

REVIEW ARTICLE

Intestinal organoids: Reshaping the paradigm of inflammatory bowel disease research and therapy

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Abstract

The advent of intestinal organoid technology has brought a revolutionary breakthrough to inflammatory bowel disease (IBD) research, enabling more precise *in vitro* simulation of human intestinal physiology and pathology. This article systematically reviews the latest advances in the application of intestinal organoid technology to IBD research. First, we introduce technological innovations such as synthetic hydrogels, microfluidic organ-on-a-chip systems, and polarity-reversal culture. These approaches overcome limitations of traditional two-dimensional cultures and greatly expand the applicability of organoid models. Second, we focus on research utilizing patient-derived organoids to unveil novel mechanisms of IBD pathogenesis, highlighting their unique advantages in dissecting intrinsic epithelial defects (e.g., impaired barrier function, abnormal mucus secretion) and dynamically simulating host-microbe interactions. Furthermore, we highlight clinical translation: high-throughput drug screening for personalized medicine and organoid transplantation for mucosal regeneration. Finally, we summarize current challenges regarding standardization, vascularization, and immune microenvironment reconstruction of organoids, and discuss their broad prospects for driving innovation in IBD diagnosis and treatment strategies.

Keywords: Intestinal organoids; Inflammatory bowel disease; Intrinsic epithelial defects; Mucosa-microbiota interaction; Drug screening; Regenerative medicine

1. Introduction: The role of intestinal organoids in studying mucosa-microbiota interactions in inflammatory bowel disease

Inflammatory bowel disease (IBD) comprises a group of chronic, refractory intestinal inflammatory disorders of unclear etiology, distinguished by relapsing courses, multiple complications, and a substantial disease burden.¹ For decades, IBD treatment has largely focused on suppressing abnormal immune activation. From 5-aminosalicylic acid to glucocorticoids, and further to anti-tumor necrosis factor- α monoclonal antibodies, integrin inhibitors, and Janus kinase (JAK) inhibitors, therapeutic strategies have continually evolved. However, clinical outcomes remain unsatisfactory: approximately 30% of patients show primary non-response to biologics,

and nearly half experience secondary loss of response within one year. Moreover, the clinical application of biologics carries risks such as increased susceptibility to infections and malignancies.^{2,3} In fact, the pathogenesis of IBD is highly complex (Figure 1A), involving the interplay of genetics, environment, gut microbiota, immune imbalance, and intestinal mucosal barrier dysfunction.⁴ Genome-wide association studies suggest that as of 2023, over 300 IBD risk loci have been identified.⁵ Many of these genes exert critical functions primarily in intestinal epithelial cells rather than immune cells. For instance, risk variants in *NOD2* and *ATG16L1* lead to abnormal Paneth cell granules and reduced α -defensin secretion, resulting in gut dysbiosis and barrier defects.^{6,7} XBP1, a core transcription factor of endoplasmic reticulum stress response, when dysfunctional, can induce goblet cell apoptosis and mucosal

barrier collapse.^{8,9} Therefore, an in-depth investigation into epithelium-related pathogenic mechanisms is crucial for exploring IBD therapeutic strategies.

Notably, even when inflammation is controlled, the gut microbiota of many patients fails to return to a healthy state.¹⁰ Conversely, some healthy individuals can persistently carry traditionally defined “pathogens” (such as adherent-invasive *Escherichia coli*) without developing disease.¹¹ These observations suggest that early mucosa-microbiota interactions in IBD may shape the threshold for immune activation and the extent of tissue damage, thereby leading to heterogeneity in disease progression and treatment response. Thus, studying intestinal mucosa-microbiota interactions is paramount. Research on mucosa-microbiota interactions currently faces significant challenges. Traditional models are inadequate because of their inherent limitations. For instance, conventional two-dimensional (2D) monolayer cultures lack physiological architecture and cellular diversity,^{12,13} whereas animal models (e.g., interleukin [IL]-10 knockout mice and dextran sulfate sodium-induced colitis models) differ substantially from humans in terms of microbiota composition and immune response.

The emergence of intestinal organoid technology has provided a breakthrough. In 2009, Sato *et al.*¹⁴ from the Netherlands successfully cultured three-dimensional (3D) mini-guts with crypt-villus structures from leucinerich repeat-containing G-protein-coupled receptor 5-positive intestinal stem cells in Matrigel for the first time. Unlike traditional primary cells or cell lines, organoids possess significant advantages in cellular complexity. They can replicate the multicellular structure of animal or human tissues, thereby retaining partial physiological characteristics of the organ. This allows for the simulation of disease states and microenvironments, making them invaluable for exploring disease mechanisms, particularly showing immense potential in clinical drug development and screening. Intestinal organoids feature a 3D cellular structure (Figure 1B), with the apical side facing inward towards the lumen and the basal side facing the extracellular matrix (ECM). They contain all major cell types of the intestinal epithelium, including absorptive enterocytes, goblet cells, Paneth cells, enteroendocrine cells, and stem cells located at the crypt base. They recapitulate partial physiological functions of the *in vivo* intestine, such as barrier formation, mucus secretion, and nutrient absorption.^{15,16} Therefore, intestinal organoids represent a highly promising research platform.

Intestinal organoids can be directly established from patient biopsies or surgical specimens, retaining individual genetic backgrounds and disease characteristics while lacking immune cells, nerves, or vascular components. Together, these features provide a controllable human-

derived platform for studying epithelial biology and epithelial interactions with microbes and drugs.¹⁷ In IBD research, intestinal organoids can be derived directly from patients with ulcerative colitis (UC) or Crohn's disease, preserving individual genetic susceptibility backgrounds (e.g., risk gene variants in *NOD2*, *ATG16L1*) and disease-associated epithelial phenotypes,^{18,19} such as reduced goblet cells, thinner mucus layers, abnormal Paneth cell function, and increased barrier permeability. Consequently, they allow for the independent assessment of whether intrinsic functional defects exist in the IBD intestinal epithelium. On the other hand, traditional animal models struggle to directly observe physical contact, colonization dynamics, and functional responses between bacteria and the intestinal epithelium. Organoids, by reconstructing a polarized, secretory, and barrier-competent mucosal epithelium, enable these processes to be visualized and quantified *in vitro*, allowing direct observation of their interactions with gut microbiota. Beyond mechanistic dissection, intestinal organoids hold great potential in IBD therapy. They can serve as platforms for drug sensitivity testing to perform personalized drug screening, predict clinical efficacy, and assist clinical decision-making.²⁰ Additionally, they can be transplanted into damaged intestinal mucosa to reconstruct crypt-villus units.²¹⁻²³ Thus, intestinal organoids are poised to transition from the laboratory to the clinic, becoming a key link in the closed loop of precision IBD treatment.

This review emphasizes the significance of gut organoids in IBD mucosa-microbiota interactions, systematically outlining intestinal organoid culture technologies and their applications in simulating the gut microenvironment, establishing IBD models, conducting drug screening, and exploring regenerative medicine (Figure 1C). Additionally, we introduce advanced multicellular models, organ-on-a-chip systems, microfluidics, 3D printing, and hydrogel technologies aiding intestinal organoid research. This review aims to provide a conceptual framework and guidance for the broader application of intestinal organoids.

2. Investigating intrinsic defects of the intestinal epithelium using advanced organoid technologies

The pathogenesis of IBD is heterogeneous, yet current clinical treatment strategies primarily revolve around the core mechanism of “immune dysregulation.” While there are numerous options ranging from 5-aminosalicylic acid and glucocorticoids to biologics (infliximab, adalimumab, vedolizumab, etc.) and small molecule drugs (JAK inhibitors, S1P receptor modulators, etc.), clinical outcomes remain unsatisfactory.^{2,3} Mucosal barrier injury is another hallmark of IBD. One hypothesis posits that mucosal defects or microbial abnormalities lead to barrier impairment, which subsequently triggers abnormal immune activation.²⁴

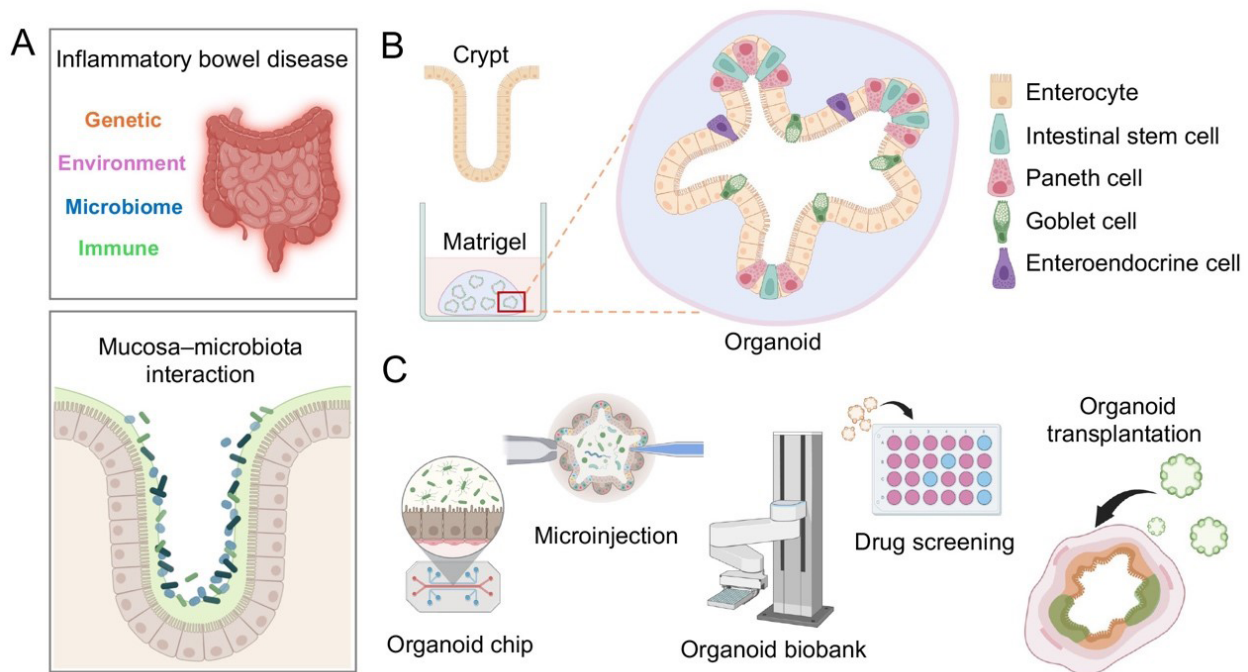


Figure 1. Applications of organoids in inflammatory bowel disease (IBD). (A) The pathogenesis of IBD is heterogeneous, with mucosa-microbiota interaction playing a central role. (B) Construction of intestinal organoids and the cellular composition of mature intestinal organoids. (C) Latest technological advances in using organoids to investigate IBD pathogenesis and therapeutic responses. Created in BioRender. hu, X. (2026) <https://BioRender.com/gt5dmyz>.

This could explain why immunomodulatory therapy can temporarily control disease activity but fails to cure IBD. However, the wide variety of cell types in the intestinal mucosa and the complex composition of the gut microbiota have severely constrained related research.

Organoid culture technology is currently a focal point of research. It allows the isolation of intestinal epithelial cells from IBD patients from the complex *in vivo* environment for study, overcoming the limitations of traditional cell lines and becoming a vital tool for studies of disease mechanisms and translational drug research. The reliability and reproducibility of organoid research heavily depend on their 3D scaffold microenvironment. Traditional 3D intestinal organoid cultures rely on animal-derived ECM. Although these natural hydrogels are biocompatible, they suffer from undefined composition (containing various undefined proteins and growth factors), substantial batch-to-batch variability, poorly controlled mechanical properties, and contamination risk.²⁵⁻²⁹ These factors may interfere with the reproducibility and accuracy of experimental results, leading to unstable organoid phenotypes.^{25,27,30,31}

The mechanical properties of 3D scaffolds have been shown to affect epithelial differentiation and phenotype. Synthetic or semi-synthetic polymer hydrogels such as polyethylene glycol (PEG) and gelatin methacryloyl

(GelMA) are now widely applied in organoid culture, becoming ideal alternatives to animal-based matrices.^{27,30-32} Compared to animal matrices, materials like PEG and peptide-crosslinked hydrogels (e.g., PEG-4MAL) allow for the construction of ECM mimics with defined composition, precisely tunable mechanical properties (e.g., stiffness, viscoelasticity), and biochemical characteristics (e.g., adhesion ligand type, density, spatial distribution). This reduces experimental interference and achieves stable genetic and phenotypic maintenance (Figure 2A).^{27,29,30,33} For example, laminin in the ECM adjusts mucosal structure by participating in cell proliferation, migration, and differentiation.^{25,26} The boronate ester-based hydrogel developed by Hushka *et al.*,³⁴ with its high viscoelasticity, promotes intestinal organoid cells to secrete endogenous matrix proteins such as laminin, thereby supporting crypt formation without the need for exogenous addition (Figure 2B and 2C). Further research integrates bioactive components into hydrogels to achieve more complex regulation. For instance, in the calcium silicate (CS) nanowire/GelMA composite material developed by Ma *et al.*,³² CS nanowires not only enhance the matrix stiffness of the hydrogel but also continuously release bioactive ions. These properties activate the key Wnt/ β -catenin signaling pathway and improve cellular glucose uptake and ATP metabolism levels, stimulating the growth and development

of mouse intestinal organoids (Figure 2D). Defined synthetic hydrogel platforms can systematically adjust key parameters such as matrix stiffness, degradability, and adhesion ligand density and type. This not only facilitates the construction of reproducible disease models but also enables the study of the impact of physical properties on organoid formation. For example, Gjorevski *et al.*³⁰ found that specific matrix stiffness is a prerequisite for the spatial separation and differentiation of specialized cells like Paneth cells. Overall, compared to traditional Matrigel, organoids cultured in synthetic hydrogels exhibit more stable phenotypes and better reflect the inherent pathological characteristics of patient epithelial cells, providing a basis for comparing IBD and healthy epithelia.

Beyond achieving stable organoid culture, synthetic hydrogels can also simulate different extracellular microenvironments. For example, culturing organoids in high-stiffness hydrogels successfully simulated the fibrotic

environment of IBD and confirmed that this stiffening drives abnormal epithelial differentiation by inducing Yes-associated protein nuclear translocation,^{27,30} recapitulating disease characteristics of the IBD epithelium.³⁵ However, dense 3D polymer networks in hydrogels may impede the diffusion of oxygen, nutrients, cytokines, and drugs, leading to insufficient substance exchange inside organoids. This also raises the possibility that some disease-associated abnormalities observed in matrices with substantial batch variability, such as Matrigel, may be influenced by *ex vivo* mechanical conditions rather than reflecting purely cell-intrinsic defects. While matrix variations complicate the analysis, evidence suggests that core IBD pathological features can be maintained across different materials under standardized conditions.²⁷ Although direct proof that IBD phenotypes remain identical across disparate matrices is currently limited, all these studies imply the necessity of utilizing chemically defined, mechanically tunable synthetic platforms. By leveraging synthetic hydrogels to

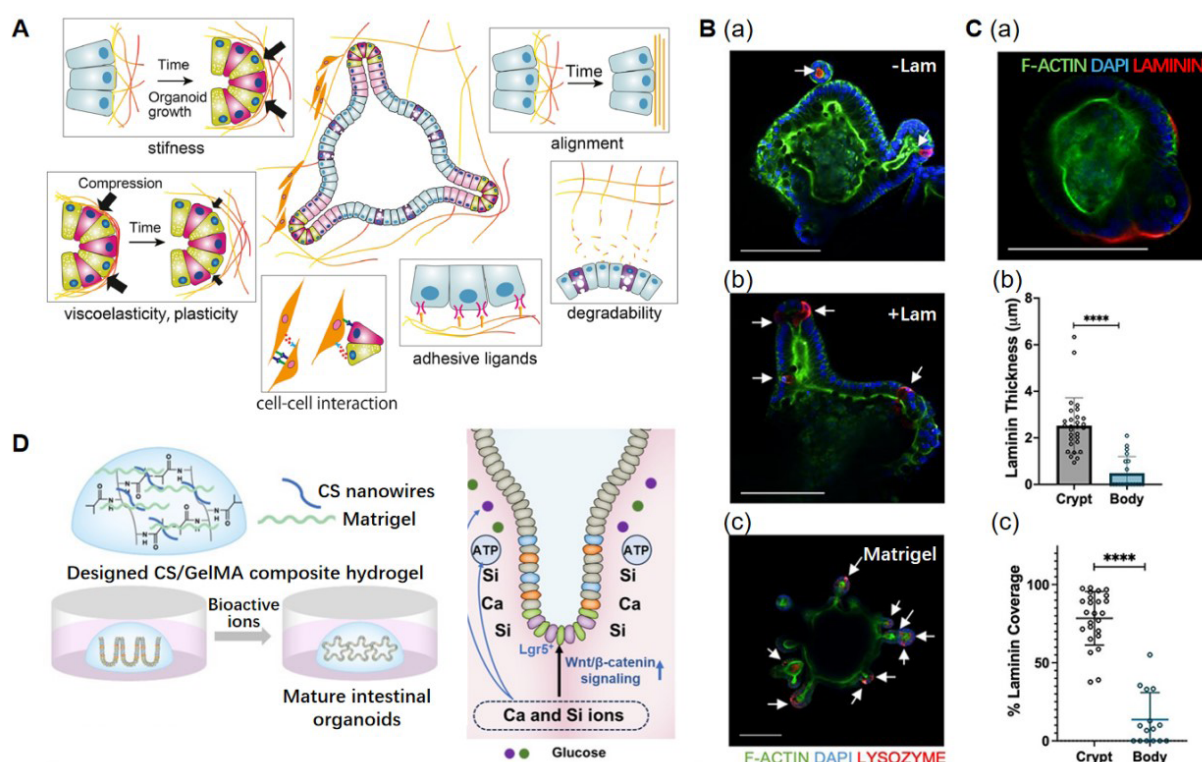


Figure 2. Synthetic hydrogel platforms simulate the gut microenvironment and recapitulate intrinsic inflammatory bowel disease epithelial defects in organoids. (A) Dynamic interactions between hydrogels and intestinal organoids. Reprinted with permission from Ref.²⁵ Copyright © 2021 Acta Materialia Inc. (B) Organoid crypt structures formed in boronate ester-based hydrogels (white arrows indicate Paneth cells). Reprinted with permission from Ref.³⁴ Copyright © 2025 Wiley-VCH GmbH. (C) Immunofluorescence staining marking abundant laminin (red) surrounding organoids in boronate ester-based hydrogel matrices, with more significant deposition in crypt regions. Reprinted with permission from Ref.³⁴ Copyright © 2025 Wiley-VCH GmbH. (D) Calcium silicate (CS)/gelatin methacryloyl (GelMA) composite hydrogels support intestinal organoid growth and development by releasing bioactive ions. Reprinted with permission from Ref.³² Copyright © 2024 Royal Society of Chemistry.

simulate the *in vivo* environment while minimizing matrix interference, researchers can effectively compare IBD and healthy organoids under identical conditions.

There are still many issues that need to be addressed in organoid research. For organoid transplantation, the degradation rate of hydrogels is difficult to precisely match the speed of *in situ* intestinal tissue regeneration, either degrading too fast to provide stable support or too slow to hinder tissue integration. Moreover, traditional 3D organoids have a closed sac-like structure, with the apical membrane facing inward, limiting material exchange, bacterial contact, and drug action, and lacking vascular, nervous, and immune components, making it difficult to simulate the *in vivo* microenvironment. All these issues are currently the focus of organoid research. In summary, the rapidly developing organoid technology has become a crucial research tool for current IBD pathology and mechanism studies.

In addition to biomaterial optimization, the combination of organoids and gene editing has become a frontier tool to verify intrinsic epithelial defects. Gene editing technologies such as the CRISPR-Cas9 system and lentivirus-mediated short hairpin RNA knockdown have been widely used for gene modification of intestinal organoids.³⁶ By co-culturing intestinal organoids with human induced pluripotent stem cell-derived mesenchymal stem cells, researchers found enhanced epithelial proliferation and mucosal repair, with TSG-6 identified as a key mediator of this effect.³⁷ Targeted silencing of the *SLFN11* gene via lentivirus transfection can effectively inhibit inflammation-induced apoptosis of intestinal epithelial cells, providing a new perspective for the study of the pathogenesis of inflammatory bowel disease.³⁸ In addition, in intestinal organoids with liposome-mediated *circHIPK3* overexpression, proliferation-related genes are significantly upregulated, and tissue repair is accelerated, offering a new strategy for gene therapy of intestinal diseases.³⁹ However, there are still many challenges in the research on gene-edited organoids. In terms of safety issues, viral transduction has the risk of insertional tumorigenesis, while non-viral methods such as CRISPR may still cause off-target effects. Therefore, to realize the wide application of gene-edited organoids, it is still necessary to solve key issues such as transfection efficiency, safety, tissue maturity, and system standardization.

Based on current advanced organoid technologies, researchers have found that patient-derived organoids (PDOs) from IBD patients still exhibit significant epithelial functional abnormalities under culture conditions without exogenous pro-inflammatory factors and immune cells.⁴⁰ However, when interpreting the abnormal epithelial phenotypes observed in IBD organoids, it is essential to clarify whether these abnormalities stem from persistent inherent defects or long-lasting epigenetic memory

(inflammatory imprinting) induced by the patient's prior *in vivo* inflammatory environment. In-depth investigations have revealed that the complex and persistent pathological phenotypes in IBD organoids are the product of synergistic effects between both mechanisms.

On the one hand, inherent defects driven by genetic variants (e.g., risk alleles in *NOD2*, *ATG16L1*, and *IL10RB*) form the genetic foundation of IBD pathogenesis, the functional impacts of which have been extensively validated in organoid models. In PDOs from UC patients, the number of goblet cells is significantly decreased, and their secretion of mucin 2 (MUC2) is reduced. This leads to a thinner, loosely structured, and discontinuous mucus layer, thereby disrupting the physical barrier and antimicrobial function of the intestinal epithelium.^{41,42} Studies have also found that in PDOs from Crohn's disease patients, Paneth cells exhibit abnormal morphology and reduced numbers, with significantly diminished capacity to synthesize and secrete core antimicrobial peptides such as α -defensins, leading to weakened antimicrobial activity.⁴³ This defect is directly related to intrinsic genetic regulatory abnormalities in epithelial cells. For example, dysregulation of the Wnt/ β -catenin signaling pathway affects Paneth cell differentiation,^{43,44} while loss-of-function mutations in the IL-22 co-receptor *IL10RB* can lead to a complete absence of Paneth cells in organoids.⁴⁵ These genetically driven Paneth cell abnormalities can be observed in early passages of PDOs and do not depend on exogenous inflammatory stimulation, confirming the existence of stable intrinsic defects in epithelial cells. On the other hand, mounting evidence suggests that ISCs develop persistent epigenetic imprinting after chronic or high-intensity cytokine exposure. Hamdan *et al.*⁴⁶ found that even after long-term expansion *ex vivo*, prior-inflamed (PI) organoids retain a "primed" chromatin state associated with stress response, repair, and inflammatory genes. Upon secondary challenge, PI organoids exhibit pathologically heightened transcriptional responses and accelerated wound closure, confirming the existence of a retained inflammatory memory program.⁴⁶ This discovery provides a mechanistic explanation for site-specific IBD relapse and suggests that the interplay between epigenetic imprinting and genetic predispositions is the root cause of sustained epithelial dysfunction. Current research protocols typically integrate matched-pair comparisons with functional genetic validation to decouple these mechanisms. First, by establishing paired organoids derived from matched PI and never-inflamed (NI) regions of the same IBD patient, researchers define acquired inflammatory imprinting as phenotypes persisting exclusively in PI organoids.⁴⁶ Conversely, abnormalities consistently present in both PI and NI lines (compared to healthy controls) are indicative of patient-wide inherent epithelial defects.⁴² Furthermore,

CRISPR/Cas9-mediated gene correction has emerged as a powerful tool for verifying intrinsic genetic causality; for instance, correction of specific risk alleles (e.g., *NOD2* or *ATG16L1*) in PDOs can restore normal cellular functions.^{45,47}

Additionally, intrinsic defects in the IBD epithelium include increased barrier permeability and heightened stress sensitivity. Using polarized monolayer epithelia formed by dissociating PDOs (e.g., Transwell models), barrier function can be quantified. Studies show that in monolayer epithelia derived from IBD sources, transepithelial electrical resistance (TEER) is consistently lower than in healthy controls, and baseline permeability to fluorescent markers is higher.⁴⁸ Reduced expression of tight junction proteins (e.g., ZO-1, occludin) in IBD organoid cultures further confirms impaired barrier function.¹⁸ When exposed to the same low dose of tumor necrosis factor-alpha, Crohn's disease PDOs exhibit faster and more severe barrier damage.⁴⁹ This heightened stress sensitivity suggests that stress pathways (e.g., endoplasmic reticulum stress, autophagy) within IBD epithelial cells themselves may be intrinsically dysregulated.

In summary, by isolating intestinal epithelial cells from IBD patients from the complex *in vivo* environment via organoid culture systems, disease-associated abnormal phenotypes persist stably. By combining strategies such as paired sample comparisons and gene editing technologies, it is possible to effectively distinguish long-term inflammatory imprinting from intrinsic epithelial defects. This strongly proves that intrinsic defects and long-lasting epigenetic imprints exist in the IBD intestinal epithelium rather than being merely transient inflammatory effects; therefore, targeting immune dysregulation alone cannot cure the disease.

3. Investigating inflammatory bowel disease mucosa-microbiota interactions using advanced organoid technologies

Gut microbiota dysbiosis is an important pathogenic factor in IBD. Numerous metagenomic studies have shown that IBD is associated with marked alterations in gut microbial composition and reduced microbial diversity. Beneficial commensals (such as butyrate-producing *Faecalibacterium prausnitzii*) are depleted, whereas potential pathogens (such as adherent-invasive *E. coli*) are enriched. This ecological imbalance can weaken the mucus barrier and activate abnormal host immune responses.⁵⁰ Notably, dysbiosis often persists in the early stages of the disease and even during clinical remission,^{51,52} suggesting it is not merely a consequence of inflammation but may participate in disease initiation and maintenance. Therefore, clarifying the details of gut microbiota-intestinal mucosa interactions is critical

for studying IBD pathogenesis and drug development.⁵³

However, traditional research models have obvious flaws: 2D cell line models (e.g., Caco-2) cannot autonomously secrete and form a complete mucus layer, while animal models have complex *in vivo* environments, making it difficult to simulate human host-microbe interactions.^{54,55} Intestinal organoids cultured from human adult stem cells provide a more reliable platform for studying intrinsic epithelial defects and epithelium-microbiota interactions. Nevertheless, microbiota research based on organoids still faces many difficulties. For instance, most gut microbiota are strict anaerobes, while traditional 3D organoids have a closed luminal structure, making it difficult to simulate intestinal fluid shear stress, mechanical stretching, and oxygen gradients, and thereby limiting long-term co-culture with strict anaerobes.⁵⁶

Microinjection technology allows for the introduction of bacteria into the lumen of 3D organoids. This technique maintains the 3D structure of organoids and enables quantitative delivery of bacteria, often used to study pathogen infection and commensal colonization mechanisms (Figure 3A and 3B).⁵⁷ For example, in studying the pathogenesis of Shiga toxin-producing *E. coli* O157:H7, microinjection was used to inject bacteria into the lumen of human intestinal organoids (HIOs), successfully establishing an infection model. This revealed the pathogenic process where O157:H7 promotes toxin expression and induces host epithelial production of reactive oxygen species, thus disrupting the cytoskeleton and epithelial barrier integrity.⁵⁸

Additionally, polarity-reversal organoids break through the limitations of closed lumens, providing a pathway for microbiota to directly contact the apical membrane of the epithelium. Studies have found that when culturing organoids in synthetic hydrogels (such as GelMA), spontaneous and controllable "apical-out" inversion occurs, exposing the apical membrane directly to the external environment. This provides a convenient model for studying bacterial adhesion and invasion mechanisms (Figure 3C and 3D).^{31,57} For instance, researchers found in apical-out organoids that *Salmonella* Typhimurium can trigger cytoskeletal rearrangements similar to those *in vivo*,^{57,59} while probiotics (such as *Lactobacillus acidophilus*) exhibit protective effects on the epithelial barrier.⁶⁰

Two-dimensional organoid culture directly avoids the problem of inaccessible apical membranes caused by the closed lumen of 3D organoids. It allows for quantitative assessment of barrier function and high-resolution dynamic imaging, providing a more flexible platform for studying IBD epithelium-microbiota interactions. By seeding organoid cells onto Transwell membranes coated with synthetic hydrogels, 2D monolayer epithelia with

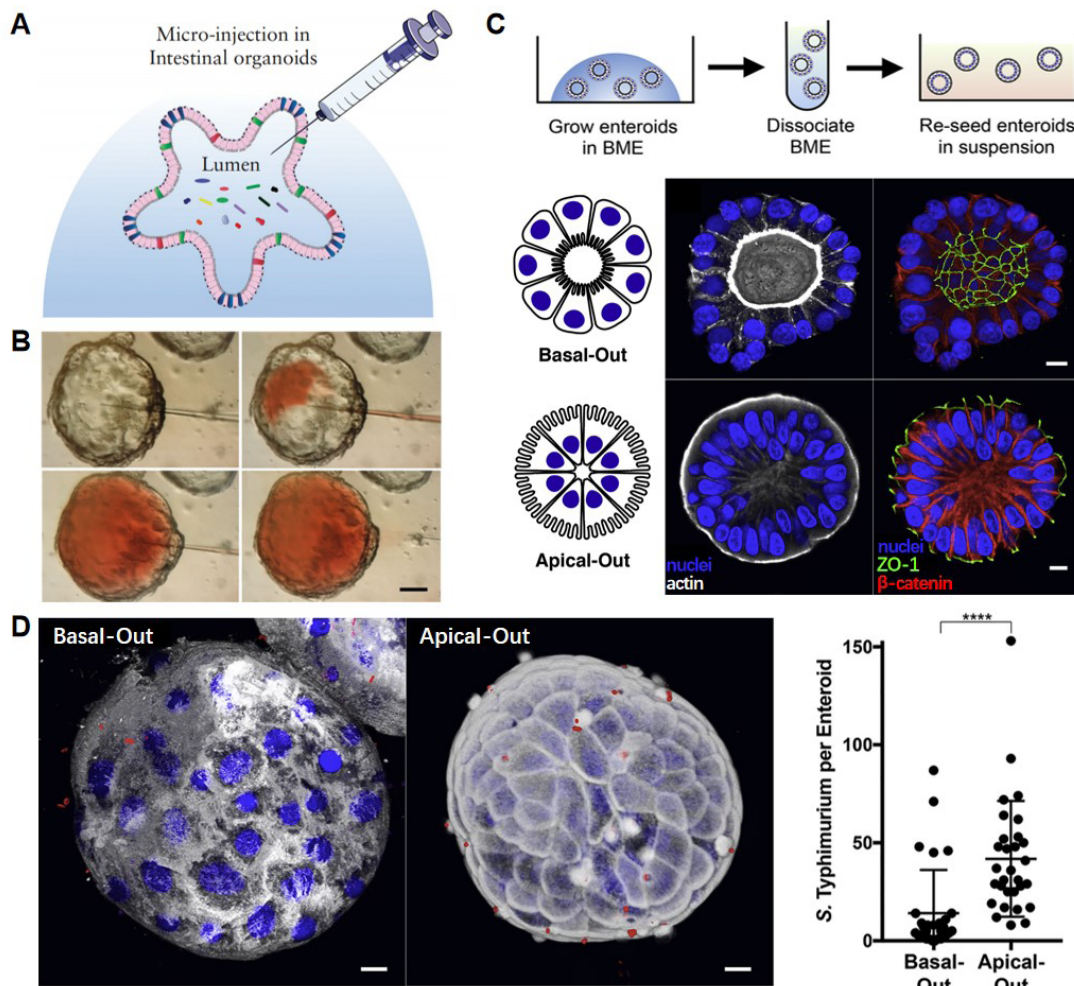


Figure 3. 3D Organoid technology platforms for studying epithelium-microbiota interactions. (A) Introducing bacteria into 3D organoids via microinjection. Reprinted with permission from Ref.⁶¹ Copyright © 2020, The Author(s). (B) Microscopic observation of injecting non-toxic edible dye into pig colon organoids. Reprinted with permission from Ref.⁶² Copyright © 2021, The Author(s). (C) Construction and structure of apical-out and basal-out intestinal organoids. Suspension culture induces polarity reversal in basal-out organoids, forming apical-out organoids with the apical membrane facing outward. Immunofluorescence staining shows the apical membrane directly exposed to the external environment. Reprinted from Ref.⁵⁹ (D) Susceptibility analysis of apical-out and basal-out intestinal organoids to *Salmonella* Typhimurium. Reprinted from Ref.⁵⁹ Abbreviation: BME: Basement membrane extract.

clear apical-basal polarity can be formed (Figure 4A).⁶³⁻⁶⁵ This highly reproducible epithelial barrier model enables comparison of barrier function between organoid-derived epithelia from healthy individuals and patients with IBD.^{48,65} Furthermore, the 2D monolayer model has advantages in studying bacterial trans-epithelial translocation. By staining lipoteichoic acid, a component of the bacterial cell wall, bacterial translocation across small-intestinal 2D monolayers can be observed (Figure 4B).⁶⁶ However, while 2D Transwell monolayers offer a highly stable environment for TEER measurements, the lack of dynamic stimuli typically results in an attenuated mucus layer (<36 μm) that lacks the physiological stratified architecture.⁶⁷

Additionally, the expression levels of antimicrobial peptides in conventional 3D or 2D static cultures often remain at basal levels.⁶⁸ Furthermore, these static culture models lack fluid exchange and aeration control, resulting in the transport of nutrients and metabolites being entirely dependent on passive diffusion. This prevents the formation of a hypoxic microenvironment characteristic of intestinal crypts, making it difficult to simulate the dynamic intestinal environment and maintain strict anaerobic conditions, which limits the long-term co-culture with strict anaerobes when used alone.

In recent years, advanced microfluidic organ-on-a-chip systems have overcome these limitations through multi-

dimensional biomimicry. The gut-on-a-chip developed by Kim *et al.*⁶⁹ successfully induced the formation of 3D villus-like structures by simulating fluid shear stress and periodic mechanical stretching, thereby enhancing barrier function (Figure 4C). This dynamic perfusion system not only mimics intestinal peristalsis but also achieves more efficient nutrient supply and metabolic waste clearance than static models through continuous media replacement, thereby maintaining long-term cellular metabolic homeostasis.⁶⁹ Meanwhile, under fluid and mechanical stimulation, a mucus barrier with physiological characteristics can form within the chip, providing a key tool for investigating bacterial penetration behavior.⁷⁰ Finally, microfluidic chips utilizing gas-permeable materials such as polydimethylsiloxane, combined with dual-channel designs, establish stable trans-epithelial oxygen gradients by controlling gas perfusion to maintain an extremely low oxygen environment in the lumen (Figure 4D).⁵⁴ Research by Jalili-Firoozinezhad *et al.*⁵⁴ confirmed that the establishment of such physiological oxygen gradients is crucial for maintaining intestinal barrier function and microbial diversity. This allows for the long-term co-culture of human intestinal epithelium with over 200 unique human gut microbial species, including high

abundances of obligate anaerobes, maintaining microbial ratios similar to those observed in human feces.

Furthermore, using genetically encoded fluorescent proteins (e.g., green fluorescent protein [GFP]) to label live bacteria allows for real-time tracking of bacterial behavior in organoid or chip models.⁷¹ For example, the Williamson team developed a high-throughput organoid microinjection platform, microinjecting GFP-labeled *E. coli* into the organoid lumen and achieving long-term, high-resolution dynamic imaging and bacterial quantification (Figure 4E).⁷² In summary, these technologies provide a feasible strategy for studying the mechanisms of microbiota penetrating the mucus layer, contacting the epithelium, and triggering subsequent immune responses in IBD.

Compared to static models, the gut-on-a-chip produces TEER values that more closely mirror the high-impedance environment *in vivo*, forms a significantly thicker (~50 μm) stratified mucus barrier, and partially restores the physiological secretion of antimicrobial peptides.⁵⁴ Consequently, absolute quantitative readouts—such as mucus thickness, TEER, and antimicrobial peptide secretion—exhibit platform-dependent variations,

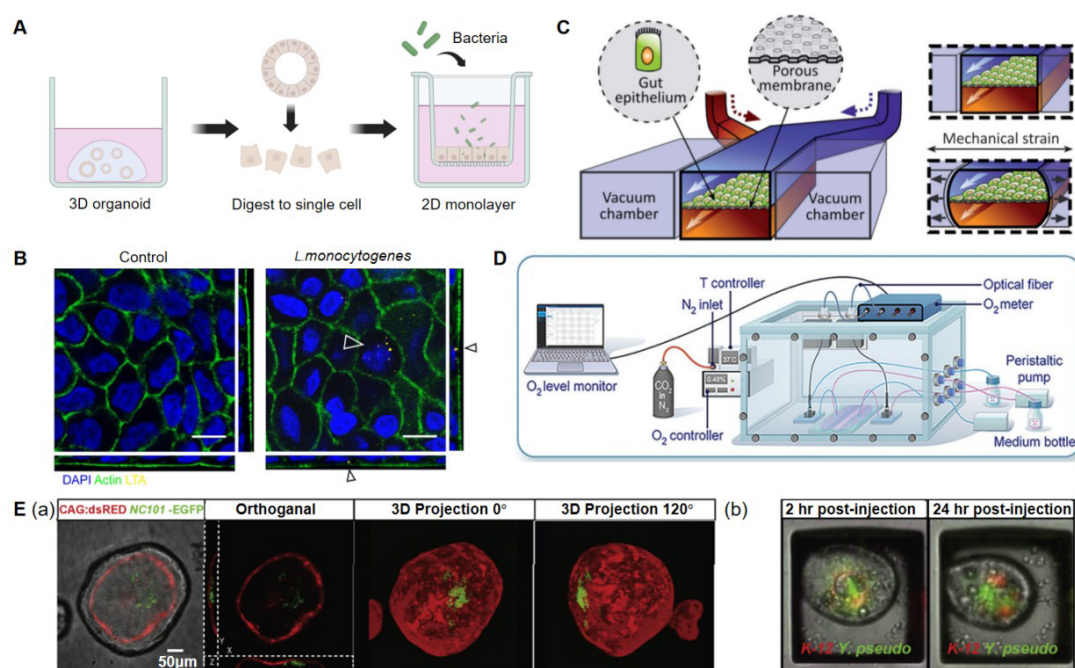


Figure 4. Organoid-derived 2D models and microfluidic chip platforms for epithelium-microbiota interaction and real-time imaging analysis. (A) Converting 3D organoids into 2D monolayers to study epithelium-microbiota interactions. Created in BioRender. hu, X. (2026) <https://BioRender.com/ea9fx4u>. (B) Studying mucosal barriers and bacterial translocation using 2D monolayers. Immunofluorescence observation of 2D monolayers uninfected (left) and infected (right) with *Listeria monocytogenes*. Reprinted from Ref.⁶⁶ (C) Schematic of a microfluidic gut chip device simulating fluid shear stress and periodic mechanical stretching. Reprinted with permission from Ref.⁶⁹ Copyright © Royal Society of Chemistry. (D) Schematic of a dual-channel microfluidic organ chip with an oxygen gradient. Reprinted with permission from Ref.⁵⁴ Copyright © 2019, The Author(s). (E) Real-time imaging and quantitative analysis of fluorescently labeled bacteria within the organoid lumen. Reprinted with permission from Ref.⁷² Copyright © 2018 The Authors.

reflecting the varying degrees of physiological fidelity across different systems. Nevertheless, as demonstrated by Arnauts *et al.*,⁴⁸ the core pathological trends observed in healthy versus IBD samples remain highly consistent across platforms, ensuring the robustness of the fundamental biological conclusions.

Current research utilizes advanced technologies such as intestinal organoids and organ-on-a-chip models to dissect mucosa–microbiota crosstalk in controlled, complex biomimetic environments. In-depth studies suggest that the intestinal epithelium is not a passive recipient of microbiota activity. Instead, it participates in shaping the microbiota structure by constructing a complex microenvironment composed of physical barriers, chemical gradients, and biological factors. Healthy intestinal epithelium creates favorable survival conditions for commensals by maintaining an intact mucus barrier, sustaining a low-oxygen microenvironment, and exerting antimicrobial effects. Under culture conditions simulating intestinal fluid shear stress, healthy organoids can differentiate into goblet cells and secrete a functional mucus layer rich in MUC2 protein.^{73,74} This mucus layer is crucial for maintaining commensal microbiota; the dense inner layer structure directly prevents most direct bacterial contact with the epithelium,⁴⁸ while the loose, mucin-rich outer layer provides a habitat and nutrients for strict anaerobic commensals (such as Firmicutes, Bacteroidetes).^{41,48,75} This function of the epithelium, providing key metabolic support for commensals, has been demonstrated in co-culture models using organoid-derived epithelia and microfluidic technology, where *F. prausnitzii* can stably colonize and metabolize host-derived chemicals.⁷⁶ Colon epithelial cells maintain an extremely low oxygen environment at the epithelial surface through rapid oxygen consumption, providing a competitive advantage for obligate anaerobic commensals. This physiological function has been directly validated in organoid models. For example, in an intestinal organoid physiological oxygen co-culture system, organoid-derived monolayer epithelial cells effectively clear apical oxygen, creating strict anaerobic conditions, thereby successfully supporting the colonization and interaction of various strict anaerobic commensals.⁵⁶ Meanwhile, antimicrobial peptides such as α -defensins play an important role in regulating gut microbiota and defending against pathogens. Studies confirm that in small intestinal organoid models, α -defensins can effectively limit the growth of pathogens (such as *Salmonella*), supporting the role of antimicrobial peptides in shaping microbiota structure.^{68,77}

On the other hand, intrinsic defects existing in the intestinal epithelium of IBD patients impair their ability to shape a healthy microbiota. Studies have found that in organoids derived from UC patients, IBD-characteristic

changes occur, including reduced goblet cells, thinner mucus, and abnormal mucus composition.^{78,79} Functional experiments show that the pathobiont *E. coli* p19A can penetrate the mucus layer, invade the epithelium, and induce a strong inflammatory response.⁷⁹ Simultaneously, due to the thinning of the mucus layer and disruption of tight junctions,^{18,78} luminal oxygen can more easily reach the epithelial surface, disrupting the low-oxygen environment on the epithelial side. A slight rise in oxygen concentration provides proliferation opportunities for facultative anaerobes, altering microbiota composition. Regarding antimicrobial function, Paneth cell dysfunction in IBD leads to a shortage of core antimicrobial peptides like α -defensins, further weakening the epithelium's ability to clear invading bacteria and shape a normal microbiota.⁴³

Compared to healthy intestinal epithelium, IBD intestinal epithelium exhibits multiple intrinsic defects: reduced goblet cells leading to thinner mucus layers and decreased MUC2 expression; Paneth cell dysfunction resulting in reduced α -defensin secretion; disrupted tight junctions increasing barrier permeability; and abnormal epithelial oxygen consumption causing apical oxygen leakage, which disrupts the physiological low-oxygen microenvironment. These defects collectively create a microenvironment conducive to the overgrowth of facultative anaerobes and their direct contact with the epithelium. Dysbiosis further exacerbates epithelial damage, forming a vicious cycle, highlighting the active contribution of intrinsic epithelial defects to IBD pathogenesis (Figure 5).

In summary, intrinsic defects in the IBD epithelium (such as impaired barrier function, elevated oxygen concentration, and lack of antimicrobial peptides) create a pro-inflammatory microbiota microenvironment, thereby driving the vicious cycle of IBD. The combination of organoid and hydrogel technologies enables researchers to isolate intrinsic epithelial defects in controlled *in vitro* systems, dynamically analyzing how they enrich harmful microbiota and trigger sustained inflammatory attacks.

4. Translational applications of organoids in inflammatory bowel disease therapy

Intestinal organoid technology has gradually evolved from a basic research tool into a key bridge connecting mechanistic dissection with clinical intervention. The core advantage of organoids lies in their ability to directly expand miniature intestinal structures containing a complete lineage of epithelial cells from living tissues. They maintain donor genetic and phenotypic characteristics long-term *in vitro* while excluding confounding influences from the immune or nervous systems. These characteristics give them unique value in the precision treatment of IBD.

Current clinical or pathological classifications of IBD fail

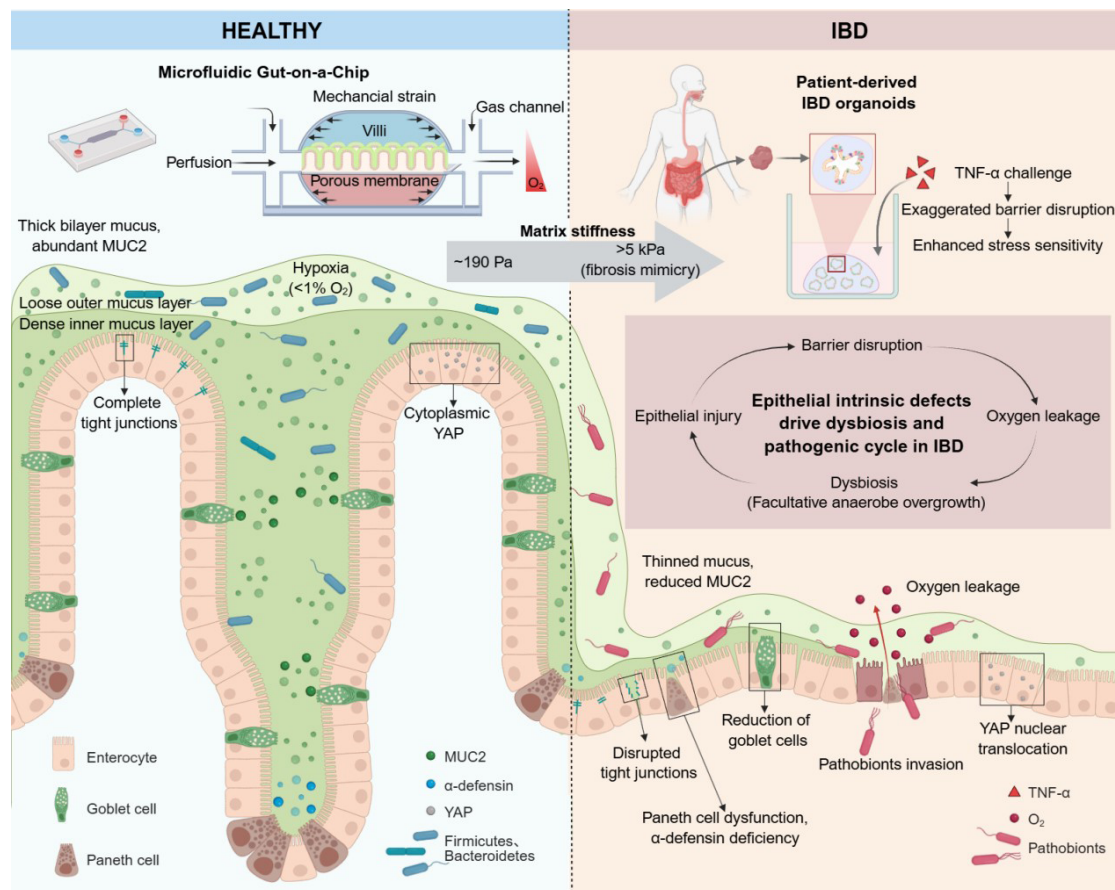


Figure 5. Organoid models reveal mechanisms by which intrinsic inflammatory bowel disease (IBD) epithelial defects drive dysbiosis. Created in BioRender. hu, X. (2026) <https://BioRender.com/ygjj8vo>.

to explain the substantial variation in patient responses to the same therapy. Increasing evidence suggests that the intestinal epithelium of IBD patients may itself harbor inherent, persistent functional defects. Researchers have established organoids from tissues of UC patients in endoscopic remission and found that even in an *in vitro* environment without exogenous inflammatory factor stimulation, their gene expression profiles differed significantly from those of healthy controls. They exhibited continuous dysregulation of epithelial secretion and absorption-related pathways and showed an abnormal differentiation trend shifting toward an “upper gastrointestinal” phenotype.⁴⁷ This suggests that the pathological basis of IBD may be rooted at the stem cell level. Such alterations of the epithelium may lead to permanent changes in the mucosa, subsequently transforming absorption and secretion functions, thereby fueling long-term disease development. Therefore, systematic assessment of intestinal function based on patient organoids—such as organoid formation speed, goblet cell differentiation capacity, mucus layer quality, and barrier integrity—can evaluate whether a patient’s intestinal inflammation is driven by immune

dysregulation or mucosal functional defects. This function-oriented classification not only helps understand disease heterogeneity but also provides a theoretical basis for subsequent targeted interventions.

The translational application of organoids in IBD therapy is a current major research direction (Figure 6). Establishing patient intestinal organoid banks can be used for personalized precision treatment, drug screening, pharmacological characteristic studies (e.g., toxicity, absorption, metabolism, excretion), and exploring regenerative medicine strategies based on organoid transplantation. Drug development for IBD often faces challenges such as high costs, low response rates, and potential side effects. Two-dimensional cell line cultures are simple and low-cost but cannot truly reflect *in vivo* conditions. There are species differences between model animals and humans, meaning animal experimental results may differ from actual clinical outcomes.^{80,81} In contrast, drug screening based on intestinal organoids offers advantages of speed, high throughput, and strong clinical relevance, driving a shift from traditional screening models to more precise and efficient directions. The

highthroughput screening platforms integrated with organoids have been increasingly applied in IBD drug discovery, enabling largescale compound evaluation and mechanism exploration with improved efficiency and clinical relevance.⁸²

In a study on the JAK inhibitor tofacitinib for treating UC, it was found that the *in vitro* sensitivity of UC PDOs to tofacitinib was highly correlated with patient clinical response.⁸³ Patients whose organoids were classified as tofacitinib-sensitive (UC-S) showed greater clinical improvement after treatment. MATE1 (a cationic transporter protein on which tofacitinib uptake depends) was expressed at higher levels in UC-S organoids, and inhibition of MATE1 reduced tofacitinib efficacy in these organoids, suggesting that MATE1 expression may serve as a biomarker of response to tofacitinib.⁸³ Based on data from this cohort of 48 UC patients, Jang *et al.*⁸² further quantified the predictive performance of this model, demonstrating that organoids achieved a specificity of up to 100% in identifying clinical non-responders, whereas the sensitivity for predicting responders was approximately 50–60%. These findings underscore the significant clinical value of organoids in averting ineffective treatments, though their role as independent decision-making tools remains constrained by current technical limitations. This methodology of utilizing patient-specific organoids to

elucidate or predict drug responsiveness holds immense clinical potential. However, most current evidence in the IBD field is derived from single-center, small-scale retrospective studies and lacks the large-scale, prospective multi-center validation established in fields such as colorectal cancer.⁸⁴ To enhance predictive dimensionality and reproducibility, the establishment of large-scale organoid biobanks integrated with machine learning has become a prevailing trend. For instance, Dennison *et al.*¹⁹ established a substantial biobank comprising 312 intestinal epithelial organoids from IBD patients to develop prognostic epigenetic molecular signatures. Similarly, Penrose *et al.* were the first to utilize PDOs as prospective predictive tools in Crohn's disease, identifying two distinct molecular subtypes and their associations with real-world clinical outcomes.⁸⁵ In summary, organoids can be used as a rapid preliminary screening and supplementary predictive tool, but they cannot fully replace clinical decision-making at present.

On the other hand, the combination of high-throughput screening platforms and organoid technology has greatly improved efficiency. In a series of studies from the University of Basel, Switzerland, single intestinal stem cells were expanded into organoids and used for large-scale compound screening. Analysis of 2,789 compounds, combined with RNA sequencing and quantitative imaging,

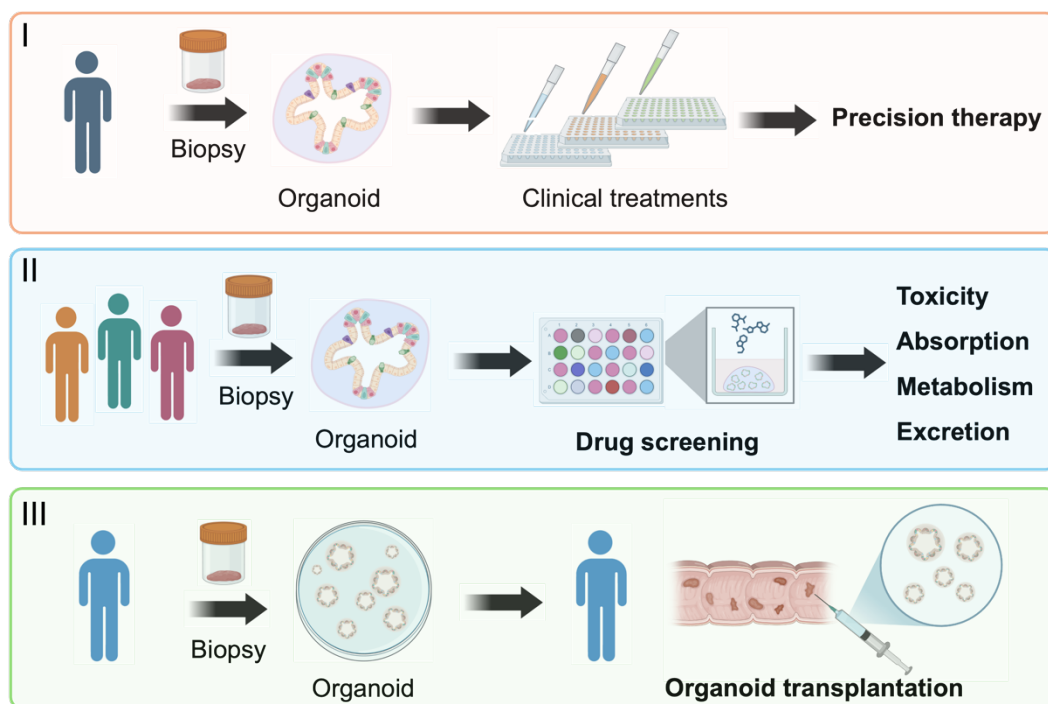


Figure 6. Applications of organoids in inflammatory bowel disease treatment. Created in BioRender. hu, X. (2026) <https://BioRender.com/mf6hs7v>

identified 7 phenotypes and 15 phenotype clusters, highlighting a role for intrinsic retinoic acid metabolism in epithelial lineage regulation.^{86,87}

Bioengineering techniques can address many limitations of high-throughput analysis. Lavickova *et al.*⁸⁸ proposed an integrated microfluidic platform (Figure 7A and 7B) for the high-throughput preparation of intestinal organoids growing within hydrogel droplets/microparticles. This solves the deficiencies of traditional matrix dome cultures in terms of reproducibility, scalability, and individual analysis, which is particularly important for enhancing the application potential in personalized medicine as well as drug screening. Furthermore, organoid-on-a-chip technology enables researchers to evaluate drug absorption, distribution, and metabolism characteristics under dynamic conditions. By integrating microfluidic systems of intestinal and liver organoids, first-pass metabolism processes can be simulated, more accurately predicting drug bioavailability.^{89,90} Such systems can also study drug-induced intestinal toxicity, such as intestinal mucosal damage caused by non-steroidal anti-inflammatory drugs.⁹¹

For some refractory IBD patients, especially those with extensive mucosal damage or even structural remodeling due to long-term chronic inflammation, simple immunosuppression may be insufficient to restore normal intestinal physiological function. In such cases, regenerative medicine strategies that transplant healthy epithelium to repair damaged areas become an extremely attractive treatment approach. As epithelial “seeds” with self-renewal and multi-lineage differentiation potential, organoids show great promise in this field. The first successful transplantation of healthy intestinal organoids was published in 2012, in which Yui *et al.*⁹² found that 4 weeks post-transplantation, donor-derived cells successfully reconstituted a monolayer epithelium, generating self-renewing crypts that were normal in function and histology. Currently, HIO models can be generated either from adult stem cells or from pluripotent stem cells.^{14,93} However, organoid transplantation still faces technical barriers, such as how to deliver them and ensure organoid survival, which urgently need to be resolved. Currently, researchers are attempting to use biomedical materials to solve problems like culture transfer during organoid transplantation. For example, using synthetic hydrogels as delivery carriers for intestinal organoids to participate in intestinal injury repair provides a new strategy for IBD treatment.^{25,27,94,95} Cruz-Acuña *et al.*²⁷ used a novel hydrogel that not only supported the stable growth and expansion of HIOs but also served as an injectable carrier to directly deliver HIOs to sites of intestinal injury, promoting HIO engraftment and mucosal repair. Additionally, Sugimoto *et al.*,⁹⁶ by transplanting ileum-derived organoids into the rat colon, successfully constructed a “small intestinalized colon” with

small intestinal villus structures and absorptive function (Figure 7C), significantly improving the nutritional status and survival rate of short bowel syndrome model animals. This study provides an important reference for mucosal regeneration strategies in IBD treatment. Although human applications are still in the early exploratory stage, the success in animal models has laid a solid foundation for their clinical translation.

In summary, intestinal organoids are profoundly reshaping the landscape of IBD research and treatment. Deep mechanistic exploration via organoids can touch the essence of the disease; *in vitro* drug sensitivity screening using organoids holds the promise of bidding farewell to empirical medication and moving towards precision medicine; and organoid transplantation makes it possible for IBD treatment to shift fundamentally from controlling symptoms to restoring tissue function. Although challenges remain in model complexity, technical standardization, and clinical validation, with the deepening of multidisciplinary cross-fusion, organoid technology is bound to bring a new era of more effective, safe, and personalized treatment for IBD patients.

5. Current limitations

Although intestinal organoids show immense potential in IBD research, their transition from mechanistic dissection to clinical translation still faces multiple challenges. While traditional intestinal organoid models have advantages in studying autonomous epithelial cell functional defects, the pathogenesis of IBD involves multifactorial interactions among immunity, mucosa, microbiota, and the environment. Existing models struggle to comprehensively simulate the complex pathological microenvironment of IBD. Most PDOs contain only epithelial components, lacking key constituents such as immune cells (e.g., T cells, macrophages), stromal cells (e.g., fibroblasts), nerves, and blood vessels. Immune-cell-free intestinal organoids should not be regarded as deficient models; rather, they are controllable, simplified systems for isolating epithelial behavior. Phenotypes observed in these systems should be interpreted as the “structural vulnerability” of the intestinal epithelium—the initiating link that drives the IBD inflammatory cascade. However, it must be acknowledged that immune-cell-free models cannot fully recapitulate the integrated immune-epithelial-microbiota cascade *in vivo*, and their conclusions should be confined to the epithelial level. To further simulate the bidirectional communication between immune compartments and the epithelium in IBD, some studies have attempted to co-culture autologous peripheral blood immune cells with organoids to simulate immune attacks. These systems are mostly short-term and static, struggling to maintain physiological ratios and spatial distributions. Additionally, vascularization represents a

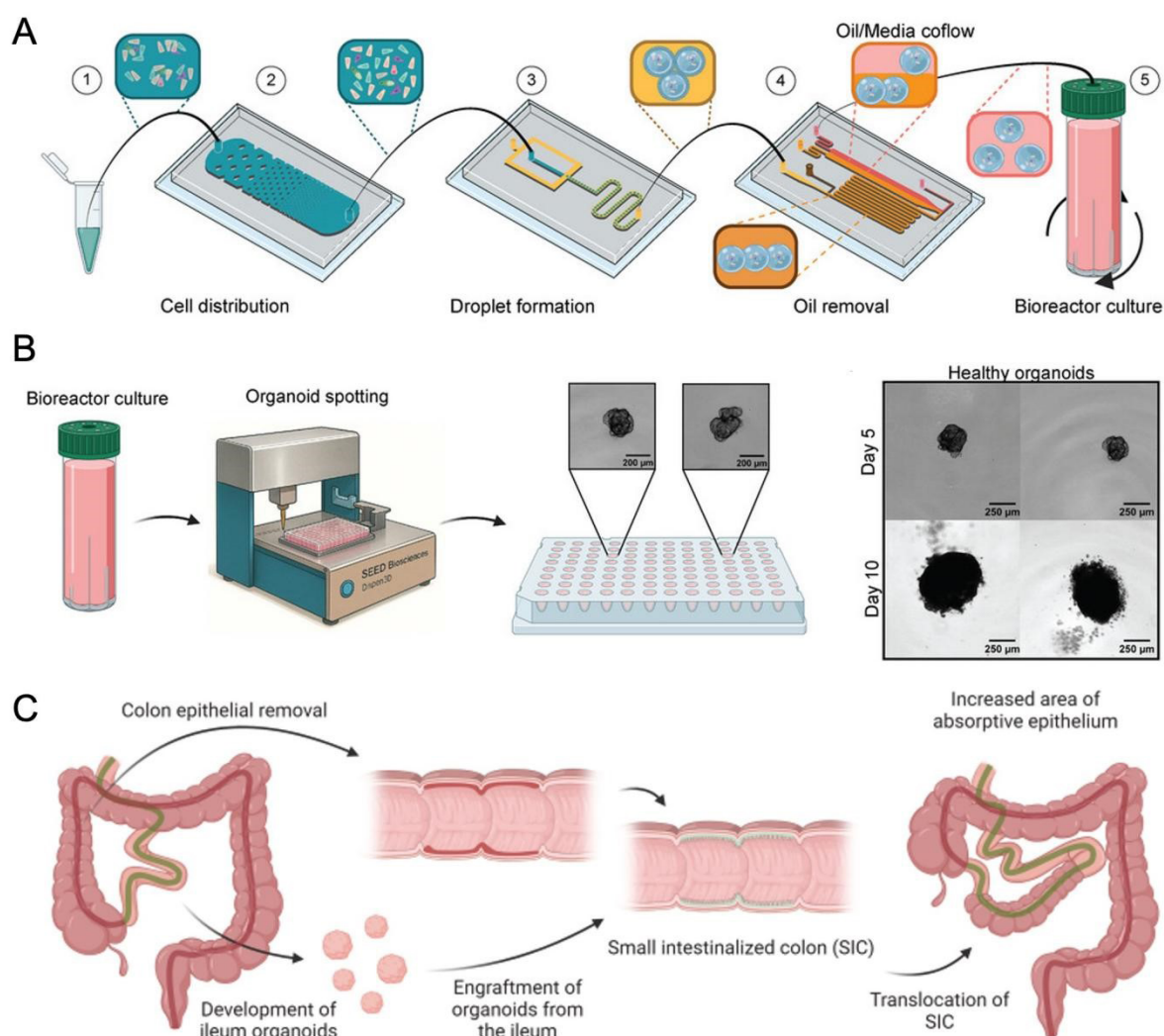


Figure 7. Advances of gut organoids in therapy development. (A) Schematics of the microfluidic chips and oil removal device used for the generation of organoid droplets. Reprinted from Ref.⁸⁸ Copyright © 2026 The Author(s). (B) Schematic of organoid distribution from the bioreactor to the well plate by organoid spotting. Reprinted from Ref.⁸⁸ Copyright © 2026 The Author(s). (C) Schematic of a small intestinalized colon. Reprinted with permission from Ref.²¹ Copyright: © 2024 The Author(s).

major challenge: conventional organoids lack endothelial cells and perfusable vascular networks, co-culture of epithelium and vascular cells is difficult due to mismatched microenvironmental requirements, *in vitro* vascular lumen formation is inefficient without hemodynamic shear stress, and integration between donor organoid vessels and host vasculature is slow after transplantation. Furthermore, organoids are typically cultured under normoxic conditions, whereas intestinal crypts reside in a hypoxic environment. This discrepancy may affect the authenticity of epithelial metabolic states and microbiota interactions.

On the other hand, current technical protocols for organoid culture research lack unified standards. From tissue acquisition and stem cell expansion to differentiation

induction, there are significant differences in culture media formulations (e.g., concentrations of different growth factors), matrix materials (Matrigel vs. synthetic hydrogels), passaging methods, and even differentiation protocols across different laboratories. Matrigel, in particular, as an animal-derived matrix, shows substantial batch-to-batch variability and contains undefined growth factors, which may interfere with drug or microbiota responses. Although synthetic hydrogels have been used as alternatives to Matrigel in recent years due to their defined composition and tunable mechanical properties, their effectiveness in supporting long-term culture and maintaining cellular heterogeneity still requires large-scale validation. Regarding the clinical translation of intestinal organoids,

although some teams have reported using organoids to predict IBD patient responses to biologics or probiotics, these are mostly retrospective small-sample studies lacking prospective, multi-center clinical trial validation. The organoid culture cycle is relatively long, and the functional stability after cryopreservation and recovery has not been fully resolved, hindering the clinical translation of organoid transplantation strategies. Additionally, healthcare systems have not yet established certification standards for organoid drug sensitivity testing, making it difficult to incorporate them into routine diagnostic and treatment workflows.

Routine clinical application of intestinal organoids, particularly for regenerative transplantation, currently faces significant technological and regulatory barriers. At the technological level, achieving scale-up production in compliance with Good Manufacturing Practice (GMP) is a primary prerequisite. This necessitates a complete shift from undefined animal-derived matrices toward chemically defined synthetic hydrogels or xeno-free culture systems.^{97,98} Furthermore, the lack of standardized evaluation frameworks hinders the unification of quality control. Initiatives such as the Organoid Standards Initiative in Korea and the Human Intestinal Organoids group standard (2023) in China have begun to establish norms for terminology and technical requirements.^{99,100} Regarding engraftment safety, critical challenges such as delivery methods, engraftment efficiency, and long-term stability must be addressed. Notably, evidence confirming the non-tumorigenicity of normal intestinal stem cell-derived organoids provides a crucial safety foundation for organoid-based regenerative medicine.¹⁰¹ From a regulatory perspective, intestinal organoids are typically classified as tissue-engineered products under the framework of Advanced Therapy Medicinal Products in international jurisdictions, requiring rigorous clinical trials and marketing authorization.¹⁰² In March 2026, the autologous organoid therapy ATORM-C received Investigational New Drug approval from the Ministry of Food and Drug Safety in Korea to initiate Phase 1 clinical trials, marking the world's first regulated clinical validation for an organoid-based regenerative therapy.¹⁰³ Nevertheless, challenges remain regarding the absence of unified global filing standards and imperfect traceability systems for individualized products.

6. Future prospects

Future efforts should focus on further enhancing the physiological relevance of intestinal organoid models. By integrating microfluidic chip technology with autologous immune cells, multicellular systems with oxygen gradients and mechanical stimulation can be developed to better recapitulate the intestinal mucosal microenvironment and support long-term co-culture with strict anaerobes. For clinical translation, the standardization of organoid culture

protocols should be accelerated, including the application of fully defined synthetic hydrogels and serum-free culture systems. Relevant authorities and professional institutions are expected to establish unified operational and evaluation standards, covering key functional indicators such as barrier function, mucus secretion, and antimicrobial peptide production. To address cross-platform variability, efforts are underway to establish standardized frameworks and incorporate real-time monitoring technologies,^{104,105} thereby improving comparability across organoid platforms. Meanwhile, clinical validation must be strengthened: largescale organoid biobanks should be established that integrate genotypes, functional phenotypes, and clinical data; prospective clinical trials are warranted to verify the reliability of organoid-based drug sensitivity testing; and rapid detection strategies (such as short-term drug response evaluation using unexpanded primary epithelial sheets) will help realize personalized treatment.

In addition, several key challenges remain to be addressed, including the stable reconstruction of the multicellular immune microenvironment, efficient vascularization, GMP-compliant manufacturing, engraftment safety, long-term therapeutic efficacy, as well as ethical and regulatory improvements. The integration of intestinal organoids with gene editing, high-throughput screening, and artificial intelligence will further promote the dissection of disease mechanisms, identification of drug targets, and prediction of individual therapeutic responses.

7. Conclusion

In conclusion, with the continuous advancement of technologies including organ-on-a-chip, high-throughput screening, and multicellular integration, coupled with the progressive establishment of unified global regulatory frameworks and standardized GMP manufacturing systems, intestinal organoids are expected to become a core platform for precision medicine in IBD, providing a new paradigm for illuminating pathogenesis, developing novel therapeutics, achieving mucosal regeneration, and implementing personalized patient management.

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Conflict of interest

The authors declare they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

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Availability of data

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