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Review Article

The Application of Intestinal Stem Cell Organoids in the Treatment of Inflammatory Bowel Disease

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Abstract

Inflammatory Bowel Disease (IBD) comprises chronic inflammatory disorders affecting the gastrointestinal tract, such as Crohn's Disease (CD) and Ulcerative Colitis (UC). The escalating global incidence of IBD is multifactorial, involving genetic, microbial, environmental and immunological factors. Despite current therapies emphasizing immune suppression, sustained efficacy remains elusive. Recently, Stem Cell Therapy (SCT) has emerged as a potential avenue for inducing remission in IBD patients. Intestinal Stem Cells (ISCs) have gained attention for their recent emergence in research and potential cultivation into organoids, offering a promising source for IBD treatment. This review focuses on the potential of ISC SCT, emphasizing its organoid culturing capabilities. It highlights organoids' applications in IBD research, monitors advancements in animal and human trials and examines current limitations and future directions in organoid research for IBD treatment.

Keywords: Intestine; Stem Cells; Organoids; Treatment

Abbreviations

CD: Crohn's Disease; DSS: Dextran Sulfate Sodium; EGFP: Enhanced Green Fluorescent Protein; ESC: Embryonic Stem Cells; GI: Gastrointestinal; HSC: Hematopoietic Stem Cells; iPSCs: Induced Pluripotent Stem Cells; IBD: Inflammatory Bowel Disease; IL: Interleukin; IECs: Intestinal Epithelial Cells (IECs); JAK: Janus Kinase Inhibitors; LGR5: Leucine-rich Repeat-containing G Protein Coupled Receptor 5; MSCs: Mesenchymal Stem Cells; PGE2: Prostaglandin E2; SBS: Short Bowel Syndrome; SCT: Stem Cell Therapy; SCs: Stem Cells; TNF: Tumor Necrosis Factor; UC: Ulcerative Colitis; VEO: Very Early-Onset IBD; WAE: Wound-Associated Epithelial Cells

Introduction

Inflammatory Bowel Disease (IBD) is a group of chronic inflammatory diseases of the Gastrointestinal (GI) tract [1]. It includes both Crohn's Disease (CD) and Ulcerative Colitis (UC), which are characterized by repeated inflammation and intestinal mucosal injury [2]. CD affects any part of the GI tract and is more commonly associated with abscesses and fistulas resulting from transmural inflammation [3]. In contrast, UC is limited to the large intestine and is associated with mucosal inflammation extending from the rectum to the proximal colon [4]. A third category, indeterminate colitis, is IBD with features of both CD and UC [5]. IBD presents challenges to patients as repeated inflammation can cause obstruction, GI bleeding, abdominal pain and complications such as intestinal cell cancerization and death [1]. Over time, intestinal damage can occur which necessitates surgery to remove. The incidence of IBD is growing worldwide, specifically in Asia, Africa and South America [1].

IBD is thought to have genetic, microbial, environmental and immunological etiologies [3]. As of 2018, 240 high-risk gene loci have been associated with IBD, mainly involved in regulating adaptive immunity and intestinal epithelial function [6]. Additionally, studies have shown a reduced biodiversity and stability of the fecal microbiome in IBD patients compared to healthy controls [3]. Environmental factors, such as smoking, vitamin deficiency, air pollution and stress, may also influence the pathogenesis of IBD [3]. Finally, dysfunction of innate and adaptive immunity pathways, specifically the T cell response, have dominated IBD studies [3]. Commonly used medications today aim to suppress immune inflammatory responses thought to be generated by the systemic immune system. These therapies typically use monoclonal antibodies that target inflammatory cytokines, such as Tumor Necrosis Factor a (TNFa), Interleukin (IL)-23 and IL-17 [7]. More recently, small molecules such as Janus Kinase (JAK) inhibitors have been developed to increase immune suppression [7]. However, these options rarely generate long-term remission through complete mucosal healing [1].

One promising treatment with the aim of generating long-term remission for IBD patients is Stem Cell Therapy (SCT). Stem Cells (SCs) have the potential for unlimited proliferation into organs and tissues. They can be classified as unipotent, pluripotent, or totipotent, differentiating into one cell type, many cell types and all cell types respectively. SCs are regulated by their microenvironment. If there are changes in this microenvironment, SC malfunction, cancerization and death can occur. SCT involves transplanting exogenous stem cells into patients with the goal of revitalizing their own SCs to treat or cure disease. The main pathogenesis of IBD involves the loss of integrity of the intestinal mucosa and disruption of immune homeostasis, which could result from the absence abnormal functioning of intestinal resident stem cells. SCT could help patients with IBD through restoration of SC function to repair the mucosal barrier, decrease chronic inflammation and enhance the intestine microenvironment. Several types of SCs have been used and evaluated for IBD treatments, including Hematopoietic Stem Cells (HSCs), Mesenchymal Stem Cells (MSCs), Intestinal Stem Cells (ISCs) and induced Pluripotent Stem Cells (iPSCs). ISCs are particularly promising due to their recent emergence in research and their potential to be cultured into organoids, offering an excellent source of SCs for IBD treatment. This review, therefore, will focus on the potential of ISC SCT to treat IBD through leverage of its organoid culturing capabilities [1,8].

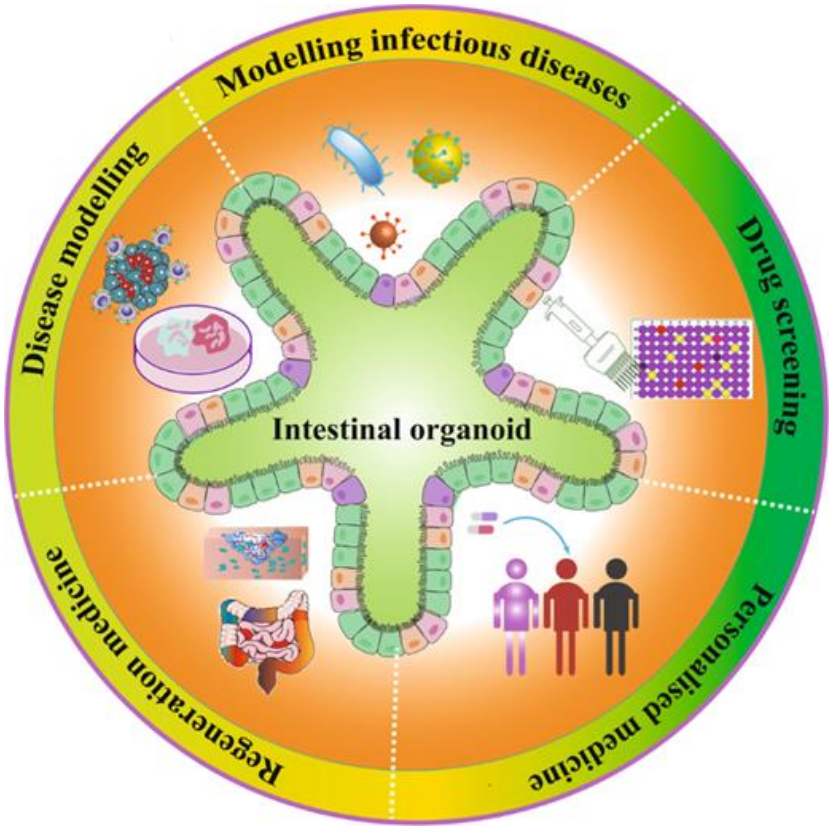

Figure 1: Applications of intestinal organoids [9].

Discussion

ISCs, situated in the lining of the GI tract, particularly in the small intestine and colon, are responsible for the continuous turnover and regeneration of the intestinal lining [1]. Their pluripotent nature enables them to replenish various types of Intestinal Epithelial Cells (IECs), including absorptive cells, goblet cells, Paneth cells and enteroendocrine cells, ensuring the proper function and integrity of the intestine [10]. Moreover, ISCs give rise to resident immune cells, such as T cells, B cells and dendritic cells, contributing to the dynamic balance of the GI tract [1].

The intestinal epithelium, comprised of a monolayer of specialized IECs, undergoes constant replenishment through the division of ISCs found within the mucosal crypts of both the small and large intestines [1]. Within a specialized microenvironment containing mesenchymal cells, Paneth cells and extracellular matrix components, ISCs divide into progenitor cells [11]. ISC proliferation plays a crucial role in maintaining the intestinal lining and repairing damage caused by injury or inflammation [10]. ISC dysfunction emerges as a significant factor in the development of IBD and other GI disorders [2]. In the context of IBD, disruptions to the intestinal microbiota and barrier mechanisms permit pathogenic bacteria invasion, triggering inflammation [4]. Activated macrophages and specific immune cells in the lamina propria contribute to heightened intestinal inflammation by producing pro-inflammatory cytokines [4].

Leucine-rich repeat-containing G protein coupled receptor 5 is an important marker of ISCs. The main types of ISCs include LGR5+ SCs, which are most common and are located at the base of intestinal crypts and +4SCs, which are reserve SCs thought to regenerate LGR5+ SCs in times of stress. LGR5+ ISCs are heavily regulated by the Notch and Wnt/β-catenin signaling pathways. Investigation of these pathways and the transplantation of ISCs in the form of intestinal organoids have been focal points of multiple studies on IBD outcomes [2,4].

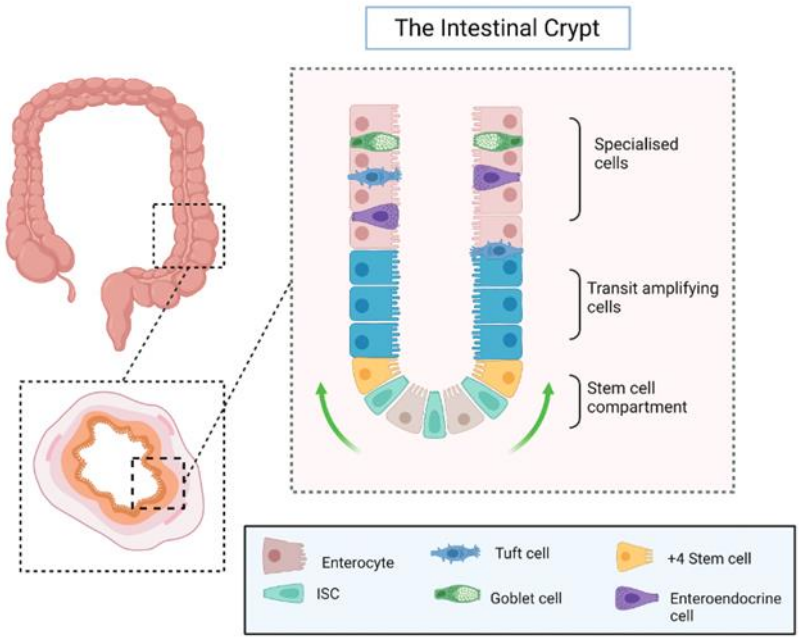

Figure 2: The structure of the intestinal epithelium, organized into crypts and villi. LGR5+ ISCs at the base divide and replicate while +4 stem cells act as reserves. ISCs progress along the crypt-villus axis, entering the transit-amplifying zone, differentiate into specialized cells and ultimately are shed into the lumen [7].

In recent years, the development of 3D culture techniques for intestinal organoids has opened new avenues for basic and translational research in gastroenterology [12]. These hollow spheres, formed by ISCs, mimic the structural intricacies of intestinal epithelial crypts, regulating stem cell differentiation and promoting the physiological function of IECs [9]. LGR5+ ISCs have been instrumental in forming murine and human intestinal organoids with a structure closely resembling the *in-vivo* intestinal epithelium [13,14]. Recreating the ISC microenvironment in organoids has enabled successful *in-vitro* extraction,

presenting a promising alternative to traditional animal models by offering easier manipulation and ethical advantages [9]. Compared to 2D cultures, 3D cultures can self-organize into functional organ-specific structures and differentiate into various cell types found in the GI tract, making them more adept at modeling the complex interactions within the intestinal tissue [15].

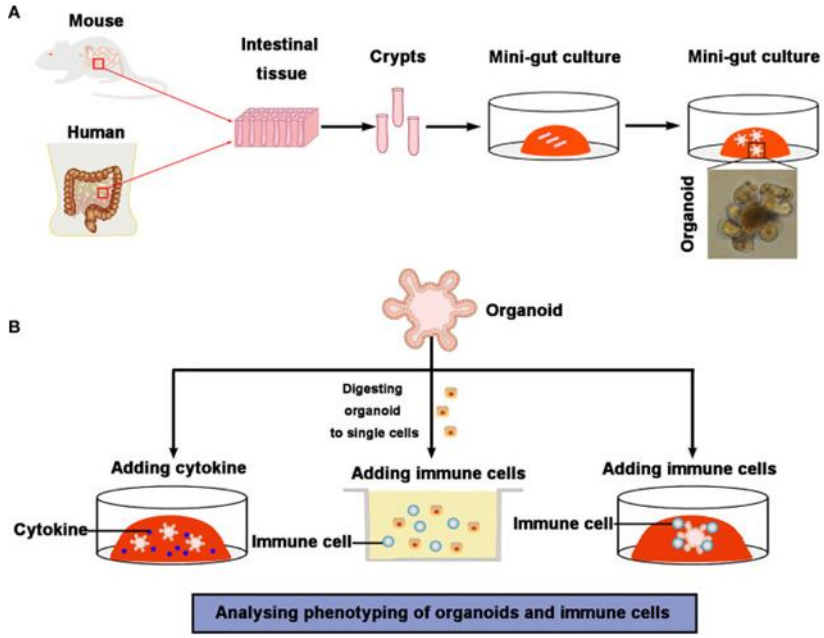

Figure 3: Intestinal organoid-immune cell co-culture models. (A) Intestinal organoids are formed through isolation of crypts from intestinal tissue and embedding into Matrigel with culture medium. (B) Intestinal organoids, immune cells and cytokines are co-cultured to explore their interactions. Cytokine-treated organoids assess the impact on immune cell-derived cytokines on IECs, particularly ISCs. Organoids digested to single cells and co-cultured with immune cells reveal insights into immune-epithelial interactions. Activated immune cells, like lymphoid or T cells, added to complete organoids, help evaluate epithelial-immune cell interactions [16].

Intestinal organoids are a valuable tool for studying the complex origins of IBD, allowing examination of epithelial homeostasis and host-microbial interactions in an environment that mimics the *in-vivo* GI tract [17]. While ISCs serve as the main cell source for these organoids, Pluripotent Stem Cells (PSCs), Embryonic Stem Cells (ESCs) and induced Pluripotent Stem Cells (iPSCs) have also found applications in constructing 3D models [1]. Several protocols currently exist that outline how various organoid types, such as those derived from normal tissue, colorectal cancers, pluripotent stem cells and cancer-associated mutations, can be generated and transplanted to answer clinical questions [18,19]. Application of organoids allow for detailed investigations into the structure, growth, development, function and interactions of the intestinal epithelium with surrounding cells [1,9]. The creation of an epithelial injury model using ISC organoids provides a valuable approach for investigating the cellular and molecular mechanisms underlying inflammation in IBD. This model allows for the exploration of factors such as mucus sialylation dysfunction, enzyme deficiency or mutation and disturbances in modulators of cell cycle progression [20-22]. Research on intestinal organoids has revealed the stimulatory impacts of molecules such as IL-22, IL-6 and Prostaglandin E2 (PGE2), fostering the expansion and differentiation of ISCs [4]. ISC organoids provide a versatile platform for studying IBD origins and pathogenesis.

In addition to contributing to knowledge on IBD processes, ISC organoids present potential clinical applications and therapeutic strategies. They are powerful tools for evaluating the efficacy of existing treatments [15]. These models could reveal the effects and targets of investigational drugs in IECs under inflammatory conditions, potentially more efficiently than animal models for drug development [23]. ISC organoids can be regenerated from IBD patients' stem cells, thus retaining individual genetic and transcriptomic profiles [15]. Testing of current medications on patient-specific organoids will optimize treatment for patients, reduce therapy failure and mitigate adverse effects [15]. Organoids can be used to produce colonic monolayers where the individual effects of immune cells and bacteria can be assessed in CD patients [24]. Additionally, organoids offer a new

therapeutic approach to reconstruct the epithelial barrier in the inflamed mucosa of IBD patients to accelerate healing through engraftment onto damaged epithelium [16,19]. Patient-derived organoids have proven efficacy in exploring gene abnormalities responsible for variations in IBD, including Very Early-Onset (VEO) IBD affecting infants and young children [4]. Metabolic programming strategies, such as mitochondrial antioxidant therapy, have been applied to organoids *in-vitro* to correct factors contributing to IBD pathogenesis [25].

Researchers have successfully replicated IBD conditions in organoids from healthy patients, allowing the study of genetic-phenotypic relations, exploration of pathogenesis and improvement of gene expression [19]. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas 9 technology has been employed to introduce mutations into healthy organoids, shedding light on the effect on the intestinal epithelium [4]. It enables precise manipulation of gene loci within intestinal organoids, facilitating the editing of Single Nucleotide Polymorphisms (SNPs) associated with the risk of IBD [26]. CRISPR/Cas 9 has been harnessed to correct genetic defects, enhance genetic susceptibility factors associated with IBD, avert carcinogenesis and suppress tumor growth [9,26]. It holds promise for transplantation using engineered ISC organoids to achieve deeper molecular remission in IBD treatment [26].

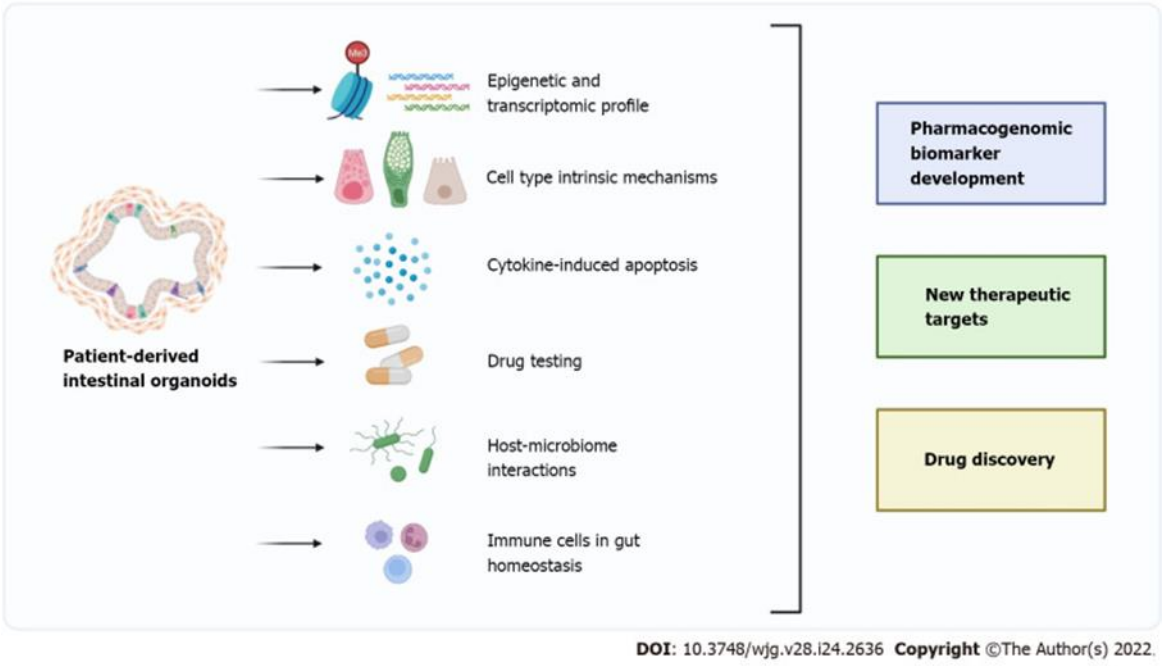

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Figure 4: Patient-derived intestinal organoids offer a precise in vitro model for studying Inflammatory Bowel Disease (IBD). With disease-specific defects, these structures aid in exploring epigenetic profiles, new disease mechanisms and drug testing. Co-culturing with microbiota or immune cells enhances IBD pathogenesis studies, potentially revealing biomarkers, targets and drugs for precision medicine in IBD [15].

The application of organoids extends to preclinical trials and clinical studies, exemplified by various experimental protocols and trials. In 2012, Yui and colleagues developed a protocol to isolate LGR5+ colon stem cells from EGFP transgenic mice and cultured 3D organoid spheres with cytokines and growth factors [27]. These organoids adhered to and covered damaged tissue in DSS-induced acute colitis models, leading to higher body weights in recipient mice compared to controls [27]. Fordham, et al., suggested the possibility of patient-specific regeneration, as fetal mouse small intestinal organoids differentiated into colon-like epithelial tissue after transplantation into a colonic injury model [28]. In contrast, Fukuda, et al., found that organoids cultured from mouse small intestine ISCs retained their characteristics long-term when transplanted into the colon [29]. These findings suggest that differentiation ability of organoids may be based on the site of the lesion. Rat small intestine organoids have shown promise in treating complications resulting from IBD, such as Short Bowel Syndrome (SBS) [4]. To bridge the gap between mouse and human intestinal models, organoids have been derived from canine IBD models, preserving ISCs through cryopreservation for future research [30]. Human application reached a significant milestone in 2018 when Sugimoto, et al., successfully

transplanted typical human colonic organoids into the colons of mice with compromised immune systems, preserving key features of the human colon [31]. The human intestinal organoids were able to reconstruct the damaged mucosa of the mice [31]. In 2022, Watanabe, et al., adapted the DSS-grafting assay originally employed by Yui, et al., in 2012 for the treatment of UC mice [19]. This innovative approach involved inducing colitis-like epithelial injury in the distal colon of mice using DSS [19]. Post-DSS, organoids were infused directly into the luminal space through the anus [19]. The infused organoids exhibited a remarkable ability to localize lesions caused by UC and initiate the reconstruction of the damaged epithelial crypt [19]. The versatility of this assay has been demonstrated through its successful application in organoids derived from both wild-type and genetically altered epithelial cells from the adult colon and adult and fetal small intestine [19].

Building upon this success, the protocol used by Watanabe et al., has become the foundation for the first in-human clinical trial by the Tokyo Medical and Dental University (TMDU) [19]. This clinical trial is ongoing and aims to assess the therapeutic benefits of transplanting colonic organoids as a regenerative therapy for patients with UC who have not responded to conventional treatments [19]. The procedure involves culturing organoids from the patient's intestinal mucosa and endoscopically transplanting them to the colon lesion sites, resulting in positive outcomes [9]. Subsequent medical examinations of the patient at four- and eight-week intervals post-procedure, along with a year-long monitoring period, confirmed the efficacy of the approach [32]. There is potential for eight additional participants to undergo organoid transplantation as part of the ongoing trial [32]. The trial is registered and more details can be found on the official registry website [33].

Challenges and limitations still exist in the use of ISC derived organoids for the treatment of IBD. Perfecting the ecological niche of ISCs poses a challenge- organoids lack tissues and their components to supplement intestinal epithelium, such as blood vessels, smooth muscle cells and immune cells [9]. Traditional organoid systems primarily reflect stem cells rather than a mix of stem cells and terminally differentiated cells seen *in-vivo*, which limits the ability to study changes in barrier function and the effects of inflammation on differentiated intestinal epithelial cells [34]. ISCs differentiated may differ from *in-vivo* surface markers and functions [1]. More verification in clinical settings is needed to understand these differences. Laboratory procedures need to be standardized for the culture of intestinal organoids as there is no uniform protocol and ISC sources used in individual studies are diverse [9]. Generating healthy organoids in large batches to be used clinically will present additional challenges in scaling, standardization, automation and method of application [7]. Developing quality-assurance and control tests for donor organoids could prevent adverse events from pathogens and optimize culture conditions [19]. Techniques used for transplantation such as endoscopic technology are complex with high costs [9]. Expanding IBD research involves in-depth model studies and rigorous testing of novel therapeutic methods [9]. This additional research will enable better delivery methods for organoids, promote engraftment and identify favorable host mucosal conditions for engraftment [27,31]. These efforts are crucial for advancing the field and improving outcomes for IBD patients.

Summary and Conclusion

IBD, comprising CD and UC, poses significant challenges, necessitating alternative treatments. SCT, specifically using ISCs cultured into organoids, has emerged in recent years as a promising avenue for treatment. ISCs play a pivotal role in maintaining GI integrity and immune balance. Organoids offer a powerful model for studying IBD, enabling evaluations of treatment efficacy and personalized medicine. Successful preclinical and clinical studies, including an ongoing trial, highlight the potential of ISC organoid transplantation for regenerative therapy in IBD patients. Despite challenges, ISC organoids offer a transformative approach for deeper molecular remission in IBD treatment, highlighting the need for ongoing research to optimize their clinical application and improve outcomes.

Conflict of Interest

The authors have no conflict of interest to declare.

References

1. Tian CM, Zhang Y, Yang MF, Xu HM, Zhu MZ, Yao J, et al. Stem cell therapy in inflammatory bowel disease: a review of achievements and challenges. J Inflamm Res. 2023;16:2089-119.
2. Zheng L, Duan SL. Molecular regulation mechanism of intestinal stem cells in mucosal injury and repair in ulcerative colitis. World J Gastroenterol. 2023;29(16):2380-96.

3. Zhang YZ, Li YY. Inflammatory bowel disease: Pathogenesis. World J Gastroenterol. 2014;20(1):91-9.
4. Wakisaka Y, Sugimoto S, Sato T. Organoid medicine for inflammatory bowel disease. Stem Cells. 2022;40(2):123-32.
5. Johns Hopkins Medicine. Inflammatory bowel disease. [Last accessed on: February 17, 2024] https://www.hopkinsmedicine.org/health/conditions-and-diseases/inflammatory-bowel-disease#:~:text=If%20medications%20do%20not%20calm,intestine%20damaged%20by%20prolonged%20inflammation
6. Wawrzyniak M, Scharl M. Genetics and epigenetics of inflammatory bowel disease. Swiss Medical Weekly. 2018;148(3738):w14671.
7. Rutherford D, Ho GT. Therapeutic potential of human intestinal organoids in tissue repair approaches in inflammatory bowel diseases. Inflammatory Bowel Dis. 2023;29(9):1488-98.
8. Okamoto R, Shimizu H, Suzuki K, Kawamoto A, Takahashi J, Kawai M, et al. Organoid-based regenerative medicine for inflammatory bowel disease. Regenerative Ther. 2020;13:1-6.
9. Tian CM, Yang MF, Xu HM, Zhu MZ, Yue NN, Zhang Y, et al. Stem cell-derived intestinal organoids: a novel modality for IBD. Cell Death Discovery. 2023;9(1):255.
10. Umar S. Intestinal stem cells. Current Gastroenterol Rep. 2010;12(5):340-8.
11. Sailaja BS, He XC, Li L. The regulatory niche of intestinal stem cells. The J Physiol. 2016;594(17):4827-36.
12. Sittipo P, Lee YK. The application of intestinal organoids and their co-culture systems in the study of gastrointestinal diseases. Organoid. 2022;2.
13. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van Den Brink S, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma and Barrett's epithelium. Gastroenterol. 2011;141(5):1762-72.
14. Sato T, Vries RG, Snippert HJ, Van De Wetering M, Barker N, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009;459(7244):262-5.
15. Lucafò M, Muzzo A, Marcuzzi M, Giorio L, Decorti G, Stocco G. Patient-derived organoids for therapy personalization in inflammatory bowel diseases. World J Gastroenterol. 2022;28(24):2636-53.
16. Hou Q, Huang J, Ayansola H, Masatoshi H, Zhang B. Intestinal stem cells and immune cell relationships: potential therapeutic targets for inflammatory bowel diseases. Frontiers in Immunol. 2021;11:623691.
17. Ghorbaninejad M, Asadzadeh-Aghdaei H, Baharvand H, Meyfour A. Intestinal organoids: A versatile platform for modeling gastrointestinal diseases and monitoring epigenetic alterations. Life Sciences. 2023:121506.
18. Gopalakrishnan S, Bakke I, Hansen MD, Skovdahl HK, Granlund AV, Sandvik AK, et al. Comprehensive protocols for culturing and molecular biological analysis of IBD patient-derived colon epithelial organoids. Frontiers in Immunol. 2023;14:1097383.
19. Watanabe S, Kobayashi S, Ogasawara N, Okamoto R, Nakamura T, Watanabe M, et al. Transplantation of intestinal organoids into a mouse model of colitis. Nature Protocols. 2022;17(3):649-71.
20. Yao Y, Kim G, Shafer S, Chen Z, Kubo S, Ji Y, et al. Mucus sialylation determines intestinal host-commensal homeostasis. Cell. 2022;185(7):1172-88.
21. Wang R, Li H, Wu J, Cai ZY, Li B, Ni H, et al. Gut stem cell necroptosis by genome instability triggers bowel inflammation. Nature. 2020;580(7803):386-90.
22. Salvi PS, Cowles RA. Butyrate and the intestinal epithelium: modulation of proliferation and inflammation in homeostasis and disease. Cells. 2021;10(7):1775.
23. Nishimura R, Shirasaki T, Tsuchiya K, Miyake Y, Watanabe Y, Hibiya S, et al. Establishment of a system to evaluate the therapeutic effect and the dynamics of an investigational drug on ulcerative colitis using human colonic organoids. Journal of gastroenterology. 2019;54:608-20.
24. Angus HC, Urbano PC, Laws GA, Fan S, Gadeock S, Schultz M, et al. An autologous colonic organoid-derived monolayer model to study immune: bacterial interactions in Crohn's disease patients. Clin Translational Immunol. 2022;11(8):e1407.
25. Jackson DN, Panopoulos M, Neumann WL, Turner K, Cantarel BL, Thompson-Snipes L, et al. Mitochondrial dysfunction during loss of prohibitin 1 triggers Paneth cell defects and ileitis. Gut. 2020;69(11):1928-38.
26. Boye TL, Steenholdt C, Jensen KB, Nielsen OH. Molecular manipulations and intestinal stem cell-derived organoids in inflammatory bowel disease. Stem Cells. 2022;40(5):447-57.
27. Yui S, Nakamura T, Sato T, Nemoto Y, Mizutani T, Zheng X, et al. Functional engraftment of colon epithelium expanded *in-vitro* from a single adult Lgr5+ stem cell. Nature Medicine. 2012;18(4):618-23.

28. Fordham RP, Yui S, Hannan NR, Soendergaard C, Madgwick A, Schweiger PJ, et al. Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury. Cell stem cell. 2013;13(6):734-44.
29. Fukuda M, Mizutani T, Mochizuki W, Matsumoto T, Nozaki K, Sakamaki Y, et al. Small intestinal stem cell identity is maintained with functional Paneth cells in heterotopically grafted epithelium onto the colon. Genes Develop. 2014;28(16):1752-7.
30. Chandra L, Borcherding DC, Kingsbury D, Atherly T, Ambrosini YM, Bourgois-Mochel A, et al. Derivation of adult canine intestinal organoids for translational research in gastroenterology. BMC biology. 2019;17(1):1-21.
31. Sugimoto S, Ohta Y, Fujii M, Matano M, Shimokawa M, Nanki K, et al. Reconstruction of the human colon epithelium *in-vivo*. Cell Stem Cell. 2018;22(2):171-6.
32. The Tokyo Medical and Dental University (TMDU) team succeeded with the world's first Mini Organ transplantation to a patient with "Ulcerative Colitis (UC)" Tokyo Medical and Dental University, National University Corporation. (2022, July 7). Tokyo Medical and Dental University. [Last accessed on: February 17, 2024] https://www.tmd.ac.jp/english/press-release/20220707-1/
33. https://rctportal.niph.go.jp/en/detail?trial_id=jRCTb032190207 [Last accessed on: February 17, 2024]
34. Crawford E, Mentrup HL, Novak EA, Mollen KP. Studying the epithelial effects of intestinal inflammation *in-vitro* on established murine colonoids. J Visualized Experim. 2023;2(196):e64804.