ACCSCIENCE PUBLISHING

OR

REVIEW ARTICLE

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

Hua Liu[1,2†], Xiaoyu Hu[1†], Jie Zhong[1*], and Zhengting Wang[1*]

[1]Department of Gastroenterology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

[2]Department of Geriatrics, Medical Center on Aging, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

***Corresponding authors:** Jie Zhong (zj10455@rjh.com.cn); Zhengting Wang (zhengtingwang@shsmu.edu.cn)

[†]*These authors contributed equally to this work.*

Citation: Liu H, Hu X, Zhong J, Wang Z. Intestinal organoids: Reshaping the paradigm of inflammatory bowel disease research and therapy. *Organoid Res.* 2026;2(3):026090014. doi: 10.36922/OR026090014

Received: February 28, 2026

Revised: April 10, 2026

Accepted: April 15, 2026

Published online: May 12, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, which provided that the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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-alpha 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 repeatcontaining Gproteincoupled 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]

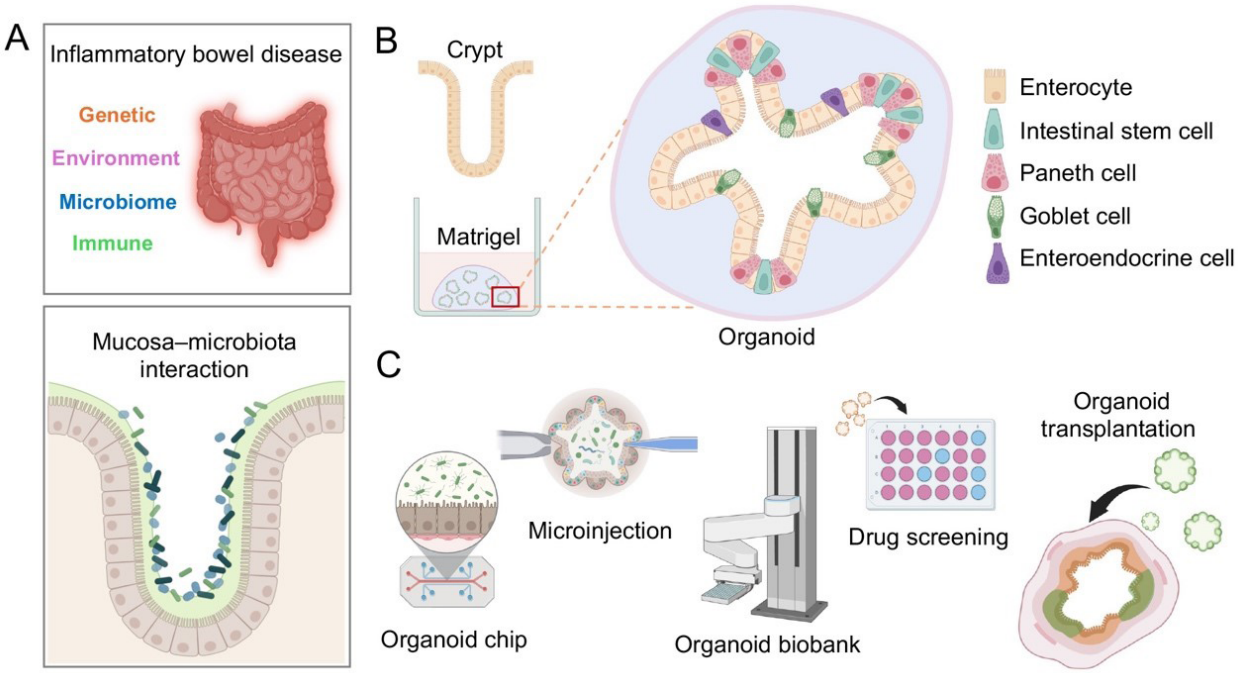

Figure 1. Applications of organoids in inflammatory bowel disease (IBD). (A) The pathogenesis of IBD is heterogeneous, with mucosa–microbiota interaction playing a central role. (B) Construction of intestinal organoids and the cellular composition of mature intestinal organoids. (C) Latest technological 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

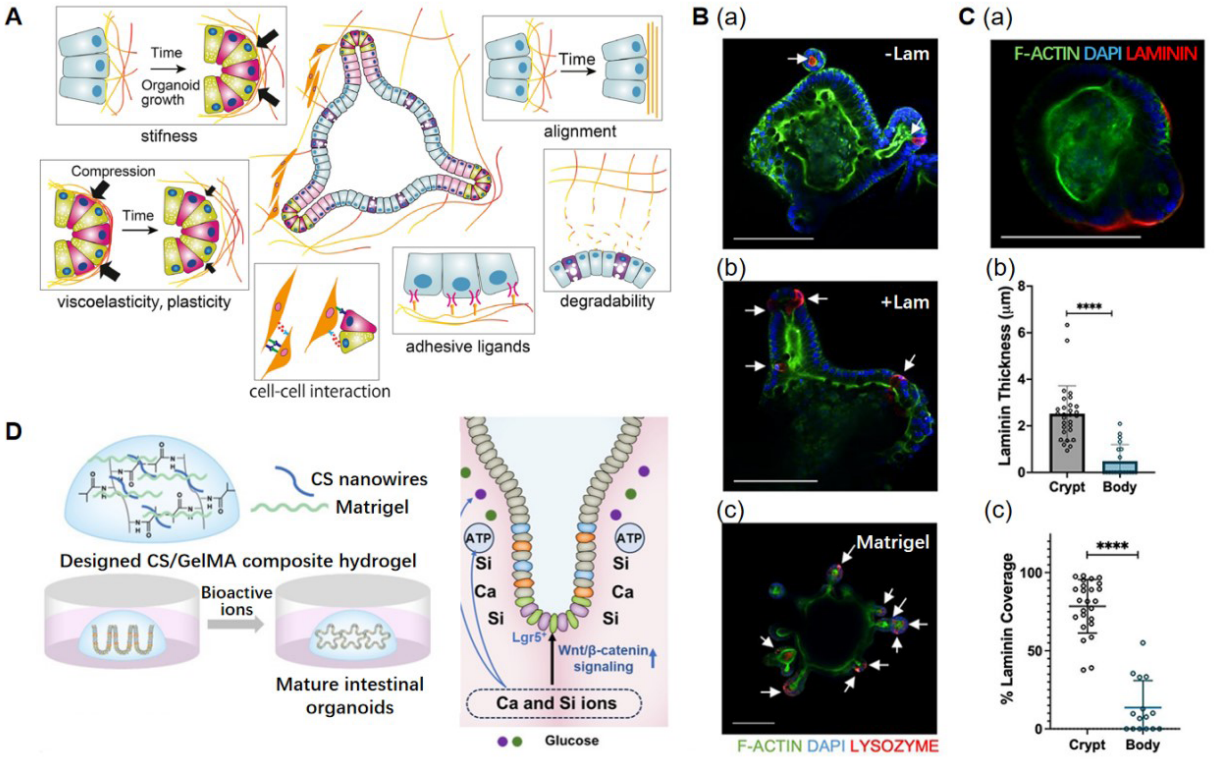

Figure 2. Synthetic hydrogel platforms simulate the gut microenvironment and recapitulate intrinsic inflammatory bowel disease epithelial defects in organoids. (A) Dynamic interactions between hydrogels and intestinal organoids. Reprinted with permission from Ref.[25] Copyright © 2021 Acta Materialia 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

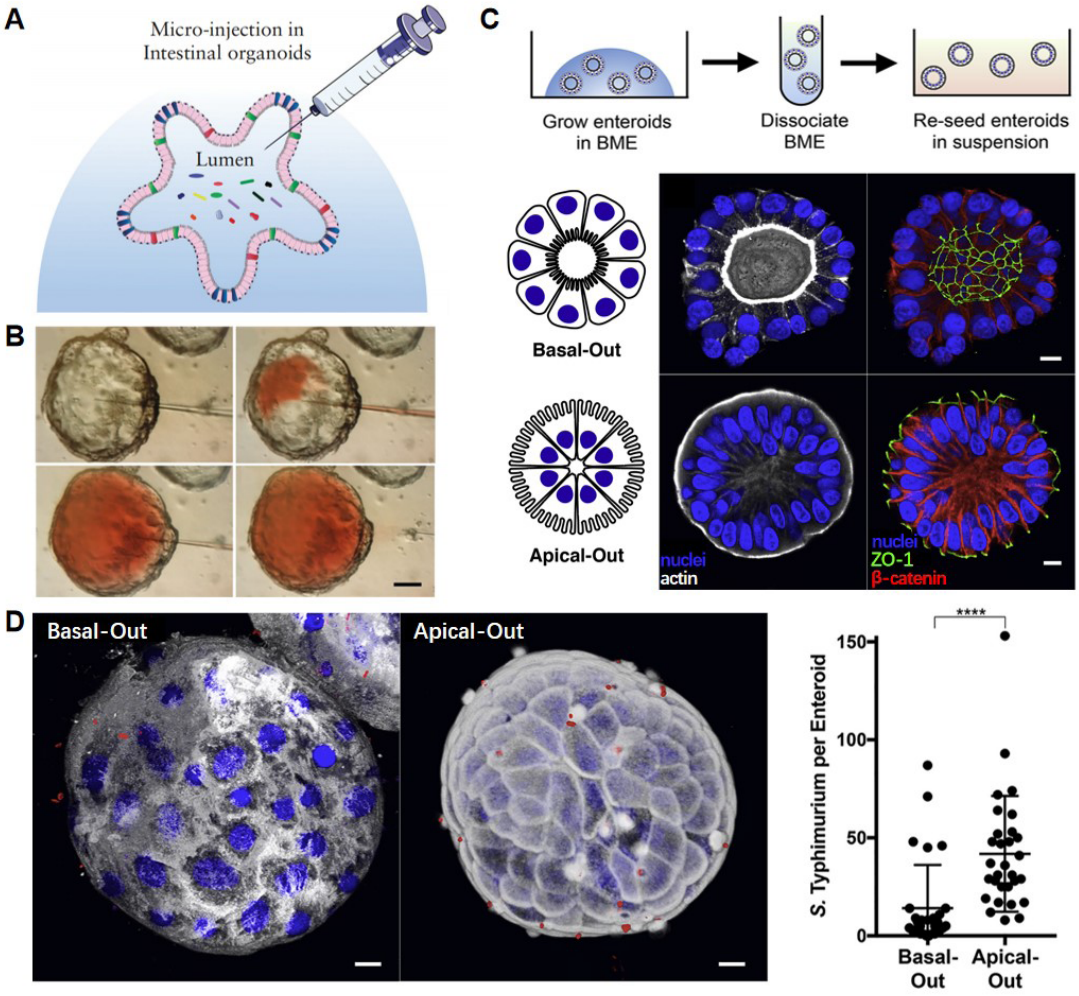

Figure 3. 3D Organoid technology platforms for studying epithelium–microbiota interactions. (A) Introducing bacteria into 3D organoids via microinjection. Reprinted with permission from Ref.[61] Copyright © 2020, The Author(s). (B) Microscopic observation of injecting non-toxic edible dye into pig colon 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,

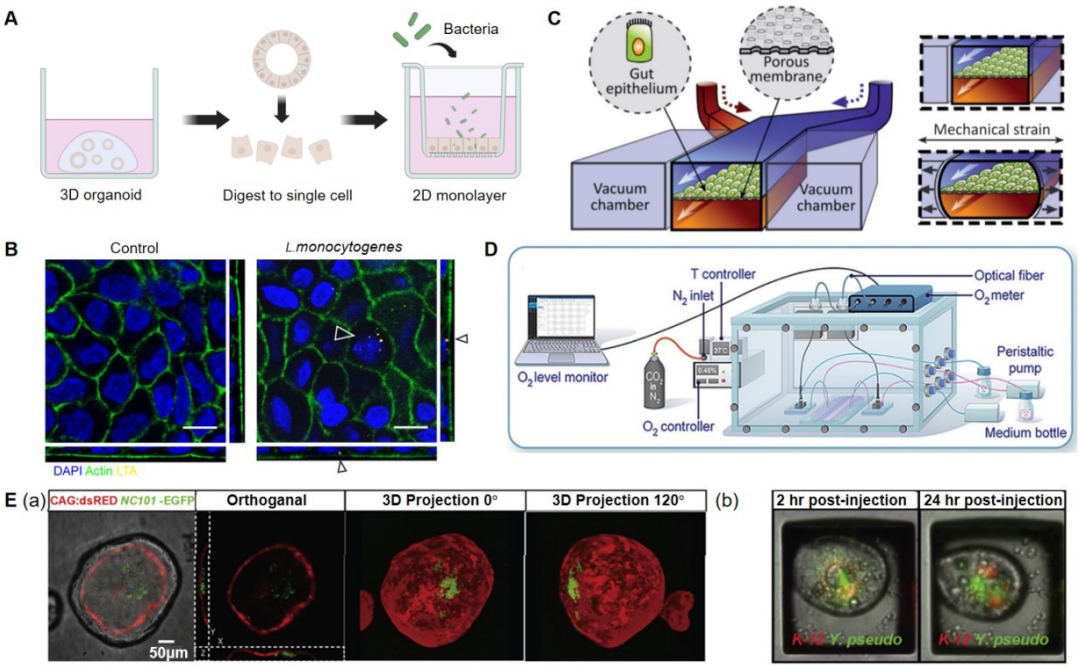

Figure 4. Organoid-derived 2D models and microfluidic chip platforms for epithelium–microbiota interaction and real-time imaging analysis. (A) Converting 3D organoids into 2D monolayers to study epithelium-microbiota interactions. Created in BioRender. hu, X. (2026) https://BioRender.com/ea9fx4u. (B) 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.

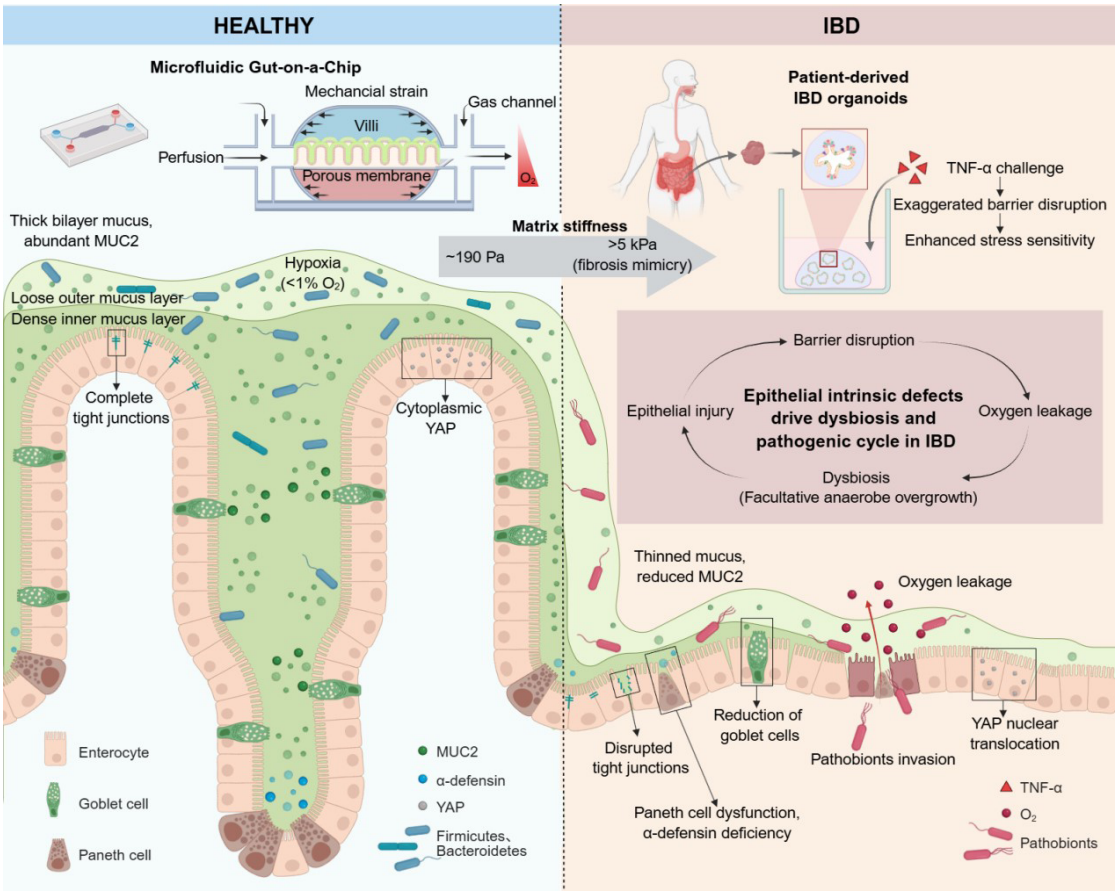

Figure 5. Organoid models reveal mechanisms by which intrinsic inflammatory bowel disease (IBD) epithelial defects drive dysbiosis. Created in BioRender. hu, X. (2026) https://BioRender.com/ygjj8vo.

Current clinical or pathological classifications of IBD fail 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,

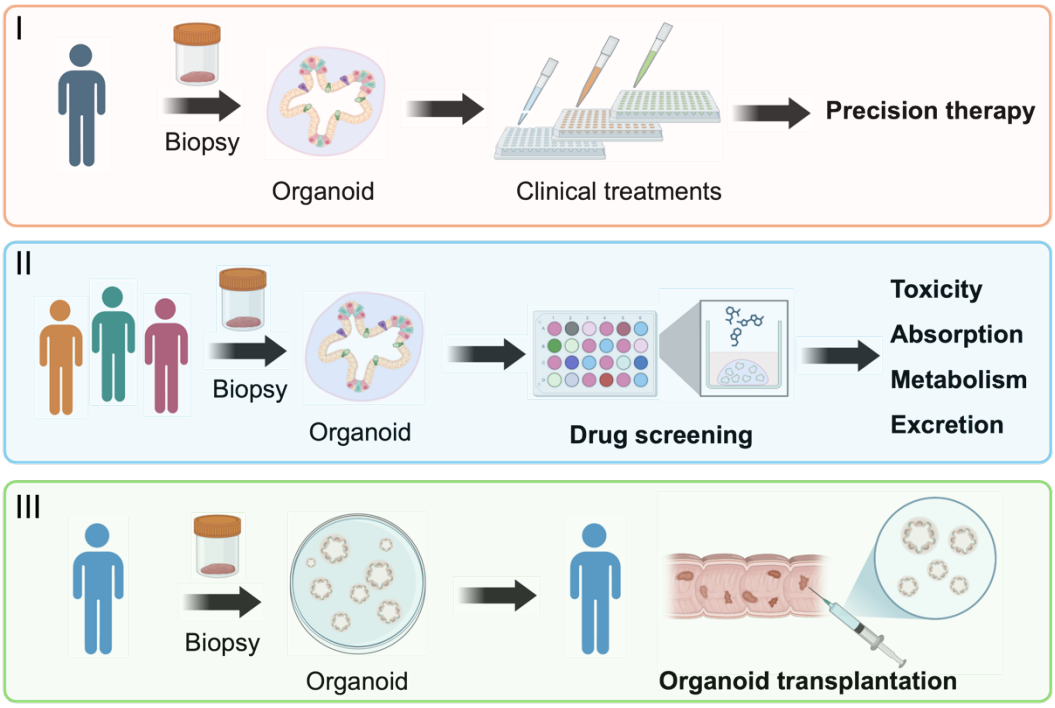

Figure 6. Applications of organoids in inflammatory bowel disease treatment. Created in BioRender. hu, X. (2026) https://BioRender.com/mf6hs7v

combined with RNA sequencing and quantitative imaging, 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

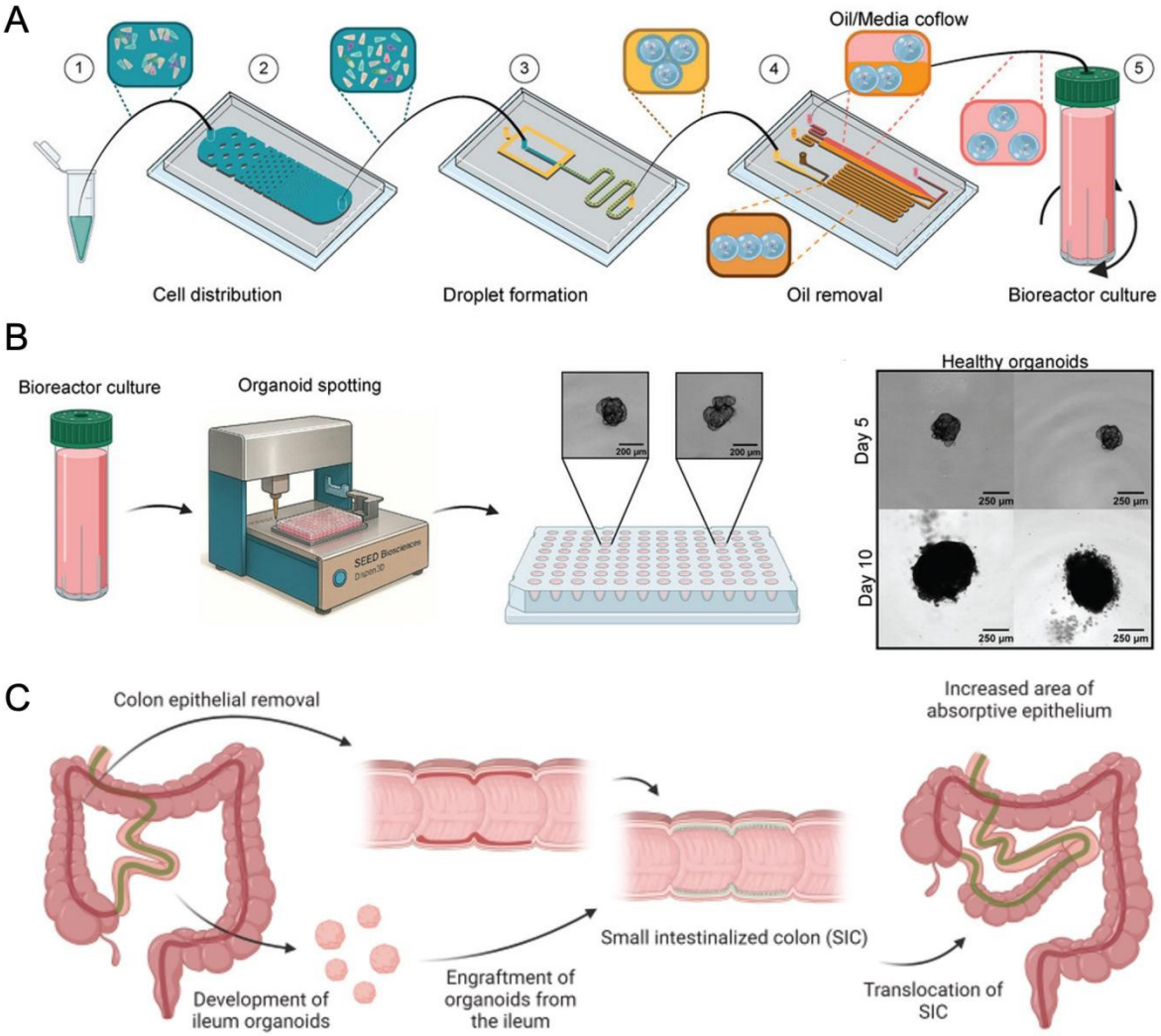

Figure 7. Advances of gut organoids in therapy development. (A) Schematics of the microfluidic chips and oil removal device used for the generation of organoid droplets. Reprinted from Ref.[88] Copyright © 2026 The Author(s). (B) Schematic of organoid distribution from the bioreactor to the well plate 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).

distributions. Additionally, vascularization represents a 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 serumfree 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 organoidbased drug sensitivity testing; and rapid detection strategies (such as shortterm 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, GMPcompliant manufacturing, engraftment safety, longterm therapeutic efficacy, as well as ethical and regulatory improvements. The integration of intestinal organoids with gene editing, highthroughput 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 organonachip, highthroughput 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.

Acknowledgments

Parts of the illustrations were created using BioRender.com. We acknowledge BioRender for providing the scientific illustration platform that assisted in the visual representation of this work.

Funding

This work was supported by the National Natural Science Foundation of China (32470951, 82500660), China Postdoctoral Science Foundation (2025M782139), and Shanghai Municipal Health Planning Commission (202340092).

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Zhengting Wang
Visualization: Xiaoyu Hu
Writing–original draft: Hua Liu, Xiaoyu Hu
Writing–review & editing: Jie Zhong, Zhengting Wang

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

References

1. Ng SC, Shi HY, Hamidi N, *et al*. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet*. 2017;390(10114):2769-2778.

   doi: 10.1016/s0140-6736(17)32448-0

2. Hirten RP, Iacucci M, Shah S, Ghosh S, Colombel JF. Combining Biologics in Inflammatory Bowel Disease and Other Immune Mediated Inflammatory Disorders. *Clin Gastroenterol Hepatol*. 2018;16(9):1374-1384.

   doi: 10.1016/j.cgh.2018.02.024

3. Ahmed W, Galati J, Kumar A, *et al*. Dual Biologic or Small Molecule Therapy for Treatment of Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol*. 2022;20(3):e361-e379.

   doi: 10.1016/j.cgh.2021.03.034

4. Ramos GP, Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. *Mayo Clin Proc*. 2019;94(1):155-165.

   doi: 10.1016/j.mayocp.2018.09.013

5. Sazonovs A, Stevens CR, Venkataraman GR, *et al*. Large-scale sequencing identifies multiple genes and rare variants associated with Crohn's disease susceptibility. *Nat Genet*. 2022;54(9):1275-1283.

   doi: 10.1038/s41588-022-01156-2

6. VanDussen KL, Liu TC, Li D, *et al*. Genetic Variants Synthesize to Produce Paneth Cell Phenotypes That Define Subtypes of Crohn's Disease. *Gastroenterology*. 2014;146(1):200-209.

   doi: 10.1053/j.gastro.2013.09.048

7. Lin JD, Devlin JC, Yeung F, *et al*. Rewilding Nod2 and Atg16l1 Mutant Mice Uncovers Genetic and Environmental Contributions to Microbial Responses and Immune Cell Composition. *Cell Host Microbe*. 2020;27(5):830-840.e4.

   doi: 10.1016/j.chom.2020.03.001

8. Kaya GG, Schwarzer R, Dannappel M, *et al*. Unfolded protein response transcription factor XBP1 suppresses necroptosis-induced colitis by reinforcing the mucus barrier. *Immunity*. 2025;58(9):2208-2225.e6.

   doi: 10.1016/j.immuni.2025.07.023

9. Stengel ST, Fazio A, Lipinski S, *et al*. Activating Transcription Factor 6 Mediates Inflammatory Signals in Intestinal Epithelial Cells Upon Endoplasmic Reticulum Stress. *Gastroenterology*. 2020;159(4):1357-1374.e10.

   doi: 10.1053/j.gastro.2020.06.088

10. Pierce R, Jan NJ, Kumar P, Middleton J, Petri WA, Marie C. Persistent dysbiosis of duodenal microbiota in patients with controlled pediatric Crohn's disease after resolution of inflammation. *Sci Rep*. 2024;14(1):11567.

    doi: 10.1038/s41598-024-63299-y

11. Rahmouni O, Vignal C, Titécat M, *et al*. High carriage of adherent invasive E. coli in wildlife and healthy individuals. *Gut Pathog*. 2018;10(1):23.

    doi: 10.1186/s13099-018-0248-7

12. Pearce SC, Coia HG, Karl JP, Pantoja-Feliciano IG, Zachos NC, Racicot K. Intestinal in vitro and ex vivo Models to Study Host-Microbiome Interactions and Acute Stressors. *Front Physiol*. 2018;9:1584.

    doi: 10.3389/fphys.2018.01584

13. Rahman S, Ghiboub M, Donkers JM, *et al*. The Progress of Intestinal Epithelial Models from Cell Lines to Gut-On-Chip. *Int J Mol Sci*. 2021;22(24):13472.

    doi: 10.3390/ijms222413472

14. Sato T, Vries RG, Snippert HJ, *et al*. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*. 2009;459(7244):262-265.

    doi: 10.1038/nature07935

15. Beumer J, Clevers H. Cell fate specification and differentiation in the adult mammalian intestine. *Nat Rev Mol Cell Biol*. 2020;22(1):39-53.

    doi: 10.1038/s41580-020-0278-0

16. Nyström EEL, Martinez-Abad B, Arike L, *et al*. An intercrypt subpopulation of goblet cells is essential for colonic mucus barrier function. *Science*. 2021;372(6539):eabb1590.

    doi: 10.1126/science.abb1590

17. Kollmann C, Buerkert H, Meir M, *et al*. Human organoids are superior to cell culture models for intestinal barrier research. *Front Cell Dev Biol*. 2023;11:1223032.

    doi: 10.3389/fcell.2023.1223032

18. d'Aldebert E, Quaranta M, Sébert M, *et al*. Characterization of Human Colon Organoids From Inflammatory Bowel Disease Patients. *Front Cell Dev Biol*. 2020;8:363.

doi: 10.3389/fcell.2020.00363

19. Dennison TW, Edgar RD, Payne F, *et al*. Patient-derived organoid biobank identifies epigenetic dysregulation of intestinal epithelial MHC-I as a novel mechanism in severe Crohn's Disease. *Gut*. 2024;73(9):1464-1477.

doi: 10.1136/gutjnl-2024-332043

20. Mao Y, Wang W, Yang J, *et al*. Drug repurposing screening and mechanism analysis based on human colorectal cancer organoids. *Protein Cell*. 2023;15(4):285-304.

doi: 10.1093/procel/pwad038

21. Hammerhøj A, Chakravarti D, Sato T, Jensen KB, Nielsen OH. Organoids as regenerative medicine for inflammatory bowel disease. *iScience*. 2024;27(6):110118.

doi: 10.1016/j.isci.2024.110118

22. Miura S, Suzuki A. Generation of Mouse and Human Organoid-Forming Intestinal Progenitor Cells by Direct Lineage Reprogramming. *Cell Stem Cell*. 2017;21(4):456-471.e5.

doi: 10.1016/j.stem.2017.08.020

23. Sugimoto S, Ohta Y, Fujii M, *et al*. Reconstruction of the Human Colon Epithelium In Vivo. *Cell Stem Cell*. 2018;22(2):171-176.e5.

doi: 10.1016/j.stem.2017.11.012

24. Cadwell K, Loke P. Gene–environment interactions shape the host–microbial interface in inflammatory bowel disease. *Nat Immunol*. 2025;26(7):1023-1035.

doi: 10.1038/s41590-025-02197-5

25. Hirota A, AlMusawi S, Nateri AS, Ordóñez-Morán P, Imajo M. Biomaterials for intestinal organoid technology and personalized disease modeling. *Acta Biomater*. 2021;132:272-287.

doi: 10.1016/j.actbio.2021.05.010

26. Kleinman HK, Martin GR. Matrigel: Basement membrane matrix with biological activity. *Semin Cancer Biol*. 2005;15(5):378-386.

doi: 10.1016/j.semcancer.2005.05.004

27. Cruz-Acuña R, Quirós M, Farkas AE, *et al*. Synthetic hydrogels for human intestinal organoid generation and colonic wound repair. *Nat Cell Biol*. 2017;19(11):1326-1335.

doi: 10.1038/ncb3632

28. Hughes CS, Postovit LM, Lajoie GA. Matrigel: A complex protein mixture required for optimal growth of cell culture. *Proteomics*. 2010;10(9):1886-1890.

doi: 10.1002/pmic.200900758

29. Capeling MM, Czerwinski M, Huang S, *et al*. Nonadhesive Alginate Hydrogels Support Growth of Pluripotent Stem Cell-Derived Intestinal Organoids. *Stem Cell Rep*. 2019;12(2):381-394.

doi: 10.1016/j.stemcr.2018.12.001

30. Gjorevski N, Sachs N, Manfrin A, *et al*. Designer matrices for intestinal stem cell and organoid culture. *Nature*. 2016;539(7630):560-564.

doi: 10.1038/nature20168

31. Vanhove L, Van Gansbeke T, Devriendt B, Van der Meeren R, Dmitriev RI, Okkelman IA. Lack of biochemical signalling in GelMA leads to polarity reversion in intestinal organoids independent from mechanoreciprocity. *J Tissue Eng*. 2025;16:20417314251345000.

doi: 10.1177/20417314251345000

32. Ma W, Zheng Y, Yang G, *et al*. A bioactive calcium silicate nanowire-containing hydrogel for organoid formation and functionalization. *Mater Horiz*. 2024;11(12):2957-2973.

doi: 10.1039/d4mh00228h

33. Madl CM, Heilshorn SC, Blau HM. Bioengineering strategies to accelerate stem cell therapeutics. *Nature*. 2018;557(7705):335-342.

doi: 10.1038/s41586-018-0089-z

34. Hushka EA, Blatchley MR, Macdougall LJ, *et al*. Fully Synthetic Hydrogels Promote Robust Crypt Formation in Intestinal Organoids. *Adv Mater*. 2025;37(43):e2509672.

doi: 10.1002/adma.202509672

35. He S, Lei P, Kang W, *et al*. Stiffness Restricts the Stemness of the Intestinal Stem Cells and Skews Their Differentiation Toward Goblet Cells. *Gastroenterology*. 2023;164(7):1137-1151.e15.

doi: 10.1053/j.gastro.2023.02.030

36. Zhang Q, He J, Zhu D, *et al*. Genetically modified organoids for tissue engineering and regenerative medicine. *Adv Colloid Interface Sci*. 2025;335:103337.

doi: 10.1016/j.cis.2024.103337

37. Yang H, Feng R, Fu Q, *et al*. Human induced pluripotent stem cell-derived mesenchymal stem cells promote healing via TNF-α-stimulated gene-6 in inflammatory bowel disease models. *Cell Death Dis*. 2019;10(10):718.

doi: 10.1038/s41419-019-1957-7

38. Watanabe S, Nishimura R, Shirasaki T, *et al*. Schlafen 11 Is a Novel Target for Mucosal Regeneration in Ulcerative Colitis. *J Crohns Colitis*. 2021;15(9):1558-1572.

doi: 10.1093/ecco-jcc/jjab032

39. Xiao L, Ma XX, Luo J, *et al*. Circular RNA CircHIPK3 Promotes Homeostasis of the Intestinal Epithelium by Reducing MicroRNA 29b Function. *Gastroenterology*. 2021;161(4):1303-1317.e3.

doi: 10.1053/j.gastro.2021.05.060

40. D'Addio F, Amabile G, Assi E, *et al*. TMEM219 signaling promotes intestinal stem cell death and exacerbates colitis. *J Clin Invest*. 2025;135(10):e185783.

doi: 10.1172/jci185783

41. Kang Y, Park H, Choe BH, Kang B. The Role and Function of Mucins and Its Relationship to Inflammatory Bowel Disease. *Front Med*. 2022;9:848344.

doi: 10.3389/fmed.2022.848344

42. Ding W, Marx OM, Mankarious MM, Koltun WA, Yochum GS. Disease Severity Impairs Generation of Intestinal Organoid Cultures From Inflammatory Bowel Disease Patients. *J Surg Res*. 2024;293:187-195.

doi: 10.1016/j.jss.2023.08.027

43. Koslowski MJ, Beisner J, Stange EF, Wehkamp J. Innate antimicrobial host defense in small intestinal Crohn's disease. *Int J Med Microbiol*. 2010;300(1):34-40.

doi: 10.1016/j.ijmm.2009.08.011

44. Armbruster NS, Stange EF, Wehkamp J. In the Wnt of Paneth Cells: Immune-Epithelial Crosstalk in Small Intestinal Crohn's Disease. *Front Immunol*. 2017;8:1204.

doi: 10.3389/fimmu.2017.01204

45. He GW, Lin L, DeMartino J, *et al*. Optimized human intestinal organoid model reveals interleukin-22-dependency of paneth cell formation. *Cell Stem Cell*. 2022;29(9):1333-1345.e6.

doi: 10.1016/j.stem.2022.08.002

46. Hamdan FH, Farhadipour, *et al*. Intestinal Stem Cells From Patients With Inflammatory Bowel Disease Retain an Epigenetic Memory of Inflammation. *Cell Mol Gastroenterol Hepatol*. 2026;101774.

doi: 10.1016/j.jcmgh.2026.101774

47. Dotti I, Mora-Buch R, Ferrer-Picón E, *et al*. Alterations in the epithelial stem cell compartment could contribute to permanent changes in the mucosa of patients with ulcerative colitis. *Gut*. 2016;66(12):2069-2079.

doi: 10.1136/gutjnl-2016-312609

48. Arnauts K, Sudhakar P, Verstockt S, *et al*. Microbiota, not host origin drives ex vivo intestinal epithelial responses. *Gut Microbes*. 2022;14(1):2089003.

doi: 10.1080/19490976.2022.2089003

49. Lee C, Hong SN, Kim ER, Chang DK, Kim YH. Epithelial Regeneration Ability of Crohn's Disease Assessed Using Patient-Derived Intestinal Organoids. *Int J Mol Sci*. 2021;22(11):6013.

doi: 10.3390/ijms22116013

50. Schirmer M, Garner A, Vlamakis H, Xavier RJ. Microbial genes and pathways in inflammatory bowel disease. *Nat Rev Microbiol*. 2019;17(8):497-511.

doi: 10.1038/s41579-019-0213-6

51. Gevers D, Kugathasan S, Denson LA, *et al*. The Treatment-Naive Microbiome in New-Onset Crohn's Disease. *Cell Host Microbe*. 2014;15(3):382-392.

doi: 10.1016/j.chom.2014.02.005

52. Rimmer P, Zhang F, Scott G, *et al*. The Gut Microbiome at the Onset of Inflammatory Bowel Disease: A Systematic Review and Unified Bioinformatic Synthesis. *Gastroenterology*. 2026;170(3):539-556.

doi: 10.1053/j.gastro.2025.09.014

53. Zheng L, Duan SL, Wang K. Research progress concerning the involvement of the intestinal microbiota in the occurrence and development of inflammatory bowel disease. *World J Gastroenterol*. 2025;31(42):113170.

doi: 10.3748/wjg.v31.i42.113170

54. Jalili-Firoozinezhad S, Gazzaniga FS, Calamari EL, *et al*. A complex human gut microbiome cultured in an anaerobic intestine-on-a-chip. *Nat Biomed Eng*. 2019;3(7):520-531.

doi: 10.1038/s41551-019-0397-0

55. Floor E, Su J, Chatterjee M, *et al*. Development of a Caco-2-based intestinal mucosal model to study intestinal barrier properties and bacteria–mucus interactions. *Gut Microbes*. 2024;17(1):2434685.

doi: 10.1080/19490976.2024.2434685

56. Fofanova TY, Karandikar UC, Auchtung JM, *et al*. A novel system to culture human intestinal organoids under physiological oxygen content to study microbial-host interaction. *PLoS One*. 2024;19(7):e0300666.

doi: 10.1371/journal.pone.0300666

57. Han X, Mslati MA, Davies E, Chen Y, Allaire JM, Vallance BA. Creating a More Perfect Union: Modeling Intestinal Bacteria-Epithelial Interactions Using Organoids. *Cell Mol Gastroenterol Hepatol*. 2021;12(2):769-782.

doi: 10.1016/j.jcmgh.2021.04.010

58. Karve SS, Pradhan S, Ward DV, Weiss AA. Intestinal organoids model human responses to infection by commensal and Shiga toxin producing Escherichia coli. *PLoS One*. 2017;12(6):e0178966.

doi: 10.1371/journal.pone.0178966

59. Co JY, Margalef-Català M, Li X, *et al*. Controlling Epithelial Polarity: A Human Enteroid Model for Host-Pathogen Interactions. *Cell Rep*. 2019;26(9):2509-2520.e4.

doi: 10.1016/j.celrep.2019.01.108

60. Tan H, Chen X, Wang C, *et al*. Intestinal organoid technology and applications in probiotics. *Crit Rev Food Sci Nutr*. 2023;65(6):1055-1069.

doi: 10.1080/10408398.2023.2288887

61. Poletti M, Arnauts K, Ferrante M, Korcsmaros T. Organoid-based Models to Study the Role of Host-microbiota Interactions in IBD. *J Crohns Colitis*. 2020;15(7):1222-1235.

doi: 10.1093/ecco-jcc/jjaa257

62. Beaumont M, Blanc F, Cherbuy C, *et al*. Intestinal organoids in farm animals. *Vet Res*. 2021;52(1):33.

doi: 10.1186/s13567-021-00909-x

63. Moon C, VanDussen KL, Miyoshi H, Stappenbeck TS. Development of a primary mouse intestinal epithelial cell monolayer culture system to evaluate factors that modulate IgA transcytosis. *Mucosal Immunol*. 2014;7(4):818-828.

doi: 10.1038/mi.2013.98

64. Wang Y, DiSalvo M, Gunasekara DB, *et al*. Self-renewing Monolayer of Primary Colonic or Rectal Epithelial Cells. *Cell Mol Gastroenterol Hepatol*. 2017;4(1):165-182.e7.

doi: 10.1016/j.jcmgh.2017.02.011

65. VanDussen KL, Marinshaw JM, Shaikh N, *et al*. Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays. *Gut*. 2014;64(6):911-920.

doi: 10.1136/gutjnl-2013-306651

66. Roodsant T, Navis M, Aknouch I, *et al*. A Human 2D Primary Organoid-Derived Epithelial Monolayer Model to Study Host-Pathogen Interaction in the Small Intestine. *Front Cell Infect Microbiol*. 2020;10:272.

doi: 10.3389/fcimb.2020.00272

67. Sontheimer-Phelps A, Chou DB, Tovaglieri A, *et al*. Human Colon-on-a-Chip Enables Continuous In Vitro Analysis of Colon Mucus Layer Accumulation and Physiology. *Cell Mol Gastroenterol Hepatol*. 2020;9(3):507-526.

doi: 10.1016/j.jcmgh.2019.11.008

68. Eng SJ, Nonnecke EB, de Lorimier AJ, *et al*. FOXO inhibition rescues α-defensin expression in human intestinal organoids. *Proc Natl Acad Sci USA*. 2023;120(47):e2312453120.

doi: 10.1073/pnas.2312453120

69. Kim HJ, Huh D, Hamilton G, Ingber DE. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip*. 2012;12(12):2165.

doi: 10.1039/c2lc40074j

70. Wang CM, Oberoi HS, Law D, *et al*. Human mesofluidic intestinal model for studying transport of drug carriers and bacteria through a live mucosal barrier. *Lab Chip*. 2025;25(12):2990-3004.

doi: 10.1039/d4lc00774c

71. Ginga NJ, Slyman R, Kim GA, *et al*. Perfusion System for Modification of Luminal Contents of Human Intestinal Organoids and Realtime Imaging Analysis of Microbial Populations. *Micromachines*. 2022;13(1):131.

doi: 10.3390/mi13010131

72. Williamson IA, Arnold JW, Samsa LA, *et al*. A High-Throughput Organoid Microinjection Platform to Study Gastrointestinal Microbiota and Luminal Physiology. *Cell Mol Gastroenterol Hepatol*. 2018;6(3):301-319.

doi: 10.1016/j.jcmgh.2018.05.004

73. Almalla A, Alzain N, Elomaa L, *et al*. Hydrogel-Integrated Millifluidic Systems: Advancing the Fabrication of Mucus-Producing Human Intestinal Models. *Cells*. 2024;13(13):1080.

doi: 10.3390/cells13131080

74. Lindner M, Laporte A, Block S, Elomaa L, Weinhart M. Physiological Shear Stress Enhances Differentiation, Mucus-Formation and Structural 3D Organization of Intestinal Epithelial Cells In Vitro. *Cells*. 2021;10(8):2062.

doi: 10.3390/cells10082062

75. Wang X, He L, Dong Y, *et al*. Mucosa-associated bacteria and metabolites in inflammatory bowel disease: from inside to insight. *NPJ Biofilms Microbiomes*. 2026;12(1):15.

doi: 10.1038/s41522-025-00887-4

76. Huang YJ, Lewis CA, Wright C, *et al*. Faecalibacterium prausnitzii A2-165 metabolizes host- and media-derived chemicals and induces transcriptional changes in colonic epithelium in GuMI human gut microphysiological system. *Microbiome Res Rep*. 2024;3(3):30.

doi: 10.20517/mrr.2024.14

77. Wilson SS, Tocchi A, Holly MK, Parks WC, Smith JG. A small intestinal organoid model of non-invasive enteric pathogen–epithelial cell interactions. *Mucosal Immunol*. 2015;8(2):352-361.

doi: 10.1038/mi.2014.72

78. Sarvestani SK, Signs S, Hu B, *et al*. Induced organoids derived from patients with ulcerative colitis recapitulate colitic reactivity. *Nat Commun*. 2021;12(1):262.

doi: 10.1038/s41467-020-20351-5

79. Gilliland A, Chen Y, Ng I, *et al*. Biopsy-derived colonoid air-liquid interface monolayers reveal persistent mucosal defects in ulcerative colitis patients. *Inflamm Bowel Dis*. 2026;32(3):526-541.

doi: 10.1093/ibd/izaf318

80. Xu X, Zhang Y, Huang G, Perekatt A, Wang Y, Chen L. Advances and applications of gut organoids: modeling intestinal diseases and therapeutic development. *Life Med*. 2025;4(2):lnaf012.

doi: 10.1093/lifemedi/lnaf012

81. Day CP, Merlino G, Van Dyke T. Preclinical Mouse Cancer Models: A Maze of Opportunities and Challenges. *Cell*. 2015;163(1):39-53.

doi: 10.1016/j.cell.2015.08.068

82. Yang Z, Chen J, Xiao Y, *et al*. Digital Barcodes for High-Throughput Screening. *Chem Bio Eng*. 2024;1(1):2-12.

doi: 10.1021/cbe.3c00085

83. Jang KK, Hudesman D, Jones DR, *et al*. Tofacitinib Uptake by Patient-Derived Intestinal Organoids Predicts Individual Clinical Responsiveness. *Gastroenterology*. 2024;167(7):1453-1456.e5.

doi: 10.1053/j.gastro.2024.07.035

84. Smabers LP, Wensink GE, Verissimo CS, *et al*. Patient-

Derived Organoids Predict Treatment Response in Metastatic Colorectal Cancer. *Clin Cancer Res*. 2025;31(23):5015-5026.

doi: 10.1158/1078-0432.ccr-25-1564

85. Penrose HM, Sinha S, Tindle C, *et al*. New Approach Methodologies in Crohn's Disease Link Molecular Disease Subtypes to Clinical Outcomes. *bioRxiv*. Posted online April 3, 2025.

doi: 10.1101/2025.04.01.25325058

86. Serra D, Mayr U, Boni A, *et al*. Self-organization and symmetry breaking in intestinal organoid development. *Nature*. 2019;569(7754):66-72.

doi: 10.1038/s41586-019-1146-y

87. Lukonin I, Serra D, Challet Meylan L, *et al*. Phenotypic landscape of intestinal organoid regeneration. *Nature*. 2020;586(7828):275-280.

doi: 10.1038/s41586-020-2776-9

88. Lavickova B, Kronabitter H, Cervera-Negueruela M, *et al*. Integrated Microfluidic Platform for High-Throughput Generation of Intestinal Organoids in Hydrogel Droplets. *Adv Sci*. 2026;13(13):2516507.

doi: 10.1002/advs.202516507

89. Esch MB, Mahler GJ, Stokol T, Shuler ML. Body-on-a-chip simulation with gastrointestinal tract and liver tissues suggests that ingested nanoparticles have the potential to cause liver injury. *Lab Chip*. 2014;14(16):3081-3092.

doi: 10.1039/c4lc00371c

90. Arakawa H, Sugiura S, Kawanishi T, *et al*. Kinetic analysis of sequential metabolism of triazolam and its extrapolation to humans using an entero-hepatic two-organ microphysiological system. *Lab Chip*. 2020;20(3):537-547.

doi: 10.1039/c9lc00884e

91. Trietsch SJ, Naumovska E, Kurek D, *et al*. Membrane-free culture and real-time barrier integrity assessment of perfused intestinal epithelium tubes. *Nat Commun*. 2017;8(1):262.

doi: 10.1038/s41467-017-00259-3

92. Yui S, Nakamura T, Sato T, *et al*. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5+ stem cell. *Nat Med*. 2012;18(4):618-623.

doi: 10.1038/nm.2695

93. Spence JR, Mayhew CN, Rankin SA, *et al*. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature*. 2010;470(7332):105-109.

doi: 10.1038/nature09691

94. Deng F, Wu Z, Zou F, Wang S, Wang X. The Hippo–YAP/TAZ Signaling Pathway in Intestinal Self-Renewal and Regeneration After Injury. *Front Cell Dev Biol*. 2022;10:894737.

doi: 10.3389/fcell.2022.894737

95. Mulero-Russe A, García AJ. Engineered Synthetic Matrices for Human Intestinal Organoid Culture and Therapeutic Delivery. *Adv Mater*. 2023;36(9):2307678.

doi: 10.1002/adma.202307678

96. Sugimoto S, Kobayashi E, Fujii M, *et al*. An organoid-based organ-repurposing approach to treat short bowel syndrome. *Nature*. 2021;592(7852):99-104.

doi: 10.1038/s41586-021-03247-2

97. Kasendra M, Troutt M, Broda T, *et al*. Intestinal organoids: roadmap to the clinic. *Am J Physiol Gastrointest Liver Physiol*. 2021;321(1):G1-G10.

doi: 10.1152/ajpgi.00425.2020

98. Kozlowski MT, Crook CJ, Ku HT. Towards organoid culture without Matrigel. *Commun Biol*. 2021;4(1):1387.

doi: 10.1038/s42003-021-02910-8

99. Ahn SJ, Lee S, Kwon D, *et al*. Essential Guidelines for Manufacturing and Application of Organoids. *Int J Stem Cells*. 2024;17(2):102-112.

doi: 10.15283/ijsc24047

100. Wang Y, Lin H, Zhao L, *et al*. Standard: Human intestinal organoids. *Cell Regen*. 2023;12(1):19.

doi: 10.1186/s13619-023-00168-5

101. Sugimoto S, Kobayashi E, Kanai T, Sato T. In Vivo Intestinal Research Using Organoid Transplantation. *Keio J Med*. 2022;71(4):73-81.

doi: 10.2302/kjm.2022-0019-ir

102. Hanna E, Rémuzat C, Auquier P, Toumi M. Advanced therapy medicinal products: current and future perspectives. *J Mark Access Health Policy*. 2016;4(1):31036.

doi: 10.3402/jmahp.v4.31036

103. Lambda Biologics. Organoids Enter the Clinic: A Milestone for Regenerative Medicine. Lambda Biologics. Published March 27, 2026. Available from: https://afs.lambda-bio.com/blog/organoids-enter-the-clinic-a-milestone-for-regenerative-medicine/ [Last accessed on April 2, 2026].

104. Ouahoud S, Giugliano F, Muncan V. Monitoring Intestinal Organoid–Derived Monolayer Barrier Functions with Electric Cell–Substrate Impedance Sensing (ECIS). *Bio Protoc*. 2024;14(1340):e4947.

doi: 10.21769/bioprotoc.4947

105. Lee H, Yang S, Lee KJ, *et al*. Standardization and quality assessment for human intestinal organoids. *Front Cell Dev Biol*. 2024;12:1383893.

doi: 10.3389/fcell.2024.1383893