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Generation of an Artificial Intestine for the Management of Short Bowel Syndrome

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Abstract

Purpose of review—To discuss the current state of the art in artificial intestine generation in the treatment of short bowel syndrome.

Recent findings—Short bowel syndrome defines the condition in which patients lack sufficient intestinal length to allow for adequate absorption of nutrition and fluids, and thus need parenteral support. Advances towards the development of an artificial intestine have improved dramatically since the first attempts in the 1980s, and the last decade has seen significant advances in understanding the intestinal stem cell niche, the growth of complex primary intestinal stem cells in culture, and fabrication of the biomaterials that can support the growth and differentiation of these stem cells. There has also been recent progress in understanding the role of the microbiota and the immune cells on the growth of intestinal cultures on scaffolds in animal models. Despite recent progress, there is much work to be done before the development of a functional artificial intestine for short bowel syndrome is successfully achieved.

Summary—Continued concerted efforts by cell biologists, bioengineers and clinician-scientists will be required for the development of an artificial intestine as a clinical treatment modality for short bowel syndrome.

Keywords

artificial intestine; intestinal organoids; intestinal stem cells; short bowel syndrome

Introduction

One of the last great frontiers in the field of tissue engineering is the development of an artificial intestine, which in many respects represents one of the greatest challenges in

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modern medicine [1]. Barriers towards the successful development of a functional intestine include not only the need to absorb fluid and nutrients, but also the need to achieve the goal of developing a synthetic surface area that is sufficient to allow for efficient absorption, while still being of a size that can be placed within the abdominal cavity. Despite these challenges, several investigators have made important advances in determining the requisite components for a functional engineered intestinal prototype, and have performed studies in small and large animal models that suggest that the development of a functional engineered intestine may be an achievable goal. These studies have shed light on the progenitor cells that are required to constitute the engineered intestine, as well success in the development of the scaffold and matrix components to support these progenitor cells. This review will highlight progress in the field towards the development of an artificial intestine, will review the medical conditions that would benefit most from such a development, and will define a pathway forward in order to address these challenges on behalf of patients who suffer from the inability to successfully absorb and digest nutrients.

Filling a critical need: what disease would an artificial intestine treat?

In general terms, an artificial intestine would offer treatment for patients who lack the ability to absorb nutrients through their gastrointestinal tracts, and who are therefore dependent on parenteral nutrition. This disorder, termed short bowel syndrome (SBS), is a significant problem for infants and children, and is estimated to affect 24.5 infants per 100,000 live births [2]. There are several causes of SBS, all of which involve the loss of significant portions of the intestinal tract, and include volvulus (in which the intestine twists and cuts off the blood supply), intestinal atresia (in which the intestine fails to develop), or necrotizing enterocolitis – a severe and debilitating disorder affecting newborn infants in which portions of the intestine develop areas of necrosis in response to formula feeding [3]. In general, infants and adults are thought to be at risk of SBS if they have less than 75 cm and 150 cm of small intestine remaining respectively [4]. Treatment of SBS is challenging. The mainstays of treatment consist of parenteral nutrition (PN), intestinal lengthening procedures, and intestinal transplant. All of these treatment modalities are associated with significant complications. For instance, parenteral nutrition is extremely expensive (\$100,000 - \$150,000 per patient per year) [4], and is associated with liver injury due to the toxicity of the nutritional formula, and septic complications from central line associated infections [5]. Intestinal lengthening procedures often require multiple operations and are successful in only carefully selected cases [6]. Intestinal transplant is considered definitive treatment, yet given the tremendous scarcity of donor organs and the high failure rate, intestinal transplant is typically reserved for patients who experience serious complications such as line-associated infections, venous stenosis, or liver failure [7]. In view of these limitations with current treatment approaches, it is clear that there is a pressing need for specific treatment of SBS with an autologous, engineered intestine that could be used to increase absorptive area. In the past few decades there has been significant progress in the development of artificial intestine using tissue engineering and regenerative medicine techniques, as will be discussed in detail below.

The anatomic and physiological barriers that must be overcome for the successful development of an artificial intestine

A variety of anatomic and physiological barriers must be overcome in order to generate a synthetic artificial intestine that achieves the functional properties of the native organ [8]. These barriers are a reflection of the complex nature of the intestine, which in order to accomplish its signature task of absorbing nutrition and water must possess a surface area that is sufficient to allow this to occur without being too large to fit into the abdominal cavity. Of equal importance, the design of an artificial intestine must possess adequate motility in order to allow for efficient contact of luminal contents with the absorptive surface area and for appropriate elimination of waste. Other functions of the intestine, including neural and endocrine signaling of satiety, release of immunoglobulins and antimicrobial peptides are important, but likely not essential in the development of a functional organ [9]. An effective artificial intestine should also serve to overcome the limitations of small bowel transplant and should therefore be derived from autologous cells derived from the recipient, thus obviating the need for immunosuppressant drugs, and eliminating the risk of immune-mediated rejection. The successful development of an artificial intestine requires three processes to be successfully achieved, and are described in **Figure 1**: first, the isolation and differentiation of stem cells with the capacity to differentiate into each of the four epithelial cells that are found lining the epithelium (Paneth cells, goblet cells, enteroendocrine cells and enterocytes); second, the use of an appropriate scaffold that can be implanted into the host without rejection and which can support the growth, differentiation and absorption of an engineered intestinal mucosa; and third, strategies which recruit a functional blood supply to support the absorption of nutrients. Each of these items is discussed in turn, below.

The location and function of the intestinal stem cells

Central to the development of an artificial intestine is the ability to isolate intestinal stem cells, and to maintain these cells in culture, as we and others have achieved. The intestinal stem cells are located at the base of the intestinal crypts in the small intestine, which are invaginations of the folded intestinal epithelium that extend below the lamina propria [10]. The extensive proliferative capacity of the intestinal stem cells is highlighted by the fact that the intestinal epithelium turns over approximately every five to seven days in the adult, and more frequently in the infant [10-12]. Asymmetric division of the intestinal stem cells results in renewal of the stem cell and the formation of a transit-amplifying cell that undergoes further divisions [13]. The transit-amplifying cells ultimately differentiate into the four main types of intestinal epithelial cells (*i.e.* enterocytes, goblet cells, enteroendocrine cells and tuft cells) [13]. As the epithelial cells differentiate, they migrate towards the tip of the villus where they ultimately undergo apoptosis and slough off into the lumen of the intestine [10-12,14].

There has been tremendous progress in the identification of signature molecules that define the intestinal stem cell, and in the development of techniques that permit the maintenance of intestinal stem cells in tissue culture see [8,14,15] for recent review, see representative image of cultured human intestinal stem cells from our lab in **Figure 2**. While a full description of each of the studies regarding the characterization of the intestinal stem cell is beyond the scope of the current work, the reader is referred to recent reviews by Barker and

Rookmaaker *et al.*, which provide a more complete description [13,15]. Perhaps the most important recent advance in the identification and isolation of molecules that define the intestinal stem cell involves the work of Hans Clevers and colleagues [10,15], who defined the expression of the wingless (Wnt) target gene leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5) as a signature feature of the intestinal stem cell within the intestinal crypts [10]. Lgr5 is a receptor for the chemokine r-spondin, which is a key regulator of the Wnt/ β -catenin signaling pathway within the intestine [16]. Lgr5-positive intestinal stem cells are located within the niche at the crypt base, which is rich in factors released by intestinal myofibroblasts [14,17], Notch, and epidermal growth factor (EGF) [14,18]. As the daughter cells differentiate and divide, they continue to migrate out of the crypt towards the villus, and the pathways that regulate enterocyte migration *in vitro* and *in vivo* have been carefully studied [19-21]. This migration serves to position the differentiated cells in the villus near the gut lumen where they can interact with the luminal contents, but also contributes to the differentiation of the daughter cells as they are no longer exposed to Wnt, Notch and EGF signals in the crypt base [11,12]. The crypt base is maintained primarily via Paneth cells, which reside in the crypt base and secrete Wnt and EGF as well as promote Notch signaling via direct contact with Lgr5-positive stem cells [11,12,14,22]. Epithelial-mesenchymal interactions also contribute to the niche via subepithelial myofibroblasts, which secrete growth factors and cytokines that promote epithelial proliferation [11]. There has also been recent interest in the use of induced pluripotent stem cells for the development of an artificial intestine. These cells, which can be obtained from the skin or from fibroblasts, can be induced to differentiate into intestinal cells with proliferative properties, and with the ability to differentiate into all major epithelial cells that line the gastrointestinal tract, and could therefore offer a novel approach in the development of an artificial intestine [23,24].

Scaffold materials and the recruitment of a blood supply in the use of an engineered intestine

There have been several important advances in the design and fabrication of biomaterials, which have allowed for the support of intestinal stem cells both *ex vivo* and within a host. Early studies by Vacanti *et al.* utilized synthetic biodegradable polymers, such as polyglactin, polyorthoesters and polyanhydride [25]. More recently, different groups have used a polyester scaffold (typically poly-glycolic acid [PGA] coated with poly-L lactic acid [PLLA]) fashioned into a tube [26,27]. Totonelli *et al.* demonstrated a method for intestinal decellularization that maintained its original extracellular matrix architecture including that of the villus and crypt [28,29]. This approach should provide a useful scaffold for supporting an artificial intestine as it maintains not only the crypt and villus architecture, but potentially also the vascular network of the intestine. The decellularized intestine is also easy to handle which offers advantages regarding surgical implantation. In additional studies, Chen *et al.* [30] described the use of tubular scaffolds derived from silk that were either ridged (to simulate villi) or flat, and seeded them using established intestinal cell lines (Caco-2, HT29-MTX and human intestinal myofibroblasts). The ridged constructs more closely resembled native intestine in terms of mucus production, alkaline phosphatase activity, villin expression, and sucrose-isomaltase expression compared to the flat versions. Our group has recently utilized a polyvinylidene fluoride (PVDF) membrane in a multi-step fabrication

technique to produce a biodegradable tubularized scaffold, which can have 100 μm -long micro-projections into the lumen, and which approximates the surface area of the intact intestine [31,32], see **Figure 3**. The successful implantation of an engineered intestine requires the recruitment of a supportive vascular network, to permit the absorption of nutritive elements [33]. Recruitment of a vascular network appears to be largely dependent on the site of implantation, as placement of a scaffold within the omentum [31,33,34], capsule of the kidney [23], or the retroperitoneum [35] seem to most effectively facilitate the recruitment of a vascular network [34]. Our group recently described that the implantation of a PGA rich scaffold resulted in the recruitment of vascular endothelial stem cells, which facilitate the development of an intact vascular network when the scaffold and overlying intestinal stem cells are implanted into the omentum of mice, **Figure 3**. Further studies in the appropriate design of biomaterials as well as techniques that promote efficient angiogenesis [1,27] are required in order to advance the development of a functional organ.

Growth and development of intestinal stem cell cultures

Several investigators have described techniques for the maintenance of intestinal stem cells in culture, which is central to the development of a functional artificial intestine [12,36]. For instance, Ootani *et al.* demonstrated that mouse intestinal organoids could be maintained in culture for up to 350 days when obtained from neonatal mice by using an air-liquid interface, collagen as a 3D substrate and stimulating the Wnt signaling pathway with Rsp1-Fc (a Wnt agonist) [37]. The ability to maintain primary organoid cultures *in vitro* for this length of time could prove invaluable for expansion of cells for tissue engineering purposes. A related study by Spurrier *et al.* demonstrated that intestinal organoids could be suspended via vitrification, a cryopreservation technique that prevents cell damage by rapid cooling of cells or tissues [38,39], therefore allowing long term frozen storage and later use while maintaining approximately 90% viability for both murine and human organoids [39]. This process could potentially facilitate a process by which intestinal stem cells would be harvested and subsequently banked from the healthy margins of resected necrotic bowel (for instance in the setting of necrotizing enterocolitis) for later artificial intestine formation once the acute illness has resolved. Further work by McCracken *et al.* described a technique of differentiating human embryonic stem cells (hESCs) into human intestinal organoids (HIOs) *in vitro* [40], which raises the possibility of creating autologous induced pluripotent stem cells (iPSCs) and differentiating them into HIOs for therapeutic use. Additional work in this area by Watson *et al.*, has demonstrated that HIOs can engraft *in vivo* to form mature intestinal epithelium that differentiates into the various cell lineages, express functional brush-border enzymes and display digestive functions (*i.e.* peptide uptake) [23]. In related studies, Liu *et al.* described a technique to optimize the isolation of crypt cells from the villus cells in organoid isolation based on cell size using a filtration system [41], and determined that the crypt cells tended to be in the 25 - 70 micrometer fraction whereas the villus cells tended to be 100 - 200 micrometer range. This technique may provide quicker and most cost effective approaches for intestinal stem cells isolation that could prove useful for scaling up as would be required for clinical applicability, although this technique is disadvantaged by the inclusion of a heterogeneous population of isolated cells. Taken in aggregate, these studies provide a framework for the establishment of intestinal cultures that

when placed on an appropriate scaffold may be used in the implantation of an artificial intestine, as described below.

Early success in the development of an artificial intestine

Several investigators have been able to successfully replicate the growth of intestinal mucosa in culture, based upon an understanding of location of the intestinal stem cell within the crypts; studies that became significantly more successful after the components of the stem cell niche were elucidated [13,17,31]. Early attempts at growth of intestinal stem cells on a bioscaffold were described by Vacanti *et al.* in 1988, in which intestinal cells from crypts were seeded onto polymer fibers and implanted into either the omentum, the interscapular fat pad or the bowel mesentery of rats [25]. Further studies by the Vacanti group advanced the culture of small pieces of intestine from mice, so called “intestinal organoids”, which were seeded on a variety of scaffolds [42-47]. While histologic evidence of an engineered intestine was achieved, these studies did not reveal significant functional capacity and were limited by the small size of the tissue that could be achieved. More recent work by Grikscheit *et al.* has demonstrated the feasibility of implantation of organoid-based engineered intestine into rat omentum, which showed improved weight gain in a model of massive small bowel resection compared to those rats that did not receive engineered intestine [48]. To date the work of several groups, including the ones led by Drs. Stelzner, Dunn, Helmrath and Wells, have led to significant improvements in the optimization of culture techniques and demonstrated that implanted organoids can thrive and display architectural and functional features reminiscent of the native intestine [23,24,49,50]. More recent studies have shed light on the ability of intestinal stem cells to be maintained in culture on a synthetic scaffold *in vivo*. For instance, Grant *et al.*, using primary organoid cultures and nonwoven PGA/PLLA scaffolds, developed artificial intestine from mice and human organoids that demonstrated digestive function upon explantation after incubation for 4 weeks in the omentum of either genetically identical mice or immune deficient mice (for human-based organoids) [51]. This group also showed that multiple absorptive enzymes (Na-K ATPase, Na-Hydrogen exchanger protein, alkaline phosphatase, sucrose isomaltase, and aquaporin 7) localized at the apical side of the epithelium as well as demonstrated disaccharidase digestive function after explantation. This study also demonstrated the feasibility of using human organoids in an immune deficient mouse for study of human artificial intestine formation. Further, Wieck *et al.* demonstrated the ability of cultured murine enteric neurospheres to form enteric nervous system components when co-seeded with organoids isolated from mice with aganglionic colon and implanted in an omental model [52]. While not performed in the small intestine, this study is important as a first step towards engineering an intact enteric nervous system in artificial intestine. Finkbeiner *et al.* investigated the use of hESCs with different scaffolds as components for artificial intestine [53]. This study consisted of three groups: undifferentiated hESCs seeded on decellularized porcine or human small intestine, HIOs differentiated from hESCs seeded on decellularized small intestine or HIOs seeded on nonwoven PGA/PLLA scaffolds. They concluded that decellularized scaffolds could not direct differentiation of hESCs and that HIOs are needed for artificial intestine formation. They also found that despite the very good seeding of the HIOs on the decellularized small intestine *in vitro*, upon implantation in immunocompromised mice, only 2/7 scaffolds maintained cells of human lineage. In

contrast, HIOs that were seeded on PGA/PLLA scaffolds thrived *in vivo* and resembled native intestine after 12 weeks. More recently, our group used a synthetic porous PLGA scaffold to demonstrate that co-culture of organoids with macrophages and intestinal myofibroblasts enhanced organoid growth and differentiation. The constructs were also co-cultured with the probiotic bacteria *Lactobacillus rhamnosus*, which enhanced organoid proliferation and differentiation of Paneth cells. In contrast, co-culture with bacteria from the stool of infants with severe inflammatory disease adversely affected growth and differentiation. These constructs were implanted in the omenta of mice (using both murine and human organoids) and shown to support villi like structures within a tubular lumen that approximates the native intestine (See Figure 4 for a representative image). We also demonstrated that these constructs could be used in a canine mucosectomy model to restore mucosal integrity, and showed the constructs allowed for water absorption [31]. This study, along with Wieck *et al.*[52] are the first studies that have begun to perform co-cultures and experiments with IECs and other intestinal components.

Ongoing Challenges and Unanswered Questions

Although there have been significant strides made towards the ultimate goal of artificial intestine formation, there are many ongoing challenges in the field. As previously discussed, the ideal intestinal construct should be autologous and easily obtained, and would in practice be obtained at intestinal resection or during intestinal biopsy. Off the shelf intestinal lining – akin to that used for skin grafting – could be achieved, providing key antigenic components are neutralized [54]. Human Intestinal Organoids (HIOs) differentiated from induced Pluripotent Stem Cells (iPSCs) may provide additional advantages as the primary cell source for engineered intestine, largely due to the fact that these cells could be obtained from skin cell progenitors or fibroblasts of patients without requiring an intestinal biopsy. The ideal scaffold for intestinal engineering needs to be off the shelf, customizable, and easily produced, and must possess ideal properties for the diffusion for oxygen and nutrients. The integration of additional cell types within the artificial intestine in order to promote absorption, immune tolerance, and appropriate microbial communities, requires further study.

Conclusion

The development of an artificial intestine represents an important treatment for SBS towards the ultimate goal of restoring nutrient absorption and achievement of enteral autonomy. There has been recent progress towards this end with the greatest successes resulting from the discovery of the intestinal stem cell and its niche. Further studies are required for the development of an ideal scaffold, as well as understanding the integration of the immune system, the nervous system and the microbiome towards achievement of this goal for the benefit of these patients.

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Abbreviations

SBS	Short bowel syndrome
PN	parenteral nutrition
CBCs	crypt-based columnar cells
hESCs	human embryonic stem cells
HIOs	human intestinal organoids
iPSCs	induced pluripotent stem cells

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Key Points

- Short bowel syndrome is the inability to absorb adequate nutrition to support growth and maintenance of homeostasis.
- Current treatments for short bowel syndrome are often inadequate or have high complication rates, which is the impetus for research in the development of an artificial intestine.
- The success of artificial intestine formation to date has been due to identification of the Lgr5-positive crypt based columnar cell as the intestinal epithelial stem cell and an understanding of the niche in which it resides.
- Continued advancement in artificial intestine formation will rely on continued understanding of intestinal cell biology and the addition of system complexity to recapitulate all functions of the intestine and not just the absorptive function.

Figure 1.
Schematic diagram displaying the work-flow for the generation of a personalized artificial intestine for a patient with short bowel syndrome.

Figure 2. Growth and differentiation enteroids derived from normal human intestine
Representative micrograph of enteroid isolated from healthy neonatal small intestine at the time of ostomy closure stained for the goblet cell marker muc2 (red), nuclei (blue) and absorptive enterocytes (green).

Figure 3. Microscopic evidence of the growth and differentiation of intestinal stem cells on a bioscaffold

A: Scanning EM showing bioscaffold on which intestinal stem cells had been plated 48 h prior (arrows); **B:** confocal scan of cultured intestinal cells lining the bio-scaffold; **C:** Scanning EM of native mouse intestine showing remarkable similarity to bio-scaffold.

Figure 4. Implantation of a novel intestinal scaffold into mouse omentum maintains cell growth, which is donor derived

A: Representative H&E micrographs of the omentum from a mouse 28 days after implantation of synthetic scaffold that had been seeded with mouse-derived progenitor cells showing intact villous structure (**i**); higher magnification of the demarcated area is shown (**Aii**).