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REVIEW

Extracellular Matrix Bioscaffolds for Building Gastrointestinal Tissue

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SUMMARY

The development of decellularization techniques to preserve structure and biochemical composition of the extracellular matrix (ECM) has greatly facilitated the use of ECM bioscaffolds as an in vitro substrate to maintain physiologically relevant cell phenotypes. In addition, preclinical and human studies have shown promising results in the use of ECM bioscaffolds as an inductive substrate for tissue engineering applications in the gastrointestinal tract.

Regenerative medicine is a rapidly advancing field that uses principles of tissue engineering, developmental biology, stem cell biology, immunology, and bioengineering to reconstruct diseased or damaged tissues. Biologic scaffolds composed of extracellular matrix have shown great promise as an inductive substrate to facilitate the constructive remodeling of gastrointestinal (GI) tissue damaged by neoplasia, inflammatory bowel disease, and congenital or acquired defects. The present review summarizes the preparation and use of extracellular matrix scaffolds for bioengineering of the GI tract, identifies significant advances made in regenerative medicine for the reconstruction of functional GI tissue, and describes an emerging therapeutic approach. *(Cell Mol Gastroenterol Hepatol 2018;5:1–13; http://dx.doi.org/10.1016/j.jcmgh.2017.09.004)*

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The fundamental role of a biomaterial in tissue remodeling is to provide structural support and a microenvironmental niche that modulates cell attachment and cell behavior. Nature's template for such a biomaterial is the extracellular matrix (ECM); the composite of structural and functional molecules secreted by resident cells in every tissue and organ. The composition and ultrastructure of ECM is tissue-specific, but generally consists of a complex mixture of structural components (eg, collagen and laminin) and soluble growth factors.[1] Once thought to exist for the primary purpose of providing structural support to tissues, the ECM now is recognized as a complex milieu that has a dramatic effect on cell behavior.[2] During homeostatic maintenance and in response to injury, the ECM is subject to extensive and continuous remodeling. Proteolytic degradation of the ECM, as part of the remodeling process, provides morphogenic cues that influence cell survival, proliferation, migration, polarization, and differentiation.[3–5] The ECM is in a state of dynamic reciprocity with resident cells; that is, ECM provides signaling and biophysical cues that influence cell morphology and phenotype.[6–8] In turn, cells modify their secreted ECM products in response to microenvironmental signals including mechanical stimuli, oxygen, and nutrient concentration.[4]

Biologic scaffolds derived from ECM have been developed as inductive substrates for functional tissue remodeling in multiple anatomic sites,[9–15] including the GI tract,[16–22] and are associated with at least partial restoration of functional, site-appropriate tissue; a process referred to as "constructive remodeling."[23] Among the varied and intertwined components of the host response associated with ECM-induced, constructive tissue remodeling are angiogenesis,[24] innervation,[25–27] stem cell recruitment,[28,29] and, perhaps most importantly, modulation of the innate immune response.[30] Arguably, the major determinant of downstream functional remodeling outcome is the early innate immune response to ECM bioscaffolds.[31–33]

ECM bioscaffolds typically have been used as an implantable physical scaffolding to bridge or reinforce a defect site. Diseased or defective tissue is removed and the ECM scaffold subsequently is placed at the site of tissue resection to induce deposition of appropriately organized tissue. However, recent studies have suggested that this paradigm is only one means by which ECM scaffolds can be used in GI tract applications. Hydrogels can be prepared from solubilized ECM and have been shown to be deliverable by minimally invasive methods and favorably change the default response to tissue injury toward a more constructive and functional outcome.[34] Moreover, recent research has shown that whole-organ engineering using a 3-dimensional (3D) ECM scaffold provides an ideal transplantable scaffold with all the necessary signaling cues for cell attachment, differentiation, vascularization, and

Abbreviations used in this paper: **ECM, extracellular matrix; GI, gastrointestinal; IBD, inflammatory bowel disease; iPSC, induced pluripotent stem cell; MBV, matrix-bound nanovesicle; SIS, small intestinal submucosa; 3D, 3-dimensional; 2D, 2-dimensional; UBM, urinary bladder matrix.**

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function.[35] Although much attention has been given to the direct clinical applications of scaffold-based systems, reports also have implicated the use of ECM bioscaffolds in other areas of biomedical research, such as the establishment of in vitro physiological models to study disease pathogenesis.[36–38] The present review summarizes the preparation and use of ECM bioscaffolds for bioengineering of the gastrointestinal (GI) tract, and identifies significant advances made in regenerative medicine for the reconstruction of functional GI tissue.

ECM Bioscaffold Production

Methods for tissue decellularization have been described for almost every tissue type,[24] including regions of the GI tract, such as the esophagus,[39–42] stomach,[43–48] small intestine,[49–51] and colon.[52] Although a detailed discussion on decellularization methods is beyond the scope of this review, a significant body of literature is devoted to decellularization agents, techniques, sterilization, and storage of ECM bioscaffolds.[53–55] In general, decellularization techniques are tailored to the distinctive physical and biochemical characteristics of the tissue of interest including thickness, matrix density, and 3D configuration. Decellularization of source tissue typically involves a combination of mechanical, chemical, and enzymatic strategies to remove the cellular component while maintaining the molecular composition and ultrastructure of the ECM.[56] For example, chemical solutions, freeze-thaw cycles, and enzymatic treatment can be used to disrupt cell membranes. Cytosolic and nuclear components can be solubilized using a variety of detergents such as Triton X-100 (Sigma, St. Louis, MO), sodium deoxycholate, or sodium dodecyl sulfate. Alternatively, mechanical removal of the histomorphologic layers of the GI tract can be used. For example, in the preparation of small intestinal submucosa (SIS), porcine jejunum is split horizontally and superficial layers of the mucosa, serosa, and muscularis are removed mechanically, leaving the submucosa and basilar portions of the mucosa[49] (Figure 1). ECM bioscaffolds fabricated as multilaminate sheets are used clinically as a surgical mesh or patch graft. However, ECM bioscaffolds also can be processed into tubular grafts, comminuted forms (powders),[57] and hydrogels[34,58] (Figure 1). In addition, perfusion decellularization can be used to generate acellular whole-organ scaffolds. Delivery of decellularization reagents via the native vasculature of cadaveric organs effectively can remove cellular components while maintaining the vascular and lymphatic networks critical for subsequent recellularization.[53,59–63]

Although the objective of any decellularization protocol is the removal of cellular components and the preservation of the native ECM ultrastructure and biochemical composition, all methods of decellularization invariably disrupt the ECM to some degree. Inefficient decellularization or the use of harsh decellularization may lead to detrimental remodeling effects after implantation.[64,65] There is a delicate balance between maintaining native ECM structure/composition and the removal of cellular components such as nucleic acid, membrane lipids, and cytosolic proteins. Studies have shown that these remnant cellular components can elicit an adverse inflammatory response and inhibit constructive remodeling if not removed adequately.[31,66] In addition, the use of chemical cross-linking agents to increase the strength of ECM bioscaffolds has been shown to disrupt the ligand landscape of the material significantly and prevent the degradation of the scaffold material and release of bound signaling molecules after implantation.[67,68] Numerous studies have shown that degradation of the scaffold material and release of ECM components, such as matricryptic peptides,[69] is necessary and critical for functional constructive remodeling outcomes (Table 1). For example, enzymatic cleavage of the collagen IIIα molecule and the release of the carboxy-terminal telopeptide region was shown to be chemotactic for progenitor cells and capable of initiating angiogenesis and mitogenesis.[69–72] Additional degradation products of ECM bioscaffolds include growth factors stored within the matrix[73-76]; structural molecules such as collagen,[9,77] laminin,[77] fibronectin,[78] and glycosaminoglycans[79]; and matrix-bound nanovesicles (MBV), nanometer-sized membranous vesicles that are similar in size and structure to exosomes.[80,81] Although exosomes exist exclusively in body fluids, MBV are bound within the collagen network of the ECM.[81] MBV were shown to be sufficient (ie, in an ECM-independent manner) to recapitulate phenotypical and functional effects attributed to ECM bioscaffolds, as assessed by in vitro cell culture studies.[80,81]

Recent work has described the potential benefits of ECM bioscaffolds derived from homologous tissue vs heterologous tissue when used in selected anatomic locations.[29,82–90] However, the necessity or preference for site-specific ECM remains unknown for many therapeutic applications. Although tissue specificity may not be necessary for all therapeutic applications,[9,91,92] some studies have shown that site-specific ECM can better maintain tissue-specific cell phenotypes,[82–85] promote cell proliferation,[84,86] induce tissue-specific differentiation,[87] and enhance the chemotaxis of lineage-directed progenitor cells[29,88,89] compared with ECM derived from heterologous tissue sources. Zhang et al[86] have shown that ECM derived from liver, skin, and skeletal muscle increase the proliferation and differentiation potential for site-matched cell types. Sellaro et al[82,82] have shown that ECM derived from liver improves the maintenance of sinusoidal endothelial cell phenotype and the function of hepatocytes in vitro compared with nonhepatic ECM substrates. More recently, myocardial ECM has been shown to improve cardiac progenitor cell function in vitro.[85] Seif-Naraghi et al[93] have shown that injection of a hydrogel form of cardiac ECM after myocardial infarct improves cardiac function and results in increased cardiac muscle mass. Regarding the GI tract, ECM derived from the esophageal mucosa was shown to enhance the migration of esophageal stem cells and promote the formation of 3D organoids better than ECM derived from SIS or urinary bladder matrix (UBM).[94]

In Vitro Culture Systems

In vitro culture of gastrointestinal cell types can be used for a wide range of potential applications, including drug development, basic research, disease modeling, and as a source of cells for whole organ re-seeding. Successful use of these in vitro models, however, requires maintenance of the

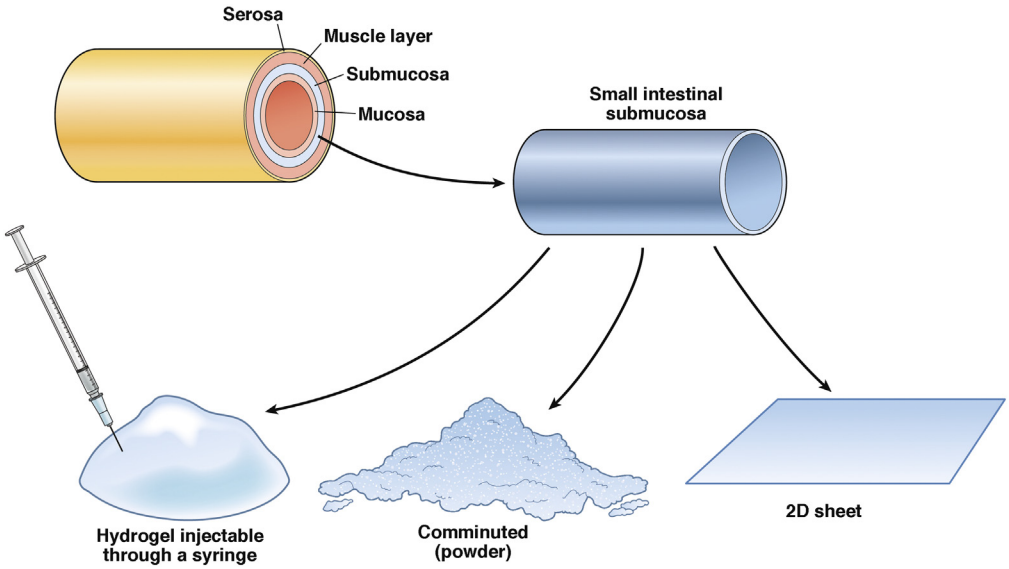

Figure 1. Overview of SIS ECM scaffold decellularization and processing. Porcine jejunum is split horizontally and superficial layers of the tunica mucosa, tunica serosa, and tunica muscularis are removed mechanically, leaving the tunica submucosa and basilar portions of the tunica mucosa. Biologic scaffolds composed of ECM have been fabricated primarily as multilaminate sheets, which are used clinically as surgical mesh materials or patch grafts. However, ECM bioscaffolds also can be processed into tubular grafts, comminuted forms (powders), and hydrogels.

appropriate cell phenotype and function. Certain GI cell types are notoriously difficult to culture (eg, hepatocytes,[95] sinusoidal endothelial cells,[82] and intestinal epithelial cells[96]), and undergo rapid dedifferentiation after isolation and culture on plastic or on substrates consisting of single ECM components such as collagen. ECM bioscaffolds contain biochemical cues that better mimic the native microenvironment and therefore are being investigated as a tool to maintain physiologically relevant cell phenotypes.

Primary Cell Expansion

Primary rat esophageal epithelial cells seeded on decellularized rat esophageal matrix form a stratified epithelium consisting of multiple cell layers.[39,40,97] Ozeki et al[97] observed a mostly keratinized 3- to 4-cell-layer–thick epithelium with cell morphology, polarization, and localization of proliferating cells similar to that of the native esophagus after 1 week of culture. By using esophageal ECM, Bhrany et al[39] also showed a stratified epithelium with a thick keratin layer after 11 days. Consistent with the Ozeki et al[97] study, the basal layer contained proliferating cells with a rounded morphology and cell polarization similar to native tissue.

Loneker et al[98] investigated the effects of solubilized ECM as a media supplement on 2-dimensional (2D) culture of primary rat hepatocytes. ECM derived from human,

Table 1. Bioactive Components of ECM Scaffolds That Play a Role in Constructive Tissue Remodeling Outcomes

ECM component	Examples	Function	References
Structural proteins	Collagens I, III, IV, V, VI, VII Laminin Fibronectin	Provide tensile strength to tissues Cell adhesion molecules	9,77,78
Glycosaminoglycans	Heparin Heparan sulfate Chondroitin sulfate Hyaluronic acid	Modulation of enzyme activity Assembly and organization ECM Regulation of cell growth	79
Matricryptic peptides	Carboxy-terminal telopeptide region of the collagen IIIα molecule	Chemoattractant for progenitor cells Capable of initiating angiogenesis and mitogenesis	69–72
MBV	MBV contain microRNA, protein, and lipid cargo	Promote macrophage polarization and stem cell differentiation	81,82
Growth factors	VEGF, TGFβ, bFGF	Promote angiogenesis, mitogenesis, and cellular differentiation	73–76

bFGF, basic fibroblast growth factor; TGFβ, transforming growth factor β; VEGF, vascular endothelial growth factor.

canine, rat, and porcine livers were compared with porcine SIS and porcine UBM to determine both species- and tissue-specific effects of ECM on hepatocyte phenotype. Treatment with porcine and canine liver ECM resulted in increased albumin secretion and bile production compared with the other ECM materials.[98] Theoretically, human liver ECM may be the ideal scaffold for culture of primary human hepatocytes, however, a shortage of donor organs limits this application. To overcome this, the ability of spleen ECM to maintain hepatocyte function has been investigated.[99,100] Primary rat hepatocytes were seeded into the spleen matrix by perfusion through the splenic artery with an engraftment efficiency of almost 75%. Expression of key genes related to hepatocyte function was greater in culture of hepatocytes on spleen matrix than in the standard collagen sandwich configuration, although it was lower than hepatocytes cultured on a liver matrix. Hepatocytes seeded on both spleen and liver scaffolds produced similar amounts of albumin and urea, however, they were significantly lower than levels produced in sandwich culture.[100] The mechanisms by which the ECM is able to modulate the expression of tissue-specific genes are not fully understood, but it is thought that the release of growth factors, such as hepatocyte growth factor, from the ECM plays a role.[100] Cell–cell and cell–matrix interactions also have been shown to be important in determining hepatocyte phenotype.[101]

Stem Cell Differentiation

ECM has been used as a substrate to maintain or enhance the phenotype of isolated organoids and induce differentiation of stem cells for multiple organs of the GI tract, including the esophagus, small intestine, and liver. Keane et al[94] showed that ECM derived from esophageal mucosa enhanced the migration of esophageal stem cells and promoted the formation of 3D organoids better than ECM derived from SIS or UBM. In a study by Schweinlin et al,[102] intestinal organoid structures containing epithelial and progenitor cells were seeded as single cells on a decellularized SIS scaffold in a Transwell-like configuration. After 7 days in co-culture with fibroblasts, the cells formed an intact and stable epithelial barrier and some cells differentiated into goblet cells, Paneth cells, enteroendocrine cells, and enterocytes. Human bone marrow stem cells seeded onto an SIS scaffold in a perfusion bioreactor formed intact mucosa, villi and crypts containing goblet cells, as well as blood vessels lined with endothelial cells.[103]

The ability of the ECM to maintain phenotype or enhance differentiation to hepatocytes has been shown for multiple cell sources, culture conditions, and ECM configurations. Induced pluripotent stem cell (iPSC)-derived hepatocytes seeded onto rat liver scaffolds maintained viability, formed bile canaliculi structures, and had better gene expression and metabolic activity as compared with iPSC-derived hepatocytes cultured on collagen or Matrigel (Corning, Corning, NY).[104] Zhang and Dong[105] conducted a direct comparison of adipose-derived mesenchymal stem cells cultured on a 2D layer of liver ECM, collagen, fibronectin, and Matrigel. Cells cultured on liver matrix differentiated into mature hepatocytes with enhanced hepatocyte-specific gene expression and function compared with the other materials. Recently, Bao et al[106] evaluated the influence of 2D vs 3D spheroid culture on the hepatogenic differentiation of bone marrow–derived mesenchymal stem cells seeded on liver ECM compared with tissue culture plastic. Culture of bone marrow–derived mesenchymal stem cell spheroids on liver ECM produced differentiated hepatocytes with significantly better gene expression and function than all other culture methods examined, although the use of liver ECM as the substrate improved the outcomes in 2D single-cell culture as well. Park et al[107] showed that media supplementation with solubilized liver ECM enhances iPSC commitment to a hepatic lineage, producing hepatocytes with greater maturity and cell-specific function. The cumulative results of these studies show that decellularized ECM retain tissue-specific architecture and biochemical composition and provide a biomimetic environment for differentiation of stem cells in vitro. However, further studies are required to fully interrogate the biochemical components within ECM that mediate hepatogenic differentiation of stem cells, and the signaling mechanisms involved therein.

ECM as an In Vitro Model of Cancer

Changes in the mechanical or biochemical cues provided by the ECM are capable of altering tumor growth and differentiation. ECM hydrogel derived from metastatic human colon tumors had a different protein composition and a 3-fold higher stiffness than an ECM hydrogel derived from normal human colon.[108] Endothelial cells cultured in the tumor ECM hydrogel formed a tumor-like vasculature and colon tumor cells had significantly faster growth as compared with normal colon ECM hydrogels.[108] Similarly, comparison of ECM derived from the mucosa of normal colon, perilesional area, or colorectal cancer, showed differential effects on the proliferation and phenotype of transformed epithelial cells.[37]

An alternative approach to creating an in vitro model of cancer is to recellularize an ECM scaffold from normal tissue with malignant cancer cells or mutated cells.[38,109] Unlike Caco-2 colon cancer cells, malignant SW480 cells destroyed the basement membrane and formed tightly associated tumor-like structures when in co-culture with fibroblasts on a normal small intestinal submucosa and mucosa ECM scaffold.[109] Epithelial cells with mutations in key genes implicated in colorectal cancer cultured on healthy colon ECM induced a transition from dysplasia to noninvasive neoplasia and finally to an invasive submucosal tumor within 4 weeks of culture.[36] In vitro models of both colon and liver cancer have shown a response to therapeutics consistent with known in vivo effects.[109,110] Models of the tumor microenvironment can be useful tools to allow isolation of specific signaling molecules involved in cancer progression.[36]

GI Tissue Engineering

The GI tract is a structurally complex tubular system with diverse functions ranging from a transit tube (esophagus), to digestion (stomach), nutrient and water absorption

(intestine), and excretion of waste (rectum). Thus, tissue engineering strategies for the creation of segments of the GI tract requires the consideration of not only ECM composition, but also the selection of an appropriate scaffold configuration (eg, multilaminate sheets, tubular grafts, or hydrogels). An outline of the various ECM scaffold configurations and their use in the GI tract is illustrated in Figure 2. Recent advancements in ECM-mediated approaches have shown great promise in repair of GI tissue damaged by neoplasia, inflammatory bowel disease, and congenital or acquired defects. A summary of the preclinical and human studies evaluating the use of ECM bioscaffolds to repair GI tissue is provided in Table 2.

GI Neoplasia

Gastric cancer is the third leading cause of cancer death worldwide.[111] Gastrectomy (removal of part of the stomach) is the current standard of care for stomach cancer and is associated with postsurgical complications including anastomotic leakage and intra-abdominal abscesses.[112–114] Similarly, esophageal adenocarcinoma is one of the most lethal malignancies of the digestive tract with the greatest increase in incidence worldwide,[115] often requiring esophagectomy (esophageal resection), a procedure associated with high morbidity and a decreased quality of life.[116,117] ECM bioscaffolds have been investigated for their ability to stimulate regeneration of gastric and esophageal mucosa after surgical resection. For example, preclinical studies have shown that SIS bioscaffold sheets can be used to patch gastric wall defects.[118,119] Implantation of an SIS bioscaffold sheet into a full-thickness defect created in the rodent stomach resulted in the formation of smooth muscle, peripheral nerve, and gastric parietal cells 12 months after implant, suggesting that ECM scaffolds have the potential to promote physiological and site-specific regeneration of gastric muscosal tissue accompanied by intrinsic nerve migration.[118,120] Although, to date, applications for repairing gastric defects have remained at the preclinical stage of development, the use of tubular ECM grafts for the repair of esophageal mucosa have advanced to human studies. Early studies using rodent models in which gastric acellular matrix[48] or SIS[121] bioscaffolds were implanted into patch defects created in the esophagus showed restoration of a stratified squamous

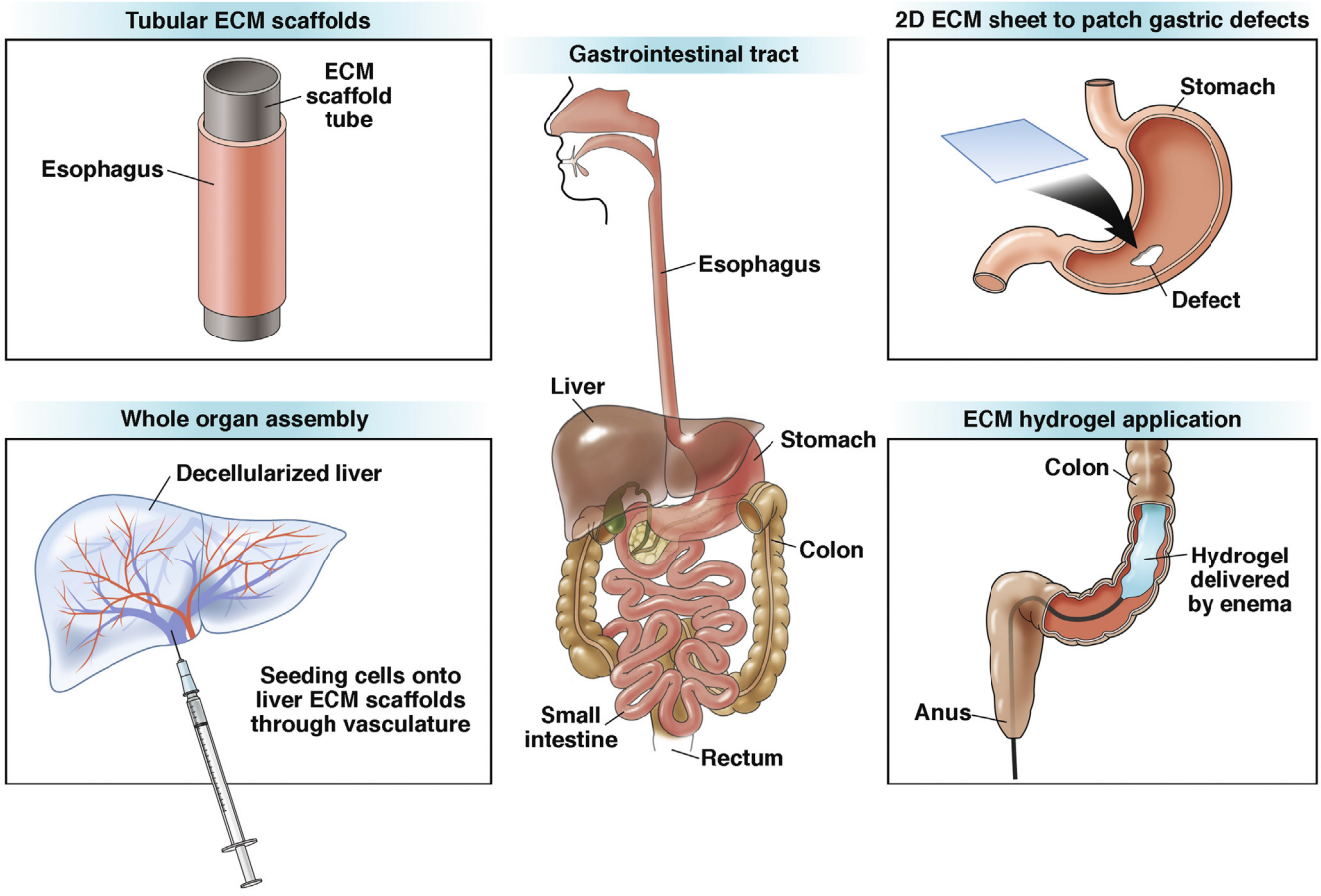

Figure 2. ECM scaffold configurations and their use in the GI tract. ECM scaffolds can be processed into tubular grafts to regenerate esophageal submucosa and mucosa. ECM patch grafts have been used in preclinical models to repair defects in the stomach. Perfusion-based decellularization and reseeding with host cells is being explored as a method to engineering whole organs for transplantation. ECM hydrogels have been shown to be adhesive to colonic mucosa when delivered via enema and have been shown to restore epithelial cell barrier function while mitigating the proinflammatory response during experimentally induced ulcerative colitis.

Table 2. Preclinical and Human Studies Evaluating the Use of ECM Bioscaffolds to Repair GI Tissue

Organ system	Objective	ECM substrate	Model	Results	References
Esophagus	Repair of patch defect created in the abdominal esophagus	GAM patch	Rat	Regeneration of a keratinized, stratified squamous mucosa without the occurrence of stenosis or dilation	48
	Repair of a semicircumferential defect in the cervical or abdominal esophagus	SIS patch	Rat	Restoration of the keratinized stratified squamous epithelium, and complete regeneration of muscle fibers, with no evidence of fistula, stenosis, or diverticula	121
	Repair of a critically sized, short-segment, circumferential defect in the esophagus	Tubular UBM graft	Canine	Restoration of esophageal histomorphology and function, with minimal stricture formation	17
	Remodeling the anastomotic site at the cervical esophagus and gastroesophageal junction after an esophageal transection and gastric pull-up procedure	Tubular UBM graft	Canine	Restoration of a mature epithelium and regeneration of muscle tissue	18
	Repair of an aggressive, long-segment, circumferential esophageal resection	Tubular UBM graft	Canine	Esophageal mucosal remodeling without stricture formation	19
	Repair of an endoscopic long-segment, circumferential sleeve resection of the mucosa and submucosa on 5 human patients with mucosal-confined (T1A) esophageal adenocarcinoma (nonsurgical candidates for esophagectomy owing to comorbidities)	Tubular SIS graft	Human cohort study	Restitution of normal esophageal mucosa, no recalcitrant stricture formation, and no recurrence of neoplasia	92
Stomach	Repair of a circular, full-thickness defect created on the antrum of the rodent stomach	SIS patch	Rat	Regeneration of normal gastric mucosa was seen at the periphery of the defect after 21 days Nerve migration to the graft occurred in the rodent stomach 6 months after implantation Smooth muscle, peripheral nerve, and gastric parietal cells were observed 1 year after implantation	118–120
Small intestine	Repair of a partial defect created by resection of a portion of the small bowel	SIS patch	Canine	Regeneration of the mucosal epithelial layer, smooth muscle tissue, and the serous membrane with no evidence of intestinal dysfunction or stenosis	22
	Placement of tubular porcine SIS bioscaffolds after an ileostomy	Tubular SIS graft	Rat	Rapid regeneration of mucosa, smooth muscle, and serosa	123
	Placement of tubular porcine SIS bioscaffolds after an ileostomy	Tubular SIS graft	Rat	Partial restoration of structural features of the normal intestine, including mucosal thickness, villus height, and crypt depth	125
	Repair a jejunal incisional defect	SIS patch	Rabbit	Complete coverage of the SIS graft with columnar epithelium by 4 weeks after implantation, and the presence of organized mucosal and submucosal tissues (including goblet cells and villus-like configurations) were observed at 6 weeks after implantation	124

Table 2. Continued

Organ system	Objective	ECM substrate	Model	Results	References
Colon	ECM hydrogel therapy to accelerate tissue regeneration in a rodent model of ulcerative colitis	SIS hydrogel	Rat	Hydrogel delivered by enema was adhesive to colonic tissue and resulted in a marked reduction in the clinical and histologic signs of the disease Application of the ECM hydrogel showed restoration of colonic epithelial barrier function and mitigation of the proinflammatory macrophage phenotype	136
Anus	Closure of anal fistulas	ADM plug	Porcine	ECM plugs rapidly were vascularized, accompanied by the formation of organized bundles of muscle at the site of the anal fistulas	135

ADM, acellular dermal matrix; GAM, gastric acellular matrix.

epithelium and complete regeneration of muscle fibers with no evidence of fistula or significant stenosis. A preclinical study in a canine model showed that short-segment circumferential esophageal defects could be repaired by a tubular UBM graft with minimal stricture formation and near-normal restitution of the esophageal histomorphology and function, whereas long-segment circumferential defects required the presence of at least portions of the muscularis externa to prevent intractable stricture.[17] Moreover, a study of esophageal transection designed to evaluate reinforcement of the anastomosis of a gastric pull-up procedure showed restoration of a mature epithelium and regeneration of muscle tissue in the abluminal muscularis externa layer.[18] In addition, the use of a tubular UBM scaffold as an inductive substrate also was shown in a preclinical canine model of aggressive, long-segment circumferential esophageal resection.[19] Results from these preclinical studies showed that implantation of ECM bioscaffolds induced a fundamental change in the default healing response from the expected inflammation/scarring response toward a restorative tissue formation (ie, constructive remodeling) paradigm. The promising results of these preclinical studies were the basis of a human cohort study involving 5 patients with mucosal-confined adenocarcinoma (stage T1a). These patients were nonsurgical candidates for esophagectomy owing to comorbidities and were treated with endoscopic, long-segment, circumferential sleeve resection of the mucosa and submucosa and placement of a tubular SIS graft over the site of the resected tissue. A follow-up period of 15–35 months showed restitution of normal esophageal mucosa, no recalcitrant stricture formation, and, importantly, no recurrence of neoplasia.[92]

Short-Bowel Syndrome

Short-bowel syndrome is a complex disease that can result from anatomic or functional loss of portions of the small intestine.[122] The loss of segments of the intestine owing to congenital or acquired defects, or from surgical resection of inflamed, necrotic, or cancerous intestinal tissue, results in malnutrition, fluid and electrolyte disturbances, and malabsorption. The current therapy for short-bowel syndrome includes surgical approaches to increase the absorptive surface area, which mostly have been unsuccessful, and small-bowel transplantation, which is limited by immunologic challenges and is associated with high morbidity.[123] Preclinical studies evaluating the use of ECM bioscaffolds as an inductive substrate for in situ intestinal regeneration and bowel-lengthening surgeries have shown promising results. The use of SIS as a patch graft to repair a partial wall resection of small bowel in a canine model showed that by 3 months after implant, the ECM bioscaffold was fully resorbed and by 6 months showed that the multilayered tissue of the remodeled wall contained mucosa, submucosa, smooth muscle, and serosa, with minimal architectural differences between the native and the regenerated bowel.[22] An SIS patch also was evaluated for the ability to repair a jejunal incisional defect in a rabbit model. Results from this study showed that 6 weeks after implantation, the graft consisted of mucosal and submucosal tissue and a complete columnar epithelial layer with

villus-like architecture.[124] In addition to use as a patch graft, tubular ECM constructs also have been evaluated for their ability to support regeneration of neointestine. For example, a tubular SIS graft inserted with a bilateral anastomosis in an isolated ileal loop showed that by 12 weeks, the luminal surface of the graft was completely covered by a mucosal layer. At 24 weeks, the neointestine showed layers of mucosa, submucosa, muscularis externa, and serosa. The neomucosa showed typical small-bowel morphology characterized by a columnar epithelial cell layer with goblet cells, Paneth cells, enterocytes, and enteroendocrine cells, but intestinal absorption and metabolic function were not examined.[123,125] Results from these preclinical studies, and others,[46,126] suggest that an appropriately configured ECM bioscaffold may be useful as an inductive substrate for regeneration of neointestine for patients suffering from short-bowel syndrome.

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD), which includes ulcerative colitis and Crohn's disease, is a worldwide health problem.[127] These debilitating chronic relapsing diseases typically consist of acute flares followed by periods of healing.[128] The specific etiology of IBD is unknown but genetic predisposition and immunologic factors are known contributors. Generally, IBD is characterized by an aberrant immune response with associated defects in intestinal epithelial cell barrier function.[129] Tissue damage associated with IBD has long been considered a downstream effect of disease and not a contributing causative factor. This interpretation has led to the development of numerous treatments that solely target inflammation, but all treatments to date have shown limited efficacy. Although its role often is overlooked, the ECM is a critical component of intestinal inflammation and progression of IBD. Macroscopic tissue damage and clinical signs of IBD are preceded by changes in the ECM. Changes in collagen microarchitecture and thickening of ECM at crypt regions are evident in the colonic mucosa of patients with IBD.[130] IBD can progress in diametrically opposing directions based on the balance of ECM deposition or degradation. For example, Crohn's disease can advance toward stricture or penetrating disease. Stricture, or fibrostenosis, is the result of excessive ECM deposition.[131–133] In contrast, penetrating disease is characterized by ECM destruction and fistula formation.[132] ECM bioscaffolds fabricated into plugs have been used successfully in human patients for the closure of Crohn's anorectal fistulas.[134] An experimental porcine model of fistula-in-ano showed that ECM plugs derived from acellular dermal matrix are vascularized rapidly and accompanied by the formation of organized bundles of muscle at the site of the anal fistulas.[135] Although the use of ECM-based fistula plugs address the downstream damage caused by IBD, a recent study sought to evaluate the ability of an ECM hydrogel therapy to accelerate tissue regeneration in a rodent model of ulcerative colitis.[136] Results from this study showed that an SIS hydrogel delivered by enema was adhesive to colonic tissue and resulted in a marked reduction in the clinical and histologic signs of the disease. Application of the SIS hydrogel showed restoration of colonic epithelial barrier function and mitigation of the proinflammatory macrophage phenotype. Overall, results from this study, and others,[33,80,137–139] have shown that ECM possesses immunomodulatory properties, a process shown to be a critical determinant of downstream constructive and functional tissue-remodeling outcomes.

Conclusions

Tissue engineering strategies to repair the gastrointestinal tract have made significant advancements over the past 2 decades from in vitro and benchtop studies to a clinically translatable therapy. The development of decellularization techniques to preserve the structure and biochemical composition of native ECM, and the fabrication of tubular ECM grafts and ECM hydrogels, have greatly facilitated the site-specific applications of ECM bioscaffolds in the GI tract. Apart from the use of ECM bioscaffolds as an in vitro physiological model to study disease pathogenesis, numerous preclinical studies have shown promising results in the use of ECM bioscaffolds as an inductive substrate that can be applied as a therapy to a wide array of GI pathologies including short-bowel syndrome, inflammatory bowel disease, and congenital defects. However, further studies are required for the widespread clinical translation of ECM bioscaffolds.

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Conflicts of interest
The authors disclose no conflicts.