Bioengineering of Trachea and Esophagus

Soichi Shibuya, Natalie Durkin, Matías Garrido, Paola Bonfanti, and Paolo De Coppi

Contents

Soichi Shibuya, Natalie Durkin and Matias Garrido Flores contributed equally with all other contributors.

S. Shibuya · N. Durkin
Stem Cell and Regenerative Medicine Section, Great Ormond Street Institute of Child Health, University College London, London, UK

M. Garrido · P. Bonfanti
Epithelial Stem Cell Biology and Regenerative Medicine Laboratory, The Francis Crick Institute, London, UK

P. De Coppi (✉)
Stem Cell and Regenerative Medicine Section, Great Ormond Street Institute of Child Health, University College London, London, UK

Specialist Neonatal and Paediatric Surgery, Great Ormond Street Hospital, London, UK
e-mail: p.decoppi@ucl.ac.uk; paolo.decoppi@gosh.nhs.uk

D. Eberli et al. (eds.), *Organ Tissue Engineering*, Reference Series in Biomedical Engineering, https://doi.org/10.1007/978-3-030-44211-8_18

Abstract

Tissue engineering offers huge potential as a novel strategy to treat complex congenital and acquired conditions of both the trachea and the esophagus where standard therapy has failed or current options for organ replacement fall short. Existing approaches have involved the use of scaffolds, cells, or a combination of both to reconstruct damaged organs, however increasing evidence suggests that hybrid techniques with exogenous cell delivery enhances tissue regeneration while reducing inflammation. While many cell lines have been used, increasing focus on the use of stem cells of mesenchymal origin holds promise, although the mechanism through which remodeling occurs remains unclear.

In vivo animal models have provided real insights into the use of these techniques for human therapy. While initial success in partial-thickness and patch defects models has been translated into clinical studies in humans, repair of circumferential defects remains altogether more challenging due to difficulties with stenosis, luminal collapse, and anastomotic leak. More work is required to establish safe and standardized methods for circumferential organ transplantation in this field prior to advancement to human candidates with open reporting of outcomes. Here, we review current approaches, evaluate the existing evidence, and discuss the future of tissue engineering of the trachea and esophagus.

1 Introduction

In the past decade, translation of tissue-engineered organs into clinical practice is more promising than ever owing to rapid progress in research focusing on cell biology and tissue regeneration. The major goal of bioengineering is to fabricate a biocompatible organ as a viable substitute for transplantation into patients in whom no other treatment options exist. This novel strategy has huge potential to save and improve the quality of life of patients suffering from either congenital or acquired disease. Simultaneously, however, it can pose ethical and economical concerns, particularly in clinical trials developed on a compassionate basis. In this chapter, we describe developing research on bioengineering of the trachea and the esophagus.

2 Tracheal Bioengineering

2.1 Introduction

The trachea is a vital organ which cannot be replaced by any other. Its dysfunction results in difficulty breathing, which is directly associated with mortality. A lack of alternative treatment for end-stage tracheal failure raises demands for novel

treatment strategies, such as tracheal bioengineering. In adults, the most frequent cause of losing a healthy trachea is cancer treatment requiring bulk resection due to massive tumor invasion emerging from the esophagus or lung. If the length of resected trachea is greater than 50%, it is considered impossible to reconstruct by end-to-end anastomosis (Chiang et al. 2016). In children, the most significant indication for tracheal reconstruction is congenital tracheal malformation. This includes tracheal agenesis, tracheal stenosis, and severe tracheomalacia, all of which require intensive treatment immediately after birth and can result in devastating consequences despite painstaking medical efforts. Over the past decade, slide tracheoplasty has become the most reliable surgical treatment for tracheal stenosis, but despite significant improvement in mortality compared to other options it is technically demanding and can result in postoperative bronchomalacia, which is occasionally lethal (Butler et al. 2014). In addition, accidental events such as foreign body ingestion, especially of lithium button battery, and swallowing of caustic substances can devastate tracheal function. Due to the severity and urgency of these scenarios, compassionate application of innovative therapy is more likely to be adopted in the emergency setting than in chronic disease. Clinical trials in tracheal tissue engineering have been performed on a compassionate basis and have significantly contributed to the progress of this field. The first tracheal reconstruction was introduced in 1950 (Belsey 1950). A large defect in the trachea due to massive tumor resection was restored by the combination of steel wire scaffold and a free fascial graft from the thigh. Although eventual outcomes were not satisfactory, with collapse of the structure and granulation in the lumen, respiratory function was temporally maintained and epithelization on the luminal surface was observed. Current literature suggests that tracheal reconstruction using bioengineered grafts is complicated and potentially risky but not impossible if adequate tissue regeneration is induced by a biocompatible method (Delaere and Van Raemdonck 2016).

In the following sections, we discuss the fundamental knowledge, which is a prerequisite to formulating a bioengineering strategy, and the current achievements in clinical translation of the tissue-engineered trachea.

2.2 Anatomy and Embryology

The size of the trachea in an adult human is approximately 12–15 cm in length and 2–3 cm in diameter. The lumen is covered with a mucous membrane layer, composed of pseudostratified columnar epithelia resting on the basal lamina. The tracheal epithelium bears cilia on its apical surface, 0.25 nm hair-like projections which expel external particles through a polarized beating movement. Scattered throughout the cilia are mucus secreting goblet cells which help maintain the humidity in the airway and trap microorganisms. There are numerous blood and lymphatic vessels running throughout the lamina propria between the mucosa and the tracheal wall. The function of the vessels includes supply of oxygen and nutrition, control of intraluminal heat and removal of harmful substances trapped by epithelia. The wall of the tracheal tube is composed of elastic and collagen fibers, supported by

horseshoe-shaped cartilages, which contributes to the contractility and stability. The tracheal cartilages cover only the ventral and lateral side of the trachea, leaving the dorsal side open, which allows the esophagus to bulge as food passes through. Muscle fibers running alongside the cartilage control air flow to the lungs by changing the length and the diameter of the trachea in synchronization with respiration. These muscles are controlled by the coordination of the specific autonomic nerves, namely, sympathetic fibers from the thoracic sympathetic trunk and parasympathetic fibers from the vagus nerves and their recurrent laryngeal branches. Furthermore, the muscle fibers also function as a barrier against harmful particles by triggering strong constriction when such substances are inhaled or aspirated, resulting in coughing.

Understanding normal embryonic development is important to establish a strategy for organ bioengineering. The development of airway begins in the fourth week of gestation in humans, where a laryngotracheal diverticulum emerges from the foregut endoderm just caudal to the level of the fourth pharyngeal pouches. The laryngotracheal diverticulum is a pouch-like structure located at the ventral side of the foregut, from which the respiratory bud sprouts and elongates caudally, giving rise to primordial lungs. Subsequently, the trachea becomes a distinctive structure from the original foregut tube, but the mechanism of separating the trachea from the esophagus is not well understood. There have been roughly two contrasting theories for explaining this process. One theory suggests that the respiratory system develops as a result of rapid outgrowth of the laryngotracheal diverticulum. This theory considers the tracheal primordium buds as a separate structure from the foregut during the subsequent stages of development. An alternative theory hypothesizes the growth of mesenchymal ridges, which appear between the laryngotracheal diverticulum and the foregut. Fusion of the ridges creates the tracheoesophageal septum which divides the foregut into the ventral and dorsal part, differentiating the trachea and the esophagus. Several studies using animal models have attempted to resolve the controversy and it is likely that the physical separation of the foregut tube plays a key role in the organogenesis, but this assumption is yet to be proven. After separation from the foregut, the tracheal primordium starts formulating the functional airway. The endodermal cells lining the tracheal lumen differentiate to the epithelium and the glands, while the mesodermal cells give rise to cartilage, connective tissue, and muscle.

2.3 Strategy for Tissue Engineering

The main role of the trachea is to allow filtered air to pass into the lungs while trapping and clearing harmful particles. This unique function is achieved by the characteristic cell layer covering the luminal surface of the tube. As such, the fundamental idea of tracheal bioengineering is to generate a conduit lined with ciliated mucosa capable of mucus secretion and ciliary movement. In addition, the graft needs to be airtight to prevent air leakage and robust enough to maintain its structure in the mediastinum. These requirements raise two major questions; how to

create a three-dimensional (3D) tube-shaped scaffold and how to populate it with cells.

In order to facilitate clinical translation, biocompatibility of the graft is essential. Due to its function as a barrier between external airflow into the internal environment of body, the trachea is constantly exposed to foreign substances. As such, the immune system is more active than in other organs, making allogenic tracheal transplantation complicated; severe immune rejection frequently occurs, leading to a poor success rates. Any graft therefore needs to be immune tolerant, while retaining the immunological barrier function. The other crucial factor for successful graft implantation is adequate vascularization, especially if the graft covers a circumferential defect. Unlike lung, liver, or intestine, the trachea does not have a truncal blood supply, with a segmental vascular network providing nutritional and immune capabilities. This lack of major vessel connecting the trachea would not allow a graft to draw sufficient blood by vascular anastomosis. Therefore, techniques to promote vascularization, such as a pericardial patch, muscular patch, or prevascularization in vivo, is likely to be mandatory for preventing necrosis and collapse of the graft.

In summary, tracheal bioengineering for clinical application consists of three parts; obtaining biocompatible scaffolds, populating this with cells able to generate a functional mucosal layer, and establishing a proper blood supply.

2.4 Scaffolds

The major role of scaffolds is to provide a footing where cells can adhere and colonize. The scaffold must be mechanically robust to maintain structural integrity in order to resist extrinsic pressure induced by neck flexion and negative pressure during inspiration. Conversely, it needs to be flexible enough to fit in the narrow space without damaging neighboring vulnerable structures, such as the esophagus and aorta. Currently, materials commonly chosen as potential sources can be categorized into two types, synthetic and biologically derived scaffolds.

The advantage of synthetic scaffolds is the adjustability of the size according to recipient need and prompt availability when required. In early attempts, solid prostheses were directly used to reconstruct the trachea but this resulted in unacceptably high mortality associated with severe stenosis and fistula formation (Neville 1982; Toomes et al. 1985). These complications were secondary to severe inflammatory reactions of adjacent tissues causing granuloma and erosions. As such, solid prostheses are no longer used for clinical trials and many researchers began to use porous constructs, which can facilitate ingrowth and migration of indigenous cells. However, reconstruction using porous tubes has achieved limited success in the proximal airway while incidence of erosion and fistula formation remains high in the distal trachea (Maziak et al. 1996). Based on these results, the focus has shifted to biodegradable polymers, which gradually degrade in vivo, accommodating the surrounding environment. Biodegradable materials may especially benefit pediatric patients, allowing tissue growth beyond

the initial size of the graft once this has completely degraded. Traditionally, Poly (glycolic acid) (PGA), poly(lactic acid) (PLA), Poly(lactic-co-glycolic acid) (PLGA), and polydioxanone (PDS) have been used as biodegradable scaffolds. PGA is the simplest linear polyester, with a high melting point, high solubility in water, and low solubility in organic solvents. Due to its hydrophilic nature, constructs made of PGA quickly uptake water and lose their mechanical strength. PLA has less hydrolytic properties than PGA and thus its degradation is slow. This characteristic is advantageous in terms of sustainability of mechanical strength, but can be problematic by remaining too long after implantation. PLGA is hybrid polymer of PGA and PLA, which enables tuning of the degradation parameters according to the ratio of glycolic acid and lactic acid. PDS is a unique material commonly used as a surgical suture. The tensile strength of PDS is lower than other synthetic materials; however it is more durable with a significantly slower degradability. All these materials have the advantage of being FDA-approved and are already in use as surgical sutures and implants, making their use in clinical translation straightforward. The major limitation of polymer structures is their hydrophobic nature and smooth surface, which may prevent adherence of cells to the scaffold. Recently, 3D printing technology combined with electro spinning methods enables nano-level surface modification of synthetic materials. In this state-of-the-art technique, polycaprolactone (PCL) has been the preferred choice due to its strength and long absorption time despite a low porosity (Jang et al. 2014; Park et al. 2019). Nevertheless, synthetic materials should be adopted only following thorough evaluation of their safety and biocompatibility in preclinical studies.

Biologically derived polymeric materials, such as collagen, fibrin, and hyaluronic acid sponges, have been used in a number of preclinical studies for partially repairing tracheal defects. However, grafts solely made from these materials lack the durability to maintain the mechanical strength required when implanted into a circumferential tracheal defect. Recently, scaffolds derived from natural organs by decellularization, in which indigenous cells are removed through perfusion or circulation of detergent and enzymatic solutions, have emerged as promising alternative (Conconi et al. 2005). By removing native cells, the expression of the major histocompatibility complex (MHC) becomes almost null, significantly decreasing immunoreactivity of non-autologous tissues. This technique, which is applicable to either donated human cadavers or size-matched animals, can convert xenogenic organs into natural scaffolds without the need for immunosuppression. Decellularized organs conserve structured extracellular matrix (ECM), which arguably retains cytokines and growth factors containing essential information for cell growth and maturation. Composition of the ECM is generally organ-specific, thus, using a decellularized trachea is likely the most efficient to establish proper differentiation of seeded cells. It is also reported that decellularized scaffolds tolerate cryopreservation for prolonged periods, indicating the possibility of an off-the-shelf availability, which is important for universal clinical translation and potential commercial distribution (Urbani et al. 2017). The downside of decellularized scaffolds is the inability to modify the mechanical properties. Donor organs tend to become less

durable after decellularization; over-treatment should therefore be avoided as scaffolds lose mechanical strength with degradation of the ECM resulting in technical difficulty at anastomosis. Residual chemical reagents are also harmful, suppressing cell ingrowth and even causing cell death, necessitating vigorous washout during the procedure. Consequently, the decellularization protocol for the trachea tends to take long time (Aoki et al. 2019; Butler et al. 2017; Conconi et al. 2005). Conversely, however, insufficient treatment can leave native cells in scaffolds as tracheal chondrocytes are relatively stable and cartilage is hard to perfuse due to its density which could result in immune rejection on implantation. Therefore, optimization of the protocol as well as careful quality control is crucial to make decellularized scaffold to be satisfactory for clinical use.

2.5 Cells

In order to enhance biocompatibility, it is important to achieve sufficient growth of cells on the graft prior to transplantation. Use of recipient autologous cells is undoubtedly advantageous with potential to make the graft immune-tolerant. The dominant cell types in the native trachea are epithelial cells lining the luminal aspect and chondrocytes inside the cartilaginous matrices. If possible, constructing a muscle and nerve network is desirable to establish the physiological function of controlling air flow and the defense reaction to noxious aspiration. It is however challenging, because it not only demands a population of smooth muscle and nerve cells, but also connection of this with the autonomous nervous system.

Types of cells used in previous studies vary from mature cells to stem cells. Mature cell lineages, such as epithelial cells, chondrocytes, and skeletal muscle cells, have an advantage of already being differentiated and functional, whereas stem cells, such as mesenchymal stromal/stem cells (MSCs) and induced pluripotent stem cells (iPSCs), retain a high proliferative potential and the ability to differentiate into different types of cells. Although there is no consensus on the most efficient combination of cell types to be seeded, recapitulating the epithelial–mesenchymal interaction observed in embryonic development by co-seeding both endoderm- and mesoderm-derived cells is conceivably valuable. However, there is a lack of reliable method to appraise colonization of cells after seeding. Neocartilage formation, which is an area of persistent chondrocytes surrounded by remodeled ECM, is often reported as an index of fresh colonization of chondrocytes, but its association with functionality is hard to determine (Maughan et al. 2017; Park et al. 2019; Zhu et al. 2016).

The source of epithelial cells can be from biopsy of either nasal or bronchial mucosa. Owing to the good availability of autologous epithelial cells, seeding epithelial cells on the tracheal lumen has already been performed in several studies, demonstrating prevention of mucus plugging and distal airway infection. However, it is unclear whether seeded cells can survive long-term or just temporarily cover the surface of airway until recruited endogenous cells overlay the lumen. Clinical cases

report that the engrafted bioengineered trachea eventually obtains a functional mucosal layer, but the process is slow and takes months to complete (Elliott et al. 2012). This suggests that the ultimate epithelial coverage was gained by migration of the recipient's native cells rather than ingrowth of seeded cells.

Chondrocytes, the other principal cell type, play an important role in maintaining integrity and elasticity of the graft by generating cartilage. Considering native tracheal cartilage is composed of hyaline cartilage, it is reasonable to isolate cells from body parts enriched with hyaline cartilage, such as the trachea itself, ribs, knees, and nasal septum. The ear contains elastic cartilage that has also been shown to be possible source of chondrocytes, which can ultimately produce hyaline cartilage (Fuchs et al. 2002). Obtaining a specimen from these anatomical sites, however, requires invasive procedures, which represents a significant hurdle for clinical translation. In an effort to overcome this problem, researchers have recently shifted to focus on pluripotent cells as a cell source of chondrocytes.

MSCs are widely used for tissue engineering, as they have the potential to differentiate into various mesenchymal lineages, including osteoblasts, chondroblasts, and myoblasts. Ease of accessibility is a significant advantage, as isolation is possible from adipose tissue, bone marrow, and amniotic fluid, which can be obtained without highly invasive procedures. In addition, many preclinical and clinical trials have demonstrated the safety of using MSCs in in vivo experiments. Furthermore, MSCs are not only useful as a source of chondrocytes, but also appear to be promotors of engraftment by interaction with the endogenous immune system (Elliott et al. 2017; Seguin et al. 2013). The variability of chondrogenic potential depending on the source of MSCs is, however, an ongoing issue, and how MSCs contribute to tissue regeneration is yet to be fully understood (Diekman et al. 2010).

There is increasing interest in the implication of patient-derived iPSC in bioengineering, which have the potential to be expanded indefinitely in an undifferentiated state retaining the pluripotency. Studies demonstrate differentiation of iPSCs into epithelial cells and chondrocytes in response to tissue-specific culture conditions, successfully generating hyaline cartilage in animal models (Diekman et al. 2012; Zhu et al. 2016). One of the major limitations of the use of iPSC is the time required to obtain pluripotency and to induce proper differentiation, which could be a practical problem on translation to clinical applications. For antenatally diagnosed congenital tracheal malformations, utilization of cells derived from amniotic fluid is likely to be a promising alternative. Amniotic fluid contains abundant cells including stem cells (AFSCs), which have been shown to differentiate into multiple cell lineages, and can be easily obtained from amniocentesis between prenatal diagnosis and delivery, allowing enough time for cell growth and preparation of a graft.

In addition to choice of cell, the technical method for seeding cells on the scaffold is important, as well as the maturation period prior to in vivo transplantation. Wide variation in seeding techniques has been reported ranging from superficial delivery to micro injection. The number of cells to be seeded is a further variable which may impact outcome. It is generally acknowledged that at least 1 x 10^6 cells/cm^2

epithelial cells are required to cover the intraluminal area of the scaffold. Nevertheless, the type of the scaffold may impact cell proliferation and colonization, so this must be optimized according to individual protocol (Butler et al. 2017). Some researchers have developed tailored bioreactors which can generate a continuous flow of culture media, which may facilitate cells to grow in vitro. Populated grafts are generally kept in a static condition before entering a dynamic culture for the purpose of allowing cells to adhere on the scaffold.

2.6 Animal Models

Bioengineered grafts are thought to contribute to organ restoration in two separate fashions. One is through direct colonization of implanted, exogenous cells, which remodel surrounding native tissues, and the other is through a paracrine effect via cytokines secreted by seeded cells or preserved in natural scaffolds, recruiting endogenous cells to the implanted site. The paracrine effect also potentially promotes remodeling and vascularization of the graft by stimulating immune cells in vivo. Animal studies have been performed attempting to understand how each mechanism contributes to tissue repair, ultimately aiming to maximize the effect of tissue-engineered grafts on tissue remodeling.

The first in vivo transplantation of a tissue-engineered trachea in an animal model was reported by Vacanti et al. (Vacanti et al. 1994). Seeding of chondrocytes obtained from the shoulder of newborn calves on a sheet of nonwoven PGA mesh produced cylindrical cartilages. After subcutaneous implantation in a mouse for 4 weeks, the grafts were used to substitute a circumferential defect created in immune deficient rats. Death of all animals from respiratory distress was assumed to be secondary to an inability to clear secretions, highlighting the necessity of functional epithelium. A fetal lamb model where the trachea was augmented by a tissue-engineered cartilage patch was subsequently developed as a possible fetal approach for congenital tracheal stenosis (Fuchs et al. 2002). In this model, chondrocytes were derived from either fetal ear or tracheal ring and seeded on PGA sheet, followed by in vitro maturation in bioreactors for 6–8 weeks. This had promising results, with fetal survival, engraftment of the patch, and epithelialization on the luminal side.

Partial tracheal resection models in rabbits have been widely utilized to evaluate the biocompatibility of newly invented materials (Grimmer et al. 2004; Shin et al. 2015). Advantages of this model include simplicity of the surgical technique and higher survival rate compared to rodent models. Park et al. fabricated a multilayered implant containing epithelial cells derived from nasal mucosa on the inner layer and chondrocytes derived from auricular cartilage on the outer layer by utilizing a 3D printing technique (Park et al. 2019). They repaired a semicircumferential defect created in the rabbit trachea with the artificial patch and obtained a trachea-like ciliated epithelial layer 6 months after surgery. However, cartilage formation was limited even after 12 months, suggesting the patency of the

airway was maintained by the original trachea rather than the patched neocartilage.

Pig models are useful for assessing the feasibility of circumferential transplantation prior to human clinical translation due to their robustness and anatomical comparability to humans. Go et al. compared outcomes of tracheal implantation in pigs using decellularized homologous tracheas with bone marrow-derived MSC (BM-MSC) and epithelial cell seeding (Go et al. 2010). Maintained graft integrity was seen in the cell-seeded group. The graft without MSCs resulted in severe luminal collapse and the graft without epithelium was significantly contaminated with bacterium and fungus, suggesting the importance of repopulation with both chondrocytes and epithelial cells.

2.7 Clinical Trials

Several clinical trials for substituting a severely impaired trachea with an engineered graft have been reported. Failing to achieve appropriate tissue regeneration results in stricture and collapse of the graft. Initially, pioneers struggled to overcome the period immediately after transplantation during which the graft is vulnerable to cell ischemia and external pressure. Pre-vascularization of grafts in a rich vascular environment, such as the omentum and pericardial membrane, has been shown to be an effective option to improve adaptation of grafts. Delaere et al. revascularized a cadaver allograft in the recipient's forearm using the radial artery and successfully transplanted this into a 4.5 cm tracheal defect with vascular anastomosis (Delaere et al. 2010). However, the time taken to obtain a sufficient blood supply means this process may not be feasible in emergency settings. In such cases, interposition of a pedicled wrap is likely to be the solution to shorten the pre-vascularization period. In terms of prevention of early disintegration, securing the lumen by placing a stent is arguably essential until tissue remodeling progresses and the structure of the graft becomes strong enough. Despite a number of trials, the best procedure to accomplish this goal is yet to be validated.

The first case of circumferential replacement of the proximal airway with a tissue-engineered graft was performed on an adult patient affected with severe bronchial stenosis and malacia due to post-tuberculous chronic bronchitis (Macchiarini et al. 2008). The scaffold was obtained by decellularization of the trachea retrieved from a human cadaver and repopulated with nasal epithelial cells and MSC-derived chondrocytes. The stenotic left main bronchus was replaced with the graft and vascularization promoted by interposition of an omental wrap. In this patient, signs of an establishing vascular supply were observed 4 days after implantation with subsequent restoration of the mucosal layer.

The first clinical application in a pediatric patient was led by a group in the UK in an 11-year-old-boy with long-segment congenital tracheal stenosis. This was initially repaired by an autologous patch tracheoplasty, but scarring of the patch and severe bronchomalacia required a stent placement, which resulted in creation of an

aortotracheal fistula at 3 years old. Repair by implantation of a homologous graft was successful, but required ongoing stents for recurrent stenosis, which eventually lead to an acute hemorrhage due to stent erosion. Repeated failure of treatment and the devastating condition of the patient rendered him a candidate for tissue-engineering therapy. The scaffold was obtained by decellularization of the cadaveric trachea from a 30-year-old donor, matched to the recipient's trachea size. Because of the urgent clinical need, repopulation of the graft was performed during the surgery with hematopoietic stem cells derived from bone marrow aspiration. Human recombinant erythropoietin (hrEPO), G-CSF, and transforming growth factor β (TGF-β) were injected into the graft to promote angiogenesis, indigenous MSC recruitment, and chondrocyte differentiation. The omentum was mobilized and interposed between the transplanted trachea and the heart to prevent perforation and increase the vascularity of the graft. Bleeding from the luminal mucosa of the graft was observed 1 week postoperatively, suggesting an established blood supply to the mucosa. Although the intratracheal stent was periodically replaced after surgery, the graft eventually became stable allowing him to be stent free (Elliott et al. 2012; Hamilton et al. 2015). After some minor alterations, this resulted in a good manufacturing practice (GMP) compliant method to produce a decellularized tracheal scaffold with cell-seeding (Elliott et al. 2017). Sadly, however, the subsequent application of this method in a 15-year old girl with congenital tracheal stenosis resulted in a lethal acute airway obstruction postoperatively. The lack of a stent was deemed pivotal, and based on their experiences prolonged stenting posttransplantation is now advocated. This case emphasizes the requirement of a significant evidence base before further clinical use and full disclosure of negative as well as positive clinical outcomes.

2.8 Conclusion

For many decades, surgeons and scientists have striven for a solution to devastating tracheal disorders. Significant progress in cell biology and scaffold manufacture has realized clinical translation with long-term reports demonstrating adequate epithelialization and stabilization of grafts. These primary successes seemed a promising novel alternative for patients with limited other treatment options. Unfortunately, however, not all cases have been successful. This has led researchers back to the laboratory to find the solution; a deeper understanding of the mechanism of tissue regeneration will be key to help refine and improve existing techniques. Clinical lessons from these early successes and failures must also be harnessed. As the trachea is a truly vital organ, luminal collapse and occlusion debilitates the patient's condition immediately. As such, close monitoring for signs of stricture and blockage is crucial to prevent graft failure during the precarious postoperative period. Finally, trials of novel tissue-engineered therapies in humans must not be rushed and multidisciplinary discussion and ethical committees are essential prior to planning surgery, even when limited or no alternatives are available.

3 Esophageal Bioengineering

3.1 Introduction

Complex congenital and acquired esophageal pathologies may require esophageal substitution to restore anatomical continuity. This continues to present a significant challenge to both pediatric and adult surgeons as traditional surgical techniques carry significant morbidity. Tissue engineering techniques offer a promising alternative to treat these conditions, with different strategies required depending on the nature and depth of damage caused to the esophagus.

In the pediatric population, the primary indication for esophageal replacement is esophageal atresia. Faulty embryonic separation of the esophagus and trachea results in an abnormal connection between the two organs. In the majority of cases this results in a tracheoesophageal fistula (TEF) in which a primary anastomosis is usually possible. In approximately 10% of patients, however, limited distal esophagus exists (van der Zee et al. 2017). In this circumstance, primary anastomosis may not be feasible due to a large tissue deficit, resulting in the requirement for an esophageal substitute. Even patients undergoing primary anastomosis may require an esophageal substitute regardless in the eventuality of recurrent anastomotic leak or recurrent fistula, albeit infrequently. Although less common, severe esophageal strictures refractory to endoscopic intervention may also require consideration of esophageal replacement, secondary to caustic injury, significant gastro-esophageal reflux, ischemia, radiation exposure or anastomotic stricture.

Despite the advent of organ transplantation in the 1950s, current techniques for esophageal allografts are limited entirely by the segmental vascular supply to the esophagus from multiple arteries, rendering this extremely technically challenging in adults and impossible in neonates. The combination of this and other transplant-associated issues such as scarcity of organ availability, need for immunosuppression and infection risk precludes orthotopic donor transplantation from being a viable treatment option for esophageal replacement. The concept of esophageal replacement is long standing and initial attempts in children began in the first half of the twentieth century with jejunal, colonic, and gastric constructs. Over the last 50 years, while refinements have been made and outcomes have improved, techniques for surgical replacement remain essentially unchanged. All three techniques have respective weaknesses; gastric pull-ups are associated with worse reflux, colonic transpositions have a higher risk of redundancy and delayed emptying with an unknown malignancy risk, and jejunal interpositions have a significant risk of anastomotic leak and graft loss (Gallo et al. 2012). Additionally, in rare situations, failure of these replacements with no appropriate esophageal substitute precludes further reconstruction and renders the patient unable to feed orally.

While esophageal malignancy in children is extremely rare, esophageal cancer is the seventh most common cancer worldwide in adults with over 500,000 new cases in 2018 (World Cancer Research Fund; https://www.wcrf.org/dietandcancer/cancer-trends/esophageal-cancer-statistics). Where resection is possible, the standardized surgical option is esophagectomy with reconstruction. Gastric tubularization remains

the primary reconstructive technique due to technical ease and safety, although colonic transpositions or free or pedicalized jejunal grafts are also used when use of the stomach is not possible. Regardless of technique, this is a major undertaking; even with the introduction of minimally invasive techniques, in a meta-analysis of over 14,000 patients, in-hospital-mortality was 4%. This reflects both the underlying health status of this population and gravity of the operation (Zhou et al. 2015). As such, overall and disease-free survival have been the primary aims with less focus on long-term functional outcomes despite over 70% of patients reporting long-term dysphagia in a recent a systematic review, with additional troublesome symptoms of reflux, dumping syndrome, and delayed gastric emptying (Irino et al. 2017). With ongoing improvements in oncological treatments and minimally invasive techniques, however, adult patients can expect to live longer with their esophageal substitute, meaning long-term functional results are increasingly as important as short-term outcomes.

Esophageal tissue engineering has huge potential to provide an alternative approach, critically without the loss of function of another gastrointestinal organ. Huge advances in this field have allowed for successful replacements of partial thickness esophageal defects in humans. While whole organ replacement is far from possible at present, animal models show increasing promise for imminent implementation of full thickness patch and circumferential replacements which could change the face of esophageal pathology.

3.2 Anatomy, Physiology, and Development

Bioengineering of the esophagus presents distinct challenges from that of the trachea. In adults, the esophagus is approximately 20–25 cm in length, corresponding to 8–10 cm in a neonate. It crosses three anatomical planes during its course to the gastro-esophageal junction; the neck, thorax, and abdomen. As such, it is intimately related to key mediastinal structures including the trachea, aorta, recurrent laryngeal, and vagus nerves, making approaches operatively challenging with high morbidity. Due to its length, the esophageal blood and lymphatic supply is segmental with the upper, middle, and lower thirds supplied by differing branches of regional vasculature.

While also acting primarily as a conduit, unlike the trachea the motility of the esophagus is essential. Gravity alone is insufficient to propel a food bolus from the oropharynx to the stomach and it is the complex interplay of neural and muscular mechanisms which enables coordinated peristalsis and enteral autonomy. To facilitate this, the esophageal wall is composed of four main layers; the mucosa, submucosa, muscularis externa, and adventitia. The *mucosa* comprises of three distinct regions. On the luminal aspect is stratified non-keratinized squamous epithelium. The presence of a highly proliferative basal epithelial layer can readily replenish the well-differentiated, flattened superficial layer when injury occurs, protecting the esophagus from both mechanical and chemical injury during passage of food. Deep to this, the lamina propria is composed of loose connective tissue, vessels,

and sensory nerves with numerous cell types including fibroblasts, endothelial, and smooth muscle cells. Finally, the muscularis mucosae bestows the overall mucosal shape and contracts to move the luminal folds during the passage of food. The *submucosa* is composed of a dense layer of loose connective tissue, rich in collagen and elastin. The orientation of ECM fibers in this layer allow for the high circumferential distensibility of the esophagus without compromising longitudinal strength (Bonavina et al. 1995). Glands secrete mucous directly into the lumen via transmucosal ducts, allowing for lubrication of food. The submucosal nerve plexus regulates the secretion of these ducts, in addition to contraction of the muscularis mucosa. The orientation of inner circular and outer longitudinal layers of muscle in the *muscularis externa* gives rise to radial contractions and longitudinal shortening resulting in peristalsis and is coordinated by the myenteric nerve plexus, which lies between the two muscular layers. In the human esophagus, this changes from striated skeletal muscle to smooth muscle at approximately one third of the cranial to caudal distance. Peristalsis is therefore under voluntary control in the proximal one third, and autonomic control distally, however this distance is variable among species. Below the diaphragm, the musculature specializes to produce a physiological rather than anatomical lower esophageal sphincter at the gastro-esophageal junction. This reduces reflux of gastric contents due to a higher intrinsic spontaneous tone than that found in the musculature of the esophageal body, which relaxes on swallowing. The diaphragmatic crural muscle fibers surround the lower esophageal sphincter to act as a pinchcock, supplementing this anti-reflux mechanism.

3.3 Strategy and Challenges for Esophageal Tissue Engineering

Esophageal tissue engineering has traditionally involved three approaches; scaffold alone, cells alone, or a combination of scaffold and cells. The use of these techniques is dependent on clinical need; mucosal defects may only require epithelial cells for reconstruction whereas full thickness defects clearly require all layers of the esophageal wall. The location and size of the defect may also have implications for replacement requirements depending on the anatomy and function of that region. For example, a more proximal replacement should be skeletal rather than smooth muscle with less requirement for the epithelium to be reflux resistant. Similarly innervation, and therefore peristalsis of an esophageal patch, does not appear to be integral to global function whereas in a circumferential replacement this may be vital.

Evidence now suggests that successful full thickness esophageal tissue engineering requires three key components; a biocompatible scaffold, cells able to differentiate into epithelial and muscular tissues, and a vascular supply. The presence of these components alone, however, is not sufficient and organization to resemble a multi-striated tube is key for function. Significant challenges include avoiding stricture and coordinating peristalsis, with the absence of either resulting in dysphagia, regurgitation, or aspiration. Coordinated peristalsis requires communication of the enteric nervous system with a continuous bi-directional muscular layer. The

biomechanical and elastic properties of the native esophagus must also be replicated to withstand the pressure of passage of a food bolus without perforation, for which appropriate choice of scaffold is essential. Finally, the ability to be resistant to chemical or mechanical injury from the passage of food and gastric acid requires an epithelial barrier, which also appears to be integral to protection against stricture.

The other main challenge for clinical translation is vascularization. The use of bioreactors prior to transplantation ensures transport of oxygen and nutrients to the scaffold to promote cell adherence, proliferation and migration, tissue maturation, and angiogenesis. Various "natural" bioreactors have been used including the greater omentum, latissimus dorsi, or thyroid gland flaps and results in animal models have been promising. Pre-vascularization with omental wrapping or in vitro bioreactor time clearly had a beneficial effect on tissue regeneration in circumferential cervical esophageal replacements with cellularized hybrid scaffolds compared to controls in a rat model (Kim et al. 2019). However, the effect of duration of pre-transplantation maturation still needs to be addressed with regard to mechanical strength and cell survival. Luc et al. attempted to identify the optimal duration of omental in vivo maturation with seeded and unseeded decellularized matrices in a murine model. While vascularization was present at 2 weeks, this was at the expense of a marked inflammatory infiltrate of mononuclear cells, suggesting this time period is too short. By 4 weeks, vascularization persisted with weaning of inflammatory infiltrate; however some degradation of the scaffold was seen and by 8 weeks, more than half of the matrix had degraded with no discernable layers (Luc et al. 2018). Omental maturation does not come without risks; it requires two operations rather than one and this has been reflected in lower weight gain in animal models with pre-vascularization compared to controls in porcine models (Luc et al. 2018). In addition, several studies found that epithelial cells specifically did not survive short omental pre-implantation periods (Nakase et al. 2008; Poghosyan et al. 2015). Further studies are required to understand whether there is a clinical advantage in pre-vascularization.

3.4 Scaffold

Minimum requirements for clinical translation require a scaffold to be biocompatible, non-immunogenic, and nontoxic. It must be porous enough to allow cell delivery, adherence, migration, and differentiation, while facilitating permeability of nutrients prior to neovascularization. Finally, it must retain elasticity while being mechanically robust. In addition to this, desirable qualities include biodegradability to prevent mismatch with patient growth and low inflammatory potential. The ideal scaffold would also be replicable, easy to store, and readily available in a variety of sizes.

Currently, as with the trachea, both synthetic and biologically derived scaffolds have been utilized for esophageal tissue engineering, in combination with, or without, cellular seeding. More recently, advances in hybrid or "intelligent matrices" may be a promising future alternative.

3.4.1 Synthetic Scaffolds

Nonabsorbable synthetic scaffolds have been used for esophageal replacement for over a century with initial unsuccessful attempts using materials including ivory, metal, rubber, and plastic tubes. Regeneration of full-thickness cervical esophageal defects in dogs was compared with one of the three materials; lyophilized dura mater (biological), polytetrafluoroethylene (PTFE), and polyethylene terephthalate (Dacron), both non-absorbable synthetic materials. While anastomotic leak was high in all three groups, significantly increased rates of foreign body reactions, delayed epithelialization, and circumferential stenoses were seen in the synthetic material group (Freud et al. 1999). Formulation of muscle generation was also absent, suggesting that while they provide mechanical support, they are unable to promote tissue regeneration. Results such as these paved the way for development of biodegradable synthetic polymers as absorbable scaffolds, which degrade in situ with absorption of by-products, leaving an entirely new biological layer. As with the trachea, similar materials have been used including PLGA, PLA, PCL, and PLLA, either in isolation or in combination with a protein coating.

The main advantages of absorbable synthetic scaffolds are the ability to customize them to the requirements of the patient with respect to size and biomechanical strength, with an "off-the-shelf" availability. The main limitation, however, remains biocompatibility; close attention must also be paid to the fine balance between early degradation resulting in loss of mechanical stability versus delayed degradation inhibiting tissue remodeling. Some studies have suggested acidic microenvironments on degradation of biodegradable scaffolds may result in a locally toxic environment, resulting in poorer cell adherence and contributing to a high stricture incidence (Ceonzo et al. 2006; Kohn et al. 2002). The final criticism of absorbable scaffolds for esophageal reconstruction is that they are predominantly used to "bridge the gap," rather than to reconstruct the complex native esophageal layers. One group attempted to address this issue with an electrospun polycarbonate polyurethane polymer (PCU) multilayered scaffold with broad pore layers on the luminal and exterior surface with an intervening small pore layer. This prevented mixing of different seeded cell populations while allowing for diffusion of nutrients and oxygen with promising results (Soliman et al. 2019). While this technology is still in proof of concept phase, other groups have shifted their focus to both natural and, more recently, hybrid alternatives, which are increasing in popularity.

3.4.2 Natural Scaffolds

For organ-specific tissue engineering, decellularized xenogenic matrices offer an attractive prospect; a ready-made, multilayered, three-dimensional scaffold with in situ polysaccharides, proteins encouraging cell repopulation, and tissue-appropriate cytokines to guide regeneration. Decellularized tissues commonly used for esophageal tissue engineering include small intestinal submucosa (SIS), urinary bladder (UBM), and esophageal matrices. Decellularized porcine esophagus appears to be a logical choice. It has similar anatomical dimensions to the human esophagus and a precedent exists for use of porcine decellularized matrix in humans with cardiac valves. In addition, using the organ-specific matrix may have advantages for

effective tissue-specific recellularization as previously shown in the liver (Faulk et al. 2015). A reproducible technique for clinical grade porcine decellularized matrices for esophageal replacement has shown the scaffold remains structurally intact, with similar transverse tensile strength to that found in the native esophagus (Arakelian et al. 2019). While longitudinal strength appeared to be stiffer in decellularized specimens, this remained easily suturable in vivo, findings echoed by Luc et al. in 40 decellularizations of porcine esophagus (Luc et al. 2018).

3.4.3 Composite/Hybrid Scaffolds

The relative weaknesses of both biological and synthetic scaffolds have led to increased investigation into a combined approach with composite or "hybrid" scaffolds. Synthetic scaffolds can be coated with biological compounds to improve biocompatibility and cell adherence or biological scaffolds can be reinforced with synthetic materials to improve strength. PLGA scaffolds grafted with collagen and fibronectin demonstrated significant improvements in smooth muscle proliferation and epithelial regeneration, respectively, with no difference in the tensile strength of the scaffolds (Zhu et al. 2005; Zhu et al. 2007). Similarly, reinforcing a tubular SIS construct with electrospun PLGA nanofibers led to improved mechanical properties compared to SIS alone. This was also found to improve muscle cell alignment with limited inflammation in a subcutaneous rat model and allowed for delivery of implanted bioactive molecules of vascular endothelial growth factor (VEGF), which improved angiogenesis (Syed et al. 2014).

Nanoparticles will likely have an increasing role in the future of "intelligent matrices," either by delivering bioactive molecules to promote tissue regeneration as above or to improve structural integrity. When compared to decellularized esophagi alone, those conjugated with silver nanoparticles had improved structural stability and biocompatibility with reduced host immune response in a subcutaneous mouse model (Saleh et al. 2019). The addition of copper, known for its angiogenic properties, to SIS scaffolds promoted reepithelialization, revascularization, and muscular regeneration compared to SIS alone in a cervical patch esophagoplasty model (Tan et al. 2014). Finally, 3D printing represents an exciting development in the field of "scaffold-free" esophageal replacements. Takeoka et al. described a technique of multicellular spheroid culture, with subsequent harvesting and arrangement into a tubular formation by a 3D printer and fusion into a continuous layer after 1 week in a bioreactor. While questionable evidence of muscle development was seen and tensile strength was not comparable to native esophagus, this model was able to withstand esophago-gastric bypass transplantation in rats with no perforation at 30 days (Takeoka et al. 2019). Clearly more work is needed but this field may represent a promising alternative to current tissue engineering techniques.

3.5 Cells

While the precise role of exogenous cell delivery on tissue remodeling is as yet unknown, it appears to be beneficial. Whether biological or synthetic in nature,

acellular scaffolds perform poorly in vivo compared to their cellularized counterparts with increased stricture rates and less muscle and epithelial regeneration (Nakase et al. 2008). Mature cells, stem cells, and muscle cell progenitors from mesenchymal and endodermal lineages have all been used to tackle scaffold cellularization. Autologous cells are preferential as these do not carry a risk of bacterial or viral transmission and do not require immunosuppression. Cells must be easy to harvest with minimal donor site morbidity and ideally have excellent proliferation and differentiation capacity once seeded. Cell selection is also dependent on the clinical need of the construct; partial thickness injury may only require one cell type for repair; for prevention of stricture formation after mucosectomy, for example, epithelial cells alone are required to regenerate the mucosa. When circumferential esophageal replacement is required, however, a combination of cells may be more effective for inducing regeneration in all layers.

3.5.1 Epithelial Cells

Epithelial cells are required as a physical barrier from mechanical stress, infection, and gastric acid. Regardless of scaffold type or presence of cells, ingrowth of endogenous epithelium appears to occur by 3 months; however this response appears to be slower and less comprehensive than in those with epithelial cell seeding (Wei et al. 2009). Interestingly, some studies have suggested decellularized scaffolds allow for a more mature, stratified epithelium than that seen on synthetic scaffolds (Beckstead et al. 2005).

Epithelial cells may be sourced from either buccal mucosal biopsy or endoscopic esophageal biopsy giving rise to oral muscosal epithelial cells (OMEC) or esophageal epithelial cells (EEC), respectively. Newer techniques for epithelial cell delivery include use of organoid units (Grikscheit et al. 2003; Spurrier et al. 2015) or epithelial cell sheets; culture of cells on thermo-responsive polymers allows the polymer to convert from a hydrophobic to hydrophilic state on temperature reduction, resulting in epithelial cell detachment without compromise to cell morphology or function (Yamato et al. 2001). This has now been extensively used with OMECs and is in clinical use for stricture prevention after submucosal dissection.

Smooth and Skeletal Muscle Progenitors

Attempts to repopulate the muscularis externa have included combinations of several different types of cell seeding. Autologous delivery of cells likely provides paracrine signals and growth factors, lowering the inflammatory response and inducing migration of host muscle cells from the edges of the implant. Saxena et al. obtained unidirectional, smooth-muscle-actin positive muscle fibers after seeding rat smooth muscle cells on collagen scaffolds in vitro (Saxena et al. 2009). In a canine model, esophageal decellularized matrix seeded with mature smooth muscle cells had muscular regeneration and significantly less inflammatory infiltrate at 3 weeks than unseeded controls (Marzaro et al. 2006). Main limitation of adult smooth muscle cells is their origin as they are obtained from either the vasculature or esophagus. In addition, they demonstrate slower expansion than other cell lines used for muscular replacement.

Skeletal muscle progenitor cells including myoblasts are easily obtained from skeletal muscle biopsies. Cultured human and porcine myoblasts on SIS produced multinucleated, desmin-positive skeletal muscle fibers with upregulation of late skeletal muscle markers including MyoD. When adapted for circumferential, full thickness use in a porcine model, evidence of circular muscle morphology was seen at 9 months (Poghosyan et al. 2016).

Mesoangioblasts (MABs)

Mesoangioblasts (MABs) are pericytes which are easy to culture, have prior approval for clinical use and have smooth and skeletal muscle differentiation potential. Seeding of MABs, fibroblasts, and neural crest cells on decellularized rat esophagi with omental maturation and subsequent EEC seeding resulted in an organized esophageal construct with a multi-stratified epithelium and mature smooth muscle layer (Urbani et al. 2018). Co-seeding with fibroblasts clearly improved the migratory potential of the MABs and cell distribution throughout the scaffold. The synergistic effect of co-seeding with fibroblasts does not appear to be a phenomenon exclusive to muscle progenitors; when EEC were co-seeded with fibroblasts, superior epithelial layer generation was seen compared to EEC alone (Miki et al. 1999).

Mesenchymal Stem/Stromal Cells (MSCs)

MSCs derived from bone marrow, adipose tissue, and amniotic fluid have all been used in in vivo esophageal models (Jensen et al. 2018; Luc et al. 2018; Tan et al. 2013; Wang et al. 2018). SIS seeded with BM-MSCs was compared to unseeded SIS in a canine patch esophagoplasty model; the presence of MSCs enhanced both epithelialization and muscularization with faster and more comprehensive generation of mucosal and muscular layers. Significant neovascularization and reduced inflammation in the BM MSC-group also suggests MSCs promote angiogenesis and tissue healing while reducing inflammation, properties previously affiliated with the role of MSCs in tissue repair (Tan et al. 2013). Pluripotency, ease of harvesting, and immunomodulatory effects continue to make MSCs an attractive option for cellular seeding.

3.5.2 Neural Progenitors

Cellular approaches have primarily focused on regeneration of epithelial or muscular layers; however neuronal structures are also required for esophageal function; the enteric nervous system is necessary for coordinated peristalsis and release of neuropeptides. Use of enteric nervous system progenitors has demonstrated promising results. When seeded with MABs and fibroblasts, neural crest cells distributed throughout the scaffold in a ring-like formation similar to that seen in the native esophagus. Subsequent differentiation into neurons and glial cells and connections with MABs represents promising evidence that this approach may result in functional neuromusculature when adapted in vivo (Urbani et al. 2018). Whether this seeding is required, however, remains to be seen. The presence of neural markers (S100B) was found in regenerated tissue despite the absence of neural cell seeding in in vivo models (Algarrahi et al. 2018; Jensen et al. 2018). Additionally, despite the absence of peristalsis on barium swallow or limited muscular regeneration on

histology, some animal models were able to grow and feed normally (Urita et al. 2007; Yamamoto et al. 1999). Therefore, although ideal, peristalsis may not be essential for short segment circumferential esophageal replacements.

3.6 Clinical Trials

While strategies for mucosal replacement are well underway with some success in human studies, attempts at full thickness replacements are currently in early stages. Animal models have provided real insights into future techniques; however any potential construct for human use requires rigorous evaluation, particularly with regard to implant integration, immune response, survivability, and long-term functionality of the graft. This is especially critical when considering use in pediatric patients who require the replacement to provide a life-long, good functional outcome.

3.6.1 Partial Thickness Defects

Partial thickness defects of the esophagus are predominantly iatrogenic. Endoscopic mucosal resection and submucosal dissection (EMR/ESD) to treat superficial esophageal cancers reduces the need for esophagectomy. Unfortunately, however, they are associated with high stricture rates due to inflammation and scarring. Strictures significantly affect quality of life, requiring treatments such as dilatation, stenting, and application of steroids. In an animal model of mucosal loss by EMR, ulcer formation, inflammatory cell invasion, and collagen hyperplasia were observed in the first week with fibrosis of the submucosa by day 28. Recommendations to reduce stricturing included reduction of the inflammatory response, promotion of epithelial regeneration, and prevention of damage to the intrinsic muscle layer (Honda et al. 2011). Regenerative medicine approaches have attempted to address these via cell or scaffold delivery to areas of mucosal loss (Table 1).

Endoscopic injection of autologous cell suspensions has been used in animal models with success. Direct injection is simple and quick, however limited by the cell number isolated from the tissue and the viability rate posttransplantation. Various cell types have been used in EMR animal models. Autologous OMECs harvested from oral mucosal biopsy induced reepithelialization by 2 weeks compared to no regeneration in controls without injection (Sakurai et al. 2007). Injected adipose tissue-derived stromal cells showed a similar result in a canine model; improved dysphagia scores, reduced mucosal contraction, and improved angiogenesis were seen in the seeded group compared to controls at 8 weeks (Honda et al. 2011). Finally, injection of autologous keratinocytes from a split skin graft in a sheep model showed no evidence of stricture at 6 months (Zuercher et al. 2013). The contribution of the exogenous cells is unclear. While labeled OMECs were still present in the defect at 2 weeks in Sakurai's model, this is too early to determine if they contribute to long-term tissue remodeling and the rapid turnover of esophageal epithelium negates effective tracing over longer time periods. What appears clear, however, is that delivery of cells directly after EMR appears to promote early reepithelialization and prevent inflammation and fibrosis.

Table 1 Summary of papers which describes replacement of esophageal mucosa

Authors	Year	Cell/Material	Model	n = x	Control	Stricture	Histology: Epithelialization	Follow-up
Cell injection								
Sakurai et al.	2007	OMEC	Porcine	4	4	Reduced vs control	++	2 weeks
Honda et al.	2011	ADSC	Canine	5	5	Reduced dysphagia scores	Unreported	8 weeks
Zuercher et al.	2013	Skin keratinocytes	Ovine	9	Nil	Nil	Unreported	6 months
Cell sheet								
Ohki et al.	2006	OMEC	Canine	3	3	Nil	+++	4 weeks
Ohki et al.	2012	OMEC	Human	9	Nil	11% stricture	++*	4 weeks
Kanai et al.	2012	Epidermal	Porcine	4	4	Reduced vs control	+	2 weeks
Jonas et al.	2016	OMEC	Human	5	Nil	60% stricture	++ **	4 weeks
Perrod et al.	2017	ADSC	Porcine	6	6	Reduced vs control (17% vs 100%)	++ **	4 weeks
Yamaguchi et al.	2017	OMEC	Human	10	Nil	40% stricture	++*	105 weeks
Covered stents								
Nieponice et al.	2009	Unseeded porcine UBM	Canine	5	5	Reduced vs controls	+++	8 weeks
Badylak et al.	2011	Unseeded SIS	Human	5	Nil	100% requiring dilatation	+++	24 months
Barrett et al.	2014	Unseeded amniotic membrane	Porcine	10	10	100% by day 35, less severe in AM group	+	5 weeks
Han et al.	2017	Acellular dermal matrix	Porcine	7	7	Less severe in ACM group	+++	4 weeks

Oral mucosal epithelial cells (OMEC); Adipose tissue-derived stromal cells (ADSC); Urinary bladder matrix (UBM); Small Intestine Submucosa (SIS). Evaluation of the outcome: + patchy/incomplete; ++ continuous; +++ mature and stratified. * endoscopic assessment; ** confocal laser endomicroscopy

Endoscopically deployed acellular ECM grafts post EMR have had mixed results. Endoscopic placement of porcine UBM resulted in development of a stratified epithelium and weight gain compared to 100% stricture in controls in a dog model (Nieponice et al. 2009); however this initial success has not been replicated in subsequent models. A similar trial in humans using SIS with stent had poor results with strictures in all patients (Badylak et al. 2011). Median stricture-free survival was significantly better in a porcine model of human amniotic membrane (HAM) and stent compared to controls with no ECM or stent delivery after ESD. The lack of difference in survival between the "HAM/stent" and a "stent only" group, however, suggests that stricture reduction may be due to the presence of a stent, rather than the contribution of the acellular ECM graft (Barret et al. 2014). More recently, acellular dermal matrix (ADM) was applied with surgical clips in a porcine model and compared to a control group with no intervention. Despite the absence of stents in the study, the ADM group had less stenosis, a more complete reepithelialization and reduced inflammatory infiltrate compared to controls (Han et al. 2017). This suggests that ECM grafts alone may have a role; however the full extent of potential clinical application is yet to be seen.

Perhaps the most promising results for translational use have been with cell sheet technology. In 2006, tissue-engineered autologous OMEC sheets were transplanted in a canine model immediately after ESD. Those transplanted with OMEC sheets had significantly better results compared to controls with no OMEC; a stratified, mature epithelium resembling the native esophageal surface was seen in the OMEC group at 4 weeks compared with patchy, immature epithelium with ulcerated areas in all controls. Marking of cell sheets indicated that the epithelial cells were entirely derived from the exogenous OMECs, suggesting exogenous cells can contribute to tissue remodeling (Ohki et al. 2006). These results have been replicated with different cell types. Transplantation of allogenic adipose tissue-derived stromal cell sheets post-ESD had a lower stricture rate and fibrosis in a porcine model compared to controls (Perrod et al. 2016) and autologous epidermal sheets were as effective as OMEC sheets in preventing esophageal stricture after ESD in porcine models (Kanai et al. 2012). Proposed mechanisms for reduced stricture rate include provision of an epithelial barrier protecting the underlying submucosa and muscularis from mechanical damage and secretion of growth factors and cytokines to recruit host epithelial cells to proliferate and migrate into the wound.

Human feasibility and safety studies have now shown cell-sheet technology to be a reproducible and safe measure to promote early reepithelialization in ESD. The first transplantation of autologous OMEC sheets in humans had a median wound healing time of 3.5 weeks, with the exception of one case transecting the gastroesophageal junction, which took significantly longer and developed a refractory stricture (Kanai et al. 2012). A study of ten patients over 2 years demonstrated a 40% stricture rate with requirement for 1.5 median balloon dilatations and no significant complications or adverse reactions reported (Yamaguchi et al. 2017). Jonas et al. reported a similar time to reepithelialization, however a 60% stricture rate in five patients undergoing a similar technique. Possible explanations included a more distal esophageal ESD in patients with known reflux and fewer numbers of cell sheets used

to cover the defect (Jonas et al. 2016). The lack of control arms in these human studies makes effectiveness compared to current standard treatments difficult to ascertain. This has paved the way for a multicenter, prospective, phase III clinical trial for which recruitment has now been completed.

Full Thickness Defects – Patch

Patch esophagoplasty, whereby a full-thickness patch of esophagus is replaced, may be a treatment option for localized perforation. Conservative management requires prolonged hospital stay, has a high failure rate, and often results in stricture formation as a sequelae of the healing process. Therapeutic endoscopic interventions including clipping, suturing, and stenting have high success rates in acute cases, however this is much lower in anastomotic leaks or chronic fistulae (Dasari et al. 2014; Mennigen et al. 2014). Where these techniques are not possible, due to failure or the size or complexity of the defect, surgery is required. Current surgical alternatives for esophageal reconstruction involve either a pedicled or free tissue flap requiring microvascular anastomoses with high morbidity (Lin et al. 2017; Sa et al. 2013). Surgical reconstruction of recalcitrant strictures refractory to endoscopic intervention is also increasingly used. In these circumstances, tissue-engineered patch esophagoplasty has the potential to allow preservation of the native esophagus and avoid complex esophageal reconstruction.

Natural scaffolds as models for patch esophagoplasty have had universally good results. When 50% patch defects were repaired with acellular SIS in dogs, survival was up to 15 months. While no evidence of stricture was seen in the patch group, all four dogs with circumferential replacements developed stricture (Badylak et al. 2000). A rat model had similar results; patch and circumferential defects were replaced with acellular SIS with survival of all animals in the patch group compared to none in the circumferential group due to obstruction, stricture, or leak (Lopes et al. 2006). Epithelialization was seen by 4 weeks with organized skeletal muscle bundles and neoinnervation in both models. Interestingly, in a patch esophagoplasty rat model with gastric acellular matrix, survival until 18 months was reported despite no evidence of muscle regeneration, suggesting peristalsis is not critical for esophageal function or survival in patch repair (Urita et al. 2007).

Despite evidence of tissue regeneration and good clinical outcomes in these models, the cell-seeded ECM approach has increased in popularity and with good reason. Cellularized patch defects appear to heal quicker compared to ECM patch esophagoplasty alone. Improved reepithelialization and skeletal muscle regeneration was seen in OMEC-seeded SIS compared to SIS alone in patch defects in a canine model, with a faster clinical recovery and better weight gain (Wei et al. 2009). This is not a result exclusive to epithelial cell seeding; smooth muscle and BM-MSC seeded ECM scaffolds also demonstrate earlier epithelialization and improved muscular regeneration compared to controls in patch esophagoplasty models (Marzaro et al. 2006; Tan et al. 2013).

Patch models on biodegradable synthetic scaffolds have had varied results. Unseeded poly-e-caprolactone (PCL) mesh used for abdominal esophageal patch replacement had a 25% 1-month mortality in 20 rabbits, with stricture in 13% of the

surviving animals and pseudodiverticula in 60% (Diemer et al. 2015). This implies degradation of the mesh was too fast to allow for proper ingrowth of new tissue, a result previously found in vicryl mesh rabbit model with an 80% anastomotic leak rate (Jansen et al. 2004). Park et al. performed the only in vivo cell-seeded patch model using a synthetic scaffold in a rabbit model. At 3 weeks, the BM-MSC seeded fibrin-coated PCL group had epithelial and smooth muscle development compared to no regeneration in unseeded PCL controls, suggesting that cell seeding has the same effect of enhancing regeneration in synthetic scaffolds as in natural scaffolds (Park et al. 2016). In addition, no strictures or pseudodiverticulae were seen, albeit at a short end point. As such, the cell-seeded synthetic scaffold approach has now been adopted into circumferential defect models.

Novel synthetic scaffolds have included subcutaneous implantation of a silicone mould for 8 weeks to create a "biotube" of collagen-based tissue. After cervical transplantation, all animals survived until 12 weeks with normal epithelial covering and some evidence of organized skeletal muscular bundles on histology (Okuyama et al. 2018). However, success in this model must not be overstated as the defect was small (1 × 2 cm) and the true stricture rate is unknown due to short follow-up period. Algarrahi pioneered the use of bilayer silk fibroin grafts; a biodegradable scaffold with a porous foam compartment for host tissue integration and a buttressing film layer preventing leakage of luminal contents (Algarrahi et al. 2015). Superior muscular and neural regeneration compared to SIS in a rat patch esophagoplasty model resulted in its use in a subsequent porcine thoracic defect model. All animals survived to 3 months with peristalsis on fluoroscopy and no strictures observed. Although underdeveloped in comparison to native esophageal tissue, organized circular and longitudinal layers of muscularis externa were reported, perhaps representing the best evidence of muscular regeneration in a patch model to date. In addition, submucosal glands were observed and the presence of synaptophysin positive boutons and CD31+ endothelial cells confirmed the presence of neo-innervation and vascularization similar to native tissue (Algarrahi et al. 2018).

What is clear from animal studies is that patch defects are less susceptible to stricture formation than circumferential models, despite the use of natural or biological scaffolds, or cellular or acellular constructs (Table 2). Relatively good clinical outcomes despite a lack of comprehensive muscular regeneration also suggests that peristalsis in patch models may not be critical to outcome. Direct extrapolation of the success of these models into human studies must be done with caution as often defects were small, definitions of stricture and muscle generation varied, and follow-up relatively short. Additionally, limited data exists for the use of patch esophagoplasty in the thoracic or abdominal esophagus where the blood supply is not as well developed. A single series of four patients with esophageal strictures has been reported whereby conventional treatments had failed. Porcine UBM was used to repair three cervical and one distal defect of varying diameters. Despite being a heterogeneous group, this study had remarkable results; only one of four patients developed a postoperative stricture, allowing all patients to recover functionality and preserve their esophagus up to 16 months postoperatively (Nieponice et al. 2014). Clearly, tissue engineering represents a promising area for the rare situation where a

Table 2 Summary of papers which describes replacement of a patch of the esophagus

Authors	Year	Scaffold	Cells	Model	n = x	Control	Location	Clinical Stricture	Anastomotic Leak	Epithelium	Muscle	Maximum Follow up
Acellular												
Badylak et al.	2000	Porcine SIS and UBM	Nil	Canine	11	0	Cervical	Nil	Nil	++	+	15 months
Isch et al.	2001	Alloderm	Nil	Canine	12	0	Cervical	Nil	Nil	++	–	3 months
Jansen et al.	2004	Vicryl vs PVDF	Nil	Rabbit	5 vs 5	0	Abdominal	Nil	80% in vircyl group	+	+	3 months
Lopes et al.	2006	SIS	Nil	Rat	34	15	Cervical	Nil	Nil	+++	++	1-6 months
Urita et al.	2006	GAM	Nil	Rat	27	0	Abdominal	Nil	11%	+++	–	18 months
Aikawa et al.	2013	BAPP	Nil	Porcine	9	0	Thoracic	Nil	Nil	+++	+	12 weeks
Tan et al.	2014	Copper SIS	Nil	Canine	12	6 SIS	Cervical	Nil	Nil	+++	+ (better in CuSIS vs SIS alone)	8 weeks
Nieponice et al.	2014	Porcine UBM	Nil	Human	4	0	Cervical 3, thoracic 1	25%	25%	+++	Not assessed	12-16 months
Diemer et al.	2015	PCL mesh	Nil	Rabbit	15	0	Abdominal	13%	60% pseudodiverticula	+	+	4 weeks
Algarrahi et al.	2015	Silk fibroin	Nil	Rat	40	22 SIS	Abdominal	Nil	5% SIS	+++	++	2 months
Okuyama et al.	2018	IBTA biosheet	Nil	Canine	4	0	Cervical	Nil	Nil	+++	+	4,12 weeks
Algarrahi et al.	2018	Silk fibroin	Nil	Porcine	6	0	Thoracic	Nil	Nil	+++	++	3 months
Cellular												

(continued)

Table 2 (continued)

Authors	Year	Scaffold	Cells	Model	n = x	Control	Location	Clinical Stricture	Anastomotic Leak	Epithelium	Muscle	Maximum Follow up
Marzaro et al.	2006	DEM	SMC	Porcine (neonatal)	3	3 (unseeded)	Thoracic	Not assessed	Not assessed	Not assessed	+ (enhanced regeneration in SMC group)	3 weeks
Wei et al.	2009	SIS	OMEC	Canine	6	6 (unseeded)	Cervical	Nil	Nil	+++	+ (enhanced regeneration in OMEC group)	8 weeks
Tan et al.	2013	SIS	BM-MSC + myoblasts	Canine	6	6 (unseeded)	Cervical	Nil	Nil	+++	+ (enhanced regeneration in BM- MSC group)	12 weeks
Park et al.	2016	Fibrin coated PCL	BM-MSC	Rabbit	3	3 (unseeded)	Cervical	Nil	Nil	Not quantified	Epithelium and smooth muscle in seeded group only	3 weeks

Polyvinylidene fluoride (PVDF); Gastric acellular matrix (GAM); Bioabsorbable polymer patch (BAPP); Decellularized esophageal matrix (DEM); Poly-e-caprolactone (PCL); Autologous Smooth muscle cells (SMC); Bone marrow mesenchymal stem cells (BM-MSC). + islets/bundles of muscle cells, incomplete, disorientated; ++ two distinct layers of muscle

patch esophagoplasty is required; however significant further investigation needs to be undertaken prior to further clinical use.

Full Thickness Defects – Circumferential

For conditions that require circumferential, full-thickness esophageal regeneration, the challenge is even more complex (Table 3). Initial attempts from a Japanese group using acellular natural scaffolds in canine models appeared positive. Cervical esophageal defects were replaced with double-layered collagen sponges with stent removal at 2, 3, and 4 weeks. Stent removal prior to 4 weeks resulted in stricture universally with no muscular regeneration by 6 months. In contrast, where the stent remained in situ for 4 weeks, epithelialization was established prior to stent removal, longitudinal and circular muscle regeneration was reported from the edges of the defect, and no strictures were seen by 12 months (Takimoto et al. 1998). The same group adapted this technique to an intrathoracic model with 4-week stent removal. While 89% survived until their defined end point, a degree of stenosis was present in all animals and muscle regeneration was less advanced; immature skeletal muscle myotubes were present, however they did not extend to the middle of the regenerated esophagus by 24 months (Yamamoto et al. 1999). They reflected that the difference in results between the cervical and thoracic esophagus may be due to the poor blood supply in the thoracic region. To address this, a final experiment with omental wrapping of the thoracic replacement was performed with stent for 8 weeks compared to controls with no wrap and 4-week stents. Surprisingly, those with omental wrapping performed worse than controls; delayed epithelial regeneration was seen with prolonged stent duration, and only 20% survived to their defined end point compared to 100% in controls (Yamamoto et al. 2000).

Subsequent circumferential reconstruction of the esophagus without cell seeding has invariably led to stricture formation and the absence of tissue remodeling. A collagen sponge scaffold with split-skin graft and latissimus dorsi muscle flap in a cervical rabbit model resulted in death of all 12 rabbits by day 16 due to aspiration (Saito et al. 2000) and SIS replacement in a cervical porcine model resulted in stricture in all but one of 14 animals by 24 days requiring early sacrifice (Doede et al. 2009). More recently, reconstruction with decellularized esophagus in a porcine 0% abdominal model also had high complication rates; 60% anastomotic leak and 40% stricture at 5 weeks. Although epithelialization was seen, there was little or no muscle regeneration with significant fibrosis and mononuclear infiltrate (Luc et al. 2018). The unifying feature in these experiments was the lack of stent, which had been used in earlier studies. Despite the use of silicone stents in an SIS intrathoracic piglet model, however, all six animals developed recurrent, symptomatic stricture post removal of stent at 4 weeks (Jönsson et al. 2011). Multiple lessons can be learnt from these early experiences. Both the presence of a stent and its duration in situ appear to be critical. Stricture is universal in both natural and synthetic acellular scaffolds after stent removal regardless of intact epithelium. This suggests the process is secondary to processes deeper in esophageal wall and it may be a stent is required until underlying muscular remodeling has occurred.

Table 3 Summary of papers which describes replacement of a circumferential esophagus

	Year	Scaffolds	Cells	Model	n = x	Control	Location
Acellular							
Takimoto et al.	1998	Collagen sponge +silicone	Nil	Canine	16	27 (early stent removal)	Cervical
Yamamoto et al.	1999	Collagen sponge +silicone	Nil	Canine	9	0	Thoracic
Yamamoto et al.	2000	Collagen sponge +silicone	Nil	Canine	5	9	Thoracic
Badylak et al.	2000	Porcine SIS/UBM	Nil	Canine	4	0	Cervical
Saito et al.	2000	Collgen sponge + STS	Nil	Rabbit	12	0	Cervical
Badylak et al.	2005	Porcine UBM	Nil	Canine	5	0	Cervical
Doede et al.	2009	SIS	Nil	Porcine (neonatal)	14	0	Cervical
Jönsson et al.	2011	SIS	Nil	Porcine (neonatal)	6	0	Thoracic
Luc et al.	2018	DEM	Nil	Porcine	3	3 no bioreactor	Abdominal
Cellular							
Nakase et al.	2008	Ham +PGA	Keratinocytes + fibroblasts	Canine	6	6 (acellular)	Thoracic
Poghosyan et al.	2015	SIS + HAM	Myoblasts + OMEC	Porcine	6	12 (acellular)	Cervical
Dua et al.	2016	Dermal matrix	PRP	Human	1	0	Cervical
Catry et al.	2017	SIS	BM-MSC	Mini-pig	10	10 (acellular)	Abdominal
Barron et al.	2018	PU	OMEC	Porcine	2	1 (acellular)	Thoracic
La Francesca et al.	2018	PU	a-MSC	Porcine	8	0	Thoracic

Stent	Bioreactor	Anastomotic Leak	Stricture	Epithelium	Muscle	Survival to endpoint	Maximum Follow-up
Silicone	Nil	0%	0% vs 81%	+++	++	100% vs 26%	12 months
Silicone	Nil	0%	100% (13-54% stenosis)	+++	+	89%	24 months
Silicone	Omental	20%	Not reported	++	Nil	20%	3 months
Nil	Nil	0%	100%	+	+	0%	15 months
Nil	Lat dorsi	0%	Insufficient survival	–	–	0%	16 days
Nil	Nil	0%	100%	–	–	0%	19 days
Nil	Nil	14%	93%	+	–	7%	4 weeks
Silicone	Nil	0%	100%	++	+	100%	17 weeks
Nil	Omental	60%	40%	+	–	83%	5 weeks
Nil	Omental	0%	33% vs 100%	+++	+	67% vs 0%	60 weeks
Polyflex	Omental	0% vs 42%%	67% vs 100%	+++	+	83% vs 8%	12 months
4 years	Nil	0%	100%	+++	Not available	100%	4 years
Polyflex	Omental	0%	100%	+++ (earlier in MSC)	+ (MSC only)	No difference between groups	3 months
Wallflex	Nil	0%	0%	+++	++	No defined endpoint	29 days
Wallflex	Nil	12.5%%	25% (no stent)	+++	++	No defined endpoint	19 months

(continued)

Table 3 (continued)

	Year	Scaffolds	Cells	Model	n = x	Control	Location
Jensen et al.	2018	PU	AF-MSC/ EEC	Porcine	- MSC,	1 (unseeded)	Thoracic
Kim et al.	2019	PU + PCL	a-MSC	Rat	21	14 (unseeded)	Cervical

Split thickness skin (STS); Human Amniotic Membrane (HAM); Polyglycolic Acid (PGA); Platelet-Rich Plasma (PRP); Polyurethane (PU); Adipose-derived mesenchymal stem cells (a-MSC); Amniotic fluid-derived mesenchymal stem cells (AF-MSC); Esophageal epithelial cells (EEC)

Stent	Bioreactor	Anastomotic Leak	Stricture	Epithelium	Muscle	Survival to endpoint	Maximum Follow-up
Biliary stent	Nil	0%	33%	+++	++ (AF > EEC > un seeded)	100%	6 months
Nil	Thyroid gland flap vs omental	Not quantified	Not quantified	++	+	0%	15 days

In parallel with results seen in patch defects, the approach in recent years has shifted to the use of pre-seeded scaffolds, which appear to result in lower stricture rates, enhanced epithelial and muscular regeneration, and a reduced inflammatory response. After 3 weeks of omental maturation, thoracic implantation of un-stented hybrid scaffolds seeded with OMEC and fibroblasts were compared to acellular controls. Within 3 weeks, stratified epithelialization was complete with polarized smooth muscle-like regeneration in contrast to acellular controls with incomplete epithelialization, significant inflammation, and complete stenosis. Stricture occurred in only one third of the cellular group, interestingly in the two animals with desquamation of the epithelial layer noted after bioreactor maturation (Nakase et al. 2008). Despite the absence of a stent, epithelial cell seeding was in some way protective of stricture formation. Both stents and epithelial cells are therefore potentially key components to stricture reduction and further work is required to understand if this effect is synergistic. While unable to show a difference in stricture rate or survival, BM-MSC on SIS also promoted epithelial regeneration compared to SIS alone in a porcine abdominal model. Mature epithelium was seen at 45 compared to 95 days in the unseeded control, with early initiation of muscle cell colonization, which was never found in the unseeded model (Catry et al. 2017). This suggests that, as in patch models, cell seeding of scaffolds accelerates both epithelial and muscle regeneration regardless of cell type. The effect of stenting on stricture rate in cell-seeded constructs also appears to be positive; all un-stented controls developed stricture compared to 50% of the stented group in a porcine cell-seeded biological scaffold cervical model. In addition, the un-stented group had a high anastomotic leak rate of 83% not seen in the stented group, highlighting additional clinical benefits of stenting (Poghosyan et al. 2015).

An alternative approach is the use of synthetic scaffolds as temporary templates to guide esophageal tissue regrowth rather than integration and degradation. Polyurethane electro-spun scaffolds seeded with autologous adipose-derived MSC were used to replace the thoracic esophagus in eight pigs. The extruded graft was removed at 3 weeks with stent exchange and platelet-rich plasma and MSC application. Subsequent stent exchange occurred every 3 weeks until 6 months. Epithelialization and organized smooth muscle were reported with symptom-free survival of two pigs at 18 and 19 months. The frequency of stent exchange in this model represents a major limitation if this approach were to be translated to humans (La Francesca et al. 2018). The same model was used to determine whether seeding with epithelial or mesenchymal cells resulted in better tissue regeneration, although numbers were small ($n = 4$). Scaffolds seeded with amniotic fluid-derived MSCs had improved muscular regeneration compared to EEC scaffolds with a bidirectional muscularis externa, however both groups were spatially disorganized. The unseeded control had incomplete epithelium at 6 months, no muscular regeneration, and significantly higher inflammatory infiltrate. Interestingly, no stricture was seen, although a stent was used for the duration of the study (Jensen et al. 2018).

Human experience is extremely limited due to the availability of alternative surgical options rather than the use of radical experimental techniques. A single case of a full thickness circumferential replacement was reported in 2016 on

compassionate grounds after extensive esophageal injury failed all conventional treatment in a 24-year old patient. The 5-cm defect in the cervical esophagus was repaired using a self-expanding metal stent covered with Alloderm, an acellular dermal matrix, coated with autologous platelet-rich plasma adhesive gel. After initial issues with stent migration and embedding, the stent remained in situ for 3 years. One-year post removal, no evidence of stricture or fistula was seen. Biopsy-confirmed squamous epithelium, endoscopic ultrasonography confirmed "normal architecture" and high resolution manometry confirmed peristaltic contractile motility in the neo-esophagus (Dua et al. 2016). Most importantly, the patient had achieved oral enteral autonomy. With a lack of histology confirming contribution of muscular regeneration in this patient, it is difficult to know the tissue remodeling processes underlying this clinically successful outcome. These results do suggest, however, that exogenous cells are not essential for full thickness regeneration in the presence of a long-term stent.

3.7 Conclusions

Esophageal replacement remains a major challenge in both children and adults. Although replacement techniques exist, all have associated morbidity and none are able to fully replicate the function of the native esophagus. There is, therefore, a tangible clinical need for a tissue-engineered esophageal construct. Huge advances in tissue engineering over the last two decades have resulted in a number of viable options for replacement of partial thickness defects using regenerative medicine techniques. The complex anatomy and physiology of the esophagus means full thickness defects present a significantly more complex reconstructive challenge with stricture prevention, a bi-directional muscular layer and coordination of peristalsis the main obstacles.

While full esophageal replacement post-esophagectomy is a distant prospect, short circumferential defects as required in esophageal atresia are a very real possibility in the near future. Current evidence from animal models offers promising results with relative success of both natural and synthetic scaffolds. It is clear that cellularization of scaffolds enhances muscular and epithelial regeneration and reduces inflammation. Future approaches should focus on these techniques. However, further work to identify optimal cell combinations is required, with cell-labeling potentially offering a more thorough understanding of the contribution of exogenous cell delivery on tissue remodeling. Stents also appear to be essential to reducing stricture formation and improving clinical outcomes, however duration must be carefully considered for optimal results. Finally, increased work using intrathoracic rather than cervical animal models, with a particular focus on the merits of bioreactors and pre-vascularization, must be undertaken.

Animal models have provided real insights into future techniques for human therapies and demonstrate the huge potential therapeutic possibilities offered by regenerative medicine. Although further work is clearly needed prior to translation in

humans, the implications of tissue engineering for both benign and malignant conditions of the esophagus is enormous.

4 Final Considerations

Recent innovation in both science and technology has encouraged the development of trailblazing research in the field of regenerative medicine, creating novel treatments for diseases in which limited or no alternative solutions exist. The promise of this cannot be underestimated; however, despite the significant progress achieved, there remains a long way to go to establish standardized and safe methods for bioengineered organ transplantation.

Research in regenerative medicine is often plagued with ethical controversy. Proof of biological mechanisms from preclinical studies is therefore imperative prior to proceeding with novel treatments in patients. Open reporting and standardization of definitions and outcomes between groups is key. The creation of an international register for preclinical trials with mandatory registration prior to publication of both positive and negative outcomes may also go some way to addressing this.

Compassionate clinical trials in patients should only be performed when all treatment options are exhausted and open discussions with both patients and ethical committees conclude the merit of the trial, particularly where the treatment may result in lethal adverse effects. Building valuable international collaborations, gathering expertise from varied specialties, and increasing the role of clinical scientists facilitating bench-to-bedside translation are crucial for the advancement of regenerative medicine techniques into viable and successful options for patients in the future.

Acknowledgments PDC is supported by Horizon 2020 grant INTENS 668294; PDC is supported by NIHR Professorship, NIHR UCL BRC-GOSH and the Oak Foundation. ND is supported by L. Spitz GOSHCC Clinical Research Training Fellowships. MG is supported by PhD scholarship Agencia Nacional de Investigación y Desarrollo(ANID)/DoctoradoBecasChile/2018 – 72190110. PB is supported by the Rosetrees Trust (M362; M362-F1; M553) and the Cystic Fibrosis Trust (SRC 006)

References

Aikawa M, Miyazawa M, Okamoto K, Okada K, Akimoto N, Sato H, Koyama I, Yamaguchi S, Ikada Y (2013) A bioabsorbable polymer patch for the treatment of esophageal defect in a porcine model. J Gastroenterol 48:822–829

Algarrahi K, Franck D, Ghezzi CE, Cristofaro V, Yang X, Sullivan MP, Chung YG, Affas S, Jennings R, Kaplan DL et al (2015) Acellular bi-layer silk fibroin scaffolds support functional tissue regeneration in a rat model of onlay esophagoplasty. Biomaterials 53:149–159

Algarrahi K, Franck D, Cristofaro V, Yang X, Savarino A, Affas S, Schäfer FM, Ghezzi C, Jennings R, Nedder A et al (2018) Bi-layer silk fibroin grafts support functional tissue regeneration in a porcine model of onlay esophagoplasty. J Tissue Eng Regen Med 12:e894–e904

Aoki FG, Varma R, Marin-Araujo AE, Lee H, Soleas JP, Li AH, Soon K, Romero D, Moriya HT, Haykal S et al (2019) De-epithelialization of porcine tracheal allografts as an approach for tracheal tissue engineering. Sci Rep 9:1–12

Arakelian L, Caille C, Faivre L, Corté L, Bruneval P, Shamdani S, Flageollet C, Albanese P, Domet T, Jarraya M et al (2019) A clinical-grade acellular matrix for esophageal replacement. J Tissue Eng Regen Med 13:2191–2203

Badylak S, Meurling S, Chen M, Spievack A, Simmons-Byrd A (2000) Resorbable bioscaffold for esophageal repair in a dog model. J Pediatr Surg 35:1097–1103

Badylak SF, Vorp DA, Spievack AR, Simmons-Byrd A, Hanke J, Freytes DO, Thapa A, Gilbert TW, Nieponice A (2005) Esophageal reconstruction with ECM and muscle tissue in a dog model. J Surg Res 128:87–97

Badylak SF, Hoppo T, Nieponice A, Gilbert TW, Davison JM, Jobe BA (2011) Esophageal preservation in five male patients after endoscopic inner-layer circumferential resection in the setting of superficial cancer: a regenerative medicine approach with a biologic scaffold. Tissue Eng - Part A 17:1643–1650

Barret M, Pratico CA, Camus M, Beuvon F, Jarraya M, Nicco C, Mangialavori L, Chaussade S, Batteux F, Prat F (2014) Amniotic membrane grafts for the prevention of esophageal stricture after circumferential endoscopic submucosal dissection. PLoS One 9:e100236

Barron MR, Blanco EW, Aho JM, Chakroff J, Johnson J, Cassivi SD, Carey WA, Wigle DA (2018) Full-thickness oesophageal regeneration in pig using a polyurethane mucosal cell seeded graft. J Tissue Eng Regen Med 12:175–185

Beckstead BL, Pan S, Bhrany AD, Bratt-Leal AM, Ratner BD, Giachelli CM (2005) Esophageal epithelial cell interaction with synthetic and natural scaffolds for tissue engineering. Biomaterials 26:6217–6228

Belsey R (1950) Resection and reconstruction of the intrathoracic trachea. Br J Surg 38:200–205

Bonavina L, Segalin A, Pavanello M, Faranda C, Cioffi U, Peracchia A (1995) Surgical treatment of esophageal stenosis caused by reflux. Ann Ital Chir 66:621–624

Butler CR, Speggiorin S, Rijnberg FM, Roebuck DJ, Muthialu N, Hewitt RJ, Elliott MJ (2014) Outcomes of slide tracheoplasty in 101 children: a 17-year single-center experience. J Thorac Cardiovasc Surg 147:1783–1790

Butler CR, Hynds RE, Crowley C, Gowers KHC, Partington L, Hamilton NJ, Carvalho C, Platé M, Samuel ER, Burns AJ et al (2017) Vacuum-assisted decellularization: an accelerated protocol to generate tissue-engineered human tracheal scaffolds. Biomaterials 124:95–105

Catry J, Luong-Nguyen M, Arakelian L, Poghosyan T, Bruneval P, Domet T, Michaud L, Sfeir R, Gottrand F, Larghero J et al (2017) Circumferential esophageal replacement by a tissue-engineered substitute using mesenchymal stem cells: an experimental study in mini pigs. Cell Transplant 26:1831–1839

Ceonzo K, Gaynor A, Shaffer L, Kojima K, Vacanti CA, Stahl GL (2006) Polyglycolic acid-induced inflammation: role of hydrolysis and resulting complement activation. Tissue Eng 12:301–308

Chiang T, Pepper V, Best C, Onwuka E, Breuer CK (2016) Clinical translation of tissue engineered trachea grafts. Ann Otol Rhinol Laryngol 125:873–885

Conconi MT, De Coppi P, Di Liddo R, Vigolo S, Zanon GF, Parnigotto PP, Nussdorfer GG (2005) Tracheal matrices, obtained by a detergent-enzymatic method, support in vitro the adhesion of chondrocytes and tracheal epithelial cells. Transpl Int 18:727–734

Dasari BVM, Neely D, Kennedy A, Spence G, Rice P, Mackle E, Epanomeritakis E (2014) The role of esophageal stents in the management of esophageal anastomotic leaks and benign esophageal perforations. Ann Surg 259:852–860

Delaere P, Van Raemdonck D (2016) Tracheal replacement. J Thorac Dis 8:S186–S196

Delaere P, Vrancks J, Verleden G, de Leyn P, van Raemdonck D (2010) Tracheal allotransplantation after withdrawal of immunosuppressive therapy. Bull Acad Natl Med 194:138–183

Diekman BO, Rowland CR, Lennon DP, Caplan AI and Guilak F (2010) Chondrogenesis of adult stem cells from adipose tissue and bone marrow: induction by growth factors and cartilage-derived matrix. In *Tissue Engineering - Part A*, pp. 523–533. Mary Ann Liebert Inc.

Diekman BO, Christoforou N, Willard VP, Sun H, Sanchez-Adams J, Leong KW, Guilak F (2012) Cartilage tissue engineering using differentiated and purified induced pluripotent stem cells. Proc Natl Acad Sci U S A 109:19172–19177

Diemer P, Markoew S, Le DQS, Qvist N (2015) Poly-ε-caprolactone mesh as a scaffold for in vivo tissue engineering in rabbit esophagus. Dis Esophagus 28:240–245

Doede T, Bondartschuk M, Joerck C, Schulze E, Goernig M (2009) Unsuccessful alloplastic esophageal replacement with porcine small intestinal submucosa. Artif Organs 33:328–333

Dua KS, Hogan WJ, Aadam AA, Gasparri M (2016) In-vivo oesophageal regeneration in a human being by use of a non-biological scaffold and extracellular matrix. Lancet 388:55–61

Elliott MJ, De Coppi P, Speggiorin S, Roebuck D, Butler CR, Samuel E, Crowley C, McLaren C, Fierens A, Vondrys D et al (2012) Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year follow-up study. Lancet 380:994–1000

Elliott MJ, Butler CR, Varanou-Jenkins A, Partington L, Carvalho C, Samuel E, Crowley C, Lange P, Hamilton NJ, Hynds RE et al (2017) Tracheal replacement therapy with a stem cell-seeded graft: lessons from compassionate use application of a GMP-compliant tissue-engineered medicine. Stem Cells Transl Med 6:1458–1464

Faulk DM, Wildemann JD, Badylak SF (2015) Decellularization and cell seeding of whole liver biologic scaffolds composed of extracellular matrix. J Clin Exp Hepatol 5:69–80

Freud E, Efrati I, Kidron D, Finally R, Mares AJ (1999) Comparative experimental study of esophageal wall regeneration after prosthetic replacement. J Biomed Mater Res 45:84–91

Fuchs JR, Terada S, Ochoa ER, Vacanti JP, Fauza DO (2002) Fetal tissue engineering: in utero tracheal augmentation in an ovine model. J Pediatr Surg 37:1000–1006

Gallo G, Zwaveling S, Groen H, Van Der Zee D, Hulscher J (2012) Long-gap esophageal atresia: a meta-analysis of jejunal interposition, colon interposition, and gastric pull-up. Eur J Pediatr Surg 22:420–425

Go T, Jungebluth P, Baiguero S, Asnaghi A, Martorell J, Ostertag H, Mantero S, Birchall M, Bader A, Macchiarini P (2010) Both epithelial cells and mesenchymal stem cell-derived chondrocytes contribute to the survival of tissue-engineered airway transplants in pigs. J Thorac Cardiovasc Surg 139:437–443

Grikscheit T, Ochoa ER, Srinivasan A, Gaissert H, Vacanti JP (2003) Tissue-engineered esophagus: experimental substitution by onlay patch or interposition. J Thorac Cardiovasc Surg 126:537–544

Grimmer JF, Gunnlaugsson CB, Alsberg E, Murphy HS, Kong HJ, Mooney DJ, Weatherly RA (2004) Tracheal reconstruction using tissue-engineered cartilage. Arch Otolaryngol - Head Neck Surg 130:1191–1196

Hamilton NJ, Kanani M, Roebuck DJ, Hewitt RJ, Cetto R, Culme-Seymour EJ, Toll E, Bates AJ, Comerford AP, McLaren CA et al (2015) Tissue-engineered tracheal replacement in a child: a 4-year follow-up study. Am J Transplant 15:2750–2757

Han Y, Guo J, Sun S, Wu W, Wang S, Ge N, Liu X, Wang G, Wang S (2017) Acellular dermal matrix for esophageal stricture prevention after endoscopic submucosal dissection in a porcine model. Gastrointest Endosc 86:1160–1167

Honda M, Hori Y, Nakada A, Uji M, Nishizawa Y, Yamamoto K, Kobayashi T, Shimada H, Kida N, Sato T et al (2011) Use of adipose tissue-derived stromal cells for prevention of esophageal stricture after circumferential EMR in a canine model. Gastrointest Endosc 73:777–784

Irino T, Tsekrekos A, Coppola A, Scandavini CM, Shetye A, Lundell L, Rouvelas I (2017) Long-term functional outcomes after replacement of the esophagus with gastric, colonic, or jejunal conduits: a systematic literature review. Dis Esophagus 30

Jang YS, Jang CH, Cho YB, Kim M, Kim GH (2014) Tracheal regeneration using poly-caprolactone/collagen-nanofiber coated with umbilical cord serum after partial resection. Int J Pediatr Otorhinolaryngol 78:2237–2243

Jansen PL, Klinge U, Anurov M, Titkova S, Mertens PR, Jansen M (2004) Surgical mesh as a scaffold for tissue regeneration in the esophagus. Eur Surg Res 36:104–111

Jensen T, Wanczyk H, Sharma I, Mitchell A, Sayej WN, Finck C (2018) Polyurethane scaffolds seeded with autologous cells can regenerate long esophageal gaps: an esophageal atresia treatment model. J Pediatr Surg

Jonas E, Sjöqvist S, Elbe P, Kanai N, Enger J, Haas SL, Mohkles-Barakat A, Okano T, Takagi R, Ohki T et al (2016) Transplantation of tissue-engineered cell sheets for stricture prevention after endoscopic submucosal dissection of the oesophagus. United Eur Gastroenterol J 4:741–753

Jönsson L, Gatzinsky V, Jennische E, Johansson C, Nannmark U, Friberg LG (2011) Piglet model for studying esophageal regrowth after resection and interposition of a silicone stented small intestinal submucosa tube. Eur Surg Res 46:169–179

Kanai N, Yamato M, Ohki T, Yamamoto M, Okano T (2012) Fabricated autologous epidermal cell sheets for the prevention of esophageal stricture after circumferential ESD in a porcine model. Gastrointest Endosc 76:873–881

Kim IG, Wu Y, Park SA, Cho H, Choi JJ, Kwon SK, Shin JW, Chung EJ (2019) Tissue-engineered esophagus via bioreactor cultivation for circumferential esophageal reconstruction. Tissue Eng - Part A 25:1478–1492

Kohn DH, Sarmadi M, Helman JI, Krebsbach PH (2002) Effects of pH on human bone marrow stromal cells in vitro: implications for tissue engineering of bone. J Biomed Mater Res 60:292–299

La Francesca S, Aho JM, Barron MR, Blanco EW, Soliman S, Kalenjian L, Hanson AD, Todorova E, Marsh M, Burnette KL et al (2018) Long-term regeneration and remodeling of the pig esophagus after circumferential resection using a retrievable synthetic scaffold carrying autologous cells. Sci Rep 8:4123

Lin YC, Hsiao JR, Lee YC (2017) Patch esophagoplasty with a free proximal lateral leg flap for focal stricture of the cervical esophagus: a case report. Microsurgery 37:426–430

Lopes MF, Cabrita A, Ilharco J, Pessa P, Paiva-Carvalho J, Pires A, Patrício J (2006) Esophageal replacement in rat using porcine intestinal submucosa as a patch or a tube-shaped graft. Dis Esophagus 19:254–259

Luc G, Charles G, Gronnier C, Cabau M, Kalisky C, Meulle M, Bareille R, Roques S, Couraud L, Rannou J et al (2018) Decellularized and matured esophageal scaffold for circumferential esophagus replacement: proof of concept in a pig model. Biomaterials 175:1–18

Macchiarini P, Jungebluth P, Go T, Asnaghi MA, Rees LE, Cogan TA, Dodson A, Martorell J, Bellini S, Parnigotto PP et al (2008) Clinical transplantation of a tissue-engineered airway. Lancet 372:2023–2030

Marzaro M, Vigolo S, Oselladore B, Conconi MT, Ribatti D, Giuliani S, Nico B, Perrino G, Nussdorfer GG, Parnigotto PP (2006) In vitro and in vivo proposal of an artificial esophagus. J Biomed Mater Res - Part A 77:795–801

Maughan EF, Hynds RE, Proctor TJ, Janes SM, Elliott M, Birchall MA, Lowdell MW, De Coppi P (2017) Autologous cell seeding in tracheal tissue engineering. Curr Stem Cell Reports 3:279–289

Maziak DE, Todd TRJ, Keshavjee SH, Winton TL, Van Nostrand P, Pearson FG, Wood DE, Grillo HC (1996) Adenoid cystic carcinoma of the airway: thirty-two-year experience. J Thorac Cardiovasc Surg 112:1522–1532

Mennigen R, Senninger N, Laukoetter MG (2014) Novel treatment options for perforations of the upper gastrointestinal tract: endoscopic vacuum therapy and over-the-scope clips. World J Gastroenterol 20:7767–7776

Miki H, Ando N, Ozavva S, Sato M, Hayashi K, Kitajima M (1999) An artificial esophagus constructed of cultured human esophageal epithelial cells, fibroblasts, polyglycolic acid mesh, and collagen. ASAIO J 45:502–508

Nakase Y, Nakamura T, Kin S, Nakashima S, Yoshikawa T, Kuriu Y, Sakakura C, Yamagishi H, Hamuro J, Ikada Y et al (2008) Intrathoracic esophageal replacement by in situ tissue-engineered esophagus. J Thorac Cardiovasc Surg 136:850–859

Neville WE (1982) Reconstruction of the trachea and stem bronchi with Neville prosthesis. Int Surg 67:229–234

Nieponice A, McGrath K, Qureshi I, Beckman EJ, Luketich JD, Gilbert TW, Badylak SF (2009) An extracellular matrix scaffold for esophageal stricture prevention after circumferential EMR. Gastrointest Endosc 69:289–296

Nieponice A, Ciotola FF, Nachman F, Jobe BA, Hoppo T, Londono R, Badylak S, Badaloni AE (2014) Patch Esophagoplasty: esophageal reconstruction using biologic scaffolds. Ann Thorac Surg 97:283–288

Ohki T, Yamato M, Murakami D, Takagi R, Yang J, Namiki H, Okano T, Takasaki K (2006) Treatment of oesophageal ulcerations using endoscopic transplantation of tissue-engineered autologous oral mucosal epithelial cell sheets in a canine model. Gut 55:1704–1710

Okuyama H, Umeda S, Takama Y, Terasawa T, Nakayama Y (2018) Patch esophagoplasty using an in-body-tissue-engineered collagenous connective tissue membrane. J Pediatr Surg 53:223–226

Park SY, Choi JW, Park JK, Song EH, Park SA, Kim YS, Shin YS, Kim CH (2016) Tissue-engineered artificial oesophagus patch using three-dimensionally printed polycaprolactone with mesenchymal stem cells: a preliminary report. Interact Cardiovasc Thorac Surg 22:712–717

Park JH, Yoon JK, Lee JB, Shin YM, Lee KW, Bae SW, Lee JH, Yu JJ, Jung CR, Youn YN et al (2019) Experimental tracheal replacement using 3-dimensional bioprinted artificial trachea with autologous epithelial cells and chondrocytes. Sci Rep 9:2103

Perrod G, Rahmi G, Pidial L, Camilleri S, Bellucci A, Casanova A, Viel T, Tavitian B, Cellier C, Clement O (2016) Cell sheet transplantation for esophageal stricture prevention after endoscopic submucosal dissection in a porcine model. PLoS One 11

Poghosyan T, Sfeir R, Michaud L, Bruneval P, Domet T, Vanneaux V, Luong-Nguyen M, Gaujoux S, Gottrand F, Larghero J et al (2015) Circumferential esophageal replacement using a tube-shaped tissue-engineered substitute: an experimental study in minipigs. Surg (United States) 158:266–277

Poghosyan T, Catry J, Luong-Nguyen M, Bruneval P, Domet T, Arakelian L, Sfeir R, Michaud L, Vanneaux V, Gottrand F et al (2016) Esophageal tissue engineering: current status and perspectives. J Chir Viscerale 153:21–30

Sa YJ, Du Kim Y, Kim CK, Park JK, Moon SW (2013) Recurrent cervical esophageal stenosis after colon conduit failure: use of myocutaneous flap. World J Gastroenterol 19:307–310

Saito M, Sakamoto T, Fujimaki M, Tsukada K, Honda T, Nozaki M (2000) Experimental study of an artificial esophagus using a collagen sponge, a latissimus dorsi muscle flap, and split-thickness skin. Surg Today 30:606–613

Sakurai T, Miyazaki S, Miyata G, Satomi S, Hori Y (2007) Autologous buccal keratinocyte implantation for the prevention of stenosis after EMR of the esophagus. Gastrointest Endosc 66:167–173

Saleh T, Ahmed E, Yu L, Kwak HH, Kang BJ, Park KM, Choi KY, Kim BM, Kang KS, Woo HM (2019) Characterization of silver nanoparticle-modified decellularized rat esophagus for esophageal tissue engineering: structural properties and biocompatibility. J Biosci Bioeng 128:613–621

Saxena AK, Kofler K, Ainödhofer H, Höllwarth ME (2009) Esophagus tissue engineering: hybrid approach with esophageal epithelium and unidirectional smooth muscle tissue component generation in vitro. J Gastrointest Surg 13:1037–1043

Seguin A, Baccari S, Holder-Espinasse M, Bruneval P, Carpentier A, Taylor DA and Martinod E (2013) Tracheal regeneration: evidence of bone marrow mesenchymal stem cell involvement. J Thorac Cardiovasc Surg 145: 1297-1304.e2

Shin YS, Choi JW, Park JK, Kim YS, Yang SS, Min BH, Kim CH (2015) Tissue-engineered tracheal reconstruction using mesenchymal stem cells seeded on a porcine cartilage powder scaffold. Ann Biomed Eng 43:1003–1013

Soliman S, Laurent J, Kalenjian L, Burnette K, Hedberg B, La Francesca S (2019) A multilayer scaffold design with spatial arrangement of cells to modulate esophageal tissue growth. J Biomed Mater Res - Part B Appl Biomater 107:324–331

Spurrier RG, Speer AL, Hou X, El-Nachef WN, Grikscheit TC (2015) Murine and human tissue-engineered esophagus form from sufficient stem/progenitor cells and do not require micro-designed biomaterials. Tissue Eng - Part A 21:906–915

Syed O, Walters NJ, Day RM, Kim HW, Knowles JC (2014) Evaluation of decellularization protocols for production of tubular small intestine submucosa scaffolds for use in oesophageal tissue engineering. Acta Biomater 10:5043–5054

Takeoka Y, Matsumoto K, Taniguchi D, Tsuchiya T, Machino R, Moriyama M, Oyama S, Tetsuo T, Taura Y, Takagi K et al (2019) Regeneration of esophagus using a scaffoldfree biomimetic structure created with biothree- dimensional printing. PLoS One 14:e0211339

Takimoto Y, Nakamura T, Yamamoto Y, Kiyotani T, Teramachi M, Shimizu Y (1998) The experimental replacement of a cervical esophageal segment with an artificial prosthesis with the use of collagen matrix and a silicone stent. J Thorac Cardiovasc Surg 116:98–106

Tan B, Wei RQ, Tan MY, Luo JC, Deng L, Chen XH, Hou JL, Li XQ, Yang ZM, Xie HQ (2013) Tissue engineered esophagus by mesenchymal stem cell seeding for esophageal repair in a canine model. J Surg Res 182:40–48

Tan B, Wang M, Chen X, Hou JL, Chen XH, Wang Y, Li-Ling J, Xie HQ (2014) Tissue engineered esophagus by copper-small intestinal submucosa graft for esophageal repair in a canine model. Sci China Life Sci 57:248–255

Toomes H, Mickisch G, Vogt-Moykopf I (1985) Experiences with prosthetic reconstruction of the trachea and bifurcation. Thorax 40:32–37

Urbani L, Maghsoudlou P, Milan A, Menikou M, Hagen CK, Totonelli G, Camilli C, Eaton S, Burns A, Olivo A et al (2017) Long-term cryopreservation of decellularised oesophagi for tissue engineering clinical application. PLoS One 12:e0179341

Urbani L, Camilli C, Phylactopoulos DE, Crowley C, Natarajan D, Scottoni F, Maghsoudlou P, McCann CJ, Pellegata AF, Urciuolo A et al (2018) Multi-stage bioengineering of a layered oesophagus with in vitro expanded muscle and epithelial adult progenitors. Nat Commun 9: 4286

Urita Y, Komuro H, Chen G, Shinya M, Kaneko S, Kaneko M, Ushida T (2007) Regeneration of the esophagus using gastric acellular matrix: an experimental study in a rat model. Pediatr Surg Int 23:21–26

Vacanti CA, Paige KT, Kim WS, Sakata J, Upton J, Vacanti JP (1994) Experimental tracheal replacement using tissue-engineered cartilage. J Pediatr Surg 29:201–205

van der Zee DC, Bagolan P, Faure C, Gottrand F, Jennings R, Laberge JM, Martinez Ferro MH, Parmentier B, Sfeir R, Teague W (2017) Position paper of INoEA working group on long-gap esophageal atresia: for better care. Front Pediatr 5:63–63

Wang F, Maeda Y, Zachar V, Ansari T, Emmersen J (2018) Regeneration of the oesophageal muscle layer from oesophagus acellular matrix scaffold using adipose-derived stem cells. Biochem Biophys Res Commun 503:271–277

Wei RQ, Tan B, Tan MY, Luo JC, Deng L, Chen XH, Li XQ, Zuo X, Zhi W, Yang P et al (2009) Grafts of porcine small intestinal submucosa with cultured autologous oral mucosal epithelial cells for esophageal repair in a canine model. Exp Biol Med 234:453–461

Yamaguchi N, Isomoto H, Kobayashi S, Kanai N, Kanetaka K, Sakai Y, Kasai Y, Takagi R, Ohki T, Fukuda H et al (2017) Oral epithelial cell sheets engraftment for esophageal strictures after endoscopic submucosal dissection of squamous cell carcinoma and airplane transportation. Sci Rep 7:17460

Yamamoto Y, Nakamura T, Shimizu Y, Matsumoto K, Takimoto Y, Kiyotani T, Sekine T, Ueda H, Liu Y, Tamura N (1999) Intrathoracic esophageal replacement in the dog with the use of an artificial esophagus composed of a collagen sponge with a double-layered silicone tube. J Thorac Cardiovasc Surg 118:276–286

Yamamoto Y, Nakamura T, Shimizu Y, Matsumoto K, Takimoto Y, Liu Y, Ueda H, Sekine T, Tamura N (2000) Intrathoracic esophageal replacement with a collagen sponge-silicone double layer tube: evaluation of omental-pedicle wrapping and prolonged placement of an inner stent. ASAIO J 46:734–739

Yamato M, Utsumi M, Kushida A, Konno C, Kikuchi A, Okano T (2001) Thermo-responsive culture dishes allow the intact harvest of multilayered keratinocyte sheets without dispase by reducing temperature. Tissue Eng 7:473–480

Zhou C, Zhang L, Wang H, Ma X, Shi B, Chen W, He J, Wang K, Liu P, Ren Y (2015) Superiority of minimally invasive oesophagectomy in reducing in-hospital mortality of patients with resectable oesophageal cancer: a meta-analysis. PLoS One 10:1371

Zhu Y, Chan-Park MB, Chian KS (2005) The growth improvement of porcine esophageal smooth muscle cells on collagen-grafted poly(DL-lactide-co-glycolide) membrane. J Biomed Mater Res - Part B Appl Biomater 75:193–199

Zhu Y, Leong MF, Ong WF, Chan-Park MB, Chian KS (2007) Esophageal epithelium regeneration on fibronectin grafted poly(l-lactide-co-caprolactone) (PLLC) nanofiber scaffold. Biomaterials 28:861–868

Zhu Y, Wu X, Liang Y, Gu H, Song K, Zou X, Zhou G (2016) Repair of cartilage defects in osteoarthritis rats with induced pluripotent stem cell derived chondrocytes. BMC Biotechnol 16:1–11

Zuercher BF, George M, Escher A, Piotet E, Ikonomidis C, Andrejevic SB, Monnier P (2013) Stricture prevention after extended circumferential endoscopic mucosal resection by injecting autologous keratinocytes in the sheep esophagus. Surg Endosc 27:1022–1028