Regenerative medicine for oesophageal reconstruction after cancer treatment

Kerm Sin Chian PhD[a], Meng Fatt Leong PhD[b], Koji Kono MD[c,d]

All authors contributed equally to this review.

[a] School of Mechanical and Aerospace Engineering, Nanyang Technological University, Nanyang Avenue, Singapore 639798

[b] Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, Singapore 138669.

[c] Department of Surgery, National University of Singapore, 1E Kent Ridge Road, Singapore 119228

[d] Cancer Science Institute of Singapore, National University of Singapore, 14 Medical Drive, Singapore 117599

Correspondence to:
Kerm Sin Chian PhD, School of Mechanical & Aerospace Engineering, Nanyang Technological University, Nanyang Avenue, Singapore 639798

Abstract
Current surgical procedure of removing malignant tissue in oesophageal cancer and replacing the excised tissues with autologous grafts from the stomach and colon is not without its problems. The need to reduce stenosis and/or anastomotic leakage after oesophagectomy remains high priority. However recent developments in tissue engineering methods and cell sheet technology have enabled improved scaffold materials for oesophageal repair. Despite the large number of publications of successful animal studies, few tissue-engineering approaches have progressed to clinical trials. In this review we shall discuss the recent status of oesophagus reconstruction after surgery. In particular, two recent trials using decellularised constructs and epithelial cell sheets to replace excised tissues after ESD/EMR procedures will be highlighted. The clinical trials showed that both decellularised graft and epithelial cell sheets prevented stenosis after ESD/EMR. In contrast, animal studies have shown that the use of tissue engineered constructs after oesophagectomy remains a challenge.

Introduction
Oesophageal cancer is the sixth most frequent cause of cancer death worldwide and affects more than 450,000 people all over the world.[1] Most patients with oesophageal cancer in Asia countries such as Japan and China have squamous cell carcinoma (SCC), while most of those in Western countries have adenocarcinoma.[2,3] In particular, the incidence of oesophageal adenocarcinoma in UK is rapidly increasing, in which the age-adjusted incidence has risen by 39·6% for men and 37·5% for women every five years.[3] Despite improvements in surgical techniques and perioperative management,[4,5] and surgery combined with chemotherapy and/or radiotherapy,[6,7] the prognosis of oesophageal cancer at advanced stage remains poor.

This is mainly due to the biologically aggressive behavior of the disease, with high incidence of lymph node and distant metastasis. The five year survival rates have been reported to be 15-25% and a majority of the patients died of disease progression or disease recurrence.[1,8] Surgical interventions are broadly divided into two main categories, namely oesophagectomy and endoscopic treatments. It is generally accepted that there is a high incidence of anastomotic leakage with oesophago-gastrostomy and stricture with endoscopic treatments.

There is a need to reduce complications such as anastomotic leakage and stenosis resulting from oesophageal surgery. Addressing these challenges would require additional tissue and/or material for surgical reconstruction to provide structural continuity and integrity, tissue integration, and anastomosis of the surgical sites. Although autologous tissue grafts such as stomach and colon are available for surgical reconstruction, it is more desirable to have a readily available replacement without additional surgical procedures. In contrast, tissue engineering can potentially offer ready-to-use constructs. These constructs, which consist of cells and/or biomaterials organized appropriately in a three-dimensional manner, can be used for surgical reconstruction to replace the excised tissues. In recent years, tissue engineering constructs have successfully been used in clinical reconstruction of bladder,[9] trachea,[10] and cartilage defects.[11]

In this review, we shall highlight some of the recent developments in oesophageal reconstruction after cancer surgery, which include: (1) to discuss the possible complications from existing surgical treatments of oesophageal cancer, (2) to highlight the basic principles of engineering a tissue construct, and (3) to identify promising tissue engineering strategies that could improve the surgical outcomes based on recent clinical trials and animal studies.

Oesophagectomy

During oesophagectomy, a gastric tube made by whole or subtotal stomach is commonly used as the reconstructed organ. Since oesophagectomy is extensive and invasive, it had been reported that there was a high mortality (23%) and morbidity (26-41%) in the past,[12] even if the tumor was curatively resected. Recent advances in surgical techniques and perioperative intensive care has reduced the mortality rate to 1·7%.[13] In particular, there is a relatively high incidence (3%–24%) of anastomotic leakage with oesophago-gastrostomy, which causes stricture after spontaneous healing or leads to fatal septic complications such as pyothorax.[14] Furthermore, dysfunctions of the reconstructed organ, such as stricture or regurgitation of enteral contents can lead to dysphasia, heartburn, and even fatal complication such as aspiration pneumonia.

Traditionally, surgical options for resection of oesophageal carcinoma include transhiatal and transthoracic approaches.[8] After oesophagectomy, a gastric tube pull-up made by whole or subtotal stomach is the preferred choice as the reconstructed organ, and colon conduit or supercharged jejunal flap are considered when the gastric tube is not available. However, the oesophagectomy procedure is complex and invasive and is often associated with high mortality and morbidity. Therefore, treatment in high-volume centres with experienced surgeons and the availability of critical-care support is associated with improved outcomes.[12]

In order to reduce complications of oesophagectomy and improve patient's quality of life, minimal invasive oesophagectomy has been introduced. A randomized trial of minimally invasive oesophagectomy compared with open oesophagectomy showed a decrease in the frequency of pulmonary complications in the minimally invasive group.[13] In this trial, minimally invasive oesophagectomy resulted in a lower incidence of pulmonary infections, a shorter hospital stay, and better short-term quality of life than did open oesophagectomy, with no compromise in the quality of the resected specimen.[13] However, even though the minimally invasive group showed the shorter hospital stay, there was still a high incident of anastomotic leakage with 12% in the group, suggesting that incidence of anastomotic leakage after oesophagectomy has not been improved in spite of lesser invasive approach.

It has been proposed that the reasons for anastomotic leakage of oesophago-gastrostomy after oesophagectomy include poor arterial inflow, insufficient venous drainage, high mechanical tension from over stretching of the stomach tube, technical problems in the suturing of the anastomosis, and poor nutritional status.[15] In order to reduce anastomotic leakage technically, several surgical options have been proposed including the use of a slender gastric tube,[16] the supercharge technique, which involves additional vascular anastomosis of the gastric tube by microsurgical techniques[17] and the use of auto-suturing techniques using a stapler device.[14] There is, however, no definite solution to overcome the anastomotic leakage, for example, meta-analysis of RCTs that compared hand-sewn anastomosis versus mechanical anastomosis using a circular stapler concluded that the use of a stapler contributed to reducing the length of the operation, but was associated with an increased risk of anastomotic strictures.[14]

Endoscopic treatments

Endoscopic submucosal dissection (ESD) or endoscopic mucosal resection (EMR) has been considered as the standard treatment for superficial oesophageal cancer[18] and Barrett's oesophagus with dysplasia.[19] Endoscopic resection for these early malignant and premalignant lesions is considered as a minimally invasive curative treatment with low morbidity. However, in most endoscopic resection treatments, there is still high incidence of stenosis, leading to dysphagia.[20] Of note, postoperative stricture in the oesophagus is frequently seen after resection of nearly circumferential lesions, in which the stricture rate after ESD was reported to be 18%[20] and those after stepwise EMR were 52-67%.[21,22]

At present, several medical interventions are proposed as the prophylactic options for the stricture after ESD or EMR;[23] intraluminal steroid injection, self-expandable metal stenting, and preventive balloon dilatation. However, there are a number of debates regarding the advantages and disadvantages of the options, and the clinical outcomes were inconsistent.[23] Patch reconstruction with tissue engineered constructs could provide a more viable solution after ESD or EMR.

Tissue engineering approach

The term "Tissue Engineering" is often used to describe, "an interdisciplinary field that applies the principles of biology and engineering toward the development of biological substitutes that restore, maintain, or improve tissue function".[24] Tissue engineering has been used synonymously with tissue regeneration because of its potential for restoring organ structure and functions. The history of tissue engineering dates back to the 1980s when the first tissue engineered skin was successfully used clinically.[25,26] The idea of growing and regenerating tissues and organs with the host's cells is clinically attractive.

Central to the tissue engineering approach is the role of the scaffold material. With the exception of the blood cells, all mammalian cells are anchorage dependent and therefore requiring scaffold support. In the human body, the extracellular matrices (ECM) are the native scaffolds. There are many types of ECM found in tissues and organs, and these include the vast family of collagens, elastic fibres, glycosaminoglycans, proteoglycans and adhesive glycoproteins.[27] The composition of the ECM varies depending on the type and function of the tissue or organ. In order to tissue-engineer a scaffold, a good understanding of the structure and functions of these ECM in the human tissues is essential.

ECM are naturally biodegradable macromolecules that provide a variety of biologically significant functions and some of these include (i) a three-dimensional (3D) environment for cell-cell and cell-matrix interaction necessary for cell attachment, migration, and proliferation,[28] (ii) provision of a stable and yet degradable structure that allows tissue remodeling in response to physiological and pathological needs,[27,29] (iii) a storage place for a range of growth factors for tissue development and functions,[30] and (iv) an environment for neovascularisation to ensure exchange of nutrients and metabolic wastes from the cells and tissues can take place. A more detailed discussion on extracellular matrices can be found elsewhere.[31]

Likewise, tissue-engineered scaffolds must have properties similar to the ECM. Some of these important scaffold properties are: (i) highly interconnecting porous structures with well-controlled pore size, (ii) provision of a 3D environment that supports cellular interactions that controls cell adhesion, migration, and proliferation, and (iii) degradable structure that provides initial structural and mechanical support during tissue development but is completely resorbable during tissue remodeling.

Porosity of the scaffold is important for cell infiltration into the scaffold necessary for tissue formation. The interconnectivity of the pores is necessary for the formation of capillaries that carry nutrients to the cells and remove metabolic wastes. Studies have shown that cells must be within 100 – 200 microns from the capillaries to remain viable.[32] Recent studies have also shown that the extent of inflammation response by macrophages and angiogenesis in scaffold are influenced by the size of the pores.[33] Scaffolds with pore size between 30 – 40 microns promote angiogenesis and reduce fibrotic response.[34,35]

Adhesion of cells on the scaffolds is the crucial step towards cell migration and proliferation. Hence, most synthetic polymer scaffolds such as poly(lactide-co-glycolide), poly(caprolactone), and fumarate-based polymers need to be modified with adhesion proteins for cell attachment.[36] Incorporation of growth factors on synthetic polymer scaffolds to promote cell growth and proliferation is also a common practice in tissue engineering.

Scaffold materials must be biodegradable and tissue compatible. The degradation product(s) of scaffold must be non-toxic and readily removed by the cells or resorbed. However, all scaffold materials, even collagen obtained from animal sources, are recognized by the host immune system as foreign bodies. Inflammatory reaction persists until all the foreign body is removed. Chronic inflammation due to foreign body invariably leads to fibrous capsule formation surrounding the scaffold.[37] In addition, the rate of scaffold degradation must also be comparable to the regeneration rate of the extracellular matrices.[38] The degradation profile of scaffold materials can differ based on different implant sites, pore structure and surface chemistry. Studies have shown that the optimal degradation half-life of scaffolds for repairing skin and peripheral nerve wounds should be around 2-3 weeks.[39,40] Further material modifications, such as crosslinking of scaffolds to achieve mechanical and structural stability, may be carried out to alter their degradation rates.[41]

Tissue engineered constructs for oesophageal reconstruction

Tissue engineered constructs can be used in the form of a patch or tubular grafts for oesophageal reconstruction (figure 1). The goal is to initially provide physical closure, and ultimately regenerate a functional oesophageal tissue. Often, long lengths of the oesophagus are removed thus requiring a long tissue engineered replacement constructs. The applications of both acellular and cellular grafts have demonstrated reasonable success in post-operative tissue replacement in animal models and pre-clinical studies. Table 1 summarises the use of tissue engineered patch or tubular constructs that have been used in oesophageal reconstruction after ESD/ EMR or oesophagectomy.

Acellular grafts

There are two kinds of acellular grafts that are used for oesophageal reconstruction research and trials. Acellular grafts can either be made from synthetic degradable polymers,[42-44] and natural sources such as collagen and decellularised tissues or organs obtained from animals.[45-48]

During surgical reconstruction, acellular grafts are used to replace the length of oesophagus removed during surgery. The success of the oesophageal reconstruction is determined by the ability of the acellular graft to regenerate a functional tissue without leakage and/or stenosis. Several graft properties that are crucial to the regeneration process include the following: (1) the ability to attract host oesophageal epithelial and smooth muscle cells into the graft, (2) the graft material must be non-toxic to the cells (3) the degradation profile of the graft must be comparable to the rate of tissue remodeling, and (4) the graft must modulate tissue integration processes such as vascularisation, scarring, wound contraction, and innervation.

Synthetic acellular polymeric grafts have the advantages of being xeno-free, readily available and reproducible. Although synthetic polymers lack bioactive molecules, the grafts can be surface modified with bioactive molecules such as collagen, laminin and fibronectin to attract and enhance cell attachment.[36,49] Early success was reported with the use of silicone and collagen hybrid tubes in canine models.[50,51] These hybrid tubes were used to bridge 5cm oesophageal defects in the animals for up to 24 months. The silicone tubing served as a stent and was endoscopically removed at various periods for up to 4 weeks. Those animals with the silicone stents removed at 4 weeks had regenerated stratified epithelium and formation of striated muscle tissue. However in animals where the silicone stents were removed before 4 weeks, stenosis, graft shrinkage, and incomplete epithelialisation and muscle cells in-growth were observed.

In a recent porcine study, porous surgical meshes made from biodegradable polyester were used for oesophageal reconstruction.[43] A temporary synthetic stent was also used to keep the lumen open and stabilize the implant. At 12 weeks after surgery, the epithelium and muscle tissues resembling the native oesophagus were found on the replacement graft. These initial evidences demonstrated that these temporary acellular grafts have promising potentials for oesophageal defect reconstruction and regeneration.

Despite the satisfactory results in the use of synthetic acellular grafts in animal models, no clinical trials have been conducted to evaluate their performance. In contrast, acellular matrices from decellularised tissues have been evaluated clinically.[47] These acellular matrices are produced by removing all the cellular and nucleus components whilst preserving the native collagenous environment, which contains physical and biochemical components of the native ECM.[52] Various methods of decellularisation have been discussed elsewhere.[53] These decellularised matrices can be resorbed *in vivo*, with the degradation profile and tissue remodeling outcomes depending upon the anatomic site.[54] The degradation profiles of porcine decellularised small intestinal submucosa (SIS) were studied by labeling the ECM with radioactive isotopes.[54] These labeled ECM were used in repairing urinary bladder and Achilles tendon in canine models.[55,56] In both studies, the SIS degraded by 40-60% in 4 weeks. Complete degradation of the SIS was observed by 60 days and 90 days in the Achilles tendon and urinary bladder studies respectively.

In a separate study involving the use of porcine SIS and urinary bladder matrix (UBM) for oesophageal patch repair, complete degradation of the decellularised matrices were observed within 30 to 60 days.[46] In addition, during degradation, these decellularised matrices also release biochemical components known to promote angiogenesis, cell migration, and proliferation.[48] Decellularised UBM implanted after circumferential EMR was shown to prevent stenosis, which is a common surgical outcome, for up to 2 months.[57] Histological analysis of the oesophageal replacement at 2 months also showed a lack of inflammation and scar tissues.

Since 2011, two clinical trials and two clinical practices to reconstruct oesophageal tissues using commercial decellularised matrices, such as porcine small intestinal submucosa (SIS,

Surgisis® - Cook Biotech)[45,47] and urinary bladder matrix (UBM, MatriStem - ACell Inc.),[48] were conducted. Details of the clinical trials will be discussed in a later section. In one of the clinical practices, a 8-layer UBM mesh was used in a patch oesophagoplasty procedure to repair the oesophageal defects in 4 patients.[48] All the patients had severely diseased oesophagi and had received varying forms of surgical augmentations but suffered from oesophageal stenosis prior to patch oesophagoplasty. The UBM mesh was layered such that its basement membrane formed the luminal surface of the patch so as to enhance the recruitment and attachment of the host epithelial cells. After the procedure all 4 patients had partially restored oesophageal functions and improved quality of life.

In the other clinical practice, SIS was used to replace excised oesophageal tissue after circumferential ESD/EMR.[45] All 3 patients with oesophageal high-grade dysplasia and superficial adenocarcinoma, but with no disease invasion to the lymph nodes, were selected for the treatment. The SIS was used with a temporary intraluminal stent that was removed between Day 9 – 19. Post-operatively, stricture occurred due to stent movement or incomplete coverage of the resected area with the SIS matrix. However, after oesophageal dilatation procedure, all 3 patients remained disease-free and without dysphagia for up to 21 months. Despite the small number of patients treated with acellular decellularised matrices, the preliminary results are nonetheless promising and offer good potential for oesophageal reconstruction and regeneration.

Cellular grafts

In contrast to acellular grafts, cellular grafts are tissue engineered constructs that are pre-seeded with host cells (figure 2). Biologically it would be ideal if the replacement cellular graft closely resembles the native anatomy of the human oesophagus. Using tissue engineering methods, oesophageal grafts with luminal epithelial cells and abluminal layers with smooth muscle, endothelial, and other stromal cells have been achieved.[58-60] Successful strategies for vascularising this full thickness cellular graft include the use of omentum[61] or latissimus dorsi[59] to provide vascular supply to the transplanted cells.

Several studies have shown that cell seeded constructs performed better than acellular scaffolds in promoting earlier tissue remodeling, reducing inflammatory response and preventing stricture in oesophageal reconstruction.[62,63] Patch replacement for porcine oesophageal defects using decellularised porcine oesophageal matrix seeded with autologous smooth muscle cells resulted in earlier tissue remodeling and less inflammatory response.[62] In a canine study, the role of the epithelial cells in promoting tissue integration and preventing stricture was also observed.[63] A tissue engineered oesophageal tissue was constructed containing oral keratinocytes, fibroblasts, and stomach smooth muscle tissue. Keratinocytes and fibroblasts were cultured on the luminal side of the basement membrane of a human amniotic membrane. The cellular membrane was then wrapped on the outside with a polyglycolic (PGA) mesh, which contains autologous smooth muscle tissues, forming a tube. A two-stage surgical procedure on dogs was used to develop the full thickness oesophageal graft. The tubular construct was first wrapped with omentum and then implanted in the abdomen of a dog for 3 weeks to establish a squamous epithelium and vascularised thick

muscle tissue. In a second surgery, the implanted cellular construct was used to replace an oesophageal defect created on the same dog. 66% of the dogs receiving constructs with regenerated epithelium did not develop stricture. In contrast, all dogs receiving the control constructs without regenerated epithelium developed stricture. These animal studies show that clinical success in oesophageal regeneration depends on the replacement graft ability to recapitulate the native anatomical structure and maintaining tissue viability.

Besides seeding oesophageal cells on 3D scaffolds, cell sheets have been used to repair oesophageal ulcerations and to prevent stricture after ESD in dogs and clinical studies.[64,65] Oral mucosal epithelial cells were cultured on thermo-responsive surfaces for 2 weeks. The cells were harvested as cell sheets, and introduced to the defect sites endoscopically in a single operation. Lateral contacts between the epithelial cells in the cell sheets were ensured and coverage of the excised submucosa was achieved immediately. Details of the clinical trial will be elaborated in a later section.

In another tissue engineering strategy, freshly harvested oesophageal organoid units were used to populate a poly(glycolic acid) tubular construct. These organoids were harvested from healthy oesophagus tissues that consist of a mixture of cells, with the epithelial cells on the periphery of a mesenchymal core.[61] Similar to the previous method, the tubular construct was first implanted with the omentum, and subsequently removed after 4 weeks for oesophageal reconstruction. The tissue-seeded constructs were used as patch and interposition grafts in rats for up to 42 days. Histological sections showed formation of a neo-oesophageal tissue that resembled the native oesophagus. Stenosis was observed in interposition grafts. Importantly, organoid units harvested from two out of three adult rats were able to reorganize into the tissue-engineered oesophagus.

The use of a decellularised matrix seeded with bone marrow-derived mesenchymal stromal cells (BMSCs) to facilitate regeneration of oesophageal tissues was reported.[66,67] Clinically, BMSCs have been used to repair cartilage defects and they have been shown to be non-tumourigenic. Compared to other autologous sources such as oesophageal cells or iPSC-derived cells, BMSCs are more readily available and could be harvested from bone marrow aspirates. Decellularised tissue matrices seeded with BMSCs have been shown to promote tissue remodeling, vascularization, and recruitment of epithelial and muscle cells in patch oesophagoplasty in canine model.[66] In a recent study, allogeneic rat BMSCs were observed to spontaneously differentiate into epithelial and muscle-like cells when cultured for 3 weeks on the adluminal and abluminal sides of decellularised rat oesophageal matrices respectively.[67] It was reported that spontaneous differentiation was induced by the presence of appropriate biochemical cues on the decellularised oesophageal matrices. The cell seeded constructs were used to replace the entire cervical oesophagus. Histological analysis of the explanted grafts after 14 days showed functional epithelium, regenerated muscle, nerve and vascular tissue structures. These studies demonstrate a new strategy involving the use of BMSCs with decellularised matrices could enhance the regeneration of the oesophagus.

Cell source

Before an engineered cellular graft can be realized, there is a need to establish reliable, readily available and clinically relevant sources of oesophageal cells. Several reports have demonstrated the feasibility of isolating and expanding two major oesophageal cell types, epithelial cells and smooth muscle cells, *in vitro*. These oesophageal cells were isolated from human,[59,68] rat,[69,70] porcine,[58] and canine[71] sources. Typically, cell isolation protocols involve firstly dislodging the epithelium from the basement membrane and then harvesting the epithelial cells. For harvesting smooth muscle cells, the extracellular matrices are treated with enzymes such as collagenase and elastase to free the muscles cells. Although it is possible to obtain cells from healthy autologous oesophageal tissues, it is often challenging as the process of obtaining biopsy samples could lead to scarring and is highly dependent on the extent of the cancer. There are also problems associated with expanding these highly differentiated adult cells consistently and rapidly for cell seeding purposes.

Recent advances in cellular reprogramming technology have made it possible to create patient-specific induced pluripotent stem cell (iPSC).[72] iPSCs are generated by treating adult cells with a cocktail of 4 specific genes for reprogramming. This technology has enabled a self-renewal human cell source, which can be further differentiated into the required oesophageal cell types. However, there is currently no established protocol to differentiate iPSCs to both oesophageal epithelial and smooth muscle cells. There are other challenges in the use of iPSCs-derived oesophageal cells and these include the purity of the cell population, their potential tumourigenicity, and the re-acceptance of the newly derived cells by the host despite their autologous origin.[73]

Clinical trials of regenerative medicine after resection of the oesophageal cancer

Two clinical trials on the prevention of oesophageal stenosis have recently been reported. In 2011, Badylak *et al.* utilized the decellularised matrix (ECM, SurgiSis®) and in 2012, Ohki *et al.* used engineered cell sheets for post- EMR and ESD treatments respectively.

In one trial, Badylak *et al.* utilized the decellularised matrix on the ulcer after multiple EMR for Barrett's oesophagus in 5 patients, followed by stents concurrently and removed later on.[47] Four months after the treatment, a nearly complete mature epithelium was positively identified by histological analysis with cytokeratin staining. Postoperative complications included small perforations and stent migration (Table 2).

Clinical trial conducted by Ohki *et al.* showed an approach in 9 patients using cell sheets cultured from autologous oral mucosal epithelial cells after ESD for superficial oesophageal cancer.[64] Cell sheets were transplanted to the mucosal defects after ESD, and a beneficial healing effect was observed in the follow-up, notably with only 1 patient experiencing stenosis (Table 2).

In the case of oesophagectomy, there has been no clinical trial and most treatments in animal models resulted in stenosis (Table 1).[46,61,63,74-76] However, observations from recent clinical trials involving patch replacement seem to suggest that stricture can be reduced when the

tissue engineered construct has complete intact layers of epithelium and smooth muscle tissues. Such a viable construct would consist of a decellularised matrix supporting the epithelium and muscle tissue, as demonstrated in a rat model presented in a recent study.[67] More developmental work needs to be done for the replacement tissue after oesophagectomy.

Conclusions

Tissue engineered constructs have been shown to be a promising and viable replacement in oesophageal reconstruction and regeneration. Two recent clinical trials and similar animal studies have shown that decellularised matrices and cell sheets tissue engineering approaches are able to reduce the incidences of stricture and to improve the outcome after ESD/EMR. However, the number of patients involved in these clinical trials is relatively small and a more extensive clinical study is needed to establish the efficacy of these approaches. Full thickness tissue replacement after oesophagectomy remains a challenge. It is hoped that advances in stem cell and cell sheet technologies could provide the ultimate solution to the current complications encountered after oesophagectomy.

Conflict of interest

The authors declared no conflict of interest.

Search strategy and selection criteria

References for this review were identified by searches of PubMed and Web of Science databases. Search criteria includes the terms “oesophageal tissue engineering”, “decellularised matrix”, “oesophageal cell”, “cell sheet engineering”, “oesophageal reconstruction”, “oesophageal cancer”, and “oesophageal surgery”. The focus of the search is on the use of tissue engineering solutions for oesophageal reconstruction in animal models or clinical trials since Year 2000. Only papers published in English were reviewed.

Acknowledgements

K.S. Chian would like to acknowledge the generous support from the Nanyang Technological University (Singapore) and the Singapore Agency for Science, Technology and Research (A*STAR) for the research funding (BMRC Grant No: 07/1/22/19/541). M.F. Leong is supported by the Institute of Bioengineering and Nanotechnology (Biomedical Research Council, Agency for Science, Technology and Research, Singapore). K. Kono is supported by a Clinician Scientist Award (CSA) and Clinician Scientist-Individual Research Grant (CS-IGR) from the National Medical Research Council of Singapore.

References

1. Enzinger PC, Mayer RJ. Esophageal cancer. *The New England journal of medicine* 2003;**349**(23):2241-52.
2. Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. *Journal of the National Cancer Institute* 2005;**97**(2):142-6.
3. Lepage C, Rachet B, Jooste V, Faivre J, Coleman MP. Continuing rapid increase in esophageal adenocarcinoma in England and Wales. *The American journal of gastroenterology* 2008;**103**(11):2694-9.
4. Pennathur A, Zhang J, Chen H, Luketich JD. The "best operation" for esophageal cancer? *The Annals of thoracic surgery* 2010;**89**(6):S2163-7.
5. Wu PC, Posner MC. The role of surgery in the management of oesophageal cancer. *Lancet Oncology* 2003;**4**(8):481-8.
6. van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. *The New England journal of medicine* 2012;**366**(22):2074-84.
7. Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *The New England journal of medicine* 2006;**355**(1):11-20.
8. Pennathur A, Gibson MK, Jobe BA, Luketich JD. Oesophageal carcinoma. *Lancet* 2013;**381**(9864):400-12.
9. Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB. Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet* 2006;**367**(9518):1241-6.
10. Macchiarini P, Jungebluth P, Go T, Asnaghi MA, Rees LE, Cogan TA, et al. Clinical transplantation of a tissue-engineered airway. *Lancet* 2008;**372**(9655):2023-30.
11. Sharma B, Fermanian S, Gibson M, Unterman S, Herzka DA, Cascio B, et al. Human Cartilage Repair with a Photoreactive Adhesive-Hydrogel Composite. *Sci Transl Med* 2013;**5**(167):9.
12. Birkmeyer JD, Siewers AE, Finlayson EV, Stukel TA, Lucas FL, Batista I, et al. Hospital volume and surgical mortality in the United States. *The New England journal of medicine* 2002;**346**(15):1128-37.
13. Biere SS, van Berge Henegouwen MI, Maas KW, Bonavina L, Rosman C, Garcia JR, et al. Minimally invasive versus open oesophagectomy for patients with oesophageal cancer: a multicentre, open-label, randomised controlled trial. *Lancet* 2012;**379**(9829):1887-92.
14. Walther B, Johansson J, Johnsson F, Von Holstein CS, Zilling T. Cervical or thoracic anastomosis after esophageal resection and gastric tube reconstruction: a prospective randomized trial comparing sutured neck anastomosis with stapled intrathoracic anastomosis. *Ann Surg* 2003;**238**(6):803-12.
15. Urschel JD. Esophagogastrostomy anastomotic leaks complicating esophagectomy: a review. *American journal of surgery* 1995;**169**(6):634-40.
16. Tabira Y, Sakaguchi T, Kuhara H, Teshima K, Tanaka M, Kawasuji M. The width of a gastric tube has no impact on outcome after esophagectomy. *American journal of surgery* 2004;**187**(3):417-21.
17. Poh M, Selber JC, Skoracki R, Walsh GL, Yu P. Technical challenges of total esophageal reconstruction using a supercharged jejunal flap. *Ann Surg* 2011;**253**(6):1122-9.
18. Altorki NK, Lee PC, Liss Y, Meherally D, Korst RJ, Christos P, et al. Multifocal neoplasia and nodal metastases in T1 esophageal carcinoma: implications for endoscopic treatment. *Ann Surg* 2008;**247**(3):434-9.

19. Shaheen NJ, Sharma P, Overholt BF, Wolfsen HC, Sampliner RE, Wang KK, et al. Radiofrequency ablation in Barrett's esophagus with dysplasia. *The New England journal of medicine* 2009;**360**(22):2277-88.
20. Ono S, Fujishiro M, Niimi K, Goto O, Kodashima S, Yamamichi N, et al. Long-term outcomes of endoscopic submucosal dissection for superficial esophageal squamous cell neoplasms. *Gastrointestinal endoscopy* 2009;**70**(5):860-6.
21. Chung A, Bourke MJ, Hourigan LF, Lim G, Moss A, Williams SJ, et al. Complete Barrett's excision by stepwise endoscopic resection in short-segment disease: long term outcomes and predictors of stricture. *Endoscopy* 2011;**43**(12):1025-32.
22. Lewis JJ, Rubenstein JH, Singal AG, Elmunzer BJ, Kwon RS, Piraka CR. Factors associated with esophageal stricture formation after endoscopic mucosal resection for neoplastic Barrett's esophagus. *Gastrointestinal endoscopy* 2011;**74**(4):753-60.
23. Rajan E, Gostout C, Feitoza A, Herman L, Knipschield M, Burgart L, et al. Widespread endoscopic mucosal resection of the esophagus with strategies for stricture prevention: a preclinical study. *Endoscopy* 2005;**37**(11):1111-5.
24. Langer R, Vacanti JP. Tissue engineering. *Science* 1993;**260**(5110):920-6.
25. Burke JF, Yannas IV, Quinby WC, Bondoc CC, Jung WK. Successful use of a physiologically acceptable artificial skin in the treatment of extensive burn injury *Ann Surg* 1981;**194**(4):413-28.
26. Bell E, Sher S, Hull B, Merrill C, Rosen S, Chamson A, et al. The reconstitution of living skin. *J Invest Dermatol* 1983;**81**(1):S2-S10.
27. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nature Biotechnology* 2005;**23**(1):47-55.
28. Hersel U, Dahmen C, Kessler H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials* 2003;**24**(24):4385-415.
29. Raeber GP, Lutolf MP, Hubbell JA. Mechanisms of 3-D migration and matrix remodeling of fibroblasts within artificial ECMs. *Acta Biomater* 2007;**3**(5):615-29.
30. Iyer AKV, Tran KT, Griffith L, Wells A. Cell surface restriction of EGFR by a tenascin cytotactin-encoded EGF-like repeat is preferential for motility-related signaling. *Journal of Cellular Physiology* 2008;**214**(2):504-12.
31. Hynes RO. The Extracellular Matrix: Not Just Pretty Fibrils. *Science* 2009;**326**(5957):1216-9.
32. Jain RK, Au P, Tam J, Duda DG, Fukumura D. Engineering vascularized tissue. *Nature Biotechnology* 2005;**23**(7):821-3.
33. Oates M, Chen R, Duncan M, Hunt JA. The angiogenic potential of three-dimensional open porous synthetic matrix materials. *Biomaterials* 2007;**28**(25):3679-86.
34. Madden LR, Mortisen DJ, Sussman EM, Dupras SK, Fugate JA, Cuy JL, et al. Proangiogenic scaffolds as functional templates for cardiac tissue engineering. *Proceedings of the National Academy of Sciences of the United States of America* 2010;**107**(34):15211-6.
35. Fleckman P, Usui M, Zhao G, Underwood R, Maginness M, Marshall A, et al. Cutaneous and inflammatory response to long-term percutaneous implants of sphere-templated porous/solid poly(HEMA) and silicone in mice. *Journal of Biomedical Materials Research Part A* 2012;**100A**(5):1256-68.
36. Zhu Y, Leong MF, Ong WF, Chan-Park MB, Chian KS. Esophageal epithelium regeneration on fibronectin grafted poly(L-lactide-co-caprolactone) (PLLC) nanofiber scaffold. *Biomaterials* 2007;**28**(5):861-8.
37. Anderson JM. Biological responses to materials. *Annual Review of Materials Research* 2001;**31**:81-110.

38. Hutmacher DW. Scaffolds in tissue engineering bone and cartilage. *Biomaterials* 2000;**21**(24):2529-43.
39. Yannas IV. Emerging rules for inducing organ regeneration. *Biomaterials* 2013;**34**(2):321-30.
40. Soller EC, Tzeranis DS, Miu K, So PTC, Yannas IV. Common features of optimal collagen scaffolds that disrupt wound contraction and enhance regeneration both in peripheral nerves and in skin. *Biomaterials* 2012;**33**(19):4783-91.
41. Leach JB, Bivens KA, Patrick CW, Schmidt CE. Photocrosslinked hyaluronic acid hydrogels: Natural, biodegradable tissue engineering scaffolds. *Biotechnology and Bioengineering* 2003;**82**(5):578-89.
42. Jansen PL, Klinge U, Anurov M, Titkova S, Mertens PR, Jansen M. Surgical mesh as a scaffold for tissue regeneration in the esophagus. *European Surgical Research* 2004;**36**(2):104-11.
43. Aikawa M, Miyazawa M, Okamoto K, Okada K, Akimoto N, Sato H, et al. A bioabsorbable polymer patch for the treatment of esophageal defect in a porcine model. *Journal of Gastroenterology* 2013;**48**(7):822-9.
44. Liang J-H, Zhou X, Zheng Z-B, Liang X-L. Long-Term Form and Function of Neoesophagus After Experimental Replacement of Thoracic Esophagus With Nitinol Composite Artificial Esophagus. *Asaio Journal* 2010;**56**(3):232-4.
45. Hoppo T, Badylak SF, Jobe BA. A Novel Esophageal-preserving Approach to Treat High-grade Dysplasia and Superficial Adenocarcinoma in the Presence of Chronic Gastroesophageal Reflux Disease. *World Journal of Surgery* 2012;**36**(10):2390-3.
46. Badylak S, Meurling S, Chen M, Spievack A, Simmons-Byrd A. Resorbable bioscaffold for esophageal repair in a dog model. *Journal of Pediatric Surgery* 2000;**35**(7):1097-103.
47. Badylak SF, Hoppo T, Nieponice A, Gilbert TW, Davison JM, Jobe BA. 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 engineering Part A* 2011;**17**(11-12):1643-50.
48. Nieponice A, Ciotola FF, Nachman F, Jobe BA, Hoppo T, Londono R, et al. Patch Esophagoplasty: Esophageal Reconstruction Using Biologic Scaffolds. *Annals of Thoracic Surgery* 2014;**97**(1):283-9.
49. Zhu Y, Chan-Park MB. Density quantification of collagen grafted on biodegradable polyester: Its application to esophageal smooth muscle cell. *Analytical Biochemistry* 2007;**363**(1):119-27.
50. Yamamoto Y, Nakamura T, Shimizu Y, Matsumoto K, Takimoto Y, Kiyotani T, et al. Intrathoracic esophageal replacement in the dog with the use of an artificial esophagus composed of a collagen sponge with a double-layered silicone tube. *Journal of Thoracic and Cardiovascular Surgery* 1999;**118**(2):276-86.
51. Takimoto Y, Nakamura T, Yamamoto Y, Kiyotani T, Teramachi M, Shimuzu Y. The experimental replacement of a cervical esophageal segment with an artificial prosthesis with the use of collagen matrix and a silicone stent. *Journal of Thoracic and Cardiovascular Surgery* 1998;**116**(1):98-106.
52. Hodde J. Naturally occurring scaffolds for soft tissue repair and regeneration. *Tissue Engineering* 2002;**8**(2):295-308.
53. Gilbert TW, Sellaro TL, Badylak SF. Decellularization of tissues and organs. *Biomaterials* 2006;**27**(19):3675-83.
54. Gilbert TW, Stewart-Akers AM, Badylak SF. A quantitative method for evaluating the degradation of biologic scaffold materials. *Biomaterials* 2007;**28**(2):147-50.

55. Record RD, Hillegonds D, Simmons C, Tullius R, Rickey FA, Elmore D, et al. In vivo degradation of C-14-labeled small intestinal submucosa (SIS) when used for urinary bladder repair. *Biomaterials* 2001;**22**(19):2653-9.
56. Gilbert TW, Stewart-Akers AM, Simmons-Byrd A, Badylak SF. Degradation and remodeling of small intestinal submucosa in canine Achilles tendon repair. *Journal of Bone and Joint Surgery-American Volume* 2007;**89A**(3):621-30.
57. Nieponice A, McGrath K, Qureshi I, Beckman EJ, Luketich JD, Gilbert TW, et al. An extracellular matrix scaffold for esophageal stricture prevention after circumferential EMR. *Gastrointestinal Endoscopy* 2009;**69**(2):289-96.
58. Leong MF, Chan WY, Chian KS, Rasheed MZ, Anderson JM. Fabrication and in vitro and in vivo cell infiltration study of a bilayered cryogenic electrospun poly(D,L-lactide) scaffold. *Journal of Biomedical Materials Research Part A* 2010;**94A**(4):1141-9.
59. Hayashi K, Ando N, Ozawa S, Kitagawa Y, Miki H, Sato M, et al. A neo-esophagus reconstructed by cultured human esophageal epithelial cells, smooth muscle cells, fibroblasts, and collagen. *Asaio Journal* 2004;**50**(3):261-6.
60. Kosoff RE, Gardiner KL, Merlo LMF, Pavlov K, Rustgi AK, Maley CC. Development and characterization of an organotypic model of Barrett's esophagus. *Journal of Cellular Physiology* 2012;**227**(6):2654-9.
61. Grikscheit T, Ochoa ER, Srinivasan A, Gaissert H, Vacanti JP. Tissue-engineered esophagus: Experimental substitution by onlay patch or interposition. *Journal of Thoracic and Cardiovascular Surgery* 2003;**126**(2):537-44.
62. Marzaro M, Vigolo S, Oselladore B, Conconi MT, Ribatti D, Giuliani S, et al. In vitro and in vivo proposal of an artificial esophagus. *Journal of Biomedical Materials Research Part A* 2006;**77A**(4):795-801.
63. Nakase Y, Nakamura T, Kin S, Nakashima S, Yoshikawa T, Kuriu Y, et al. Intrathoracic esophageal replacement by in situ tissue-engineered esophagus. *Journal of Thoracic and Cardiovascular Surgery* 2008;**136**(4):850-9.
64. Ohki T, Yamato M, Ota M, Takagi R, Murakami D, Kondo M, et al. Prevention of esophageal stricture after endoscopic submucosal dissection using tissue-engineered cell sheets. *Gastroenterology* 2012;**143**(3):582-8 e1-2.
65. Ohki T, Yamato M, Murakami D, Takagi R, Yang J, Namiki H, et al. Treatment of oesophageal ulcerations using endoscopic transplantation of tissue-engineered autologous oral mucosal epithelial cell sheets in a canine model. *Gut* 2006;**55**(12):1704-10.
66. Tan B, Wei RQ, Tan MY, Luo JC, Deng L, Chen XH, et al. Tissue engineered esophagus by mesenchymal stem cell seeding for esophageal repair in a canine model. *Journal of Surgical Research* 2013;**182**(1):40-8.
67. Sjoqvist S, Jungebluth P, Lim ML, Haag JC, Gustafsson Y, Lemon G, et al. Experimental orthotopic transplantation of a tissue-engineered oesophagus in rats. *Nat Commun* 2014;**5**:15.
68. Green N, Huang Q, Khan L, Battaglia G, Corfe B, MacNeil S, et al. The Development and Characterization of an Organotypic Tissue-Engineered Human Esophageal Mucosal Model. *Tissue Engineering Part A* 2010;**16**(3):1053-64.
69. Beckstead BL, Pan S, Bhrany AD, Bratt-Leal AM, Ratner BD, Giachelli CM. Esophageal epithelial cell interaction with synthetic and natural scaffolds for tissue engineering. *Biomaterials* 2005;**26**(31):6217-28.
70. Saxena AK, Kofler K, Ainodhofer H, Hollwarth M. Esophagus Tissue Engineering: Hybrid Approach with Esophageal Epithelium and Unidirectional Smooth Muscle

Tissue Component Generation In Vitro. *Journal of Gastrointestinal Surgery* 2009;**13**(6):1037-43.
71. Wei RQ, Tan B, Tan MY, Luo JC, Deng L, Chen XH, et al. Grafts of Porcine Small Intestinal Submucosa with Cultured Autologous Oral Mucosal Epithelial Cells for Esophageal Repair in a Canine Model. *Exp Biol Med* 2009;**234**(4):453-61.
72. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;**131**(5):861-72.
73. Hanna JH, Saha K, Jaenisch R. Pluripotency and Cellular Reprogramming: Facts, Hypotheses, Unresolved Issues. *Cell* 2010;**143**(4):508-25.
74. Badylak SF, Vorp DA, Spievack AR, Simmons-Byrd A, Hanke J, Freytes DO, et al. Esophageal reconstruction with ECM and muscle tissue in a dog model. *Journal of Surgical Research* 2005;**128**(1):87-97.
75. Lopes MF, Cabrita A, Ilharco J, Pessa P, Paiva-Carvalho J, Pires A, et al. Esophageal replacement in rat using porcine intestinal submucosa as a patch or a tube-shaped graft. *Diseases of the Esophagus* 2006;**19**(4):254-9.
76. Doede T, Bondartschuk M, Joerck C, Schulze E, Goernig M. Unsuccessful Alloplastic Esophageal Replacement With Porcine Small Intestinal Submucosa. *Artificial Organs* 2009;**33**(4):328-33.

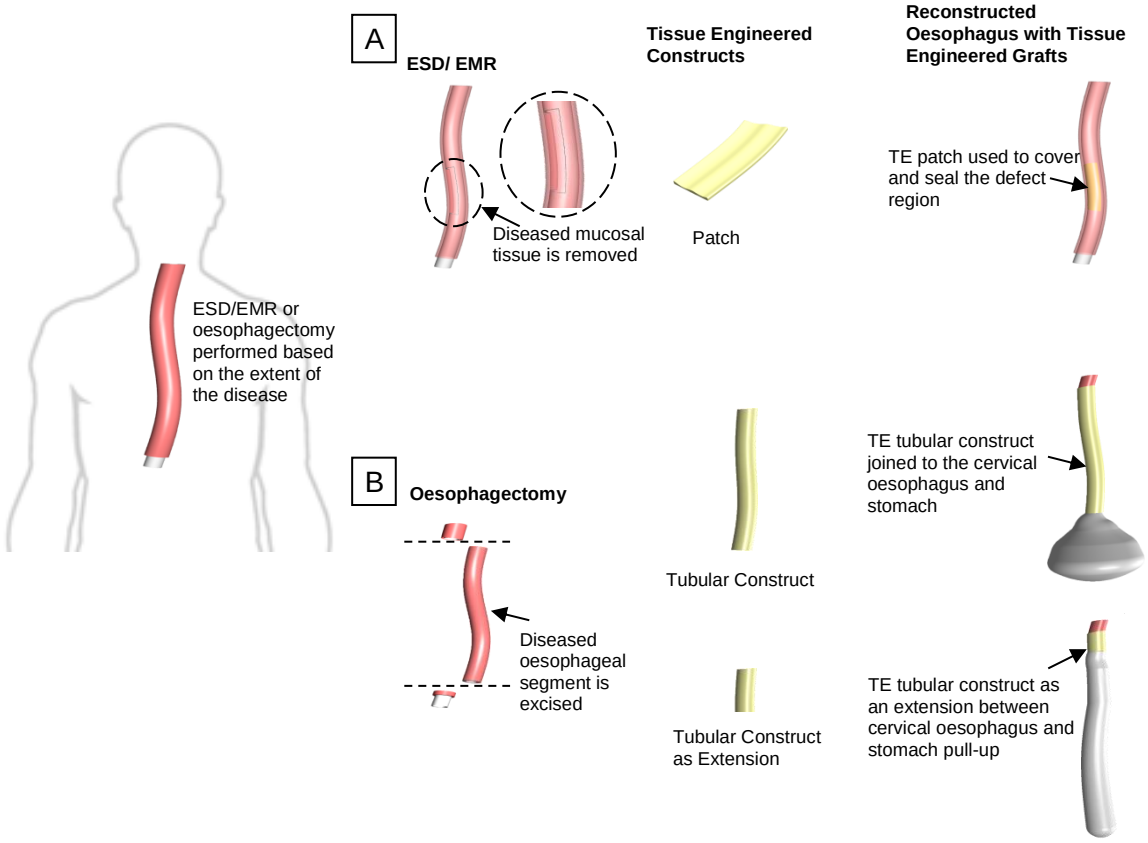

Figure 1 Tissue engineering solutions for endoscopic resections (ESD/EMR) and oesophagectomy.

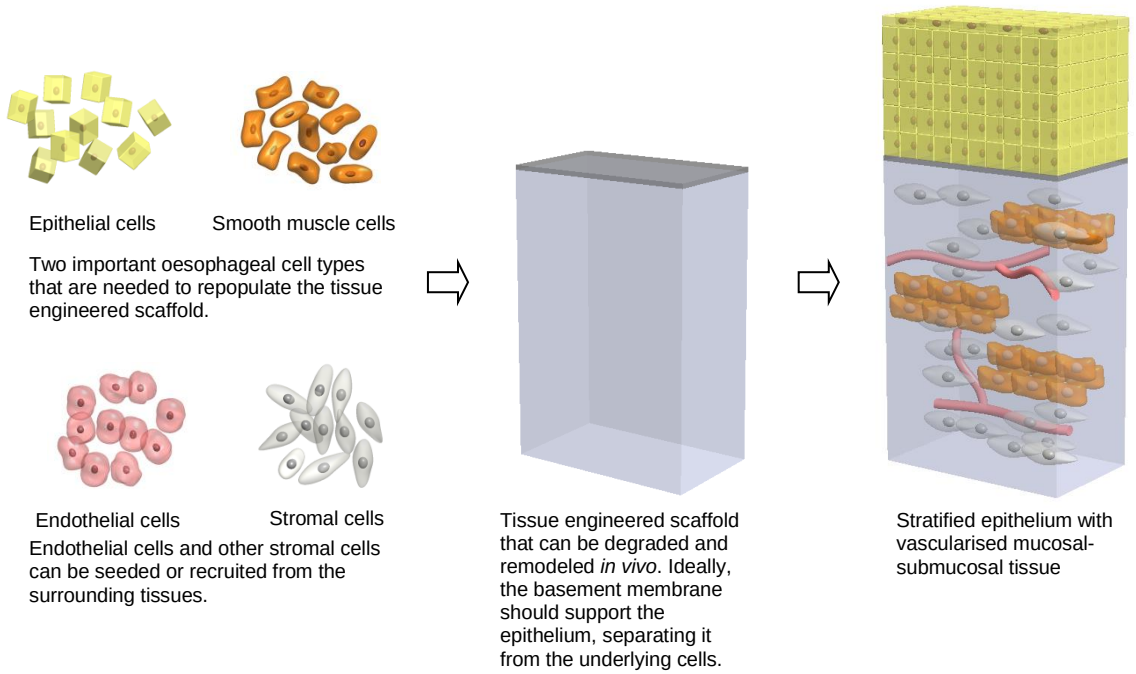

Figure 2 Tissue engineering approach in constructing a cellular oesophageal construct.

Table 1 Tissue engineering grafts used in oesophageal reconstruction since Year 2000.

			2000 – 2009	2010 - present
ESD / EMR	Acellular	Synthetic materials	Jansen et al. (2004) [42] ✦	Aikawa et al. (2013) [43]
		Decellularised matrices	Badylak et al. (2000) [46] Badylak et al. (2005) [74] ⧗ Marzaro et al. (2006) [62] Lopes et al. (2006) [75] Nieponice et al. (2009) [57]	Badylak et al. (2011) [47] ✦ ⁘ Hoppo et al. (2012) [45] ⧗ Nieponice et al. (2014) [48] ✦
	Cellular	Synthetic materials	Grikscheit et al. (2003) [61]	
		Decellularised matrices	Marzaro et al. (2006) [62] Wei et al. (2009) [71]	Tan et al. (2013) [66]
		Cell sheet	Ohki et al. (2006) [65]	Ohki et al. (2012) [54] ⧗
Oesophagectomy	Acellular	Synthetic materials		Liang et al. (2010) [44]
		Decellularised matrices	Badylak et al. (2000) [46] ⧗ Badylak et al. (2005) [74] ⧗ Lopes et al. (2006) [75] ⧗ Doede et al. (2009) [76] ⧗	
	Cellular	Synthetic materials	Grikscheit et al. (2003) [61] ⧗	
		Decellularised matrices	Nakase et al. (2008) [63] ⧗	Sjoqvist et al. (2014) [67]

***In vivo* models: Human, Rat, Dog, Pig, Rabbit**

Problems: ✦ Leakage ⧗ Stenosis ⁘ Construct dislocation / migration

Table 2 Clinical trials of regenerative medicine after oesophageal cancer treatment.

	Badylak et al. (2011)	Ohki et al. (2012)
No of patients	5	9
Age	62·2 y.o. (54-68)	68·7 y.o. (55-80)
Pathology	Barrett's Eso with HGD	ESCC
Endoscopic treatment	Multiple EMR /RFA/PDT	ESD
Resection size	8-13 cm in length	38·8 x 32·8 mm
Biological materials	Biological scaffold materials composed of xenogeneic extracellular matrix	Autologous cell sheet
Concurrent treatment	stenting	No
Postoperative stricture	No case for applied lesion 5 cases for not applied lesion	1 stricture
Major complications	1 muscle tear 1 perforation 1 stent migration	No

Eso, oesophagus; HGD, high grade dysplasia, EMR, endoscopic mucosal resection;
RFA, radiofrequency ablation; PDF, photodynamic therapy;
ESCC, oesophageal squamous cell carcinoma; ESD, endoscopic submucosal dissection