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REVIEW ARTICLE

Regenerative Medicine Strategies for Esophageal Repair

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Pathologies that involve the structure and/or function of the esophagus can be life-threatening. The esophagus is a complex organ comprising nonredundant tissue that does not have the ability to regenerate. Currently available interventions for esophageal pathology have limited success and are typically associated with significant morbidity. Hence, there is currently an unmet clinical need for effective methods of esophageal repair. The present article presents a review of esophageal disease along with the anatomic and functional consequences of each pathologic process, the shortcomings associated with currently available therapies, and the latest advancements in the field of regenerative medicine with respect to strategies for esophageal repair from benchtop to bedside.

Introduction

The human esophagus

The human esophagus is a tubular organ that extends from the epiglottis in the pharynx to the stomach. Structurally, it comprises four concentric layers: the mucosa, the submucosa, the muscularis externa, and the adventitia.[1] The mucosa lines the lumen of the esophagus and comprises a stratified squamous epithelium that serves as a protective layer for the deeper layers of the esophagus during deglutition. The submucosa consists of vascular, connective, and glandular tissues that provide mucous secretions to facilitate the passage of food. The muscularis externa comprises two distinct muscular layers organized in circumferential and longitudinal directions that function in tandem to generate esophageal peristalsis. The muscularis externa transitions from skeletal muscle in the proximal end of the esophagus to smooth muscle in the distal two-thirds of the esophagus. The skeletal muscle portion of the esophagus is innervated by lower motor neurons that course through the vagus nerve and allow voluntary initiation of the deglutition process. The distal two-thirds of the esophageal muscularis externa comprises smooth muscle and is innervated by fibers originating from the sympathetic trunk and the vagus nerve. Once the deglutition process is voluntarily initiated, esophageal peristalsis is mediated by the sympathetic and parasympathetic innervation through a series of well-orchestrated muscle contractions, including opening and closing of the lower esophageal sphincter, which allow the process of food intake to occur[2,3] (Fig. 1).

Need for Esophageal Repair

Pathologies that involve the structure and/or function of the esophagus are often life-threatening. While damage to the mucosa can result in scar tissue formation and clinical stricture, damage to the muscularis externa or injury to the innervation of the esophagus or lower esophageal sphincter can compromise peristalsis and result in achalasia.[4] Damage to the lower esophageal sphincter itself can result in gastroesophageal reflux disease (GERD), a condition that can lead to Barrett's esophagus and progress to adenocarcinoma.[5] Trauma, iatrogenic injury, and congenital malformations can have a variety of adverse consequences depending on the anatomic structures that are compromised. The most common of these adverse consequences include fistula and stricture formation.[6]

The esophagus is a complex organ comprising nonredundant tissue that does not have the ability to regenerate. Hence, surgical repair and/or replacement of the esophagus are the only feasible treatment options upon extensive structural damage. Reports of esophageal repair date back to the beginning of the 20th century. Attempts to preserve the continuity of the esophagus consisted primarily of procedures involving esophagocolostomy and esophagogastrostomy,[7] although experimentation with plastic[8] and synthetic constructs followed shortly after.[9,10] During the second half of the 20th century, esophageal replacement with different portions of the gastric tube[11–17] became the preferred treatment option, while experimentation with other alternatives such as aortic homografts[18] and prosthetic constructs[10,19,20] continued.

Advancements in surgical technique and instrumentation such as stents[21–24] have allowed for the development of techniques that are effective in restoring esophageal continuity and function. These strategies include omental wrapping of the esophagus,[25–27] gastric pull-up,[28–32] colonic and jejunal interpositions,[33–35] and deltopectoral[36–38] and pectoralis major[39–42] myocutaneous flaps. However, these techniques are still associated with significant complications and morbidity and, without exception, come at the expense of other anatomic structures.[43,44]

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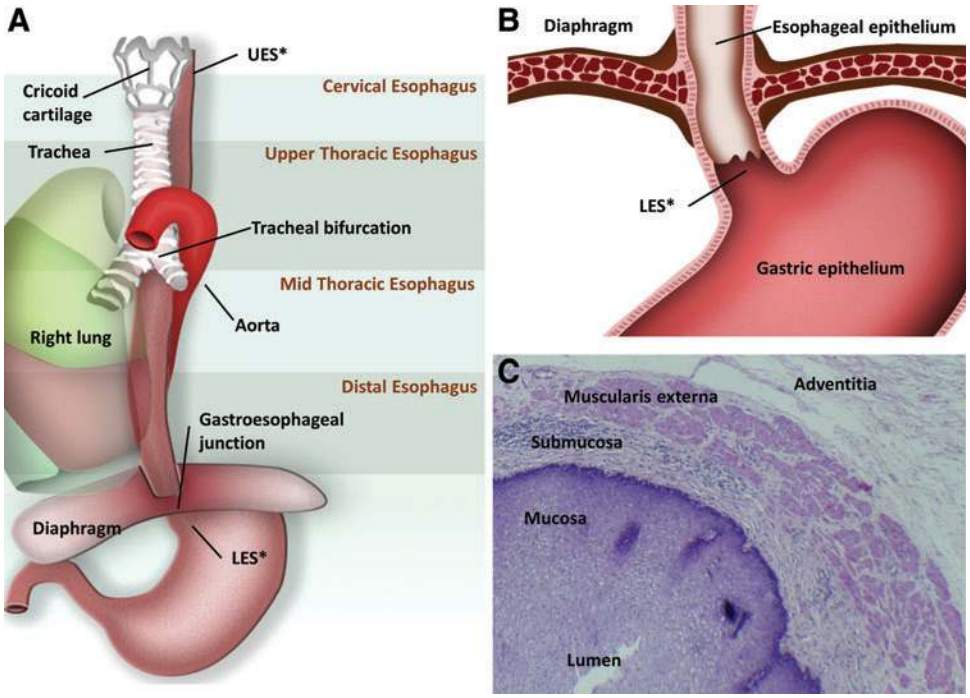


FIG. 1. The human esophagus: **(A)** The majority of the esophagus resides in the mediastinum anteriorly to the vertebral column and the descending aorta and posteriorly to the trachea, lungs, and heart. The esophagus has three natural narrowings: at the cricoid cartilage, at the tracheal bifurcation, and as it passes through the diaphragm. **(B)** The esophagus and the stomach are separated by the gastroesophageal sphincter. While the esophagus is lined by a stratified squamous epithelium, the stomach is lined by a columnar epithelium. **(C)** The esophagus comprises four concentric layers: starting from the lumen, the mucosa (stratified squamous epithelium), submucosa (glands and connective tissue), muscularis externa (two layers: circumferential and longitudinal), and adventitia (connective tissue). UES, upper esophageal sphincter; LES, lower esophageal sphincter. Color images available online at www.liebertpub.com/teb

There is an unmet clinical need for effective methods of esophageal repair. An understanding of the different diseases that affect the esophagus, the anatomic and functional consequences of each pathologic process, and the shortcomings associated with currently available therapies is necessary for the development of successful regenerative medicine strategies for esophageal repair that can be tailored to the exact dimensions of the compromised components of the esophagus (Table 1) while sparing adjacent anatomic structures.

Esophageal cancer

The incidence of esophageal cancer has shown a recent dramatic increase in the United States[53,54] and worldwide.[55] This recent increase in esophageal cancer incidence is associated with a change in the epidemiology of the two major types of esophageal cancer: adenocarcinoma and squamous cell carcinoma[45,56] (Table 2). As recently as 30 years ago, squamous cell carcinoma was responsible for more than 90% of esophageal neoplasia in the United States. However,

Table 1. Esophageal Diseases and Associated Anatomic Involvement

		Anatomic involvement					
Condition	*Incidence*	*Mucosa*	*Submucosa*	*Muscularis*	*Proximal esophagus*	*Distal esophagus*	*LES*
Adenocarcinoma	52,000/year[45] (world)	Always	Upon invasion	Upon invasion	Rarely	Mostly	Mostly
Squamous cell carcinoma	398,000/year[45] (world)	Always	Upon invasion	Upon invasion	Mostly	Rarely	Rarely
Caustic injury	5000/year[46] (US)	1st degree	2nd degree	3rd degree	Possible	Possible	Possible
Congenital deformity	1 in 3000 births[47–49]	Possible	Possible	Possible	Possible	Possible	Possible
Perforations (trauma)	Very rare[50–52]	Usually	If severe	If severe	Mostly	Rare	Rare

An understanding of the structural components of the esophagus, their function, and their involvement in disease is necessary for the development of successful strategies for esophageal repair.

LES, lower esophageal sphincter.

TABLE 2. ESOPHAGEAL CANCER: COMPARATIVE CHARACTERISTICS OF ADENOCARCINOMA VERSUS SQUAMOUS CELL CARCINOMA

	Adenocarcinoma	*Squamous cell carcinoma*
Overall incidence	Increasing	Decreasing
Geography	Predominant in the United States	Predominant outside the United States
Demographics	Predominant in white males	Predominant in black males
Anatomic location affected	Distal esophagus	Middle esophagus
Main risk factors	GERD, Barrett's esophagus	Alcohol and tobacco

Changes in the epidemiology of the two most common types of esophageal cancer, adenocarcinoma and squamous cell carcinoma, are associated with the dramatic increase in incidence of esophageal neoplasia.
GERD, gastroesophageal reflux disease.

adenocarcinoma is now more prevalent in the United States and accounts for more than 80% of esophageal cancer cases.[57] Squamous cell carcinoma remains the most prevalent form of esophageal cancer in the rest of the world.[58] Despite advances in detection, diagnosis, and treatment, the 5-year survival rate for all patients diagnosed as having esophageal cancer ranges from 15% to 20%.[57]

Adenocarcinoma. Esophageal adenocarcinoma has one of the highest rates of increased incidence among neoplastic diseases worldwide with 52,000 cases per year.[45,55,59] Esophageal adenocarcinoma is not only the most common form of esophageal cancer in the United States but also its increase in incidence is only matched by that of obesity[60,61] (Fig. 2).

Esophageal adenocarcinoma develops primarily in the distal portion of the esophagus, including the gastroesophageal junction, as a consequence of Barrett's esophagus, a pathologic process that in turn is a downstream complication of GERD. The most common cause of GERD is lower esophageal sphincter relaxation or insufficiency, a condition that can be caused by mechanical factors, such as obesity, pregnancy, or increased gastric volume, and by nonmechanical factors such as central nervous system depressants and alcohol and tobacco abuse.[57] Barrett's esophagus develops in approximately 10% of patients with GERD as a result of chronic exposure to the acidic contents of the stomach.[5] Over time, the esophageal epithelium adapts to the new acidic environment by transforming from squamous epithelium to columnar epithelium through a process known as metaplasia. Barrett's esophagus is more common in white males over the age of 40 than in the rest of the population, and once this condition develops, progression into high-grade dysplasia and adenocarcinoma is possible.[62] Hence, the increased incidence of esophageal adenocarcinoma is attributed in great part to obesity-related GERD and Barrett's esophagus.[63,64]

Squamous cell carcinoma. Squamous cell carcinoma is less common than adenocarcinoma in the United States and it typically occurs in patients over 45 years of age. It is four times more frequent in males than in females, and it is eight times more frequent in African-Americans than in Caucasians.[65] In the rest of the world, however, particularly in rural and underdeveloped areas, squamous cell carcinoma remains the most common cause of esophageal cancer.[66] As with many cancers, the main risk factors associated with squamous cell carcinoma are alcohol and tobacco use. Other factors, such as poverty, caustic injury, achalasia, human papillomavirus,[67] and consumption of hot beverages and mutagenic compounds (i.e., polycyclic hydrocarbons, nitrosamines), have also been associated with the disease.[68]

Squamous cell carcinoma has an insidious onset that typically presents with dysphagia, odynophagia, and/or esophageal obstruction.[69,70] Although both adenocarcinoma and squamous cell carcinoma begin as superficial lesions in the esophageal mucosa, squamous cell carcinoma tends to localize to the middle third of the thoracic esophagus. Early lesions typically begin as patchy thickenings that slowly develop into polyps or exophytic tumors that eventually obstruct

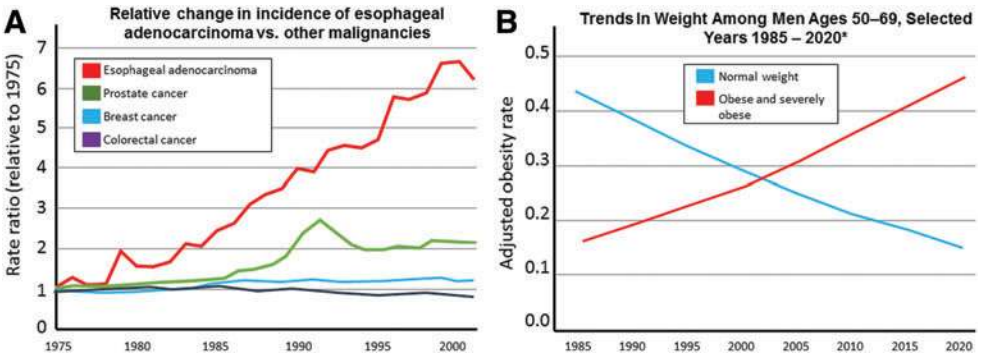
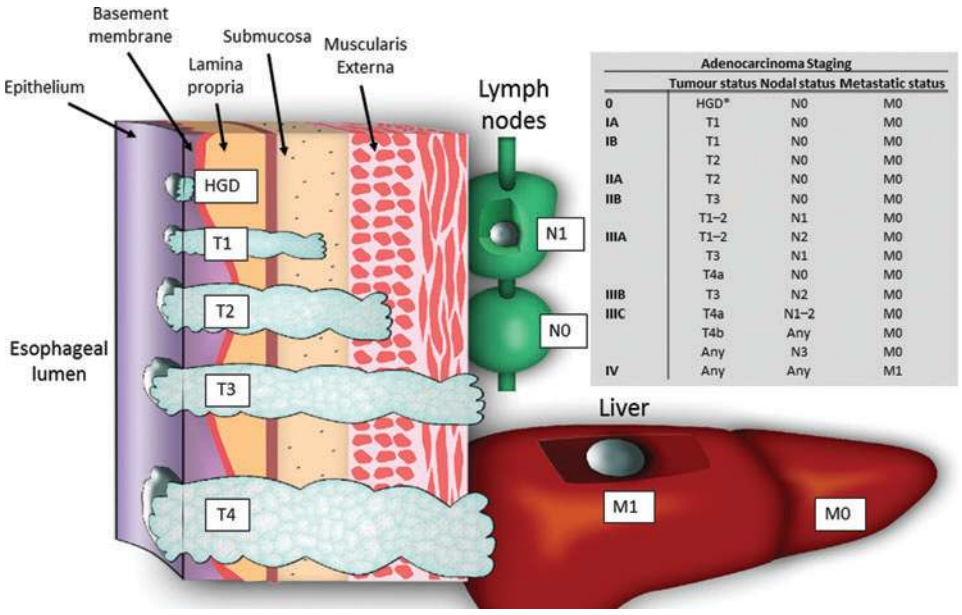


FIG. 2. Increase in incidence of esophageal adenocarcinoma and obesity: **(A)** Esophageal adenocarcinoma has one of the highest rates of increased incidence among neoplastic diseases. **(B)** The increase in the incidence rate of esophageal adenocarcinoma is only matched by that observed in obesity. *Data for 2005–2020 are extrapolated. Figures adapted from Pohl and Welch 2005[176] and Sturm *et al.*, 2004.[177] Color images available online at www.liebertpub.com/teb



Adenocarcinoma Staging			
	Tumour status	Nodal status	Metastatic status
0	HGD*	N0	M0
IA	T1	N0	M0
IB	T1	N0	M0
	T2	N0	M0
IIA	T2	N0	M0
IIB	T3	N0	M0
	T1–2	N1	M0
IIIA	T1–2	N2	M0
	T3	N1	M0
	T4a	N0	M0
IIIB	T3	N2	M0
IIIC	T4a	N1–2	M0
	T4b	Any	M0
	Any	N3	M0
IV	Any	Any	M1

FIG. 3. Esophageal cancer staging: The TNM (tumor, node, and metastasis) staging system takes into consideration a number of variables, including tumor invasion (T), the presence or absence of metastatic disease (M), and nodal invasion (N). Tumor staging will determine the clinical approach to the disease. Staging for adenocarcinoma of the esophagus is shown as an example. HGD, high-grade dysplasia. Figure adapted from Pennathur *et al.*[62] Color images available online at www.liebertpub.com/teb

the lumen of the esophagus or as ulcerated and infiltrative lesions that progressively invade all layers of the esophagus and eventually infiltrate the surrounding organs in the mediastinum (Fig. 3). Whereas invasion of the trachea, bronchi, or lungs can lead to pneumonia usually resulting in detection of the disease, invasion of the aorta and pericardium can lead to catastrophic exsanguination.[71]

Standard of care. Although recent improvements in screening, staging, surgical technique, adjuvant therapy, and patient selection have reduced morbidity and prolonged postoperative survival,[72–74] significant controversy remains over the optimal management of esophageal carcinoma.[75,76] As with many neoplastic processes, the primary objective following detection is surgical removal of the neoplastic tissue with or without adjuvant therapy. In the case of advanced disease, an esophagectomy, followed by gastric pull-up into the mediastinum and anastomosis of the gastric cardia and the proximal esophagus, remains the only viable alternative.[77] However, this procedure is associated with high morbidity, decreased quality of life, and high mortality rates.[78–80]

A number of novel alternatives for the treatment of noninvasive early stage disease are under investigation to ultimately replace traditional approaches and associated complications that can lead to esophagectomy. Minimally invasive endoscopic ablation techniques for the treatment of Barrett's esophagus with high-grade dysplasia and superficial carcinoma are among the most studied approaches. Radiofrequency ablation is now an accepted treatment for flat Barrett's esophagus. This technique offers significantly lower rates of stricture formation than other ablative techniques.[81] In cases where nodularity exists, endomucosal resection (EMR) with or without ablation has been shown to be an effective treatment that prevents recurrence.[82] These procedures have shown improved survival rates and quality of life.[83,84] However, the development of metachronous lesions after these procedures remains a common finding (21.5%). Risk factors for the development of metachronous lesions include piecemeal resection, no ablation therapy following EMR, multifocal neoplasia, and long-segment Barrett's esophagus.[85] Stepwise radical endoscopic resection, a technique being investigated for the treatment of recurrent Barrett's esophagus after radiofrequency ablation or EMR, has shown to be effective, although it usually requires a large number of therapeutic sessions and complications such as esophageal stenosis that require dilation in 50% of cases.[86]

In summary, limitations associated with these techniques include the requirement for numerous interventions, incidence of metachronous lesions, absence of a suitable tissue specimen for histologic assessment, and the unavoidable sampling error that occurs in patients with long-segment disease.[87] Furthermore, even with successful treatment, there is need for repeated postsurgical dilation in more than 50% of cases.[86,88]

Congenital abnormalities

Every year, 1 in 3000 births presents with esophageal pathology (Table 1). Congenital abnormalities can compromise all layers of the esophagus and include esophageal atresia, tracheoesophageal fistulas, and esophageal agenesis (Fig. 4). Without exception, these defects are incompatible with life.

Esophageal atresia is characterized by the replacement of a portion of the esophagus with a nonpatent esophageal segment that results in mechanical obstruction. This segment of the esophagus is typically present at or near the carina of the trachea and usually associated with a fistula connecting either the upper or lower fully developed esophageal segments to the trachea.[89] Agenesis of the esophagus is a very rare condition.[90,91]

The survival rate for patients with esophageal atresia has been approximately 95% in the last 10 years.[92,93] Depending on the specific underlying pathology, congenital abnormalities may be surgically addressed with synthetic prosthetics, flaps, or grafts. One of the major issues with congenital abnormalities is that pediatric patients outgrow prosthetic devices such as stents and, as a result, often require further intervention.

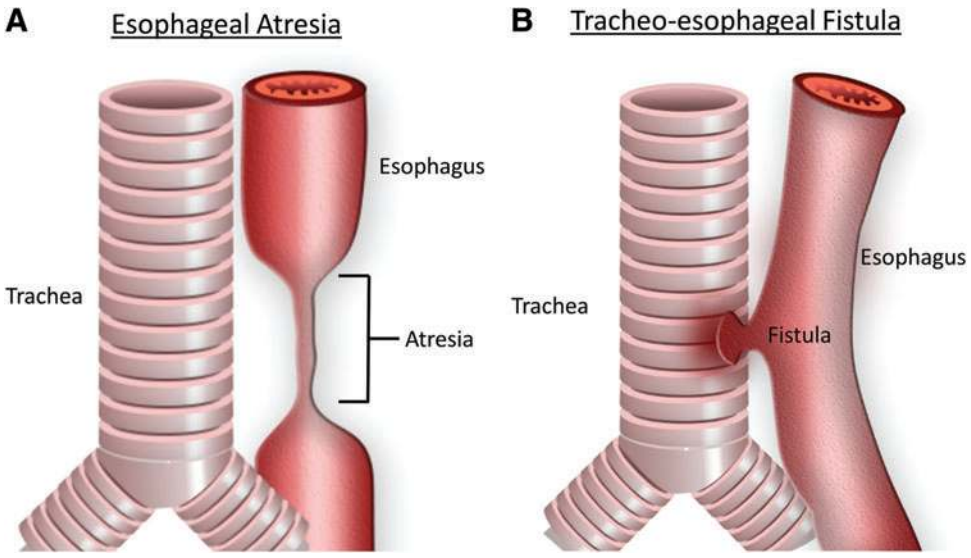


FIG. 4. Esophageal congenital abnormalities: The most common congenital abnormalities of the esophagus include **(A)** esophageal atresia and **(B)** tracheoesophageal fistula. These conditions result in mechanical obstruction of the esophagus and are incompatible with life. Detection occurs shortly after birth. Color images available online at www.liebertpub.com/teb

Esophageal injury

Despite decades of clinical experience, most perforations of the esophagus are iatrogenic and occur during endoscopy.[94–96] Mortality from iatrogenic esophageal injury approaches 20%.[89] Other important causes of esophageal injury include caustic injury, Mallory-Weiss tear, ingestion of foreign body, and acute trauma. Regardless of the etiology, severe esophageal perforation is a surgical emergency since patients can initially appear stable, but then decompensate quickly.[97–100] Decompensation usually results from esophageal and gastric contents leaking into the mediastinum with resultant necrotizing inflammation, sepsis, and ultimately multiorgan failure and death.[101,102]

The Esophagus and Regenerative Medicine

The ultimate goal of regenerative medicine is the functional restoration of lost or damaged tissues. To date, strategies for functional tissue repair have included delivery of bioactive molecules, cell-based therapies, biomaterial-based therapies, and combinations thereof.[103–105] The delivery of these technologies and their effect upon host tissues have been investigated in various anatomic locations and have shown different degrees of success.

As previously discussed, esophageal pathologies are diverse and involve different anatomic components and tissue types. For example, while regenerative medicine strategies for superficial injury and noninvasive neoplastic disease focus on mucosal restoration, invasive neoplastic disease, congenital abnormalities, and transmural caustic necrosis involve the replacement of the entire esophagus, which represents a significant challenge. The present article focuses on progress made in the field of esophageal regenerative medicine strategies for esophageal repair from benchtop to bedside.

Approach

In vitro studies and preclinical animal studies are necessary steps toward the development of novel strategies for tissue repair. Well-designed experiments permit the isolation of test variables and the establishment of necessary parameters for optimal preclinical study design. In the field of regenerative medicine, important aspects of preliminary studies include the cellular composition and architecture of target tissues and organs, the mechanical properties of biomaterials and scaffolds, the assessment of cytotoxicity and cytocompatibility of new technologies, and the biochemical properties of novel constructs. Preclinical studies permit the evaluation of new technologies *in situ*, including the different components of the host response such as the type and magnitude of the immune response, and important cellular processes, such as stem cell migration, proliferation, and differentiation. Scar tissue formation, resistance to infection, angiogenesis, and functional tissue remodeling are important processes that are also evaluated during the preclinical stage.

Esophageal architecture and stem cell populations

Several differences across multiple species have been identified in the microarchitecture of the esophagus, including the presence of a keratinized epithelium in mice, rats, pigs, and domesticated animals,[106–108] and a different distribution of striated versus smooth muscle within the muscularis externa. While striated muscle is only present in the proximal one-third of the human esophagus, striated muscle can be found in virtually the entire length of the esophagus in other mammalian species. This configuration allows these species to voluntarily regurgitate gastric contents to chew cud and/or to feed the young.[109] The differences in the cellular composition and tissue architecture of the esophagus among different species should be taken into consideration when choosing an animal model for preclinical study design and when interpreting the results from these studies.

Several groups have further characterized the esophageal epithelium as a high-turnover tissue comprising two layers: a basal layer comprising a single sheet of cells in direct contact with the basement membrane that have self-renewal capacity and a suprabasal layer that contains progressively more differentiated cell populations and lines the lumen of the esophagus.[110] The basal layer comprises two distinct zones: the papillary basal layer (PBL), which extends along papillae that invaginate the epithelium, and the interpapillary basal layer (IBL), which is located at the flat interface

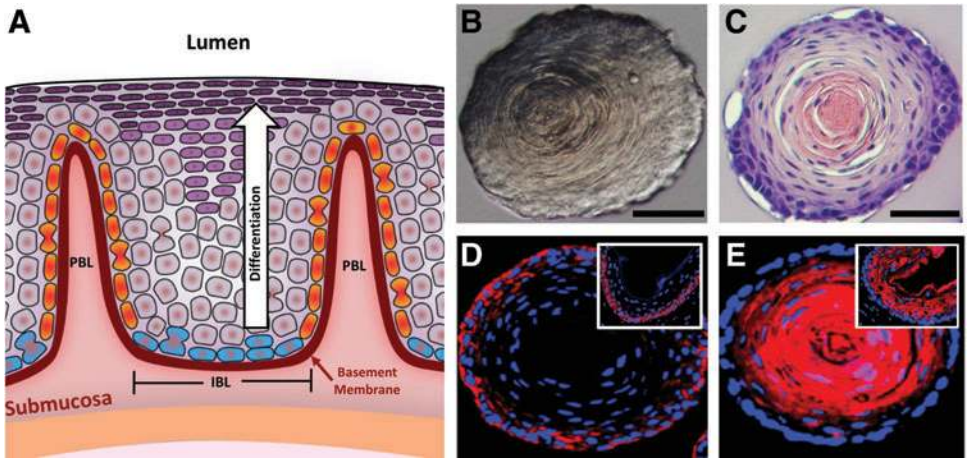


FIG. 5. Esophageal epithelium: **(A)** The architecture of the esophageal epithelium includes papillary structures located at regular intervals (PBL) separated by flat interpapillary zones (IBL). The basal cells comprise a heterogeneous population of epithelial cells with cells located in the IBL constituting the stem cell compartment (*blue*) and transit-amplifying cells residing in the PBL (*orange*). Epibasal layers (*purple*) are undergoing differentiation and can no longer divide. The *arrow* indicates the direction of differentiation. **(B)** When isolated and cultured, esophageal stem cells have shown organoid-forming capacity[114] and show similar organization to native tissues through **(C)** hematoxylin and eosin staining and **(D)** Cytokeratin 14 and **(E)** Cytokeratin 13 stains. Native tissue shown in *inset* for comparison **(D, E)**. Color images available online at www.liebertpub.com/teb

between papillae[111] (Fig. 5A). The IBL cells constitute the stem cell compartment of the esophageal epithelium and proliferate infrequently and asymmetrically.[112,113] Recent studies in the mouse esophagus have identified these cells to be $Itgb4^{High,}$ $CD73^{+}$ and having the greatest stem cell potential, whereas $CD73^{-}$ transit-amplifying cells show variation in their degree of maturation. Esophageal stem cells have been used *in vitro* to show three-dimensional organoid-forming capacity (Fig. 5B–E) and the participation of Sox2, Wnt, and bone morphogenetic protein signaling pathways in the process of esophageal epithelium self-renewal.[114]

Biomaterials for esophageal repair

The ideal biomaterial for esophageal repair remains to be determined and it is unlikely that a one-size-fits-all approach will be optimal. A number of synthetic and biologic materials have been proposed for esophageal repair (Table 3). While synthetic scaffolds can be manufactured with precision and their mechanical properties can be fine-tuned for specific applications, these materials tend to cause a well-characterized foreign body reaction.[115] In contrast, biologic materials are subject to natural variability and have less tunable properties, but tend to produce a friendlier host response and promote constructive tissue remodeling, a term that implies the deposition of site-specific functional tissue.[116]

Biologic scaffolds comprising acellular esophageal tissue have been proposed by a number of groups. Work by Bhrany *et al.*[117] presented a sodium dodecyl sulfate (SDS)-based method for decellularization of murine esophagi. The resulting scaffold showed extracellular matrix (ECM) protein preservation, the ability to support esophageal cell proliferation *in vitro*, and neovascularization with minimal inflammation after subcutaneous implantation. Although studies have shown that constructive tissue remodeling consistently occurs when chemical cross-linking of biologic scaffolds is avoided, the same group cross-linked the developed acellular matrix in additional studies with the intent of reducing antigenicity and improving collagen stability to prolong *in vivo* durability. As expected, results showed increased stability in cross-linked scaffolds, and while minimal inflammatory response was also reported upon *in vivo* implantation, inflammation was assessed only by quantification of macrophages and multinucleate giant cells at the treatment site and did not take into consideration macrophage phenotype. Interestingly, while genipin-cross-linked scaffolds supported esophageal epithelial adhesion and proliferation in this study, glutaraldehyde-cross-linked scaffolds did not support epithelial cell adherence or proliferation.[118] Inhibition of biologic scaffold degradation has been shown to prevent matricryptic peptide release and inhibit constructive scaffold remodeling in other studies.[116,119]

Recognizing the potential benefits of using biologic scaffolds derived from homologous tissues in regenerative medicine applications, a protocol for the decellularization of the porcine esophageal mucosa was developed for the treatment of noninvasive disease by Keane *et al.*[120] The protocol developed in this study avoids the use of SDS and other harsh decellularization agents and is compliant with previously established criteria for decellularization.[116,121] The resulting scaffold maintained important proteins and ultrastructure consistent with the basement membrane complex, including laminin, collagen IV, and fibronectin. Perivascular stem cells remained viable when seeded upon the porcine esophageal ECM scaffold *in vitro*, and the *in vivo* host response showed an increased number of M2 proremodeling macrophages and an associated pattern of constructive remodeling when used to repair striated muscle defects in rats.

Commercially available biologic scaffold materials such as AlloDerm have been evaluated for use in esophageal repair. In a study by Beckstead *et al.*,[122] rat esophageal epithelial cells were isolated and characterized for epithelial identity, adhesion protein preference, and *in vitro* interaction with both AlloDerm and synthetic scaffolds. Various

Table 3. Biomaterials Used in Esophageal Repair

Biomaterials for esophageal repair		
Biomaterial	*Summary of results*	*References*
Synthetics		
poly(L-lactide-co-caprolactone)	Fibronectin grafted on PLLC scaffold greatly promotes epithelium regeneration.	123
Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)-based nanofibrous scaffolds (PHBV)	Human esophageal epithelial cells seeded on PHBV present higher proliferation than those seeded in PHBV-gelatin after 7 days of culture.	124
Polyvinylidene fluoride (PVDF) and absorbable Vicryl surgical meshes	Mucosal regeneration after 3 months. Vicryl treatment group showed leakage.	146
Poly-ε-caprolactone	Ingrowth of epithelial and smooth muscle cells was observed 1 month postoperatively.	147
Biologics		
Small intestine submucosa ECM (SIS)	Used in different defect models with different degrees of success.	136,169
Urinary bladder ECM (UBM)	Used in different defect models with different degrees of success.	126,127,132,136,138
Esophageal ECM	SDS-based decellularization protocol. Supports esophageal cell proliferation *in vitro* and neovascularization upon subcutaneous implantation.	117
Gastric ECM	No stenosis or dilatation. Regeneration of keratinized stratified squamous epithelium only, not other layers.	170
Cross-linked esophageal ECM	Increased stability in genipin-cross-linked vs. noncross-linked scaffolds. Glutaraldehyde cross-linking was detrimental.	118
Esophageal mucosa ECM	Protocol avoids SDS and is compliant with decellularization criteria. Host response showed increased numbers of M2 macrophages when implanted in striated muscle defects.	120
Acellular dermal matrix	Superior epithelial organization and stratification compared with synthetic scaffolds.	122
Hybrids		
Collagen-coated Vicryl tubes	Mediastinitis within days of implantation, stenosis, and granulation tissue formation.	151
Collagen-modified PLGA	Collagen-modified PLGA increases the proliferation of the ESMCs and promotes extended morphology.	125
Collagen-coated silicone stents	Segmental defects showed stricture formation and inability to swallow when the stent was removed at 2–3 weeks. When removed at 4 weeks, no stricture was observed.	152,153
Complete decellularized esophagus with allogeneic mesenchymal stromal cells	All animals survive the 14-day study period with patent and functional grafts. Explanted grafts show regeneration of all the major cell and tissue components.	137

Synthetic and biologic materials evaluated *in vitro* and *in vivo* in esophageal repair applications.
ECM, extracellular matrix; PLLC, poly(L-lactide-co-caprolactone); PLGA, poly(lactic-co-glycolic acid); ESMC, esophageal smooth muscle cell; SDS, sodium dodecyl sulfate.

factors, including calcium concentration, scaffold composition, and pore size, were evaluated by measuring their influence on epithelial growth and differentiation. Results from this study showed superior epithelial organization and stratification on AlloDerm compared with synthetic scaffolds such as poly(lactic-co-glycolic acid) (PLGA), poly-L-lactic acid (PLLA), and polycaprolactone (PCL)/PLLA. The authors concluded that modification of the synthetic scaffold's surface properties and pore size may be necessary to improve cell behavior in these constructs.

Studies of esophageal epithelial cells have also been performed with other materials, including fibronectin-grafted

poly(L-lactide-co-caprolactone)[123] and Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)-based nanofibrous scaffolds (PHBV).[124] These studies showed that human esophageal epithelial cells seeded on PHBV present higher proliferation than those seeded in PHBV-gelatin after 7 days of culture. Cells seeded on both scaffolds present epithelial cobblestone morphology after 3 days of culture. However, ECM proteins, including collagen type IV and laminin, and expression of phenotypic markers, including cytokeratin-4 and 14, were significantly higher in cells cultured in PHBV-gelatin scaffolds than in cells cultured in PHBV scaffolds without gelatin.

Zhu *et al.*[125] studied the effect of covalent immobilization of collagen onto poly(DL-lactideco-glycolide) (PLGA) surfaces on cell behavior by seeding porcine esophageal smooth muscle cells (ESMCs) on collagen-PLGA versus unmodified PLGA and tissue culture plastic. The authors found that collagen-modified PLGA increases the proliferation of the ESMCs and promotes extended morphology.

The unifying findings of these *in vitro* studies are that although a number of materials have been found to be cytocompatible, naturally occurring biomolecules provide superior substrate properties for esophageal cells compared with synthetic materials.

Animal models for esophageal repair

A number of animal models are available for the study of regenerative medicine strategies for esophageal repair (Table 4). The use of small animal models, particularly murine species, offers a number of advantages, including cost efficiency, the ability to adequately statistically power studies, the availability of genetic modification tools that facilitate mechanistic studies, and the possibility to evaluate multiple innate physiologic variables that cannot be mimicked *in vitro*. However, small animal models are technically challenging and a great degree of expertise is required to perform surgical procedures in the murine esophagus. Large animal models, on the other hand, are technically easier to implement and permit the evaluation of technologies at their intended therapeutic physical dimensions. As a result, large animal models are a valuable tool for the optimization of surgical approaches and evaluation of feasibility and delivery of these technologies. However, large animal models are expensive and genetic modification tools are usually not available to the same degree as they are in small animal models.

Table 4. Preclinical Models for Esophageal Repair

Animal models for esophageal repair			
Model	*Application*	*Species*	*References*
Mucosal resection	Noninvasive neoplastic disease, superficial trauma	Dog	126
		Pig	129,130
		Mouse	132
Mucosal damage	Caustic injury	Rat	133
Full-thickness defect	Esophagectomy, congenital disease, trauma	Pig	126
		Dog	136
		Rat	137
Anastomosis reinforcement	Anastomosis reinforcement after segmental resection	Dog	127

Preclinical models of esophageal repair include both small and large animals and involve different anatomical components of the esophagus. Anatomical differences exist among different species and these differences should be taken into consideration when designing preclinical studies and interpreting results.

Esophageal mucosal resection models have been described in the dog,[126,127] pig,[128–131] and in rodents[132] (Fig. 6). These models are particularly important for modeling the treatment of noninvasive neoplastic disease as a mucosectomy alone can oftentimes entirely remove early stage neoplastic tissue without compromising the remaining layers of the esophagus. Caustic esophageal burn models[133,134] also study pathology localized to the mucosa and focus on integrity of the epithelium. Injury or removal of the esophageal mucosa invariably leads to stricture formation, and as a result, regenerative medicine strategies aimed toward mucosal regeneration usually focus on stricture prevention as one of the primary objectives.

Full-thickness defects, including part or the full circumference of the esophagus, have been described in the pig,[135] dog,[136] and rat.[137] Full-thickness defect models permit the investigation of treatment options for invasive neoplastic disease, congenital abnormalities involving all layers of the esophagus (e.g., tracheoesophageal fistulas), and acute trauma. In addition to full-thickness defects, anastomosis reinforcement is an important aspect of esophageal repair in these scenarios, particularly after esophagectomy. Anastomosis reinforcement models focus on leaks and dehiscence and have been described at different anatomic locations in the esophagus in the dog.[138]

The Levrat procedure is a well-established model involving an esophagojejunostomy, a procedure that produces retrograde flow of gastrointestinal contents into the distal part of the esophagus, resulting in Barrett's esophagus and eventually progressing to esophageal adenocarcinoma[139–143] (Fig. 7). The Levrat procedure will be a valuable tool in the study of regenerative medicine strategies for esophageal repair after neoplastic tissue resection with or without adjuvant radiation therapy and chemotherapy.

Preclinical studies

Molecular therapies. Molecular therapies focus on the delivery of bioactive molecules that aim to modify one or several steps of the wound healing response. Poly(adenosine diphosphate-ribose) polymerase affects the repair of DNA in damaged cells, but its activation can lead to ATP depletion and death in damaged cells.[144] With this in mind, Guven *et al.*[133] evaluated 3-aminobenzamide, a poly(adenosine diphosphate-ribose) polymerase inhibitor, in the context of caustic esophageal burn and the prevention of stricture formation in rats. This group reported a decreased stenosis

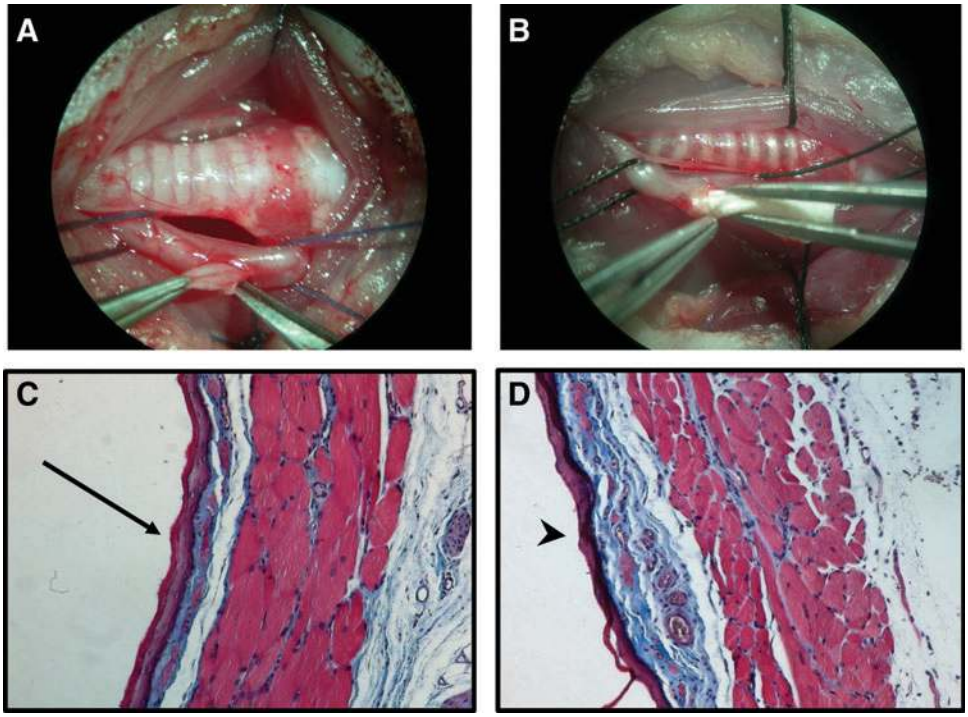


FIG. 6. Esophageal mucosal resection in the rat model: **(A)** Mucosal resection in the rat is performed by exposing the esophagus around the trachea and performing a mucosectomy through a horizontal incision in the muscularis layer of the esophagus. **(B)** Once the mucosa is removed, an extracellular matrix (ECM)-derived biomaterial is delivered *in situ* to facilitate constructive tissue remodeling. **(C)** Masson's trichrome stain of native esophageal mucosa (*arrow*) and **(D)** remodeled esophageal mucosa after biomaterial-mediated repair showing intact keratinized epithelium (*arrowhead*). Color images available online at www.liebertpub.com/teb

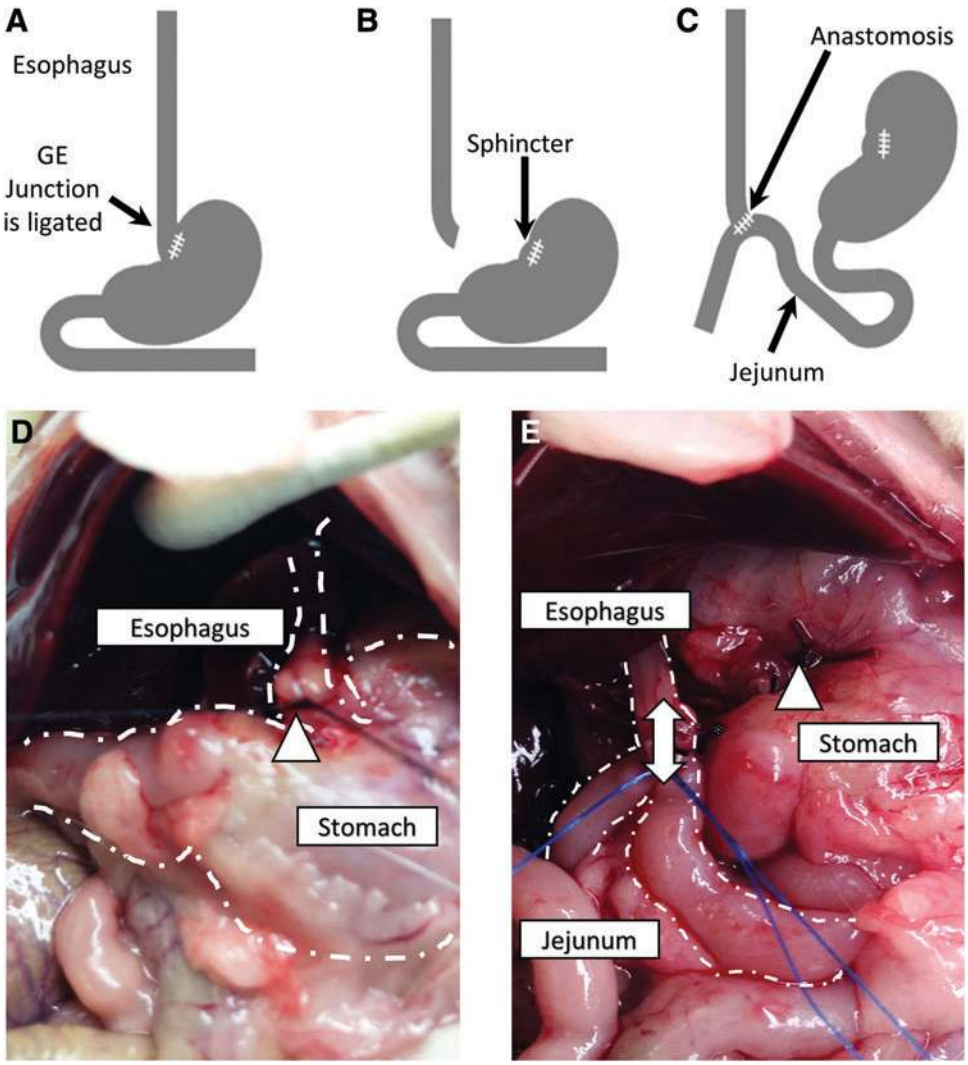


FIG. 7. Levrat model: **(A, B)** An esophagoduodenal anastomosis is performed by ligating the gastroesophageal junction and **(C)** anastomosing the distal end of the esophagus to the jejunum, creating a patent conduit that induces gastroduodenojejunal reflux. **(D, E)** The gastroesophageal sphincter is ligated and remains attached to the stomach (*arrow head*). The anastomosis between the distal end of the esophagus and the jejunum forms a patent conduit that allows free retrograde flow (*double arrow*). Color images available online at www.liebertpub.com/teb

index and histopathologic damage in the treatment group and concluded that 3-aminobenzamide has a preventive effect in the scarring of the esophagus and decreases tissue damage by increasing antioxidant enzyme activity.

Growth factors are among the most commonly investigated molecular therapies for tissue repair. In the context of esophageal disease, the effect of basic fibroblast growth factor (bFGF) on vascularization was evaluated in the canine esophagus. In this study, Hori *et al.*[145] compared an acellular collagen in sponge and gel formats supplemented with bFGF. The scaffolds were implanted in the cervical esophagus and evaluated 1 month after implantation. Histologic analysis confirmed the presence of blood vessels in significantly higher number in the bFGF-containing collagen gel group compared with the bFGF (−) control group. However, in the collagen sponge groups, no difference was observed between the bFGF (+) group and the bFGF (−) group. This study highlights the fact that structure, in addition to composition, is an important determinant of the host response to implanted biomaterials.

Synthetically derived biomaterials. A number of synthetic materials have been used in preclinical studies for esophageal repair with limited success. In a study by Lynen Jansen *et al.*,[146] nonabsorbable polyvinylidene fluoride (PVDF) and absorbable Vicryl surgical meshes were used to repair 1 cm by 2 cm semicircular defects in the rabbit and resulted in mucosal regeneration after 3 months without stricture and initial muscular regeneration in the PVDF group. However, three patch failures with consecutive anastomotic leakage were reported in the Vicryl treatment group.

In a similar study performed in the rabbit by replacing smaller, 0.6 cm by 1 cm, windows in the abdominal esophagus with poly-ε-caprolactone, ingrowth of epithelial and smooth muscle cells was observed 1 month postoperatively with an almost completely degraded mesh. However, the study had a 75% survival rate, and more than half of the surviving animals developed pseudodiverticula.[147]

The use of hybrid constructs that seek to combine the biomechanical properties of a synthetic material with the biocompatible properties of a biologic material, typically as a coating agent, is becoming increasingly popular in regenerative medicine.[148–150] This type of construct has been investigated in esophageal repair. Purushotham *et al.*[151] investigated the replacement of complete esophageal segments in the thoracic esophagus with collagen-coated Vicryl tubes. Preliminary experiments resulted in mediastinitis within days of implantation due to prosthetic leakage secondary to acid reflux and digestion of the construct. The complication was addressed thereafter by cross-linking the constructs with glutaraldehyde, which increased the resistance of the material. The animals, however, developed stenosis at a mean of 11 days postoperatively and considerable granulation tissue and scar formation was found histologically.

In addition to coating Vicryl tubes, collagen has been used to coat silicone stents by Natsume *et al.* and Takimoto *et al.*[152,153] In these studies, these groups report the use of collagen-coated silicone tubes to replace 5-cm esophageal segmental defects in dogs, followed by endoscopic removal of the inner silicone stent at weekly intervals from 2 to 4 weeks. Results showed stricture formation and inability to swallow when the stent was removed at 2–3 weeks. In the dogs, in which the stent was removed at the 4-week time point, a regenerated esophagus with stratified flattened epithelia, striated muscle, and esophageal glands was observed.

In summary, a variety of synthetic materials have been used to attempt to repair esophageal defects with different degrees of success. However, due to the synthetic nature of the materials, recurrent problems include stricture formation, inflammation, foreign body reaction, and leakage.

Biologically derived biomaterials. The advantage of using biologically derived biomaterials for esophageal repair is based on the premise that unlike synthetically derived materials, biologic scaffolds comprising an ECM have the ability to promote constructive tissue remodeling.[154,155] The mechanisms of *in vivo* tissue remodeling upon biologic scaffold implantation are reviewed elsewhere.[116] Briefly, appropriately configured biologic scaffolds have the ability to modulate different phases of the wound healing response and induce a shift from a process of inflammation and scar tissue formation to one of constructive tissue remodeling and functional tissue repair. The factors that facilitate this process during the biomaterial–host interaction are complex and involve both host-related factors (i.e., age, immunocompetence, native stem cell populations, and overall health state of the patient) and biomaterial-related factors (i.e., source and composition,[120,156–158] efficacy of the decellularization process,[121,159] postprocessing modifications such as cross-linking and solubilization,[119,160–165] source animal age,[166] and surface topography[167,168]).

Biologic scaffolds have been used in multiple large animal models to study the feasibility of biomaterial-mediated esophageal repair. Initial studies by Badylak *et al.*[136] utilized porcine-derived acellular small intestinal submucosa (SIS) and urinary bladder matrix (UBM) to repair patch defects in the dog model. The ability of these materials to repair defects measuring 5 cm in length and encompassing either 40% to 50% of the esophageal circumference or the entire circumference of the esophagus was shown as the xenogeneic scaffolds used to repair the patch defects were replaced by appropriately oriented skeletal muscle within 30–60 days and showed complete and intact squamous re-epithelialization without signs of clinical dysfunction. However, the scaffolds used to repair full-circumference segmental defects showed stricture formation within 45 days of implantation.

Given the results of stricture formation when attempting a full-thickness full-circumference defect repair, subsequent experiments by Badylak *et al.*[126] addressed the necessity of a native (i.e., host) tissue component for adequate esophageal repair without stricture formation. In these experiments, esophageal defects encompassing different portions of the esophageal circumference were repaired with UBM-ECM. Treatment groups included full-circumference full-thickness defects, full-circumference mucosal resections, and full-thickness defects with 30% intact muscularis externa. This study concluded that UBM-ECM scaffolds plus autologous muscle tissue, but not UBM-ECM scaffolds alone or muscle tissue alone, can promote constructive tissue remodeling of

segmental defects in the esophagus. Biologic scaffolds have also been shown to be effective in the reinforcement of surgical anastomoses of the esophagus in a dog model.[138]

Following these studies, endoscopic deployment of biologic scaffolds was investigated for mucosal repair after EMR in the dog. EMR is an accepted technique for the treatment of high-grade dysplasia and early neoplasia, but often leads to stricture formation when used to treat extensive (i.e., long segment) areas. In this study by Nieponice *et al.*,[127] endoscopic placement of a biologic scaffold was shown to effectively prevent esophageal stricture formation after EMR. Together, the results from these preclinical studies formed the basis for initial clinical trials of biomaterial-mediated tissue repair after neoplastic tissue resection.

Biologically derived biomaterials have also been studied in small animal models. In contrast to large animal models, the focus of small animal studies is usually to determine mechanisms of tissue repair, screen large numbers of potential therapies, and optimize treatment options by systematically modifying design parameters. For instance, a murine model of esophageal reconstruction with chimeric mice constitutively expressing green fluorescent protein (GFP) in the bone marrow was used by Nieponice *et al.*[132] to study the contribution of bone marrow-derived stem cells to biomaterial-mediated esophageal repair. In this study, animals were subjected to partial mucosal resection, followed by ECM scaffold implantation. The authors found GFP-labeled bone marrow stem cells at the treatment site and concluded that stem cells originating from the bone marrow participate in the ECM remodeling process during tissue repair after esophageal injury. However, the low number of GFP-labeled cells argues against the significant involvement of these cells in the constructive remodeling process.

In a different study, Lopes *et al.*[169] performed semicircumferential esophageal defects and segmental esophageal defects in a rat model and repaired them with an SIS patch graft and an SIS tube interposition graft, respectively. Similar to results obtained in large animal studies by Badylak *et al.*, all animals in the segmental defect group died within the first postoperative month. Surviving animals in the semicircumferential defect group showed no signs of esophageal dysfunction and returned to normal weight. There was no evidence of fistula, significant stenosis, or diverticula. No hematologic or serum biochemistry abnormalities were found. By month 5, the SIS patch had been replaced by esophageal-derived tissues.

A similar study was performed by Urita *et al.*[170] in the rat model using gastric acellular matrix for the repair of patch esophageal defects created in the abdominal esophagus. In this study, rats were sacrificed 1 week to 18 months after

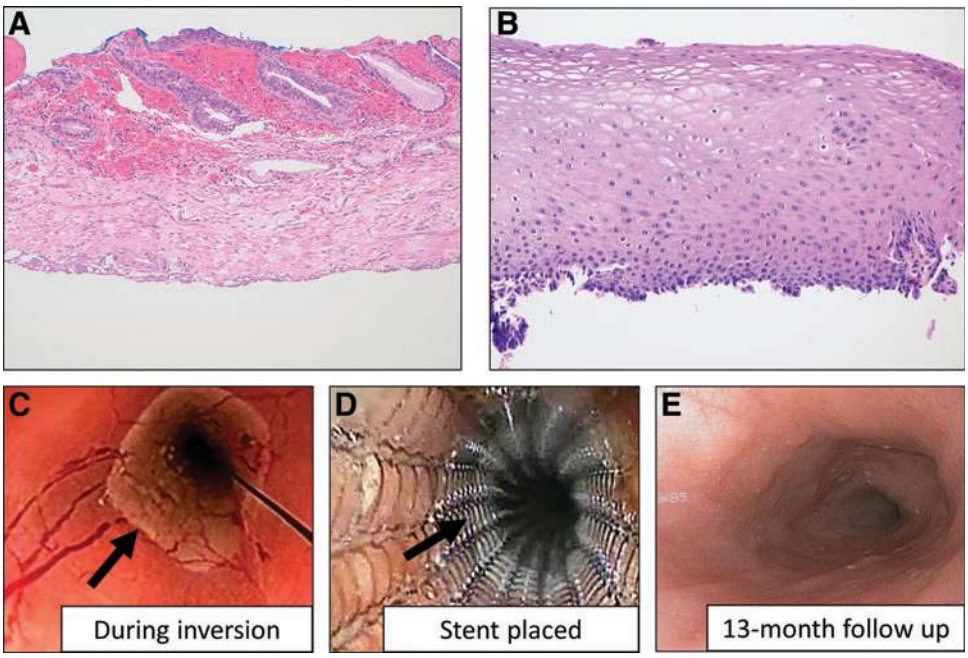


FIG. 8. Esophageal preservation in human patients after endomucosal resection in the setting of superficial cancer: The current standard of care for esophageal neoplasia is esophagectomy, a procedure associated with high morbidity and mortality. As an alternative, Badylak *et al.*[174] have implemented an entirely endoscopic method for removal of the mucosa and submucosa with subsequent placement of a biologic scaffold to promote constructive mucosal remodeling and minimize stricture formation in the setting of superficial cancer. To date, the method has been successfully used to treat eight human patients. **(A)** Diagnostic biopsy showing high-grade dysplasia. **(B)** Postoperative biopsy showing replacement of the ECM scaffold with mature, differentiated squamous epithelium. Representative endoscopic views of each stage in the procedure and follow-up: **(C)** Muscularis externa being exposed during inversion and resection of the entire sleeve of mucosal and submucosal layers (*arrow*). **(D)** Stent placed to gently compress the ECM scaffold (*arrow*) against the exposed muscularis layer. **(E)** Thirteen-month follow-up showing complete coverage of the resected area by normal esophageal epithelium without stricture formation. Color images available online at www.liebertpub.com/teb

implantation and showed an implant site free of stenosis or dilatation. Keratinized stratified squamous epithelium had regenerated in the entire construct after the 2-week time point. However, regeneration of the muscle layer or lamina muscularis mucosa was not observed.

Clinical studies

Properly designed clinical studies for regenerative medicine approaches to esophageal repair are scarce. In 2008, a case study presented by Knorr *et al.*[171] reported a 16-year-old female with a perforated esophagus after accidental ingestion of a toothbrush. Inspection of the esophagus after retrieval of the brush revealed a near-total perforation of the esophageal wall below the upper esophageal sphincter measuring approximately 1.5×2 cm, which was treated with antibiotics and no oral ingestion. Two days after the primary treatment, an area measuring 1×2 cm covered with fibrin was found through endoscopy and the area was treated with factor XIII in all four quadrants of the lesion. Eight weeks after the incident, esophagogastroduodenoscopy (EGD) showed a completely healed wound at the site of the rupture. Coagulation factor XIII was first used by Laki and Lóránd in 1948 as a fibrin-stabilizing factor[172] and has been used since as a therapy for ulceration due to pressure, large burns, sepsis, and acute liver disorders.[173]

In 2011, Badylak *et al.*[174] reported results of five male patients with adenocarcinoma of the esophagus treated by an entirely endoscopic technique for long-segment *en bloc* resection of the mucosa and submucosa, followed by placement of a biologic scaffold. Results from this study reported at 4–24-month follow-up showed restoration of normal, mature, K4+/K14+ squamous epithelium and return to a normal diet. These patients had no significant complications from the procedure. Two of five patients showed recurrent Barrett's esophagus confined to the gastroesophageal junction after 12 months, while the rest of the reconstituted esophageal mucosa remained intact. This study provided evidence that a biomaterial-based regenerative medicine strategy may enable aggressive endoscopic resection of early stage disease, avoiding the traditional approaches and associated complications of watchful wait upon Barrett's esophagus diagnosis and stepwise mucosal resection/ablation upon noninvasive early stage neoplastic disease diagnosis (Fig. 8).

In 2014, Nieponice *et al.*[175] proposed the use of an ECM scaffold as a reconstructive patch for the augmentation of the esophageal diameter during primary repair. In this study, four patients requiring esophageal reconstruction underwent patch esophagoplasty with a UBM-ECM scaffold. The full thickness of the esophagus was replaced by the scaffold by securing it to the edges of the remaining esophagus. All patients had a favorable clinical recovery and resumed normal oral intake after 7 days. One of the patients presented a microleak that closed spontaneously after drainage. Follow-up studies, including barium swallow and EGD, showed normal esophageal emptying in all patients. Complete mucosal remodeling was observed at 2 months and was indistinguishable from surrounding healthy tissue. Implant sites presented 20% area contraction, and biopsy of the treatment site showed normal esophageal epithelium.

Conclusion

The esophagus is a complex organ comprising multiple tissues that do not have the ability to regenerate. Esophageal pathologies that affect the esophagus are life-threatening and currently available treatment options are limited. This problem is compounded by the default inflammatory response and inherent mode of repair of the esophagus, typically leading to scar tissue deposition and stricture formation even when utilizing a minimally invasive endoscopic approach. Regenerative medicine strategies that utilize cell-based, scaffold-based, and bioactive molecule-based approaches for tissue repair show promise as effective alternatives for the treatment of esophageal disease. However, stricture formation remains a problem in most cases.

In addition to factors related to specific technologies (i.e., biomaterial composition and surface topography, cell seeding, concentration of bioactive molecules, among others), it is important to note that the successful implementation of regenerative medicine strategies for esophageal repair should take into consideration factors such as (1) the availability of healthy and vascularized tissue adjacent to the treatment site that can provide cellular and vascular access, as well as structural and metabolic support, (2) the local microenvironment (e.g., the reflux of gastric contents can affect the remodeling outcome of the regenerative process), and (3) host-related factors (e.g., age, immunocompetence, comorbidities such as preexisting neoplastic disease, and inherent wound healing ability).

Esophageal pathologies are diverse, and a single regenerative medicine approach is unlikely to prove effective in all settings of esophageal pathology. A thorough understanding of different pathologies that affect the esophagus, the anatomic and functional consequences of each disease, and the shortcomings associated with currently available therapies is necessary for the development of successful regenerative medicine strategies for esophageal repair.

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Disclosure Statement

No competing financial interests exist.

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