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TOPIC HIGHLIGHT

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Esophageal tissue engineering: A new approach for esophageal replacement

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Abstract

A number of congenital and acquired disorders require esophageal tissue replacement. Various surgical techniques, such as gastric and colonic interposition, are standards of treatment, but frequently complicated by stenosis and other problems. Regenerative medicine approaches facilitate the use of biological constructs to replace or regenerate normal tissue function. We review the literature of esophageal tissue engineering, discuss its implications, compare the methodologies that have been employed and suggest possible directions for the future. Medline, Embase, the Cochrane Library, National Research Register and ClinicalTrials.gov databases were searched with the following search terms: stem cell and esophagus, esophageal replacement, esophageal tissue engineering, esophageal substitution. Reference lists of papers identified were also examined and experts in this field contacted for further information. All full-text articles in English of all potentially relevant abstracts were reviewed. Tissue engineering has involved acellular scaffolds that were either transplanted with the aim of being repopulated by host cells or seeded prior to transplantation. When acellular scaffolds were used to replace patch and short tubular defects they allowed epithelial and partial muscular migration whereas when employed for long tubular defects the results were poor leading to an increased rate of stenosis and mortality. Stenting has been shown as an effective means to reduce stenotic changes and promote cell migration, whilst omental wrapping to induce vascularization of the construct has an uncertain benefit. Decellularized matrices have been recently suggested as the optimal choice for scaffolds, but smart polymers that will incorporate signalling to promote cell-scaffold interaction may provide a more reproducible and available solution. Results in animal models that have used seeded scaffolds strongly suggest that seeding of both muscle and epithelial cells on scaffolds prior to implantation is a prerequisite for complete esophageal replacement. Novel approaches need to be designed to allow for peristalsis and vascularization in the engineered esophagus. Although esophageal tissue engineering potentially offers a real alternative to conventional treatments for severe esophageal disease, important barriers remain that need to be addressed.

Key words: Esophagus; Regenerative medicine; Tissue engineering; Scaffolds; Transplantation

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INTRODUCTION

Recent years have witnessed great interest in regenerative medicine, the replacement, repair and regeneration of tissues and organs[1,2]. Particular interest has focused on the potential for this new field to offer new solutions for failing tissues and organs, and alternatives to transplantation, implants and reconstructive surgery, all of which have limitations.

Several conditions, both congenital and acquired, may require esophageal tissue replacement. In the pediatric population the primary indication for esophageal replacement is long-gap esophageal atresia (EA) with insufficient length for primary anastomosis. Patients with long-gap EA, which fail a primary repair, receive a denervated gastric pull-up or interposition graft using either jejunum or colon, with many associated early and late post-operative complications, such as stricture formation and the potentially carcinogenic effect of acid reflux[3,4]. In children, gastric transposition and intestinal interposition can also be used in esophageal strictures not responsive to dilatation following failed EA repair or caustic ingestion, or for rare neoplastic conditions such as inflammatory pseudotumor, leiomyosarcoma and teratoma[5]. By contrast, the commonest indication for esophageal replacement in adults is cancer, a condition whose incidence is escalating[6], whilst colon interposition is sometimes indicated for diffuse Barrett's esophagus, a premalignant condition. Unfortunately, all of these methods of esophageal replacement severely impair the quality of life of recipient adults and children[7,8] and present problems related to donor site morbidity. Even recent developments in endoluminal resection, which removes the diseased inner layers of the esophagus through an endoscope, whilst reducing morbidity, still results in a high rate of stenosis and consequent dysphagia[9]. Despite its 60-year history, conventional organ transplantation is not a solution for the failure of every organ, due to technical and ethical issues, and is specifically unable to address the unmet needs of esophageal replacement. Thus, regenerative medicine techniques, which extend the boundaries of reconstruction and do not, in most applications, require immunosuppression, present attractive alternatives[10].

Regenerative medicine has been used to describe the use of natural human substances, such as genes, proteins, cells, and biomaterials to regenerate diseased or damaged human tissue[11,12] in order to restore normal function[2]. Tissue engineering with the end-point of organogenesis has been successful through a combination of appropriate cells with a scaffold[13-17] as well as the use of only one of these two components, for example in the repair of urethra[18] and skin[19] (Figure 1).

We review the literature relating to esophageal tissue engineering and suggest areas where research may lead to the most rapid clinical gains.

INFORMATION COLLECTION

We searched Medline, Embase, the Cochrane Library, National Research Register and ClinicalTrials.gov databases, using the search terms stem cell and esophagus, esophageal replacement, esophageal tissue engineering, esophageal substitution. The reference lists of papers identified in this way were searched and further papers identified. All full-text articles in English of potentially relevant abstracts were reviewed. Finally, acknowledged experts in this field were contacted for information on gaps in our review and information on unpublished studies.

TWO BROAD CATEGORIES OF INTERVENTION

Seventy-four papers were identified and are reviewed in this manuscript. Two broad categories of intervention were identified: the use of scaffolds alone, and a combination of cells and scaffolds.

Acellular scaffolds

The majority of identified studies transplanted acellular scaffolds with the aim that host epithelial and smooth muscle cells will migrate to repopulate the new conduit. Acellular scaffolds studied to date conform to one of three categories: synthetic, collagen alone and decellularized matrix.

Synthetic scaffolds: Acellular synthetic scaffolds such as polyethylene plastic[20-22] and silicon[23,24] have been used for esophageal replacement, but the nature of the materials did not allow cellular migration and led to poor results in animal models. When polyvinylidene fluoride (PVDF) and polyglastin-910 (Vicryl®) were compared for the regeneration of patch defects in rabbits, PVDF was shown to lead to improved results with an absence of strictures and neoepithelialization[25]. However, in a different study, the combination of Vicryl® and collagen brought about positive results both for patch and tubular defects in dogs, with a low mortality of 8.3%[26]. The successful use of synthetic polymers in other organs such as the trachea[27], suggests that this approach may appear attractive and further development of appropriate materials is needed.

Collagen scaffolds: In a series of experiments performed by a research group in Japan, porcine dermal collagen scaffolds were used to produce porous tubular structures (Table 1)[28-33]. The general methodology involved the use of these

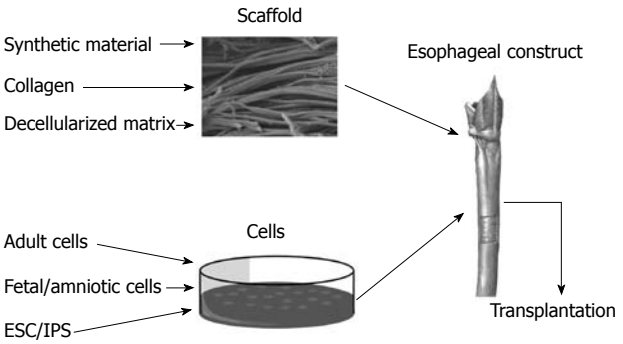

Figure 1 Esophageal tissue engineering. A tissue-engineered esophageal construct may be created by the combination of a scaffold and cells, grown in a bioreactor and transplanted in patients. A three-dimensional scaffold may be created from synthetic material, collagen or a decellularized matrix. Cells for the use of tissue engineering are derived from a number of sources such as the adult, fetus and the embryo. Additionally, non-seeded scaffolds may be transplanted with the aim of being repopulated by host cells. ESC: Embryonic stem cells; IPS: Induced pluripotent stem cells.

scaffolds to replace 5-10 cm tubular defects in the cervical or intra-thoracic portion of the esophagus in dogs. A silicon tube was used as a stent to support the scaffold until repopulation occurred. Aiming to avoid complications such as stenosis[28,29], the research group compared whether this was related to the time for which the scaffold was supported by the stent. In an experiment where three groups of dogs had a 5-cm cervical surgically created defect, the stent was removed at either 2, 3 or 4 wk. With increasing stent duration, it was observed that greater epithelial and muscle cell densities were achieved in the collagen scaffold, and this correlated with decreased stenosis and mortality[31]. However, when the collagen scaffold replaced 10-cm portions of the esophagus there was poor cellular migration in the muscular layer, suggesting that there are limitations to the size of defect that may be replaced by this methodology[30]. Moreover, when the same methods were used to replace intra-thoracic portions of the esophagus in dogs, muscular regeneration was completely absent, something the authors attributed to the lack of a vascular supply in the thorax[32]. In an attempt to address this, the scaffold was wrapped in omentum[33], as has been successfully applied to tracheal tissue engineering[34,35]. However, muscular regeneration remained absent, whilst an increase in mid-portion stenosis and mortality was observed[33].

Decellularized matrix: Decellularized matrices are derived from human and animal organs and tissues that have been treated to remove cells and immunogeneic material[36]. Importantly, however, they retain the macro- and micro-architecture of the tissue of origin, and the molecular components of its natural extracellular matrix[37-40]. They have the added hypothetical advantages over synthetic scaffolds of not producing potentially toxic degradation products or inducing inflammation characteristics that may be important in the prevention of stenosis[20,41,42]. Decellularized scaffolds that have been used for esophageal organs originated from the esophagus as well as from other tissues such as the small intestinal submucosa (SIS)[28-31,43-47].

Significant heterogeneity exists among studies, both with respect to the type of scaffold, extent of surgery and species used, which partly explains the range of results reported. Thus, regeneration of the muscularis propria layer is seen to take place in some studies[43,44,48], but not others[49]. Studies that have attempted tube-interposition with SIS report the development of esophageal stenosis and increased mortality[44,50,51]. By contrast, studies applying SIS as a patch repair demonstrated encouraging results[44,50-53]. Badylak *et al*[45] laid sheets of SIS onto the raw internal surface of esophagus following endoscopic submucosal resection in five patients with superficial cancers. With a follow-up of 4 to 24 mo, the scaffold promoted physiological remodelling as evident by endoscopy and histological characterisation following biopsy. Strictures still formed, but only at areas outside those lined by SIS, suggesting that possible technical improvements in scaffold delivery could ameliorate this. In fact, when SIS was used to completely cover a 3 cm × 5 cm mucosal defect in the cervical esophagus, there was no stenosis and endoscopy at 4 wk demonstrated good integration of the scaffold[46].

Hypothetically, decellularized esophageal tissue should retain the signals, both chemical and structural, that will direct the appropriate migration and differentiation of host cells, in a way unlikely to occur with scaffolds originating outside the esophagus, such as SIS. Ozeki *et al*[54] compared two methods of decellularization of adult rat esophagus based on deoxycholate and Triton X-100 respectively and assessed the resulting scaffolds using routine histology and biocompatibility. Those treated with deoxycholate showed superior mechanical properties, maintenance of the extracellular matrix and a lower DNA content than those treated with Triton X-100. Bhrany *et al*[55] found a combination of 0.5% sodium dodecyl sulphate and Triton X-100 to be effective in decellularization, albeit with a loss of tensile strength as measured by burst pressure studies. Our experience with the detergent-enzymatic treatment in the decellularization of the intestine[39] allowed us to use the same methodology in the esophagus (Figure 2), leading to an improved preservation in microarchitecture[56].

Cell-seeded scaffolds

To reduce complications arising from acellular approaches, some authors have seeded the scaffolds prior to transplantation. As mentioned, the two main cell types that are important for esophageal tissue engineering are those that will reconstitute the epithelium and the muscle layer on the luminal and extra-luminal sides respectively. Also important in the formation of a functional esophagus are the vascular and neuronal cell components but we could locate no studies that have studied these in engineered esophagus.

A number of *in vitro* experiments have examined the seeding and culture of esophageal epithelial cells and different scaffolds to assess the optimal combination. When a matrix composed of decellularized human skin was

Table 1 Overview of *in vivo* transplantation of acellular matrices

Animal model (*n*)	Scaffold type	Size	Results		Ref.
			Scaffold regeneration	Clinical course	
Canine (19)	Collagen with silicon stent (not removed)	5 cm circumferential gap, cervical esophagus	Partial epithelial regeneration	26% mortality	[28]
Canine (26)	Collagen with silicon stent (removed between 2 and 8 wk)	5 cm circumferential gap, cervical esophagus	Epithelial regeneration, no stenosis	0% mortality when stent dislodged after 4 wk (*n* = 4)	[29]
Canine (7)	Collagen with silicon stent (removed at 6 wk)	10 cm circumferential gap, cervical esophagus	Epithelial and partial muscular regeneration, no stenosis	29% mortality	[30]
Canine (43)	Collagen with silicon stent (removed either at 2, 3 or 4 wk)	5 cm circumferential gap, cervical esophagus	Epithelial and muscular regeneration, no stenosis	0% mortality when stent was removed at 4 wk (*n* = 16)	[31]
Canine (9)	Collagen with silicon stent (removed at 4 wk)	5 cm circumferential gap, thoracic esophagus	Epithelial but no muscular regeneration, mid-portion stenosis	11% mortality	[32]
Canine (14)	Collagen with silicon stent (removed at 4-8 wk) +/- OMPx	5 cm circumferential gap, thoracic esophagus	Epithelial regeneration, mid-portion stenosis	11% mortality in control group, 80% in OMPx group	[33]
Canine (15)	Extracellular matrix scaffold from either small intestine (*n* = 12) or urinary bladder submucosa (*n* = 3)	5 cm semi-circumferential or 5 cm circumferential, cervical esophagus	Mucosal and muscular regeneration. Stenosis in case of complete circumferential defects	0% mortality	[44]
Pigs (10)	Elastin based acellular biomaterial patch (from porcine aorta)	2-cm circular defect, abdominal esophagus	Mucosal and muscular regeneration	0% mortality. No complications reported in treatment groups	[43]
Canine (12)	Urinary bladder matrix scaffold	Complete transection with replacement of endomucosa with matrix	Mucosal and muscular regeneration	0% mortality. No complications reported in treatment groups	[48]
Rats (67)	Small intestinal submucosa patch graft	Semi-circumferential defect, cervical or abdominal esophagus	Mucosal and muscular regeneration at 150 d	94% survival at 150 d	[50]
Rats (85)	Small intestinal submucosa patch graft, or tube interposition	Semi-circumferential defect or segmental esophageal excision	Tube interposition unsuccessful. Mucosal and muscular regeneration at 150 d in patch-group	100% survival for patch-group (and no complications reported), 0% survival for tube interposition group at 28 d	[52]
Rats (27)	Gastric acellular matrix scaffold	Patch defects, abdominal esophagus	Mucosal regeneration seen at 2 wk. No muscular regeneration seen up to 18 mo	11% complication rate	[49]
Pigs (14)	Small intestinal submucosa (tubular)	4-cm defect, cervical esophagus	Prosthesis not found either macroscopically or histologically	Only 1 pig survived the full 4 wk study. The other pigs have to be sacrificed prematurely due to severe stenosis	[51]
Human (5)	Porcine small intestinal mucosa	8-cm to 13-cm en-bloc resection of mucosa and submucosa for superficial carcinoma	Restoration of normal mucosa as early as 4 mo	Strictures; perforation in one patients	[45]
Human (1)	Porcine small intestinal mucosa	5 cm × 3 cm defect cervical esophagus	Intact esophagus with normal calibre	No complications encountered	[46]

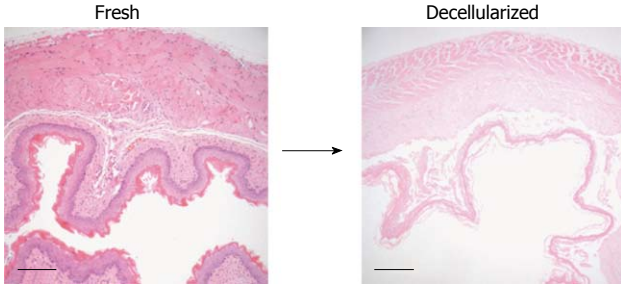

Figure 2 Production of esophageal natural acellular matrices. Decellularization involves treatment of fresh esophageal tissue with a combination of solutions that will remove the cells but maintain the structural characteristics of the native extracellular matrix. The optimal methodology of esophageal decellularization is currently under investigation. Our experience with the detergent enzymatic treatment is illustrated here with hematoxylin and eosin staining of a representative decellularized esophagus demonstrating preservation of the native architecture (Scale bar 100 μm).

compared to synthetic scaffolds *in vitro* for the capacity to support cultured epithelial cells, the decellularized scaffold exhibited cell differentiation and surface confluence similar to native esophagus, whereas synthetic scaffolds demonstrated a discontinuous epithelial lining[57]. Another study compared the growth of human esophageal squamous cells on human decellularized esophagus, porcine decellularized esophagus, human decellularized dermis, and collagen[58]. Interestingly the porcine matrix and collagen gave better results leading to the formation of a mature stratified epithelium. When rat esophageal epithelial cells (EEC) were seeded onto 3-dimensional (3-D) collagen scaffolds they were shown to be viable for up to 8 wk *in vitro* but did not fully integrate within the scaffold, remaining on the surface as individual cells or small clusters[59]. Seeding of sheep EEC on the same 3-D collagen scaffold resulted in the absence of epithelium sheet for-

mation, which was attributed to cellular penetration into the scaffold and loss of cell-to-cell contact[60]. However, when the same cells were seeded on the 2-D collagen scaffolds a single layer of epithelium was evident following 3 wk of *in vitro* culture that remained viable up to 6 wk. The same group has also performed *in vivo* studies of vascularization of the EEC-scaffold construct by omental transplantation in lambs for 8-12 wk[61]. Positive selection of the epithelial population could increase proliferative capacity as demonstrated by Kofler *et al*[62], who selected ovine EEC for expression of pancytokeratin (PCK) using fluorescence activated cell sorting. The PCK-negative subpopulation had minimal cell attachment on the collagen scaffolds, whereas the PCK-positive cells had a uniform distribution.

In vivo experiments using EEC-scaffold constructs, similarly to results in acellular approaches, have shown more promise for regeneration of partial rather than circumferential defects in rats and dogs[63-65]. An innovative approach recently described seeded cells on a temperature-responsive dish that became hydrophilic at 20 ℃ and allowed harvesting of a single-cell sheet[63]. When the cell sheets were transplanted in dogs that had undergone endoscopic submucosal resection, complete wound healing was observed at 4 wk with no signs of stricture and an intact epithelium. Wei *et al*[64] obtained mucosal epithelial cells from oral biopsy, or esophageal organoid units created following digestion of rat esophagi[65]. These were seeded onto scaffolds and implanted as complete esophageal substitutes, but histology of the resultant muscle layers showed poor architecture.

To overcome the limitations of using EEC in isolation, esophageal constructs prepared using EEC-seeded collagen scaffolds were placed on the latissimus dorsi muscle of athymic mice with the intention to harvest and tubularize the muscle once the epithelial side has matured[66,67]. Miki *et al*[68] found an increase in the number of epithelial layers from 2 when EEC seeded alone, to 18 when co-seeded with fibroblasts. A more recent study by Hayashi *et al*[69] cultured both epithelial and fibroblast cells on a bed of smooth muscle cells (SMC) embedded in a collagen gel *in vitro*, prior to transplanting them on the latissimus dorsi of athymic rats. Nakase *et al*[70] also aimed to combine different cell lines and scaffolds into one tubular structure in dogs. They used oral keratinocytes and fibroblasts cultured on human amniotic membrane and SMC seeded on poly (glycolic acid). These two scaffolds were then rolled together and implanted into the omentum for 3 wk, following which they were transplanted into a 3-cm intrathoracic esophageal defect. Both muscular and epithelial layers were present at 420 d of follow-up, although no peristaltic activity was observed.

FUTURE PERSPECTIVES

Based on the above literature, it is clear that although tissue engineering has been proposed as a solution for the current treatments of esophageal defects, currently, there is no clear strategy for recreating all the portions of the esophagus in man[71,72]. The problems that need to be solved are related to the optimal scaffold, the cell sources for the epithelial and muscular components, peristalsis and vascularization (Table 1). The stenotic changes that are the main complication encountered with esophageal constructs are likely related to poor regeneration of natural architecture.

The recent trend in organ tissue engineering has been to use decellularized scaffolds. It has been suggested that they would be an advantageous choice due to their enhancement of cellular proliferation, migration and differentiation. However, the lack of positive results when trying to replace a tubular defect, confirms that the use of biomaterials alone as a means of esophageal repair is unsuccessful. We envisage a point where "smart polymers" may replace scaffolds of biological origin and facilitate an "off-the-shelf" approach to esophageal tissue-engineering. Our group and collaborators in Sweden have used polyhedral oligomeric silsesquioxane-poly (carbonate-urea) urethane, a synthetic material used in clinical trials of vascular grafting, as an alternative to biologic scaffolds in the generation of tracheal scaffolds[73]. These have the added advantages of being tailor-made and retain biomechanical properties indefinitely, whilst there is no need for an organ donor, with all the attendant convenience, infection and ethical issues of the latter. However, early experience shows that these scaffolds do not epithelialize or vascularize easily[27]. The study of cell-scaffold interactions is likely to substantially inform the development of better biomaterials for organ and tissue regeneration. Ritchie *et al*[74] found that esophageal muscle cells seeded onto collagen membranes required mechanical stimulation to retain normal contractile properties in a bioreactor, showing the importance of a multidisciplinary engineering approach to this problem, but we could find no other references to the application of bioreactors to esophageal tissue-engineering. *Ex vivo* models, such as bioreactors and microfluidic organotypic chambers, are urgently required in order to explore the effects of varying stem cell/cell-scaffold-signaling combinations in the generation of functional esophageal tissue pre-implantation.

The general consensus indicates a significant advantage in repopulating scaffolds with cells prior to implantation. Studies that have seeded EEC have had positive results in repopulating the epithelial layer, both as an onlay patch[63,64] and as a total interposition graft[65]. Nevertheless, as with cell-free approaches, in cases in which only the lumen was seeded, there was a poor regeneration of the muscular layer, indicating a need for co-seeding with SMC. This is not a surprise, since esophageal strictures can be managed clinically easily with an intestinal patch (free graft) as partial substitution while they have very high chance of recurrence when such material is used to repair the whole circumference. Studies are required to identify the optimal cell types and sources to repopulate esophageal scaffolds. Ideally, cell sources should be autologous, easy to harvest, highly proliferative, and should have the

ability to differentiate into many specialized cell types.

Equally important to muscular regeneration is the challenge of replicating peristaltic contractility and a vascular supply in an artificial esophagus. Watanabe *et al*[75] developed nickel-titanium, shaped-memory, alloy coils, which were placed in an annular manner on a Gore-Tex vascular graft for esophageal replacement. Interestingly, low-voltage electrical current passing through the coils generated peristaltic movements in the artificial esophagus implanted in a goat model, suggesting that re-provision of appropriate muscular stimuli, either by enhanced neural regeneration or by electrical means, may be a profitable route for investigation if functionally normal swallowing is to be achieved. What is more, we propose that the physiological contribution of neural crest cells is a prerequisite for functional peristalsis.

Regarding the vascular component, the esophagus holds an additional challenge due to the tenuous intrinsic vascular anatomy of the esophagus in man and the association of stenosis with poor vascularization. Wrapping the engineered esophagus in the omentum prior to thoracic transplantation is one potential solution, as proposed by Nakase *et al*[70]. However, results in this instance as well as in our use of omental wrapping for transplantation of tissue-engineered tracheas in humans[35] were sub-optimal. More preclinical work on revascularization strategies is required. The use of intraluminal stents is another solution to avoid stenosis. Where collagen scaffolds were used in the above studies, the stenosis and mortality was inversely correlated to the length of stay of the intraluminal stent[29,31]. The use of stents allows time for epithelial and muscular migration onto the cell-free scaffolds. In our recent pediatric tissue engineered trachea transplant[35] we also used bio-absorbable stents, which were engineered using large mesh that allows epithelial ingrowth and persists for about 6 wk before complete degradation[76,77].

CONCLUSION

In the near future, tissue engineering may represent a valid therapeutic alternative to treat severe congenital or acquired esophageal disorders. We present possible lines for investigation that could indicate what such products will look like, but propose that, in the short- to medium-term, a combination of decellularized scaffolds with muscle and epithelial cells of autologous (including autologous stem cell) origin are likely to be the most expeditious route. Major questions of vascularity, cell-cell and cell-scaffold interaction, and motility remain outstanding, however, before the bioengineered neo-esophagus becomes an established, effective treatment for complex congenital and acquired malformations in adults and children.

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