Published in final edited form as:

Expert Opin Biol Ther. 2019 June ; 19(6): 527–537. doi:10.1080/14712598.2019.1595579.

Bioengineering and regeneration of gastrointestinal tissue: where are we now and what comes next?

Elie Zakhem^{1,2}, Shreya Raghavan³, and Khalil N. Bitar^{1,2,4}

¹Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston Salem, NC

²Section on Gastroenterology, Wake Forest School of Medicine, Winston Salem, NC

³Department of Materials Science and Engineering, University of Michigan Ann Arbor, MI

⁴Virginia Tech-Wake Forest School of Biomedical Engineering Sciences, Winston-Salem, NC

Abstract

Introduction: The field of tissue engineering and regenerative medicine has been applied to the gastrointestinal (GI) tract for a couple decades. Several achievements have been accomplished that provide promising tools for treating diseases of the GI tract.

Areas covered: The work described in this review covers the traditional aspect of using cells and scaffolds to replace parts of the tract. Several studies investigated different types of biomaterials and different types of cells. A more recent approach involved the use of gut-derived organoid units that can differentiate into all gut cell layers. The most recent approach introduced the use of organ-on-a-chip concept to understand the physiology and pathophysiology of the GI system.

Expert Opinion: The different approaches tackle the diseases of the GI tract from different perspectives. While all these different approaches provide a promising and encouraging future for this field, the translational aspect is yet to be studied.

Keywords

gastrointestinal tract; scaffolds; biomaterials; organoids; organ-on-a-chip

1. Introduction:

The gastrointestinal (GI) tract is a continuous hollow muscular organ that extends from the esophagus all the way down to the rectum, passing by the stomach, small intestine and large intestine (Figure 1). The GI tract is structured for most part as a tubular organ, except for the stomach. The muscular component of the GI tract is composed of 2 muscle layers, an outer

Declaration of Interests

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer Disclosures

Peer reviewers on this manuscript have no relevant financial relationships or otherwise to disclose.

longitudinal muscle layer that runs parallel to the axis of the tract and an inner circular muscle layer that runs circumferentially around the tubular structure of the tract. The muscular component is the primary effector of gut motility, i.e. the significant contribution to peristalsis leading to propulsion of luminal contents uni-directionally [1]. The contraction of the longitudinal muscle layer leads to the shortening of the length of the gut and the contraction of the circular muscle layer causes the narrowing of the lumen. The musculature is under the regulation of the enteric nervous system (ENS) neurons and the interstitial cells of Cajal (ICC). The ENS functions independently of the central nervous system but works in coordination with it. The ENS is divided primarily into 2 plexi (a collection of ganglia); the myenteric plexus and the submucosal plexus. The myenteric plexus is located between the longitudinal and the circular muscle layer. The myenteric plexus is rich in excitatory and inhibitory motor neurons that regulate smooth muscle function through the release of neurotransmitters. The submucosal plexus is located between the inner circular muscle and the mucosa. Neurons of the submucosal plexus are responsible primarily for sensory functions, but can also mediate significant regulation of mucosal activity and coordinate the motility of the muscularis mucosa. The ICCs also play a role in regulating smooth muscle function by modulating the intrinsic electrical activity inside the smooth muscle cells. A coordinated interaction between all these components of the contractile apparatus ensures unidirectional movement of luminal contents, digestion, and elimination of waste contributing to overall gut function. In addition to peristalsis, the GI tract is responsible of secretion and absorption of nutrients. The highly specialized epithelium that lines the lumen of the GI tract takes care of this function [2]. Absorption of nutrients is facilitated by the villi structures that line the lumen of the intestine. Four different types of epithelial cells exist with specific functions, reviewed extensively elsewhere [3, 4]. Goblet cells are responsible for secretion of mucins for the protection of the luminal wall. Enterocytes are responsible for absorption of nutrients. Enteroendocrine cells are responsible for secretion of hormones. Paneth cells are responsible for providing immunity to the gut. The cells of the epithelium are in a continuous dynamic state, with cells shedding and proliferating as part of their self-renewal property. The GI tract can be more complicated when taking the lymphatic system and the microbiome into account. All those factors make the regeneration or reconstruction of the GI tract a complex task despite the fact that it is structurally a simple hollow tubular organ system. The GI tract is susceptible to multiple diseases – whether congenital or acquired later in life. Several pathologies of the GI tract including Hirschsprung's disease, age-related dysfunction, colorectal cancer, inflammatory bowel disorders, etc. significantly alter the quality of life of an individual owing to varying changes in gut function. GI dysfunction is also secondary to diabetes, and Parkinson's disease. Surgical repair and pharmacological symptom management have been the mainstays to treat GI dysfunction, but these solutions are often thought to be temporary without addressing the root of the occurring pathologies. Tissue engineering and regenerative medicine provides an alternative attractive approach that can be a more suitable long term solution for the patients. Cell and tissue engineering based therapies are an emerging therapeutic paradigm to replace dysfunctional segments of cells or tissues with physiologically functional, optimally structurally designed pieces of gut to augment gut function, with a focus on remedying the underlying cellular pathology.

In this review, we present perspectives of tissue engineering within the GI tract, and approaches utilized to regenerate and recapitulate structural and physiological aspects of the gut. The review is divided into four sections of different most common approaches applied to the GI tract. First, we discuss the concept of using cells and scaffolds which is considered as the traditional concept. A lot of work has been performed using this approach and we discuss some of the studies that have high impact in the field. Second, we discuss the use of acellular approaches to reconstruct and regenerate the gut. This approach includes the use of synthetic, natural and decellularized extracellular matrices. This has been gaining a great attention and research in this field is still on going. Third, we discuss the use of organoid units to regenerate GI tissues. Last, we discuss the new emerging field of organ-on-a-chip as applied to the GI tract. The organ-on-a-chip is considered as a sophisticated system to study not only the physiology of the system but also the pathophysiology to help understand how the system functions.

2. Traditional cell/scaffold approach:

The concept of tissue engineering has been introduced to the field of gastrointestinal tract for couple of decades. Like other tissue and organ systems in the body, regenerating the gastrointestinal system started with the traditional concept of combining cells and scaffolds. The GI tract has a unique structure in being a hollow tubular muscular organ. Even though engineering tubular replacements could be structurally and architecturally simple, the cellular complexity of the GI tract adds to the complexity and makes its replacement a challenging process. The different cell layers, and their specific architecture and alignment are essential to their physiological function, and is an important that must be taken into account while designing replacements for the GI tract. Functional replacement tissues will ideally recreate or facilitate the interplay of: (1) peristalsis and digestion performed by the smooth muscle, enteric neurons and interstitial cells of Cajal [1], (2) absorption and secretion functions performed by the highly specialized epithelium [2], and (3) fluid homeostasis, lipid uptake and immune defense functions performed by the lymphatic and vascular systems [5].

2.1 Cellular Considerations

Several questions need to be answered during the design process of gut regeneration. What types of cells are needed? Are all the different cell types required as starting material to fully reconstruct the gut? Is expansion of the cells a pre-requisite for their use in regeneration? Not only is the cellular diversity of the gut a significant challenge, but sourcing the appropriate type and numbers of cells are also a major challenge. The source of the cells could be tissue specific (i.e. the gut) or it could be other tissues in the body. For example, the potential use of mesenchymal stem cells (MSCs) to promote the regeneration of the muscular component of the stomach has been demonstrated in vivo in rat models when seeded onto small intestine submucosa (SIS) scaffolds [6]. Results have shown that the regenerated smooth muscle lacked any traces of seeded MSCs. This required further investigation into the mechanism of action of MSCs in smooth muscle regeneration. The use of primary smooth muscle cells seeded onto SIS also showed the potential for small intestinal musculature regeneration in a rat model [7]. Further characterization of the

architecture of the regenerated muscle is needed. The regeneration of the musculature of the GI tract requires a full characterization of the musculature component. One of the main challenges in tissue engineering the musculature of the GI tract is the plasticity of smooth muscle phenotype. In normal physiological conditions, smooth muscle cells acquire a mature contractile phenotype. It is important to maintain this phenotype following cell seeding onto scaffolds and following their implantation. The alignment of both smooth muscle layers, survival of the smooth muscle and the differentiated phenotype of the smooth muscle are important components for function and peristalsis. A recent in vitro study investigated the advantage of using smooth muscle strips as opposed to individually isolated cells [8]. Smooth muscle cells obtained from strips showed a superior mature phenotype. To help maintain smooth muscle phenotype, a different approach aimed to align the cells in a way similar to the native alignment [9]. This maintained cell-cell interaction on an ECM component.

The function of the smooth muscle requires additional regulation provided by the enteric innervation of the gut and the pacemaker cells. The myenteric plexus of the enteric nervous system is rich in excitatory and inhibitory neurons that regulate smooth muscle function [10]. In addition, interstitial cells of Cajal (ICCs) play an important role in the regulatory apparatus [11]. Tissue engineering applications that focus on the enteric neurons and ICCs have been scarce. Studies have shown the therapeutic potential of neural progenitor cells following their injection into animal models [12–15]. The gut is a rich source of neural progenitor cells, which provides a tool to treat diseases of the gut [16]. Cells can proliferate, migrate and differentiate into functional neurons [13, 17]. There are well established protocols for isolation of gut-derived neural progenitor cells [18, 19]. Clinically, a biopsy can be obtained to isolate those cells which can then be expanded in vitro. Another easily accessible source of neural progenitor cells in the gut is the appendix. A comparative study of appendix-derived versus small intestine-derived neural progenitor cells showed that cells from both sources have similar capacity to differentiate into functional excitatory and inhibitory neurons in vitro [20]. Additional studies are required to optimize the in vitro expansion of those cells prior to their use. Expanding the cells to the required number is another challenge in tissue engineering. Additionally, cryopreservation of the neural progenitor cells requires further optimization. Despite the challenges, the appendix provides an alternative easier source for neural progenitor cells. In vitro studies have looked at optimizing neural progenitor cell differentiation. Different extracellular matrix components can direct the differentiation of the cells into a specific phenotype [9]. Collagen IV, laminin and heparan sulfate are some of the major ECM components of the gut. Different combinations of ECM components can direct cells into either a cholinergic-enriched population or nitrergic-enriched population. Additionally, the use of soluble factors such as BMP-2 can also direct neural progenitor cell differentiation into inhibitory neurons [21]. These findings are important when it comes to treating diseases that are the result of loss or decreased specific neural population. The focus on regenerating the neuro-muscular apparatus alone originates from the fact that epithelial cells have the potential to migrate following injury to the adjacent gut segments. Therefore, migrating epithelial cells can colonize the engineered gut segment following its implantation. The neuro-musculature dictates peristalsis in the gut and the propulsion of luminal content. A functional regenerated

musculature is an essential component of the gut. When talking about functionality of the musculature, it is important to note the plasticity of smooth muscle cells and the change in their phenotype. Smooth muscle cells can switch from a contractile mature physiological phenotype to a proliferative synthetic phenotype. Functional smooth muscle cell requires a mature contractile phenotype. Studying the smooth muscle component in a 3D environment is important to predict its success in vivo. However, when engineering a full gut segment, all the cells of the gut must be studied for architecture and function.

In addition to the importance of regenerating the innervated musculature of the gut, regenerating the epithelial component is as critical [22, 23]. The presence of epithelium in any regenerated tissue is required to avoid stenosis [24]. In cellular approaches of regeneration the epithelium, isolation of epithelial cells, their expansion in vitro prior to seeding onto scaffolds and their survival and differentiation on the scaffolds are main challenges. Therefore optimizing the respective protocols is a major step to overcome this challenge. A study investigated the potential of combining enzymatic digestion of tissues along with explant culture to yield higher number of cells [25]. Epithelial cells were seeded at different densities and duration onto scaffolds for esophagus reconstruction. Even though initial cell loss was observed, cell viability and phenotype were then maintained over time. Several attempts were investigated to enhance cell survival and attachment onto scaffolds. One chemical approach looked at the effect of hyaluronic acid to enhance cell attachment and proliferation [26]. A mechanical approach looked at designing scaffolds with villi structures to facilitate epithelial cell attachment and proliferation [27, 28]. Epithelial cell regeneration is tricky due to the fact that the regenerated epithelial cells must maintain the native characteristics of epithelial cells in terms of stemness and proliferative. The differentiation of the cells into the 4 major epithelial cell types is important for function of the newly generated gut tissues. Therefore, in vitro and in vivo studies testing for the presence of the epithelial cells and their function is a critical step for success. The complexity of the epithelial component could also benefit from physical modification of the scaffolds. Micropatterned scaffolds with structures that resemble 3D villi structures provide a better environment for epithelial cell differentiation in a fashion similar to native intestine [29, 30]. This allows the epithelial cells to acquire characteristics specific to different regions of the villi of the intestine. The complexity of the epithelium and its high turnover rate, it is important that this characteristic is replicated in the regenerated gut tissue. Given the challenge of isolating and maintaining epithelial cells in vitro, the search has shifted to the use of other sources of cells such as organoid units (discussed separately in the section below).

2.2 Material Considerations

Outside of cellular considerations, scaffold material choice and design considerations are also important to be able to facilitate appropriate cell-cell interactions, and to promote the behavior of mechanically competent tissue layers, while allowing for adequate vascularization. In the case of porous scaffolds, porosity plays an important factor in survival of the seeded smooth muscle. Optimizing the porosity of the scaffolds for better vascularization following implantation is an important design process [31]. Given the complexity and the importance of the specific alignment of the cells, certain modifications

(physical and chemical) have been applied to improve on the current techniques. In terms of physical modifications, work has focused on designing scaffolds that would allow the cells to acquire a similar alignment as in vivo. The circular muscle alignment has been studied and approaches to mimic this alignment have been successful. Growing smooth cells on different matrices (collagen or agarose) in wells with a central cylindrical post guides the smooth muscle cells to acquire a circumferential alignment in vitro [32–34]. Mimicking the longitudinal muscle layer was also studied. Micropatterned surfaces have been engineered and used as a surface to grow smooth muscle cells with a longitudinal alignment [35]. Micropatterned thermoresponsive surfaces were also developed to grow cells in a particular alignment [36]. Cells were seeded at a specific temperature and then lifted as one layer of aligned cells at a different temperature. The benefit of growing cells as an aligned layer is the maintenance of the cells' secreted matrix in vitro.

Several natural biomaterials have been employed in gut engineering. Collagen is known to have an excellent biocompatibility and to support cell growth and survival. Previously, stomach-derived smooth muscle cells were seeded onto collagen sponges [37]. After up to 12 weeks post implantation in rat small intestine, smooth muscle cells acquired a circumferential alignment similar to the circular smooth muscle layer of the gut and exhibited a contractile phenotype. Gut-derived smooth muscle cells also maintained a contractile phenotype in vitro when seeded onto a bi-layered silk fibroin scaffold [38]. Further in vivo testing is required for confirmation of phenotype and function. One of the main drawbacks of collagen is its weak mechanical strengths. However, it can be used in combination with other biomaterials as a boost for biocompatibility. Other forms of natural materials include the use of decellularized tissues (discussed further in the acellular section). Those tissues are obtained through chemical or mechanical processing to remove the cellular components. Decellularized esophagi seeded with mesenchymal stem cells were used to reconstruct the defected esophagus [39]. Histologically, the different cells layers including the muscle and the epithelium were regenerated. Even though the seeded cells were not detected following implantation, further characterization of their mechanism of action would be beneficial for future studies.

Chitosan has been also investigated for its potential as matrix for gut tissue engineering. Tubular chitosan scaffolds were engineered and tested for their biocompatibility to support smooth muscle and neuronal survival, differentiation and function [40–42]. The tissues were further evaluated in vivo in rat models for survival, vascularization and function [43, 44]. When anastomosed to the native intestine of the rats, the engineered chitosan-based intestine maintained a functional innervated musculature and acquired an epithelium layer [45].

The use of natural materials is usually accompanied by the disadvantage of weak mechanical properties. Enhancing the mechanical properties is important especially following implantation of the tissue. Following implantation, the ability of the engineered tissue to withstand the pressure of the gut and to degrade in a timely manner is essential for successful outcomes. In a previous study, tubular chitosan scaffolds were mechanically reinforced using chitosan fibers prepared by the extrusion/gelation technique [42]. The tensile properties of the reinforced scaffolds were within physiologic range compared to the non-reinforced scaffolds. Electrospinning technique has also employed to enhance

mechanical properties of tubular scaffolds [46]. Controlling the electrospun fibers diameter and orientation plays an important role in determining the mechanical properties of the scaffold. While advancement has been achieved in designing scaffolds for gut regeneration, studying the mechanical properties of the scaffolds hasn't gained great attention in this field yet. It is important that the mechanical properties of the engineered guts match those of the native one. The gut is subject to high luminal pressure that differs at different stages of the gut which must be taken into consideration while designing different parts of the gut. It is also important to keep in mind that the mechanical properties of the gut differ with aging and with different diseases and the engineered tissues must be compliant with those cases.

3. Acellular-based approach:

Regenerating the GI tract is not limited to the use of cellular approaches. Using scaffolds without any cellular component has been studied and showed promising outcomes. The rationale is that following implantation, the acellular scaffolds provide a temporary matrix for the host cells to infiltrate, populate and build their own matrix as part of the regeneration process. Different acellular scaffolds have been studied such as decellularized extracellular matrices, synthetic scaffolds and natural scaffolds.

Naturally-derived extracellular matrix (ECM) represents the 3D structural environment where cells sit and interact with each other and with the matrix. ECM provides mechanical and biochemical cues that allow cell proliferation, migration and differentiation [47]. Ideally, tissue specific ECM represents a good replacement scaffold as it maintains the original architecture and proteins of the tissue. ECM can maintain the binding sites that enhance cell attachment and differentiation. Additionally, ECM maintains the growth factors and proteins that are important for the cells and for accelerating the regeneration process. The methods of obtaining decellularized ECM have been well established and they have been applied to almost all organs of the body [48–50]. Mechanical or chemical processing of the tissues aims to eliminate the cellular components. This comes with the challenge of maintaining the intrinsic proteins in the tissues. The cellular content and protein content in the decellularized tissues determine the behavior of the ECM in vivo and their ability to promote reconstructive remodeling [51].

One of the very first decellularized tissues used for tissue reconstruction was the small intestine submucosa (SIS). SIS was shown to support epithelial and musculature regeneration. As a patch, SIS supported esophageal reconstruction [52] however SIS as a tubular graft remains to be tested for its compliance and mechanical strength following implantation [53]. In small intestinal repair, SIS was shown to have promising results. SIS was successfully anastomosed with the small intestine and regenerated the different cellular layers [54]. Vascularization of the graft was also demonstrated. Every scaffold used in tissue reconstruction still requires characterization of the functionality of the tissue to ensure appropriate regeneration. The use of decellularized tissues has also been employed in clinical applications. Porcine small intestine ECM scaffolds were used for esophageal reconstruction [55]. The mucosa was regenerated without evidence of stenosis. The strictures seen following the attempts to reconstruct the esophagus represent a major challenge. Urinary bladders matrix were also successfully used to reconstruct the esophagus

with promising positive results [56]. Those naturally-derived decellularized materials support reconstructive remodeling and restoration of function [57]. The presence of the proteins, growth factors and tissue-specific ECM components facilitate the reconstruction process and promote vascularization. Urinary bladder matrices were also further tested in preclinical animal models to reconstruct the esophagus. The combination of decellularized ECM scaffolds with seeded cells was also tested. Following a defect in the esophagus, the urinary bladder matrices regenerated the epithelium and maintained the luminal patency of the esophagus [58].

Synthetic materials also take part in the acellular approach of gut tissue engineering. Acellular scaffolds were tested in vivo for their capacity to regenerate different GI tissues. In cases when synthetic materials were used in an acellular approach, poly- ϵ -caprolactone was used as a mesh to repair an in vivo defect in the esophagus of rabbits [59]. This resulted in regeneration of all layers of the esophagus (smooth muscle, striated muscle and epithelium). Acellular hybrid synthetic scaffolds were also tested to reconstruct a gastric wall defect in a porcine model [60]. Studies were followed for up to 12 weeks. Functional epithelium was demonstrated through staining studies, however studying the muscular component is yet to be determined. Synthetic materials have also been used as coating materials to strengthen natural biomaterials [61]. Some of the main complications of using synthetic materials include stricture at the site of implant and the extended degradation rates of the materials. One of the main limitations of using synthetic materials is their lower biocompatibility. Chemical modifications of the surfaces of the scaffolds in multiple applications have shown to improve their biocompatibility. Increasing the hydrophilicity of the scaffolds through the addition of functional groups enhances cells attachment and proliferation [62, 63]. On the other side, the addition of adhesion peptides such as fibronectin and other growth factors can guide stem cell differentiation [64]. Determining the appropriate modification and its nature are important parameters for optimal results.

Natural biomaterials were also used in an acellular setting for gut tissue engineering. In an attempt to repair a gastric wall defect (4×4 cm), collagen sponge scaffolds were used in a canine model [65]. Following 16 weeks of implantation, regeneration of epithelial component and a non-functional muscle component was observed. Chitosan hydrogel has also been investigated for colon reconstruction [66]. Chitosan hydrogel was compared to SIS in vivo and showed a faster regeneration. Following 60 days of implantation, chitosan hydrogel supported muscular and epithelial regeneration. The scaffolds were compared over a 1×2 cm defect in the colon wall. It would be interesting and beneficial to have a follow up study that looks at a full tubular defect and the ability of chitosan hydrogel to repair the defect.

4. Organoids concept:

While addressing the cellular complexity required making holistic models of the gut, intestinal organoids are a very promising approach. Organoids are literally units of the organ of their origin, and can recapitulate biological diversity with high fidelity [67]. Protocols for isolation of gut-derived organoid units have been well established in the literature [68, 69]. Till date the focus of intestinal organoids has been directed towards the regeneration of

epithelial components, which differentiate into functional units in the absence of WNT signaling [70]. The building blocks of such epithelial organoids have been pluripotent, or embryonic stem cells, as well as, adult stem cells [71–73]. Fundamental limitations of the organoid approach without help from biofabrication and engineering components remain the lack of architectural organization. Engineering strategies that have been used to overcome this include provision of scaffolding with polymers, ECM and ECM-mimics [74–76], and using applied biomaterial strategies [77] to deliver morphogenic cues, or provide biomechanical cues for organization. The traditional use of organoid units involves their isolation from different gut segments, seeding them onto polymeric scaffolds, and finally transplanting them back for gut regeneration (Figure 2). The organoid units isolated from healthy patients and diseased patients can also be used in vitro for drug screening and engineering tools prior to their transplantation. The biggest advantage of organoids have remained that they can form functional units of tissue depending on their source of origin, i.e. full thick intestinal organoids generate both epithelium and the mesenchyme, including layers of muscularis propria and the ENS and improve colonic functionality in murine models [78–81]. The same investigators also demonstrated the regeneration of jejunal sections using autologous swine organoid units [82]. Additional in vivo studies proved that the organoid units differentiated into the different cellular layers with functional outcomes [83, 84]. The omentum represents an optimal incubator for the seeded organoids to mature and differentiate prior to their in situ transplantation [85]. The translational aspect of using organoid has been explored as well. Stomach organoid units were isolated from murine stomach, seeded onto polymeric scaffolds and then tested in vivo in murine models for their potential to regenerate the stomach [83, 86]. Following harvest, the tissue engineered stomach acquired architecture similar to that of native stomach. Those gastric wall patches composed of stomach organoid units and a biodegradable material have shown the potential to repair a gastric wall defect in rats after 24 weeks of transplantation [87]. Stomach organoid units have also been tested in larger animal models [88]. In a preclinical porcine model, scaffolds seeded with stomach organoid units demonstrated the ability to differentiate into a stomach-like structure in vivo. While the focus was mainly on the epithelial component, the muscular component requires further investigation. Human small intestines also demonstrated to be a successful source of organoids that can be seeded onto scaffolds and that can differentiate into the different required cell layers in vivo in murine models [89]. The study demonstrated the viability and growth of the human sourced organoid units in vivo. Additionally, the differentiated epithelial cells exhibited functional outcomes characterized by the presence of channels and transporters and by the absorptive/digestive capacity [90]. Organoid units differentiate into the different cell layers and can then be implanted in continuity with the native tissue. Some of the biggest successes in intestinal regeneration using organoids in the past few years have been models that have looked beyond the epithelial unit, into functional recapitulation of contractility [91], immune function [92], as well as, a functional integration of enteric neural function [93]. Improving the utility of intestinal organoids geared towards gastrointestinal regenerative medicine must consider holistic approaches, where the functionality and integration of multiple layers. Organoids have also been used as 3D model systems to study gastrointestinal diseases.

In most of the cases of using organoid units for gut regeneration, the focus has been on characterizing the regenerated epithelial component in terms of architecture and function. However, there is little studied about the regenerated smooth muscle component. Studies have shown that there is presence of a musculature but it hasn't been well characterized. The alignment and function are yet to be determined given that the musculature will provide the ability of the regenerated gut to perform peristalsis and hence the propulsion of luminal content. Ideally, the organoid units produce a full gut with all layers, the question that remains is whether organoids isolated from one part of the gut can regenerate any other part of the gut.

5. Organ-on-a-chip concept:

The gastrointestinal system is a complicated system that performs functions beyond just digestion and absorption of food. As mentioned earlier, the lymphatic system plays a role in lipid absorption and in immune defense. The gut microbiome is another component that plays a role in the defense system. There has been little emphasis on the regeneration of the lymphatic system and the microbiome. Regenerating the gastrointestinal system requires understanding the physiology and pathophysiology of the system. Ex vivo models have their limitation in terms of their lifetime in culture. In vitro 3D models represent a sophisticated culture system to study multiple aspects of the gut function.

Organ-on-a-chip is an emerging new paradigm in modeling gastrointestinal disease, with limited but significant application towards regenerative medicine. Tremendous progress has been made to create increasingly complex human intestinal organ on chip systems [94]. Typically, organ-on-a-chip approaches use microfluidic devices to generate and maintain organoid units, with the aim of achieving in vivo micro-physiology [95]. The biggest utility of organ-on-a-chip systems has been in drug discovery, toxicology, and pharmacokinetic studies, with well-outlined methods [96, 97]. Gut-on-a-chip was recently demonstrated by Kim et al., where subjecting Caco-2 cells to a peristaltic force generated intestinal villi spontaneously [98]. Organ-on-a-chip systems have been derived from small intestine and colon biopsies [99, 100], and have even included microbiota and demonstrated spontaneous peristalsis like motions [101, 102]. To date, most of these systems, like organoid units, have focused on the epithelial component of the gastrointestinal tract, with minimal to no focus on the muscularis propria or the enteric nervous system. Strategies used for vascular tissue engineering and engineering vascular smooth muscle on a chip can be co-opted to engineer intestinal smooth muscle on-a-chip as well [103–105].

Micro-engineered culture devices like organ-on-a-chip systems are a powerful 3D in vitro tool for predictive drug discovery, and can have significant utility in regenerative medicine. These tools must overcome common challenges including: i) devising reliable methods of using primary cells for manufacturing and maintaining organoid units; ii) integrating engineering systems used in these micro-devices to produce reproducible physiologically relevant intestinal organoid units that allow for controlled and reliable differentiation of all aspects of the intestine.

Organ-on-a-chip models allow studying drug absorption and epithelial permeability, the interaction of the microbiome with the intestinal cells, and studying intestinal pathophysiology. Figure 3 summarizes the design concept of the gut-on-a-chip as previously described by Kim and Kasendra et. al [106–110]. The significance of this model is in its ability to study digestion and secretion properties of the epithelium, the intestinal barrier function, the relation between the microbiome and pathophysiology, drug uptake and kinetics, and studying diseased models. Caco-2 cells have also demonstrated to be reliable models to study absorptive properties of the epithelium [111, 112]. Caco-2 cell line grown onto Transwell polycarbonate membranes that were coated with collagen exhibited similar absorptive and barrier characteristics as the native tissue [113]. Microbiome interaction with epithelial cells has also been modeled in vitro. In vitro static models provide short term models to study the effect of microbiome on intestinal cells. This is challenged by the fact that microbial cells overgrow and contaminate the culture. Microfluidic devices have been successfully developed to mimic the native lumen-capillary interface [114]. Caco-2 cells were grown in the chip followed by microbial cells inoculation. This co-culture chip system allows to study the effect of microbiome in healthy gut environments and in human disease models. Designing the microfluidic devices is critical and dependent on the purpose of the study. Typically, the devices consist of microchannels divided by porous membranes. An intestinal cell line (most commonly Caco-2 cells) is seeded on one side. Through this system, drug permeability, absorption and barrier function can be studied [115–117]. Even though the organ-on-a-chip field is still considered new and requires further optimization, it is considered as a critical aspect to study the physiology and pathophysiology of the GI system.

6. Conclusion:

Looking at the achievements made in the field of gut tissue engineering and regenerative medicine, there are so many. The field has evolved over the last 3 decades, starting with stem cell therapy, moving into biomaterials and scaffolds and now into organ-on-a-chip. All the advances seen in this field are great prerequisites to move forward into clinical applications. There have been no reported cases of using lab grown tissue engineered gut tissues in humans. Even though a lot has been done, there's a lot more to accomplish for many of these applications to reach clinical trials. When using cells and scaffolds, there's always the question of cell source and cell types. Are autologous cells the best option? Cell differentiation and phenotype in vitro versus in vivo is another challenge. In the case of autologous source, how many biopsies are required from the patient? In the case of organoid units, it is important to test their in vitro expansion capacity. Optimizing the number of those organoids needed for larger scale (human) is another factor that must be studied. Organ-on-a-chip provides a new approach to study the physiology and pathophysiology of the GI tract. The field of GI regeneration is not in its infancy anymore, however the complexity of the tract imposes more and more challenges. The GI tract has its own nervous system that is connected to the central nervous system. Ensuring the integrity of this connection must be taken into account during the regeneration process. The lymphatic system and the microbiome are 2 main components of the GI tract and there has been a timid attempt to

study them in GI regeneration. This review covered the different major aspects of regenerating the GI tract but many key issues remain to be discussed.

Funding

The work of the authors was supported by STTR NIH/NIDDK R42DK105593 to Cellf-Bio, LLC and by NIH/NIDCR T32DE00007057-41, 42 (SR).

References:

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

- [1]. Sanders KM, Koh SD, Ro S, Ward SM, Regulation of gastrointestinal motility—insights from smooth muscle biology, *Nature reviews Gastroenterology & hepatology* 9(11) (2012) 633. [PubMed: 22965426]
- [2]. Kiela PR, Ghishan FK, Physiology of intestinal absorption and secretion, *Best Practice & Research Clinical Gastroenterology* 30(2) (2016) 145–159. [PubMed: 27086882]
- [3]. Van der Flier LG, Clevers H, Stem cells, self-renewal, and differentiation in the intestinal epithelium, *Annual review of physiology* 71 (2009) 241–260.
- [4]. Goto Y, Kiyono H, Epithelial barrier: an interface for the cross-communication between gut flora and immune system, *Immunological reviews* 245(1) (2012) 147–163. [PubMed: 22168418]
- [5]. Alexander J, Ganta VC, Jordan P, Witte MH, Gastrointestinal lymphatics in health and disease, *Pathophysiology* 17(4) (2010) 315–335. [PubMed: 20022228]
- [6]. Nakatsu H, Ueno T, Oga A, Nakao M, Nishimura T, Kobayashi S, Oka M, Influence of mesenchymal stem cells on stomach tissue engineering using small intestinal submucosa, *Journal of tissue engineering and regenerative medicine* 9(3) (2015) 296–304. [PubMed: 23913876]
- [7]. Qin HH, Dunn JC, Small intestinal submucosa seeded with intestinal smooth muscle cells in a rodent jejunal interposition model, *Journal of Surgical Research* 171(1) (2011) e21–e26. [PubMed: 21937060]
- [8]. Walthers CM, Lee M, Wu BM, Dunn JC, Smooth muscle strips for intestinal tissue engineering, *PloS one* 9(12) (2014) e114850. [PubMed: 25486279]
- [9]. Raghavan S, Bitar KN, The influence of extracellular matrix composition on the differentiation of neuronal subtypes in tissue engineered innervated intestinal smooth muscle sheets, *Biomaterials* 35(26) (2014) 7429–7440. [PubMed: 24929617]
- [10]. Hao MM, Young HM, Development of enteric neuron diversity, *Journal of cellular and molecular medicine* 13(7) (2009) 1193–1210. [PubMed: 19538470]
- [11]. Sanders KM, Koh SD, Ward SM, Interstitial cells of Cajal as pacemakers in the gastrointestinal tract, *Annu. Rev. Physiol.* 68 (2006) 307–343. [PubMed: 16460275]
- [12]. Micci MA, Kahrig KM, Simmons RS, Sarna SK, Espejo-Navarro MR, Pasricha PJ, Neural stem cell transplantation in the stomach rescues gastric function in neuronal nitric oxide synthase-deficient mice, *Gastroenterology* 129(6) (2005) 1817–1824. [PubMed: 16344050]
- [13]. Hotta R, Stamp LA, Foong JP, McConnell SN, Bergner AJ, Anderson RB, Enomoto H, Newgreen DF, Obermayr F, Furness JB, Transplanted progenitors generate functional enteric neurons in the postnatal colon, *The Journal of clinical investigation* 123(3) (2013) 1182–1191. [PubMed: 23454768]
- [14]. Liu W, Wu R, Dong Y, Gao Y, Neuroepithelial stem cells differentiate into neuronal phenotypes and improve intestinal motility recovery after transplantation in the aganglionic colon of the rat, *Neurogastroenterology & Motility* 19(12) (2007) 1001–1009. [PubMed: 17973630]
- [15]. Metzger M, Caldwell C, Barlow AJ, Burns AJ, Thapar N, Enteric nervous system stem cells derived from human gut mucosa for the treatment of aganglionic gut disorders, *Gastroenterology* 136(7) (2009) 2214–2225. e3. [PubMed: 19505425]
- [16]. Metzger M, Bareiss PM, Danker T, Wagner S, Hennenlotter J, Guenther E, Obermayr F, Stenzl A, Koenigsrainer A, Skutella T, Expansion and differentiation of neural progenitors derived from the

- human adult enteric nervous system, *Gastroenterology* 137(6) (2009) 2063–2073. e4. [PubMed: 19549531]
- [17]. Anitha M, Joseph I, Ding X, Torre ER, Sawchuk MA, Mwangi S, Hochman S, Sitaraman SV, Anania F, Srinivasan S, Characterization of fetal and postnatal enteric neuronal cell lines with improvement in intestinal neural function, *Gastroenterology* 134(5) (2008) 1424–1435. [PubMed: 18471518]
- [18]. Almond S, Lindley RM, Kenny SE, Connell MG, Edgar DH, Characterisation and transplantation of enteric nervous system progenitor cells, *Gut* 56(4) (2007) 489–496. [PubMed: 16973717]
- [19]. Raghavan S, Gilmont RR, Bitar KN, Neuroglial differentiation of adult enteric neuronal progenitor cells as a function of extracellular matrix composition, *Biomaterials* 34(28) (2013) 6649–6658. [PubMed: 23746858]
- [20]. Zakhem E, Rego SL, Raghavan S, Bitar KN, The appendix as a viable source of neural progenitor cells to functionally innervate bioengineered gastrointestinal smooth muscle tissues, *Stem cells translational medicine* 4(6) (2015) 548–554. [PubMed: 25873745]
- [21]. Rego SL, Raghavan S, Zakhem E, Bitar KN, Enteric neural differentiation in innervated, physiologically functional, smooth muscle constructs is modulated by bone morphogenic protein 2 secreted by sphincteric smooth muscle cells, *Journal of tissue engineering and regenerative medicine* 11(4) (2017) 1251–1261. [PubMed: 25926098]
- [22]. Vrana NE, Lavalle P, Dokmeci MR, Dehghani F, Ghaemmaghami AM, Khademhosseini A, Engineering functional epithelium for regenerative medicine and in vitro organ models: a review, *Tissue Engineering Part B: Reviews* 19(6) (2013) 529–543. [PubMed: 23705900]
- [23]. Paz AC, Soleas J, Poon JC, Trieu D, Waddell TK, McGuigan AP, Challenges and opportunities for tissue-engineering polarized epithelium, *Tissue Engineering Part B: Reviews* 20(1) (2013) 56–72. [PubMed: 23789630]
- [24]. Ohki T, Yamato M, Murakami D, Takagi R, Yang J, Namiki H, Okano T, Takasaki K, Treatment of oesophageal ulcerations using endoscopic transplantation of tissue-engineered autologous oral mucosal epithelial cell sheets in a canine model, *Gut* 55(12) (2006) 1704–1710. [PubMed: 16709659]
- [25]. Macheiner T, Kuess A, Dye J, Saxena AK, A novel method for isolation of epithelial cells from ovine esophagus for tissue engineering, *Bio-medical materials and engineering* 24(2) (2014) 1457–1468. [PubMed: 24642973]
- [26]. Shabafrooz V, Mozafari M, Köhler GA, Assefa S, Vashae D, Tayebi L, The effect of hyaluronic acid on biofunctionality of gelatin–collagen intestine tissue engineering scaffolds, *Journal of Biomedical Materials Research Part A* 102(9) (2014) 3130–3139. [PubMed: 24132994]
- [27]. Costello CM, Hongpeng J, Shaffiey S, Yu J, Jain NK, Hackam D, March JC, Synthetic small intestinal scaffolds for improved studies of intestinal differentiation, *Biotechnology and bioengineering* 111(6) (2014) 1222–1232. [PubMed: 24390638]
- [28]. Yu J, Peng S, Luo D, March JC, In vitro 3D human small intestinal villous model for drug permeability determination, *Biotechnology and bioengineering* 109(9) (2012) 2173–2178. [PubMed: 22488418] * This paper describes a novel method to recapitulate the villi structure to facilitate epithelial cell differentiation and to study drug permeability.
- [29]. Wang Y, Gunasekara DB, Reed MI, DiSalvo M, Bultman SJ, Sims CE, Magness ST, Allbritton NL, A microengineered collagen scaffold for generating a polarized crypt-villus architecture of human small intestinal epithelium, *Biomaterials* 128 (2017) 44–55. [PubMed: 28288348]
- [30]. Yi B, Shim KY, Ha SK, Han J, Hoang H-H, Choi I, Park S, Sung JH, Three-dimensional in vitro gut model on a villi-shaped collagen scaffold, *BioChip Journal* 11(3) (2017) 219–231.
- [31]. Walthers CM, Nazemi AK, Patel SL, Wu BM, Dunn JC, The effect of scaffold macroporosity on angiogenesis and cell survival in tissue-engineered smooth muscle, *Biomaterials* 35(19) (2014) 5129–5137. [PubMed: 24695092]
- [32]. Somara S, Gilmont RR, Dennis RG, Bitar KN, Bioengineered internal anal sphincter derived from isolated human internal anal sphincter smooth muscle cells, *Gastroenterology* 137(1) (2009) 53–61. [PubMed: 19328796]
- [33]. Hecker L, Baar K, Dennis RG, Bitar KN, Development of a three-dimensional physiological model of the internal anal sphincter bioengineered in vitro from isolated smooth muscle cells,

American Journal of Physiology-Gastrointestinal and Liver Physiology 289(2) (2005) G188–G196. [PubMed: 15774939]

- [34]. Gwyther TA, Hu JZ, Billiar KL, Rolle MW, Directed cellular self-assembly to fabricate cell-derived tissue rings for biomechanical analysis and tissue engineering, *Journal of visualized experiments: JoVE* (57) (2011).
- [35]. Raghavan S, Lam MT, Foster LL, Gilmont RR, Somara S, Takayama S, Bitar KN, Bioengineered three-dimensional physiological model of colonic longitudinal smooth muscle in vitro, *Tissue Engineering Part C: Methods* 16(5) (2010) 999–1009. [PubMed: 20001822]
- [36]. Takahashi H, Shimizu T, Nakayama M, Yamato M, Okano T, The use of anisotropic cell sheets to control orientation during the self-organization of 3D muscle tissue, *Biomaterials* 34(30) (2013) 7372–7380. [PubMed: 23849343]
- [37]. Nakase Y, Hagiwara A, Nakamura T, Kin S, Nakashima S, Yoshikawa T, Fukuda K-I, Kuriu Y, Miyagawa K, Sakakura C, Tissue engineering of small intestinal tissue using collagen sponge scaffolds seeded with smooth muscle cells, *Tissue engineering* 12(2) (2006) 403–412. [PubMed: 16548698]
- [38]. Franck D, Chung YG, Coburn J, Kaplan DL, Estrada CR Jr, Mauney JR, In vitro evaluation of bi-layer silk fibroin scaffolds for gastrointestinal tissue engineering, *Journal of tissue engineering* 5 (2014) 2041731414556849. [PubMed: 25396043]
- [39]. Sjöqvist S, Jungebluth P, Lim ML, Haag JC, Gustafsson Y, Lemon G, Baiguera S, Burguillos MA, Del Gaudio C, Rodríguez AB, Experimental orthotopic transplantation of a tissue-engineered oesophagus in rats, *Nature communications* 5 (2014) 4562.
- [40]. Zakhem E, Raghavan S, Gilmont RR, Bitar KN, Chitosan-based scaffolds for the support of smooth muscle constructs in intestinal tissue engineering, *Biomaterials* 33(19) (2012) 4810–4817. [PubMed: 22483012]
- [41]. Zakhem E, Raghavan S, Bitar KN, Neo-innervation of a bioengineered intestinal smooth muscle construct around chitosan scaffold, *Biomaterials* 35(6) (2014) 1882–1889. [PubMed: 24315576]
- [42]. Zakhem E, Bitar KN, Development of chitosan scaffolds with enhanced mechanical properties for intestinal tissue engineering applications, *Journal of functional biomaterials* 6(4) (2015) 999–1011. [PubMed: 26473937]
- [43]. Zakhem E, Elbahrawy M, Orlando G, Bitar KN, Successful implantation of an engineered tubular neuromuscular tissue composed of human cells and chitosan scaffold, *Surgery* 158(6) (2015) 1598–1608. [PubMed: 26096562]
- [44]. Zakhem E, El Bahrawy M, Orlando G, Bitar KN, Biomechanical properties of an implanted engineered tubular gut-sphincter complex, *Journal of tissue engineering and regenerative medicine* 11(12) (2017) 3398–3407. [PubMed: 27882697]
- [45]. Zakhem E, Tamburrini R, Orlando G, Koch KL, Bitar KN, Transplantation of a Human Tissue-Engineered Bowel in an Athymic Rat Model, *Tissue Engineering Part C: Methods* 23(11) (2017) 652–660. [PubMed: 28653858] ** This paper describes an approach to reconstruct the small intestine using natural biomaterials with engineered innervated smooth muscle. Following anastomosis, the epithelial component was also regenerated.
- [46]. Ju YM, San Choi J, Atala A, Yoo JJ, Lee SJ, Bilayered scaffold for engineering cellularized blood vessels, *Biomaterials* 31(15) (2010) 4313–4321. [PubMed: 20188414]
- [47]. Brown BN, Badylak SF, Extracellular matrix as an inductive scaffold for functional tissue reconstruction, *Translational Research* 163(4) (2014) 268–285. [PubMed: 24291155]
- [48]. Crapo PM, Gilbert TW, Badylak SF, An overview of tissue and whole organ decellularization processes, *Biomaterials* 32(12) (2011) 3233–3243. [PubMed: 21296410]
- [49]. Badylak SF, Taylor D, Uygun K, Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds, *Annual review of biomedical engineering* 13 (2011) 27–53.
- [50]. Gilbert TW, Sellaro TL, Badylak SF, Decellularization of tissues and organs, *Biomaterials* 27(19) (2006) 3675–3683. [PubMed: 16519932]
- [51]. 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* 30(8) (2009) 1482–1491. [PubMed: 19121538]

- [52]. Badylak S, Meurling S, Chen M, Spievack A, Simmons-Byrd A, Resorbable bioscaffold for esophageal repair in a dog model, *Journal of pediatric surgery* 35(7) (2000) 1097–1103. [PubMed: 10917304]
- [53]. Doede T, Bondartschuk M, Joerck C, Schulze E, Goernig M, Unsuccessful alloplastic esophageal replacement with porcine small intestinal submucosa, *Artificial organs* 33(4) (2009) 328–333. [PubMed: 19335409]
- [54]. Wang ZQ, Watanabe Y, Toki A, Experimental assessment of small intestinal submucosa as a small bowel graft in a rat model, *Journal of pediatric surgery* 38(11) (2003) 1596–1601. [PubMed: 14614707]
- [55]. 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 Engineering Part A* 17(11–12) (2011) 1643–1650. [PubMed: 21306292]
- [56]. Nieponice A, Ciotola FF, Nachman F, Jobe BA, Hoppo T, Londono R, Badylak S, Badaloni AE, Patch esophagoplasty: esophageal reconstruction using biologic scaffolds, *The Annals of thoracic surgery* 97(1) (2014) 283–288. [PubMed: 24266951]
- [57]. Badylak SF, Valentin JE, Ravindra AK, McCabe GP, Stewart-Akers AM, Macrophage phenotype as a determinant of biologic scaffold remodeling, *Tissue Engineering Part A* 14(11) (2008) 1835–1842. [PubMed: 18950271] * This paper describes the importance of using decellularized matrices for tissue regeneration. The paper further studies the macrophage response and the effect of the material on macrophage polarization that favors remodeling.
- [58]. Nieponice A, Gilbert TW, Johnson SA, Turner NJ, Badylak SF, Bone marrow–derived cells participate in the long-term remodeling in a mouse model of esophageal reconstruction, *Journal of Surgical Research* 182(1) (2013) e1–e7. [PubMed: 23069684]
- [59]. Diemer P, Markoew S, Le DQS, Qvist N, Poly-ε-caprolactone mesh as a scaffold for in vivo tissue engineering in rabbit esophagus, *Diseases of the Esophagus* 28(3) (2015) 240–245. [PubMed: 24446895]
- [60]. Miyazawa M, Aikawa M, Watanabe Y, Takase K.-i., Okamoto K, Shrestha S, Okada K, Koyama I, Ikada Y, Extensive regeneration of the stomach using bioabsorbable polymer sheets, *Surgery* 158(5) (2015) 1283–1290. [PubMed: 25964027]
- [61]. Araki M, Tao H, Sato T, Nakajima N, Hyon SH, Nagayasu T, Nakamura T, Development of a new tissue-engineered sheet for reconstruction of the stomach, *Artificial organs* 33(10) (2009) 818–826. [PubMed: 19839991]
- [62]. Cheng Q, Lee BL-P, Komvopoulos K, Yan Z, Li S, Plasma surface chemical treatment of electrospun poly (L-lactide) microfibrinous scaffolds for enhanced cell adhesion, growth, and infiltration, *Tissue Engineering Part A* 19(9–10) (2013) 1188–1198. [PubMed: 23281641]
- [63]. Ravichandran R, Venugopal JR, Sundarajan S, Mukherjee S, Sridhar R, Ramakrishna S, Composite poly-L-lactic acid/poly-(α, β)-DL-aspartic acid/collagen nanofibrinous scaffolds for dermal tissue regeneration, *Materials Science and Engineering: C* 32(6) (2012) 1443–1451. [PubMed: 24364944]
- [64]. Yang K, Lee JS, Kim J, Lee YB, Shin H, Um SH, Kim JB, Park KI, Lee H, Cho S-W, Polydopamine-mediated surface modification of scaffold materials for human neural stem cell engineering, *Biomaterials* 33(29) (2012) 6952–6964. [PubMed: 22809643]
- [65]. Hori Y, Nakamura T, Kimura D, Kaino K, Kurokawa Y, Satomi S, Shimizu Y, Functional analysis of the tissue-engineered stomach wall, *Artificial organs* 26(10) (2002) 868–872. [PubMed: 12296927]
- [66]. Denost Q, Adam J-P, Pontallier A, Montembault A, Bareille R, Siadous R, Delmond S, Rullier E, David L, Bordenave L, Colorectal tissue engineering: a comparative study between porcine small intestinal submucosa (SIS) and chitosan hydrogel patches, *Surgery* 158(6) (2015) 1714–1723. [PubMed: 26275832]
- [67]. Fatehullah A, Tan SH, Barker N, Organoids as an in vitro model of human development and disease, *Nature cell biology* 18(3) (2016) 246–54. [PubMed: 26911908]

- [68]. van Rijn JM, Schneeberger K, Wiegerinck CL, Nieuwenhuis EE, Middendorp S, Novel approaches: Tissue engineering and stem cells–In vitro modelling of the gut, *Best Practice & Research Clinical Gastroenterology* 30(2) (2016) 281–293. [PubMed: 27086891]
- [69]. Choi R, Preliminary studies of tissue-engineered intestine using isolated epithelial organoid units on tubular synthetic biodegradable scaffolds, *Transplant Proc* 29 (1997) 848–851. [PubMed: 9123551]
- [70]. Huch M, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, Sato T, Hamer K, Sasaki N, Finegold MJ, Haft A, Vries RG, Grompe M, Clevers H, In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration, *Nature* 494(7436) (2013) 247–50. [PubMed: 23354049]
- [71]. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, Hoskins EE, Kalinichenko VV, Wells SI, Zorn AM, Shroyer NF, Wells JM, Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro, *Nature* 470(7332) (2011) 105–9. [PubMed: 21151107] ** The work described in this paper provides a new approach of establishing intestinal organoids using pluripotent stem cells that can be used to study intestinal physiology and pathophysiology.
- [72]. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, Clevers H, Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche, *Nature* 459(7244) (2009) 262–5. [PubMed: 19329995]
- [73]. Ootani A, Li X, Sangiorgi E, Ho QT, Ueno H, Toda S, Sugihara H, Fujimoto K, Weissman IL, Capecchi MR, Kuo CJ, Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche, *Nature medicine* 15(6) (2009) 701–6.
- [74]. Jabaji Z, Brinkley GJ, Khalil HA, Sears CM, Lei NY, Lewis M, Stelzner M, Martin MG, Dunn JC, Type I collagen as an extracellular matrix for the in vitro growth of human small intestinal epithelium, *PloS one* 9(9) (2014) e107814. [PubMed: 25222024]
- [75]. Buske P, Przybilla J, Loeffler M, Sachs N, Sato T, Clevers H, Galle J, On the biomechanics of stem cell niche formation in the gut--modelling growing organoids, *The FEBS journal* 279(18) (2012) 3475–87. [PubMed: 22632461]
- [76]. Gjorevski N, Sachs N, Manfrin A, Giger S, Bragina ME, Ordonez-Moran P, Clevers H, Lutolf MP, Designer matrices for intestinal stem cell and organoid culture, *Nature* 539(7630) (2016) 560–564. [PubMed: 27851739]
- [77]. Costello CM, Hongpeng J, Shaffiey S, Yu J, Jain NK, Hackam D, March JC, Synthetic small intestinal scaffolds for improved studies of intestinal differentiation, *Biotechnology and bioengineering* 111(6) (2014) 1222–32. [PubMed: 24390638]
- [78]. Grikscheit TC, Ogilvie JB, Ochoa ER, Alsberg E, Mooney D, Vacanti JP, Tissue-engineered colon exhibits function in vivo, *Surgery* 132(2) (2002) 200–4. [PubMed: 12219012]
- [79]. Grikscheit TC, Ochoa ER, Ramsanahie A, Alsberg E, Mooney D, Whang EE, Vacanti JP, Tissue-engineered large intestine resembles native colon with appropriate in vitro physiology and architecture, *Annals of surgery* 238(1) (2003) 35–41. [PubMed: 12832963]
- [80]. Yip HYK, Tan CW, Hirokawa Y, Burgess AW, Colon organoid formation and cryptogenesis are stimulated by growth factors secreted from myofibroblasts, *PloS one* 13(6) (2018) e0199412. [PubMed: 29928021]
- [81]. Grikscheit TC, Siddique A, Ochoa ER, Srinivasan A, Alsberg E, Hodin RA, Vacanti JP, Tissue-engineered small intestine improves recovery after massive small bowel resection, *Annals of surgery* 240(5) (2004) 748. [PubMed: 15492554] * This paper described the use of gut-derived organoid units to reconstruct a model of short bowel syndrome. The organoid units differentiated into the different cell layers following its anastomosis.
- [82]. Sala FG, Kunisaki SM, Ochoa ER, Vacanti J, Grikscheit TC, Tissue-engineered small intestine and stomach form from autologous tissue in a preclinical large animal model, *The Journal of surgical research* 156(2) (2009) 205–12. [PubMed: 19665143]
- [83]. Grikscheit T, Srinivasan A, Vacanti JP, Tissue-engineered stomach: a preliminary report of a versatile in vivo model with therapeutic potential, *Journal of pediatric surgery* 38(9) (2003) 1305–1309. [PubMed: 14523810]

- [84]. Grikscheit T, Ochoa ER, Srinivasan A, Gaissert H, Vacanti JP, Tissue-engineered esophagus: experimental substitution by onlay patch or interposition, *The Journal of thoracic and cardiovascular surgery* 126(2) (2003) 537–544. [PubMed: 12928655]
- [85]. Spurrier RG, Speer AL, Hou X, El-Nachef WN, Grikscheit TC, Murine and human tissue-engineered esophagus form from sufficient stem/progenitor cells and do not require microdesigned biomaterials, *Tissue Engineering Part A* 21(5–6) (2014) 906–915. [PubMed: 25298083]
- [86]. Maemura T, Ogawa K, Shin M, Mochizuki H, Vacanti J, Assessment of tissue-engineered stomach derived from isolated epithelium organoid units, *Transplantation proceedings*, Elsevier, 2004, pp. 1595–1599.
- [87]. Maemura T, Kinoshita M, Shin M, Miyazaki H, Tsujimoto H, Ono S, Hase K, Saitoh D, Assessment of a Tissue-Engineered Gastric Wall Patch in a Rat Model, *Artificial organs* 36(4) (2012) 409–417. [PubMed: 22040317]
- [88]. Sala FG, Kunisaki SM, Ochoa ER, Vacanti J, Grikscheit TC, Tissue-engineered small intestine and stomach form from autologous tissue in a preclinical large animal model, *Journal of Surgical Research* 156(2) (2009) 205–212. [PubMed: 19665143]
- [89]. Levin DE, Barthel ER, Speer AL, Sala FG, Hou X, Torashima Y, Grikscheit TC, Human tissue-engineered small intestine forms from postnatal progenitor cells, *Journal of pediatric surgery* 48(1) (2013) 129–137. [PubMed: 23331805]
- [90]. Grant CN, Mojica SG, Sala FG, Hill JR, Levin DE, Speer AL, Barthel ER, Shimada H, Zachos NC, Grikscheit TC, Human and mouse tissue-engineered small intestine both demonstrate digestive and absorptive function, *American Journal of Physiology-Gastrointestinal and Liver Physiology* 308(8) (2015) G664–G677. [PubMed: 25573173]
- [91]. DiMarco RL, Su J, Yan KS, Dewi R, Kuo CJ, Heilshorn SC, Engineering of three-dimensional microenvironments to promote contractile behavior in primary intestinal organoids, *Integrative biology : quantitative biosciences from nano to macro* 6(2) (2014) 127–142. [PubMed: 24343706]
- [92]. Chen Y, Zhou W, Roh T, Estes MK, Kaplan DL, In vitro enteroid-derived three-dimensional tissue model of human small intestinal epithelium with innate immune responses, *PloS one* 12(11) (2017) e0187880. [PubMed: 29186150]
- [93]. Workman MJ, Mahe MM, Trisno S, Poling HM, Watson CL, Sundaram N, Chang CF, Schiesser J, Aubert P, Stanley EG, Elefanti AG, Miyaoka Y, Mandegar MA, Conklin BR, Neunlist M, Brugmann SA, Helmrath MA, Wells JM, Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system, *Nature medicine* 23(1) (2017) 49–59.
- [94]. Bein A, Shin W, Jalili-Firoozinezhad S, Park MH, Sontheimer-Phelps A, Tovaglieri A, Chalkiadaki A, Kim HJ, Ingber DE, Microfluidic Organ-on-a-Chip Models of Human Intestine, *Cellular and molecular gastroenterology and hepatology* 5(4) (2018) 659–668. [PubMed: 29713674]
- [95]. Bhatia SN, Ingber DE, Microfluidic organs-on-chips, *Nature biotechnology* 32(8) (2014) 760–72.
- [96]. Huh D, Kim HJ, Fraser JP, Shea DE, Khan M, Bahinski A, Hamilton GA, Ingber DE, Microfabrication of human organs-on-chips, *Nature protocols* 8(11) (2013) 2135–57. [PubMed: 24113786]
- [97]. Lefebvre DE, Venema K, Gombau L, Valerio LG Jr., Raju J, Bondy GS, Bouwmeester H, Singh RP, Clippinger AJ, Collnot EM, Mehta R, Stone V, Utility of models of the gastrointestinal tract for assessment of the digestion and absorption of engineered nanomaterials released from food matrices, *Nanotoxicology* 9(4) (2015) 523–42. [PubMed: 25119418]
- [98]. Kim HJ, Ingber DE, Gut-on-a-Chip microenvironment induces human intestinal cells to undergo villus differentiation, *Integrative biology : quantitative biosciences from nano to macro* 5(9) (2013) 1130–40. [PubMed: 23817533]
- [99]. Kasendra M, Tovaglieri A, Sontheimer-Phelps A, Jalili-Firoozinezhad S, Bein A, Chalkiadaki A, Scholl W, Zhang C, Rickner H, Richmond CA, Li H, Breault DT, Ingber DE, Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids, *Scientific reports* 8(1) (2018) 2871. [PubMed: 29440725]

- [100]. Wang Y, Ahmad AA, Sims CE, Magness ST, Allbritton NL, In vitro generation of colonic epithelium from primary cells guided by microstructures, *Lab on a chip* 14(9) (2014) 1622–31. [PubMed: 24647645]
- [101]. Huh D, Torisawa YS, Hamilton GA, Kim HJ, Ingber DE, Microengineered physiological biomimicry: organs-on-chips, *Lab on a chip* 12(12) (2012) 2156–64. [PubMed: 22555377]
- [102]. Kim HJ, Huh D, Hamilton G, Ingber DE, Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow, *Lab on a chip* 12(12) (2012) 2165–74. [PubMed: 22434367]
- [103]. Grosberg A, Nesmith AP, Goss JA, Brigham MD, McCain ML, Parker KK, Muscle on a chip: in vitro contractility assays for smooth and striated muscle, *Journal of pharmacological and toxicological methods* 65(3) (2012) 126–35. [PubMed: 22521339]
- [104]. Zhang B, Montgomery M, Chamberlain MD, Ogawa S, Korolj A, Pahnke A, Wells LA, Masse S, Kim J, Reis L, Momen A, Nunes SS, Wheeler AR, Nanthakumar K, Keller G, Sefton MV, Radisic M, Biodegradable scaffold with built-in vasculature for organ-on-a-chip engineering and direct surgical anastomosis, *Nature materials* 15(6) (2016) 669–78. [PubMed: 26950595]
- [105]. Rodriguez-Rodriguez R, Munoz-Berbel X, Demming S, Buttgenbach S, Herrera MD, Llobera A, Cell-based microfluidic device for screening anti-proliferative activity of drugs in vascular smooth muscle cells, *Biomedical microdevices* 14(6) (2012) 1129–40. [PubMed: 22773184]
- [106]. Huh D, Torisawa Y.-s., Hamilton GA, Kim HJ, Ingber DE, Microengineered physiological biomimicry: organs-on-chips, *Lab on a Chip* 12(12) (2012) 2156–2164. [PubMed: 22555377]
- [107]. Kim HJ, Huh D, Hamilton G, Ingber DE, Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow, *Lab on a Chip* 12(12) (2012) 2165–2174. [PubMed: 22434367] ** This paper describes the development of a gut-on-a-chip device that recapitulates the cellular complexity of the gut. When exposed to cyclic strain and microfluidic flow, the polarized epithelium provides a model to study absorption, transport, epithelial barrier function, drug uptake and microbiome role.
- [108]. Kim HJ, Ingber DE, Gut-on-a-Chip microenvironment induces human intestinal cells to undergo villus differentiation, *Integrative Biology* 5(9) (2013) 1130–1140. [PubMed: 23817533]
- [109]. Kim HJ, Li H, Collins JJ, Ingber DE, Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip, *Proceedings of the National Academy of Sciences* 113(1) (2016) E7–E15.
- [110]. Kasendra M, Tovaglieri A, Sontheimer-Phelps A, Jalili-Firoozinezhad S, Bein A, Chalkiadaki A, Scholl W, Zhang C, Rickner H, Richmond CA, Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids, *Scientific reports* 8(1) (2018) 2871. [PubMed: 29440725]
- [111]. Artursson P, Karlsson J, Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells, *Biochemical and biophysical research communications* 175(3) (1991) 880–885. [PubMed: 1673839]
- [112]. Artursson P, Epithelial transport of drugs in cell culture. I: A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells, *Journal of pharmaceutical sciences* 79(6) (1990) 476–482. [PubMed: 1975619]
- [113]. Hidalgo JJ, Raub TJ, Borchardt RT, Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability, *Gastroenterology* 96(3) (1989) 736–749. [PubMed: 2914637]
- [114]. Kim HJ, Lee J, Choi J-H, Bahinski A, Ingber DE, Co-culture of Living Microbiome with Microengineered Human Intestinal Villi in a Gut-on-a-Chip Microfluidic Device, *Journal of visualized experiments: JoVE* (114) (2016).
- [115]. Gao D, Liu H, Lin J-M, Wang Y, Jiang Y, Characterization of drug permeability in Caco-2 monolayers by mass spectrometry on a membrane-based microfluidic device, *Lab on a chip* 13(5) (2013) 978–985. [PubMed: 23340920]
- [116]. Imura Y, Asano Y, Sato K, Yoshimura E, A microfluidic system to evaluate intestinal absorption, *Analytical Sciences* 25(12) (2009) 1403–1407. [PubMed: 20009325]

- [117]. ávan der Meer AD, JungáKim H, ávan der Helm MW, den Berg A, Measuring direct current trans-epithelial electrical resistance in organ-on-a-chip microsystems, *Lab on a Chip* 15(3) (2015) 745–752. [PubMed: 25427650]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Expert opinion:

The field of GI tissue engineering and regenerative medicine has evolved over the last 3 decades. Several approaches have been investigated including tissue specific cells, stem cells, acellular and cellular scaffolds. New technologies were then introduced to this field including organoids and organ-on-a-chip. There has been a great amount of work performed and great advancement in this field, however little is seen in the clinic. When using cells and scaffolds, there's always the question of cell source and cell types. Are autologous cells the best option? Cell differentiation and phenotype in vitro versus in vivo is another challenge. In the case of autologous source, how many biopsies are required from the patient? In the case of organoid units, it is important to test their in vitro expansion capacity. Optimizing the number of those organoids needed for larger scale (human) is another factor that must be studied. Organ-on-a-chip provides a new approach to study the physiology and pathophysiology of the GI tract. The field of GI regeneration is not in its infancy anymore, however the complexity of the tract imposes more and more challenges. The GI tract has its own nervous system that is connected to the central nervous system. Ensuring the integrity of this connection must be taken into account during the regeneration process. The lymphatic system and the microbiome are 2 main components of the GI tract and there has been a timid attempt to study them in GI regeneration. Due to the complexity of the GI tract and the interconnection between the different cells, the restoration of this connectivity is essential for a fully functional gut segment. The phase of feasibility has been performed and completed and this has given a strong background. Next is the fine tuning and connecting the dots. The use of organ-on-a-chip will be very helpful in the future for cure and diagnostic purposes. Utilizing the findings obtained from this technique will allow us to determine which approach will be the most suitable to reconstruct or regenerate parts of the GI tract. The complexity of the GI tract forces the existence of multiple approaches and not a single universal approach to treat all GI diseases. Studying the microbiome and its interaction with the cells of the gut will be an important indicator and predictor of regeneration. It is important to start including those factors in the design process. Functions of the GI tract go beyond propulsion of food, digestion and absorption. The interaction with the lymphatic system and with the central nervous system must be studied and followed for a long term successful outcome. This field has advanced so far, and the advancements have opened up new areas and focuses that shouldn't be ignored.

Article Highlights box:

- This review covers recent advances in the field of GI regeneration using cells and scaffolds. A lot of work has been performed in this area which focused on regenerating the musculature and the epithelium. Studying the phenotype and function are key factors for success.
- Different biomaterials have been studied and optimized to promote cell survival and differentiation. In vitro results do not necessarily translate into in vivo therefore animal studies are necessary to confirm the results in the correct animal models.
- Extracellular matrices and their decellularization is a technique that has been applied to regenerate all organs of the body. The GI tract benefited from this technique by using decellularized tissue-specific and other tissues for gut reconstruction. ECM maintains the architecture and proteins needed for a reconstructive remodeling.
- Organoid units have gained special attention due to their ability of differentiating in vivo into the different cell layers of the gut. Those units can be isolated from various parts of the gut and seeded onto scaffolds for implantation.
- Organ-on-a-chip technique has been introduced to the field of GI regeneration. This highly sophisticated technique allows studying the physiology and pathophysiology of the system. Absorption of drugs, interaction of epithelium with the microbiome and the barrier function of the epithelium can be studied through this system.

Figure 1: An overview of the complexity of the GI tract

The intestinal tract is a continuous hollow tubular organ from the esophagus to the anus, with smooth muscle structures being the primary effectors of intestinal motility. As the tract flows from one organ structure to the other, flow is interrupted by high-pressure occlusive sphincteric structures that limit the flow of luminal content unidirectionally. Sphincteric smooth muscle structures are biochemically different from smooth muscle cells in other parts of the gut. The intestine has a similar basic structure with regional variations in the orientation of smooth muscle, and the organization and composition of the epithelial layers. The enteric nervous system is the intrinsic innervation of the gut, embedded within the smooth muscle and epithelial layers of the gut. Enteric nerves do not form conventional neuromuscular junctions, but still release neurotransmitters to regulate smooth muscle and epithelial function. Together, all the layers of the GI tract function to coordinate peristalsis propelling luminal content from mouth to anus, allowing for digestion, absorption of nutrients and elimination of waste.

Figure 2: Organoid concept

The use of organoids in tissue engineering and regenerative medicine involves the isolation of organoid units from different segments of the gut (esophagus, stomach, small intestine and large intestine), seeding them onto polymeric scaffolds followed by transplantation back in the GI tract. In vitro, the organoid units can be used as tools to model diseases, compare healthy to diseased patients and for drug screening.

Figure 3: Schematic of the gut-on-a-chip concept

The gut-on-a-chip is a multichannel 3D microfluidic system. On one side (top) epithelial cells are seeded and on the other side (bottom) endothelial cells are seeded. Cells are separated by a permeable porous membrane. This system allows studying the interaction of different cells and the interaction of the cells with drugs, microbiome or immune cells. The channels are subjected to strain by side vacuum channels to mimic the in vivo peristalsis. This system also allows a longer culture time in vitro.