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Review

Clinical challenges of short bowel syndrome and the path forward for organoid-based regenerative medicine☆

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ABSTRACT

Short bowel syndrome (SBS) is a rare condition, the main symptom of which is malabsorption following extensive resection of the small intestine. Treatment for SBS is mainly supportive, consisting of supplementation, prevention and treatment of complications, and promotion of intestinal adaptation. While development of parenteral nutrition and drugs promoting intestinal adaptation has improved clinical outcomes, the prognosis of patients with SBS remains poor. Intestinal transplantation is the only curative therapy but its outcome is unsatisfactory. In the absence of definitive therapy, novel treatment is urgently needed. With the advent of intestinal organoids, research on the intestine has developed remarkably in recent years. Concepts such as the "tissue-engineered small intestine" and "small intestinalized colon," which create a functional small intestine by combining organoids with other technologies, are potentially novel regenerative therapeutic approaches for SBS. Although they are still under development and there are substantial issues to be resolved, the problems that have prevented establishment of the complex function and structure of the small intestine are gradually being overcome. This review discusses the current treatments for SBS, the fundamentals of the intestine and organoids, the current status of these new technologies, and future perspectives.

Contents

Abbreviations: CBCs, crypt base columnar cells; CRBSI, catheter-related blood stream infection; ENS, enteric nervous system; GLP-2, glucagon-like peptide-2; HIOs, human intestinal organoids; IF, intestinal failure; IFALD, intestinal failure-associated liver disease; IRP, intestinal rehabilitation program; ISCs, intestinal stem cells; LILT, longitudinal intestinal lengthening and tailoring; NEC, necrotizing enterocolitis; PN, parenteral nutrition; PSC, pluripotent stem cell; SBS, short bowel syndrome; SIBO, small intestinal bacterial overgrowth; SIC, small intestinalized colon; SILT, spiral intestinal lengthening and tailoring; STEP, serial transverse enteroplasty; TESI, tissue-engineered small intestine.

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1. Background

Short bowel syndrome (SBS) is a rare condition characterized by malnutrition and growth retardation after extensive surgical resection of the small intestine. The clinical manifestation of SBS is intestinal failure (IF), the severity of which varies widely according to type and the length of remaining small intestine [1]. A common issue is the need for supplemental treatment providing elements such as water, electrolytes, macronutrients (carbohydrates, proteins, and fats), and micronutrients (vitamins and minerals) owing to the shortened small intestine. Parenteral nutrition (PN) plays a vital role in compensating for these factors and improving the prognosis of patients with SBS [2]. However, long-term use of PN has disadvantages as well as benefits. Severe and life-threatening complications associated with long-term use of PN include IF-associated liver disease (IFALD), bacterial translocation, and catheter-related bloodstream infection (CRBSI) [3,4]. The recent challenges in treating SBS have focused on how to wean a patient from PN. Glucagon-like peptide-2 (GLP-2) analogs, which have recently become available as a novel treatment for SBS, can reduce the requirements for PN in PN-dependent patients and may help to improve their prognosis. However, intestinal transplant remains the only curative therapy for severe SBS. Nevertheless, the worldwide prognosis of post-intestinal transplant is disappointingly poor, with 5-year and 10-year survival rates of 58% and 47%, respectively, and a similar prognosis in Japan [5,6]. Therefore, development of novel fundamental treatments is urgently needed.

The intestine is a complex organ composed of multiple layers. Furthermore, its epithelium plays a variety of roles in digestion, absorption, and formation of an immune/mechanical barrier, and even secretes the hormone that affects all body systems. The intestinal epithelium maintains homeostasis by self-renewal of a small number of intestinal epithelial stem cells located at the bottom of the crypt [7]. Despite the difficulty of culturing intestinal stem cells (ISCs) *in vitro*, Sato et al. have succeeded in constructing three-dimensional tissue structures that mimic the structure of the intestinal epithelium and allow permanent self-renewal of ISCs and formation of organoids [8]. The epoch-making invention of organoids has led to rapid progress in many research fields, including elucidation of pathological conditions and development of therapeutic agents [9,10]. Furthermore, in recent years, development of organoid-based regenerative medicine has come into the spotlight as a new treatment for SBS [11,12]. Regenerative medicine involving the small intestine, once thought to be impractical in view of its complex structure and function, is now considered a feasible approach, while there are still many challenges to overcome. This review provides an overview of SBS, intestinal organoids, and the future potential of regenerative medicine for SBS, primarily focusing on therapeutic use of organoids.

2. Short bowel syndrome

2.1. What is SBS?

SBS in adults is defined by the European Society of Enteral and Parenteral Nutrition as “The clinical condition associated with the remaining small bowel in continuity of less than 200 cm” However, the definition varies from country to country and between different societies and papers. The definition of SBS in children is even more inconsistent. Such inconsistency has made it difficult to estimate the exact incidence, prevalence, and mortality of SBS in children [13,14]. To address this knowledge gap, an International Intestinal Transplant Registry has been established to improve our knowledge about the epidemiology of SBS. A Canadian study found that the incidence of SBS in children was approximately 24.5 per 100,000 live births [15]. The prevalence of SBS in adults is generally estimated based on the number of patients who receive long-term PN for IF [16]. The annual prevalence of SBS in Europe and the US has been reported to be 1.4 and 30 per million, respectively [17,18].

The diseases that cause SBS are manifold. Necrotizing enterocolitis (NEC) is the most common cause of SBS in children, accounting for at least 30% of reported cases [4]. NEC is a severe disease that occurs most frequently in preterm infants with a birth weight of less than 1500 g [19,20]. The number of patients with SBS secondary to NEC is expected to increase in the future because developments in neonatal intensive care have allowed resuscitation of extremely premature infants. Other common causes of pediatric SBS are congenital diseases such as intestinal atresia, gastroschisis, malrotation with volvulus, and Hirschsprung's disease of the extensively aganglionosis type [4,21–23]. In adults, the leading causes of SBS are Crohn's disease, mesenteric ischemia, and surgical complications, which account for the majority of cases [1,21,24]. The numbers of patients requiring mass resection of the intestine and multiple surgeries have been decreasing as a result of recent advances in immunomodulatory therapy for Crohn's disease, but IF still occurs [3,25,26]. Severe mesenteric ischemia requires massive intestinal resection and often causes SBS [27]. Arterial embolism accounts for 40%–50% of all cases of mesenteric ischemia, while mesenteric thrombosis accounts for 20%–35% and nonocclusive mesenteric ischemia for 5%–15% [28,29].

The mortality rate in patients with SBS varies from study to study, ranging from 5% to 50% [4]. This wide range in reported mortality rates may partly reflect patient selection bias in the different studies, considering that many factors contribute to the clinical course of SBS. These factors include the length of the intestine, the function and adaptability of the remaining intestine, the underlying cause of SBS, and the presence or absence of the ileocecal valve and colon [13,30].

2.2. Therapeutic approaches

Clinical management is essential to maintain quality of life in patients with SBS. The therapeutic approach for SBS can be broadly divided into two categories: one is a management strategy, which includes supplementing factors involved in malabsorption and preventing/treating complications, and the other is a treatment strategy aiming at achieving enteral autonomy by treatment, such as promoting intestinal adaptation, intestinal lengthening procedures, and intestinal transplantation (Fig. 1). The goal of enteral autonomy is to improve quality of life by weaning patients from PN and improves the prognosis by reducing the risk of PN-related complications and ameliorating IF. Intestinal transplantation may also be performed in cases where severe complications or loss of a central venous catheter access route necessitate urgent enteral autonomy. The goal of enteral autonomy contributes to management by reducing the risk of complications, while effective management promotes intestinal adaptation and brings the patient closer to enteral autonomy. Thus, management and treatment aiming at enteral autonomy are not separate but overlap with each other, and the intestinal rehabilitation program (IRP) is recognized as extremely important for comprehensive implementation of these treatments. The IRP is an integrated care and treatment strategy for patients with SBS, involves multiple health care professionals, including physicians, pharmacists, nurses, dietitians, therapists/physical therapists, transplant coordinators, psychologists, and social workers, and has become the standard of care for patients with IF since its usefulness was first demonstrated by Kocher et al., in 2000 [31,32]. This stepwise and multidisciplinary program aims to enhance remaining intestinal function and wean patients from PN and has dramatically improved the prognosis of SBS and decreased mortality [33,34].

2.2.1. Management

Control of diarrhea, which is a common symptom in patients with SBS, has a significant impact on patients' quality of life and survival. Effective antimotility agents include loperamide and diphenoxylate/atropine, which inhibit intestinal smooth muscle contractions [2]. However, control of diarrhea is often challenging, especially in the acute phase. PN plays an important role in providing the nutrients necessary for survival in patients with SBS, who have reduced intestinal absorption [2]. However, long-term PN carries the risk of complications, such as CRBSI, IFALD, and growth retardation in children. Therefore, it is important to combine enteral nutrition with oral supplementation whenever possible, while taking precautions to avoid these serious complications.

Complications of SBS can be divided into two types: those caused by SBS, such as diarrhea, small intestinal bacterial overgrowth (SIBO), renal stones/failure, cholecystolithiasis, osteoporosis, and gastroduodenal ulcer, and those caused by long-term PN, such as CRBSI, occlusions, thrombosis, and IFALD. An overview of the main complications is shown in Fig. 1. CRBSI is a common complication in patients receiving long-term PN. The overall CRBSI rate is 0.87–1.35 per 1000 catheter days [35,36]. Patients who develop CRBSI need to be hospitalized for antimicrobial treatment and the central venous catheter often requires replacement. Access routes to the central venous system are often limited in patients with SBS, making removal of a catheter placed for CRBSI a critical concern. Measures to prevent CRBSI include ethanol or antibiotics lock during periods of non-use to maintain catheter patency and reduce the risk of infection [37,38]. A randomized controlled trial has shown that a taurolidine-based catheter lock solution significantly reduces the incidence of CRBSI [39].

IFALD is another serious complication associated with long-term PN for conditions such as SBS and has a significant impact on patient outcomes. Although the etiology of this disease remains unclear, background factors for IFALD include inadequate nutritional management, prolonged use of PN, and prematurity in neonates; infections, in particular CRBSI, and enteritis can also trigger development of IFALD [40,41]. Previously known as PN-related liver disease, this condition is now known as IFALD because it is not

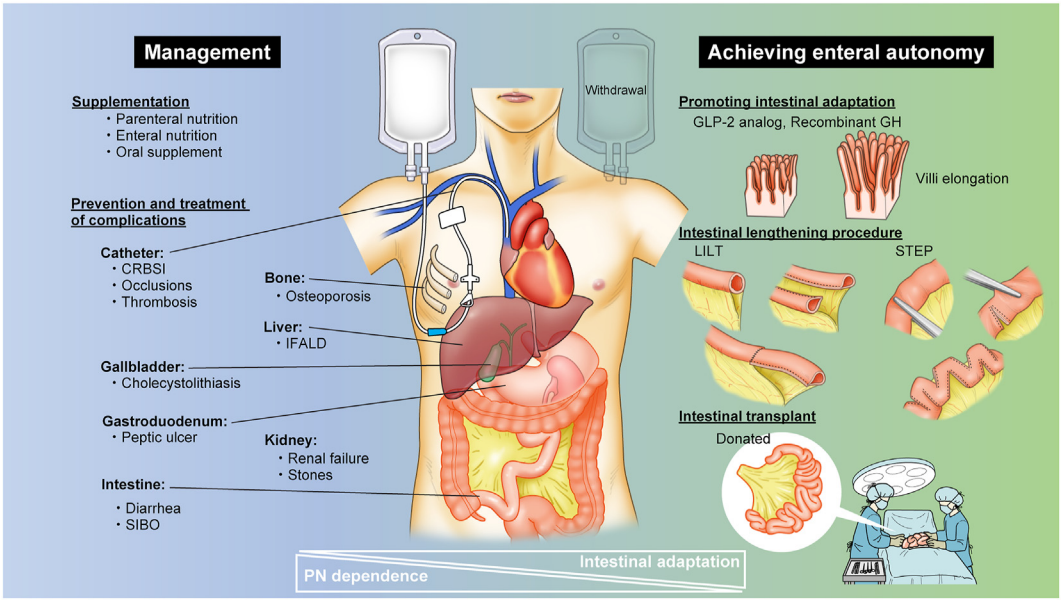

Fig. 1. Current treatment approaches for patients with SBS. Treatment of SBS includes management with the aim of enteral autonomy. Management is essential for survival and includes supplementation of nutrients and prevention/treatment of complications. Complications are manifold, with some associated with PN and some with SBS itself. Weaning off PN is crucial to prevent complications such as IFALD and CRBSI. Treatment for achieving enteral autonomy and withdrawing PN includes promoting intestinal adaptation, intestinal lengthening procedures, and intestinal transplant, which is the only curative therapy. Given that withdrawal of PN helps to reduce the risk of complications, management and treatment aimed at enteral autonomy cannot be clearly separated. CRBSI, catheter-related blood stream infection; GH, growth hormone; GLP-2, glucagon-like peptide-2; IF, intestinal failure; IFALD, intestinal failure-associated liver disease; LILT, longitudinal intestinal lengthening and tailoring; PN, parenteral nutrition; SBS, short bowel syndrome; SIBO, small intestinal bacterial overgrowth.

necessarily associated with PN. The incidence of IFALD has been reported to be 20% in pediatric patients receiving long-term PN, with 10% of cases progressing to end-stage liver disease [42]. The disease usually stabilizes and improves when intestinal adaptation is achieved, but when the disease progresses to end-stage liver disease, combined liver and intestinal transplantation is required for survival. Hence, prevention and treatment of IFALD is of significant importance. Omegaven, a fish-oil-based emulsion containing omega-3 fatty acids, is effective for prevention and/or treatment of IFALD [43,44]. However, long-term use of Omegaven alone can lead to a deficiency of essential fatty acids. Therefore, SMOF lipid, a fatty mixture of soybean, coconut, olive, and fish oils, has recently been used in Europe [45].

SIBO is another well-known complication in patients with SBS and is caused primarily by motility disorders and anatomical changes. SIBO leads to inflammation of the mucosa, resulting in malabsorption and deficiencies of essential nutrients, including fat-soluble vitamins, calcium, and iron [46]. Furthermore, decreased local immunity contributes to increased gut permeability, exposing the patient to risks of bacterial translocation, sepsis, and D-lactic acidosis [47]. Antibiotics and promotion of intestinal motility are effective in improving SIBO. Extensive removal of the small intestine generally reduces the negative feedback mechanism of the intestine that inhibits gastric acid secretion, which may further impair digestion and absorption and increase the risk of developing peptic ulcer disease. To address this problem, proton pump inhibitors should be used to suppress secretion of gastric acid [48–50]. Conversely, decreased gastric acidity may increase the proliferation of intestinal bacteria which lead to SIBO and requires careful consideration [46].

2.2.2. Treatment to achieve enteral autonomy

Intestinal adaptation is the intestinal response to restore total gastrointestinal absorption of nutrients and fluids to preoperative conditions after intestinal resection [51]. This is achieved by an increase in the area of absorption in the remaining intestine and slowing gastrointestinal transit. Drugs that promote intestinal adaptation through the action of hormones include somatropin, a recombinant human growth hormone, and teduglutide, a GLP-2 analog [2]. Growth hormone in combination with glutamine and other dietary treatments for 4 weeks has been shown to significantly reduce PN requirements [52]. GLP-2 is thought to be produced by L-cells in the terminal ileum and colon in response to stimulation by nutrients in the intestinal lumen [53]. The effects of GLP-2 on the intestinal tract include an increase in villus height, proliferation of crypt cells, an increase in intestinal blood flow, maintenance of the mucosal barrier, and inhibition of gastrointestinal motility and gastric acid secretion [54–57]. In view of GLP-2 being an endocrine hormone with a short half-life of 7 min, a GLP-2 analog, teduglutide, which is more resistant to degradation of dipeptidyl peptidase-4, has been developed [58,59]. Apraglutide and glepaglutide, which have longer half-lives, are under clinical trials [60,61]. The long-term prognosis of GLP-2 analogs is unknown, and even if PN can be reduced or discontinued, nutritional status and adverse events should continue to be monitored.

Intestinal lengthening is a surgical approach that has been shown to promote enteral feeding and wean the patient from PN, especially in pediatric SBS [30,62]. Two well-known intestinal lengthening procedures are available; one is longitudinal intestinal lengthening and tailoring (LILT), first reported by Bianchi et al., in 1980, and the other is serial transverse enteroplasty (STEP) by Kim et al., in 2003 [63,64]. LILT is a novel technique that involves dividing the expanded remaining small intestine in a longitudinal direction, which results in doubling of the length of the intestine. STEP, the intestinal tract is lengthened by making alternating zigzag incisions perpendicular to the longitudinal axis of the remaining intestinal tract [65]. These operations help to improve the efficiency of nutrient absorption and also reduce abnormal growth of intestinal bacteria, resulting in promotion of enteral autonomy [66]. Recently, spiral intestinal lengthening and tailoring (SILT), another intestinal lengthening technique, was proposed by Cserni et al. [67,68]. SILT has the advantage of being able to be performed for the small intestine with a lesser degree of dilation [69]. However, the outcome of this technique has not been well studied, and an adequate comparative study for these intestinal lengthening procedures is needed. There are no clear criteria with regard to the indications for intestinal lengthening; however, it should be considered as an important means of IRP for the purpose of reducing repeated SIBO and dependence on PN.

The development of medical therapies such as IRP and the advent of innovative lipid emulsions has dramatically decreased the mortality rate in patients with IF on the transplant waiting list and reduced the need for intestinal transplantation [33]. The number of intestinal transplants has been declining after peaking at 198 per year in 2007 in the US and 270 per year in 2008 worldwide [70–72]. However, intestinal transplantation remains the only curative therapy for severe SBS. A statement from the American Society of Transplantation, revised in 2019, states that all patients with permanent IF should be managed with IRP and that intestinal transplantation should be considered for patients with progressive IFALD, those with progressive loss of central venous access, and those with repeated and severe life-threatening CRBSI [73]. The results of intestinal transplantation have improved greatly over the last two decades, owing mainly to the advent of immunosuppressive agents [71,74]. Based on data from the worldwide Intestinal Transplant Registry in 2015, patient and graft survival rates were reported to be 58% and 50%, respectively, at 5 years and 47% and 41% at 10 years [5]. Nutritional outcomes are favorable, with most recipients being able to be weaned from PN and achieving enteral autonomy once engraftments are successful [74]. Therefore, immunosuppressants contribute greatly to treatment outcomes, but transplant recipients are required to continue taking immunosuppressive drugs lifelong. Immunosuppressive drugs have potentially lethal risks, including opportunistic infections and post-transplant lymphoproliferative disorders [75]. Moreover calcineurin inhibitors, which are commonly used for immunosuppression after intestinal transplant, may cause renal impairment [76]. Despite the remarkable improvement in transplantation outcomes owing to the advent of immunosuppressive drugs, the prospects for further improvement in outcomes are not promising, and the disadvantages of immunosuppressive agents cannot be ignored. For these reasons, innovative treatment methods are urgently needed.

3. Intestinal research

3.1. Intestinal anatomy and function

The intestine is a multilayered organ consisting of a mucosa with epithelium, lamina propria and muscularis mucosae as the internal uppermost layer, a submucosa with a network of lymphovascular vessels, a muscle layer consisting of inner circular and outer longitudinal muscle layers, a subserosa, and an outermost layer known as the serosa (Fig. 2). However, its components are much more diverse and include the mucosal epithelium, which provides a mechanical and immune barrier to the external environment and is responsible for digestion and absorption, mesenchymal cells, including fibroblasts and myofibroblasts, which provide the niche necessary for the diversity of the mucosal epithelium, muscles, and the enteric nervous system (ENS), which are essential for peristalsis and efficient transport of the intestinal

contents, and blood vessels and lymphatics, which are essential for feeding and absorption [77]. The ENS is not only involved in motility but also plays a critical role in digestion, immunity, and homeostasis by acting on various cells to regulate secretion, intestinal blood flow, release of hormone, and interaction with the microbiome [78,79]. There are some differences in the functions and structures of the small intestine (duodenum, jejunum, and ileum) and large intestine (colon and rectum) (Fig. 2). The most noticeable difference is in the mucosa, which has a major role in absorption. The mucosa of the small intestine forms a ring-shaped fold, which consists of circular folds (valves of Kerckring) that help to increase the absorption area. The epithelium of the small intestine is composed of luminally protruding structures called villi and concave structures called crypts, which promote efficient absorption. The epithelium of the large intestine lacks villi and instead has deeper crypt structures. The structures of the small and large intestines, other than the mucosa, are generally similar, although their thicknesses are different [80,81].

3.2. The impact of organoids on the intestinal research

The intestinal epithelium is one of the tissues with the most rapid cell renewal cycle and has both absorption and barrier functions, being in contact with food for nutrient absorption as well as being exposed to a variety of microorganisms, including pathogenic bacteria and viruses. The role of ISCs, which possess differentiation and long-term self-renewal capacities, is crucial in maintaining the complex functions and structures of the intestine [82]. It has long been known that there are stem cells in the intestinal epithelium but they have not been specifically identified, resulting in limited availability of appropriate research tools. In 2007, crypt base columnar cells (CBCs) labeled with Lgr5 were eventually confirmed to function as ISCs [7]. This landmark discovery has led to a better understanding of ISCs and research into the niche that regulates their functions. Subsequently, Sato et al. found that by embedding ISCs from mouse small intestine in the extracellular matrix Matrigel and culturing them in a medium that contained niche factors, including an epithelial growth factor, Noggin (a bone morphogenic protein inhibitor), and R-spondin1 (a Wnt activator), they were able to build three-dimensional structural organoids that can be cultured *in vitro* for a long time [8]. The niche for generating these organoids varies among species and organs. For example, in the mouse colon, Wnt-3a and a transforming growth factor-β inhibitor are required in addition to the culture medium for the small intestine [83]. Furthermore, addition of insulin-like growth factor-1 and fibroblast growth factor-2 can form human ISC-derived organoids that maintain gene expression patterns that are highly homologous to the intestinal epithelium *in vivo* [84]. Therefore, organoids can represent complex and diverse cellular functions and are now intensive research tools for the intestinal epithelium.

Intestinal epithelial cells are broadly divided into absorptive and secretory cells, all of which originate from CBCs [85]. CBCs in the small intestine reside at the base of the crypts and are surrounded by Paneth cells, which are one of the types of secretory cells, providing a necessary niche as stem cells [86,87]. Paneth cells also produce other antimicrobial molecules, including defensin family proteins and Regenerating islet-derived protein 3 and have a barrier role in the intestine [88]. Other secretory cells include goblet cells, which produce mucus that acts as a barrier and lubricates the intestinal surface; chemosensory tuft cells, which are involved in taste and the immune response; and enteroendocrine cells, which secrete hormones involved in various biological reactions, including intestinal peristalsis, gastric and pancreatic fluids, and insulin secretion [85,89]. Absorptive cells, which are involved in digestion and absorption, are characterized by brush borders with dense microvilli to increase absorption efficiency and comprise the majority of differentiated cells [90]. Microfold (M) cells play a key role in intestinal immunosensing by transporting antigens in the intestinal lining and activating immune cells [91]. More recently,

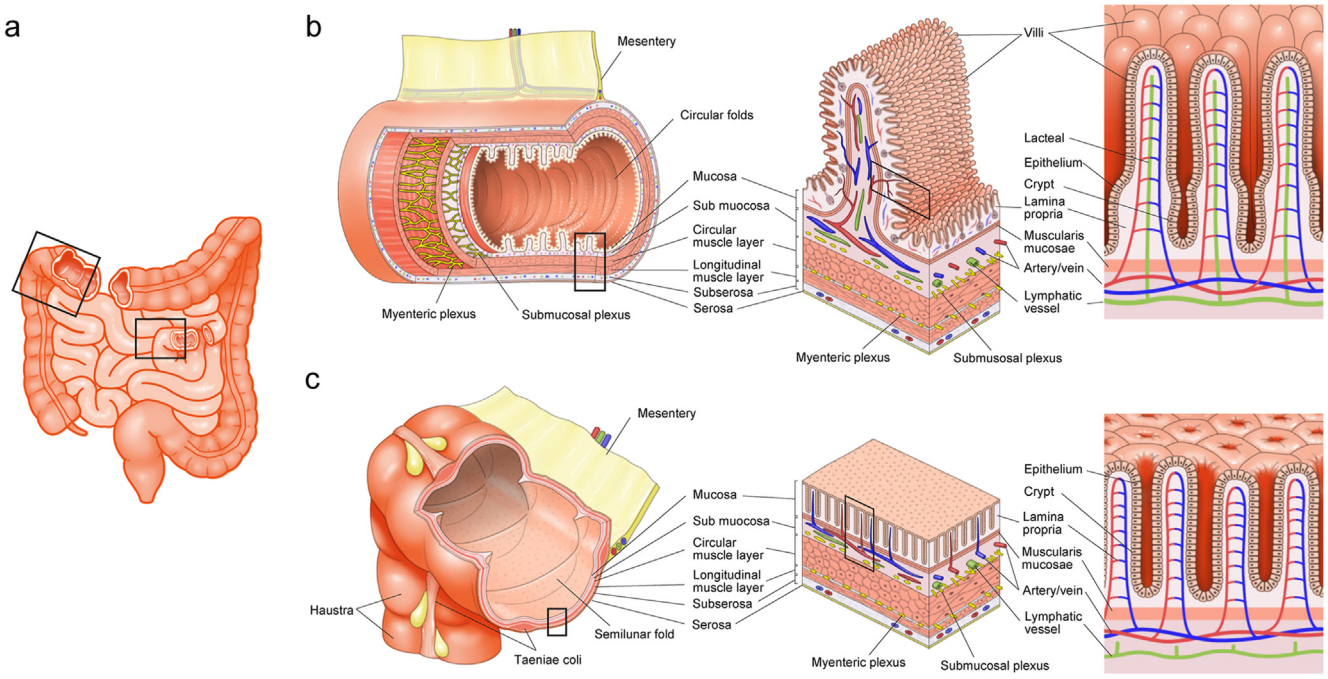

Fig. 2. Schematic of construction of the small intestine and colon. (a) The lower gastrointestinal tract includes the small intestine and the large intestine. The major difference between (b) the small intestine and (c) the colon is in the mucosa; otherwise, the structures are very similar. The mucosa of the small intestine has villi containing lacteals whereas the colon has deep crypts without villi.

single-cell analysis has identified BEST4/OTOP2 cells as a cell type in the absorptive lineage in humans [92]. That research suggested that BEST4/OTOP2 cells may be involved in regulation of intestinal pH, but their function is still not known in detail. The component cells of the colonic epithelium are similar to those of the small intestine, with some differences. Paneth cells are absent in normal colonic epithelium. In the murine colon, the CBC is surrounded by deep crypt secretory cells that serve as the niche for CBCs [93], but cells with a similar function have not yet been identified in the human colon. In the large intestine, which contains far more intestinal bacteria than does the small intestine, the number of goblet cells is much higher and two layers of thick mucus cover the intestinal epithelial cells, preventing foreign invasion [94].

4. Regenerative medicine: a therapeutic approach for SBS

Regenerative medicine using organoids is attracting attention as a future treatment for SBS, for which there are currently few curative treatments. As previously described [8,84], organoids can reproduce the epithelium of the small intestine but cannot generate the small intestine itself with use of only organoids as cells. Therefore, several concepts that involve combining organoids with other technologies to produce small intestinal tissue have been suggested (Fig. 3). For example, there have been a report of tissue-engineered small intestine (TESI) created by seeding organoids on a decellularized scaffold of patient-derived colon [95]. In that study, small intestine-like structures were generated using a decellularized colon scaffold and organoids derived from patients with IF. Other concepts proposed by Brassard et al. combine bioprinting technology, which allows for controlled placement of cells in three-dimensional space, with organoids to create the small intestine [96]. They succeeded in creating centimeter-scale tubular intestinal epithelium with crypt-villus-like morphology, connective tissues, and vascular networks by three-dimensionally printed human umbilical vein endothelial cells, mesenchymal stem/progenitor cells, and intestinal organoids in a systematic manner. However, as mentioned above, although some of these intestinal structures could be constructed, the intestine is a multilayered organ with very complex structures and functions, and it remains challenging to create all these structures using the current regenerative technologies. This technical hurdle prompted us to establish a novel concept of the “small intestinalized colon” (SIC), in which the epithelium of the large intestine is removed and replaced by transplanted organoids derived from the small intestine (Fig. 3c). This section provides an overview of the current TESI technology and the emerging concept of SIC and discusses their strengths and weaknesses.

4.1. Generation of functional TESI

The combination of organoids and biodegradable tissue scaffolds such as polyglycolic acid, polycaprolactone, chitosan, collagen, and decellularized tissue with organoids has enabled the construction of intestinal epithelia [97–101]. Despite this significant step towards building the epithelium, there remain vital issues pertinent to organ complexity, such as harnessing muscle layers, the neural network, and lymphovascular structures to TESI. For example, non-epithelial cells, such as myofibroblasts, endothelial cells, macrophages, and the ENS, not only play a pivotal role in supporting the intestinal structures but also form a niche to maintain ISCs [102]. These niche cells produce essential niche factors used in organoid culture, including Wnt proteins, R-spondins, and bone morphogenetic protein inhibitors [8]. Despite their importance, the installation of the mesenchymal components into TESI has yet to be achieved, which raises concerns regarding the maintenance of stem cells in TESI. This is in contrast with the

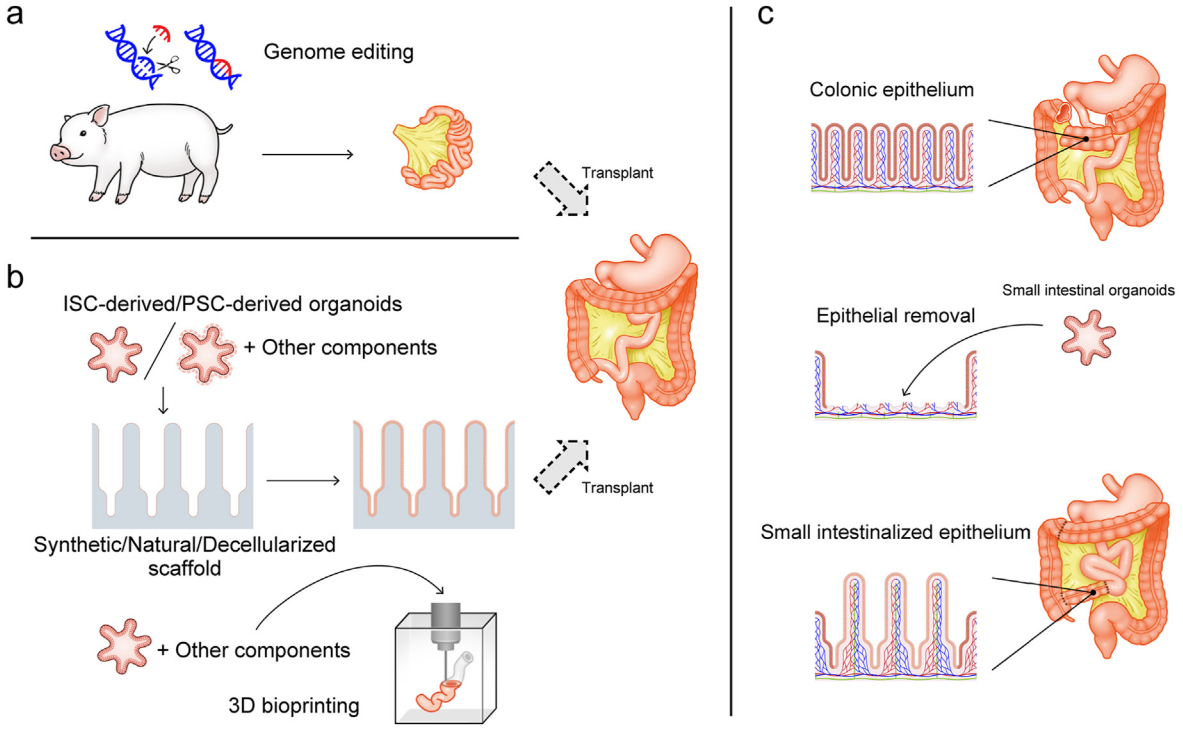

Fig. 3. Future therapeutic strategies for patients with SBS. (a) Xenotransplantation using genetically modified porcine small intestine, (b) TESI using small intestinal organoids and various components to create small intestine by seeding on scaffolds or three-dimensional bioprinting, and (c) SIC using intestinal organoids to replace the epithelium of the colon with small intestinal epithelium are expected to be future treatments for patients with SBS. ISC, intestinal stem cell; PSC, pluripotent stem cell; SBS, short bowel syndrome; SIC, small intestinalized colon; TESI, tissue-engineered small intestine.

pluripotent stem cell-derived human intestinal organoids (HIOs) that develop into both intestinal epithelium and surrounding mesenchyme and stably self-organize their structures upon transplantation [103].

The blood supply for TESI is provided by angiogenesis from the recipient-derived tissues [97,104]. This *in vivo* angiogenesis from the recipient is not controllable and requires further improvement. There has been a recent report of a potential solution to this problem, which entails generation of decellularized intestine from the superior mesenteric artery using detergent perfusion. This method preserves a villous vascular network scaffold that could be lined by human umbilical vein endothelial cells seeded from the vascular scaffold [105]. Importantly, this vascularized TESI has shown an ability to absorb glucose in recipient rats [105]. TESI also requires the ENS and muscles for normal function of the intestine. Although the combination of pluripotent stem cell-derived enteric neural crest cells and HIOs have generated intestinal structures with neural innervation [106–108], similar complete functions has not been achieved in TESI.

4.2. Small intestinalized colon

It is extremely difficult to reconstruct the complex structure of the intestine to a stage where it can be transplanted into humans, and a different approach is necessary. Therefore, we focused on the similarity between the colon and the small intestine below the submucosa (Fig. 2) and hypothesized that the colon could be repurposed as the small intestine by replacing only the epithelium of the colon with the small intestine by transplantation of small intestinal organoids [109]. Transanal transplantation of mouse colonic organoids into epithelium-disrupted mouse colon has been shown to form functionally and histologically normal crypts [110]. Another study found that transanal transplantation of mouse small intestinal organoids into mouse colon could replace the epithelium with the small intestinal organoids in a small portion of the colon near the anus [111]. Although the potential of epithelial replacement was suggested, it was obvious that this method would not be effective in treating SBS because the replaced epithelium had only a small area and, most importantly, the villi of the small intestine were not formed sufficiently to function in the same way as the native small intestine. Considering that villus formation is essential for construction of a functional small intestine, we focused on factors that promote formation of villi and, in particular, investigated the possibility that luminal flow might be mandatory [109]. The water content ratio of the intestinal contents is higher in the small intestine, and transit flow in the lumen is more rapid than that in the large intestine. Indeed, patients who have been subjected to dietary restrictions or undergone stoma creation have reduced flow because food and water cannot pass through the small intestine, resulting in villus atrophy [112,113]. Conversely, villi will develop if the flow is restarted [114]. With this clinical knowledge in the background, we speculated that if the transplanted small intestinal organoids were exposed to a high-flow environment similar to that of the small intestine, a normal small intestinal epithelium with well-developed villi could be reconstructed. We were able to confirm the formation of villi-like structures in two-dimensional-cultured small intestinal epithelium by adding artificial flow, which supported this hypothesis [109].

For *in vivo* validation, we isolated a 4-cm-long segment of the ascending colon in a rat model and transplanted small intestinal organoids into the site where the epithelium was removed. When the graft was constructed as a stoma on both the oral and anal sides to prevent passage of stool and luminal flow, engraftment of small intestinal organoid-derived epithelium was observed on the graft, as expected, but without sufficient villi formation. The immature SIC was then anastomosed to the end of the ileum, placing the SIC in the same flow environment as the original small intestine. Consequently, as we hypothesized, small intestinal villi formed in the epithelium of the SIC interposed between the jejunum and the terminal ileum. The mature SIC formed lacteals, blood vessels, and nerve connections and performed the essential functions of the small intestine, including absorption of lipids, sugars, and peptides and peristalsis. In a rat model of short bowel syndrome, SIC contributed to improved survival [109]. At present, no other regenerative medicine approach can improve the survival rate even in rodents with SBS. This strategy for creating SIC is considered more feasible than conventional TESI because it has the very strong advantage of using the submucosa and deeper layers of the colon, thereby eliminating the need to construct non-epithelial layers *de novo*. This strategy has some limitations, including the fact that the small intestine is created using patient-derived colon, which makes this method unsuitable for patients with SBS and a short available colon. Nevertheless, it is a treatment approach that has the potential to become an option for patients with SBS in the future.

5. Future perspectives

We have discussed the limitations of PSC/ISC-derived organoid-based TESI and SIC and the challenges that need to be overcome. However, the fundamental question may be whether organoids can be used to treat human diseases. Induced PSC can cause cancers and human embryonic stem cells involve ethical controversial. In this regard, development of regenerative medicine for colorectal disease would be instructive. ISC-derived human colonic organoids have been successfully transplanted orthotopically into the immunodeficient mice colon [115]. Interestingly, transplanted human colonic organoids were reconstructed in the mouse colon as human colonic crypts with mucus and other properties that differed from those of the mouse. Furthermore, the transplanted human colonic organoids grew tumor-free for more than 10 months, providing the first data indicating that human ISC-derived organoids do not form tumors *in vivo* and a basis for confirming the safety of therapeutic use of organoids. This ability has not only become a powerful tool for the study of human colonic epithelium *in vivo* [116], but also for *in vivo* analysis of other types of organoids [117,118]. Most importantly, it has increased the likelihood of transplantation therapy of intestinal organoids in humans. Recently, a first-in-human clinical trial was designed and approved in Japan based on the concept of organoid transplantation for dextran sulfate sodium-induced colitis [110], and organoid transplantation for patients with ulcerative colitis has been realized [119]. Organoid transplantation therapy in humans is already a reality, and the future of organoid-based therapy seems promising. Although many challenges remain, we believe that our therapeutic concept of SIC could also be clinically applicable in the future. Despite the ethical issues of human embryonic stem cells and the possible carcinogenicity of PSCs, PSC-derived transplants and allogeneic transplants, which have advantages in terms of acquiring non-existent tissue, may find their way in the future as research progresses. The successful transplantation of PSC-derived HIOs into the colon of immunodeficient mice [120], and the stocking plan for human induced PSCs with a homozygous human leukocyte antigen, which is characterized by less immune rejection, may lead to the future PSC-derived organoid therapy [121].

In addition to the organoid-based TESI and SIC already mentioned, xenotransplantation of small intestines obtained from genetically modified pigs, for example, is one promising solution for the future treatment of SBS (Fig. 3a). Hyperacute rejection, the primary challenging of this model, can be avoided by using pigs

with knockout of alpha-1,3-galactosyltransferase, as demonstrated in a 54-h kidney xenotransplantation experiment in a brain-dead patient [122]. However, the long-term outcome is unknown, and owing to the complex immune defenses of the small intestine, rejection is more difficult to suppress than with other organ transplants. Indeed, graft survival rates are markedly worse than for other organs [123]. Therefore, the hurdles to achieving xenotransplantation of the small intestine are still considered to be high. Even if this difficulty can be overcome, xenotransplant recipients would not be free from immunosuppressive therapy and would still be at constant risk of rejection and infection. The benefit will be limited to the elimination of the donor shortage. If the need for immunosuppression of xenografts could be eliminated, this would be a very significant step forward.

In conclusion, regenerative therapies, such as TESI and SIC, and xenotransplantation are being investigated as future treatments for patients with SBS, although all approaches have various issues to be resolved. No definitive treatment for SBS has been established as yet, but we are hopeful that further research in this field will lead to development of innovative treatments for patients with SBS and improve their prognosis.

Declaration of competing interest

T.S. is the holder of several patents related to organoid culture. The remaining authors disclose that they have no conflicts of interest related to this article.

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