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The Engineered Gut: Use of Stem Cells and Tissue Engineering to Study Physiological Mechanisms and Disease Processes

Gut bioengineering strategies for regenerative medicine

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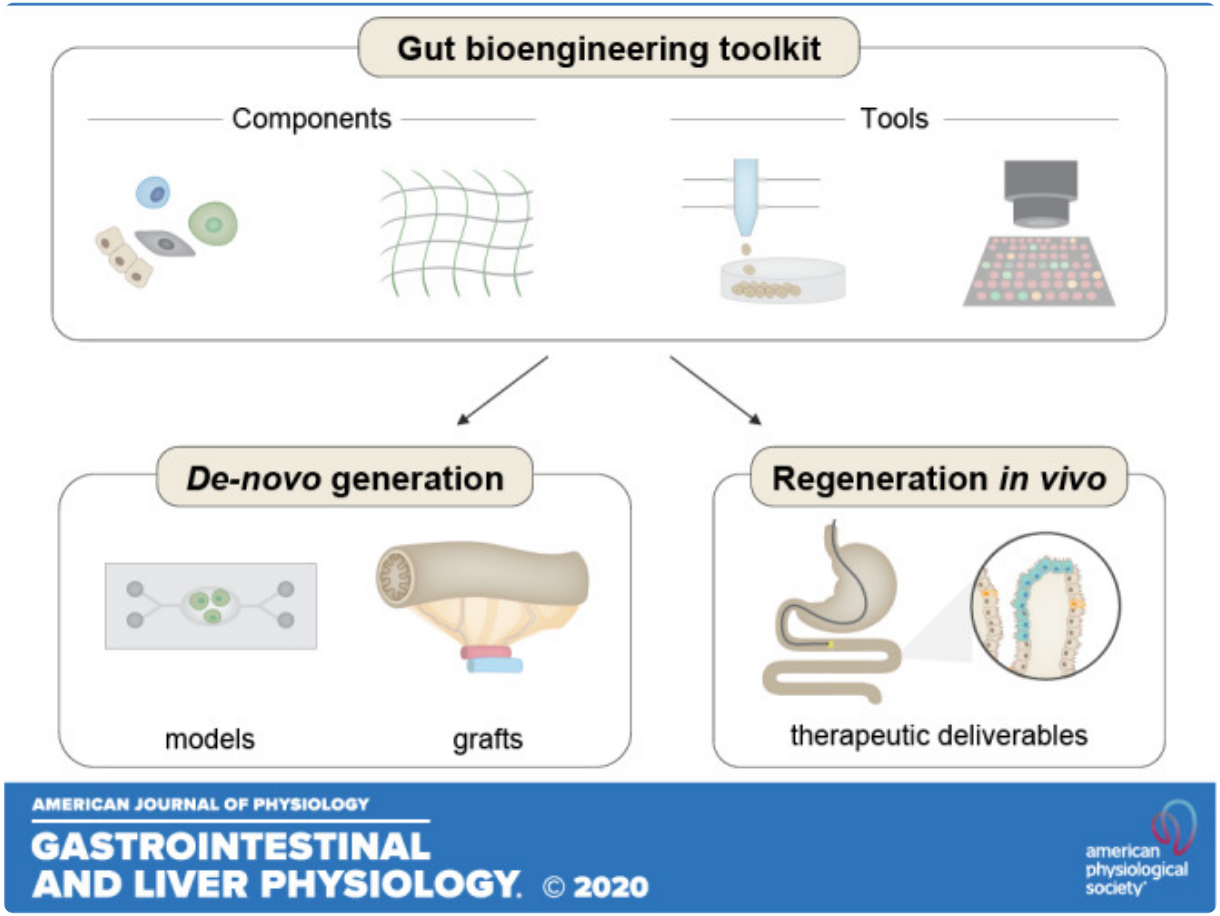
Abstract



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Abstract

Gastrointestinal disease burden continues to rise in the United States and worldwide. The development of bioengineering strategies to model gut injury or disease and to reestablish functional gut tissue could expand therapeutic options and improve clinical outcomes. Current approaches leverage a rapidly evolving gut bioengineering toolkit aimed at *1*) de novo generation of gutlike tissues at multiple scales for microtissue models or implantable grafts and *2*) regeneration of functional gut in vivo. Although significant progress has been made in intestinal organoid cultures and engineered tissues, development of predictive in vitro models and effective regenerative therapies remains challenging. In this review, we survey emerging bioengineering tools and recent methodological advances to identify current challenges and future opportunities in gut bioengineering for disease modeling and regenerative medicine.

INTRODUCTION

Gastrointestinal disease affects ~70 million people, and causes over 230,000 deaths every year in the United States alone (1). Annual health care costs exceed $136 billion (2). To alleviate this immense socioeconomic burden, gut bioengineering aims to develop predictive models and regenerative therapies for treating intestinal injury and disease.

Structure–function relationships in gut physiology are highly complex and present significant biological and engineering challenges. The intestinal tract performs an array of vital functions, serving as a barrier to the external environment and supporting propulsion, digestion, absorption, secretion, excretion, immunity, tolerance, and communication with the central nervous system (3). The intestinal surface has cryptic architecture that creates a large surface area roughly the size of a tennis court (~250 m^2) (4). The intestinal wall comprises mucosal, submucosal, and muscular layers with integrated vascular, muscular, and nervous structures that coordinate gut functions. Tissue morphology varies regionally and corresponds to specialized functions along the cephalocaudal and crypt–villus axes (3).

Diverse cell types from all three germ layers reside and interact in different regions of the intestine (5). The mucosa serves as a selective absorption barrier lined with an active monolayer of epithelial cells that includes intestinal stem cells and at least six other cell types [enterocytes, enteroendocrine cells, goblet cells, microfold (M) cells, Paneth cells, and tuft cells] (6). The submucosa contains smooth muscle (myocytes, myofibroblasts), endovascular networks (endothelial cells, pericytes), a nervous plexus (neurons, glial cells), and a reservoir of resident immune cells (dendritic cells, macrophages, plasma cells, T cells) that collectively coordinate transport, communication, and immune functions (3). The gut also hosts a dynamic microbiome comprised of trillions of commensal microbes. Notably, the spectrum of intestinal disease includes a wide range of disorders that may involve loss of intestinal function resulting from inflammation, cellular dysregulation, aberrant changes to the extracellular matrix, and microbiome dysbiosis (7, 8). Development of therapeutic deliverables such as cell-derived factors and patient-specific cells could offer personalized alternatives to treat intestinal diseases through regeneration in vivo.

In this review, we focus on bioengineering of the small intestine and colon and distinguish two major aims: *1*) in vitro generation of gutlike tissues at multiple scales from microtissue models to implantable grafts and *2*) regeneration of functional gut in vivo (Fig. 1). We survey recently established and emerging tools, highlight key technical advances, and identify current challenges and future opportunities in gut bioengineering for regenerative medicine.

GUT BIOENGINEERING TOOLKIT

The gut bioengineering toolkit (Fig. 2) comprises a continually evolving set of components and tools used for bioengineering the gut.

Cells

Immortalized cells (e.g., Caco-2, HT-29, T84) offer practical utility for absorption, transport, metabolism (9, 10), and coculture (11, 12) studies but have low physiological relevance and translational potential due to their malignant origin. Primary cells offer higher physiological relevance but often have limited viability, proliferation, and phenotypic maintenance ex vivo. Nevertheless, culture protocols have been established for a variety of primary intestinal cell types,

including intestinal epithelial cells (13), intestinal microvascular endothelial cells (14), enteric myofibroblasts, enteric neurons, enteric glia (15), smooth muscle cells, interstitial cells of Cajal (16), and monocyte-derived macrophages (17).

Organoids, multicellular tissue-mimicking structures, recapitulate many of the molecular, morphological, and functional characteristics of tissues and represent a significant advance beyond cultivation of primary or immortalized cell types in mono- or cocultures. Since the term "organoids" has been used generically for different types of organotypic structures in vitro, clearly defined and standardized terminology is needed. Here we define organoids as "heterotypic structures that *1*) can be reproducibly generated from single cells or cell clusters derived from somatic tissue or pluripotent stem cells, *2*) self-assemble through cell–cell and cell–matrix interactions, and *3*) share features of the original tissue" (18). Furthermore, we distinguish intestinal organoids derived from intestinal tissue ("crypt-derived organoids") and induced pluripotent stem cells ("iPS organoids"). Through directed differentiation via mitogens, morphogens, and cytokines, stem cells (intestinal crypt or iPS) generate three-dimensional (3-D), luminal structures with budding outgrowths that resemble intestinal crypts and contain intestinal stem cells and other differentiated intestinal cell types. Organization along the cryptlike outgrowth axis is similar to that of intestinal crypts: self-renewing leucine-rich repeat-containing G protein receptor 5 ($Lgr5^+$) intestinal stem cells and Paneth cells localize at the base, and differentiated enterocytes advance along the outgrowth from the base toward the lumen (19).

The advantages and disadvantages of crypt-derived and iPS organoids have been extensively reviewed elsewhere (20–22). Regardless of derivation, organoids have closed lumens that restrict access to the apical surface, retain secretions and sloughed epithelial cells, and preclude incorporation of mechanical stimuli (e.g., hydrodynamic flow, shear stress, mechanical strain). Organoids also lack a vascular network and intestinal tissue–tissue interfaces. Despite these limitations, crypt-derived organoids are highly relevant and useful for modeling normal or diseased intestinal tissue in vitro (23–27), especially physiological processes involving intestinal epithelium. Stable maintenance with serial passaging is achievable for longer than 1 yr, enabling significant expansion of diseased intestinal epithelium from small tissue biopsies—a major advantage over prior tissue culture methodologies. Crypt-derived organoids also recapitulate different absorptive and digestive functions according to their origin along the intestinal proximal–distal axis (28). In contrast, iPS cells derived from a sample of blood or skin can be used to generate intestinal iPS organoids (29–31), enabling modeling of intestinal development and disease and potential treatment of intestinal disorders with personalized cell sources. For applications in intestinal repair and regeneration, iPS organoids offer several advantages, including *1*) patient specificity for target identification, drug testing, or cell-based therapies and *2*) unlimited proliferative potential for scalable biomanufacturing of therapeutic cells (32). Notably, iPS organoids display more fetuslike phenotype and contain mesenchymal cell populations that may support or enhance integration and repair in damaged or diseased intestinal tissue following therapeutic delivery (33). Widespread reliance on Matrigel to generate intestinal organoids in vitro precludes clinical translation because of the diseased origin of tumor-derived substrates, prompting the need for alternative substrates with translational potential (e.g., synthetic or natural matrix materials).

A variety of immune cells, including B cells, dendritic cells, natural killer cells, plasma cells, subepithelial macrophages, and T cells, also reside in the intestine and are critical for gut homeostasis and response to injury. Resident immune cells have developed specialized phenotypes to support normal intestinal function, moderate commensal gut flora, and prevent pathogenic dysregulation (34). Examples of specialized phenotype and function of resident intestinal immune cells include B cell and plasma cell coordination to maintain the interface between intestinal flora and the epithelial surface, dendritic cell homing to Peyer's patches to actively monitor bacterial growth, and subepithelial macrophage response, which despite retention of phagocytic activity is not proinflammatory in the presence of normal intestinal bacteria. Integrated models of intestinal epithelial and mucosal immune populations, including lymphocytes, macrophages, and neutrophils, have been developed to study gut immunological interactions in vitro (17, 35, 36).

As the human gut contains >1,900 bacterial species (34), intestinal microtissue models increasingly incorporate immune cells (e.g., $CD4^+$ T cells) and both commensal (e.g., *Lactobacillus gasseri*) (37) and pathogenic (38) [e.g., *Salmonella typhimurium, Escherichia coli* (enteropathogenic), *Listeria*, *Pseudomonas aeruginosa*, *Yersinia pseudotuberculosis*] microbes to investigate normal and pathological cell–microbe interactions. Disruption of immune–microbiome homeostasis can contribute to development of inflammatory bowel disease (IBD), a broad classification of disorders characterized by severe inflammation of the intestine (e.g., Crohn's disease, ulcerative colitis). Microtissue systems that incorporate relevant bacterial populations and immune cells can be used to model inflammatory bowel disease and other intestinal diseases and support discovery-driven multi-omics investigations of host–microbiome interactions (39).

Biomaterials

Biomaterials provide structural, mechanical, and biochemical cues to induce or support cell phenotype and function. A variety of biomaterials are used in gut bioengineering, including synthetic and natural polymers, native extracellular matrix, and matrix components in compatible formats such as hydrogels (hydrated networks of hydrophilic polymers). For cell culture applications, biomaterial scaffolds offer numerous advantages over traditional two-dimensional substrates, including the ability to support cells in three-dimensional environments with stiffnesses similar to those of human tissues. Although selection criteria for biomaterials vary by application and technical objective, primary considerations should include mechanical stiffness, chemical composition, cell compatibility (e.g., adhesion, viability), and quality [e.g., research grade, Good Manufacturing Practices (GMP) grade]. Biomaterials can be generally classified as synthetic, natural, or hybrid based on origin.

Synthetic materials are advantageous because of their low cost, tunability (e.g., pore size, stiffness, degradability), and high availability, lot-to-lot consistency, and scalability. Synthetic biomaterials including polyethylene glycol (PEG), poly(lactic-co-glycolic) acid (PLGA), and polyethylene-co-vinyl-acetate (PEVA) have been applied across gut bioengineering to culture organoids, mimic crypt–villus architecture, and deliver therapeutic cells and organoids to the gut (40–42). Low cell attachment can often be improved by decorating synthetic polymers with functional groups (43). Nevertheless, synthetic materials have minimal physiological relevance, lack proteocleavable sites, and can potentially yield harmful degradation products.

Natural materials are highly biocompatible and may contain intrinsic molecules that promote cell attachment. Fibrin-based hydrogels have been used for gut bioengineering applications and can be formulated within the appropriate stiffness range for intestinal cells (44). Other natural polymers have demonstrated utility in gut bioengineering applications, including alginate (45), chitosan (46), and silk (47–49), and have been used to generate scaffolds that supported multiple intestinal cell types in physiological three-dimensional constructs. Natural materials are attractive because of their biological origin, high availability, and biocompatibility but are often not endogenous to the human body and have limited ability to reproduce complex physicochemical features of the native intestinal environment.

Extracellular matrix (ECM) components, which are widely used for in vitro models, contain natural cell adhesion sites such as Arg-Gly-Asp (RGD) peptides. Although singular ECM proteins such as collagen type I, fibronectin, and hyaluronic acid are abundant in the intestines, use of individual ECM proteins is relatively simplistic and fails to account for the biological complexity of intestinal tissue. Basement membrane extracts such as Matrigel have been used to establish intestinal organoids but are tumor derived and have no translational potential. Synthetic alternatives to basement membrane extracts have also been developed (40, 43), but they lack important tissue-specific signals. Intestinal ECM can be isolated from animal or human tissues by using decellularization to remove cells while maintaining original tissue structure, molecular composition, and biomechanics (50, 51). Intestinal ECM materials can be prepared in various

formats, including decellularized tissue scaffolds and reconstituted hydrogels, which offer a supportive physiological milieu with high fidelity to the human intestinal environment. Proteomic analyses of intestinal ECM reveal complex tissue-specific compositions with components that provide bioactive signaling for cell attachment, proliferation, differentiation, and maturation. More than 100 intestinal ECM proteins have been identified, including multiple collagens (type I, II, III, IV, V, VI, XII, XIV, XXI) and glycoproteins (fibrillins, laminins) (52). Furthermore, over 600 exosomal proteins have been identified, suggesting that ECM provides a supportive network for cells and serves as a storage reservoir for soluble factors. Intestinal ECM hydrogels can be prepared at appropriate stiffnesses (similar to Matrigel), which support intestinal cell and organoid development. Because of proteomic and viscoelastic profiles similar to endodermal tissue with enrichment of key ECM proteins, intestinal ECM hydrogels supported cell clustering within the derivative germ layer during organoid formation (53), suggesting that ECM functions as both a physical scaffolding and a fundamental regulator of tissue specification.

Hybrid materials combine natural–natural or synthetic–natural materials to obtain novel sets of target attributes. For example, hybrid polyacrylamide and intestinal tissue-derived ECM hydrogel produced a tunable gel with physiologically relevant signals that supported intestinal organoid cultures (41).

Bioactive Factors

Proteins that activate or inhibit cellular pathways (e.g., growth factors, cytokines) are used to maintain or differentiate intestinal stem cells (e.g., EGF, Noggin, R-spondin) or recreate pathological conditions in vitro (47, 54–56). Small molecules that function as inhibitors of BMP (LDN193189) or GSK-3 (CHIR99021) are increasingly used in conjunction with chemically defined, serum-free culture media (57). Cell-derived paracrine factors, in particular cell-secreted vesicles (i.e., exosomes) that contain regulatory microRNAs, can be harnessed as therapeutic deliverables with innate homing and immunomodulatory effects (58).

Biofabrication

Built on the principles of additive manufacturing, biofabrication enables assembly of building block components—cells, biomaterials, bioactive factors—into bioengineered systems (e.g., bioengineered tissue constructs) with increasing control and spatial resolution to emulate structural and functional complexity of intestinal microenvironments. Electrospinning is a common scaffold fabrication technique that applies high electrical voltage to molten or solution biomaterial polymers to obtain thin fibers with defined or random alignment (59, 60). Bioprinting enables patterning and assembly of cells and biomaterials into predefined two- and three-dimensional structures. Printable bioinks comprised of synthetic or natural polymers, matrix proteins, or their combinations are mixed with cells during or after printing (61, 62).

Bioreactors

In gut bioengineering, bioreactors are used to recapitulate controlled, dynamic parameters that match the physiological intestinal environment and provide stimuli including perfusion (flow), oxygenation (mass transport), shear stress (propulsion), and mechanical stress and strain (peristalsis). Controlled environments at multiple scales, from microfluidic chips to whole organ bioreactors, investigate normal or pathological physiology or support the development of implantable grafts. Biochemical and biophysical signals can stimulate, regulate, and maintain mature phenotypes in bioreactors. Porous scaffolds and microfluidic channels support nutrient transport, cell viability, proliferation, differentiation, tissue assembly, and overall function (63–65).

Bioanalytics

Mathematical in silico models informed by biological data can approximate normal and diseased intestinal motility and electrophysiology at the cell and tissue levels (66). Computational fluid dynamics and mass transport models can predict how mixing, shear stress, and biochemical gradients affect cells and tissues over time. Real-time monitoring of bioengineered tissues such as online sensors of chemical gradients and metabolites, noninvasive imaging techniques, and electrical resistance measurements can inform the development, application, and quality assessment of model systems. Notably, multi-omics (e.g., transcriptomics, proteomics, metabolomics) and single-cell analysis can elucidate comprehensive molecular profiles and pathways in bioengineered gut systems. Pharmacogenomics and microbiomics have revealed high interpatient variability in gut microbes and can help predict disease susceptibility and drug efficacy in personalized medicine (67, 68).

DE NOVO GENERATION OF BIOENGINEERED GUT MODELS AND GRAFTS

High-fidelity approaches that recapitulate key features of the native gut milieu are needed for de novo generation of gutlike tissues for intestinal disease modeling or implantable grafts. Critical design considerations include cellular diversity, tissue-specific biochemical and mechanical cues, morphology, topography, and patient specificity.

The intestine is home to highly diverse populations of epithelial, stromal, neural, and immune cells and microbes that together regulate the intestinal environment. In vitro intestinal models need to support multiple mature, phenotypically stable cell types with different microenvironmental requirements in appropriate spatial configurations. Localized biochemical signals that permit human cells and aerobic and anaerobic microbes to coexist include intestinal extracellular matrix-bound and soluble growth factors, oxygen gradients, and mucus that lubricates and protects the intestinal wall. In the healthy gut, enzymes, hormones, chemoreceptors, and antimicrobial peptides enable normal digestive functions but may be disrupted by aberrant intestinal cell signaling or inflammatory mediators in dysfunctional or diseased gut. Micro- and macroscale mechanical signals include matrix stiffness, mixing of intestinal contents, shear stresses on the apical epithelium due to flow, and cyclical stresses and strains due to peristalsis (69). Maintenance of the intestinal mucosa involves dynamic regulation of $Lgr5^+$ intestinal stem cells that proliferate and differentiate into mature absorptive cell types along the crypt–villus axis (56).

Recent insights into the gut microbiome reveal a high degree of patient-to-patient variability dependent on genetic, environmental, and dietary factors (70). In vitro systems that combine patient-specific intestinal cells, microbiota, and tissue-derived intestinal ECM may be valuable for studying disease, developing effective therapies, and generating nonimmunogenic transplantable grafts. Beyond biomimetic design criteria, practical considerations for experimentation include scalable production of standardized gut tissues for high-throughput studies, maintenance of cell phenotype in prolonged cultures (weeks to months), and noninvasive multimodal assessments of cell function.

Bioengineered Gut Models

Tissue bioreactors and organ-on-chip technologies allow for fine control over culture conditions (e.g., spatiotemporal distribution of cells and factors, fluid dynamics, mechanical stress) but still largely rely on tumor-derived, immortalized epithelial cell lines that are less physiological than primary stem cells used to form organoids. Efforts to provide physiological growth conditions similar to the regulatory niche in vivo include 3-D printed gut topographies (9), intestinal

ECM substrates (71), mechanical and morphogen gradients (72), pulsatile peristaltic movement (49), and coculture with enteric neural tissue (73). Together, these advances have led to development of gut tissues capable of epithelial barrier formation, microvilli assembly, and mucus secretion. Recent efforts have focused on integrating biologically complex organoid cell populations with tightly controlled mechanical environments by exposing organoids to uniaxial deformation, shear flow, and microvascular perfusion, to recapitulate transcriptional, morphological, and functional capabilities of the native gut (14, 69).

The role of the microbiome in maintaining gut homeostasis motivates epithelial and microbial cocultures for modeling human gut pathophysiology. However, incorporating commensal microbes while avoiding bacterial overgrowth presents a major bioengineering challenge that typically limits culture times to <1 day (74). To extend culture times, microfluidic gut-on-chip platforms offer spatiotemporal regulation of luminal and basal compartments with differential oxygen tensions to maintain self-sustaining oxygen gradients in the cellular niche (39, 75). Coculture of aerobic and anaerobic microorganisms and intestinal epithelium for up to 5 days was achieved by using a human–microbiota coculture system with gutlike epithelial barrier function, mucus secretion, and nutrient digestion (76).

Applications of gut tissue models include studies of nutrition (77), infectious disease (78), radiation injury (39), and inflammatory diseases (47). Cessation of peristalsis-like movement and luminal flow in vitro may lead to bacterial overgrowth and inflammation, similar to inflammatory pathologies in vivo (79). As the gut is increasingly recognized for interactions with other organs, impressive efforts have been made to develop multiorgan in vitro model systems to study gut–brain (80), gut–immune (77), gut–liver (47, 54), and other gut–organ interactions (81, 82). In the future, clever combinations of established and emerging components and tools in the gut bioengineering toolkit (Fig. 2) will enable patient-specific studies of diseases such as cystic fibrosis with complex, multiorgan pathologies using integrated multiorgan “patient-on-chip” platforms.

Bioengineered Implantable Grafts

For patients with short bowel syndrome or intestinal failure, bioengineered intestine could serve as an implantable graft to restore intestinal function. Grafts suitable for implantation would ideally be lined with intestinal epithelium and smooth muscle, innervated, and vascularized to connect to host vascular supply and bowel lumen. Although grafts comprised of synthetic scaffolds have been successfully cellularized and implanted, robust vascular perfusion remains a significant challenge (42). The complexity of the mesentery vascular plexus has prompted the use of decellularized intestinal tissues as scaffolds with natural mesenteric vascular architecture as a basis for generating bioengineered intestinal grafts. Decellularized intestinal tissue scaffolds can retain the biochemical, mechanical, and morphological properties of native intestine, thereby reducing the challenge of synthesizing scaffolds with such complex features.

A humanized intestinal graft generated by repopulating decellularized rat intestine with human epithelial and endothelial cells demonstrated glucose and fatty acid absorption and vascular perfusion (83). Integrating jejunal organoids with decellularized human intestine produced grafts that matured after heterotopic transplantation and showed disaccharidase digestive activity (84). Innervated muscle constructs have also been developed from decellularized human intestinal tissue with neural progenitor and smooth muscle cells and demonstrated response to neurotransmitters and electrical field stimulation for neuromuscular replacement therapy in the gut (85). Tubular grafts comprised of chitosan scaffolds and smooth muscle and neural progenitor cells were implanted in the omentum to mature in vivo before subsequent orthotopic transplantation to the intestine (46). Although decellularized intestinal matrixes served as useful scaffolds for implantable grafts in these applications, fundamental regenerative biomanufacturing challenges remain toward the scalable production of scaffolding biomaterials and well-characterized cell types with phenotypic stability.

GUT REGENERATION IN VIVO

Endoscopic delivery of therapeutic cell-derived factors (e.g., growth factors, cytokines, exosomes), acellular biomaterials, or patient-specific cells/organoids represents nonsurgical strategies to treat intestinal diseases through personalized tissue regeneration in vivo (Fig. 1*C*). Such therapeutic modalities can stimulate endogenous repair mechanisms, modulate inflammation, reverse pathology, and/or support long-term functional recovery. Translational considerations include patient specificity, safety, immune response, long-term efficacy, and biomanufacturing.

Personalized medicine approaches study individual genetic and molecular profiles for diagnostic and therapeutic purposes. The majority of identified gut microbial genes are present in <1% of patients (86). As individual variations have functional consequences for healthy and diseased intestine, personalized medicine offers valuable insights to guide approaches for gut regeneration. Multi-omics (e.g., combination of pharmacogenomic and microbiomic data sets) may reveal individualized disease mechanisms and therapeutic targets, and autologous cells and organoids (e.g., iPS derived) may provide personalized cell therapies. For clinical translation of emerging bioengineered therapies, demonstration of a robust safety profile is required.

In vitro models of human gut and in vivo animal models can aid in screening therapies for safety, efficacy, and off-target effects. Gut is a challenging destination for exogenous therapeutic cargo because of its complex role in regulating immunostasis and precluding autoimmunity and dysbiosis (87). Regenerative medicine strategies that edit the host genome in situ by gene delivery or use autologous approaches such as iPS-derived cells/organoids and immunomodulatory mesenchymal cells can avoid immunogenicity and promote beneficial immune responses.

Achieving long-term effects with cell therapies may be challenging in the gut because the entire intestinal epithelium is replenished every 4–5 days (88). A high rate of cellular turnover presents unique challenges and opportunities for regenerative therapies. Strategies to achieve long-term therapeutic effect could lever the ubiquitous activity of intestinal stem cells that continuously proliferate to maintain the intestinal epithelium. Biomanufacturing requirements may vary by therapy, but key considerations include product formulation, purification, preservation, and quality control. Biologically sourced therapeutics (e.g., cells, matrix grafts) originate from variable sources (e.g., human, animal tissues) and therefore necessitate robust procurement and processing pipelines to ensure quality and consistency. Defining and adhering to strict in-process and quality control metrics is necessary to ensure safety and minimize lot-to-lot variability (89).

Therapeutic Cell-Derived Factors

Bioactive cell-derived factors such as growth factors, cytokines, nucleic acids, or exosomes can mediate cellular cross talk in vivo and have lower regulatory burden for therapeutic delivery than cell-based therapies. Notably, exosomes can serve as biocompatible delivery vehicles with innate homing ability to therapeutic targets (58). Compared to therapeutic cells, which involve extensive lead times for manufacturing at scale, cell-derived therapeutics can be more readily manufactured and standardized as off-the-shelf medicines.

Therapeutic Biomaterials

Acellular biomaterials can foster regenerative environments that favorably alter the inflammatory cascade to promote migration, proliferation, and differentiation of endogenous progenitor cells and provide structural support during tissue remodeling. Matrix biomaterials derived from native tissues are approved for clinical use and widely used because of their tissuelike composition, mechanics, structure, matrix-bound growth factors, and biodegradability (90). In the gut, detergent/enzyme decellularization has been used to generate intestinal biomaterial scaffolds that promote angiogenesis and retain mechanical properties critical for peristalsis and gut function (91). Alternatively, synthetic biomaterials can offer other advantages including standardization, ease of manufacturing, and long-term stability. For example, a synthetic PEG-based hydrogel was used as an injectable cell carrier to repair injured colon mucosa (40). Interaction between delivered biomaterials and the host immune system can also be harnessed to promote healing by designing materials that elicit proregenerative macrophage phenotypes (92). Therapeutic biomaterials from allogeneic and xenogeneic sources must undergo rigorous safety testing to eliminate risks of disease transmission.

Therapeutic Cells

Although biomaterials alone may provide some regenerative benefits, therapeutic cells offer sophisticated mechanisms of action that are not achievable with biomaterials or drugs. Despite evidence of efficacy, the high incidence of adverse events associated with hematopoietic stem cell delivery into the intestine has hindered their therapeutic use. In contrast, mesenchymal stromal cells (MSC) derived from bone marrow or lipoaspirates have shown broad safety profiles and been extensively evaluated for a variety of indications in the gut and other organs because of their potent immunomodulatory and anti-inflammatory properties. Currently, more than 550 MSC therapies are in clinical trials (93). Notably, despite poor engraftment and short-term retention in the gut, MSCs have demonstrated efficacy at treating Crohn's, celiac, and malabsorptive diseases, suggesting that therapeutic mechanisms of action may involve paracrine signaling. After localized cell delivery alone or in combination with a biomaterial carrier, intestinal repair may be mediated by cross talk between exogenous therapeutic cells and endogenous epithelium (94, 95). Optimal cell dosing, time course delivery, and methods for identifying patients who may be most responsive to MSC therapy remain unclear and require further investigation.

Development of in vitro methods to differentiate iPS cells into intestinal stem cells and organoids has motivated preclinical studies of intestinal stem cell-based therapies for intestinal injury and disease. Furthermore, iPS cells could provide an unlimited autologous source that avoids the adverse immune effects associated with allogeneic cells. Intestinal organoids represent particularly interesting therapeutic candidates for cell replacement (Fig. 3). In a murine model of ulcerative colitis, intestinal organoids attached to damaged colon regions, differentiated, and continued to self-renew for up to 25 wk (96,97). Intestinal organoids have also been shown to contain interstitial cells of Cajal, whose pacemaker functions are impaired or lost in gut motility disorders but may be restored by delivery of therapeutic organoids (83). Parallel advances in gene editing technologies could enable autologous corrective cell therapy for patients with monogenic or polygenic forms of intestinal disease. Interestingly, intestinal organoids derived from CRISPR-corrected cystic fibrosis iPS cells demonstrated improved function over cystic fibrosis intestinal organoids that did not undergo CRISPR editing (98).

However, therapeutic use of iPS cells prompts concerns that delivery could lead to teratoma formation, so iPS-derived cell therapies have not been investigated clinically. Engraftment and retention also remain major obstacles in intestinal cell therapies (96,97, 99). Codelivery of cells with intestinal matrix components may promote cellular attachment and retention in injured sites. New developments in biofabrication, in situ imaging, and online sensors may facilitate generation of deliverable organoids and implantable grafts and allow for real-time monitoring of cell engraftment after delivery. Among the various therapeutic approaches for intestinal regeneration, cell therapies may hold the greatest promise but still face challenging translational paths to patients because of safety, regulatory, and biomanufacturing considerations.

FUTURE PERSPECTIVES

Despite the immense challenges in gut bioengineering, emerging tools and recent advances create new opportunities for (re)generating functional gut tissues. Expanding interdisciplinary collaborations between cell biologists, microbiologists, bioengineers, gastroenterologists, immunologists, surgeons, and other domain experts will open new horizons in gut bioengineering. Efforts continue to focus on contextual understanding of intestinal and gut microbiome physiology using patient-specific multiorgan models and scalable tissue platforms for rapid deployment and high-throughput drug screening. Studies of novel infectious diseases such as COVID-19 and the development of personalized regenerative therapies call for effective interdisciplinary approaches to basic and translational studies. New gut bioengineering strategies will undoubtedly continue to emerge from the clever integration of tools, techniques, and disciplines to yield deeper insights and better treatment options for patients across the spectrum of intestinal disease.

DISCLOSURES

J.D.O. and G.V. have financial interest in Xylyx Bio, Inc., a company that markets extracellular matrix biomaterials. None of the other authors has any conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

J.D.O., M.R.P., B.A.G., and G.V. drafted manuscript; J.D.O., M.R.P., B.A.G., and G.V. edited and revised manuscript; G.V. approved final version of manuscript.

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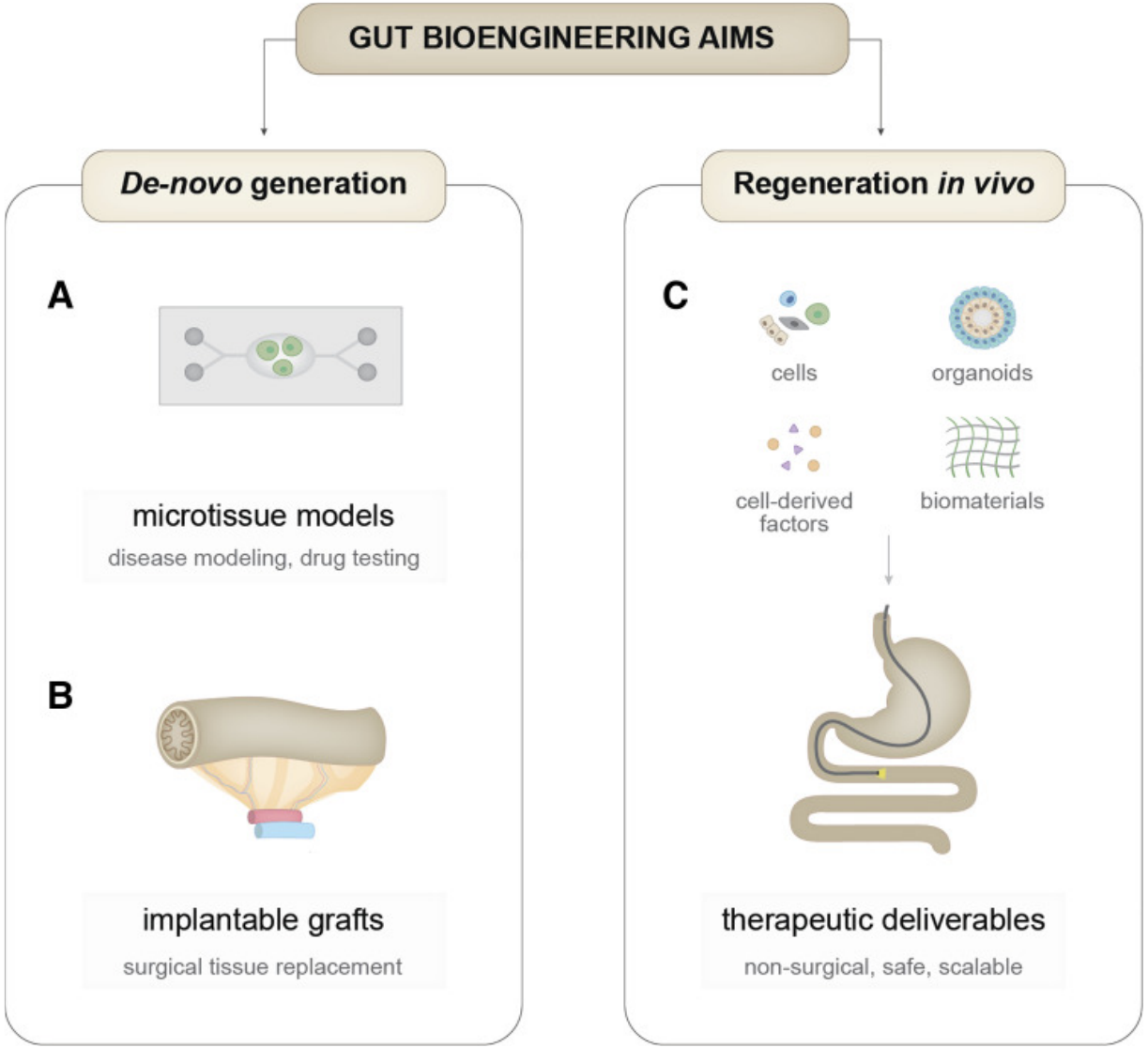
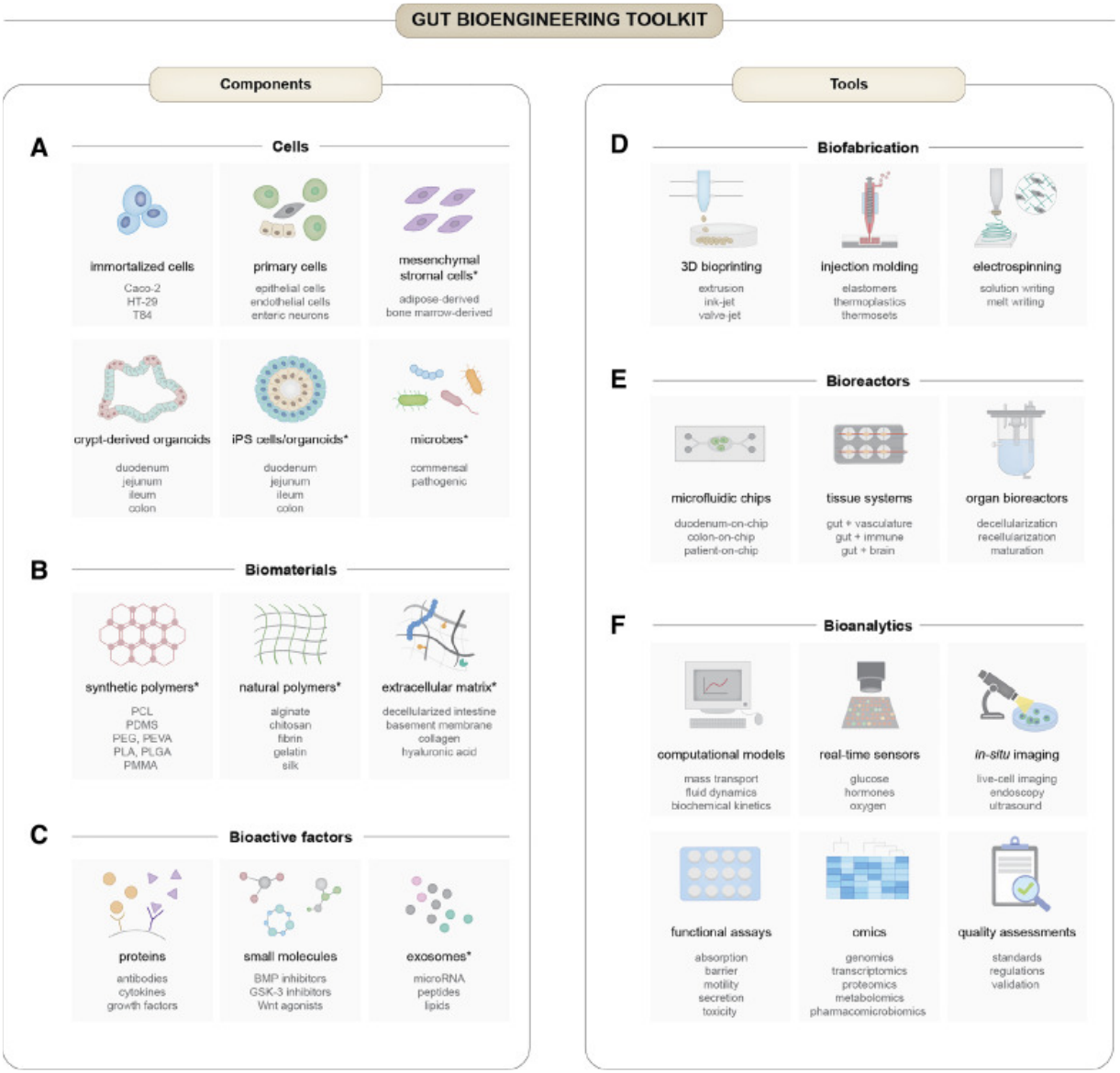
Figure 1.



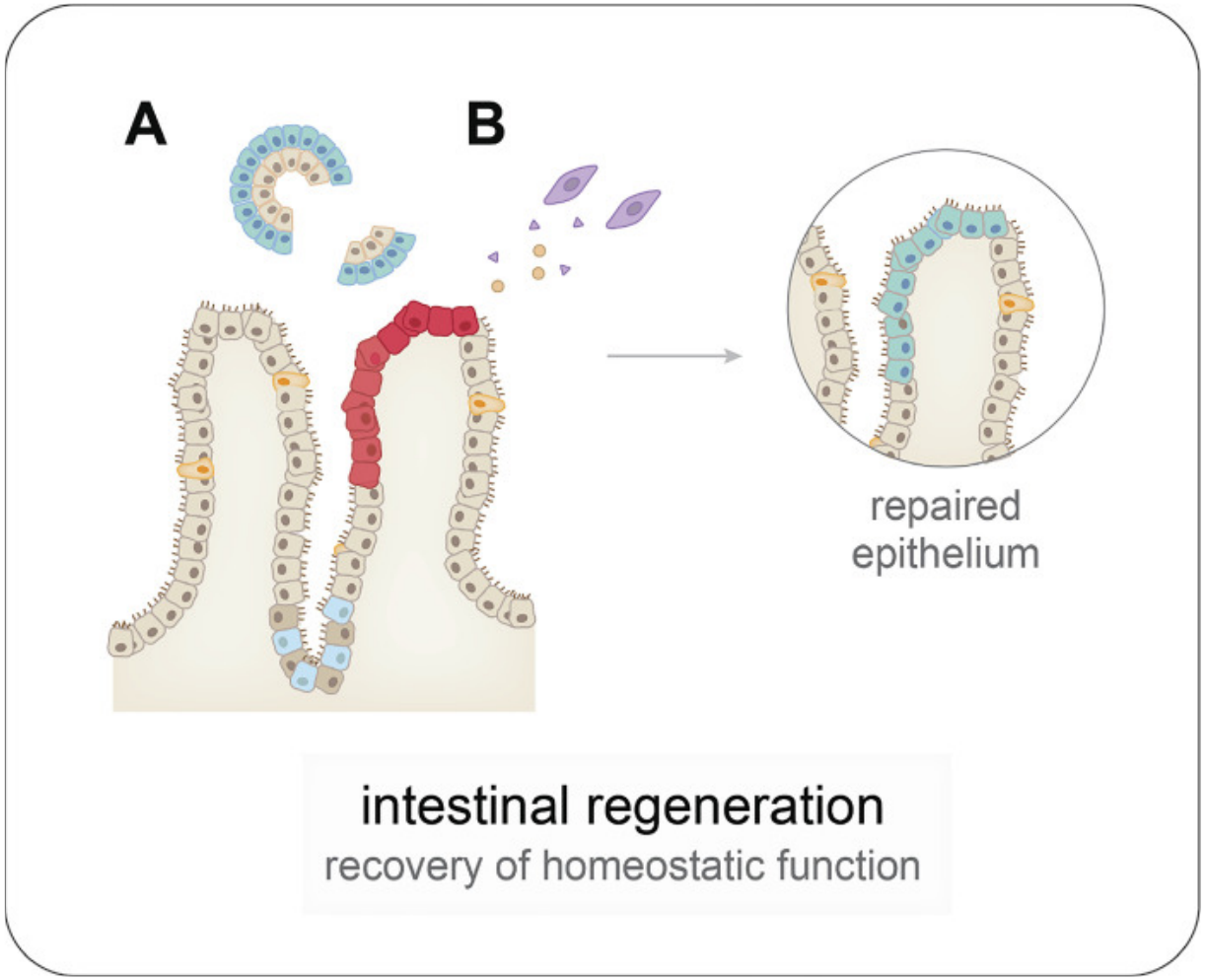
Gut bioengineering aims. *A* and *B*: de novo generation of gutlike tissues at multiple scales for microtissue models (*A*) or implantable grafts (*B*). *C*: regeneration of functional gut in vivo via therapeutic deliverables that drive tissue repair.

Figure 2.



Gut bioengineering toolkit. *A–C*: building block components include cells (*A*), biomaterials (*B*), and bioactive factors (*C*). *D–F*: tools include biofabrication techniques for bioassembly (*D*), bioreactors at multiple scales (*E*), and bioanalytics through various modalities (*F*). BMP, bone morphogenetic protein; GSK-3, glycogen synthase kinase 3; PCL, polycaprolactone; PDMS, polydimethylsiloxane; PEG, polyethylene glycol; PEVA, polyethylene vinyl acetate; PLA, polylactic acid; PLGA, poly(lactic-co-glycolic acid); PMMA, poly(methyl methacrylate). *Potential bioengineered therapeutic or therapeutic carrier.

Figure 3.



Regeneration of functional gut in vivo by cell therapy. Delivery of organoids (*A*) or mesenchymal stromal cells (*B*) to repair intestinal epithelium.