to maintain the biochemical composition, tissue structure, and mechanical properties of the remaining extracellular matrix (ECM) at a sufficient level. For synthetic scaffold applications, issues of design, material selection, manufacturing, and introduction of bioactive molecules should be considered. The large number of patients in need of organ transplant restricts the use of donor-dependent biological scaffolds. This highlights the need for useful synthetic scaffolds, which can be endowed with a greater potential of reproducibility and availability. The advantages and disadvantages of the biological and synthetic scaffolds are presented in Table 2.

Biological Scaffolds

Tissue-engineered biological scaffolds can be used as frameworks for constructed organs and then later transplanted into a patient. However, the process of engineering a biological scaffold is fraught with challenges. The first challenge involves identifying key antigens that play crucial roles in eliciting an immune response. The second involves exploring feasible methods of eliminating or inactivating those key antigens. As biological scaffolds are from cadaver organs, it is essential to avoid immunocompetence. Therefore, these organs are decellularized to remove the major histocompatibility complexes (MHCs) I and II and other cellular components. This can be obtained using different techniques, such as chemical agents (eg, Triton X-100, sodium deoxycholate, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate, and sodium-dodecyl-sulfate), physical processes (eg, agitation), or mechanical processes (eg, perfusion).[11] Although the cellular components

Mayo Clin Proc. ■ October 2013;88(10):1151-1166 ■ http://dx.doi.org/10.1016/j.mayocp.2013.03.011
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TABLE 1. Estimated Mortality Rates and NCD-Associated Costs in Health Care

NCDs	Distribution of top 3 NCD-related deaths in 2008 (%)	Annual No. of deaths (in millions)		Estimated costs for care of NCD in 2009 (in billion US $)	Global economic burden of NCDs		
					Cost-of-illness approach (in billion US $)		WHO EPIC model in 2010 (in billion US $)
		2008	2030		2010	2030	
Cardiovascular disease[1-8]	48	17	30	183	863	1040	15.6
Cancer[1,2,9]	21	7.6	13	…			8.3
Lung					52	83	
Esophageal					6.5	14	
COPD[2,10]	12	4.2	…	833	2100	4800	4.8

COPD = chronic obstructive pulmonary disease; EPIC = European Prospective Investigation into Cancer and Nutrition; NCD = noncommunicable disease; WHO = World Health Organization.

are removed successfully, it is vital that the ECM of the organs and tissues retains both the architecture and the strength of a native organ. The ECM of a decellularized organ provides the essential bioactive proteins to create a microenvironment for cells to maintain adhesion, proliferation, migration, and differentiation during the recellularizing process. Although there are promising results that support the biological scaffold engineering of different organs, such as the trachea, lung, heart valve, and larynx, in preclinical animal studies and human clinical trials, there are still many challenges that lie ahead with the use of this technology.[12-18] It is still donor dependent, and engineering processing in most cases is long, cost intensive, and labor-intensive. The considerable amounts of handling of the organ or tissues increase the contamination risks, which makes it unattractive for routine clinical application.

Synthetic Scaffolds

Synthetic scaffolds represent a novel alternative concept to biological scaffolds because they are not donor dependent and can be tailor-made. Unfortunately, their clinical application is extremely challenging. Thus, they would need to not only mimic the native tissue/organ architecture but also be nontoxic, noncarcinogenic, and nonimmunogenic. They would also have to be biocompatible (ie, capable of supporting host cells once seeded with autologous cells, such as mesenchymal stromal cells) and contain or produce bioactive molecules to promote vascularization to avoid graft necrosis.

In general, bioartificial scaffolds aim to provide a 3-dimensional (3D) substrate for specific host cells to populate and appropriately function in. To serve this purpose, applied biomaterials should be biocompatible (preferably approved by the Food and Drug Administration), with controlled degradation rate with respect to remodelling of the target tissue or organ. Their mechanical, structural, and biological properties should also be engineered based on the regeneration site. To fulfill such requirements,

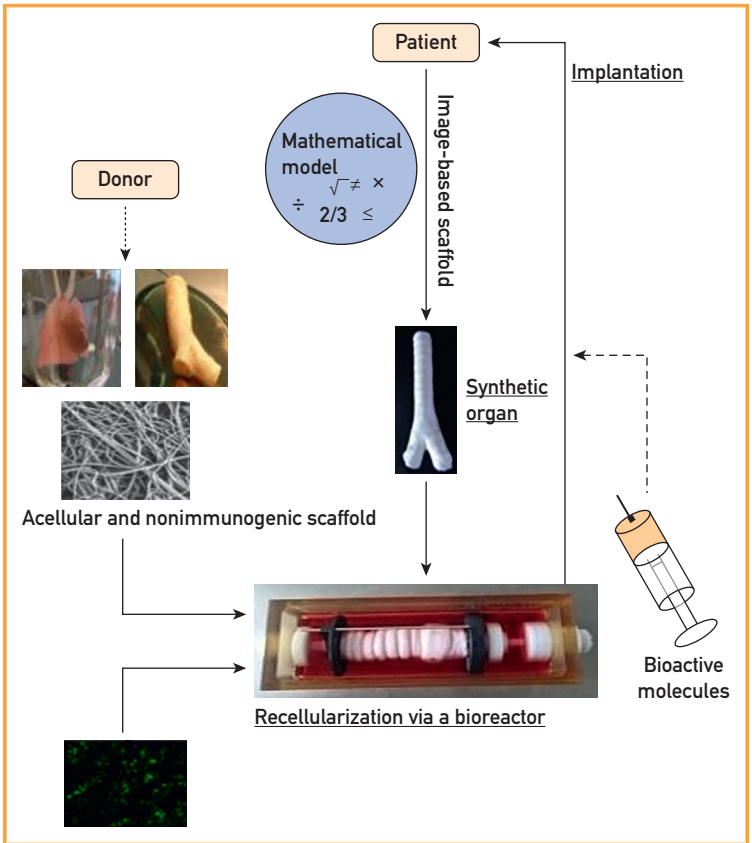

FIGURE 1. Interactions of the different disciplines involved in tissue engineering with a clinical implication.

TABLE 2. Advantages and Disadvantages of Biological and Synthetic Scaffolds

Biological		Synthetic	
Advantage	Disadvantage	Advantage	Disadvantage
…	Organ donor dependent	Donor independent	…
…	Organ dimension dependent on donor	Made to measure	…
…	Long processing time and contamination risk	Short processing time	…
Nonimmunogenic, no MHC I and II	…	Nonimmunogenic	…
Preserved ECM	…	Precoat or manufacture scaffold with specific bioactive molecules or proteins	…
Contains bioactive molecules	…	…	Lack of bioactive molecules
Supports angiogenesis	…	…	No vascularization
Have the potential to grow with the patient	Risk of quicker biodegradation, can lose mechanical stability[12]	…	Scaffold stiffness and rigidity may not grow with the patient

ECM = extracellular matrix; MHC = major histocompatibility complex.

synthetic polymers have appeared as appropriate candidates for several reasons: (1) their reproducibility facilitates control over the chemical and mechanical properties, (2) they can be processed through different scalable manufacturing methods to form complex 3D structures, and (3) surface treatment and/or biomolecule additions can be applied to improve cell-scaffold interaction.

For a clinical use, designing the 3D target organ is the first step. Usually, the 3D geometry of the patient organ, available on either computed tomography or magnetic resonance images, is delivered to a manufacturing group to decide on the right material and fabrication method. Special attention has to be paid to the anatomical shape, porosity, layer-based orientations, and interconnectivity of the organ. Currently, different manufacturing techniques can be used, such as solvent casting (particularly leaching), phase separation, electrospinning, freeze drying, and rapid prototyping or a combination of these techniques, depending on the complexity of the target organ. To have an integer cell-seeded scaffold, improvements of biological cues of the engineered synthetic scaffolds are performed. Translation of the scaffold to preclinical studies can be considered as the next step. Focusing on application of bioartificial scaffolds in thoracic surgery, it is worth mentioning that despite progressive developments with synthetic scaffolds in animal studies, there are still considerable scientific challenges involved in bringing bioengineered products to the clinic.

CELLS

An ideal engineered tissue or organ is one that possesses a desired specific cell population that demonstrates homogeneity, ability to survive, efficient engraftment capacity, and low immunogenicity. In these aspects, stem cells play an important role during the TE processing of the biological or synthetic scaffolds. The bone marrow contains abundant amounts of autologous stem cells, such as hematopoietic stem cells and mesenchymal stem cells (MSCs). Bone marrow and peripheral blood-derived MSCs are proliferative in nature, are capable of differentiating into mesenchymal derivatives, and are relatively immune privileged compared with other somatic cells in the human body.[19,20] They can release several growth factors and anti-inflammatory cytokines, which regulate endothelial and epithelial permeability and thus reduce severity of inflammation. Preclinical animal models and patient studies have found the capabilities of MSCs in terms of repairing and regenerating injured tissues and organs.[21,22] Ensuring the preservation of the organ matrix during TE processing is essential. This is because bioactive molecules, such as collagen, fibronectin, and laminin, on the matrices can cross-interact with stem cells to remodel damaged tissues or organs through cell adhesion, proliferation, homing, migration, and differentiation processes. To improve the outcomes of seeding tissues or organs, the method of cell delivery can play an important role. One technique involves delivering cells in low-viscosity solutions

Mayo Clin Proc. ■ October 2013;88(10):1151-1166 ■ http://dx.doi.org/10.1016/j.mayocp.2013.03.011
www.mayoclinicproceedings.org

or cross-linking them with molecules or binding agents. Another strategy is to deliver cell patches that can adhere to the surface of the wound. The microenvironment in tissue and organs produced by the scaffold can protect, activate, guide, and direct differentiation of implanted cells.

BIOREACTOR

The aim of using a bioreactor is to provide ideal conditions for tissue and organ regeneration and support the requirements of the native tissue. Basically, one can use the organism of the recipient as an in vivo bioreactor or ex vivo system.[23,24] The ex vivo device is designed to enable controlled standardized conditions to engineer tissues or more complex organs.[25] The bioreactor system needs to include physiologic, metabolic, and biomechanical parameters suited for the target tissue type that is being engineered. Ideally, monitoring systems (eg, camera, fluid-handling hardware, sensors, and appropriate software) are useful tools for aiding the engineering process. It should also incorporate other motorized technologies: cell delivery, "feeding" media or solutions, and other systems, such as perfusion or agitation. Other general features to include for a clinically applicable bioreactor include the following:

- Ability to sterilize the bioreactor and its components or environmental-friendly disposables
- Ability to customize organ or tissue chamber
- Allow standardized manufacturing of the bioreactor
- Simple and safe handling
- Possibilities to adjust parameters due to measured parameters
- Safety and control functions

From the ongoing research in this field, specific requirements and needs will dictate the manufacturing of certain elements in the coming next-generation tissue or organ bioreactors. Application of TE will most probably become routine in the clinical treatment of various chest disorders and diseases. Further efforts in the development of bioreactors must be made to improve the process of engineering tissue and organs.

BIOACTIVE MOLECULES

In the field of transplantation medicine, the use of either a conventional donor source or engineered tissues or organs constantly causes problems. Three common problems are:

- Early or chronic ischemic conditions due to poor vascularization
- Immunologic responses by the host
- Graft contamination and infection

To overcome these hurdles and reach clinical success, additional strategies are necessary. Different pharmacologic agents and bioactive molecules, administered systemically or locally, can activate, promote, and support the endogenous self-regeneration capacity. Examples of such substances are proangiogenic factors, such as epidermal growth factor and basic fibroblast growth factor, homing factors such as SDF-1, or nanoparticles with antibiotic or antimycotic properties. However, to make their applications for TE safe, it is important to fully understand the organ-specific biological effects of pharmacologic agents that are administered in a clinical setting. Erythropoietin has anti-inflammatory and antiapoptotic effects on cells when administrated at high doses but could have potential adverse effects because it belongs to a class of potent pharmaceuticals.[26] Another agent that may be used for TE application is granulocyte colony-stimulating factor. It functions by mobilizing progenitor cells such as endothelial progenitor cells and hematopoietic stem cells from the bone marrow into the peripheral blood. Mesenchymal stem cells have also been reported to possess anti-inflammatory characteristics.[23,27]

Previous studies have demonstrated that the addition of growth factors, hormones, or other bioactive substances has a strong impact and a beneficial effect on the outcome of a tissue or organ transplant.[27] Ongoing research regarding underlying functions and pathways involved in cell-cell interaction, proinflammatory or anti-inflammatory responses, cell homing, and differentiation will likely help to reinforce endogenous regenerative mechanisms.

UPPER AND LOWER RESPIRATORY SYSTEM

Larynx

Total laryngectomy is a common treatment in patients with severe laryngeal disorders. A conventional allotransplant of the larynx is highly challenging and associated with lifelong immunosuppression. In fact, few cases of such a transplant have been documented, and the clinical acceptance of the method is poor because of

the difficult procedure.[28] Therapies based on TE, using either biological or synthetic scaffolds, are therefore desired. Recently, the human larynx has been decellularized and characterized for its anatomical, physiologic, and biomechanical properties.[18] Fishman et al[29] decellularized the dorsal cricoarytenoid muscle of a rabbit and investigated its potential use for laryngeal regeneration. The technology to develop a larynx exists, but further efforts must be made before a clinical translation will be possible. The development of a bioartificial cricoarytenoid unit to be implanted in patients who need a total laryngectomy would probably be the most clinically relevant undertaking.

Trachea

In the trachea, both benign and malignant diseases may be curable by surgical resection of the affected segment and a subsequent end-to-end anastomosis.[30] Unfortunately, this standard approach is only possible if the diseased portion of the airway does not exceed approximately half of the total length of the trachea in an adult or one-third of the total length in a child.[30] A clinically relevant (>6 cm) tracheal transplant is surgically complicated because of the delicate network of the blood supply. The first clinical application of a fully tissue-engineered tracheal patch was performed in 2003 using a decellularized porcine jejunum as scaffold, which was seeded with autologous muscle cells and fibroblasts.[31] However, this method is rather inappropriate for full circumferential defects because of the lack of mechanical strength of the transplant.

Different experimental approaches have been evaluated, and to date the most promising and clinically applicable method is the use of a biological scaffold treated with detergents, including deoxycholate and DNase, for decellularization.[32] The obtained scaffolds preserve nearly native mechanical characteristics and retain proangiogenic factors, such as vascular endothelial growth factor and fibroblast growth factor 2. In 2008, the first clinical transplant using a biological scaffold was performed to replace the left main bronchus in a patient.[33] Since then several other transplants following that protocol have been successful in both adults and children.[34] A method of allogeneic tracheal graft transplant, originally described by Rose et al[35] in 1979, was modified in 2010 by Delaere et al.[36] The tissue was processed in a heterotopical position. The patient was treated initially with immunosuppressants, and the graft was later moved after reepithelialization into an orthotopic position. However, the need for multiple operations, long processing times, and immunosuppressants during the initial phase leaves this as an unattractive option to treat patients.

In 2011, the first synthetic-based, Y-shaped scaffold was used to replace the entire distal trachea, including the carina and both main bronchi.[27] The graft was customized based on the patient's computed tomogram and seeded with autologous stem cells. To eliminate contamination and/or graft necrosis, a novel concept was used to increase the regenerative capacity of the organism and the bioactivity of the artificial scaffold by using different growth and boosting factors, such as erythropoietin, granulocyte colony-stimulating factor, and others.[23,27] Although these initial experiences strongly suggest that TE can play a major role in the clinical treatment of tracheal disorders, we are far from any routine application, and further extensive experimental research must still be done.

Lung

For patients with end-stage lung disease, drug administration can slow the degeneration process, but the only other treatment option would be allogeneic organ replacement. The lung is a complex 3D structure composed of many different cell types. It has a delicate vascular and bronchial network, which is difficult to mimic in the laboratory. Another fundamental issue to consider when attempting to construct an artificial lung for transplant is whether it can handle an adequate gas exchange to allow the survival of the patient. This requires mature cells at the time of implantation. Huh et al[37] developed a laboratory lung-on-a-chip. This chip, which is based on a porous polydimethylsiloxane membrane, has been demonstrated to support cell seeding of human lung epithelial cells and capillary endothelial cells. Mechanical forces can be applied to the membrane and allow a more physiologic condition for the cells. The lung-on-a-chip can be used to study bacterial and nanoparticle penetration in the lung and perform drug testing for pathologic conditions, such as pulmonary edema.[38] The lung-on-a-chip concept has certainly advanced the possibilities for investigating basic lung functions. The

Mayo Clin Proc. ■ October 2013;88(10):1151-1166 ■ http://dx.doi.org/10.1016/j.mayocp.2013.03.011
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drawback to this technology is the inability to reach the complexity of an entire lung because the device cannot support a sufficient surface area for gas exchange that resembles that in a clinical setting.

Currently, there are also a number of studies that have investigated the architecture of a whole lung in animals models and human cadavers.[11,15,39,40] In experimental studies in rats, tissue-engineered lungs have been created that were able to participate in gas exchange for several hours, and repopulation of cells was observed on scaffolds.[15,25] Thus, the recent development in lung TE demonstrates some promising results, but many challenges and obstacles remain and require further research before clinical translations can become a reality.

UPPER DIGESTIVE SYSTEM

The esophagus is a conduit that connects the pharynx to the stomach. Although it might seem like a simple tube at first glance, it is an intricate organ and dysfunctions commonly have large negative effects on the patients' quality of life. Surgical removal of the esophagus and replacement by stomach or intestine tissue is the current option for many disorders, such as cancer, congenital defects, and traumatic injuries. This surgery is extensive and associated with an approximately 2.5% mortality and 26% to 41% morbidity.[41] Endoscopic treatments for early cancers have less mortality, but a common complication is stenosis.[42]

Tissue engineering graft techniques may help to overcome difficulties associated with traditional surgical therapies. Tan et al[43] recently made progress in full-wall replacements by transplantation of MSC-seeded acellular small intestine submucosa (SIS). Esophageal defects were replaced with either seeded SIS or SIS alone. The seeded SIS showed faster recovery, re-epithelialization, and less inflammatory response.[43] Approaches with full-circumference replacements by stenting with nonseeded SIS have been burdened by complications such as mediastinitis, lack of re-epithelialization, stent migration, and stricture formations.[44] An earlier approach with SIS without stenting also revealed a high incidence of stricture.[45]

Recently, 2 clinical trials have examined the regeneration of the esophagus, both focusing on preventing stenosis after endoscopic removal of early cancers. Badylak et al[46] used tubularized xenogeneic ECM (SurgiSis), and stents were initially placed on the ulcer and removed 9 to 18 days after surgery. Four months after surgery, histologic analysis and cytokeratin staining demonstrated a nearly complete mature epithelium. These results suggest that ECM can be used to induce lesion healing. However, in all patients strictures occurred locally in the region where the esophagus was uncovered by the ECM. Other complications included small perforations and stent migration.[46] The other clinical trial, conducted by Ohki et al,[47] performed an approach in 9 patients using cell sheets grown from autologous oral mucosal epithelial cells. Cell sheets were transplanted to the wound bed after mucosal dissection, and a beneficial healing effect was observed in the follow-up, notably with only 1 patient experiencing a stenosis.

Decellularized esophagi to function as transplantable grafts have been evaluated in rodent and porcine models.[48-50] All studies have demonstrated angiogenesis induction, ability to host seeded cells, and low in vivo immune response after subcutaneous transplant. To date, no orthotopic replacement of a decellularized esophagus has been realized. In conclusion, TE for the partial replacement of the esophagus is an exciting and promising field. Initial attempts have found stenosis prevention and accelerated mucosal healing. However, more research, which also takes neural and muscular function into consideration, is needed before a whole esophagus can be reconstructed.

CARDIOVASCULAR SYSTEM

Heart

An alternative strategy to current treatments of advanced heart failure would be to stimulate recovery of the myocardial function by implanting autologous stem cells. Initial clinical studies conducted by Orlic et al[51] and others[52-54] found that heart function was modulated, but the mechanisms behind this remain unclear. Other clinical trials that used autologous bone marrow mononuclear cells or MSCs to treat ischemic heart disease could not elucidate any beneficial effects when compared with controls.[55] The assumption that stem cells can survive after implantation in the absence of supporting tissues and that implanted cells spontaneously differentiate into a desired cell type is apparently inaccurate.

In more recent studies, it was demonstrated that the heart cell matrix could play a key role in supporting maintenance and differentiation of progenitor cells. Human embryonic stem cell–derived cardiomyocytes survived in an intact myocardium after transplantation.[56] However, in ischemic conditions, cells could only survive when coinjected with a commercially available matrix (Matrigel). In another TE approach, Miyahara et al[57] produced a cell sheet of MSC and reported an improved myocardial function when this was transplanted on the surface of the infarcted heart of rats. It has been reported that at least 2 types of multipotent progenitor cells (c-kit$^+$ and Islet-1$^+$) are involved in the regeneration of the adult rat heart in response to ischemia reperfusion.[58] Both cell types were found to be present in the adult human heart as well and can potentially be differentiated into most cell types of the heart.[59] c-kit$^+$ cells are currently being used in phase 1 clinical trials in patients with ischemic cardiomyopathy (Stem Cell Infusion in Patients with Ischemic Cardiomyopathy trial).[60] However, Islet-1$^+$ cells from an autologous source still remain to be tested. At present, it appears that these cell types in combination with cardiac matrix proteins provide the best combination for future successful cardiomyoplasty.

Taylor et al[61] succeeded in decellularizing rat hearts and found that the architecture of the coronary vessels and all other compartments of the heart was preserved. Recellularization of the heart muscle with neonatal cardiac cells and the coronary vessels with aortic endothelial cells demonstrated that the support tissue of the heart could guide the development of a functioning beating heart. This indicates that the decellularized heart still contains key proteins and also sustains a specific 3D architecture for maintaining cell survival and differentiation of transplanted cells.

Aortic Valve

There is a need for novel strategies to tissue engineer heart valves. The decellularized heart valve has been extensively studied in small and large animal models. Long-term functionality follow-up has revealed maintained physiologic hemodynamic properties for allogeneic valve prostheses.[61-63] In 2010, da Costa et al[64] described initial orthotopic implantations of decellularized aortic homografts in 41 patients. Their 3-year follow-up showed low rates of calcification, adequate hemodynamics, and stable structural integrity. However, further observations are still needed to determine the long-term functional impact. Cebotari et al[65] demonstrated a significant improvement in 38 patients treated with a decellularized homograft (pulmonary valve) in pulmonary position compared with conventional glutaraldehyde-fixed bovine jugular vein and cryopreserved homografts. The evaluation (up to 5 years) revealed that there were no signs of an increase of the transvalvular gradient, cusp thickening, or aneurysmatic dilatation. Xenografts remain suboptimal because decellularized valves provoke an immune response with consecutive degradation processes.[66]

A potential alternative would involve novel synthetic materials and process modeling.[67,68] The current trends in synthetic heart valve concepts engage biodegradable polymeric solutions that gradually dissolve and are replaced by endogenous tissue.[67,69] Recently, hybrid heart valves were studied more extensively using electrospinning and decellularization technology.[70,71] However, no in vivo data are available as yet. Further studies are necessary to improve the currently available methods and materials.

Diaphragm

The diaphragm is the most important respiratory muscle. For optimal respiratory function, it is obviously important that both hemidiaphragms are intact.[72] Failure of the diaphragm is usually due to congenital or acquired hernia or dysfunctions in innervation.

Various biological scaffolds have been used for diaphragm repair. These scaffolds include bovine fascia lata and pericardium, human lyophilized dura, human cadaver dermis (Alloderm), and an acellular sheet of porcine dermal collagen.[73-75] Absorbable biosynthetic materials offer lower risks of infection and the ability to grow with the patient, but the drawbacks of these scaffolds are incomplete muscular ingrowth and recurrent hernia formation.[76,77] Currently, the most commonly used synthetic materials for diaphragm replacement are nonbiodegradable (eg, polytetrafluoroethylene [Gore-Tex] or polypropylene mesh [Marlex]).[78] These prosthetic patch closures are still inadequate because there are deformities and an increased incidence of bowel obstruction.[78] Autologous structure, such as prerenal fascia,

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rib structures, and various thoracic and abdominal wall muscle flaps, can be alternatives. However, the use of local muscle flaps can reduce the abdominal domain. This may leave a large thoracic cavity on the side of the hernia, with an associated risk of atrophy of a denervated muscle.[79-82]

Engineered diaphragm muscle tissue must provide a patch of functional skeletal muscle, and it should withstand atrophy and carry a low risk of infection. It is essential to find the appropriate cell source. Skeletal muscle regeneration relies on a cell source with myogenic potential.[82,83] Amniotic fluid–derived stem cells can be a safe and abundant source of such cells.[84] Fuchs et al[85] have developed mesenchymal amniocyte cell–based engineered tendons for partial diaphragmatic replacement. This method used human cadaveric dermis (AlloDerm) and SIS. The results indicated that these matrices induced neovascular formation, thus providing means for survival of implanted myoblasts.[85] There are also a few studies that have investigated decellularized diaphragms per se. These studies indicate that that the ECM of this muscle has a unique composition and structure. In summary, although there have been some encouraging studies that have attempted to use both biological or synthetic scaffold, more ongoing research into the reconstruction of a diaphragm is necessary. The various cell types used to investigate TE in different thoracic tissues and organs are listed in Table 3 and shown in Figure 2.

CELL DELIVERY

Studies of TE of whole organs, such as the heart and lung, have produced remarkable successes.[15,86] Nonetheless, there is a huge need for further investigations on, for example, optimal cell delivery methods and culture conditions to produce an adequate functional recellularized organ. Determining the optimal number of stem cells required for a functional organ remains a challenge.[87] Hence, it is important to quantify the ultimate degree of cellularization of the tissue in terms of the number of seeded cells.

A useful theoretical tool for this purpose is mathematical modeling. It predicts cell density at a given point in the tissue by solving a set of mathematical equations. These equations embody the biological and physical processes operating during the reseeding and incubation time of the organ (ex vivo), such as cell attachment, migration, proliferation, and apoptosis. The

TABLE 3. Summary of Different Cell Types Used in TE of Thoracic Tissues or Organs

System	Organ	Engineering	Cells	Outcome
Respiratory	Trachea	Tissue	Muscle cells and fibroblasts[31]	Not applicable for fully circumferential defects due to lack of mechanical strength[17]
		Organ	Epithelial cells, chondrocytes,[33] and MSCs[27]	Nine clinical applications in humans, degradation of the ECM architecture during long-term storage
	Lung	Organ	Human alveolar epithelial and endothelial cells,[37] human umbilical cord endothelial cells[15]	Successful repopulation of the scaffold was observed; lungs established gas exchange in vivo for several hours
Upper digestive	Esophagus	Tissue	Bone marrow MSCs[43]	Quicker recovery from surgery, faster re-epithelialization, and fewer inflammatory responses
		Tissue	Autologous oral mucosal epithelial cells[47]	Clinical trial in 9 patients, treatment of mucosal defects
Cardiovascular	Heart	Tissue	Human bone marrow mononuclear cells,[55] MSCs,[57] Isl1+ cells,[58,59] cKit+ cells,[58,59] hemapoietic stem cells[53]	Heart function was modulated or no beneficial effects to placebo
	Aortic valve	Tissue	Endothelial cells[62]	Less calcification or thrombotic formation
Other	Diaphragm	Tissue	Mesenchymal amniocytes, MSCs[85]	Repair of diaphragm lesions in rats and lamb
		Tissue	Myogenic progenitor cells[83]	Repair of diaphragm lesions in rats

ECM = extracellular matrix; MSC = mesenchymal stem cell.

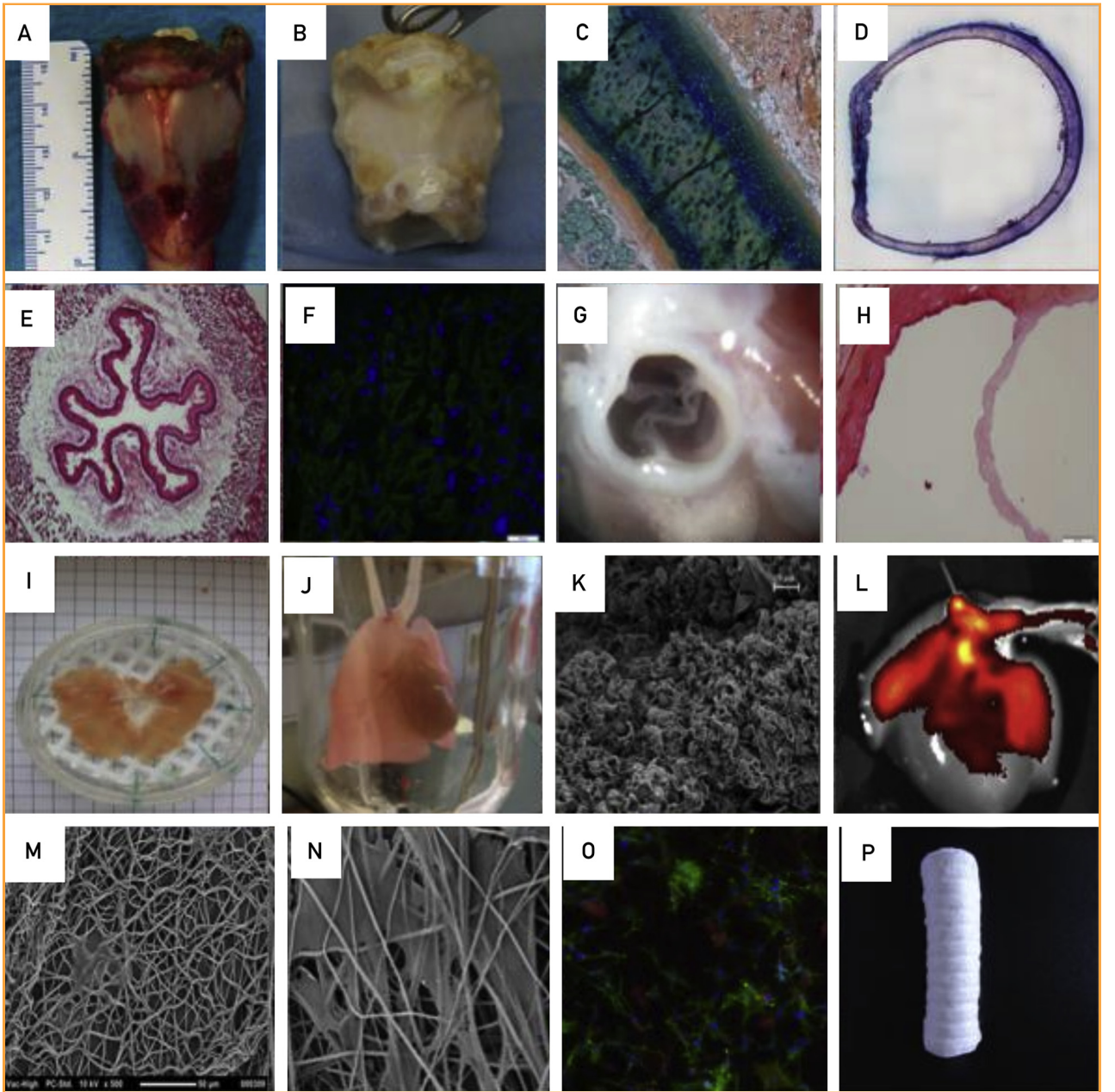

FIGURE 2. Native (A) and decellularized (B) human larynx. Movat-stained (C) and Masson trichrome–stained (D) decellularized trachea from a rat. Native esophagus from a rat stained with hematoxylin-eosin (E) and 4′,6-diamidino-2-phenylindole (DAPI) and phalloidin (F). Macroscopic view (G) and hematoxylin-eosin staining (H) of a decellularized aortic valve from a rat. Native diaphragm from rat (I), with partly decellularized heart and lung, macroscopic (J) and scanning electron microscopic (SEM) (K) views. L, Reseeding process: bioluminescence image (IVIS Spectrum imager) showing genetically labeled cells in decellularized heart and lungs. M, An SEM view showing the nanostructure of a decellularized rat trachea. N, An SEM view of reseeded synthetic nanofibers. O, Confocal image of a reseeded synthetic trachea scaffold with DAPI-labeled (blue) and phalloidin (green)–labeled cells. P, Macroscopic view of a clinical graded synthetic-based tracheal scaffold.

Mayo Clin Proc. ■ October 2013;88(10):1151-1166 ■ http://dx.doi.org/10.1016/j.mayocp.2013.03.011
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equations contain parameters whose values characterize the rate of these processes, such as perfusion flow rate, cell doublings, and cell migration. Mathematical models can predict how many cells are retained within the organ after reseeding when experimental conditions vary. To date, mathematical models have been applied in the field of TE to focus on tissues with relatively simple structure, such as cartilage and bone.[88,89] To achieve realistic mathematical models for cell delivery in complex thoracic organs, such as the heart and lung, is considerably more challenging. Larger numbers of different cell types must be delivered in a spatially structured manner through the bulk of the tissue. Another important factor to consider during cell delivery to whole organs is the loss of cells encountered when they are infused through the preexisting vasculature. Ott et al[86] demonstrated in a reseeded heart that nearly 50% of the seeded cells were lost within 20 minutes. A mathematical model that describes the flow of media during the injection and the kinetics of binding of cells to the vessel walls may help identify the infusion pressure and cell concentration at which the seeding efficiency is optimal. These models will prove useful for the development of therapies for the airway when cells are injected directly into the bronchus.[90] However, the way seeded cells repopulate an organ and self-assemble into a 3D functional tissue still remains to be understood and requires a firmer theoretical basis.

Mathematical modeling is clearly useful for thoracic TE, as shown in the case of the trachea TE (Figure 3), because so much important information can be addressed simultaneously. First, it can predict the optimal culture conditions that will maximize the extent of cellularization. Second, it can provide an estimated number of seeded stem cells that are essential for mobilizing the natural regenerative processes of the body. This is needed to attain functional integration of the organ with the host tissue. It can also provide a wider understanding of the enhanced self-renewal and migration characteristics that give stem cells their reparative properties.

IMMUNOGENICITY

The most important cell surface molecules involved in tissue or organ rejection are MHC I and II.[27,32,91] Major histocompatibility complex I is expressed on all human cells, whereas MHC II is located on antigen-presenting cells, such as monocytes, which activate a T-cell–mediated immune response.[92] In xenograft (animal to humans) transplantation, cell surface antigens from animals that are not expressed in humans, such as the oligosaccharide α-Gal, are introduced, and hyperacute immune responses with a functional failure of the organ or tissue occurs within minutes.[93] By decellularizing organs or tissues, it is expected that these become nonimmunogenic. Quantitative criteria for a sufficient decellularized tissue have been presented as remaining DNA of less than 50 ng of double-stranded DNA per milligram of dry weight, DNA fragment length less than 200 base pairs, and a lack of visible nuclei.[94] However, the importance of remaining DNA is controversial because studies have reported lack of immunologic responses despite residual DNA while promoting cell homing, migration, and differentiation.[95]

The decellularization concept, using various methods, is a powerful tool that aims for the reduction of the scaffold's immunogenicity. However, the decellularization method can provoke an immunologic reaction on its own, due to residual detergent.[96]

Organ and tissue rejection is a complex mechanism and much remains to be understood. Some novel insights have emerged regarding immunologic mechanisms during the initial period after implantation. The crucial cell subpopulations that appear to be involved are M1 and M2 mononuclear macrophages. M1 and M2 differ considerably in their characteristics. M1 cells release a high amount of proinflammatory bioactive substances, such as interleukin (IL) 1, IL-6, IL-12, and IL-23 and tumor necrosis factor. When recruited to a site of transplantation, this release causes further tissue damage and may lead to organ or tissue rejection. In contrast, M2 cells are a regenerative cell population. M2 cells produce high levels of IL-10, suppress IL-12 and IL-23, and inhibit the release of proinflammatory cells and their proliferation.[97] Several studies have found that the presence of intact cells or cell remnants on biological scaffolds leads to an M1-promoted inflammatory response. In contrast, decellularized tissue elicits no similar reaction but rather a constructive tissue remodeling with an increased M2 population. It is likely that an unknown threshold

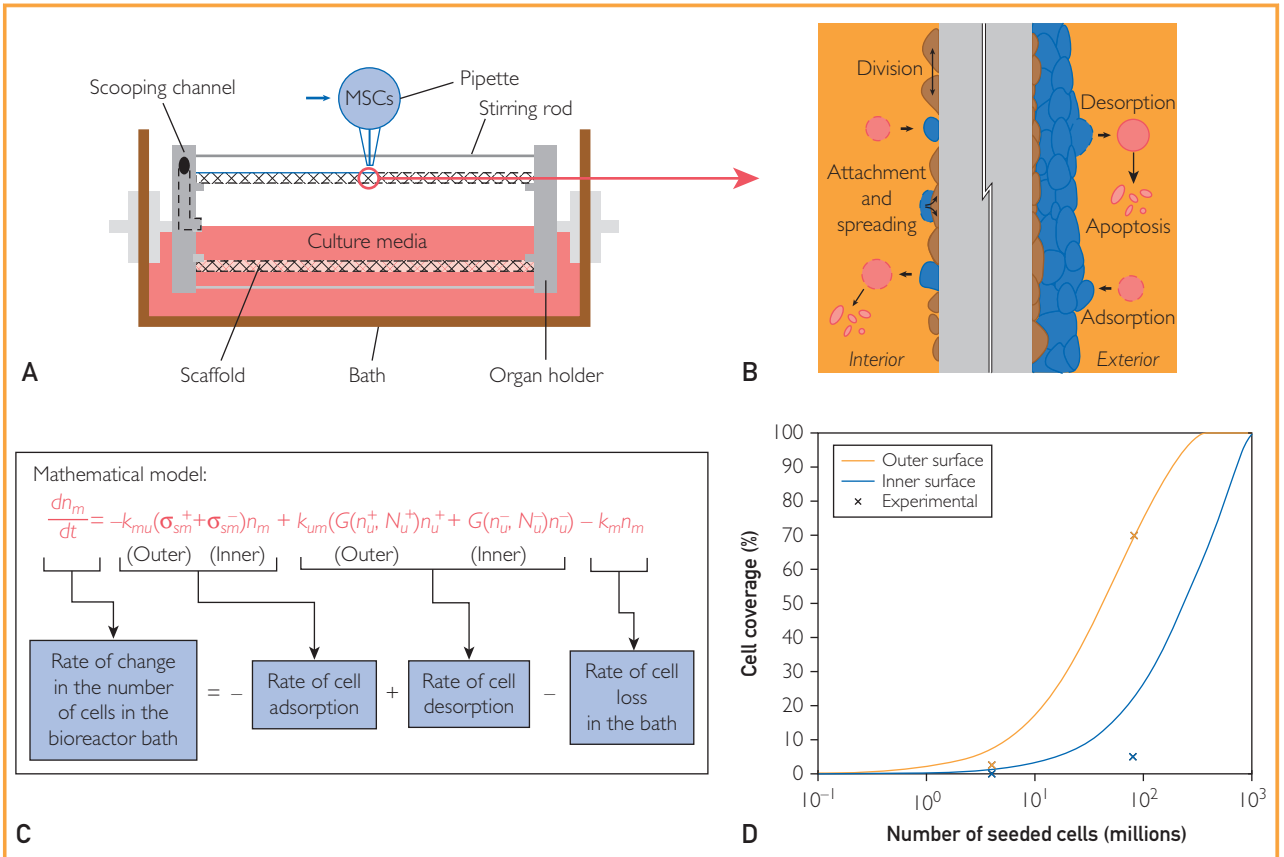

FIGURE 3. A, Mathematical modeling of seeding and incubation of tracheal scaffolds using the *in-breath* bioreactor (Harvard Bioscience Inc). Consideration of the processes of cell adsorption, desorption, proliferation, and apoptosis (B) was used to derive the mathematical model. C, The equation used to calculate the number of cells in the bioreactor bath. D, The mathematical model was used to predict the degree of cell coverage on the inner and outer surfaces of the scaffold at the end of the incubation, as a function of the initial seeding density.

level for cellular material exists that influences whether M1 or M2 populations become dominant.[98]

A potential immune response is dependent not only on the exposure time (host to the foreign antigen) but also on the processing method and the types of material and species involved. Further strategies must be developed and underlying pathways detected to prevent acute or chronic inflammation in transplanted organs or tissue. To date, there are no existing clinical, immunologic, long-term results regarding tissue-engineered organs or tissues.

ETHICAL CHALLENGES

Three main ethical issues must be addressed in the field of tissue and cell engineering research. First, it is inherent that current work is performed in accordance with the highest standards of research ethics. Second, recruitment of study participants must be made with the utmost care to ensure the safety and integrity of individuals. Lastly, adequate registries must be established together with published studies to provide a basis for the transition from research and innovation to therapy.

The shortfall of organs for transplant, lack of options for transplanting some tissues, and the accompanying adverse event profile associated with this treatment modality suggest that there likely is a substantial number of persons who will give their consent to become involved in cell and TE research. The willingness of individuals to participate underlines the importance of competent teams of researchers, institutional

Mayo Clin Proc. ■ October 2013;88(10):1151-1166 ■ http://dx.doi.org/10.1016/j.mayocp.2013.03.011
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review board or human ethics committee approvals, and the ability and readiness to disseminate findings in this area of clinical science.

Care must be taken in recruiting study participants to avoid misconceptions—participants must understand that research holds no guarantees of success and that there are risks and unknowns involved.[99] The recruitment of participants must balance the need to be able to ascertain adverse events and complications against the duty to provide fair access to all who wish to participate. This may mean involving somewhat healthier participants in initial trials and then moving on to those with greater comorbidities and psychosocial challenges.

Finally, it is important that investigators share the findings of their work, especially because this is a part of the implicit contract they have with human study participants. Registries are important in maintaining the best up-to-date knowledge both to present to potential new study participants and to provide a basis for deeming TE and cell engineering of particular organs and systems no longer experimental. Researchers must be willing to condemn the premature therapeutic use of TE as unethical.[100]

CONCLUSION

To date, the only optimal therapeutic solution for end-stage organ failure is allogeneic organ transplant. However, the shortage of donor organs and the need of immunosuppressive medication are major drawbacks in this concept.

Use of TE technologies to develop tissues and organs for clinical transplant appears to be the next promising alternative. Biological scaffolds fulfill all essential requirements of an ideal matrix for cell seeding and subsequent in vivo application. However, major improvements are still needed, for example, to avoid the need for donor tissue, to establish rapid production systems, and to customize grafts. Theoretically, synthetic materials have the potential to overcome all disadvantages of natural scaffolds. However, further studies of natural tissues and organs are needed to mimic their properties and characteristics. Mathematical models can serve as useful tools in this context because of their predictive ability and for their role in putting the seeding, incubation, and reparative processes of tissue-engineered organs on firm theoretical foundations.

Abbreviations and Acronyms: **3D** = 3-dimensional; **ECM** = extracellular matrix; **IL** = interleukin; **MHC** = major histocompatibility complex; **MSC** = mesenchymal stem cell; **NCD** = noncommunicable disease; **SIS** = small intestine submucosa; **TE** = tissue engineering

Grant Support: This work was supported by European Project FP7-NMP-2011-SMALL-5 grant 280584-2 (Biomaterials for Tracheal Replacement in Age-related Cancer via a Humanly Engineered Airway), ALF Medicine grant LS1101-0042 (Stockholm County Council) (Transplantation of Bioengineered Trachea in Humans), the Swedish Heart-Lung Foundation (trachea TE), Dr Dorka Stiftung (bioengineering of tracheal tissue), and the Government of the Russian Federation grant 11.G34.31.0065.

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