Extracellular Matrix Bioscaffolds for Building Gastrointestinal Tissue

George S. Hussey,1,2 Madeline C. Cramer,1,3 and Stephen F. Badylak1,2,3

1McGowan Institute for Regenerative Medicine, 3Department of Bioengineering, 2Department of Surgery, School of Medicine, University of Pittsburgh Medical Center Presbyterian Hospital, University of Pittsburgh, Pittsburgh, Pennsylvania

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.

Keywords: Extracellular Matrix; Bioscaffolds; Regenerative Medicine; Tissue Engineering; 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)

Keywords: Extracellular Matrix; Bioscaffolds; Regenerative Medicine; Tissue Engineering; Gastrointestinal Tract.

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.1 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.4 In turn, cells modify their secreted ECM products in response to microenvironmental signals including mechanical stimuli, oxygen, and nutrient concentration.6

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,14 innervation,20–27 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.

© 2018 The Authors. Published by Elsevier Inc. on behalf of the AGA Institute. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). 2352-345X

http://dx.doi.org/10.1016/j.jcmgh.2017.09.004
function. 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. 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, including regions of the GI tract, such as the esophagus, stomach, small intestine, and colon. 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. 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. 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 (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, comminated forms (powders), and hydrogels (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.

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. 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. 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. Numerous studies have shown that degradation of the scaffold material and release of ECM components, such as matricryptic peptides, 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. Additional degradation products of ECM bioscaffolds include growth factors stored within the matrix; structural molecules such as collagen; laminin, fibronectin, and glycosaminoglycans; and matrix-bound nanovesicles (MBV), nanometer-sized membranous vesicles that are similar in size and structure to exosomes. Although exosomes exist exclusively in body fluids, MBV are bound within the collagen network of the ECM. 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.

Recent work has described the potential benefits of ECM bioscaffolds derived from homologous tissue vs heterologous tissue when used in selected anatomic locations. 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, some studies have shown that site-specific ECM can better maintain tissue-specific cell phenotypes, promote cell proliferation, induce tissue-specific differentiation, and enhance the chemotaxis of lineage-directed progenitor cells compared with ECM derived from heterologous tissue sources. Zhang et al 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 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. Seif-Naraghi et al 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).

**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...
appropriate cell phenotype and function. Certain GI cell types are notoriously difficult to culture (e.g., hepatocytes, sinusoidal endothelial cells, and intestinal epithelial cells), 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. Ozeki et al. 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. also showed a stratified epithelium with a thick keratin layer after 11 days. Consistent with the Ozeki et al. study, the basal layer contained proliferating cells with a rounded morphology and cell polarization similar to native tissue.

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

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 III 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. 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.

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. 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. Cell–cell and cell–matrix interactions also have been shown to be important in determining hepatocyte phenotype.

**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. 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., 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 Transwell-like 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. 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. Cell–cell and cell–matrix interactions also have been shown to be important in determining hepatocyte phenotype.

**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. 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. 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.

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 mutant cells. 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. 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. In vitro models of both colon and liver cancer have shown a response to therapeutics consistent with known in vivo effects. Models of the tumor microenvironment can be useful tools to allow isolation of specific signaling molecules involved in cancer progression.

**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 (e.g., 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. 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. Similarly, esophageal adenocarcinoma is one of the most lethal malignancies of the digestive tract with the greatest increase in incidence worldwide, often requiring esophagectomy (esophageal resection), a procedure associated with high morbidity and a decreased quality of life. 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. 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 mucosal tissue accompanied by intrinsic nerve migration. 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 or SIS 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.](image-url) 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.
| 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 | Restoration 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 | 118–120    |
|              | 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 | |
| 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        |
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. 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. 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. 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.

Short-Bowel Syndrome

Short-bowel syndrome is a complex disease that can result from anatomic or functional loss of portions of the small intestine. 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. 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. 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. In addition to use as a patch graft, tubular ECM constructs also have been evaluated for their ability to support regeneration of neo-intestine. 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. Results from these preclinical studies, and others, suggest that an appropriately configured ECM bioscaffold may be useful as an inductive substrate for regeneration of neo-intestine 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. These debilitating chronic relapsing diseases typically consist of acute flares followed by periods of healing. 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. 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. Macrosopic 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. 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. In contrast, penetrating disease is characterized by ECM destruction and fistula formation. ECM bioscaffolds fabricated into plugs have been used successfully in human patients for the closure of Crohn’s anorectal fistulas. 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 fistula. 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 to accelerate tissue regeneration in a rodent model of ulcerative colitis. 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, 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.

References

1. Mecham RP. Overview of extracellular matrix. Curr Protoc Cell Biol 2012;Chapter 10:Unit 10.
2. Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. J Cell Sci 2008;121:255–264.
3. Midwood KS, Williams LV, Schwarzauer JE. Tissue repair and the dynamics of the extracellular matrix. Int J Biochem Cell Biol 2004;36:1031–1037.
4. Nelson CM, Bissell MJ. Of extracellular matrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. Annu Rev Cell Dev Biol 2006;22:287–309.
5. Steffensen B, Häkkinen L, Larjava H. Proteolytic events of wound-healing—coordinated interactions among matrix metalloproteinases (MMPs), integrins, and extracellular matrix molecules. Crit Rev Oral Biol Med 2001;12:373–398.
6. Schultz GS, Davidson JM, Kirsner RS, Bornstein P, Herman IM. Dynamic reciprocity in the wound microenvironment. Wound Repair Regen 2011;19:134–148.
7. Bornstein P, McPherson J, Sage H. Synthesis and secretion of structural macromolecules by endothelial cells in culture. Pathobiol Endothelial Cell 1982;6:215–228.
8. Bissell MJ, Hall HG, Parry G. How does the extracellular matrix direct gene expression? J Theor Biol 1982;99:31–68.
9. Badylak SF, Tullius R, Kokini K, Shelbourne KD, Klootwyk T, Voytik SL, Kaine MR, Simmons C. The use of xenogeneic small intestinal submucosa as a...
biomaterial for Achilles tendon repair in a dog model. J Biomed Mater Res 1995;29:977–985.

10. Mase VJ Jr, Hsu JR, Wolf SE, Wenke JC, Baer DG, Owens J, Badylak SF, Walters TJ. Clinical application of an acellular biologic scaffold for surgical repair of a large, traumatic quadriceps femoris muscle defect. Orthopedics 2010;33:511.

11. Kropp BP, Epley BL, Prevel C, Rippy M, Harruff R, Badylak S, Adams M, Rink R, Keating M. Experimental assessment of small intestinal submucosa as a bladder wall substitute. Urology 1995;46:396–400.

12. Kochupura PV, Azeloglu EU, Kelly DJ, Doronin SV, Badylak SF, Krukenkamp IB, Cohen IS, Gaudette GR. Tissue-engineered myocardial patch derived from extracellular matrix provides regional mechanical function. Circulation 2005;112:144–149.

13. Knoll LD. Use of small intestinal submucosa graft for the surgical management of Peyronie’s disease. J Urol 2007;178:2474–2478.

14. Gilbert TW, Nieponice A, Spievack AR, Holcomb J, Gilbert S, Badylak SF. Repair of the thoracic wall with an extracellular matrix scaffold in a canine model. J Surg Res 2008;147:61–67.

15. Dejardin LM, Arnczky SP, Ewers BJ, Haut RC, Clarke RB. Tissue-engineered rotator cuff tendon using porcine small intestine submucosa. Am J Sports Med 2001;29:175–184.

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

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

18. Nieponice A, Gilbert TW, Badylak SF. Reinforcement of esophageal anastomoses with an extracellular matrix scaffold in a canine model. Ann Thorac Surg 2006;82:2050–2058.

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

20. Hoppo T, Badylak SF, Jobe BA. A novel esophageal-preserving approach to treat high-grade dysplasia and superficial adenocarcinoma in the presence of chronic gastroesophageal reflux disease. World J Surg 2012;36:2390–2393.

21. Hoepner J, Cnogorac V, Marjanovic G, Jütte R, Karcz W, Weiser H-F, Hopt UT. Small intestinal submucosa as a bioscaffold for tissue regeneration in defects of the colonic wall. J Gastrointest Surg 2009;13:113–119.

22. Chen MK, Badylak SF. Small bowel tissue engineering using small intestinal submucosa as a scaffold. J Surg Res 2001;99:352–358.

23. Badylak SF. The extracellular matrix as a biologic scaffold material. Biomaterials 2007;28:3587–3593.

24. Londono R, Badylak SF. Biologic scaffolds for regenerative medicine: mechanisms of in vivo remodeling. Ann Biomed Eng 2015;43:577–592.

25. Swinehart IT, Badylak SF. Extracellular matrix bioscaffolds in tissue remodeling and morphogenesis. Dev Dyn 2016;245:351–360.

26. Turner NJ, Badylak JS, Weber DJ, Badylak SF. Biologic scaffold remodeling in a dog model of complex musculoskeletal injury. J Surg Res 2012;176:490–502.

27. Sicari BM, Agrawal V, Siu BF, Medberry CJ, Dearth CL, Turner NJ, Badylak SF. A murine model of volumetric muscle loss and a regenerative medicine approach for tissue replacement. Tissue Eng Part A 2012;18:1941–1948.

28. Agrawal V, Johnson SA, Reing J, Zhang L, Tottey S, Wang G, Hirschi KK, Brauhnhut S, Gudas LJ, Badylak SF. Epimorphic regeneration approach to tissue replacement in adult mammals. Proc Natl Acad Sci U S A 2010;107:3351–3355.

29. Beattie AJ, Gilbert TW, Guyot JP, Yates AJ, Badylak SF. Chemoattraction of progenitor cells by remodeling extracellular matrix scaffolds. Tissue Eng Part A 2008;15:1119–1125.

30. Brown BN, Ratner BD, Goodman SB, Amar S, Badylak SF. Macrophage polarization: an opportunity for improved outcomes in biomaterials and regenerative medicine. Biomaterials 2012;33:3792–3802.

31. Brown BN, Valentin JE, Stewart-Akers AM, McCabe GP, Badylak SF. Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component. Biomaterials 2009;30:1482–1491.

32. Badylak SF, Valentin JE, Ravindra AK, McCabe GP, Stewart-Akers AM. Macrophage phenotype as a determinant of biologic scaffold remodeling. Tissue Eng Part A 2008;14:1835–1842.

33. Dziki JL, Huleihel L, Scarritt ME, Badylak SF. Extracellular matrix bioscaffolds as immunomodulatory biomaterials. Tissue Eng Part A 2017. Epub ahead of print.

34. Saldin LT, Cramer MC, Velankar SS, White LJ, Badylak SF. Extracellular matrix hydrogels from decellularized tissues: structure and function. Acta Biomater 2016;49:1–15.

35. Yagi H, Soto-Gutierrez A, Kitagawa Y. Whole-organ regeneration: a regenerative medicine approach in digestive surgery for organ replacement. Surg Today 2013;43:587–594.

36. Chen HJ, Wei Z, Sun J, Bhattacharya A, Savage DJ, Serda R, Mackeyev Y, Curley SA, Bu P, Wang L, Chen S, Cohen-Gould L, Huang E, Shen X, Lipkin SM, Copeland NG, Jenkins NA, Shuler ML. A decellularized human colon model identifies cancer driver genes. Nat Biotechnol 2016;34:845–851.

37. Genovesi L, Zawada L, Tosoni A, Ferri A, Zerbi P, Allevi R, Nebuloni M, Alfano M. Cellular localization, in-dwnteraction, and turnover are differently influenced by healthy and tumor-derived extracellular matrix. Tissue Eng Part A 2014;20:2005–2018.

38. Lü W-D, Zhang L, Wu C-L, Liu Z-G, Lei G-Y, Liu J, Gao W, Hu Y-R. Development of an acellular tumor extracellular matrix as a three-dimensional scaffold for tumor engineering. PLoS One 2014;9:e103672.
39. Bhrany AD, Beckstead BL, Lien CJ, Futran ND, Muni NH, Giachelli CM, Ratner BD. Development of an esophagus acellular matrix tissue scaffold. Tissue Eng 2006;12:319–330.

40. Bhrany AD, Beckstead BL, Lang TC, Farwell DG, Giachelli CM, Ratner BD. Crosslinking of an esophageal acellular matrix tissue scaffold. J Tissue Eng Regen Med 2008;2:365–372.

41. Keane TJ, Londono R, Carey RM, Carruthers CA, Reing JE, Deaeth CL, D’Amore A, Medberry CJ, Badylak SF. Preparation and characterization of a bio-logic scaffold from esophageal mucosa. Biomaterials 2013;34:6729–6737.

42. Totonelli G, Maghsoudlou P, Georgiades F, Garriboli M, Koshy K, Turmaine M, Ashworth M, Sebire NJ, Pierro A, Eaton S, De Coppi P. Detergent enzymatic treatment for the development of a natural acellular matrix for oesophageal regeneration. Pediatr Surg Int 2013;29:87–95.

43. Lun S, Irvine SM, Johnson KD, Fisher NJ, Floden EW, Negron L, Dempsey SG, McLaughlin RJ, Vasudevanurthy M, Ward BR, May BC. A functional extracellular matrix biomaterial derived from ovine forestomach. Biomaterials 2010;31:4517–4529.

44. Floden EW, Malak SF, Basil-Jones MM, Negron L, Fisher JN, Lun S, Dempsey SG, Haverkamp RG, Ward BR, May BC. Biophysical characterization of ovine forestomach extracellular matrix biomaterials. J Biomed Mater Res B Appl Biomater 2011;96:67–77.

45. Irvine SM, Cayzer J, Todd EM, Lun S, Floden EW, Negron L, Fisher JN, Dempsey SG, Alexander A, Hill MC, O’Rourke A, Gunningham SP, Knight C, Davis PF, Ward BR, May BC. Quantification of in vitro and in vivo angiogenesis stimulated by ovine forestomach matrix biomaterial. Biomaterials 2011;32:6351–6361.

46. Parmigotto PP, Marzaro M, Artusi T, Perrino G, Conconi MT. Short bowel syndrome: experimental approach to increase intestinal surface in rats by gastric homologous acellular matrix. J Pediatr Surg 2000;35:1304–1308.

47. Sutherland RS, Baskin LS, Hayward SW, Cunha GR. Regeneration of bladder urothelium, smooth muscle, blood vessels and nerves into an acellular tissue matrix. J Urol 1998;156:571–577.

48. Urita Y, Komuro H, Chen G, Shinya M, Kaneko S, Kaneko M, Ushida T. Regeneration of the esophagus using gastric acellular matrix: an experimental study in a rat model. Pediatr Surg Int 2007;22:21–26.

49. Badylak SF, Lantz GC, Coffey A, Geddes LA. Small intestinal submucosa as a large diameter vascular graft in the dog. J Surg Res 1989;47:74–80.

50. Maghsoudlou P, Totonelli G, Loukogeorgakis SP, Eaton S, De Coppi P. A decellularization methodology for the production of a natural acellular intestinal matrix. J Vis Exp 2013;80:e50658.

51. Totonelli G, Maghsoudlou P, Garriboli M, Riegler J, Orlando G, Burns AJ, Sebire NJ, Smith WV, Fishman JM, Ghionzoli M, Turmaine M, Birchall MA, Atala A, Soker S, Lythgoe MF, Seifalian A, Pierro A, Eaton S, De Coppi P. A rat decellularized small bowel scaffold that preserves villus-crypt architecture for intestinal regeneration. Biomaterials 2012;33:3401–3410.

52. Keane TJ, Dziki J, Castelton A, Faulk DM, Messerschmidt V, Londono R, Reing JE, Velankar SS, Badylak SF. Preparation and characterization of a biologic scaffold and hydrogel derived from colonic mucosa. J Biomed Mater Res B Appl Biomater 2017;105:291–306.

53. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. Biomaterials 2011;32:3233–3243.

54. Gilbert TW, Sellaro TL, Badylak SF. Decellularization of tissues and organs. Biomaterials 2006;27:3675–3683.

55. Keane TJ, Swinehart IT, Badylak SF. Methods of tissue decellularization used for preparation of biologic scaffolds and in vivo relevance. Methods 2015;84:25–34.

56. Badylak SF. Xenogeneic extracellular matrix as a scaffold for tissue reconstruction. Transplant Immunol 2004;12:367–377.

57. Gilbert TW, Stolz DB, Biancanello F, Simmons-Byrd A, Badylak SF. Production and characterization of ECM powder: implications for tissue engineering applications. Biomaterials 2005;26:1431–1435.

58. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: structure and function. Acta Biomater 2009;5:1–13.

59. Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. Ann Rev Biomed Eng 2011;13:27–53.

60. Sabetkish S, Kajbafzadeh AM, Sabetkish N, Khorraramouz R, Akbarzadeh A, Seyerian SL, Pasalar P, Orangian S, Beigi RSH, Aryan Z. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix liver scaffolds. J Biomed Mater Res Part A 2015;103:1498–1508.

61. Baptista PM, Orlando G, Mirmalek-Sani S-H, Siddiqui M, Atala A, Soker S. Whole organ decellularization—a tool for bioscaffold fabrication and organ bioengineering. Conf Proc IEEE Eng Med Biol Soc 2009;1:6526–6529.

62. Napierala H, Hillebrandt K-H, Haep N, Tang P, Tintemmann M, Gassner J, Noesser M, Everwien H, Seiffert N, Kluge M. Engineering an endocrine neopancreas by repopulation of a decellularized rat pancreas with islets of Langerhans. Sci Rep 2017;7:41777.

63. Goh S-K, Bertera S, Olsen P, Candiello JE, Halfter W, Uechi G, Balasubramani M, Johnson SA, Sicari BM, Kollar E. Perfusion-decellularized pancreas as a natural 3D scaffold for pancreatic tissue and whole organ engineering. Biomaterials 2013;34:6760–6772.

64. Reing JE, Brown BN, Daly KA, Freund JM, Gilbert TW, Hsiong SX, Huber A, Kullas KE, Tottey S, Wolf MT. The effects of processing methods upon mechanical and biologic properties of porcine dermal extracellular matrix scaffolds. Biomaterials 2010;31:8626–8633.

65. White LJ, Taylor AJ, Faulk DM, Keane TJ, Saldin LT, Reing JE, Swinehart IT, Turner NJ, Ratner BD,
January 2018

ECM Bioscaffolds and GI Tissue 11

66. Keane TJ, Londono R, Turner NJ, Badylak SF. Consequences of ineffective decellularization of biologic scaffolds on the host response. Biomaterials 2012; 33:1771–1781.

67. Costa A, Naranjo J, Turner N, Swinehart I, Kolich B, Shaffey S, Londono R, Keane T, Reing J, Johnson S. Mechanical strength vs. degradation of a biologically-derived surgical mesh over time in a rodent full thickness abdominal wall defect. Biomaterials 2016; 108:81–90.

68. Brown BN, Barnes CA, Kasick RT, Michel R, Gilbert TW, Beer-Stolz D, Castner DG, Ratner BD, Badylak SF. Surface characterization of extracellular matrix scaffolds. Biomaterials 2010;31:428–437.

69. Davis GE, Bayless KJ, Davis MJ, Meininger GA. Regulation of tissue injury responses by the exposure of matricryptic sites within extracellular matrix molecules. Am J Pathol 2000;156:1489–1498.

70. Agrawal V, Tottey S, Johnson SA, Freund JM, Siu BF, Badylak SF. Recruitment of progenitor cells by an extracellular matrix cryptic peptide in a mouse model of digit amputation. Tissue Eng Part A 2011; 17:2435–2443.

71. Li F, Li W, Johnson S, Ingram D, Yoder M, Badylak S. Low-molecular-weight peptides derived from extracellular matrix as chemoattractants for primary endothelial cells. Endothelium 2004;11:199–206.

72. Agrawal V, Brown BN, Beattie AJ, Gilbert TW, Badylak SF. Evidence of innervation following extracellular matrix scaffold-mediated remodelling of muscular tissues. J Tissue Eng Regen Med 2009; 3:590–600.

73. Voytik-Harbin SL, Brightman AO, Kraine MR, Waisner B, Badylak SF. Identification of extractable growth factors from small intestinal submucosa. J Cell Biochem 1997; 67:478–491.

74. McDevitt CA, Wildey GM, Cutrone RM. Transforming growth factor-β1 in a sterilized tissue derived from the pig small intestine submucosa. J Biomed Mater Res Part A 2003;67:637–640.

75. Hiles HJED. An investigation of the long-term bioactivity of endogenous growth factor in OASIS Wound Matrix. J Wound Care 2005;14:23.

76. Hodde J, Record R, Liang H, Badylak S. Vascular endothelial growth factor in porcine-derived extracellular matrix. Endothelium 2001;8:11–24.

77. Brown B, Lindberg K, Reing J, Stolz DB, Badylak SF. The basement membrane component of biologic scaffolds derived from extracellular matrix. Tissue Eng 2006; 12:519–526.

78. Hodde J, Record R, Tullius R, Badylak S. Fibronectin peptides mediate HMEC adhesion to porcine-derived extracellular matrix. Biomaterials 2002;23:1841–1848.

79. Hodde JP, Badylak SF, Brightman AO, Voytik-Harbin SL. Glycosaminoglycan content of small intestinal submucosa: a bioscaffold for tissue replacement. Tissue Eng 1996;2:209–217.

80. Huleihel L, Bartolacci J, Dziki JL, Vorobyov T, Arnold B, Scarratt M, Pineda Molina C, LoPresti S, Brown B, Badylak SF. Matrix bound nanovesicles recapitulate extracellular matrix effects on macrophage phenotype. Tissue Eng Part A 2017. Epub ahead of print.

81. Huleihel L, Hussey GS, Naranjo JD, Zhang L, Dziki JL, Turner NJ, Stolz DB, Badylak SF. Matrix-bound nanovesicles within ECM bioscaffolds. Sci Adv 2016; 2:e1600502.

82. Sellaro TL, Ravindra AK, Stolz DB, Badylak SF. Maintenance of hepatic sinusoidal endothelial cell phenotype in vitro using organ-specific extracellular matrix scaffolds. Tissue Eng 2007;13:2301–2310.

83. Sellaro TL, Ranade A, Faulk DM, McCabe GP, Dorko K, Badylak SF, Strom SC. Maintenance of human hepatocyte function in vitro by liver-derived extracellular matrix gels. Tissue Eng Part A 2010; 16:1075–1082.

84. Allen RA, Selz LM, Jiang H, Kasick RT, Sellaro TL, Badylak SF, Ogilvie JB. Adrenal extracellular matrix scaffolds support adrenocortical cell proliferation and function in vitro. Tissue Eng Part A 2010; 16:3363–3374.

85. French KM, Boopathy AV, DeQuach JA, Chingozha L, Lu H, Christman KL, Davis ME. A naturally derived cardiac extracellular matrix enhances cardiac progenitor cell behavior in vitro. Acta Biomater 2012; 8:4357–4364.

86. Zhang Y, He Y, Bharadwaj S, Hammam N, Carnegie K, Myers R, Atala A, Van Dyke M. Tissue-specific extracellular matrix coatings for the promotion of cell proliferation and maintenance of cell phenotype. Biomaterials 2009;30:4021–4028.

87. Cortiella J, Niles J, Cantu A, Brettler A, Pham A, Vargas G, Winston S, Wang J, Walls S, Nichols JE. Influence of acellular natural lung matrix on murine embryonic stem cell differentiation and tissue formation. Tissue Eng Part A 2010;16:2565–2580.

88. Brennan EP, Tang XH, Stewart-Akers AM, Gudas LJ, Badylak SF. Chemoattractant activity of degradation products of fetal and adult skin extracellular matrix for keratinocyte progenitor cells. J Tissue Regen Med 2008;2:491–498.

89. Crapo PM, Medberry CJ, Reing JE, Tottey S, van der Merwe Y, Jones KE, Badylak SF. Biologic scaffolds composed of central nervous system extracellular matrix. Biomaterials 2012;33:3539–3547.

90. Medberry CJ, Crapo PM, Siu BF, Carruthers CA, Wolf MT, Nagarkar SP, Agrawal V, Jones KE, Kelly J, Johnson SA, Velankar SS, Watkins SC, Modo M, Badylak SF. Hydrogels derived from central nervous system extracellular matrix. Biomaterials 2013; 34:1033–1040.

91. Wolf MT, Daly KA, Reing JE, Badylak SF. Biologic scaffold composed of skeletal muscle extracellular matrix. Biomaterials 2012;33:2916–2925.

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

93. Seif-Naraghi SB, Singelyn JM, Salvatore MA, Osborn KG, Wang JJ, Sampat U, Kwan OL, Strachan GM, Wong J, Schup-Magoffin PJ, Braden RL, Bartels K, DeQuach JA, Preul M, Kinsey AM, DeMaria AN, Dib N, Christman KL. Safety and efficacy of an injectable extracellular matrix hydrogel for treating myocardial infarction. Sci Transl Med 2013;5:173ra25.

94. Keane TJ, DeWARD A, Londono R, Saldin LT, Castleton AA, Carey L, Nieponice A, Lagasse E, Badylak SF. Tissue-specific effects of esophageal extracellular matrix. Tissue Eng Part A 2015;21:2293–2300.

95. Hu C, Li L. In vitro culture of isolated primary hepatocytes and stem cell-derived hepatocyte-like cells for liver regeneration. Protein Cell 2015;6:562–574.

96. Chopra DP, Dombkowski AA, Stemmer PM, Parker GC. Intestinal epithelial cells in vitro. Stem Cells Dev 2010; 19:131–142.

97. Ozeki M, Narita Y, Kagami H, Ohmiya N, Itoh A, Hirooka Y, Niwa Y, Ueda M, Goto H. Evaluation of decellularized esophagus as a scaffold for cultured esophageal epithelial cells. J Biomed Mater Res A 2006; 79:771–778.

98. Loneker AE, Faulk DM, Hussey GS, D’Amore A, Badylak SF. Solubilized liver extracellular matrix maintains primary rat hepatocyte phenotype in-vitro. J Biomed Mater Res A 2016;104:1846–1847.

99. Gao R, Wu W, Xiang J, Lv Y, Zheng X, Chen Q, Wang H, Wang B, Liu Z, Ma F. Hepatocyte culture in autologous decellularized splenic matrix. Organogenesis 2015; 11:16–29.

100. Zheng X-L, Xiang J-X, Wu W-Q, Wang B, Liu W, Gao R, Dong D-H, Lv Y. Using a decellularized splenic matrix as a 3D scaffold for hepatocyte cultivation in vitro: a preliminary trial. Biomed Mater 2015;10:045023.

101. Ben-Ze’ev A, Robinson GS, Bucher N, Farmer SR. Cell-cell and cell-matrix interactions differentially regulate the expression of hepatic and cytoskeletal genes in primary cultures of rat hepatocytes. Proc Natl Acad Sci U S A 1988;85:2161–2165.

102. Schweinlin M, Wilhelm S, Schwedhelm I, Hansmann J, Rietscher R, Jurowich C, Walles H, Metzger M. Development of an advanced primary human in vitro model of the small intestine. Tissue Eng Part C Methods 2016; 22:873–883.

103. Patil PB, Chougule PB, Kumar VK, Almström S, Bäckdahl H, Banerjee D, Herlenius G, Olausson M, Sumitrnan-Holgersson S. Recellularization of acellular human small intestine using bone marrow stem cells. Stem Cells Transl Med 2013;2:307–315.

104. Wang B, Jakus AE, Baptista PM, Soker S, Soto-Gutierrez A, Abecassis MM, Shah RN, Wertheim JA. Functional maturation of induced pluripotent stem cell hepatocytes in extracellular matrix—a comparative analysis of bioartificial liver microenvironments. Stem Cells Transl Med 2016;5:1257–1267.

105. Zhang X, Dong J. Direct comparison of different coating matrix on the hepatic differentiation from adipose-derived stem cells. Biochem Biophys Res Commun 2015;456:938–944.

106. Bao J, Wu Q, Wang Y, Li Y, Li L, Chen F, Wu X, Xie M, Bu H. Enhanced hepatic differentiation of rat bone marrow-derived mesenchymal stem cells in spheroidal aggregate culture on a decellularized liver scaffold. Int J Mol Med 2016;38:457–465.

107. Park K-M, Hussein KH, Hong S-H, Ahn C, Yang S-R, Park S-M, Kweon O-K, Kim B-M, Woo H-M. Decellularized liver extracellular matrix as promising tools for transplantable bioengineered liver promotes hepatic lineage commitments of induced pluripotent stem cells. Tissue Eng Part A 2016;22:449–460.

108. Romero-López M, Trinh AL, Sobrino A, Hatch MM, Keating MT, Fimbres C, Lewis DE, Gershon PD, Botvinick EL, Digman M. Recapitulating the human tumor microenvironment: colon tumor-derived extracellular matrix promotes angiogenesis and tumor cell growth. Biomaterials 2017;116:118–129.

109. Nietzsche S, Baur F, Sieber S, Hansmann J, Schwarz T, Stoffer C, Hänfer H, Gasser M, Waaga-Gasser AM, Walles H. Mimicking metastases including tumor stroma: a new technique to generate a three-dimensional colorectal cancer model based on a biological decellularized intestinal scaffold. Tissue Eng Part C Methods 2016;22:621–635.

110. Hussein KH, Park KM, Ghim JH, Yang SR, Woo HM. Three dimensional culture of HepG2 liver cells on a rat decellularized liver matrix for pharmacological studies. J Biomed Mater Res Part B Appl Biomater 2016;104:263–273.

111. Tarazona N, Gambardella V, Huerta M, Roselló S, Cervantes A. Personalised treatment in gastric cancer: myth or reality? Curr Oncol Rep 2016;18:41.

112. Orditura M, Galizia G, Sforza V, Gambardella V, Fabozzi A, Laterza MM, Andreozzi F, Ventriglia J, Savastano B, Mabilia A. Treatment of gastric cancer. World J Gastroenterol 2014;20:1635.

113. Blondi A, Persiani R, Cananzi F, Zoccali M, Vigorita V, Tufo A, D’Ugo D. R0 resection in the treatment of gastric cancer: room for improvement. World J Gastroenterol 2010;16:3358.

114. Ichikawa D, Kurioka H, Yamaguchi T, Koike H, Okamoto K, Otsuji E, Shirou K, Shioka Y, Ikeda E, Mutoh F. Postoperative complications following gastrectomy for gastric cancer during the last decade. Hepatogastroenterology 2004;51:613–617.

115. Runge TM, Abrams JA, Shaheen NJ. Epidemiology of Barrett’s esophagus and esophageal adenocarcinoma. Gastroenterol Clin North Am 2015;44:203–231.

116. Londono R, Badylak SF. Regenerative medicine strategies for esophageal repair. Tissue Eng Part B Rev 2015; 21:393–410.

117. Londono R, Jobe BA, Hoppe T, Badylak SF. Esophagus and regenerative medicine. World J Gastroenterol 2012; 18:6894–6899.

118. de la Fuente SG, Gottfried MR, Lawson DC, Harris MB, Mantyh CR, Pappas TN. Evaluation of porcine-derived small intestine submucosa as a biodegradable graft for gastrointestinal healing. J Gastrointest Surg 2003; 7:96–101.
119. Ueno T, de la Fuente SG, Abdel-Wahab Ol, Takahashi T, Gottfried M, Harris MB, Tatewaki M, Uemura K, Lawson DC, Mantyhr CR, Pappas TN. Functional evaluation of the grafted wall with porcine-derived small intestinal submucosa (SIS) to a stomach defect in rats. Surgery 2007;142:376–383.

120. Nishimura T, Ueno T, Nakatsu H, Oga A, Kobayashi S, Oka M. In vivo motility evaluation of the grafted gastric wall with small intestinal submucosa. Tissue Eng Part A 2010;16:1761–1768.

121. Lopes MF, Cabrita A, Ilharco J, Pessa P, Patricio J. Grafts of porcine intestinal submucosa for repair of visceral and abdominal esophageal defects in the rat. J Invest Surg 2006;19:105–111.

122. O’Keefe SJ, Buchman AL, Fishbein TM, Jeejeebhoy KN, Jeppesen PB, Shaffer J. Short bowel syndrome and intestinal failure: consensus definitions and overview. Clin Gastroenterol Hepatol 2006;4:6–10.

123. Wang ZQ, Watanabe Y, Toki A. Experimental assessment of small intestinal submucosa as a small bowel graft in a rat model. J Pediatr Surg 2003;38:1596–1601.

124. Demirbilek S, Kanmaz T, Ozardali I, Edali MN, Yucesan S. Using porcine small intestinal submucosa in intestinal regeneration. Pediatr Surg Int 2003;19:588–592.

125. Wang ZQ, Watanabe Y, Noda T, Yoshida A, Oyama T, Toki A. Morphologic evaluation of regenerated small bowel by small intestinal submucosa. J Pediatr Surg 2005;40:1896–1902.

126. Ansaloni L, Bonasoni P, Cambrini P, Catena F, De Cataldis A, Gagliardi S, Gazzotti F, Peruzzi S, Santini D, Taffurelli M. Experimental evaluation of Surgisis as scaffold for neonointestine regeneration in a rat model. Transplant Proc 2006;38:1844–1848.

127. Hanauer SB. Inflammatory bowel disease: epidemiology, pathogenesis, and therapeutic opportunities. Inflamm Bowel Dis 2006;12(Suppl 1):S3–S9.

128. Molodecky NA, Soon S, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 2012;142:46–54, e42.

129. De Souza HS, Fiocchi C. Immunopathogenesis of IBD: current state of the art. Nat Rev Gastroenterol Hepatol 2016;13:13.

130. Shimshoni E, Yablecovitch D, Baram L, Dotan I, Sagi I. ECM remodelling in IBD: innocent bystander or partner in crime? The emerging role of extracellular molecular events in sustaining intestinal inflammation. Gut 2015;64:367–372.

131. Rieder F, Fiocchi C. Intestinal fibrosis in IBD—a dynamic, multifactorial process. Nat Rev Gastroenterol Hepatol 2009;6:228–235.

132. Rieder F, de Bruyn JR, Pham BT, Katsanos K, Annese V, Higgins PD, Magro F, Dotan I. Results of the 4th scientific workshop of the ECCO (group II): markers of intestinal fibrosis in inflammatory bowel disease. J Crohns Colitis 2014;8:1166–1178.

133. Latella G, Rogler G, Bamias G, Breynaert C, Fiorholmen J, Pellino G, Reif S, Silvia S, Lawrance IC. Results of the 4th scientific workshop of the ECCO (I): pathophysiology of intestinal fibrosis in IBD. J Crohns Colitis 2014;8:1147–1165.

134. Sarzo G, Finco C, Mungo B, Gruppo M, Cadrobbi R, Polese L. Anal fistula repair with acellular dermal matrix plug: description of a novel technique and early results. J Surg 2013;1:1–6.

135. Han JG, Xu HM, Song WL, Jin ML, Gao JS, Wang ZJ, Yang XQ. Histologic analysis of acellular dermal matrix in the treatment of anal fistula in an animal model. J Am Coll Surg 2009;208:1099–1106.

136. Keane TJ, Dziki J, Sobieski E, Smoulder A, Castleton A, Turner N, White LJ, Badyak SF. Restoring mucosal barrier function and modifying macrophage phenotype with an extracellular matrix hydrgel: potential therapy for ulcerative colitis. J Crohns Colitis 2017;11:360–368.

137. Sadtler K, Estrellas K, Allen BW, Wolf MT, Fan H, Tam AJ, Patel CH, Luber BS, Wang H, Wagner KR. Developing a pro-regenerative biomaterial scaffold microenvironment requires T helper 2 cells. Science 2016;352:366–370.

138. Sadtler K, Sommerfeld SD, Wolf MT, Wang X, Majumdar S, Chung L, Kelkar DS, Pandey A, Elisseefh JH. Proteomic composition and immunomodulatory properties of urinary bladder matrix scaffolds in homeostasis and injury. Semin Immunol 2017;29:14–23.

139. Sicari BM, Dziki JL, Siu BF, Medberry CJ, Dearth CL, Badyak SF. The promotion of a constructive macrophage phenotype by solubilized extracellular matrix. Biomaterials 2014;35:8605–8612.