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Review

Organoids as regenerative medicine for inflammatory bowel disease

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SUMMARY

Inflammatory bowel disease (IBD) is a chronic disorder with an increasing global prevalence. Managing disease activity relies on various pharmacological options. However, the effectiveness of current therapeutics is limited and not universally applicable to all patients and circumstances. Consequently, developing new management strategies is necessary. Recent advances in endoscopically obtained intestinal biopsy specimens have highlighted the potential of intestinal epithelial organoid transplantation as a novel therapeutic approach. Experimental studies using murine and human organoid transplantations have shown promising outcomes, including tissue regeneration and functional recovery. Human trials with organoid therapy have commenced; thus, this article provides readers with insights into the necessity and potential of intestinal organoid transplantation as a new regenerative therapeutic option in clinical settings and explores its associated challenges.

INTRODUCTION

Inflammatory bowel disease (IBD) encompasses chronic disorders of the gastrointestinal tract and is characterized by relapsing and remitting phases. Ulcerative colitis (UC)[1] and Crohn's disease (CD)[2] are the two most prevalent IBD subtypes, followed by microscopic colitis in older adults.[3] Despite unchanged diagnostic criteria, the incidence and occurrence of IBD have been steadily increasing for decades, with global prevalence increasing from 3.3 million individuals in 1990 to approximately 10 million in 2024 – a figure that continues to rise.[4,5] This increase is particularly pronounced in newly industrialized countries, particularly in East Asia.[6] The etiology of IBD includes a defect in the single layer of intestinal epithelial cells (IECs) that separates the external environment (i.e., substances in the intestinal lumen) from the immune system of the host,[7] resulting in a condition known as "leaky gut"[8] (Figure 1). These defects in the intestinal barrier enable the luminal microbiota and dietary antigens to gain access to the intestinal lamina propria, thereby triggering an undesirable intestinal immune response in the affected host.[9]

IBD typically requires lifelong pharmaceutical intervention to induce and maintain remission.[10] However, no single therapeutic strategy currently meets the diverse needs of patients. Certain existing therapeutics for IBD are associated with adverse events, including an elevated risk of infectious and neoplastic complications.[11–13] Consequently, an unmet need remains for therapeutics that can complement the existing therapies that rely heavily on immunosuppression to control the disease. A potential solution might be the use of stem cell therapy with the transplantation of IECs grown *in vitro* into the affected area of the intestine, a concept that is detailed in this review.

INTESTINAL BARRIER

The intestinal barrier, which serves as a defense line between the host and the environment, plays a pivotal role in the interplay between the gut microbiota and host immune system.[14] The intestinal wall features pocket-like structures called crypts, in which cells are protected from the chemical and mechanical stresses of luminal contents. Stem cells fuel tissue renewal at the bottom of each intestinal crypt, producing different intestinal cellular lineages. When stem cells are depleted by injury or ablation, the differentiated cells undergo dedifferentiation to replenish lost stem cells within a few days.[15,16] However, the persistent depletion of intestinal stem cells may result in regeneration failure,[14] which may severely compromise the intestinal barrier and homeostasis. One potential cause of IBD is the breakdown of a well-functioning intestinal barrier, leading to a "leaky gut."[17] This is illustrated in Figure 1.

Due to the importance of a functional intestinal barrier, epithelial wound healing is a major target goal of IBD therapies.[18,19] This encompasses both mucosal healing[19,20] and deep remission.[21] UC is restricted to the submucosa of the colon,[1] and does thus not involve deep remission, whereas CD may vary in its anatomical extension throughout the GI tract and further through all the layers of the intestinal wall.[2,20] Therapeutic use of intestinal organoids may directly contribute to epithelial restitution, heavily contributing to mucosal healing, and is therefore therapeutically considered more relevant for the management of UC. Although autologous intestinal organoids consist exclusively of epithelial cells (see *"Human organoids" section*) they are accordingly unable to directly facilitate deep remission or transmural

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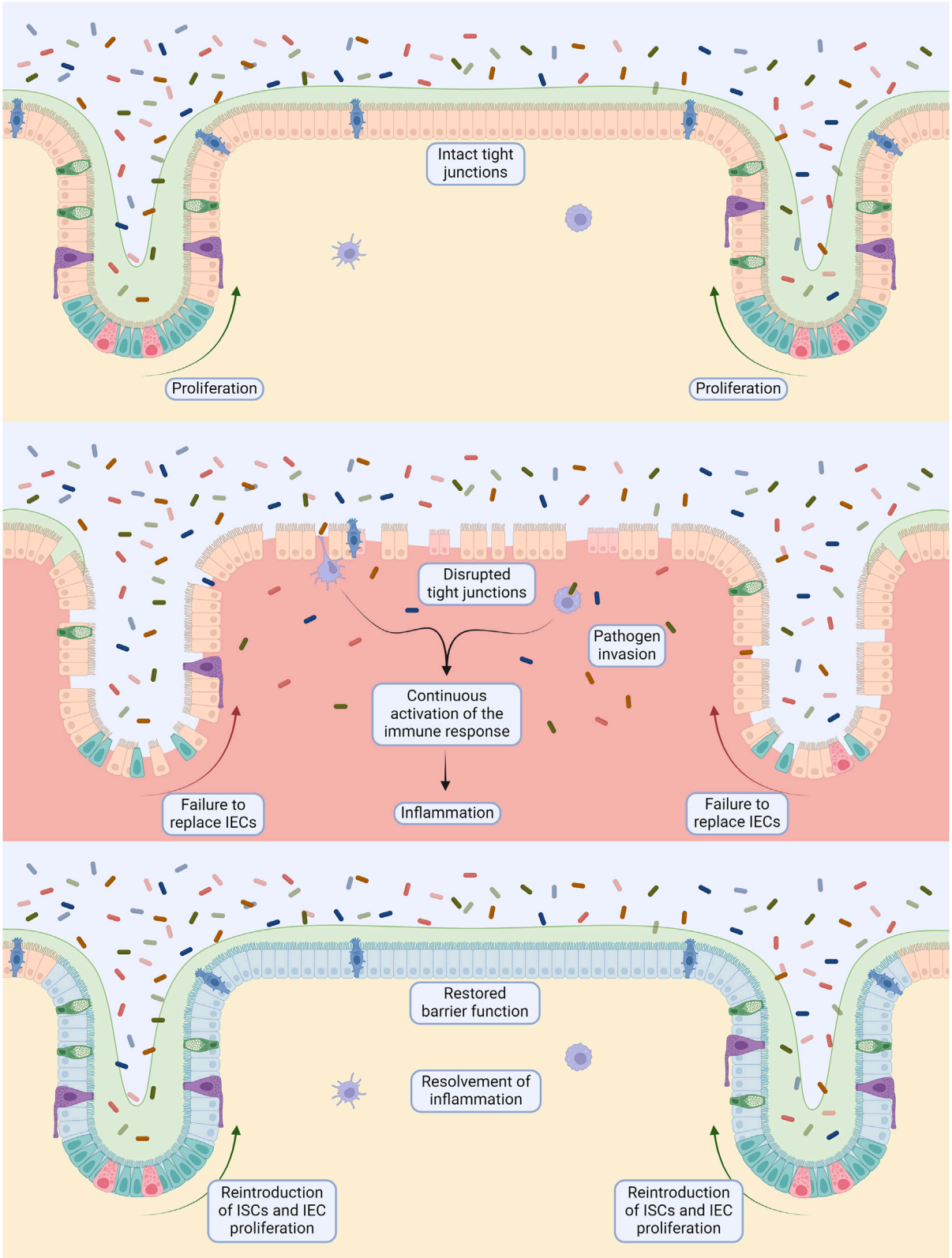
Intact tight junctions
Proliferation
Proliferation
Disrupted tight junctions
Pathogen invasion
Continuous activation of the immune response
Inflammation
Failure to replace IECs
Failure to replace IECs
Restored barrier function
Resolvement of inflammation
Reintroduction of ISCs and IEC proliferation
Reintroduction of ISCs and IEC proliferation

Figure 1. Colonic barrier

Under normal circumstances, the colonic epithelial barrier provides protection against pathogens (diet and microbiome) of the intestinal lumen. The cells of the healthy epithelial barrier are continuously replenished by intestinal stem cell differentiation and proliferation. However, during inflammation, the intestinal stem cells may be broken down. Thus, chronic inflammation may result in the depletion of intestinal stem cells and an inability to replace the epithelial cells and maintain the colonic barrier. Breakdown of the intestinal barrier (i.e., "leaky gut") is involved in several intestinal conditions, including UC and CD. Engraftment of intestinal organoids will, in addition to provide new intestinal stem cells, restore the barrier function and resolve the chronic inflammation.

healing, which involves all layers of the intestinal wall. Nonetheless, superficial wound healing facilitates deeper healing and remission through reduced exposure to external irritants, suppression of inflammation, and accelerated healing in deeper tissues, which, to some degree, also makes organoid transplantation relevant to CD, albeit in a more indirect manner.

THERAPEUTIC OPTIONS FOR IBD

Classical therapeutic options for controlling inflammation in IBD include glucocorticoids, sulphasalazine/5-aminosalicylic acid, thiopurines (i.e., azathioprine and mercaptopurine), and methotrexate (all considered as first-generation nonspecific immunomodulators). In the late 1990s, second-generation pathway-based biologics were introduced, first with TNF inhibitors, followed by anti-integrins and then interleukin blockers. Management with biologics have revolutionized outcomes for patients with IBD.[10,22,23] However, certain drawbacks, such as immunogenicity (i.e., development of anti-drug antibodies) and the requirement of specialized staff, among others, prompted the introduction of a third generation of drugs based on small molecules, e.g., JAK inhibitors and sphingosine-1-phosphate receptor agonists.[22,24–27]

Anticipating future fourth-generation therapies, regenerative medicine and cellular therapy have, however, emerged as promising options for certain subsets of patients with IBD. Protocols facilitating the culture of IECs from biopsies offer the potential to expand these cells *in vitro*, thus enabling their transplantation into the affected intestinal areas[28] (Figure 2). These cell cultures, referred to as intestinal organoids, have shown promise in experimental mouse colitis models.[29,30] Transplanted epithelial cells rapidly form a thin living monolayer lining that potentially covers ulcerations and restores the intestinal barrier. This may prevent the activation of the host innate and adaptive immune systems via luminal antigen-specific effectors, thus resulting in a reduction in amplified intestinal inflammation. Although implementing this novel therapeutic strategy requires tedious steps, it is anticipated to offer a viable mechanism for controlling the clinical activity of IBD, thereby becoming a new and complementary therapeutic option. Organoid-based therapy in IBD operates through different mechanisms distinct from conventional therapeutics. This fundamental difference suggests the potential to help patients with refractory IBD, for which biologics and other therapies fail to relieve symptoms. Accordingly, stem cell transplantation has already shown positive results in treatment of fistulas in CD.[31,32] Additionally, organoid transplantation may synergize when integrated together with conventional therapies. Thus, combining the immunomodulatory effects of current medications with the repair properties of organoids may hold the potential to enhance therapeutic outcomes by addressing multiple factors of importance for the clinical course of IBD. Moreover, organoid therapy offers the advantage of personalized medicine, aligning with a growing trend in medicine toward precision or individualized care. As research progresses, and trials yield more data, the role of organoid-based medicine in IBD will become clearer, which may contribute to a more tailored care of individuals with these conditions. In the following sections, we discuss the state-of-the-art knowledge regarding the application of organoids in the future management of IBD.

INTESTINAL ORGANOIDS

Under appropriate *in vitro* conditions, intestinal stem cells can develop into multicellular 3D-structures referred to as organoids.[33,34] Cultured intestinal stem cells (or isolated crypts containing these) are embedded in a hydrogel, such as Matrigel, which provides a supportive environment for self-organization into 3D-structures. To promote stem cell proliferation, migration, and differentiation, as well as maintaining the stem cell pool, the matrix is supplemented with specific factors (e.g., Wnt, R-spondin, Noggin, Jagged1, and EGF) of importance for various signaling pathways.[35] To expand organoids, they are periodically passaged to allow for the generation of a larger number of organoids. This involves splitting organoids (into smaller pieces) and reseeding them in a fresh matrix. Analyzing human intestinal organoid models has demonstrated that organoids contain all cell types observable *in vivo*, including enterocytes and goblet, enteroendocrine, and tuft cells.[36,37] These organoid structures can be maintained *in vitro* for extended periods, constituting miniature versions (i.e., "mini guts" or avatars) of the donor intestinal epithelium for clinical use.[38] Thus, the possibility to grow "mini guts" in the long term may offer numerous possibilities, ranging from personalized medicine to disease modeling, which could further elucidate fundamental questions underlying the pathogenesis of IBD. Consequently, the use of organoids, serving as *ex vivo* models, has gathered considerable interest since their discovery.[39]

Cultured intestinal organoids offer a versatile approach for the identification of potential therapeutic targets using various methods.[40] Disease modeling may be performed by obtaining intestinal stem cells from patients with a specific disease, which can uncover the mechanisms and genetic factors involved.[41–43] Moreover, intestinal organoids can be used to explore the interactions between microbes (bacteria, viruses, or parasites) and the epithelium following microinjection into the lumen of intestinal[44–46] or monolayered organoids.[47] Organoids can be engineered using genome editing to address the cause of specific mutations,[48–50] which may offer significant advances in drug screening and toxicity testing.[51–55] Furthermore, patient-derived organoids retain the genetic and epigenetic backgrounds of individual patients, rendering them valuable in modeling patient-specific traits as avatars with greater precision and relevance than other available disease models. This could have profound implications not only in IBD management, but also in understanding its pathogenesis and developing new

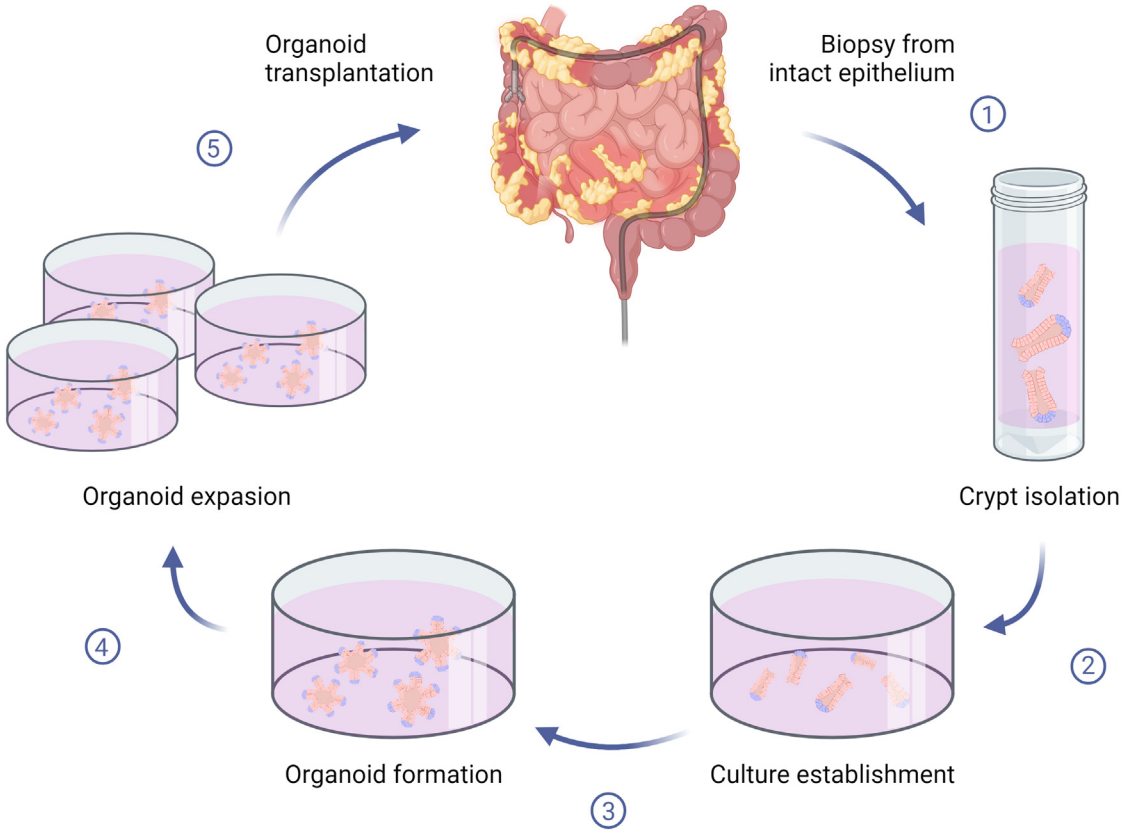

Figure 2. Phases of intestinal organoids as regenerative therapy

Using a colonoscope, biopsy specimens are acquired from healthy sections of the colonic epithelium. From these specimens, intestinal stem cell-containing crypts are isolated and cultured *in vitro*. After organoid formation and subsequent expansion, organoids are transplanted back to the diseased intestinal areas to repair and regenerate the damaged epithelium.

therapies.[39,56] Despite these advantages, current organoid technologies using patient tissue specimens lack complexity regarding spatial cues and relevant interaction with surrounding cells and organs via systemic circulation.[57]

In addition to serving as a method for studying IECs *in vitro*, organoid technology constitutes a mode of tissue regeneration via the expansion of IECs for subsequent transplantation into damaged intestinal segments. Such transplantation studies have already provided proof-of-principle research for future autologous transplantation studies (i.e., healthy stem cells harvested from a specific patient are subsequently used to replace malfunctional diseased cells with healthy stem cells expanded as organoids in the same individual) in patients with intestinal ulcerations.[58–62] In addition, transplantation studies provide a new tool for studying epithelial cells that have been manipulated *in vitro* in an *in vivo* setting, as detailed below.

INTESTINAL ORGANOID TRANSPLANTATION AS A THERAPEUTIC OPTION

Animal organoids

To explore the therapeutic potential of intestinal organoid transplantation, the first study describing the successful transplantation of healthy murine intestinal organoids was published in 2012.[29] In that study, immunocompromised mice were administered dextran sodium sulfate (DSS), a sulfated polysaccharide that induces acute chemically induced experimental colitis.[63] The colons of the DSS-treated animals exhibited certain resemblance to the signs and symptoms observed during flares of the human disease UC, including weight loss, diarrhea, bloody stools, and epithelial damage to the distal part of the colon. In this experimental setting, the transplantation of healthy murine colonic organoids into damaged areas enhanced tissue regeneration.[29] The engrafted organoids comprised a single-layer epithelium, forming both functionally and histologically normal self-renewing crypts. Moreover, intestinal organoids derived from fetal mice demonstrated cell differentiation identical to that of surrounding recipient tissues.[30] Thus, both fetal and adult intestinal epithelial organoids may serve as sources of cells for generating a functional epithelium in the gastrointestinal tract.

Organoids preserve their regional identity *in vitro*,[64] and cultured adult-derived small intestinal organoids have been shown to retain the characteristic features of the small intestine, even when transplanted into the colon.[65] These features include the formation of villus-like

structures, Paneth cells, and a reduced number of goblet cells. Additionally, the colonic marker protein, carbonic anhydrase-II, is not detected in the transplanted tissue. These changes were observed both *in vitro* and *in vivo* following engraftment in the murine colon.[65]

The retention of location-specific characteristics may prove beneficial for the future management of IBD. Sulfomucin, an intestinal mucin that serves as a mucosal barrier against colonic inflammation,[66] is downregulated in the inflamed epithelium of UC.[67] The synthesis of sulfomucin is impaired in the distal part of the colon as compared to the proximal part, which may partially explain why UC typically spreads proximally from the rectum.[67] Notably, murine colonic organoids obtained from the cecal region (i.e., highly sulfomucin-producing cells) when transplanted heterotopically into the distal colon, have been shown to maintain elevated sulfomucin production in their new environment.[67] Therefore, the heterotypic transplantation of colonic cells may further enhance the mucosal barrier.

Human organoids

When developing organoids from human tissues, two options can be utilized: 1) adult stem cells (ASCs) or 2) pluripotent stem cells (PSCs). ASC-derived intestinal organoids were first developed in 2011[68] and constructed by extracting Lgr5+ adult stem cells from the intestinal wall, which were subsequently cultured with appropriate growth factors. When cultured as organoids, ASCs differentiate into epithelial cells such as enterocytes, goblet, enteroendocrine, tuft, and Paneth cells. ASC-derived intestinal organoids recapitulate their tissue of origin regarding composition, plasticity, and maturity.[69] Intestinal organoids derived from PSCs, referred to as human intestinal organoids (hIOs), were also first reported in 2011.[70] Compared to ASCs, which are tissue-restricted, PSCs are able to give rise to all germ layers and thereby tissues in the human body. This is also reflected in hIOs, which contain not only epithelial cells, but also co-derived mesenchymal cells and blood cells. However, hIOs without additional manipulation, such as transplantation, retain a fetal identity compared to the more mature state observed in ASC-derived intestinal organoids.[71] Given the ease at which organoids can be derived from endoscopically obtained biopsies, stem cells as an autologous source for cellular therapy are receiving a growing interest for regenerative medical purposes. PSC-derived hIOs could be very relevant as a cellular source of material for patients suffering from deep tissue damage due to the fact that they contain not only epithelial cells but also the supportive stroma. Unfortunately, it is currently not feasible to generate autologous material,[72] since this requires derivation of clinical-grade PSC lines from individual patients. As an alternative, immune-privileged PSCs (stealth cells) could be utilized as an allogeneic cell source for hIOs, where specific immune-modulatory genes have been modified to enhance immune-compatability.[73]

Experiments using human cells have been conducted to reliably demonstrate the regenerative potential of intestinal organoid transplantation. To address the issue that hIOs do not naturally mature *in vitro*, they were transplanted under the renal capsules of mice.[74] In these experiments, hIO transplantation resulted in the differentiation of intestinal cell lineages in the presence of brush-border enzymes. The engraftment exhibited a crypt-villus architecture and laminated human mesenchyme supported by the vascularity of the host. Additionally, subepithelial and smooth muscle layers were observed to develop. These experiments demonstrated an overall expansion and maturation of *in vivo* engraftment compared to organoids cultured *in vitro*. Moreover, permeability and peptide uptake studies confirmed the digestive functions of the transplanted tissues. The responsiveness of grafted cells to systemic signals from the recipient organism has been confirmed by ileocecal resection and a subsequent measurement of proliferative activity through villus height, crypt depth, and smooth muscle layer thickness at the graft site.[74] Furthermore, maturation into an adult-like state was confirmed at both transcriptional and phenotypic levels.[74]

In 2017 and 2018, the first successful transplantations of human intestinal organoids into a mouse colon were reported.[58,75] Both studies utilized an injury-based mouse model, and the transplantation of organoids resulted in colonic epithelium regeneration. Lineage tracing confirmed that the engrafted stem cells were multipotent and self-renewing. Notably, the crypts formed by human-derived cells were significantly longer than murine crypts, reflecting their original size. Compared to murine colonic stem cells, human colon stem cells may have a much lower division and replacement rate.[58,75]

The above-mentioned experiments highlight the promising potential of intestinal organoid transplantation in future clinical settings as a regenerative therapeutic option to restore intestinal barrier defects during flaring (Figure 2).

In addition, "humanized" murine intestines have been constructed by transplanting human organoids and immune cells into murine intestines, which may serve as a viable alternative to traditional animal models, as they more closely resemble the human intestine in structure, cellular interactions, and immunology.[60,76–78] Although *in vitro* studies examining human intestinal development and tumorigenesis have already emerged,[78–80] all findings must be supported by subsequent *in vivo* investigations in humans.

Organoid–organ hybrids

The aggravated disease course in CD may involve multiple surgeries that may culminate in a condition known as short bowel syndrome (SBS). This condition entails a functional loss of the absorptive capacity of the small intestine, which is crucial for absorbing dietary elements such as lipids, carbohydrates, peptides, and vitamins.[28] Currently, the only therapeutic option for patients with SBS is transplantation of a segment of the small intestine, which is associated with a relatively high risk of graft rejection (~34% within one year after transplantation[81] and ~50% within 5 years[82]), as well as the lifelong administration of immunosuppressive drugs.[83] Artificial intestines from an autologous source could represent a novel and steady therapeutic option in the future.[82,84] The potential use of organoids for the treatment of SBS is currently under investigation. In a proof-of-principle study,[85] a small intestinalized colon (SIC) was developed in mouse and rat models to explore the prospects of addressing SBS via autologous transplantation (Figure 3). However, the colonic function for absorption was insufficient,[86] because the colon is typically preserved in patients with SBS. Therefore, the colon can be exploited as a scaffold to build an artificial small intestine to restore absorptive capacity. In this study, the removal of the colonic epithelium in rats and its subsequent replacement with ileum-derived organoids demonstrated the preservation of regional identity (i.e., characteristic features such as surface molecules and structures facilitating

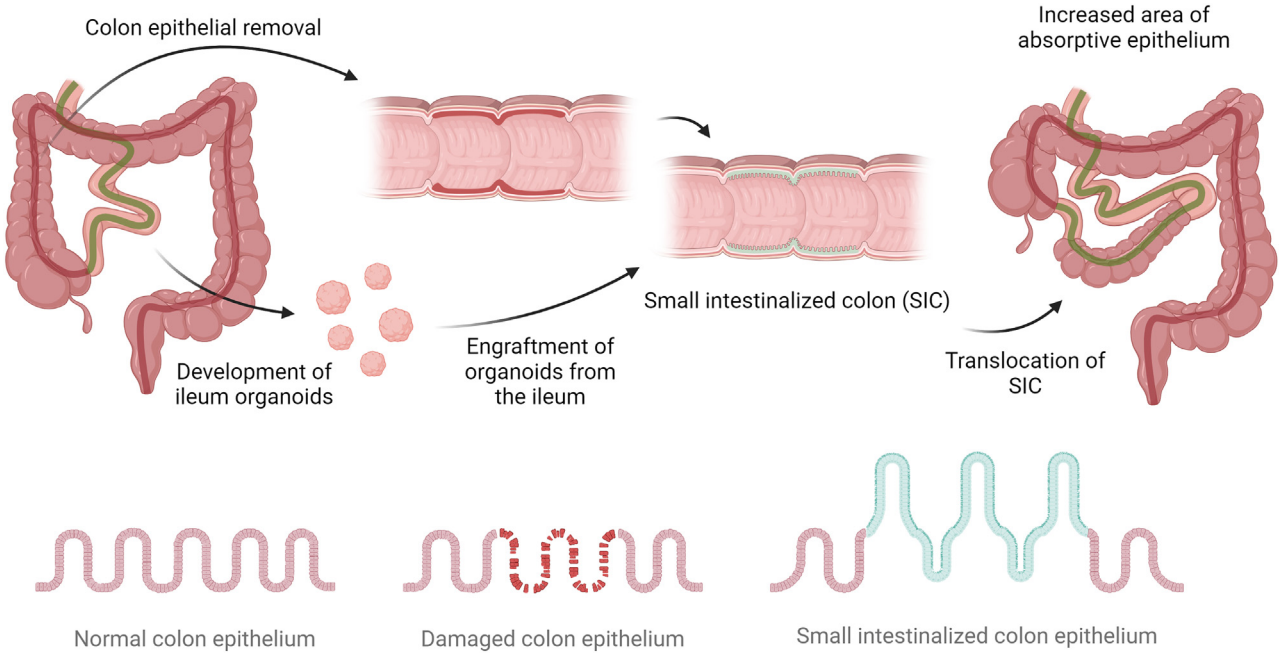

Figure 3. Proposed phases of construction of a small intestinalized colon

Intestinal organoids are developed from biopsies of the ileum. These organoids will develop ileal epithelium and preserve ileal characteristics following engraftment in a section of the colon, where the colonic epithelium has been removed. This will result in a section of the colon with ileal epithelium (i.e., a small intestinalized colon (SIC)). Subsequently, the SIC is surgically translocated to the ileum to enable the complete formation of villi and lacteals, thus promoting the absorption of nutrients.

absorptive capabilities) and formation of nascent villus structures within the engraftment in the colon.[85] Anatomical relocation of the SIC to the ileocecal junction provided the transplant with luminal mechanistic flow crucial for complete villus and lacteal formation. SIC enables the absorption of food constituents, such as carbohydrates, fats, and proteins, which are distinct features of the small intestine.[85] Furthermore, the interposition of SIC in rat models of SBS resulted in an increased survival rate ($p = 0.016$) compared to models in which the colon epithelium was replaced with colonic intestinal epithelial organoids.[85] Thus, this novel technique may represent a feasible approach for treating patients with disabling SBS.

Using a different approach, another research group developed a functional human-derived small intestine in mice.[87] In a proof-of-concept experiment, human intestinal epithelial organoids were infused into a decellularized human intestinal scaffold.

The possibility of using autologous organoids in SICs or organoid-coated scaffolds as supplements for small intestine transplantation for the management of SBS may have the potential to become a new therapeutic option[33] (Figure 3). Although experimental procedures that involve hybrid small intestines in larger animals, such as pigs, are currently underway,[83] the safety of human procedures still needs to be assessed further in human-to-human organoid engraftments. Moreover, considerations such as whether it will be necessary to apply organoids with an adhesive substrate (e.g., fibrin glue) to fasten cells onto ulcerated regions still remains an open question.

FIRST IN-HUMAN TRIAL

The research presented above paved the way for the first in-human testing of organoids as a regenerative therapy (Clinical Trial ID: jRCTb032190207). On July 5th, 2022, researchers at Tokyo Medical and Dental University performed the world's first clinical transplantation of organoids into the colon of the first of eight patients with UC in Japan to repair the damaged intestinal epithelium. Autologous transplantation was performed using a standard colonoscopic spray tube and engrafted by spreading the organoids on the mucosal surface rather than using direct injection. Medical examinations were scheduled at regular intervals for the following 12 months to ensure the efficacy and safety of the procedure, as well as endoscopic and histological improvement. To date (June 2024), the research team has not disclosed any data regarding this clinical trial.

ENSURING THE CONTINUATION OF REMISSION AFTER ORGANOID THERAPY

While autologous transplantation holds a lower risk of rejection than allogeneic transplantation, the immunogenicity of autologous engraftment still needs to be thoroughly assessed. Although experiments have shown promise for intestinal organoids as a therapeutic option, all

published studies have been performed using experimental injury models.[72] However, human intestinal epithelial organoids introduced into the inflammatory environment of IBD may respond differently to those observed in artificial experimental models.

In an *in vitro* experiment,[88] human intestinal epithelial organoids derived from inflammatory or non-inflammatory sections of patients with IBD, and control organoid cultures obtained from healthy individuals (controls) were compared. Initially, organoids from inflammatory intestinal sections showed characteristics of inflammation when assessing gene expression, as opposed to organoids derived from non-inflammatory sections, which more closely resembled that of the controls.[88] The organoids of inflamed origin gradually lost their characteristic inflammatory gene properties, and after four weeks, gene expression associated with the inflamed tissue-derived organoids was indistinguishable from that of the organoids of non-inflamed tissue and instead clustered based on patient identity. Upon exposure to a mixture containing inflammation-associated factors, all organoids, regardless of origin, displayed the gene expression characteristics of inflammation, thus demonstrating that they may have the capacity to respond to available stimuli. The results of this *in vitro* study[88] were further interpreted.[72] Organoids derived from healthy tissues may fail to reduce intestinal inflammation in IBD but are able to develop into a more inflammatory state due to changes in the local environment in intestinal sections affected by flaring disease.[88] However, with restoration of the epithelial layer, inflammation is reduced; thus, the engrafted cells might conform to a non-inflamed milieu. Nevertheless, efforts to enable the engrafted material to restore barrier function and eliminate constant exposure to luminal antigens and/or pro-inflammatory mediators still need further exploration.

Although a strong genetic disposition exists for CD, including the involvement of NOD2, ATG16L1, and IL-23R,[89,90] environmental factors may be of importance in UC.[91,92] However, the risk of developing IBD from autologous organoid engraftment still emerges, as previously described, owing to organoid retention of the genetic signature of the donor. One possible outcome is that autologous organoid transplantation may not be sufficient to reliably cure conditions with significant genetic susceptibility. Thus, IEC transplantation might be a more promising therapeutic option for UC than for CD.[93]

Genome editing (e.g., CRISPR/Cas9) is widely used in combination with intestinal epithelial organoids in experimental studies and could be applied in the cases of patients carrying specific risk alleles. Although complex conditions, such as IBD, comprise numerous susceptibility genetic loci (> 241[94]) to be feasibly targeted, epithelial stem cells obtained from patients with susceptibility to hereditary conditions that involve only a few genes can be genetically altered to eliminate the specific genetic risk factor(s) in question. Additionally, recent studies have revealed the accumulation of somatic mutations in the IL-17 signaling pathway, which may modulate the pathophysiology of IBD.[95–97] Thus, genetically modified organoids developed from patient-specific stem cells might assist in mitigating the gene-related risks associated with certain conditions outside the sphere of IBD. Nonetheless, safety concerns arise when combining genome editing with human tissue, owing to potential off-target effects.[98]

LIMITATIONS OF THE STUDY

So far, research in intestinal organoids in the context of regenerative therapy has focused on autologous as opposed to allogeneic transplantation. Allogeneic transplantation allows for a wider source of intestinal stem cells and could potentially enable "off-the-shelf" production. However, allogeneic transplantation as a viable option is hindered by its high rejection risk, but also due to a malignant potential. Thus, donor cells hold the risk of being recognized as a foreign substance by the recipient's immune cells, which may initiate an immune response targeting the engrafted cells. This response may be lessened or avoided with the co-administration of immunosuppressive drugs; however, prolonged immunosuppression may on the other hand give rise to a series of issues.[99,100] Furthermore, due to differences in the genetic make-up of donor cells, transplantation may harbor a genetic disposition to cancer or other ailments considered to be potentially harmful to the recipient. Additionally, *in vitro* culturing of stem cells has been shown to carry a greater risk of point-mutations compared to *in vivo* mutational rates[101] – a concern relevant to all types of cellular therapies. The annual mutation accumulation of *in vitro*-grown ASCs has been found to be nearly 40-fold higher than that of their *in vivo* counterparts,[101,102] while the mutation rate in PSCs was slightly lower than that of ASCs.[101] Thus, a 3-month organoid expansion for transplantation induces mutations equivalent to the number the donor intestinal epithelium that would be expected to accumulate over 10 years. Given the decade-long mutation load, the donor patients should therefore undergo regular surveillance colonoscopy after the transplantation procedure. However, stem cell transplantation studies in both healthy humans and mice have never reported the development of any tumors, strongly supporting that although mutational rates might be higher, this procedure does not seem to be associated with the accumulation of mutations in oncogenes or tumor suppressors.

Although organoids demonstrate various cell types from their native tissue, they still consist only of stem cells and their target cell types, making this lack of interaction especially relevant to ASC-derived organoids, which exclusively contain epithelial cell types. PSC-derived organoids do produce mesenchymal cells around epithelial cells, which is, however, limited as compared to the complexity observed in the *in vivo* intestine. In general, both types of organoids lack intricate mesenchymal diversity and structure, as well as vascularization, neuronal connections, and interactions with immune cells and the intestinal microbiota. Thus, co-culturing of intestinal organoids with cell types such as endothelial,[103] mesenchymal,[104] immune,[105] and glial[106] cells to examine the role of these cells when interacting with epithelial cells has been performed. Yet, existing co-culture systems remain relatively simple, incorporating only one or few cell types.[107] These assays have the advantage of simplicity in addressing the true interactions between selected cell types but fail to simulate the vast complexity of the intestine *in vivo*. In addition, mechanical characteristics such as stiffness and degradability are considered to play a crucial role in the development, sustenance, and specialization of organoids in a cultured environment.[108] However, traditional organoid culture currently falls short in incorporating essential elements such as *in vivo* growth factors, the intricate extracellular matrix composition, and fully optimized biomechanical properties within their surroundings.[109]

Table 1. Important accomplishments in the research of intestinal organoids as a regenerative therapy

Description	Year	Reference
First description of intestinal organoid culture	2009	Sato et al.[36]
First successful transplant of mouse intestinal organoids in mouse intestines to remedy colitis	2012	Yui et al.[29]
First successful transplant of human intestinal organoids in mouse into kidney capsule	2014	Watson et al.[74]
First successful transplant of human intestinal organoids in mouse intestines to remedy colitis	2017	Miura and Suzuki[75]
First transplant of autologous intestinal organoids in humans with ulcerative colitis	2022	–

Another consideration regarding clinical safety is the choice of hydrogel. Matrigel is the most optimal extracellular matrix for intestinal organoid culturing, but its mouse-based preparation hinders GMP-compliance. Matrigel is derived from Engelbreth-Holm-Swarm mouse sarcoma cells and contains an ill-defined composition; it exhibits batch-to-batch variability,[110] and has a declared risk for pathogen transmission, which has not been documented. Traditional organoid culture relies on Matrigel, providing essential extracellular components, but the issues associated with Matrigel prompt the exploration of synthetic hydrogel alternatives. Therefore, efforts have been directed toward identifying synthetic xenogeneic-free alternatives. Collagen, fibrin, and gelatin among others are current alternatives to Matrigel based on clinical safety.[111] Animal-free synthetic matrices have also been developed for organoid culture. Despite these efforts, no matrix format has promoted an organoid growth comparable to that achieved with Matrigel.[112] Reports have claimed growth promoting effect of synthetic gel comparable to Matrigel,[113,114] although such results have not been reproduced. One factor that has limited synthetic hydrogels is recombinant Wnt-3a, as it has limited Wnt-signal activation potential due to its instability in serum-free liquid solution. However, afamin, a serum-derived Wnt-stabilizing protein, has enabled the preparation of serum-free potent Wnt ligands.[115]

REMAINING RESEARCH GOALS

While initial clinical results are promising, the long-term outcomes of intestinal organoid transplantation remain to be established. Thus, thorough follow-up studies are essential to determine the sustained efficacy, potential complications, and safety of this therapeutic approach.

Although organoid transplantation has shown regenerative potential, the exact mechanisms underlying its effectiveness in restoring barrier function and controlling inflammation are not yet understood. Further research is required to elucidate the interactions between transplanted organoids, host tissues, and the immune system.

As with any medical intervention, not all patients with IBD may be suitable candidates for organoid transplantation. Criteria for selecting patients who would benefit the most from this therapeutic option have not yet been defined. Patient-specific factors, such as disease severity and genetic predisposition, also need to be addressed and integrated into the decision-making process, as is also the case for any other type of medication currently used in the clinic for patients with IBD. In this review, we have pointed toward UC as a target of organoid therapy, and the first in-human trials have been performed on patients with this disease. However, as the mechanisms of organoid-based regeneration are uncovered, the factors most relevant to these mechanisms will shed light on how to best evaluate which patients will most likely benefit from therapy with transplantation of organoids.

Because IBD is a multifactorial condition, combining organoid transplantation with existing therapeutic approaches, such as immunomodulatory drugs, could potentially achieve better outcomes. How these combined strategies can be optimized to provide synergistic effects remains to be analyzed. Identifying the most effective combinations and determining the timing of interventions will be crucial for enhancing treatment outcomes.

As organoid technology advances, efforts to standardize organoid production, quality control, and transplantation procedures become imperative. These processes need to be optimized to ensure consistent and reproducible results on a larger scale, rendering organoid transplantation a practical therapeutic option. Developing efficient and reliable transplantation procedures and identifying the best methods for delivering and integrating transplanted organoids into diseased intestinal tissues is vital for clinical success. How should the challenges of maintaining long-term viability and functionality of the engrafted organoids be addressed?

CONCLUDING REMARKS

The rapidly advancing field of regenerative medicine has ushered in an exciting new frontier, with the application of intestinal organoids in the management of IBD (Table 1). The increasing global prevalence of IBD, coupled with the shortcomings of current therapeutic strategies, has spurred the exploration of new therapeutic alternatives. Here, intestinal organoids have emerged as an attractive platform for diverse clinical applications ranging from disease modeling and drug testing to personalized medicine and tissue regeneration. Their ability to recapitulate the complexities of the intestinal epithelium, combined with advancements in genome editing technologies, provides new avenues for tailored treatment approaches for individual patients, including those with IBD. The promising outcomes of experimental animal models and *in vitro* studies, and the recent milestone of the first human colonic organoid transplants, have highlighted the feasibility and potential efficacy of using organoids in clinical settings.

While challenges persist, the integration of intestinal organoids into the landscape of molecular medicine holds significant promise for the management of this chronic disorder. In the near future, driven by the need for a next-generation therapy, research is expected to position this

novel transplantation procedure as a compelling and practical option for patients worldwide. As organoid research progresses, collaboration between scientists, clinicians, and ethicists will be crucial to ensure the responsible and effective translation of these advancements into meaningful clinical outcomes, offering new hope for patients with challenges such as IBD or other complex diseases.

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AUTHOR CONTRIBUTIONS

Study conceptualization, design, and overall supervision: O.H.N. and A.H.; Literature review: A.H. and O.H.N.; Writing – original draft: A.H.; Writing – review and editing: A.H., O.H.N., K.B.J., T.S., and D.C.; Project administration: O.H.N.

DECLARATION OF INTERESTS

T.S. is an inventor of several patents related to organoid culture. The remaining authors disclose no conflicts of interests.

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