

Review

Revolutionizing IBD research with on-chip models of disease modeling and drug screening

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Inflammatory bowel disease (IBD) comprises chronic inflammatory conditions with complex mechanisms and diverse manifestations, posing significant clinical challenges. Traditional animal models and ethical concerns in human studies necessitate innovative approaches. This review provides an overview of human intestinal architecture in health and inflammation, emphasizing the role of microfluidics and on-chip technologies in IBD research. These technologies allow precise manipulation of cellular and microbial interactions in a physiologically relevant context, simulating the intestinal ecosystem microscopically. By integrating cellular components and replicating 3D tissue architecture, they offer promising models for studying host–microbe interactions, wound healing, and therapeutic approaches. Continuous refinement of these technologies promises to advance IBD understanding and therapy development, inspiring further innovation and cross-disciplinary collaboration.

Advancing IBD research through high-fidelity simulation models

IBD, including **Crohn's disease (CD)** (see [Glossary](#)) and **ulcerative colitis (UC)**, affects nearly seven million people globally. These chronic immune-mediated disorders cause cyclical inflammation, with symptoms varying by location and severity, commonly presenting as diarrhea, abdominal pain, weight loss, fever, rectal bleeding, perianal disease, malnutrition, abdominal masses, and growth retardation in youth. IBD results from disrupted host–microbial interactions influenced by genetic, environmental, and immunological factors ([Box 1](#) and [Figure 1A,B](#)) [1–3]. Despite advancements in understanding the clinical manifestations of IBD, a significant portion of patients (25–30%) do not respond to standard treatments, necessitating a deeper comprehension and more effective therapies.

In practice, the treatments primarily aim to mitigate inflammation by targeting the immune response; but there is an increasing focus on correcting the altered gut **microbiota** that perpetuates inflammation and hampers wound healing. Inadequate treatment leading to unhealed mucosa can potentially trigger adverse responses, including the risk of gastrointestinal (GI) adenocarcinoma, intestinal fibrosis resulting in strictures and fistulas, and a shift in microbial populations [4]. Animal models lack precise control over IBD-specific factors like epithelial barrier function [5], impeding drug screening and evaluation [6]. Research on IBD patients faces ethical and logistical obstacles, complicating controlled studies [7]. Moreover, the limited availability and lifespan of *ex vivo* models [8], coupled with the inability of standard *in vitro* Transwell® systems to imitate essential 3D cell arrangements, underscore the need for new, accurate models for IBD research.

Highlights

The scarcity of standardized, easily manipulable models for inflammatory bowel disease (IBD) research underscores significant challenges in understanding the complexity of the disease and developing effective treatments.

Microfluidic and on-chip technologies enhance IBD research by mimicking the intricate human intestinal microenvironment, facilitating in-depth analysis of intestinal regeneration.

By replicating 3D tissue structures and cellular dynamics, these innovative platforms provide unparalleled insights into IBD mechanisms and offer a highly effective means of evaluating therapeutic approaches.

A paucity of standardization and integration challenges constrained the widespread adoption of microfluidic systems, underscoring the urgent need for collaborative efforts to refine these innovative technologies.

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Box 1. Dissecting the multifactorial etiology of IBD pathogenesis

Genetic research has identified over 240 loci linked to IBD risk, suggesting shared pathophysiological mechanisms through variants affecting innate and adaptive immunity (e.g., IL23R and IL10), epithelial barrier function (e.g., ECM1), microbial product recognition (e.g., NOD2), and autophagy (e.g., ATG16L1) [83]. Despite that, these genetic variants account for only a small portion of disease risk variance (8–14% for CD, 4–8% for UC) [84]. Additionally, a wide array of environmental factors has also been identified as contributing to the pathogenesis of these diseases. There is a strong link between the use of antibiotics and an increased risk of developing IBD, with odds ratios as high as 5.01 [85]. Meanwhile, non-steroidal anti-inflammatory drugs (NSAIDs) are associated with a heightened risk of both the onset and flare-ups of IBD [85]. Conversely, breastfeeding in early life shows a protective effect against IBD in adulthood [86], and diet plays a significant role, with high fiber intake and n-3 polyunsaturated fatty acids (PUFA) reducing risk, in contrast to n-6 PUFA, which increases risk [87]. Despite limitations, current evidence often allows formulation of plausible hypotheses regarding the underlying mechanisms, often hinting at direct effects (e.g., n-3 PUFA reducing TNF production) [88] or indirect effects (e.g., antibiotics or dietary effects) of these factors on the immune system.

The most important arbitrator of our health–disease equilibrium is the intestinal microbiota, which constitute 50% of our total cells and 99% of our genetic material. This symbiotic relationship profoundly influences every organ system [89], aiding in digestion, producing essential nutrients [90,91], fighting host to fight against pathogenic invaders [92], and metabolizing (toxic) substances before they enter our bloodstream [93], influencing the immune system [94–96] and metabolism of toxins [97,98]. Research is intensifying into the role of microbiota in IBD pathogenesis, revealing distinct profiles in IBD patients versus non-IBD individuals, during flare-ups versus remission, and associations with disease trajectory and treatment efficacy [90].

Many mechanistic studies with animal models have demonstrated the decisive role of gut microbiota in modulating inflammation and pathological immune reactions, while simultaneously influencing these processes. Notably, the NOD2 locus, an intracellular microbial sensor detecting bacterial wall peptidoglycan, affects cytokines like IL-8 and TNF- α via NF- κ B signaling, implicating NOD2 in both immune defense and gut stability [99,100].

Microfluidics technology – facilitating the manipulation of fluids at the microliter scale, represents a burgeoning frontier for simulating complex biological systems with high fidelity [9]. It is designed to emulate the human intestinal microenvironment at a microscale, where distinct tissues and microorganisms can be cultivated in the same milieu, thereby offering a precise surrogate to traditional animal models [10]. Such advancements are particularly vital in studying IBD, where **dysbiosis** and immune responses to gut microbiota play critical roles [11].

Herein, we highlight the transformative impact of microfluidics and on-chip technologies in advancing intestinal wound healing in IBD research. We delve into their developmental trajectory, utilization in disease emulation, and inherent constraints. Furthermore, we examine their capacity to mimic the intricate milieu of the intestinal tract, replicate epithelial damage and subsequent repair processes, and scrutinize reactions to on-chip wound recovery mechanisms. Additionally, we expound upon nascent trends and prospective breakthroughs in the IBD field.

Cellular and molecular drivers of intestinal regeneration in IBD

The intestinal mucosa milieu, a tripartite ecosystem comprising trillions of immune cells, epithelial–mesenchymal structures, and **symbiotic** commensal microbiota, functions as a vital barrier with two primary roles: it selectively permits nutrient absorption while protecting the host from luminal toxins, antigens, and microbes that could initiate disease [12]. This consortium, covering ~100 m² of intestinal epithelium formed by columnar intestinal epithelial cells (IECs) interconnected by junctional complexes, orchestrates mutual sustenance and gut homeostasis [13].

Damage to the intestinal barrier, often worsened by pathological inflammation as in IBD, increases the permeability, allowing microbial antigens to penetrate deeper mucosal layers, which triggers immune cell recruitment and cytokine release and subsequently disrupts homeostasis [14,15]. In response, the intestinal environment of the host, working alongside the immune system, attempts to rebuild the structural integrity of a strong barrier. This barrier helps prevent bacterial movement

Glossary

Crohn's disease (CD): a chronic inflammatory bowel disease that specifically affects any part of the digestive tract with sporadic, deep lesions.

2D cell culture: this method involves growing cells *in vitro* on a flat surface, where they typically LPS adhere and expand into a 2D monolayer as they proliferate.

3D cell culture: a structured environment that supports the growth of biological cells in three dimensions, facilitating more natural cell interactions and behaviors.

Dysbiosis: an imbalance in the microbial populations of the body, particularly in the gut, where beneficial microbes are diminished and potentially harmful ones are increased, potentially leading to health problems.

IBD-on-a-chip: this microfluidic system mimics the environment of the human intestine to research IBD. Intestinal epithelial cells, immunological cells, ECM, microbiological elements, and dynamic fluid movement are important components. Together, these components mimic the inflammatory processes and physiological settings of IBD, facilitating treatment testing and disease modeling.

Lab-on-a-chip (LoC): LoC devices consolidate multiple laboratory functions onto a compact chip, varying from a few square millimeters to square centimeters in size. These devices support miniaturized, automated, integrated, and parallel chemical and/or biological analyses, providing cost-effective, rapid, controlled, and superior performance in biochemical assays on a smaller scale than traditional laboratories.

Matrines: small peptides derived from the breakdown of ECM proteins. These peptides are typically released during tissue remodeling, injury, or inflammation. They play crucial roles in various biological processes by interacting with cell surface receptors to influence cell behavior. Their functions include promoting cell migration, proliferation, and differentiation, and they are also involved in the regulation of inflammation and wound healing.

Microbiota: the complex community of microorganisms that reside on and within various parts of the body, such as the skin, mouth, gut, and other surfaces exposed to the external environment. These microbial communities consist of

and the resulting immune response, a process often known as mucosal or wound healing. Microscopic mucosal healing is essential for long-term remission and avoiding severe complications like GI adenocarcinoma, intestinal strictures, fistulas, and microbial dysbiosis, which are often linked to prolonged inadequate treatment or chronic inflammation.

The intestinal wound healing is an intricate process governed by an array of regulatory peptides including growth factors and cytokines [16]. The process has three intertwined phases (Figure 1C): restitution, proliferation, and differentiation/maturation (Box 2).

The limitations of traditional methods in studying gut inflammation and intestinal healing, such as the inaccessibility of the GI tract and the inability of fecal samples to fully represent gut microbiota, necessitate innovative research approaches. *In vitro* models like organoids and **organ-on-a-chip (OoC)** technology, which utilize microfluidic capabilities to simulate 3D tissue geometries and dynamic gut conditions, offer more accessible and accurate platforms for studying gut physiology and disease mechanisms, including intestinal wound healing in IBD. These advanced models facilitate disease modeling and drug testing, potentially hastening the application of research findings to clinical settings.

On-a-chip technology in biomedical research

The evolution of on-a-chip systems

Micro-electro-mechanical systems (MEMS) have swiftly become integral miniature platforms across various biological research domains such as tissue engineering, drug delivery, biosensors, diagnostics, and therapeutic applications [17]. These integrated device networks, commonly known as **'lab-on-a-chip' (LoC)** or 'micro-total analysis systems' (μ TAS), have captured the interest of biomedical and life science researchers [18,19]. Recent advancements in the field have witnessed the seamless integration of microfluidics technology, renowned for its precise control over fluid volumes in cellular environments, with traditional cell biology and tissue engineering methods [20,21]. This integration has been a catalyst for the development of innovative micro-engineered cell culture platforms, significantly advancing the realm of *in vitro* diagnostics by allowing for the analysis of minuscule samples with minimal human intervention [22–24]. The integration of automation in these processes reduces human intervention, thereby enhancing the reliability of the analyses [25].

Parallel to the evolution of LoC, OoC technology has emerged as a sophisticated *in vitro* biomimetic platform that effectively simulates human organ physiology to an unprecedented degree [26,27]. Sparked by the 'human-on-a-chip' initiative in 2011, these platforms have seen wide adoption due to their advantages in drug development, physiological testing, and toxicological assessments, including miniaturization, seamless integration, and precise control over biological parameters [27–31].

Despite these advancements, traditional **2D cell cultures** remain prevalent for studying cell proliferation or differentiation under controlled conditions [32]. Maintaining cells in a regulated environment mirroring physiological conditions allows for their growth, supporting investigations into various aspects of human physiology [33]. From its establishment, 2D cell culture has been employed to investigate several aspects of human physiology, including stem cells, illnesses, cell–cell interactions, tissues, medicines, toxicity, and drug metabolism [34,35]. However, traditional 2D culture methods lack the ability to replicate the complex conditions of living organisms, leading to inaccurate drug effect assessments and contributing to failed drug development trials. Additionally, oxygen distribution in 2D cultures differs from *in vivo* conditions, affecting the ability to mimic tumor physiology [36–38]. From a practicability perspective, the pros of 2D cultures

bacteria, fungi, viruses, and other microscopic organisms.

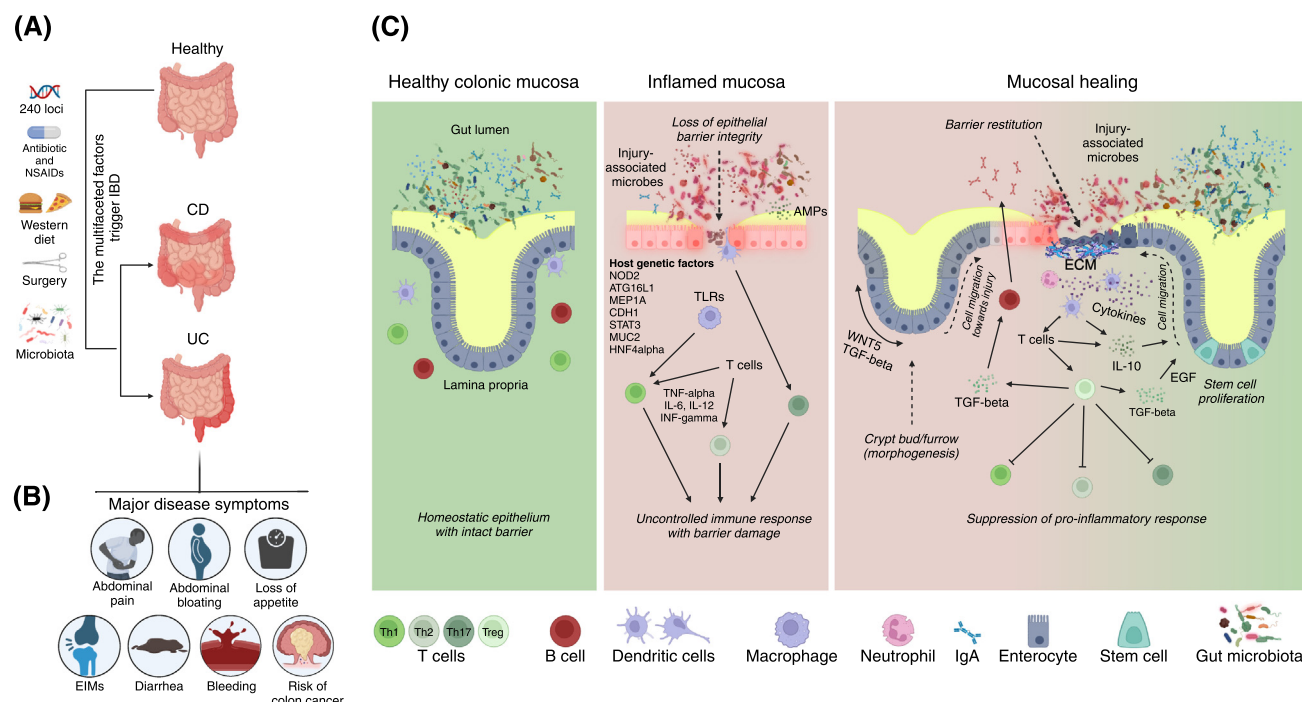
Microfluidics: the science and technology of manipulating fluids at a small scale, typically in channels with dimensions on the order of micrometers.

Organ-on-a-chip (OoC): a micro-scale, *in vitro* platform that models human organ physiology by integrating cell biology, engineering, and materials science to recreate tissue conditions found in the body.

Short-chain fatty acids (SCFAs): fatty acids with fewer than six carbon atoms, typically produced through the fermentation of dietary fibers by gut microbiota in the colon. SCFAs, such as acetate, propionate, and butyrate, are crucial for various bodily functions, including maintaining gut health, regulating immune responses, and providing energy to colon cells.

Symbiotic: a mutually beneficial relationship between different organisms.

Ulcerative colitis (UC): a chronic inflammatory bowel disease that specifically affects the lining of the large intestine (colon) and rectum.



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Figure 1. Central coordinator(s) of intestinal homeostasis and wound healing in inflammatory bowel disease (IBD). (A) IBD can lead to a wide spectrum of complications primarily in the colon and rectum; yet it can also impact other regions of the body. In Crohn's disease (CD) and ulcerative colitis (UC), various sections of the intestine may be involved, with multiple factors playing a role in the pathogenesis of these conditions. (B) Common IBD symptoms are shown. (C) Under normal healthy conditions, the intestinal epithelium facilitates nutrient absorption, manages microbial populations, and mitigates infection risks. Its regulatory capacity extends beyond intrinsic mechanisms, influenced by interactions with the commensal microbiota and signals from adjacent leukocytes. The disruption of the mucosal barrier by inflammatory lesions leads to immune activation, cytokine release, and exacerbated inflammation due to microbial invasion. During mucosal healing, circulating restitutive immune cells migrate to the wound area, accompanied by the release of repairing cytokines and interaction among the extracellular matrix and epithelial cells. This coordination promotes cell proliferation and migration, while concurrently, the alteration of microbiota and cytokines facilitates mucosal healing and functional crypt regeneration. Abbreviations: AMPs, antimicrobial peptides; EGF, epidermal growth factor; EIMs, extraintestinal manifestations; ECM, extracellular matrix; IL, interleukin; IFN, interferon; NSAIDs, non-steroidal anti-inflammatory drugs; TGF, transforming growth factor; TLR, Toll-like receptor; TNF, tumor necrosis factor. Created with [BioRender.com](https://www.biorender.com).

Box 2. Distinct phases of wound healing

As a first step, epithelial cells migrate to cover the wound area, a rapid process known as epithelial restitution, not reliant on cell proliferation but significantly stimulated by transforming growth factor β (TGF- β) within intestinal mucosa, produced by different cell types including epithelial cells, stromal cells, regulatory T cells (Tregs), dendritic cells, and macrophages [101–103]. Upregulation of specific genes, including *Slc28a2*, *Tubb2a*, and *Cpe*, and matrix metalloproteinases enhance cell migration in this phase [104]. Nutrients like amino acids and mediators such as IL-33/ST2, through miR-320, and Desmocollin-2 play crucial roles in epithelial repair and migration [105]. Following restitution, the cell proliferation step re-surfaces the wound, driven by specific growth factors [e.g., epidermal growth factor (EGF), keratinocyte growth factor (KGF), and fibroblast growth factor (FGF)] and cytokines ultimately activating essential pathways (e.g., STAT1, STAT3) for immune regulation and intestinal healing [106–108]. Of note, it has been demonstrated that TLR2 plays a pivotal role *in vivo* by inhibiting apoptosis in IECs through the regulation of trefoil factor 3 expression [109,110]. This action aids intestinal wound healing by modulating epithelial connexin-43 expression. In differentiation with the maturation stage, Lgr5+ intestinal stem cells at the base of mucosal crypts differentiate into short-lived transit-amplifying progenitors, then into absorptive and secretory cells, guided by Hedgehog, Noggin, Notch, and Wnt signaling [16,111]. During this phase, epithelial cells undergo epithelial-mesenchymal transition, gaining mobility and the ability to produce extracellular matrix for tissue repair. This process, driven by transcription factors, RNAs, and TGF- β proteins, compensates for fibroblast dysfunction, enhancing barrier restoration and potentially leading to complications like fistula formation [112,113]. Additionally, SCFAs from gut commensals promote epithelial proliferation and differentiation, strengthening barrier integrity and facilitating wound healing [114].

include familiarity with the scientific community, established imaging procedures, ease of imaging cells in the x-y plane, abundant comparative literature, and cost-effectiveness [39].

However, **3D cell culture** technologies offer a more accurate replication of the biological environment, fostering proper cell–cell and cell–extracellular matrix (ECM) interactions [40,41]. This not only enhances the accuracy of drug development and disease modeling with accurate gene expression profiling but also advances research ethics by reducing the dependence on animal models [42].

Advantages of using on-a-chip models in biomedical studies

In biomedical science, the relevance of findings from animal models, particularly those from mice to human conditions is frequently debated. While cell-based *in vitro* methods offer an alternative, they often fall short in accurately simulating physiological variables and intercellular communication [43]. Traditional 2D cultures do not adequately reflect the complexity of biological systems, limiting their usefulness in predicting human responses [44]. In response, biochip-embedded tissue cultures have been developed to create a more comprehensive physiological milieu *in vitro* (Table 1). These systems ensure an optimal supply of nutrients and oxygen, efficient removal of metabolic wastes, and maintenance of physiological cell polarization and communication within tissues [45]. Such advancements present promising avenues for integrative research, bridging the gap from cellular mechanisms *in vitro* to phenotypic expressions *in vivo*. This is particularly effective when on-a-chip (OoC) models are coupled with reliable *in vivo* models, facilitating a more holistic understanding of biological processes.

The advanced capabilities of OoC platforms significantly improve the predictability of complex drug interactions in living systems. These platforms enable concurrent testing of multiple drugs within a controlled environment, offering substantial benefits for pharmaceutical development. This approach not only mitigates the ethical concerns associated with human volunteer studies

Table 1. Comparative analysis of cell culture methods

Cell culture methods	Advantages	Disadvantages	Scalability	Biological complexity	Refs
2D cell culture	- Cost-effective - Well established methodology - Easy to work	- Poor cell–cell and cell–ECM interactions, niches or <i>in vivo</i>-like surroundings - Oxygen, metabolites, signaling molecules, and nutrition, are unlimited	Limited scalability due to surface area constraints	Low biological complexity, typically single cell type cultures	[35,36,74–77]
3D cell culture	- Oxygen, metabolites, signaling molecules, and nutrients, have varying degrees of accessibility - Effective cell–cell and cell–ECM interactions, and proper niches - Offers a drug testing methodology that is more reflective of human physiology	- Expensive - Unable to create a dynamic microenvironment - Deficient in fluid perfusion - Issues with microscopy and testing	Moderate scalability with specialized culture vessels	Medium to high biological complexity, suitable for multicellular cultures	[36,75,76,78–80]
Organ-on-a-chip (OoC) cell culture platforms	- Fluid flow capability - Accurate manipulation of the cellular microenvironment and produces dynamic culture - Nutrient and drug diffusion gradient - Potential integration with sensors and mechanical stimuli - Analysis in real-time and on-chip - Designed specifically for the user	- Problems related to manufacturing process - Non-standard protocols - Bubble formation inside channels	Limited scalability due to complex fabrication	High biological complexity, mimics organ-level functions	[18,45,47,49,81,82]

but also expedites the drug testing process [46]. OoCs possess the capability to regulate fluid flow, allowing for the replication of various mechanical stimuli present in natural bodily flows such as blood circulation and interstitial flow. In contrast, 3D static culture models lack the capacity for fluid flow [47] (Table 1).

OoCs also stand out due to their 3D structure, which more accurately recreates the tissue micro-environment and improves the reproducibility of test results [48]. The microengineering makes these devices easy to use and portable, making physiological assessments easier. Small micro-fluidic chips allow several systems to be merged into one chip, increasing efficiency and lowering costs. This compact design integrates multiple microfluidic systems into one portable unit, saving money and improving practicality.

Limitations and challenges in current on-a-chip technologies

Despite its advancements, OoC technology faces several challenges that hinder its broader adoption. One major challenge is developing an effective blood mimic that can fulfill the diverse nutritional and growth requirements of various cell types, ensuring that the cells thrive as they would in a natural environment [49]. The lack of defined techniques and materials for building OoC devices makes experiment replication and production scalability challenging. Uniform standards across laboratories would improve repeatability and accelerate the introduction of this technology into mainstream research by facilitating partnerships [50]. Promoting standardization across laboratories could boost collaboration and hasten the adoption of this technology. Moreover, the fabrication of chips requires meticulous calibration of organ sizes, fluid transit rates between organs, liquid-to-cell ratios, and the simulation of dynamic organ responses to different stimuli [51].

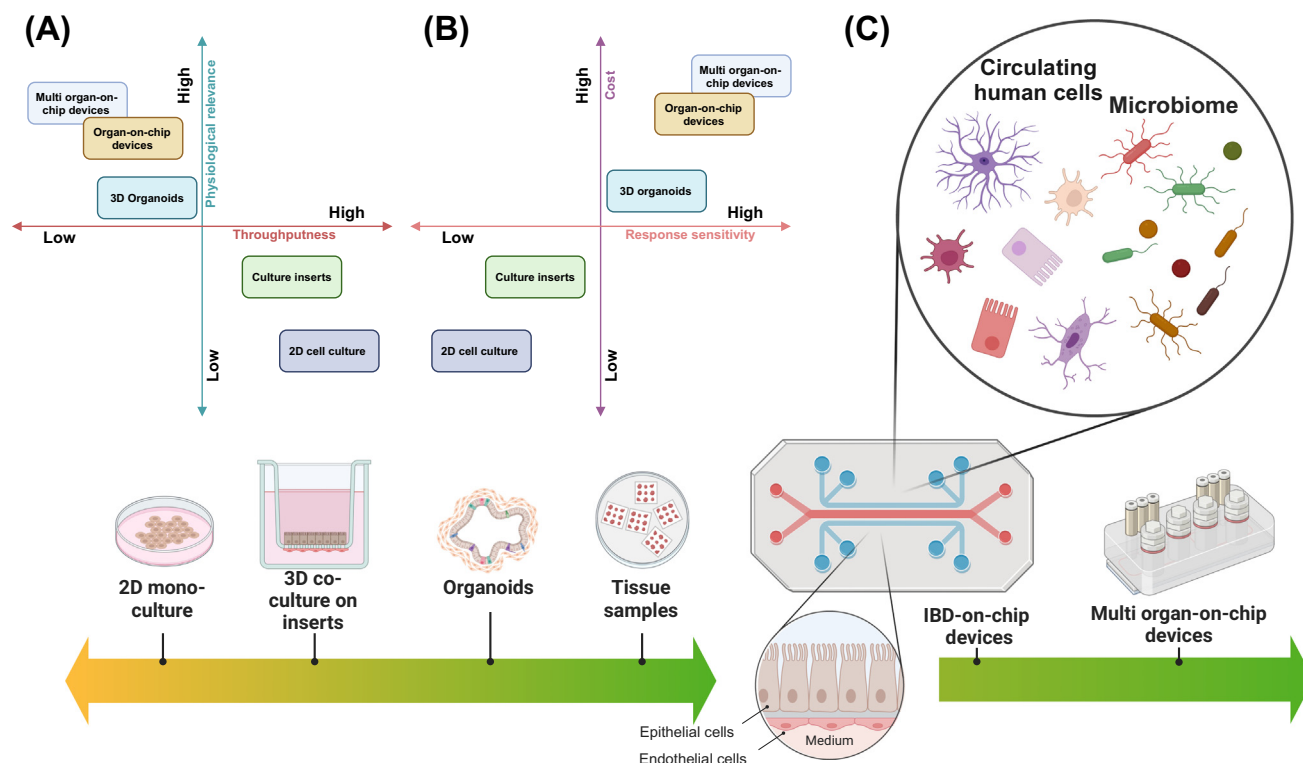
Despite the challenges, the outlook for OoCs remains promising, especially as entities like the US Food and Drug Administration (FDA) move towards phasing out mandatory animal testing in favor of more ethical and sophisticated approaches. Support organizations such as the European Organ-on-Chip Society (EUROoCS) and the UK Organ-on-a-Chip Technologies Network play an active role, providing guidance to academic researchers as they navigate through the regulatory and industrial landscapes [52,53]. The potential of OoCs to incorporate patient-specific cells holds transformative possibilities for biomarker identification and drug testing, particularly for rare diseases [54]. Furthermore, the integration of OoCs with cutting-edge analytical and imaging technologies is driving the creation of sophisticated multiorgan models. This advancement not only enriches our understanding of disease mechanisms but also enables personalized treatment approaches and improves the evaluation of therapeutic efficacy.

Models of IBD on-a-chip

Replicating the intestinal environment: innovations and approaches

The intestine, as the central hub for metabolite absorption, not only enables communication between organs but also, in conjunction with its microbial inhabitants, enhances the complexity of the gut–organ axis [55]. Disturbances in this system can lead to gut inflammation, one of the main readouts in IBD. To devise effective therapies to control inflammation, understanding the architecture of the intestine and its functional mechanisms is vital. This necessitates modeling gut functions on a chip, integrating both metabolic and mechanistic aspects.

In vitro models used in IBD research are becoming increasingly complex and physiologically relevant (Figure 2). The comparison of 2D monolayers, 3D organoids, and OoC technologies illustrates their varying degrees of physiological relevance and throughput (Figure 2A). Although multi-OoC devices offer higher physiological relevance, they typically have lower throughput. A



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Figure 2. Comparative analysis of *in vitro* models for inflammatory bowel disease (IBD) research. (A) An overview of the physiological significance and capacity for high volume testing of several *in vitro* models, such as 2D cell culture, 3D organoids, organ-on-chip devices, and multiorgan-on-chip devices. Organ-on-chip devices achieve more physiological relevance, even if they have lesser throughput. (B) A cost versus response sensitivity comparison demonstrates that organ-on-chip devices exhibit greater sensitivity but also have a higher price tag compared with conventional 2D and 3D culture systems. (C) This diagram illustrates an IBD-on-chip device, which is able to recreate the conditions of the intestine. It accurately represents the important interactions between circulating human cells, the microbiota, and the development of IBD. Created with [BioRender.com](https://www.biorender.com).

cost-benefit analysis highlights the sensitivity-cost trade-off of these systems, with OoC platforms offering greater sensitivity but at a higher cost (Figure 2B). Additionally, the IBD-on-chip system demonstrates the capability to simulate intestinal interactions (Figure 2C). To accurately mimic IBD pathophysiology, the inclusion of circulating human cells and microbiota components is essential.

Initial gut models typically involve 2D Transwell® systems harboring cultures of human-immortalized colon carcinoma cells [56]. Over time, these cells can form monolayers resembling the intestine with tight junctions. As a primary approach, Caco-2 human epithelial cells cultured with mucus-producing HT29 cells presented a more realistic structure for the intestine. Although these cells are easy to maintain and widely used in IBD research, they do not perfectly mirror human intestinal metabolism [57].

The field has evolved to include primary patient-derived cells and adult stem cells, due to their countless advantages including physiological relevance and patient-specific response in drug tests [58]. Besides, growth conditions and maintenance protocols for these cell lines advanced in the recent years for optimized experimental protocols to incorporate these cell lines in IBD-on-chip research. Induced pluripotent stem cells (iPSCs), including HT29, and T84 cell lines, are also being tested for on-chip systems. Developments in stem cell research also benefit

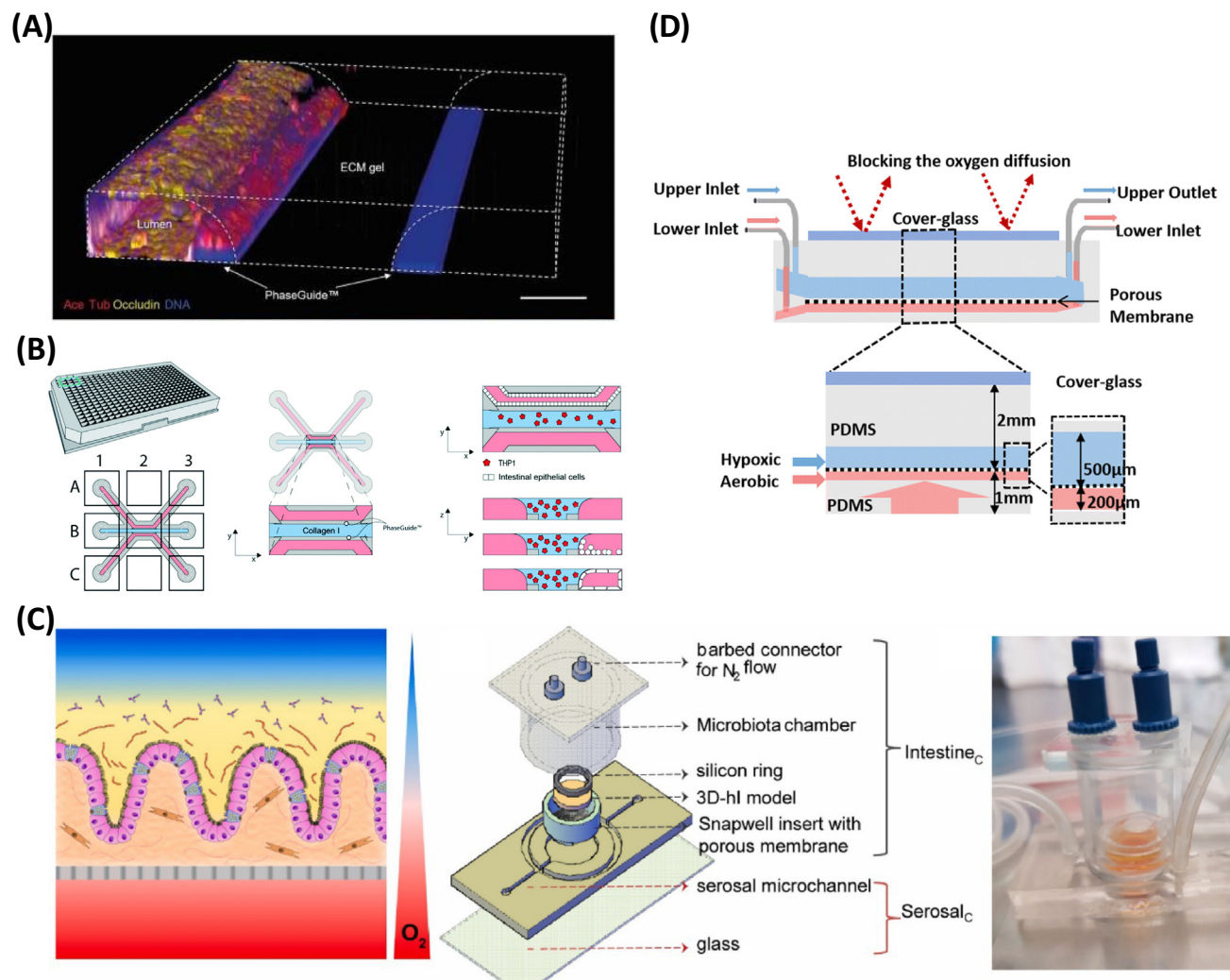
IBD-on-chip models by providing engineered cell lines that secrete differentiation signals directly into the medium and can reduce the overall effort for the experiments [59]. Moreover, integrating macrophages into these models enhances their ability to replicate real-world scenarios of intestinal inflammation [60]. Although 2D cell culture systems are traditionally used to design high throughput experiments and widely incorporated in IBD research as disease models, growing intestinal structures in 2D cultures lack two essential characteristics of the intestine: (i) 2D models cannot replicate the complex 3D structures of the human intestine for hosting the microbiome; and (ii) peristaltic movements and shear forces in the intestine are not represented in 2D models. To tackle these limitations, 3D systems are modified for multidimensional growth and differentiation of stem cells into intestinal organoids [56]. Besides, high throughput experimental protocols are also developed for 3D cell cultures in IBD-on-chip research to complete physiological relevance of the disease models.

The primary advantage of organoid models lies in their capacity to mimic the native architecture and cellular diversity of the intestine under controlled *in vitro* conditions. This is facilitated by self-organizing intestinal stem cells into complex structures that closely resemble the *in vivo* environment. Two prevalent methodologies in intestinal 3D culture are scaffold-free and scaffold-based systems. Scaffold-free cultures allow cells to naturally aggregate and differentiate into self-organizing formations such as spheroids, tissue strands, and cell sheets. Conversely, scaffold-based cultures utilize predefined geometric frameworks made from various natural or synthetic materials. These scaffolds are often augmented with ECM components to promote cell adhesion and growth, thereby forming intestine-like structures. The construction of 3D intestinal chips requires meticulous optimization of cell types and scaffold materials to accurately replicate the mechanical and structural conditions of the *in vivo* intestinal environment. The incorporation of dynamic fluid flow within these systems has been demonstrated to significantly enhance the differentiation and maturation of intestinal structures, leading to more physiologically relevant models. Key physiological features of the human intestine environment can be mimicked using a OoC microfluidic device. Methods include microfluidic flow modeling of peristalsis and shear stress, mechanical and biological cue integration to replicate *in vivo* circumstances, and co-cultivation of several cell types to simulate gut interactions. To replicate the circumstances in the small and large intestines, respectively, flow is crucial. The small intestine requires rapid perfusion rates, whereas the big intestine necessitates slower transit times and robust microbial cultures. One way to examine the pathology of IBD is to induce it in these models. This can be done by using proinflammatory cytokines or pathogenic microorganisms. The promising results of these 3D intestinal modeling efforts demonstrate how OoC technologies may help in disease modeling and drug discovery by connecting *in vitro* studies with the complicated nature of IBD [61].

Comparative analysis of different IBD on-a-chip models

Various IBD-on-chip models have been created, as highlighted in the literature. We focus on describing the models that simulate human intestinal inflammation, thereby acting as viable IBD models (Figure 3 and Table 2). In order to assess the degree of a property (low–medium–high) for cost, selectivity, sensitivity, and reliability, different parameters are considered for given studies. Machine and material cost of the production and requirement of different cell types are considered to assess cost value. Representation of whole physiological elements of the gut, including microbiome bacteria, are also used to evaluate the cost-selectivity-sensitivity column of Table 2.

In seminal contributions to the field, the research team led by Donald Ingber developed an innovative **IBD-on-a-chip** model, one of the earliest iterations designed to closely simulate the complex pathophysiology of gut inflammation [62]. This model features a mechanical motion generator for proper epithelial cell differentiation, forming essential intestinal structures like villi



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Figure 3. Inflammatory bowel disease (IBD)-on-chip designs. (A) 3D representation of gut-on-a-chip device with human Caco-2 and HT29-MTX cells. Reproduced from Gijzen and colleagues, 2020 [63]. (B) OrganoPlate platform and its multiplex chip architecture for IBD model separate epithelial and basal sides with collagen filter; the platform can be controlled independently. Reproduced from Gjorevski and colleagues, 2020 [60]. (C) A chip design for oxygen-gradient controllable IBD model is represented. Reproduced from Gregorio and colleagues, 2022 [66]. (D) Schematic illustration and cross-section of oxygen-gradient controllable IBD-on-chip model. Reproduced from Liu and colleagues, 2023 [67]. Abbreviations: ECM, extracellular matrix; PDMS, polydimethylsiloxane.

and tight junctions. It also supports the co-culture of Caco-2 cells with probiotics or enteroinvasive bacterial strains, allowing the study of dynamic inflammatory responses within the gut ecosystem. Moreover, microfluidic technologies in the model ensure precise control over fluid dynamics, simulating nutrient flow and waste removal and reflecting *in vivo* conditions closely [62].

Building on previous work, the OrganoPlate® system effectively models gut inflammation through a newly developed immunocompetent 3D perfused intestine-on-a-chip platform that successfully cultivates polarized epithelial tubular structures from enterocytes (Caco-2) and goblet cells (HT29-MTX-E12), incorporating immune cells (THP-1 and MUTZ-3) for cytokine release assessment [63]. This system effectively simulates intestinal inflammation through exposure to tumor

Table 2. An overview of the key features and design capabilities of IBD-on-chip models in the literature, serving as an introductory summary of the topic

Model	Cost	Duration	Selectivity-sensitivity-reliability	Cell types/organisms	Controllable parameters	Production/material	Refs
Mechanically active IBD-on-a-chip model with bacteria	Medium	3 days	High-high-medium	- Human intestinal - Epithelial cells (Caco-2) - Peripheral blood mononuclear cells (PBMCs) - Human endothelium - Commensal bacterial mixture	- Strain stress - Shear stress - Membrane separation	- Micropatterned silicon wafer - PDMS	[62]
Multiorgan UC model	High	2 days	High-high-high	- Primary human UC epithelium cells - Dendritic cells - Macrophages - Healthy human hepatocytes - Kupffer cells - Treg and Th17 cells	- Multiorgan model - Shear stress - Fluidic circulation - Membrane separation	- Membranes (polyurethane) - Pneumatic plates (acrylic) - Fluidic plates (polysulfone)	[64]
Intestine-on-chip model for gut inflammation	Low	10 days	Medium-high-medium	- Enterocytes (Caco-2) - Goblet cells (HT29-MTX-E12) - Immune cells (THP-1 and MUTZ-3)	- Shear stress - Membrane separation	Polystyrene and glass, OrganoPlate® platform	[63]
Neutrophilic infiltration organ-on-chip model	Low	5–7 days	Medium-high-medium	- Human intestinal epithelial cells (Caco-2) - ECM-embedded monocytes (THP1) - Circulating neutrophils	- Shear stress - Membrane separation	Polystyrene and glass, OrganoPlate® platform	[60]
IBD-on-a-chip model with bacteria	Medium	7 days	Low-medium-medium	- Human intestinal epithelial cells (Caco-2) - Commensal bacteria (<i>Bifidobacterium bifidum</i>)	- Shear stress - Oxygen gradient control - Membrane separation	- Micropatterned silicon wafer - PDMS	[67]
IBD-on-a-chip model with bacteria	Medium	12 days	Medium-medium-medium	- Human intestinal epithelial cells (Caco-2) - Primary human macrophage cells - Commensal bacteria (<i>Escherichia coli</i> 11775)	- Shear stress - Membrane separation	- Micropatterned silicon wafer - PDMS	[68]
Multiunit intestine-on-chip system	High	5 days	Medium-medium-medium	- Human intestinal epithelial cells (Caco-2) - Goblet cells (HT29-MTX-E12) - Colonic fibroblasts (CCD-18Co) - Intestinal stromal cells	- Independent cytokine stimuli - Selective inflammation - Introduction of different immune cells - Antibody treatment - Shear stress - Membrane separation	- Thermoplastic - Collagen I membrane	[65]
IBD-on-a-chip model with bacteria	High	10 days	Medium-high-high	- Human intestinal epithelial cells (Caco-2) - Human intestinal subepithelial myofibroblasts (hiSEMFs) - Bacteria (<i>Lactobacillus rhamnosus</i>, <i>Bifidobacterium longum</i>)	- Oxygen gradient control - Shear stress - Membrane separation	- Poly(methyl methacrylate) (PMMA) mold and lid - PDMS chamber - Snapwell® insert	[66]

(continued on next page)

Table 2. (continued)

Model	Cost	Duration	Selectivity-sensitivity-reliability	Cell types/organisms	Controllable parameters	Production/material	Refs
IBD-on-a-chip model with bacteria	Medium	7 days	Medium-high-high	- Human umbilical cord vein endothelial cells (HUVECs) - Human intestinal epithelial cells (Caco-2) - PBMCs, bacteria (<i>L. rhamnosus</i> and <i>Candida albicans</i>)	- Shear stress - Membrane separation	- Injection molding polystyrol chip - Polyethylene terephthalate membrane	[69]
Electrode integrated IBD-on-chip model	High	8 days	Low-high-medium	HUVECs	- Shear stress - Membrane separation - Exosome induction - Electrical measurement	- Photolithography - Polyester membrane - PDMS chamber - Silicon mold	[70]

necrosis factor (TNF)- α and interleukin (IL)-1 β , with effects quantified via transepithelial electrical resistance (TEER) and cytokine secretion, confirming impaired barrier function and increased IL-8 production. The platform's capability to test the efficacy of anti-inflammatory drugs was demonstrated with TPCA-1, which mitigated the induced inflammatory state. The model not only provides a realistic simulation of gut inflammation but also demonstrates the efficacy of the anti-inflammatory drug TPCA-1 in reducing inflammation within the system. (Figure 3A) [63].

The study by Trapecar and coworkers explores the intricate relationship between liver diseases and UC by integrating micro-physiological systems of the gut, liver, and circulating Treg and Th17 cells [64]. This model demonstrated that microbiome-derived **short-chain fatty acids (SCFAs)** can either ameliorate or exacerbate UC severity, influenced by the activity of effector CD4+ T cells. Multiomics research showed that SCFAs increase ketone body synthesis, glycolysis, and lipogenesis and decrease UC gut innate immunological responses. SCFA metabolic reprogramming increased CD4+ T cell effector activities during acute T cell-mediated inflammation. This increased inflammation, intestinal barrier disruption, and liver damage [64]. This study is also important to show that multiorgan systems could be a future direction for IBD studies and may reveal the complex nature of the disease biogenesis.

Compromised integrity of the mucosal barrier, a hallmark feature of IBD, facilitates aberrant immune responses to the microbiome, mediated primarily by dendritic cells and macrophages. Subsequently, this interaction triggers the recruitment of neutrophils, culminating in pronounced inflammatory processes within the mucosal environment [60]. Moreover, in order to establish and model neutrophilic infiltration, a multichamber microfluidic system was created which can harbor IECs (Caco-2), ECM-embedded monocytes, and circulating neutrophils. The resulting model successfully revealed the role of **matrikines** in inflammation with its close physiological relevancy to the IBD conditions (Figure 3B) [60].

The MultiU-Int platform is a versatile platform built as a multiunit thermoplastic chamber which has evaporation prevention and anti-absorption capabilities [65]. In this study, a collagen type I membrane was utilized as a scaffold to create distinct basal compartments. These compartments could be individually treated with various molecules [lipopolysaccharide (LPS), cytokines, or antibodies] and exposed to different immune cells separately [65]. In order to show the role of microbial interactions, the MhiI-oC platform was developed with a polydimethylsiloxane (PDMS)

structure that separates the apical and basal side of the intestine model with a commercial insert (Snapwell® insert) (Figure 3C) [66].

The gut microenvironment is rich in bacteria, predominantly obligate anaerobes thus, including commensal bacteria in IBD models is critical, as elements like LPS and other metabolic factors are known to influence inflammation directly. Probiotic bacteria have also been shown to mitigate gut inflammation. However, an optimal IBD model should maintain physiologically relevant oxygen levels across epithelial and basal layers. While glove box designs and nitrogen-filled chambers can achieve this, they significantly increase the operational costs and complexity of IBD-on-a-chip models. To tackle such challenges, a computationally guided design for an IBD model that includes bacteria has been developed, which also simulates physiological shear stress and cytokine-LPS induced inflammation effectively [67]. A study led by Du and colleagues presents the application of dextran sodium sulfate to induce inflammation in an IBD-on-a-chip model, which incorporates human IECs and primary human macrophages inside a PDMS microfluidic system [68]. This study demonstrated that chitosan oligosaccharide (COS) can mitigate intestinal epithelial damage by promoting expression of the mucous layer on a microfluidic chip modeling IBD induced by *Escherichia coli* 11775. COS effectively protects the intestinal and vascular endothelial barriers by reducing the adhesion and invasion of *E. coli*. Furthermore, COS diminishes the inflammatory response by lowering Toll-like receptor 4 (TLR-4) expression and decreasing NF- κ B, consistent with *in vivo* results (Figure 3D) [67].

In another study, the effect of microbial interactions was investigated with an LPS-induced inflammation model on an intestine-on-chip model [69]. Probiotics like *Lactobacillus rhamnosus* and pathogenic *Candida albicans* affect LPS-induced inflammation, slowing disease progression. A groundbreaking study examined exosome-mediated IBD progression using an electrode-integrated intestine-on-chip model. Trans-endothelial or TEER measures assessed endothelial integrity [70]. Exosomes from IBD patients reduced TEER levels – an indicator of disease progression. Although overall chip design may also include other components of the IBD-on-chip models, it could be concluded that exosome-induced inflammation on microfluidic models could be a new strategy for disease modeling and therapeutic testing.

These enhancements in IBD-on-chip models offer significant insights into the dynamics and potential treatments of the disease. They may bridge the gap between laboratory studies and actual biological complexity. However, there are still limitations that warrant careful consideration. Designing IBD-on-a-chip models in both small and large intestines requires addressing the distinct physiological characteristics of these regions. While IBD-on-a-chip models offer significant advantages in recapitulating the human intestinal inflammatory microenvironment and identifying disease-driving factors and pharmacological targets, the concept of ‘biological scalability’ needs to be considered carefully. The small intestine model requires replication of its intricate villus structures, high cell turnover rate, and rapid nutrient absorption, necessitating a microfluidic design that supports high perfusion rates, dynamic fluid flow, and nutrient gradients. Conversely, the large intestine model needs to mimic its dense microbial population, thicker mucus layer, and slower transit time, demanding a system that accommodates robust microbial cultures and prolonged luminal content exposure. Both models must incorporate and consider several parameters for accurately scaling and adapting IBD-on-a-chip models to various inflammatory conditions along the intestines (Box 3).

Future prospects promising research and clinical implications

Multiplex co-culture flexibility is vital in improving the gut-on-a-chip microsystem and enabling a more personalized model for IBD. This approach enables the construction of a model capable of

Box 3. Considerations for developing realistic primary tissue models in IBD research

The acute inflammation models, often induced by bacterial addition or cytokine exposure, may not capture the chronic and multifaceted nature of IBD on a chip model. To enhance the physiological relevance of these models, several strategies can be pursued.

Incorporation of primary cells and iPSCs

Utilizing primary intestinal epithelial cells or induced pluripotent stem cell (iPSC)-derived cells can better replicate the native cellular heterogeneity and functionality. Advances in stem cell technology and organoid cultures provide promising avenues for sourcing these cells. Organoids, which are miniaturized and simplified versions of organs produced *in vitro*, can mimic the 3D architecture and complex cell-to-cell interactions seen in actual human intestines, making them invaluable for modeling diseases like IBD [115].

Chronic inflammation simulation

It is critical to develop systems that can model chronic inflammatory states rather than acute responses. This includes long-term co-cultures with immune cells and the incorporation of patient-derived microbiota to better simulate the dysbiosis observed in IBD patients. Chronic inflammation models can involve prolonged exposure to inflammatory cytokines or the use of genetic modifications to induce a sustained inflammatory response, thereby providing a more accurate representation of IBD pathophysiology.

Advanced microfluidic designs

Enhancing microfluidic platforms to support more complex fluid dynamics, nutrient gradients, and mechanical stimuli such as peristalsis and shear stress can improve the fidelity of these models. Innovations in microfabrication techniques will be key to achieving this. These microfluidic devices can simulate the dynamic environment of the human intestine, including the flow of fluids and mechanical forces, thus providing a more realistic model for studying IBD [116].

Integration of multicellular components

A realistic IBD model should integrate various cell types, including fibroblasts, endothelial cells, and nerve cells, to reflect the full cellular complexity of the intestinal microenvironment. This also involves incorporating the extracellular matrix components to provide appropriate cell-matrix interactions. The interaction between these different cell types is crucial for maintaining intestinal homeostasis and understanding how disruptions in these interactions can lead to disease [117].

Personalized precision medicine approaches

Leveraging patient-specific cells and microbiota can enhance the relevance of personalized precision medicine models. This approach can help in understanding individual variations in disease progression and treatment response. By using cells derived from IBD patients, researchers can create models that reflect the unique aspects of an individual's disease, allowing for the development of tailored treatments and therapies [118].

simulating personalized physiological conditions relevant to IBD. Such a model holds promise for assessing novel therapeutic interventions for IBD and predicting patient responses to established treatments. The proposed methodology involves incorporating various cell types, including IECs derived from *in vitro* differentiated intestinal stem cells, immune cells, and gut microbiota sourced from diverse cohorts of IBD patients exhibiting different disease severities, stages of disease progression, treatment histories, and incidences of *Clostridium difficile* infection (CDI): we can envision a 'personalized gut-on-a-chip' [71]. As a control group, we can examine healthy individuals who do not exhibit any intestinal bowel disease symptoms. An innovative feature of the 'personalized gut-on-a-chip' will be the reconstitution of the gut microbiome. This holds significance due to the growing body of evidence suggesting a strong correlation between the microbiota responsible for producing SCFAs – a master chef regulator of the immune landscape – and the intricate orchestration of IBD pathogenesis [55]. This innovative approach holds significant potential for advancing our understanding of IBD mechanisms and optimizing personalized therapeutic strategies.

In order to assess the effectiveness of fecal microbiota transplantation (FMT), it is crucial to use the gut microbiome sample of the individuals. Using cells from healthy donors and individuals with IBD, 'Avatar chips' can be formed to transfer gut microbiota samples from one group to another. Prior to FMT therapy, this method may help to detect the abnormal expansion of

opportunistic pathogens and inflammatory events. During FMT, it may monitor the population dynamics of commensal bacteria and the improvement of recurrent CDI in patients with IBD [72]. The concept of ‘personalized gut-on-a-chip’ holds immense potential in transforming the treatment of IBD. By accurately replicating the heterogeneity of individuals, this approach can effectively replace the previous direct gut microbiota treatments such as antibiotics, probiotics, prebiotics, and synbiotics. By utilizing better patient specificities and different combinations of synbiotics, this strategy has the potential to overturn the traditional use of probiotics in IBD treatment [73]. This is due to the fact that the efficacy of probiotics and prebiotics is open to debate.

Production capacities will change on-chip technology, especially OoC systems. PDMS has been the main material for microchip manufacturing, but its dependence on mold production and the need for specialized sub-micron fabrication equipment have constrained OoC systems. However, recent 3D printing advances may overcome these constraints. Biocompatible 3D printing materials eliminate mold manufacture and enable sub-micron features. This development simplifies chip manufacture, allowing the creation of flexible and rigid whole-in-one semiconductors. 3D printing enables the direct integration of filter components into chip designs, providing researchers with versatile platforms suitable for a wide range of experiments.

Moreover, the miniaturization of consumer-grade electronics is set to empower scientists in designing microfluidic systems that closely mimic physiological conditions. This trend will enable the development of OoC models with modular architectures and intricate flow dynamics, paving the way for more physiologically relevant investigations, particularly in the realm of IBD research.

Adding machine learning (ML) algorithms to on-chip devices can improve control and adaptability. Researchers can create autonomous, self-adaptive on-chip platforms that simulate realistic IBD models using artificial intelligence (AI) and ML. These systems may dynamically modify parameters based on real-time data, making preclinical models for evaluating IBD medicines more accurate and predictive.

Concluding remarks

There is a pressing need for therapies that not only enhance wound healing in inflammatory conditions but also for methods to promptly evaluate and predict healing outcomes, enabling earlier intervention. The strides made in IBD research, particularly through the application of on-a-chip technologies, underscore a pivotal shift towards more precise and predictive biomedical investigations. This technological advancement has notably revolutionized the simulation of the human intestinal microenvironment, thereby enabling a more accurate emulation of disease processes and therapeutic responses. The microfluidic systems not only enhance our understanding of the pathophysiological nuances of IBD but also expedite the development and screening of potential therapeutic interventions, thereby mitigating the traditionally protracted timelines associated with drug discovery.

The use of OoC technologies in IBD research is a relatively new field; therefore, this review specifically highlights the developments made in the past 20 years. There is a significant expectation for on-chip models in research laboratories, especially when it comes to translational research into clinical investigations. Our comprehension of intricate disease pathways, including those seen in IBD, is expected to be greatly improved by these models. The study of complex interactions within the gut milieu, such as immune responses, microbiome dynamics, and epithelial barrier functions, can be facilitated by these technologies by offering more realistic and dynamic simulations of the human gut. By bridging the gap between laboratory research and clinical application, successful uses of on-chip models have already shown their potential in uncovering disease pathways and testing therapeutic approaches.

Outstanding questions

How can we improve the fidelity of microfluidic platforms and on-chip systems to more accurately simulate the complete human intestinal microenvironment, especially concerning the integration of immune responses and microbial dynamics?

What specific genetic, environmental, and immunological factors most critically influence the host–microbe interactions in IBD?

How can the integration of various cellular components and 3D tissue architecture in on-chip models be optimized to better simulate intestinal wound healing processes?

What novel biomarkers can be identified using microfluidics and on-chip technologies for early diagnosis and monitoring of IBD?

How can these technologies be leveraged to delve into the long-term effects of IBD treatments on the intestinal microenvironment?

How can on-chip models be used to study the role of specific microbial populations in the pathogenesis and progression of IBD?

What are the potential impacts of emerging trends in OoC technologies on the broader field of personalized medicine beyond GI research?

In what ways can we harness the full potential of on-chip models for real-time disease monitoring and management, especially using integrated sensors and feedback systems for dynamic control of microenvironmental conditions?

What are the limitations in current drug testing models using on-chip systems, and how can these be overcome to improve drug efficacy and safety evaluations?

How can patient-derived cells and tissues be better utilized in on-chip models to enhance the relevance and accuracy of findings in the context of IBD?

What steps are necessary to standardize on-chip models across research laboratories to ensure reproducibility and to streamline translational research efforts?

These innovative microchip models have the remarkable ability to replicate the intricate interactions that occur within the microenvironment of the intestines, which holds great promise for advancing our understanding of this complex system. This microenvironment includes the immune responses, microbiome, and epithelial barrier functions. The aforementioned models offer a robust basis for comprehending the complex nature of IBD. Not only can they help discover the underlying mechanisms of a disease, but they also can enable customized treatment strategies based on individual patient profiles. This is especially crucial considering the varied nature of IBD. Integrating such technologies with advanced imaging, real-time data analysis, and ML further refines their utility, making them indispensable tools in the quest for more effective and targeted IBD therapies.

As the field continues to evolve, the ongoing fusion of microengineering, cellular biology, and clinical medicine holds the promise of transforming IBD treatment paradigms. Future research should focus on enhancing the physiological relevance of these models, reducing the gap between *in vitro* studies and clinical realities. This involves refining the models to accommodate dynamic interactions among cellular and microbial communities within the gut and improving the simulation of immune responses. Moreover, developing more complex multiorgan models could facilitate a deeper understanding of systemic effects, which are often pivotal in IBD.

Integrating on-a-chip technologies in IBD research has achieved a significant milestone in gastroenterology and immunology. While this achievement paves the way for future breakthroughs that could help develop personalized medicine and offer hope for better treatment options for patients suffering from IBD, several critical questions remain (see [Outstanding questions](#)). Enhancing the simulation of the human intestinal microenvironment, identifying key factors affecting host–microbe interactions, optimizing the integration of cellular components and 3D architecture, and discovering novel biomarkers for early IBD detection and long-term effects of treatments are crucial areas needing further exploration. By continuing to harness these innovative platforms, researchers are positioned to decode the complexities of IBD more effectively, paving the way for interventions that are as precise as they are transformative.

Acknowledgments

F.I., E.G.Y., and N.H. gratefully acknowledge support from the Scientific and Technological Research Council of Turkey (TÜBİTAK) 2232 International Fellowship for Outstanding Researchers (Project No: 118C254). However, the entire responsibility of the publication/article belongs to the owner of the publication/article. The financial support received from TÜBİTAK does not mean that the content of the publication is approved in a scientific sense by TÜBİTAK. F.I. also acknowledges the support of GEBİP Award from Turkish Academy of Sciences (TÜBA). This work was also supported by BAGEP Award of the Science Academy. B.Y. is supported by Swiss National Science Foundation Starting Grant: TMSGI3_211300 and S.B.U.J. receives support from the SNSF MD-PhD programme: 323630_221867. Some of the figures were created using graphic design tool BioRender (<https://www.biorender.com/>).

Declaration of interests

The authors have no interests to declare.

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