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REVIEW

Bioengineered intestinal models: structural precision drives functional and microbial fidelity

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Abstract

The intestine is a key component of the barrier, absorption, and immune systems, contributing significantly to maintaining internal homeostasis and influencing disease progression. Its distinctive physiological functions arise from a complex interplay between its structure and microenvironment. Recent advancements in bioengineering technologies now enable the construction of in vitro intestinal models that faithfully recapitulate the organizational and functional characteristics of native tissue. This review examines the interface between in vitro models and native intestinal biology, offering insights into the replication of organ functions from a manufacturing perspective. We explore bioengineering strategies that enable the mapping of cross-scale structures and the creation of biomimetic environments essential for physiological performance. Furthermore, we discuss pragmatic optimization strategies for applying these models to both physiological and pathological studies, thereby enhancing their translational potential for drug development, disease modeling, and personalized medicine. In contrast to previous reviews, this work proposes an engineering-centered framework for linking structural fabrication strategies to functional performance across intestinal model types.

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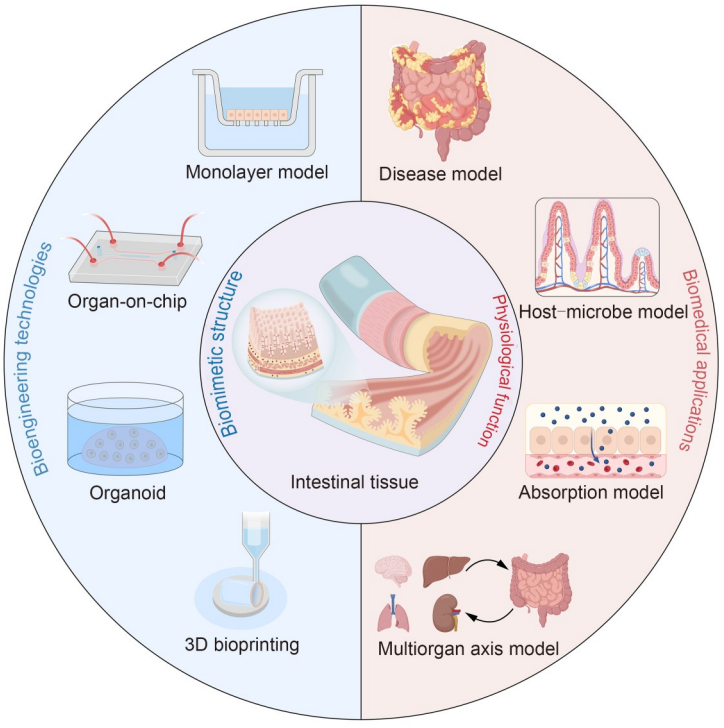
Graphical abstract



Keywords Intestinal model · Organ-on-chip · Organoid · 3D bioprinting · Biomedical application

1 Introduction

The human intestine is a critical component of the digestive system that is responsible for nutrient absorption, waste elimination, and overall health maintenance. It hosts a diverse microbiome that significantly influences immune function, metabolism, and even mental health [1–3]. Understanding the intestine's complex functions is essential for advancing knowledge of human health and disease [4–6]. The complex intestinal hierarchical structure, spanning macro- to micro-scales, provides the physiological foundation for its functions [7, 8]. The triple-level structure of circular folds, villi, and microvilli exponentially increases the intestinal surface area to optimize nutrient absorption [9]. Furthermore, crypts establish critical microenvironments by harboring intestinal stem cells (ISCs) while also functioning as the primary secretory glands for antimicrobial agents, hormones, and mucus [10, 11]. The epithelium itself ensures barrier integrity through tight junctions and secretion of a stratified mucus layer and antimicrobial proteins, which collectively restrict microbial invasion and maintain intestinal homeostasis [12]. Historically, intestinal function studies have relied on animal models [13, 14]. However, inherent anatomical and genetic differences limit the accuracy and translational relevance of such models [15, 16]. This has spurred the development of in vitro culture methods using human cells, offering innovative avenues for mimicking intestinal physiology [17]. Researchers now employ bioengineering technologies to create both two-dimensional (2D) and three-dimensional (3D) models that integrate relevant cell types [18, 19], extracellular matrices, growth factors, and controlled external stimuli to better replicate the human intestinal environment [20].

Early intestinal models utilized intestinal epithelial cells cultured on simple substrates, such as plastic or porous membranes. Although these approaches provided fundamental insights, they failed to simulate the complex interactions between cells and the surrounding matrix [21]. The introduction of Transwell systems significantly improved this approach by facilitating more physiologically relevant cell arrangements [22]. The subsequent emergence of organoid technology further advanced the field by harnessing the self-organizing behavior of intestinal stem cells to reproduce native tissue architecture [23]. In parallel, microfluidic chip technologies have refined these models by introducing dynamic culture conditions and mechanical stimulation [24].

The more recently introduced biofabrication techniques offer a powerful strategy for constructing in vitro tissues

with precise spatial organization. Through computer-aided design, these techniques generate structures that closely mimic the anatomical topology of the intestine, enhancing the simulation of physiological processes [25]. Nevertheless, replicating the full complexity of intestinal tissue remains challenging, as it requires proper cellular organization, material integration, dynamic 3D culture, niche development, and vascularization [26, 27]. Various bioengineering approaches have been developed to address these challenges, each offering unique strengths in reproducing specific physiological features (Fig. 1).

This review comprehensively examines the bioengineering technologies used to construct in vitro intestinal models and their applications in studying intestinal development, microenvironment homeostasis, and disease mechanisms. Unlike previous reviews, which often treated fabrication and biological applications separately, this review synthesizes these aspects to demonstrate the direct impact of structural decisions on functional fidelity at the manufacturing level. It highlights the transition from constructing physiologically relevant anatomical structures to restoring intestinal functions. From an engineering standpoint, this framework guides the development of appropriate in vitro models tailored to various intestinal biological applications. Furthermore, this review discusses optimization strategies for bridging the gap between structural mimicry and functional performance. Finally, by synthesizing advancements in tissue engineering, advanced manufacturing, and microfluidics, we outline future directions that will enhance the translational relevance and practical utility of in vitro intestinal models.

2 Strategies for constructing in vitro intestinal tissue models

Bioengineering technologies aim to replicate both macro- and micro-intestinal structures while mimicking the biomimetic intestinal physiological environment. These approaches encompass a wide range of scales, from micrometer-level cellular and extracellular matrix (ECM) structures to millimeter-scale tissue models (Fig. 2). Here, we summarize the characteristics, advantages, and limitations of various models, including 2D static, organ-on-chip, organoid, and 3D scaffold-based models.

2.1 2D static model

2D static models simulate specific cellular functions in a static culture environment by seeding cells onto a surface where they can migrate, proliferate, and mature [28]. These

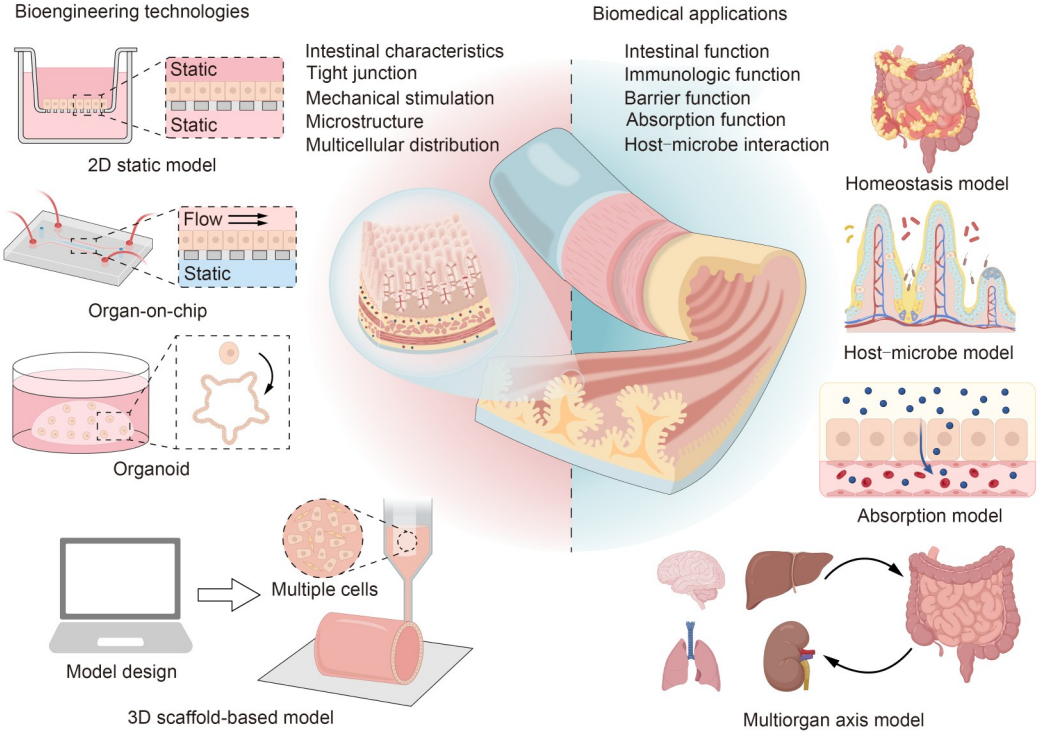


Fig. 1 Schematic of the bioengineering technology characteristics required to replicate intestinal organizational and functional characteristics in biomedical applications. Guided by intestinal physiological characteristics, various bioengineering technologies have been used to construct in vitro models with different dimensional and structural features. The resulting distinctive organizational characteristics and physiological environments constructed through these models enable in vitro studies on various physiological and pathological processes

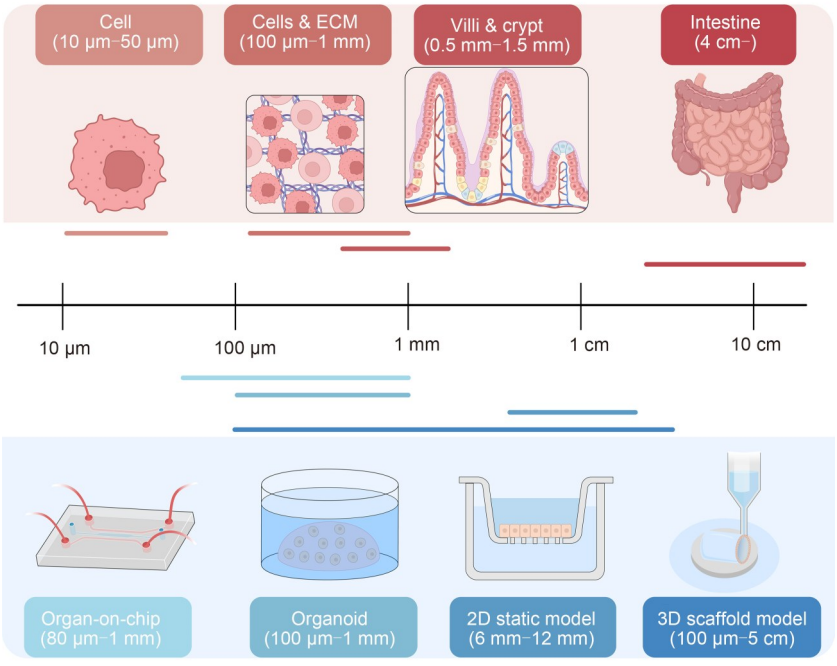


Fig. 2 Comparison of the feature size of bioengineered intestinal models with the multiscale hierarchy of native intestinal tissue

surfaces, such as culture dishes or porous membranes, are typically established in the centimeter scale, limiting cellular growth in the vertical direction. This approach offers a simplified model focusing on the basic functional replication of the interactions between intestinal epithelial cells and their environment [29]. For instance, cocultured CACO-2, HT-29, and Raji B models have demonstrated more biomimetic permeability for peptide drugs than CACO-2 monoculture models [30]. To investigate cell–microbe interactions, an oxygen gradient environment can be established using the Transwell system. The well and insert are separated by a porous membrane, which also serves as the substrate for cell seeding. The upper and lower chambers are typically filled with distinct culture media to establish different microenvironments. Furthermore, the insert can be sealed to create an anaerobic environment, while the well remains open to the atmosphere, thereby maintaining aerobic conditions. Therefore, this customizable configuration facilitates the simultaneous growth of intestinal epithelial cells and anaerobic bacteria, each within physiologically appropriate environments [31].

Although the 2D static model provides a supportive environment for the growth and proliferation of epithelial cells, it has limited ability to accurately recapitulate the unique structural characteristics and spatial organization of intestinal cells. For instance, it is unable to reproduce the native villus–crypt architecture, which is critical for establishing the appropriate epithelial niche. Furthermore, the uniform culture conditions of the 2D static model inherently restrict the possibility of dynamic culture, making it unsuitable for biomimetic fluidic stimulation and hindering its capacity for long-term culture.

2.2 Organ-on-chip

Compared with the 2D static models, organ-on-chip technology offers multiple notable advancements, such as the use of microfluidic techniques to introduce shear stress, cyclic mechanical forces, and complex spatial microenvironments [32]. By integrating these mechanical and dynamic environmental factors, organ-on-chip models overcome the absence of dynamic stimulation and restricted structural complexity, among other limitations, thereby facilitating a more accurate recapitulation of intestinal physiology [33].

Fabrication techniques are crucial in organ-on-chip development. Photolithography and etching are commonly used to create rigid glass- or silicon-based microfluidic devices, which offer excellent chemical resistance and structural stability [34]. However, these materials are unable to replicate the elasticity of native tissues, preventing the generation of relevant mechanical cues during cell culture. Soft lithography employs polydimethylsiloxane (PDMS) as a flexible mold for fabricating microfluidic channels through pattern transfer, offering high precision, optical transparency, and gas permeability [35]. However, PDMS tends to absorb hydrophobic drugs or signaling molecules, potentially altering drug absorption and interfering with intercellular interactions. Another widely adopted approach is 3D printing, which enables design customization and high-resolution rapid prototyping of complex geometries [36]. Despite

these advantages, the resolution and material constraints of 3D printing techniques may compromise channel smoothness, thereby affecting flow patterns and shear forces experienced by cells. The fabricated channels are typically >10 μm, which can sufficiently accommodate cells and provide an environment conducive to advanced cell culture systems [24, 37].

In intestine-on-chip models, microfluidic systems generate laminar flow within channels by mimicking the fluid dynamics of the intestinal lumen [38, 39]. The shear stress generated by fluid flow (range: 0.002–0.080 dyne/cm^2) promotes the self-assembly of villi-like microstructures, with epithelial cells forming projections up to 300 μm in height [40], which are still shorter than natural human intestinal villi but are significantly better than those in static models [41]. Furthermore, micromilling can fabricate 3D pores in chip designs, enabling gradient dynamic environments that guide epithelial cell morphogenesis and facilitate villi–crypt axis reconstruction [33]. Flow parameters, such as the Reynolds number (range: 0.11–0.34), also contribute critically to the replication of intestinal functions, including nutrient digestion and absorption [42, 43] (Fig. 3a). Cyclic mechanical forces applied through vacuum chambers adjacent to microfluidic devices mimic the natural peristaltic motion of the intestinal wall, further enhancing villi and crypt formation and influencing cellular behavior, such as bacterial invasion resistance [44].

Organ-on-chip systems also facilitate cocultures and interactions between multiple cell types and microbes [45]. Multilayer or parallel-flow channels enable communication between epithelial cells and other intestinal components. For example, multilayer channels simulate epithelial–endothelial interactions to promote vascularization and form cellular barriers [46–48]; these barriers exhibit more pronounced physiological characteristics under oxygen gradient conditions. Epithelial cells and enteric neurons were cultured separately in two compartments of a parallel-flow microfluidic device, which were interconnected through microgrooves (150 μm long, 2.5 μm high) [49, 50] (Fig. 3b). Over time, neuronal projections extend through the microgrooves to establish contact with the epithelial cells. Compared with monocultures, enteric neurons in the coculture system exhibited a higher rate of outgrowth and an increased density of neuronal projections. Chips incorporating immune cells, such as primary monocytes, alongside epithelial cells create an immune-active environment that mimics in vivo conditions during inflammatory responses [17, 51, 52] (Fig. 3c). The coculture of epithelial cells with anaerobic microbes was performed using a multilayer perfusion chamber containing different culture media and separated by a porous membrane [53, 54] (Fig. 3d). The basal side of the epithelial cells was exposed to cell culture medium under aerobic conditions, whereas the apical side was exposed to anaerobic conditions, thereby establishing an oxygen gradient [55]. Following the formation of a confluent epithelial monolayer, microbes were introduced for coculture, to reconstruct host–microbe interactions within a biomimetic environment.

The organ-on-chip system also enables real-time monitoring of environmental parameters and cellular states during

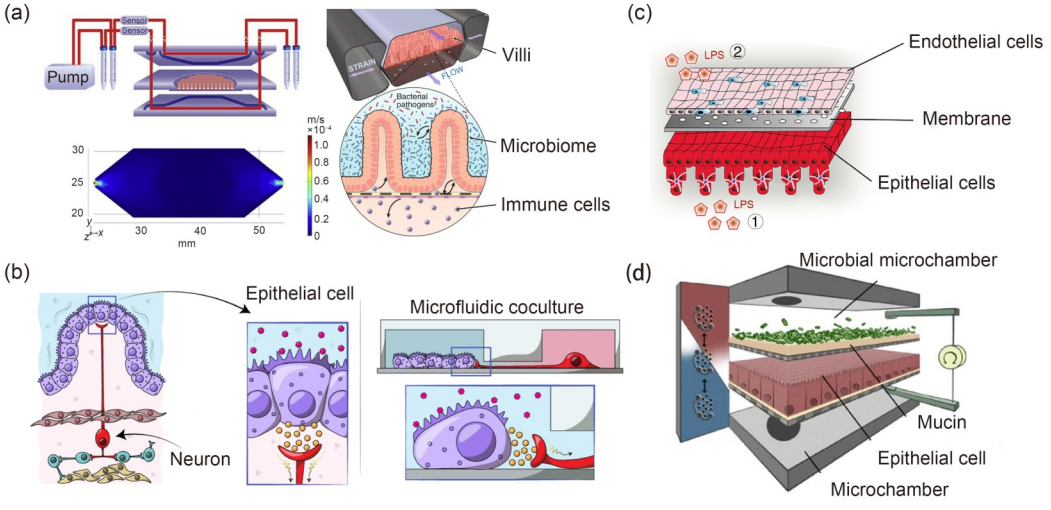


Fig. 3 Intestine-on-chip for physiological environment reconstruction and biological interaction. (a) Epithelial cells are subjected to fluid flow and exhibit self-assembly of villi-like microstructures in organ-on-chip. Reproduced from [42] (Copyright 2018, with permission from the authors, licensed under CC BY-NC-ND) and [43] (Copyright 2020, with permission from the authors, licensed under CC BY 4.0). (b) Coculture of epithelial cells and intestinal neurons in microfluidic organ-on-chip. Reproduced from [49], Copyright 2023, with permission from the authors, licensed under CC BY 4.0. (c) Cocultured epithelial cells and immune cells demonstrating immune tolerance to microbes. Reproduced from [52], Copyright 2019, with permission from Elsevier Ltd. (d) A modular microfluidics-based human–microbial coculture model featuring oxygen sensors and detection electrodes. Reproduced from [53], Copyright 2016, with permission from the authors, licensed under CC BY 4.0

culture [56]. The culture environment, including neutral pH, oxygen concentration, and temperature, can be continuously monitored using various sensors, such as non-contact optical pH, oxygen, and temperature sensors [57]. Miniaturized microscopes are used to assess cellular growth and for real-time imaging of cell morphology. Commercially available chopstick-style electrodes can be applied to measure transepithelial electrical resistance to further characterize cell growth and differentiation. Electrodes functionalized with specific antibodies can detect cell-secreted proteins, providing insights into functional protein expression [58]. The integration of these sensing modalities with organ-on-chip platforms markedly enhances their capabilities for detection and quantitative analysis.

Despite providing a more biomimetic culture environment, organ-on-chip systems confine cellular growth to a 2D plane, which imposes inherent limitations on achieving efficient multicellular interactions. First, confining different cell types to separate microchannels dilutes their paracrine signals in the culture medium, thereby limiting intercellular influence. Second, these platforms cannot replicate the native microarchitecture of intestinal villi within organ-on-chip platforms. In human intestinal tissue, the finger-like projections and invaginations created by villi and crypt structures are critical in regulating epithelial differentiation and spatial distribution. Although fluidic shear stress in organ-on-chip systems can induce epithelial cells to form rudimentary protrusions, these structures cannot fully recapitulate authentic villus–crypt topography, which compromises the fidelity of functional restoration.

Organ-on-chip platforms expand the capabilities of traditional 2D models. By integrating dynamic flow, mechanical stimulation, and multicell coculture, these platforms produce more physiologically relevant simulations of intestinal processes. Their modular design facilitates controlled-gradient studies of nutrient absorption, immune responses, and host–microbe interactions. Future integration with bioprinted architectures could provide the 3D complexity needed for villus–crypt organization and spatial microbial distribution. Furthermore, embedding real-time biosensing within chips will facilitate the continuous monitoring of metabolites, oxygen levels, and barrier integrity, while AI-driven analytics will enhance predictive modeling and adaptive control of the microenvironment. These innovations position organ-on-chip technology as a cornerstone for next-generation intestinal models.

2.3 Organoid

Organoids are a transformative advancement for in vitro modeling, offering stem cell-derived 3D structures that self-organize and differentiate into various intestinal cell types while mimicking the complexity of human intestinal tissue [59]. Compared with the 2D static model, organoids exhibit a higher degree of physiological relevance [60], replicating the villus–crypt microarchitecture and hosting various epithelial cell types, including enterocytes, goblet cells, Paneth cells, and enteroendocrine cells [61–64]. Unlike organ-on-chip models, which rely on microfluidic channels to simulate flow dynamics, organoids can closely capture the cellular heterogeneity of the intestinal epithelium [65, 66].

Currently, organoids are primarily derived from tissues or induced pluripotent stem cells (iPSCs). Tissue-derived organoids are generated by isolating and purifying stem cells or progenitor cells from tissues via optimized dissociation and purification methods [67]. In contrast, iPSC-derived organoids are constructed from well-established and comprehensively characterized iPSC lines that serve as the starting cells [68–70]. The isolated cells are seeded into biologically derived matrices, such as Matrigel or natural ECM. Subsequently, soluble factors are delivered to the cells in a spatiotemporally controlled manner to guide their differentiation toward specific tissue lineages and promote the formation of an epithelial monolayer around a central lumen. With extended culture, intestinal organoids spontaneously develop villus- and crypt-like structures, harboring ISCs and various epithelial cell types that collectively recapitulate the niche microenvironment.

Organoids offer a platform for modeling the intestinal epithelium and its interactions with other cell types through coculture systems [23, 71]. The conventional approach involves mixing organoids, other cell types, and ECMs to allow physical contact and interaction [72, 73]. However, the uncontrollable spatial distribution of these components lowers the efficiency of contact between organoids and other cells. Alternatively, organoids and other cells are cultured on opposite sides of a porous membrane, thereby enabling intercellular interactions. Coculture systems have been established by separating monocyte-derived macrophages from organoids with a porous membrane to successfully stimulate epithelial immune-related responses [74–76] (Fig. 4a). To promote the formation of tighter cell aggregates, cell adhesion is suppressed by using ultra-low-attachment culture dishes coated with hydrophilic hydrogels. The cells are then centrifuged to facilitate aggregate formation and enhance cell–cell interactions. Low-speed centrifugation can mechanically aggregate different cell types, such as organoid-derived cells and vagal neural crest cells. Cell–cell interactions facilitate measurable neuronal activity and thus the effective modeling of the enteric nervous system [77–79] (Fig. 4b). Moreover, droplet microfluidics can be integrated to coencapsulate organoids and other cell types into microgel droplets, thereby ensuring close contact [80]. In contrast to cell–cell interactions, cell–microbe interactions require an oxygen gradient to coculture organoids and microbes [81]. The central lumen of organoids is avascular and fosters a

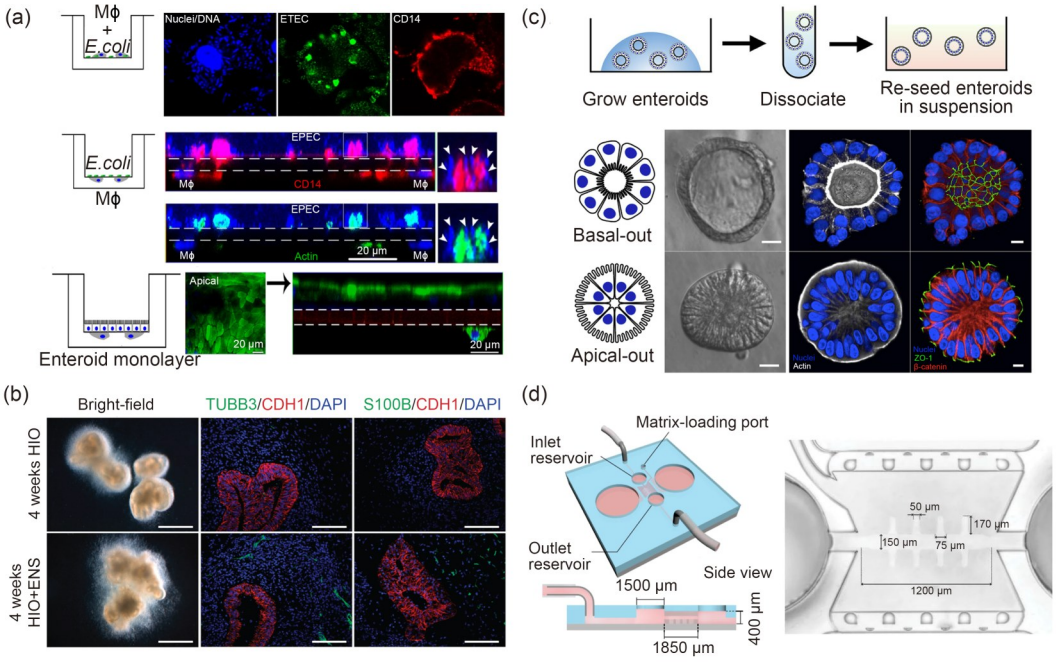


Fig. 4 Organoids for microstructure self-assembly and biological interaction. (a) Human intestinal epithelial monolayers cocultured with monocyte-derived macrophages to facilitate intercellular communication. Reproduced from [74], Copyright 2017, with permission from the authors, licensed under CC BY 4.0. (b) Mechanical aggregation of intestinal organoids and pluripotent stem cell-derived neural crest cells to form a coculture system with periodic neural activity. Reproduced from [78], Copyright 2016, with permission from Springer Nature America, Inc. (c) Suspension culture technique reversing the polarity of the intestinal organoids. Reproduced from [89], Copyright 2019, with permission from the authors, licensed under CC BY 4.0. (d) Laser ablation of the hydrogel scaffold to generate villus-like microchannels. Stem cells seeded on the channel surface proliferate and differentiate into a tubular mini-intestine within the flow environment. Reproduced from [94], Copyright 2020, with permission from the authors, under exclusive licence to Springer Nature Limited

hypoxic microenvironment driven by oxygen consumption from cellular metabolism. Moreover, the densely packed cellular architecture of organoids further restricts oxygen diffusion, thereby reinforcing and maintaining hypoxia within the organoid interior. Using microinjection technologies, symbiotic [82, 83] and pathogenic [84, 85] bacteria, intestinal viruses [86, 87], and other microbes can be introduced into the organoid inner cavity to study them in an environment that simulates the low-oxygen conditions of the intestinal lumen.

Epithelial tissues exhibit apical–basal polarity: The apical surface is specialized for absorption, secretion, and interactions with pathogens, whereas the basal surface mediates adhesion to the ECM and communication with underlying stromal and immune cells. In vivo, the apical membrane faces the intestinal lumen. However, in vitro, the apical domain is enclosed within a central lumen, rendering it inaccessible for studies requiring external induction of pathogens or drugs without invasive manipulation [88]. To address this, "apical-out" organoid configurations can be induced through mechanical inversion, suspension culture, or removal from ECM scaffolds [89] (Fig. 4c) to directly expose the apical membrane to the culture medium, thereby facilitating accessibility. Apical-out epithelial cultures enable efficient evaluation of compound transport, such as in absorption assays requiring high-throughput screening. In contrast, investigations of infection mechanisms and host–microbe interactions require apical-in epithelial culture systems, which can be induced through advanced techniques, such as microinjection, to establish coculture environments. Despite being technically more demanding, apical-in organoids remain the preferred model due to their superior physiological relevance and ability to faithfully recapitulate the luminal environment. In contrast, dissociating organoids into monolayer cultures simplifies the system by making both apical and basal membranes accessible to the external environment, thereby removing the limitations imposed by cell polarity [90].

Nevertheless, organoids face several technical and operational challenges. First, their reliance on self-organization introduces size and structure variabilities that limit reproducibility and scalability for high-throughput studies [91]. Conventional static culture conditions cannot replicate the dynamic mechanical forces present in the native intestine, thereby limiting their ability to fully recapitulate physiological functions. Currently, organoid technology can be integrated with microfluidic platforms. Specifically, organoids can be dissociated into single cells, seeded into microfluidic

chips, and subjected to fluid-induced stimulation, which facilitates an effective restoration of tissue-level functions [92–94] (Fig. 4d). Moreover, most coculture approaches simply mix the desired cell types, preventing the precise replication of the complex anatomical arrangements observed in vivo.

Organoids have significantly advanced the field of in vitro intestinal modeling, bridging some of the gaps overlooked by traditional 2D static models and organ-on-chip systems, such as insufficient recapitulation of villus morphology and inadequate cellular heterogeneity. The ability of organoids to replicate cellular heterogeneity and functional complexity makes them a valuable platform for studying intestinal biology [95]. However, their static culture conditions, reliance on self-assembly, and lack of integration with dynamic mechanical stimuli highlight their limitations. While organoids excel at modeling cellular differentiation and microarchitecture, innovations integrating dynamic mechanical stimuli and precise anatomical replication are needed to enhance their physiological relevance. Combining organoids with 3D bioprinting may overcome these constraints to obtain patterned cellular arrangements and scaffold architectures that support nutrient exchange, microbial colonization, and extended culture stability.

2.4 3D scaffold-based model

3D scaffold-based models have gained significant attention as an advanced platform that offers enhanced replication of the complex architecture, biochemical microenvironment, and functional heterogeneity of the native intestine [96–98]. Conceptually, a scaffold-based intestinal model is designed to simulate the ECM by offering structural and biochemical cues that regulate cellular attachment, polarity, proliferation, and differentiation. This strategy generates an epithelium that recapitulates in vivo morphology and physiological functions. 3D bioprinting and conventional tissue engineering techniques, such as freeze-drying, solvent casting, electrospinning, and decellularization, can be used to fabricate 3D biomimetic intestinal scaffolds using intestinal matrices or synthetic and natural polymeric materials [99–103].

3D bioprinting is an advanced additive manufacturing technology capable of creating customized architectures and replicating the inherent biological heterogeneity of intestinal tissues [104, 105]. Among the available approaches, extrusion-based printing and light-based printing are commonly used to construct in vitro intestinal models. Extrusion-based printing employs pressure-driven extrusion of bioinks through a nozzle to generate continuous filament units, which are mechanically deposited to form 3D structures. Standard extrusion printing protocols use filaments with diameters of 100–200 μm to construct scaffolds that closely approximate the dimensions of intestinal villi, thereby providing a niche for epithelial cell proliferation and differentiation [106, 107]. Advanced techniques, such as coaxial, embedded, and sacrificial printing, can facilitate a more accurate replication of epithelial functions. Coaxial printing fabricates core–shell structures that mimic multilayered tissue organization, such as epithelial and endothelial layers, to enhance epithelial absorption function [108] (Fig. 5a). Embedded printing employs a supporting medium to stabilize bioinks during deposition to fabricate 3D architectures from soft materials, such as vertically oriented microstructures resembling intestinal villi [109] (Fig. 5b). Sacrificial printing, in contrast, uses removable materials as extrudates with dimensions exceeding 500 μm to generate hollow channel structures within bulk tissues. These channels can be perfused with anaerobic medium to establish physiological oxygen gradients, with lower oxygen levels inside the channel and higher ones in the surrounding tissue [110]. Recent advancements integrate human ISCs into bioinks to construct centimeter-scale tissues and spontaneously generate villi structures [111] (Fig. 5c). These bioprinted constructs achieve greater dimensional similarity to the native intestine while maintaining greater functional complexity than organoids.

Extrusion-based bioprinting offers convenience and efficiency for multicellular printing, making it suitable for constructing vascularized intestinal models. A common strategy involves incorporating endothelial cells into bioinks to form 3D structures, followed by the seeding of epithelial cells to establish a coculture microenvironment. This strategy facilitates the flexible reconstruction of native intestinal architectures, such as vascularized circular folds, with precise control over the spatial distribution of endothelial and epithelial cells [103, 112]. However, the fabrication of large-scale tissue models remains challenging due to the inherent trade-off between the mechanical strength and biological performance of bioinks. Coaxial bioprinting, an optimized extrusion-based technique, facilitates the fabrication of vascularized intestinal models. Its concentric nozzles permit multilayered structures that contain distinct cell types and are tailored to replicate intestinal features. For example, filaments with endothelial cells in the core and epithelial cells in the shell can recreate both the structure and in vivo physiology of villi, enabling studies of vascularized nutrient absorption [108]. Alternatively, a hollow lumen can be created using epithelial cells with sacrificial materials in the core and endothelial cells in the shell. After sacrificial removal, epithelial cells are seeded along the tube to line the intestinal cavity [113]. Such tubular models can be integrated with organ-on-chip platforms for perfusion culture to closely simulate circulation and nutrient exchange [114]. Nonetheless, coaxial bioprinting mainly generates homogeneous, linear features but not the heterogeneous macrostructures of native intestines. Future studies should develop

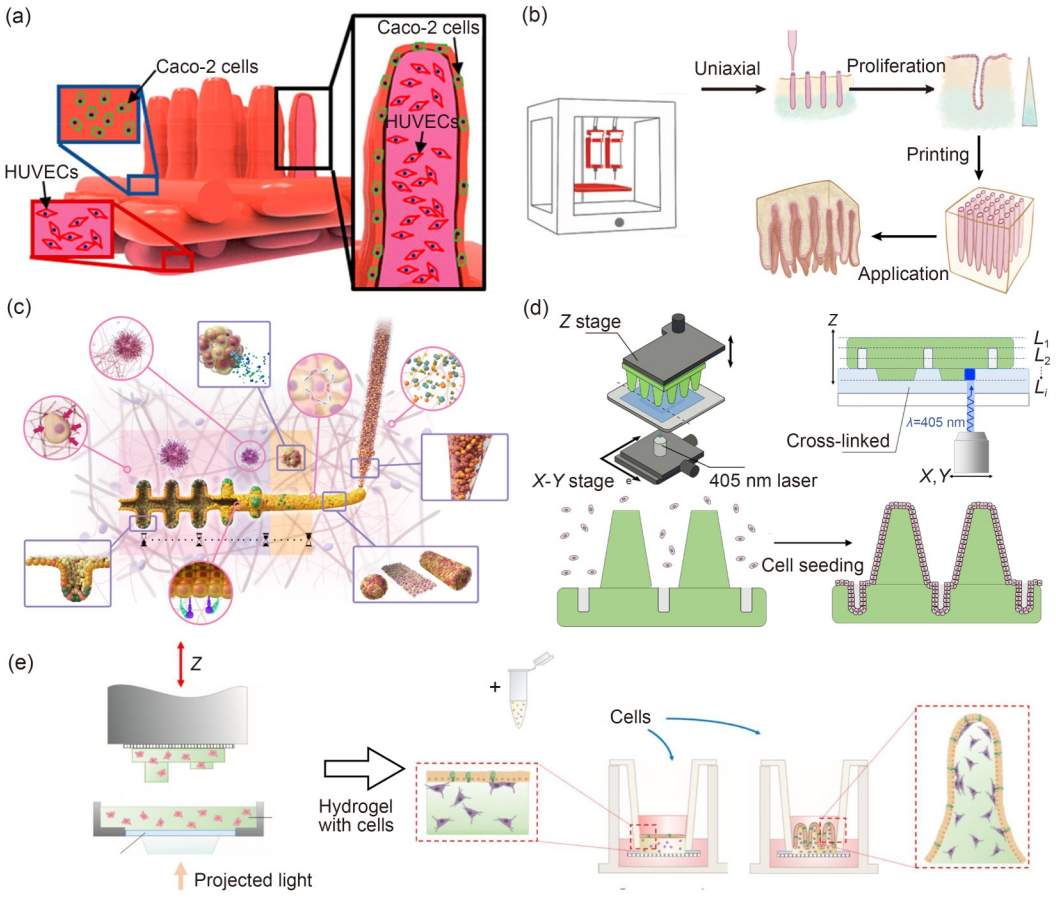


Fig. 5 3D scaffold-based models for precise structure construction and cellular distribution. (a) Villus scaffolds containing epithelial and endothelial cells constructed by coaxial extrusion-based printing technology. Reproduced from [108], Copyright 2018, with permission from the American Chemical Society. (b) Villus structures manufactured by embedded extrusion-based printing technology. Reproduced from [109], Copyright 2021, with permission from Wiley-VCH GmbH. (c) Centimeter-scale tubular intestinal tissue manufactured by extrusion-based printing. By using stem cells, the tissue spontaneously generates crypts and villi closely resembling native structures. Reproduced from [111], Copyright 2020, with permission from the authors, under exclusive licence to Springer Nature Limited. (d) The crypt and villus structures fabricated by stereolithography, providing a 5-μm resolution for 3D printing. Epithelial cells adhere and proliferate on the structure. Reproduced from [116], Copyright 2019, with permission from the authors, licensed under CC BY-NC-ND 4.0. (e) Hydrogels containing fibroblasts constructed into 3D villi-like and stromal structures by digital light processing. Epithelial cells are seeded to form a multicellular coculture system that improves barrier function and transporter activity. Reproduced from [115], Copyright 2023, with permission from the authors, licensed under CC BY-NC-ND

bioprinting strategies that support endothelial–epithelial interactions while replicating both micro- and macro-architecture of intestinal tissues.

Light-based printing, which includes techniques such as stereolithography (SLA) and digital light processing (DLP), utilizes light to crosslink photoactive materials with photopolymerization properties, enabling the layer-by-layer fabrication of complex geometries. The high resolution of light-based printing achieves precision to <100 μm, making it especially suitable for printing intricate microstructures such as villi and crypts [115]. In SLA, a computer-controlled laser beam traces 2D patterns to induce localized, layer-by-layer material curing. This approach has been used to fabricate 3D hydrogel scaffolds with villus–crypt arrays exhibiting a structural organization that is highly analogous to the intestinal epithelium, including growth factor and oxygen gradients [116] (Fig. 5d). In DLP, a digital micromirror device projects 2D pixelated patterns onto a transparent surface, thereby simultaneously curing entire resin layers and accelerating 3D fabrication [117]. Through DLP printing, fibroblasts can be incorporated within the bioink for villus fabrication, followed by epithelial cell seeding to establish uniform and precise spatial arrangements and recapitulate epithelial–stromal interactions [118] (Fig. 5e). These features facilitate biological factor exchange and ECM secretion to better enhance epithelial barrier integrity and permeability compared with 2D static models or organoid systems, which often lack this level of structural control [119].

Conventional tissue engineering techniques, such as freeze-drying, solvent casting, electrospinning, laser ablation, and

decellularization, have also been used to fabricate 3D scaffolds. Freeze-drying, solvent casting, and electrospinning are typically employed to construct highly porous scaffolds with tunable pore sizes; however, they are often limited by mechanical fragility and relatively simple morphological architectures. Currently, tubular 3D scaffolds can be fabricated using these approaches, while hollow lumens or multilayered tubular assemblies can be achieved through mold design to partially recapitulate physiological environments [120, 121]. In contrast, laser ablation and photolithography rely on light exposure and chemical reactions to process substrate materials, such as photoresists, to generate high-precision molds [122–124]. These molds can then be used for pattern transfer in fabricating tissue models using matrix materials, such as collagen and ECM. Furthermore, photopolymerization-based microfabrication techniques can directly crosslink photosensitive materials to fabricate microstructures with high aspect ratios and effectively establish 3D cell culture environments that overcome the inherent limitations of 2D static models [125]. Despite these advances, the fabrication of tissue models still requires complex manual procedures, which create difficulties in maintaining structural precision and generating microenvironment gradients [126, 127]. Decellularization offers a biologically driven approach to scaffold fabrication, in which native intestinal tissues are subjected to chemical, enzymatic, or mechanical processing to remove cellular components while retaining the structural, mechanical, and biochemical integrity of the ECM [128, 129]. Although decellularized intestinal scaffolds inherently provide a highly biomimetic environment, this technique is frequently associated with batch-to-batch variability, incomplete removal of cellular material, and the risk of eliciting immune responses, all of which constrain its broader application.

Although 3D scaffold-based models offer numerous advantages, they are still significantly limited from faithfully replicating the multiscale intestinal structural characteristics, such as the macroscopic tissue organization and the microscopic villus–crypt architecture [130]. Future investigations must focus on fully reconstructing native tissue architecture to achieve a biomimetic spatial distribution of biological cues, including cells, ECM, and cytokines. Another critical challenge is to strike a balance between bioink biocompatibility and structural integrity in 3D bioprinting, particularly for large-scale constructs [131]. Furthermore, the lack of standardized approaches for creating and maintaining physiological oxygen gradients—which influence cellular activities—in these models [132, 133] must be addressed in future research. Integrating microfluidic elements from organ-on-chip systems into 3D structures is a potential strategy for addressing this issue [114].

3D scaffold-based models offer unmatched precision in replicating villus–crypt architecture and spatial cellular organization, which directly impacts absorptive efficiency, microbial niche localization, and epithelial renewal, among other physiological functions [115, 134]. These models also enable the creation of oxygen and nutrient gradients, which support long-term microbial and epithelial coculture [135]. The repeatability and design flexibility of 3D scaffold-based models make them highly adaptable compared with other platforms. Ultimately, overcoming bioink limitations and integrating bioprinting with dynamic systems, such as organ-on-chip fluidics and real-time monitoring, will be vital to achieving physiologically robust and clinically relevant intestinal models.

We summarize the features and challenges of various bioengineering technologies used to construct in vitro intestinal models (Fig. 6), evaluating them based on technical properties and biological functions. Our framework assessed technical properties such as reproducibility, expandability, cost-effectiveness, ease-of-use, and throughput. The biological functions evaluated included environmental construction, structural fidelity, cell diversity, microbial compatibility, and biogeography. Biogeography describes the spatial distribution

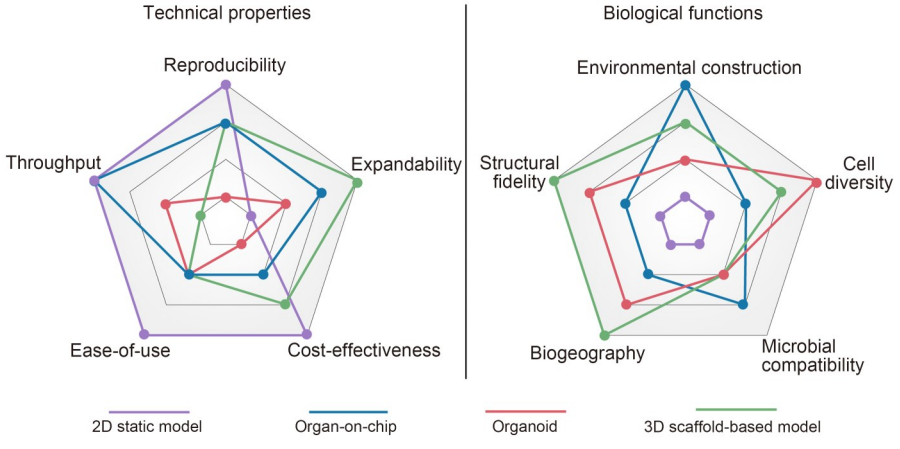


Fig. 6 Comparison of four major in vitro intestinal models based on technical properties and biological functions

patterns of multiple cellular components within in vitro models. 2D static models are of very low technical difficulty and have a low operational threshold, offering the highest cost-effectiveness. However, their simple design limits their biological relevance. Organoid models exhibit high biological complexity and closely replicate physiological functions; however, they exhibit inadequate structural precision and require extensive manual intervention and higher cultivation costs. Organ-on-chip systems replicate the micro-physiological environment using advanced methods and system simulation capabilities and offer good reproducibility. However, their fabrication requires specialized equipment for manufacturing, increasing associated costs. 3D scaffold-based models leverage structural precision to enhance biological functions, providing scalability and customization while demonstrating significant translational potential. Although these models are also constrained by materials and equipment, they offer relatively lower costs than organ-on-chip or organoid models.

Among the currently available options, the U. S. Food and Drug Administration (FDA)-endorsed CACO-2 Transwell model remains the gold standard for evaluating intestinal permeability in drug development [136, 137]. Patient-derived organoids (PDOs) are also gaining traction for drug screening and personalized medicine applications, offering more precise replication of native intestinal tissues [138]. Similarly, organ-on-chip models integrate microfluidic technology to simulate key physiological parameters, such as flow, mechanical stimulation, and oxygen gradients [139]. Although these systems effectively recreate dynamic microenvironments and controlled coculture conditions, they are limited by their complexity and small-scale design [140]. Each bioengineering method has unique strengths and limitations; however, 3D scaffold-based models stand out as the most promising approach, effectively addressing the structural and functional challenges that limit other methods. By enabling the precise spatial arrangement of multiple cell types and replication of the complex intestinal macro- and microarchitectures, 3D scaffold-based models could provide a comprehensive platform for modeling intestinal structure, function, and microbial interactions.

3 Applications of in vitro intestinal models

3.1 Intestinal homeostasis model

The intestine contains one of the most complex ecosystems in the human body, which undergoes dynamic environmental changes. Disruption of its delicate balance can lead to intestinal inflammation, such as inflammatory bowel disease (IBD) [141], which is driven by genetic predisposition, environmental influences, microbiota imbalances, altered intestinal permeability, and immune dysregulation, among other factors [142]. These mechanisms and their impact on intestinal homeostasis are explored through in vitro models, which are designed to replicate key features of the intestinal environment.

The protective mucus layer on epithelial cells serves as the primary intestinal barrier for maintaining homeostasis. It is composed of mucins secreted by the cells, primarily MUC2, MUC5AC, and MUC17. This layer fosters a symbiotic relationship between the intestine and its microbial community [143, 144]. To simulate this barrier in vitro, Transwell systems are widely used to establish monolayer polarized epithelial cells, which exhibit partial barrier functions but lack the mucin specificity characteristic of goblet cells. A coculture system of CACO-2, HT29-MTX, and Raji B cells has been developed to address this limitation. This model exhibits goblet cell characteristics [30], generating mucus layers that are 40–80 μm thick, depending on the cell types and ratios used [145] (Fig. 7a). Intestinal organoids with various epithelial cell types can be designed to secrete mucin within the central cavity. Recent innovations, such as reverse-polarity organoids, enhance mucus layer characterization by reorienting the apical surfaces of epithelial cells in space, thereby enhancing accessibility for mucus characterization [89, 146, 147]. Monolayer organoids are generated by seeding dissociated organoid-derived cells onto a specific substrate to form a 2D epithelial cell layer, which achieves similar outcomes. Compared with static models, organ-on-chip models introduce mechanical deformation and fluid shear, creating dynamic culture environments that promote villus differentiation and mucin secretion by epithelial cells [92] (Fig. 7b). Cytokines secreted by cells can diffuse into the circulating medium and subsequently act on the receptors of neighboring cells to mediate intercellular interactions. Moreover, systems constructed from patient-derived epithelial cells provide disease-specific mucus layer characteristics, enabling advanced studies into intestinal pathologies [148].

Beyond barrier function, the immune system contributes significantly to intestinal homeostasis by mediating interactions between epithelial and immune cells. Organ-on-chips, organoids, and 3D scaffold-based models can all be used to establish coculture environments of immune cells (such as macrophages and T cells derived from human THP-1 cells) and epithelial cells [149]. Such interactions enable epithelial cells to form tight junctions and regulate permeability, which more accurately reflects physiological conditions during cellular development and maturation [74, 150, 151]. Various soluble factors, such as interleukin (IL)-22, IL-2, and IL-6 [75, 76], are introduced to induce the immune response in inflammatory environments, thereby inducing multiple cell types to exhibit collective immune responses [17, 51, 52] (Fig. 7c). In organ-on-chip, cytokines

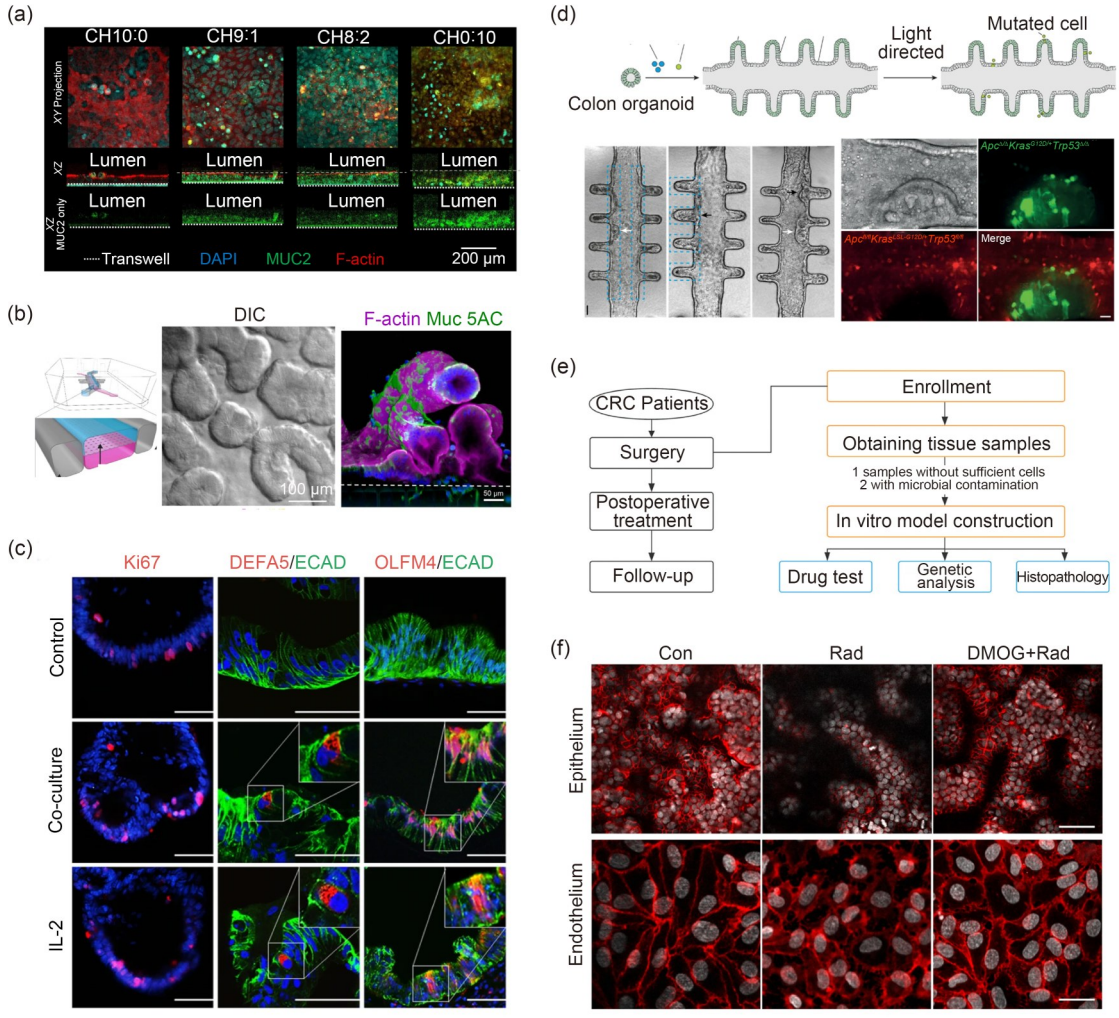


Fig. 7 Characteristics of intestinal homeostasis models. (a) Immunofluorescence images of intestinal coculture models. Reproduced from [145], Copyright 2022, with permission from the authors, under exclusive licence to Biomedical Engineering Society. (b) Morphology and mucus secretion of epithelial cells in the organ-on-chip. Reproduced from [92], Copyright 2018, with permission from the authors, licensed under CC BY 4.0. (c) Immunofluorescence of proliferative, epithelial, and mature intestinal markers in organoid models treated with IL-2. Reproduced from [76], Copyright 2018, with permission from the authors, licensed under CC BY 4.0. (d) Schematic diagram of the experimental workflow for inducing tumorigenesis in the colon. Reproduced from [153], Copyright 2024, with permission from the authors, licensed under CC BY 4.0. (e) Flow diagram demonstrating the construction of tumor models using patient-derived tumor cells through 3D printing technology. Reproduced from [134], Copyright 2023, with permission from the authors, licensed under CC BY 4.0. (f) Morphological changes in intestinal epithelial cells after exposure to radiation, resulting in the loss of cellular continuity. Reproduced from [46], Copyright 2018, with permission from the authors, licensed under CC BY 4.0

secreted by cells can diffuse into the circulating medium and subsequently act on the receptors of neighboring cells, thereby mediating intercellular interactions during the inflammatory response.

Intestinal organoids, particularly those derived from primary biopsy tissues, hold immense potential in personalized medicine. Their principal advantage lies in their ability to create patient-specific models that reflect an individual's genetic background, microbiota composition, and other factors influencing drug metabolism and disease susceptibility. This facilitates the development of highly accurate models for diseases such as cystic fibrosis, IBD, colorectal cancer, and celiac disease [152–154] (Fig. 7d). By recapitulating key processes, such as cell death, host–microbe interactions, and inflammation, PDOs provide significant advantages in elucidating the pathogenesis of diseases. Therefore, organoids can also serve as robust platforms for evaluating therapeutic efficacy, such as drug testing and screening. For example, CRISPR/Cas9-mediated gene editing can be applied to rectify patient-specific genetic defects [155]. However, the advancement of autologous organoid systems is hindered by insufficient recapitulation of the native microenvironment and limited cellular complexity. Integration with organ-on-chip technology and 3D bioprinting offers promising results

for reconstructing biomimetic micro-physiological systems, thereby enhancing the accuracy and reproducibility of existing models [134, 156] (Fig. 7e). In 3D scaffold models, cytokines secreted by cells can diffuse through the pores of the material, interact with other cells, and promote intercellular communication.

Furthermore, intestinal ecosystem imbalance can disrupt intestinal homeostasis. The induction of pathogens, such as bacteria, viruses, and other harmful substances, into in vitro models replicates processes that lead to homeostasis failure. Bacteria, such as *Salmonella typhimurium*, *Clostridium difficile*, *Vibrio cholerae*, and *Helicobacter pylori* [157–160], produce toxins that affect epithelial cells by disrupting tight junctions, reducing mucin secretion, triggering inflammatory responses, and potentially causing the expulsion of infected epithelial cells from organoids. In organ-on-chip systems, additional physical and chemical pathogenic factors, such as γ-radiation, lipopolysaccharide, and interferon-γ (IFN-γ), are administered to simulate cellular damage responses [46, 51] (Fig. 7f). Compared with organoids, organ-on-chip systems allow independent control of culture conditions, including substance gradients, oxygen levels, mechanical forces, and cell types, offering greater flexibility for experimental designs.

Overall, the genetic specificity of organoids makes them suitable for recapitulating pathological features. Advanced additive manufacturing techniques, such as 3D printing, enable more precise control over the distribution of epithelial and immune cells, facilitating the construction of a more accurate immune microenvironment. Therefore, the combination of these two approaches is potentially useful for recapitulating intestinal homeostasis and associated pathological conditions.

3.2 Intestinal host–microbe interaction models

The human intestinal microbiome is crucial to maintaining health and inducing immune regulation, nutrient absorption, and protection against pathogens [161, 162]. Disruptions in host–microbe interactions have been identified as the cause of IBD, metabolic disorders, and neurodegenerative conditions, among other diseases. Understanding these complex interactions is critical in the development of targeted therapies and the advancement of precision medicine [163]. Studying these interactions in vivo is challenging due to ethical constraints, interspecies differences in intestinal physiology, and the difficulty of isolating specific microbial contributions in complex animal models. For instance, although germ-free or gnotobiotic mice have been instrumental in elucidating host–microbe dynamics, they cannot replicate human-specific physiological and microbial characteristics, thereby limiting their translational relevance [164]. In vitro intestinal models can provide controlled environments for dissecting host–microbe interactions with greater relevance to human physiology.

The central challenge in modeling these interactions is in recreating environments that sustain both oxygen-sensitive microbes and aerobic intestinal epithelial cells. 2D static models, such as the Transwell system, fit a gas-impermeable plug into the insert to create an anaerobic internal compartment while the well remains aerobic, thereby establishing a controlled oxygen gradient across the membrane and cell monolayer [31, 165] (Fig. 8a) and achieving a coculture environment for cells and microbes. While simple and reproducible, static models lack fluid flow, mechanical stimuli, and epithelial diversity, leading to metabolite accumulation over time and limited physiological relevance.

Organoids address some of these limitations by replicating the intestinal crypt–villus architecture and epithelial development process. They can simulate cellular responses to different bacteria through invasive microinjection techniques. Symbiotic bacteria, such as *Lactobacillus reuteri* and non-pathogenic *Escherichia coli*, can interact with organoids to enhance barrier function and antimicrobial defense [82, 83, 166]. In contrast, pathogenic bacteria, such as *Clostridium difficile* and *Helicobacter pylori*, typically induce epithelial damage, such as the disruption of tight junctions and loss of barrier integrity [85, 167, 168] (Fig. 8b). Despite these advantages, organoids are unsuitable for long-term microbial coculture due to limited nutrient diffusion and waste accumulation. The closed luminal environment also complicates real-time observation and microbial manipulation, restricting their scalability for high-throughput studies.

Organ-on-chip platforms introduce dynamic conditions to better mimic physiological microenvironments [169]. These systems typically feature dual fluidic channels separated by a porous membrane, with the upper channel simulating the intestinal lumen and the lower channel mimicking the vasculature for oxygen and nutrient transport [40, 170, 171] (Fig. 8c). Mechanical cues, such as cyclic strain to mimic peristalsis, enhance bacterial invasiveness, as demonstrated in *Shigella* infection studies [44]. Moreover, specialized devices, such as the HuMix and IOAC models, establish controlled oxygen gradients to support the long-term coculture of anaerobic bacteria [53]. However, their reliance on 2D cell layers limits the replication of complex tissue architecture and spatial microbial distributions found in vivo.

3D scaffold-based models offer a promising solution by enabling the construction of macro-scale tissues with intricate architectures [110]. Techniques such as sacrificial printing create hollow channels that support oxygen diffusion, while semi-tubular scaffolds enable the modulation of luminal oxygen by orientation relative to the air interface [113]. Distinct oxygen concentrations can be applied inside the

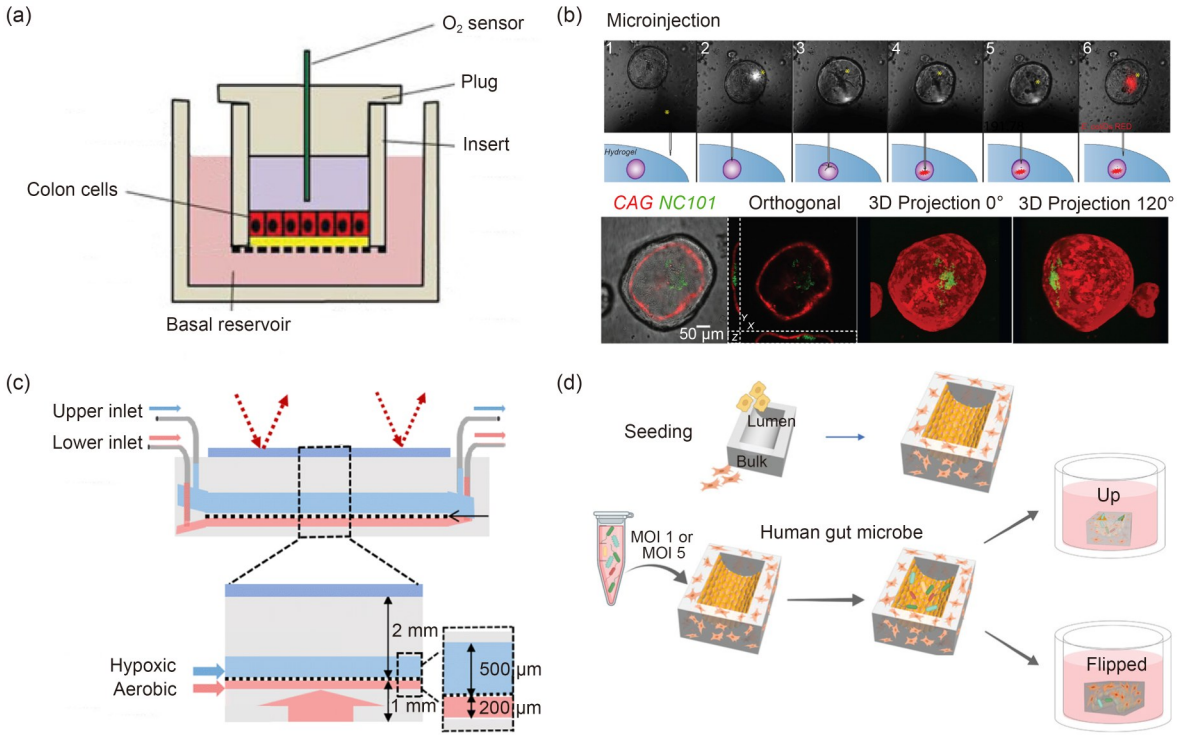


Fig. 8 Characteristics of intestinal host–microbe interaction models. (a) Schematic diagram of the oxygen gradient culture system and the coculture of cells and microbes. Reproduced from [165], Copyright 2022, with permission from the authors, licensed under CC BY 4.0. (b) Microinjection of microbes into organoids to establish a coculture microenvironment. Reproduced from [168], Copyright 2018, with permission from the authors, licensed under CC-BY-NC-ND. (c) Schematic diagram of the organ-on-chip design illustrating a controlled oxygen gradient, where epithelial cells are exposed to microbes and inflammatory factors. Reproduced from [170], Copyright 2023, with permission from The Royal Society of Chemistry. (d) Epithelial cells and intestinal microbes isolated from feces are cocultured in the half-scaffold, a system that sustains an oxygen gradient culture through tissue engineering technology. Reproduced from [172], Copyright 2022, with permission from Wiley-VCH GmbH

channels and to the surrounding tissue, thereby establishing an oxygen gradient. In contrast, semi-tubular structures generated through casting techniques can be regulated by orienting the lumen toward or away from the air interface to control the luminal oxygen level between normoxic and hypoxic conditions [172] (Fig. 8d). These approaches facilitate studies of microbial spatial organization, a critical determinant of in vivo host–microbe interactions. However, standard methods fail to maintain stable oxygen gradients, immune integration, and microbial diversity in extended cultures. Thus, combining bioprinted scaffolds with integrated flow networks helps achieve controlled oxygen microenvironments and precise host–microbe positioning, thereby fostering complexity that more closely mimics that of the native intestine ecosystem.

In conclusion, organ-on-chip systems can be used to create oxygen gradients due to their standardized and modular design, thereby facilitating the feasibility of host–microbe cocultures. Furthermore, the ability for real-time monitoring increases the reliability of assessing these interactions. Therefore, organ-on-chip platforms are especially suited for the fabrication of intestinal host–microbe interaction models.

3.3 Intestinal absorption models

The intestine is responsible for absorbing nutrients, water, and orally administered drugs [173]. However, animal intestinal models often fail to predict human-specific outcomes due to species-specific differences in epithelial transporter expression, metabolic enzyme activity, and structural complexity [174, 175]. Therefore, in vitro modeling systems must capture the mechanisms of paracellular and transcellular transport, carrier-mediated active transport, and endocytosis. These models are essential for advancing drug development, nutrition analysis, and toxicological assessments [176].

A Transwell model using immortalized epithelial cells is the gold standard for evaluating intestinal permeability and drug metabolism [177]. Its simplicity and high reproducibility facilitate the reliable measurement of permeability for compounds such as nanoparticle-mediated peptides [30] and trace elements [178] (Fig. 9a). Moreover, these absorption models enhance toxicological testing by simulating toxin release during digestion, epithelial absorption, and first-pass metabolism [179, 180]. However, the Transwell model

cannot duplicate the complex multicellular interactions, spatial organization, and mechanical stimulation that occur in vivo, limiting its predictive accuracy for absorption dynamics.

Organoids that better mimic the intestinal cellular heterogeneity have been developed, overcoming these limitations. They express transport proteins and metabolic enzymes, including key markers, such as ATP-binding cassette subfamily B member 1 and cytochrome P450 3A4 (CYP3A4), to closely simulate native tissue function [181]. Monolayer organoids derived from human PSCs exhibit enhanced absorption and metabolism compared with traditional 2D static models, offering improved pharmacokinetic predictions [182] (Fig. 9b). However, organoids lack integrated fluid flow, limiting their utility in absorption studies for evaluating the influence of shear stress on enzyme activity. Despite these shortcomings, organoids provide a valuable intermediate between static models and more physiologically dynamic systems.

Organ-on-chip platforms incorporate dynamic flow and mechanical cues that critically regulate transport protein expression and metabolic activity [183]. Dual-channel duodenum chips, which simulate the intestinal lumen and vascular interface, show enhanced CYP3A4 expression and barrier integrity under flow conditions [50] (Fig. 9c). Furthermore, NutriChip systems have been developed to simulate postprandial conditions. Thus, they can provide insights into nutrient absorption and inflammatory responses, as well as a platform for functional screening and analysis of common foods [184]. However, the planar geometry of these systems limits the replication of 3D villus structures, which would increase the absorptive surface area and substance gradient. Furthermore, compared with Transwell systems, the complexity of microfluidic setups hinders high-throughput screening, limiting their use in large-scale drug development.

Organ-on-chip systems hold significant potential for drug pharmacokinetic screening, given their ability to recapitulate the human intestinal microenvironment. Drugs are introduced into the perfusion medium to evaluate how these systems mimic the natural intestinal absorption process and characterize permeability-based absorption efficiency, facilitating the early-stage evaluation of drug candidates [185]. Moreover, the real-time monitoring capacity and modular integration of organ-on-chip platforms confer distinct advantages for investigating drug transport processes and comprehensively assessing absorption, distribution, metabolism, and excretion. Nevertheless, most organ-on-chip devices are currently fabricated manually in research laboratories using PDMS-based soft lithography. This method offers low standardization and limited throughput, preventing large-scale commercialization. Combining organ-on-chip systems with multiple bioengineering technologies could address these limitations. For example, integrating 3D printing into organ-on-chip fabrication can automate the production

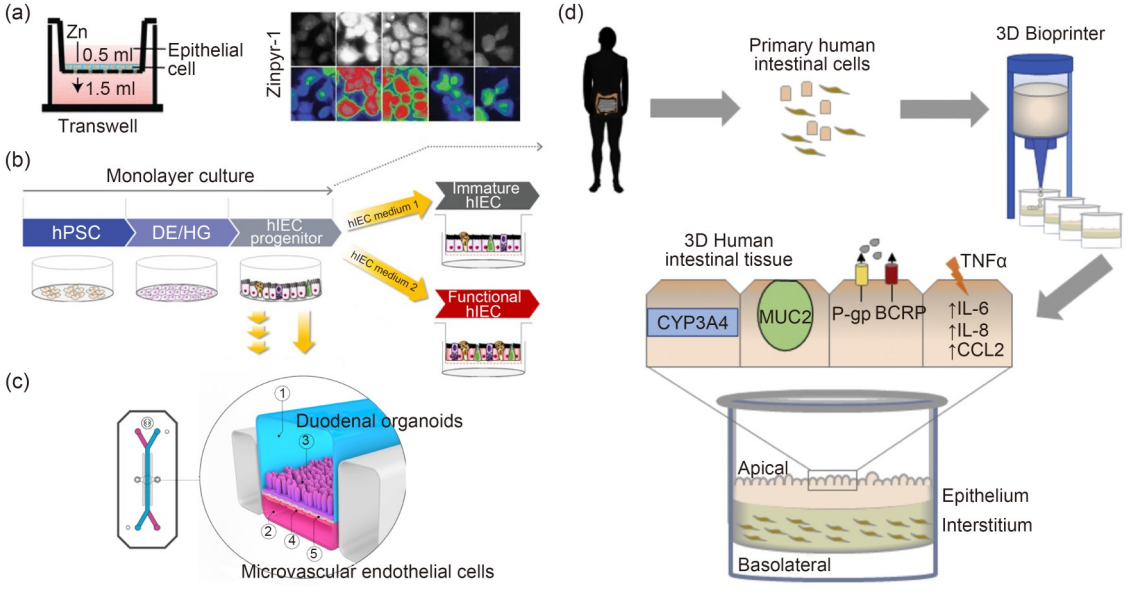


Fig. 9 Characteristics of intestinal absorption models. (a) Intestinal cells are seeded in a Transwell for Zn absorption. Zn ions in intestinal cells are stained with Zinpyr-1. Reproduced from [178], Copyright 2017, with permission from the authors, licensed under CC BY 4.0. (b) Human pluripotent stem cells differentiating into intestinal epithelial progenitor cells and forming functional monolayer intestinal epithelial organoids. Reproduced from [182], Copyright 2021, with permission from the authors, licensed under CC BY 4.0, with AAAS as the exclusive licensee. (c) Schematic diagram of duodenal organoids seeded on the organ-on-chip. Reproduced from [50], Copyright 2020, with permission from the authors, licensed under CC BY 4.0. (d) Multiple cell types constructed into 3D intestinal tissue through printing technology enable the evaluation of the absorption function. Reproduced from [107], Copyright 2018, with permission from Organovo Inc., licensed under CC-BY-NC-ND

of complex devices through predesigned modeling and high-fidelity printing [186]. In particular, two-photon printing has attracted increasing attention in precision manufacturing, owing to its higher resolution than that of DLP and SLA technologies. Furthermore, enhancing the standardization of user interfaces would facilitate multicomponent control within organ-on-chip platforms, including culture, detection, and feedback systems, thereby enhancing broad compatibility for drug testing.

Advancements in 3D bioprinting have facilitated the construction of multicellular intestinal models that closely replicate native intestinal architecture and function. Light- and extrusion-based printing techniques enable these models to incorporate epithelial cells, fibroblasts, and endothelial cells into spatially organized constructs that support the expression of CYP450 enzymes and functional transport proteins [107, 115] (Fig. 9d). However, the lack of integrated fluid dynamics in standalone bioprinted models requires the integration of hybrid systems that combine bioprinting with organ-on-chip fluidics. Bioink limitations further challenge the balance between structural integrity and biocompatibility, particularly for materials requiring epithelial–stromal interactions.

In summary, organ-on-chip platforms enhance transporter function and barrier integrity by providing dynamic flow and mechanical stimuli but are limited by their planar nature in the replication of the 3D intestinal villus–crypt structure. In contrast, 3D scaffold-based models can create more precise constructions of biomimetic structural features that simulate the epithelial cell niches and substance gradients observed in vivo. Combining both approaches enhances the accuracy of drug bioavailability predictions and the effectiveness of personalized nutritional strategies.

3.4 Intestinal multiorgan axis models

The intestine communicates with various organs through systemic circulation, creating an "intestine–*x* axis" that is essential for maintaining whole-body homeostasis [187]. Microbial metabolites and neurotransmitters produced in the intestine, such as serotonin, dopamine, and γ-aminobutyric acid, can influence the functions of distant organs [4], including the brain, liver, kidneys, lungs, and skin. Moreover, short-chain fatty acids (SCFAs) and other bioactive compounds not only exert anti-inflammatory effects but also sustain the integrity of the blood–brain and other barriers [188, 189]. Given the limitations of animal models in recapitulating human-specific organ interactions, in vitro multiorgan models are vital tools for studying these complex networks [190]. Currently, multiorgan models are constructed by combining 3D scaffold-based models and organ-on-chip platforms. The 3D scaffold enables inter-organ communication by precisely controlling the spatial distribution of multiple organ-specific cells, while the organ-on-chip replicates blood circulation through microfluidic channels that interconnect distinct organ compartments.

The significant research interest in the intestine–brain axis has facilitated the development of modular microfluidic chips for coculturing intestinal and brain endothelial cells to reproduce the intestinal and blood–brain barriers [191]. Furthermore, a 3D tubular scaffold composed of conductive polymers has been developed for the continuous monitoring of cell activity across these systems [192] (Fig. 10a). While these models capture key aspects of intestinal neural regulation, replicating the dynamic neuronal responses and precise neurotransmitter gradients observed in vivo remains challenging.

Intestine–liver axis models are designed to mimic the portal circulation that carries nutrients, microbial metabolites, and toxins from the intestine to the liver [11, 193]. Therefore, the establishment of in vitro models for the intestine–liver axis is crucial for understanding the progression of liver diseases and the hepatic metabolism of compounds. An intestine–liver chip has been developed to investigate hepatic drug pharmacokinetics. Diclofenac and hydrocortisone were administered under various experimental conditions to evaluate intestine–liver communication during hepatic metabolism [194] (Fig. 10b). Moreover, incorporating immune cells into a multiorgan-on-chip model can simulate acute T cell-mediated intestine–liver inflammation, demonstrating that SCFAs from intestine microbes can enhance the production of inflammatory cytokines [195].

Intestine–kidney axis models investigate the role of the intestine in metabolite excretion [196] and the impact of dysbiosis on renal function [197]. In vitro chips have been developed to evaluate kidney damage induced by the secretion of toxins, such as Shiga toxin, in the intestine and to explore therapeutic responses to antibiotics [198] (Fig. 10c). Although these models effectively simulate key aspects of intestine–kidney crosstalk, they require further refinement to accurately replicate the long-term effects of bacterial translocation and systemic inflammation.

Recent studies have also examined the influence of the intestinal microbiota on lung health, linking conditions like asthma and cystic fibrosis to intestinal dysbiosis [199, 200]. Intestine–lung models have been developed, some employing intestine-on-chip platforms infected with pathogens like SARS-CoV-2, to recreate intestinal barrier disruption, villi damage, and reduced tight junction integrity [201] (Fig. 10d). These systems provide valuable insights into the contributions of intestine-derived signals to respiratory diseases, although they are limited in faithfully mimicking the complexity of air–liquid interfaces and dynamic lung mechanics.

The intestine–skin axis is another emerging area of study, as allergens and fatty acids absorbed in the intestine can

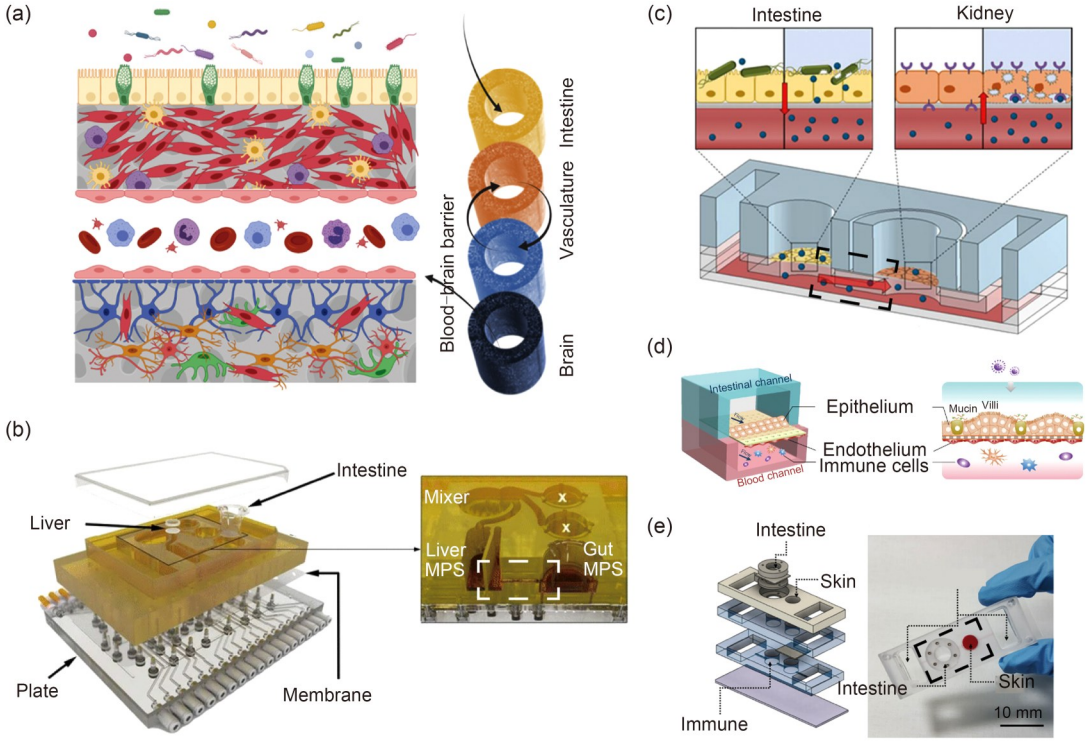


Fig. 10 Characteristics of intestinal multiorgan axis models. (a) Three-dimensional tubular scaffolds used to support cell growth or integrate monitoring and sensing functions for the intestine–brain axis. Reproduced from [192], Copyright 2021, with permission from the authors, licensed under CC BY 4.0. (b) Schematic diagram of the micro-physiological system integrating the intestine and liver. The culture medium is circulated in the intestinal system, liver system, and mixing compartment. Reproduced from [194], Copyright 2017, with permission from the authors, licensed under CC BY 4.0. (c) Schematic diagram of intestine–kidney interaction and coculture in organ-on-chip. Reproduced from [198], Copyright 2021, with permission from the authors, licensed under CC BY 4.0. (d) Organ-on-chip for SARS-CoV-2 infection including epithelial, immune, and endothelial cells. Reproduced from [201], Copyright 2020, with permission from Science China Press. (e) A dual-organ chip for evaluating the intestine and skin, which includes two cell culture chambers and a culture medium reservoir. Reproduced from [203], Copyright 2022, with permission from Wiley Periodicals LLC.

trigger dermatological reactions [202]. Intestine–skin chips have shown that compromised intestinal barriers exacerbate skin inflammation [203] (Fig. 10e). While these models offer a promising avenue for studying allergic sensitization and skin responses, the interplay between intestine-derived signals and skin pathology requires further mechanistic exploration.

A single multiorgan chip can simultaneously incorporate more than two organs, such as systems that link the intestine with the liver, kidney, brain, skeletal muscle, and skin [204, 205]. Such a platform enables simultaneous investigations of systemic drug absorption, metabolism, and toxicity that can be correlated with clinical outcomes to support comprehensive toxicity testing that can be validated in vivo [206–208]. However, integrated systems require a robust fluidic circulation network to transport cytokines and metabolites between organ modules, a task that organoids and 2D models are unable to accomplish.

In conclusion, the channel design and standardized interfaces of organ-on-chip systems make them suitable for platforms connecting different organ modules and facilitating interactions between the intestine and other organs. Thus, they are a key technology for constructing intestinal multiorgan axis models.

We summarize the applications of in vitro models and highlight the specific requirements of each (Fig. 11), as well as the advantages and limitations of the bioengineering techniques employed for different biomedical applications (Table 1). The distinct advantages of various bioengineering techniques, ranging from structural fidelity and microenvironmental characteristics to material assembly, determine their application in specific biological contexts. Enhancing the alignment between manufacturing methods and research requirements will facilitate the development of more targeted and functionally relevant in vitro models. Homeostasis models require high cellular diversity to accurately represent the complexity of native conditions. Organoids, constructed through stem cell differentiation and induction, exhibit complex cellular compositions and integrate human-relevant immune functions [153]. Therefore, organoids highly accurately recapitulate the pathogenesis of intestinal diseases, especially genetic and infectious diseases. 3D

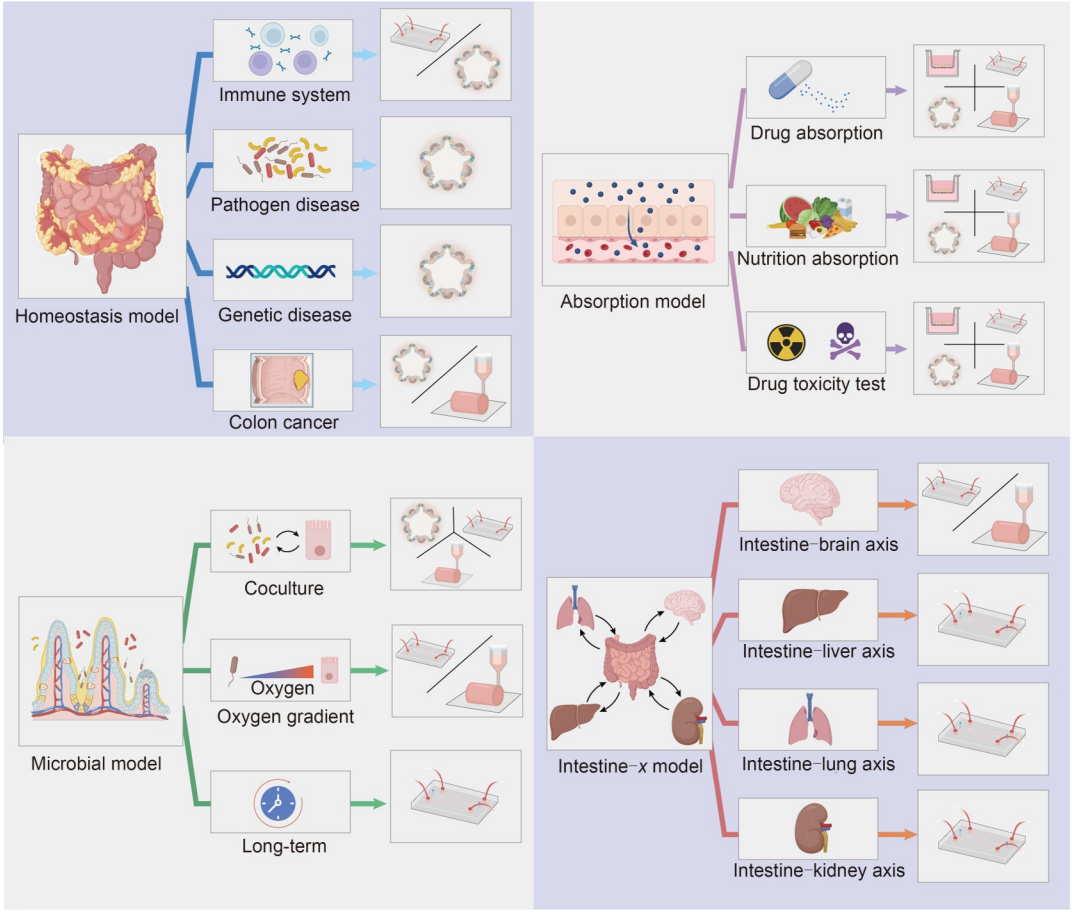


Fig. 11 Biomedical applications of in vitro intestinal models, highlighting specific application scenarios and the corresponding bioengineering technologies suitable for each

Table 1 Advantages and limitations of bioengineering techniques employed for various biomedical applications

Biomedical application	Bioengineering technology	Advantage	Limitation
Intestinal homeostasis model	Organoid	Multiple cell types; enhanced physiological fidelity	Lacks dynamic environment; insufficient reproducibility
	Organ-on-chip	Multiple cell types; dynamic microenvironment	Lacks biomimetic structures; inefficient cellular interactions
	3D scaffold-based model	Multiple cell types; controlled cellular organization; biomimetic 3D structures	High technical complexity; material constraints
Intestinal absorption model	2D static model	Reproducibility; operational simplicity	Lacks dynamic environment
	Organoid	Enhanced physiological fidelity	Lacks dynamic environment; insufficient reproducibility
	Organ-on-chip	Dynamic microenvironment	Lacks biomimetic structure
	3D scaffold-based model	Vascularized tissue; substance gradient environment	High technical complexity
Intestinal host–microbe interaction model	Organoid	Enhanced physiological fidelity; villus–crypt structure	Limitations in long-term culture
	Organ-on-chip	Dynamic microenvironment; real-time monitoring	Lacks biomimetic structures; operational complexity
	3D scaffold-based model	Substance gradient environment	High technical complexity; lacks standardized culture systems
Intestinal multiorgan axis model	Organ-on-chip	Modularized design and construction	Lacks biomimetic structures
	3D scaffold-based model	Controlled cellular organization	Lacks standardized dynamic culture systems

scaffold-based models enable system customization using patient-derived cells for personalized medicine applications. They are increasingly being employed in constructing colon cancer models. The development of absorption models requires strict maintenance of the integrity of epithelial cell tight junctions. 2D static models are the gold standard for drug absorption studies because of their reproducibility and well-established epithelial barrier function. Organ-on-chip models, organoids, and 3D scaffold-based models can integrate diverse cell types and complex architectures to form confluent epithelial layers, enabling more physiologically relevant simulations of absorption processes [209]. A key prerequisite for developing intestinal host–microbe interaction models is the successful coculture of microbes and host cells. Organ-on-chips support long-term coculture by creating dynamic microenvironments that physically separate microbial and host compartments using semi-permeable membranes [193]. 3D scaffold-based models can simulate physiologically relevant oxygen gradients using precise macrostructural designs to create essential conditions for coculturing microbes and host tissues. The organ-on-chip exhibits strong expandability, integrating multiorgan systems, which promotes its widespread application in modeling intestine–*x* axis interactions [210].

4 Conclusions and future perspectives

This review emphasizes how bioengineering strategies enable the development of in vitro intestinal models with structural and functional fidelity. By mapping structural precision to physiological outcomes, we propose an engineering-driven framework that connects manufacturing strategies with application-specific requirements, from absorption studies to host–microbe interactions. This framework provides a foundation for model selection or design for targeted biological performance. Future directions for advancing intestinal model systems include the following:

(1) Hybrid and modular platforms. Integrate 3D bioprinting with organ-on-chip technologies to combine structural complexity with dynamic microenvironments, enhancing feasibility for long-term studies and drug screening [211].

(2) Standardization and expandability. Develop automated and scalable fabrication methods alongside interoperable control systems to ensure reproducibility, regulatory compliance, and adaptability to high-throughput settings [212].

(3) Personalized and immune-competent models. Incorporate PDOs, immune components, and cocultured microbiomes to create individualized and pathologically accurate platforms for precision medicine.

(4) Data-driven optimization. Couple real-time biosensing with AI-driven analytics to enable predictive modeling, continuous feedback control, and accelerated clinical translation.

The convergence of bioengineering, material science, and computational technologies, reinforced by supportive regulations, such as the FDA Modernization Act 3.0, signals a transition toward clinically relevant, ethically responsible, and highly adaptable intestinal models [213, 214]. These platforms promise to reshape preclinical testing and precision healthcare by bridging structural fidelity with functional and microbial realism.

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Author contributions ZQG, HLD, and HZZ wrote most of the manuscript and conducted the literature review. YW and LQS assisted in writing and literature search. HYY and HZZ provided guidance and established the study framework. SYY and QL reviewed and edited the manuscript. YL and JY contributed to discussions and provided resources. HZZ and LQS conceived and supervised the study, and finalized the manuscript.

Declarations

Conflict of interest HYY is an editor-in-chief, JY is an associate editor, and HZZ is a young academic editor for *Bio-Design and Manufacturing*; they were not involved in the editorial review or the decision to publish this article. The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. The Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. Nature 486(7402):207–214. https://doi.org/10.1038/nature11234
2. Donaldson GP, Lee SM, Mazmanian SK (2016) Gut biogeography of the bacterial microbiota. Nat Rev Microbiol 14(1):20–32. https://doi.org/10.1038/nrmicro3552
3. McCallum G, Tropini C (2024) The gut microbiota and its biogeography. Nat Rev Microbiol 22:105–118. https://doi.org/10.1038/s41579-023-00969-0
4. Agirman G, Yu KB, Hsiao EY (2021) Signaling inflammation across the gut-brain axis. Science 374(6571):1087–1092. https://doi.org/10.1126/science.abi6087
5. Hsu CL, Schnabl B (2023) The gut–liver axis and gut microbiota in health and liver disease. Nat Rev Microbiol 21(11):719–733. https://doi.org/10.1038/s41579-023-00904-3
6. Mertowska P, Mertowski S, Wojnicka J et al (2021) A link between chronic kidney disease and gut microbiota in immunological

and nutritional aspects. Nutrients 13(10):3637. https://doi.org/10.3390/nu13103637
7. Chevalier NR (2022) Physical organogenesis of the gut. Development 149(16):dev200765. https://doi.org/10.1242/dev.200765
8. Harnik Y, Yakubovsky O, Hoefflin R et al (2024) A spatial expression atlas of the adult human proximal small intestine. Nature 632(8027):1101–1109. https://doi.org/10.1038/s41586-024-07793-3
9. Stojanović O, Miguel-Aliaga I, Trajkovski M (2022) Intestinal plasticity and metabolism as regulators of organismal energy homeostasis. Nat Metab 4(11):1444–1458. https://doi.org/10.1038/s42255-022-00679-6
10. Gustafsson JK, Johansson MEV (2022) The role of goblet cells and mucus in intestinal homeostasis. Nat Rev Gastroenterol Hepatol 19(12):785–803. https://doi.org/10.1038/s41575-022-00675-x
11. Beumer J, Clevers H (2021) Cell fate specification and differentiation in the adult mammalian intestine. Nat Rev Mol Cell Biol 22(1):39–53. https://doi.org/10.1038/s41580-020-0278-0
12. Paone P, Cani PD (2020) Mucus barrier, mucins and gut microbiota: the expected slimy partners? Gut 69(12):2232–2243. https://doi.org/10.1136/gutjnl-2020-322260
13. Williams CF, Walton GE, Jiang L et al (2015) Comparative analysis of intestinal tract models. Annu Rev Food Sci Technol 6:329–350. https://doi.org/10.1146/annurev-food-022814-015429
14. Jiminez JA, Uwiera TC, Inglis GD et al (2015) Animal models to study acute and chronic intestinal inflammation in mammals. Gut Pathog 7:29. https://doi.org/10.1186/s13099-015-0076-y
15. Becquemont L (2009) Pharmacogenomics of adverse drug reactions: practical applications and perspectives. Pharmacogenomics 10(6):961–969. https://doi.org/10.2217/pgs.09.37
16. Suntharalingam G, Perry MR, Ward S et al (2006) Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. N Engl J Med 355(10):1018–1028. https://doi.org/10.1056/nejmoa063842
17. Leal F, Zeiringer S, Jeitler R et al (2024) A comprehensive overview of advanced dynamic *in vitro* intestinal and hepatic cell culture models. Tissue Barriers 12(1):2163820. https://doi.org/10.1080/21688370.2022.2163820
18. Puschhof J, Pleguezuelos-Manzano C, Clevers H (2021) Organoids and organs-on-chips: insights into human gut-microbe interactions. Cell Host Microbe 29(6):867–878. https://doi.org/10.1016/j.chom.2021.04.002
19. De S, Singh N (2022) Advancements in three dimensional *in-vitro* cell culture models. Chem Rec 22(9):e202200058. https://doi.org/10.1002/tcr.202200058
20. Zushin PH, Mukherjee S, Wu JC (2023) FDA Modernization Act 2.0: transitioning beyond animal models with human cells, organoids, and AI/ML-based approaches. J Clin Investig 133(21): e175824. https://doi.org/10.1172/jci175824
21. Artursson P, Karlsson J (1991) Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. Biochem Biophys Res Commun 175(3):880–885. https://doi.org/10.1016/0006-291X(91)91647-U
22. Pan FG, Han L, Zhang Y et al (2015) Optimization of Caco-2 and HT29 co-culture *in vitro* cell models for permeability studies. Int J Food Sci Nutr 66(6):680–685. https://doi.org/10.3109/09637486.2015.1077792
23. Almeqdadi M, Mana MD, Roper J et al (2019) Gut organoids: mini-tissues in culture to study intestinal physiology and disease. Am J Physiol Cell Physiol 317(3):C405–C419. https://doi.org/10.1152/ajpcell.00300.2017
24. Ashammakhi N, Nasiri R, de Barros NR et al (2020) Gut-on-a-chip: current progress and future opportunities. Biomaterials 255:120196. https://doi.org/10.1016/j.biomaterials.2020.120196
25. Biagini F, Daddi C, Calvigioni M et al (2023) Designs and methodologies to recreate in vitro human gut microbiota models. Bio-Des Manuf 6(3):298–318. https://doi.org/10.1007/s42242-022-00210-6
26. Agarwal T, Onesto V, Lamboni L et al (2021) Engineering biomimetic intestinal topological features in 3D tissue models: retrospects and prospects. Bio-Des Manuf 4(3):568–595. https://doi.org/10.1007/s42242-020-00120-5
27. Welcome MO (2018) Structural and functional organization of the gastrointestinal tract. In: Gastrointestinal Physiology: Development, Principles and Mechanisms of Regulation (1st Ed.). Springer Cham, p.53–106. https://doi.org/10.1007/978-3-319-91056-7_2
28. Artursson P, Ungell AL, Löfroth JE (1993) Selective paracellular permeability in two models of intestinal absorption: cultured monolayers of human intestinal epithelial cells and rat intestinal segments. Pharm Res 10(8):1123–1129. https://doi.org/10.1023/A:1018903931777
29. Shim KY, Lee D, Han J et al (2017) Microfluidic gut-on-a-chip with three-dimensional villi structure. Biomed Microdevices 19(2):37. https://doi.org/10.1007/s10544-017-0179-y
30. Antunes F, Andrade F, Araújo F et al (2013) Establishment of a triple co-culture *in vitro* cell models to study intestinal absorption of peptide drugs. Eur J Pharm Biopharm 83(3):427–435. https://doi.org/10.1016/j.ejpb.2012.10.003
31. Sasaki N, Miyamoto K, Maslowski KM et al (2020) Development of a scalable coculture system for gut anaerobes and human colon epithelium. Gastroenterology 159(1):388–390.e5. https://doi.org/10.1053/j.gastro.2020.03.021
32. Bhatia SN, Ingber DE (2014) Microfluidic organs-on-chips. Nat Biotechnol 32(8):760–772. https://doi.org/10.1038/nbt.2989
33. Sibilio S, Mennella R, De Gregorio V et al (2024) A novel membrane-on-chip guides morphogenesis for the reconstruction of the intestinal crypt-villus axis. Biofabrication 16(4):045019. https://doi.org/10.1088/1758-5090/ad6599
34. Zhao YM, Wang EY, Lai FBL et al (2023) Organs-on-a-chip: a union of tissue engineering and microfabrication. Trends Biotechnol 41(3):410–424. https://doi.org/10.1016/j.tibtech.2022.12.018
35. Cao UMN, Zhang YL, Chen JL et al (2023) Microfluidic organ-on-a-chip: a guide to biomaterial choice and fabrication. Int J Mol Sci 24(4):3232. https://doi.org/10.3390/ijms24043232
36. Ren YQ, Yuan CY, Liang QM et al (2025) 3D bioprinting for engineering organoids and organ-on-a-chip: developments and applications. Med Res Rev 45(6):1630–1650. https://doi.org/10.1002/med.22121
37. Wang L, Wu J, Chen J et al (2021) Advances in reconstructing intestinal functionalities *in vitro*: from two/three dimensional-cell culture platforms to human intestine-on-a-chip. Talanta 226: 122097. https://doi.org/10.1016/j.talanta.2021.122097
38. Butler M, Ng CY, van Heel D et al (2006) Modulation of dendritic cell phenotype and function in an *in vitro* model of the intestinal epithelium. Eur J Immunol 36(4):864–874.

https://doi.org/10.1002/eji.200535497
39. Parlesak A, Haller D, Brinz S et al (2004) Modulation of cytokine release by differentiated CACO-2 cells in a compartmentalized coculture model with mononuclear leucocytes and non-pathogenic bacteria. Scand J Immunol 60(5):477–485. https://doi.org/10.1111/j.0300-9475.2004.01495.x
40. Kim HJ, Huh D, Hamilton G et al (2012) Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab Chip 12(12):2165–2174. https://doi.org/10.1039/c2lc40074j
41. Shin W, Hinojosa CD, Ingber DE et al (2019) Human intestinal morphogenesis controlled by transepithelial morphogen gradient and flow-dependent physical cues in a microengineered gut-on-a-chip. iScience 15:391–406. https://doi.org/10.1016/j.isci.2019.04.037
42. Bein A, Shin W, Jalili-Firoozinezhad S et al (2018) Microfluidic organ-on-a-chip models of human intestine. Cell Mol Gastroenterol Hepatol 5(4):659–668. https://doi.org/10.1016/j.jcmgh.2017.12.010
43. Kulthong K, Duivenvoorde L, Sun HY et al (2020) Microfluidic chip for culturing intestinal epithelial cell layers: characterization and comparison of drug transport between dynamic and static models. Toxicol Vitro 65:104815. https://doi.org/10.1016/j.tiv.2020.104815
44. Grassart A, Malardé V, Gobaa S et al (2019) Bioengineered human organ-on-chip reveals intestinal microenvironment and mechanical forces impacting *Shigella* infection. Cell Host Microbe 26(3):435–444.e4. https://doi.org/10.1016/j.chom.2019.08.007
45. Kim HJ, Li H, Collins JJ et al (2016) Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. Proc Natl Acad Sci USA 113(1):E7–E15. https://doi.org/10.1073/pnas.1522193112
46. Jalili-Firoozinezhad S, Prantil-Baun R, Jiang A et al (2018) Modeling radiation injury-induced cell death and countermeasure drug responses in a human gut-on-a-chip. Cell Death Dis 9:223. https://doi.org/10.1038/s41419-018-0304-8
47. Maaser C, Schoeppner S, Kucharzik T et al (2002) Colonic epithelial cells induce endothelial cell expression of ICAM-1 and VCAM-1 by a NF-κB-dependent mechanism. Clin Exp Immunol 124(2):208–213. https://doi.org/10.1046/j.1365-2249.2001.01541.x
48. Grootaert C, Kamiloglu S, Capanoglu E et al (2015) Cell systems to investigate the impact of polyphenols on cardiovascular health. Nutrients 7(11):9229–9255. https://doi.org/10.3390/nu7115462
49. de Hoyos-Vega JM, Yu X, Gonzalez-Suarez AM et al (2023) Modeling gut neuro-epithelial connections in a novel microfluidic device. Microsyst Nanoeng 9:144. https://doi.org/10.1038/s41378-023-00615-y
50. Kasendra M, Luc R, Yin JY et al (2020) Duodenum Intestine-Chip for preclinical drug assessment in a human relevant model. eLife 9:e50135. https://doi.org/10.7554/elife.50135
51. Beaurivage C, Kanapeckaite A, Loomans C et al (2020) Development of a human primary gut-on-a-chip to model inflammatory processes. Sci Rep 10:21475. https://doi.org/10.1038/s41598-020-78359-2
52. Maurer M, Gresnigt MS, Last A et al (2019) A three-dimensional immunocompetent intestine-on-chip model as *in vitro* platform for functional and microbial interaction studies. Biomaterials 220:119396. https://doi.org/10.1016/j.biomaterials.2019.119396
53. Shah P, Fritz JV, Glaab E et al (2016) A microfluidics-based *in vitro* model of the gastrointestinal human–microbe interface. Nat Commun 7:11535. https://doi.org/10.1038/ncomms11535
54. Brasino DSK, Speese SD, Schilling K et al (2024) A linkable, polycarbonate gut microbiome-distal tumor chip platform for interrogating cancer promoting mechanisms. Adv Sci 11(35): 2309220. https://doi.org/10.1002/advs.202309220
55. Lee J, Menon N, Lim CT (2024) Dissecting gut-microbial community interactions using a gut microbiome-on-a-chip. Adv Sci 11(20):2302113. https://doi.org/10.1002/advs.202302113
56. Yazicigil RT, Bali A, Caygara D et al (2025) Improving engineered biological systems with electronics and microfluidics. Nat Biotechnol 43(7):1067–1083. https://doi.org/10.1038/s41587-025-02709-6
57. Chen HX, Luo ZQ, Lin X et al (2023) Sensors-integrated organ-on-a-chip for biomedical applications. Nano Res 16(7): 10072–10099. https://doi.org/10.1007/s12274-023-5651-9
58. Zhang YS, Aleman J, Shin SR et al (2017) Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. Proc Natl Acad Sci USA 114(12): E2293–E2302. https://doi.org/10.1073/pnas.1612906114
59. Saraswathibhatla A, Indana D, Chaudhuri O (2023) Cell–extracellular matrix mechanotransduction in 3D. Nat Rev Mol Cell Biol 24(7):495–516. https://doi.org/10.1038/s41580-023-00583-1
60. Qi Z, Li YH, Zhao B et al (2017) BMP restricts stemness of intestinal Lgr5$^+$ stem cells by directly suppressing their signature genes. Nat Commun 8:13824. https://doi.org/10.1038/ncomms13824
61. Fatehullah A, Tan SH, Barker N (2016) Organoids as an *in vitro* model of human development and disease. Nat Cell Biol 18(3): 246–254. https://doi.org/10.1038/ncb3312
62. Clevers H (2016) Modeling development and disease with organoids. Cell 165(7):1586–1597. https://doi.org/10.1016/j.cell.2016.05.082
63. Simian M, Bissell MJ (2017) Organoids: a historical perspective of thinking in three dimensions. J Cell Biol 216(1):31–40. https://doi.org/10.1083/jcb.201610056
64. Pan FC, Evans T, Chen SB (2020) Modeling endodermal organ development and diseases using human pluripotent stem cell-derived organoids. J Mol Cell Biol 12(8):580–592. https://doi.org/10.1093/jmcb/mjaa031
65. Zhu YJ, Sun LY, Wu XY et al (2024) Engineered human organoids for biomedical applications. Adv Funct Mater 34(11):2310961. https://doi.org/10.1002/adfm.202310961
66. Yu B, Zhou DY, Wang FX et al (2025) Organoids for tissue repair and regeneration. Mater Today Bio 33:102013. https://doi.org/10.1016/j.mtbio.2025.102013
67. Sato T, Vries RG, Snippert HJ et al (2009) Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. Nature 459(7244):262–265. https://doi.org/10.1038/nature07935
68. Spence JR, Mayhew CN, Rankin SA et al (2011) Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro*. Nature 470(7332):105–109. https://doi.org/10.1038/nature09691
69. Watson CL, Mahe MM, Múnera J et al (2014) An *in vivo* model of human small intestine using pluripotent stem cells. Nat Med 20(11):1310–1314.

https://doi.org/10.1038/nm.3737
70. Finkbeiner SR, Hill DR, Altheim CH et al (2015) Transcriptome-wide analysis reveals hallmarks of human intestine development and maturation in vitro and in vivo. Stem Cell Rep 4(6):1140–1155. https://doi.org/10.1016/j.stemcr.2015.04.010
71. Holloway EM, Capeling MM, Spence JR (2019) Biologically inspired approaches to enhance human organoid complexity. Development 146(8):dev166173. https://doi.org/10.1242/dev.166173
72. Pastuła A, Middelhoff M, Brandtner A et al (2016) Three-dimensional gastrointestinal organoid culture in combination with nerves or fibroblasts: a method to characterize the gastrointestinal stem cell niche. Stem Cells Int 2016:3710836. https://doi.org/10.1155/2016/3710836
73. Jabaji Z, Brinkley GJ, Khalil HA et al (2014) Type I collagen as an extracellular matrix for the *in vitro* growth of human small intestinal epithelium. PLoS ONE 9(9):e107814. https://doi.org/10.1371/journal.pone.0107814
74. Noel G, Baetz NW, Staab JF et al (2017) A primary human macrophage-enteroid co-culture model to investigate mucosal gut physiology and host-pathogen interactions. Sci Rep 7:45270. https://doi.org/10.1038/srep45270
75. Lindemans CA, Calafiore M, Mertelsmann AM et al (2015) Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. Nature 528(7583):560–564. https://doi.org/10.1038/nature16460
76. Jung KB, Lee HN, Son YS et al (2018) Interleukin-2 induces the in vitro maturation of human pluripotent stem cell-derived intestinal organoids. Nat Commun 9:3039. https://doi.org/10.1038/s41467-018-05450-8
77. Mahe MM (2018) Engineering a second brain in a dish. Brain Res 1693:165–168. https://doi.org/10.1016/j.brainres.2018.04.015
78. Workman MJ, Mahe MM, Trisno S et al (2017) Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. Nat Med 23(1):49–59. https://doi.org/10.1038/nm.4233
79. Kaelberer MM, Buchanan KL, Klein ME et al (2018) A gut-brain neural circuit for nutrient sensory transduction. Science 361(6408):eaat5236. https://doi.org/10.1126/science.aat5236.
80. Cordero-Espinoza L, Dowbaj AM, Kohler TN et al (2021) Dynamic cell contacts between periportal mesenchyme and ductal epithelium act as a rheostat for liver cell proliferation. Cell Stem Cell 28(11):1907–1921.e8. https://doi.org/10.1016/j.stem.2021.07.002
81. Yin YB, Zhou DG (2018) Organoid and enteroid modeling of *Salmonella* infection. Front Cell Infect Microbiol 8:102. https://doi.org/10.3389/fcimb.2018.00102
82. Hill DR, Huang S, Nagy MS et al (2017) Bacterial colonization stimulates a complex physiological response in the immature human intestinal epithelium. eLife 6:e29132. https://doi.org/10.7554/elife.29132
83. Hou QH, Ye LL, Liu HF et al (2018) *Lactobacillus* accelerates ISCs regeneration to protect the integrity of intestinal mucosa through activation of STAT3 signaling pathway induced by LPLs secretion of IL-22. Cell Death Differ 25(9):1657–1670. https://doi.org/10.1038/s41418-018-0070-2
84. Rajan A, Vela L, Zeng XL et al (2018) Novel segment- and host-specific patterns of enteroaggregative *Escherichia coli* adherence to human intestinal enteroids. mBio 9:e02419-17. https://doi.org/10.1128/mbio.02419-17
85. Heo I, Dutta D, Schaefer DA et al (2018) Modelling *Cryptosporidium* infection in human small intestinal and lung organoids. Nat Microbiol 3(7):814–823. https://doi.org/10.1038/s41564-018-0177-8
86. Ettayebi K, Crawford SE, Murakami K et al (2016) Replication of human noroviruses in stem cell–derived human enteroids. Science 353(6306):1387–1393. https://doi.org/10.1126/science.aaf5211
87. Saxena K, Blutt SE, Ettayebi K et al (2016) Human intestinal enteroids: a new model to study human rotavirus infection, host restriction, and pathophysiology. J Virol 90(1):43–56. https://doi.org/10.1128/jvi.01930-15
88. Park NY, Koh A (2022) From the dish to the real world: modeling interactions between the gut and microorganisms in gut organoids by tailoring the gut milieu. Int J Stem Cells 15(1):70–84. https://doi.org/10.15283/ijsc21243
89. Co JY, Margalef-Català M, Li XN et al (2019) Controlling epithelial polarity: a human enteroid model for host-pathogen interactions. Cell Rep 26(9):2509–2520.e4. https://doi.org/10.1016/j.celrep.2019.01.108
90. Moon C, VanDussen KL, Miyoshi H et al (2014) Development of a primary mouse intestinal epithelial cell monolayer culture system to evaluate factors that modulate IgA transcytosis. Mucosal Immunol 7(4):818–828. https://doi.org/10.1038/mi.2013.98
91. Li XG, Chen MX, Zhao SQ et al (2022) Intestinal models for personalized medicine: from conventional models to microfluidic primary intestine-on-a-chip. Stem Cell Rev Rep 18(6):2137–2151. https://doi.org/10.1007/s12015-021-10205-y
92. Kasendra M, Tovaglieri A, Sontheimer-Phelps A et al (2018) Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids. Sci Rep 8:2871. https://doi.org/10.1038/s41598-018-21201-7
93. Workman MJ, Gleeson JP, Troisi EJ et al (2018) Enhanced utilization of induced pluripotent stem cell–derived human intestinal organoids using microengineered chips. Cell Mol Gastroenterol Hepatol 5(4):669–677.e2. https://doi.org/10.1016/j.jcmgh.2017.12.008
94. Nikolaev M, Mitrofanova O, Broguiere N et al (2020) Homeostatic mini-intestines through scaffold-guided organoid morphogenesis. Nature 585(7826):574–578. https://doi.org/10.1038/s41586-020-2724-8
95. Lu Y, Li G, Li YQ et al (2023) Cellulose nanofibril matrix drives the dynamic formation of spheroids. J Zhejiang Univ Sci B 24(10):922–934. https://doi.org/10.1631/jzus.b23d0003
96. Moroni L, Burdick JA, Highley C et al (2018) Biofabrication strategies for 3D in vitro models and regenerative medicine. Nat Rev Mater 3(5):21–37. https://doi.org/10.1038/s41578-018-0006-y
97. Lind JU, Busbee TA, Valentine AD et al (2017) Instrumented cardiac microphysiological devices via multimaterial three-dimensional printing. Nat Mater 16(3):303–308. https://doi.org/10.1038/nmat4782
98. Homan KA, Kolesky DB, Skylar-Scott MA et al (2016) Bioprinting of 3D convoluted renal proximal tubules on perfusable chips. Sci Rep 6:34845. https://doi.org/10.1038/srep34845
99. Shiwarski DJ, Hudson AR, Tashman JW et al (2025) 3D bioprinting of collagen-based high-resolution internally perfusable scaffolds for engineering fully biologic tissue systems. Sci Adv 11(17):eadu5905. https://doi.org/10.1126/sciadv.adu5905
100. Dutton JS, Hinman SS, Kim R et al (2019) Primary cell-derived intestinal models: recapitulating physiology. Trends Biotechnol

37(7):744–760.
https://doi.org/10.1016/j.tibtech.2018.12.001
101. Campbell J, Berry J, Liang Y (2019) Anatomy and physiology of the small intestine. In: Yeo CJ (Ed.), Shackelford's Surgery of the Alimentary Tract, 2 Volume Set (8th Ed.). Elsevier, p.817–841.
https://doi.org/10.1016/b978-0-323-40232-3.00071-6
102. Dahan A, González-Álvarez I (2021) Regional intestinal drug absorption: biopharmaceutics and drug formulation. Pharmaceutics 13(2):272.
https://doi.org/10.3390/pharmaceutics13020272
103. Li YX, Cheng SN, Shi HH et al (2024) 3D embedded bioprinting of large-scale intestine with complex structural organization and blood capillaries. Biofabrication 16(4):045001.
https://doi.org/10.1088/1758-5090/ad5b1b
104. Groll J, Boland T, Blunk T et al (2016) Biofabrication: reappraising the definition of an evolving field. Biofabrication 8(1): 013001.
https://doi.org/10.1088/1758-5090/8/1/013001
105. Pati F, Gantelius J, Svahn HA (2016) 3D bioprinting of tissue/organ models. Angew Chem Int Ed 55(15):4650–4665.
https://doi.org/10.1002/anie.201505062
106. Kim W, Hyung K (2018) An innovative cell-printed microscale collagen model for mimicking intestinal villus epithelium. Chem Eng J 334:2308–2318.
https://doi.org/10.1016/j.cej.2017.12.001
107. Madden LR, Nguyen TV, Garcia-Mojica S et al (2018) Bioprinted 3D primary human intestinal tissues model aspects of native physiology and ADME/tox functions. iScience 2:156–167.
https://doi.org/10.1016/j.isci.2018.03.015
108. Kim W, Kim G (2018) Intestinal villi model with blood capillaries fabricated using collagen-based bioink and dual-cell-printing process. ACS Appl Mater Interfaces 10(48):41185–41196.
https://doi.org/10.1021/acsami.8b17410
109. Lian LM, Zhou CP, Tang GS et al (2022) Uniaxial and coaxial vertical embedded extrusion bioprinting. Adv Healthc Mater 11(9):2102411.
https://doi.org/10.1002/adhm.202102411
110. Cheng LQ, Liu TT, Liu Q et al (2023) A 3D bioprinted gut anaerobic model for studying bacteria–host interactions. Research 6:58.
https://doi.org/10.34133/research.0058
111. Brassard JA, Nikolaev M, Hübscher T et al (2021) Recapitulating macro-scale tissue self-organization through organoid bioprinting. Nat Mater 20(1):22–29.
https://doi.org/10.1038/s41563-020-00803-5
112. Son S, Lee BY, Lim H et al (2025) Hybrid 3D-printed tricultured intestine with tubular mesh structure. Adv Funct Mater 35(16):2424495.
https://doi.org/10.1002/adfm.202424495
113. Pi QM, Maharjan S, Yan X et al (2018) Digitally tunable microfluidic bioprinting of multilayered cannular tissues. Adv Mater 30(43):1706913.
https://doi.org/10.1002/adma.201706913
114. Song H, Hong Y, Lee H (2025) Rapid automated production of tubular 3D intestine-on-a-chip with diverse cell types using coaxial bioprinting. Lab Chip 25(1):90–101.
https://doi.org/10.1039/D4LC00731J
115. Macedo MH, Torras N, García-Díaz M et al (2023) The shape of our gut: dissecting its impact on drug absorption in a 3D bioprinted intestinal model. Biomater Adv 153:213564.
https://doi.org/10.1016/j.bioadv.2023.213564
116. Creff J, Courson R, Mangeat T et al (2019) Fabrication of 3D scaffolds reproducing intestinal epithelium topography by high-resolution 3D stereolithography. Biomaterials 221:119404.
https://doi.org/10.1016/j.biomaterials.2019.119404
117. Vera D, García-Díaz M, Torras N et al (2024) A 3D bioprinted hydrogel gut-on-chip with integrated electrodes for transepithelial electrical resistance (TEER) measurements. Biofabrication 16(3):035008.
https://doi.org/10.1088/1758-5090/ad3aa4
118. Torras N, Zabalo J, Abril E et al (2023) A bioprinted 3D gut model with crypt-villus structures to mimic the intestinal epithelial-stromal microenvironment. Biomater Adv 153:213534.
https://doi.org/10.1016/j.bioadv.2023.213534
119. Hu Y, Zhu T, Cui HT et al (2025) Integrating 3D bioprinting and organoids to better recapitulate the complexity of cellular microenvironments for tissue engineering. Adv Healthc Mater 14(3):2403762.
https://doi.org/10.1002/adhm.202403762
120. Roh TT, Chen Y, Paul HT et al (2019) 3D bioengineered tissue model of the large intestine to study inflammatory bowel disease. Biomaterials 225:119517.
https://doi.org/10.1016/j.biomaterials.2019.119517
121. Zhou WD, Chen Y, Roh T et al (2018) Multifunctional bioreactor system for human intestine tissues. ACS Biomater Sci Eng 4(1):231–239.
https://doi.org/10.1021/acsbiomaterials.7b00794
122. Yu JJ, Peng SM, Luo D et al (2012) In vitro 3D human small intestinal villous model for drug permeability determination. Biotechnol Bioeng 109(9):2173–2178.
https://doi.org/10.1002/bit.24518
123. Hewes SA, Wilson RL, Estes MK et al (2020) *In vitro* models of the small intestine: engineering challenges and engineering solutions. Tissue Eng Part B Rev 26(4):313–326.
https://doi.org/10.1089/ten.teb.2019.0334
124. Wang YL, Gunasekara DB, Reed MI et al (2017) A microengineered collagen scaffold for generating a polarized crypt-villus architecture of human small intestinal epithelium. Biomaterials 128:44–55.
https://doi.org/10.1016/j.biomaterials.2017.03.005
125. Castaño AG, García-Díaz M, Torras N et al (2019) Dynamic photopolymerization produces complex microstructures on hydrogels in a moldless approach to generate a 3D intestinal tissue model. Biofabrication 11(2):025007.
https://doi.org/10.1088/1758-5090/ab0478
126. Liu TG, Gu J, Fu CL et al (2024) Three-dimensional scaffolds for intestinal cell culture: fabrication, utilization, and prospects. Tissue Eng Part B Rev 30(2):158–175.
https://doi.org/10.1089/ten.teb.2023.0124
127. Tullie L, Jones BC, De Coppi P et al (2022) Building gut from scratch: progress and update of intestinal tissue engineering. Nat Rev Gastroenterol Hepatol 19(7):417–431.
https://doi.org/10.1038/s41575-022-00586-x
128. Meran L, Massie I, Campinoti S et al (2020) Engineering transplantable jejunal mucosal grafts using patient-derived organoids from children with intestinal failure. Nat Med 26(10):1593–1601.
https://doi.org/10.1038/s41591-020-1024-z
129. Giuffrida P, Curti M, Al-Akkad W et al (2019) Decellularized human gut as a natural 3D platform for research in intestinal fibrosis. Inflamm Bowel Dis 25(11):1740–1750.
https://doi.org/10.1093/ibd/izz115
130. Mandrycky C, Wang ZJ, Kim K et al (2016) 3D bioprinting for engineering complex tissues. Biotechnol Adv 34(4):422–434.
https://doi.org/10.1016/j.biotechadv.2015.12.011
131. Kim J (2023) Characterization of biocompatibility of functional bioinks for 3D bioprinting. Bioengineering 10(4):457.
https://doi.org/10.3390/bioengineering10040457
132. Walsh DI, Dydek EV, Lock JY et al (2018) Emulation of colonic

oxygen gradients in a microdevice. SLAS Technol 23(2): 164–171.
https://doi.org/10.1177/2472630317743425
133. Yupanqui Mieles J, Vyas C, Daskalakis E et al (2024) Electrospun polyvinyl alcohol fibres incorporating an antimicrobial gel for enzymatically controlled reactive oxygen species release. Bio-Des Manuf 7(6):899–925.
https://doi.org/10.1007/s42242-024-00312-3
134. Sun H, Sun LJ, Ke XD et al (2024) Prediction of clinical precision chemotherapy by patient-derived 3D bioprinting models of colorectal cancer and its liver metastases. Adv Sci 11(2): 2304460.
https://doi.org/10.1002/advs.202304460
135. Chen Y, Lin YN, Davis KM et al (2015) Robust bioengineered 3D functional human intestinal epithelium. Sci Rep 5:13708.
https://doi.org/10.1038/srep13708
136. Zhang SY, Ong WSY, Subelzu N et al (2024) Validation of a Caco-2 microfluidic Chip model for predicting intestinal absorption of BCS Class I-IV drugs. Int J Pharm 656:124089.
https://doi.org/10.1016/j.ijpharm.2024.124089
137. Mehta M, Polli JE, Seo P et al (2023) Drug permeability - best practices for biopharmaceutics classification system (BCS)-based biowaivers: a workshop summary report. J Pharm Sci 112(7):1749–1762.
https://doi.org/10.1016/j.xphs.2023.04.016
138. Wang QY, Guo FY, Jin YT et al (2022) Applications of human organoids in the personalized treatment for digestive diseases. Signal Transduct Target Ther 7(1):336.
https://doi.org/10.1038/s41392-022-01194-6
139. Leung CM, de Haan P, Ronaldson-Bouchard K et al (2022) A guide to the organ-on-a-chip. Nat Rev Method Prime 2:33.
https://doi.org/10.1038/s43586-022-00118-6
140. Ingber DE (2022) Human organs-on-chips for disease modelling, drug development and personalized medicine. Nat Rev Genet 23(8):467–491.
https://doi.org/10.1038/s41576-022-00466-9
141. Cadinu P, Sivanathan KN, Misra A et al (2024) Charting the cellular biogeography in colitis reveals fibroblast trajectories and coordinated spatial remodeling. Cell 187(8):2010–2028.e30.
https://doi.org/10.1016/j.cell.2024.03.013
142. Mehandru S, Colombel JF (2021) The intestinal barrier, an arbitrator turned provocateur in IBD. Nat Rev Gastroenterol Hepatol 18(2):83–84.
https://doi.org/10.1038/s41575-020-00399-w
143. Lock JY, Carlson TL, Carrier RL (2018) Mucus models to evaluate the diffusion of drugs and particles. Adv Drug Deliv Rev 124:34–49.
https://doi.org/10.1016/j.addr.2017.11.001
144. Schneider H, Pelaseyed T, Svensson F et al (2018) Study of mucin turnover in the small intestine by *in vivo* labeling. Sci Rep 8: 5760.
https://doi.org/10.1038/s41598-018-24148-x
145. McCright J, Sinha A, Maisel K (2022) Generating an *in vitro* gut model with physiologically relevant biophysical mucus properties. Cell Mol Bioeng 15(5):479–491.
https://doi.org/10.1007/s12195-022-00740-0
146. Wang Y, Chiang IL, Ohara TE et al (2019) Long-term culture captures injury-repair cycles of colonic stem cells. Cell 179(5): 1144–1159.e15.
https://doi.org/10.1016/j.cell.2019.10.015
147. VanDussen KL, Marinshaw JM, Shaikh N et al (2015) Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays. Gut 64(6):911–920.
https://doi.org/10.1136/gutjnl-2013-306651
148. Bein A, Fadel CW, Swenor B et al (2022) Nutritional deficiency in an intestine-on-a-chip recapitulates injury hallmarks associated with environmental enteric dysfunction. Nat Biomed Eng 6(11):1236–1247.
https://doi.org/10.1038/s41551-022-00899-x
149. Li N, Wang DD, Sui ZG et al (2013) Development of an improved three-dimensional *in vitro* intestinal mucosa model for drug absorption evaluation. Tissue Eng Part C Meth 19(9): 708–719.
https://doi.org/10.1089/ten.tec.2012.0463
150. Biton M, Haber AL, Rogel N et al (2018) T helper cell cytokines modulate intestinal stem cell renewal and differentiation. Cell 175(5):1307–1320.e22.
https://doi.org/10.1016/j.cell.2018.10.008
151. Rogoz A, Reis BS, Karssemeijer RA et al (2015) A 3-D enteroid-based model to study T-cell and epithelial cell interaction. J Immunol Meth 421:89–95.
https://doi.org/10.1016/j.jim.2015.03.014
152. Lehle AS, Farin HF, Marquardt B et al (2019) Intestinal inflammation and dysregulated immunity in patients with inherited caspase-8 deficiency. Gastroenterology 156(1):275–278.
https://doi.org/10.1053/j.gastro.2018.09.041
153. Lorenzo-Martín LF, Hübscher T, Bowler AD et al (2024) Spatiotemporally resolved colorectal oncogenesis in mini-colons ex vivo. Nature 629(8011):450–457.
https://doi.org/10.1038/s41586-024-07330-2
154. Ghorbaninejad M, Asadzadeh-Aghdaei H, Baharvand H et al (2023) Intestinal organoids: a versatile platform for modeling gastrointestinal diseases and monitoring epigenetic alterations. Life Sci 319:121506.
https://doi.org/10.1016/j.lfs.2023.121506
155. Fujii M, Clevers H, Sato T (2019) Modeling human digestive diseases with CRISPR-Cas9–modified organoids. Gastroenterology 156(3):562–576.
https://doi.org/10.1053/j.gastro.2018.11.048
156. Gong XY, Wen Z, Liang ZX et al (2025) Instant assembly of collagen for tissue engineering and bioprinting. Nat Mater 24(8): 1307–1318.
https://doi.org/10.1038/s41563-025-02241-7
157. Karve SS, Pradhan S, Ward DV et al (2017) Intestinal organoids model human responses to infection by commensal and Shiga toxin producing *Escherichia coli*. PLoS ONE 12(6): e0178966.
https://doi.org/10.1371/journal.pone.0178966
158. Koestler BJ, Ward CM, Fisher CR et al (2019) Human intestinal enteroids as a model system of *Shigella* pathogenesis. Infect Immun 87(4):e00733-18.
https://doi.org/10.1128/iai.00733-18
159. Cervin J, Boucher A, Youn G et al (2020) Fucose-galactose polymers inhibit cholera toxin binding to fucosylated structures and galactose-dependent intoxication of human enteroids. ACS Infect Dis 6(5):1192–1203.
https://doi.org/10.1021/acsinfecdis.0c00009
160. Lawrence ALE, Berger RP, Hill DR et al (2022) Human neutrophil IL1β directs intestinal epithelial cell extrusion during *Salmonella* infection. PLoS Pathog 18(10):e1010855.
https://doi.org/10.1371/journal.ppat.1010855
161. Collins SL, Stine JG, Bisanz JE et al (2023) Bile acids and the gut microbiota: metabolic interactions and impacts on disease. Nat Rev Microbiol 21(4):236–247.
https://doi.org/10.1038/s41579-022-00805-x
162. Weiss AS, Niedermeier LS, von Strempel A et al (2023) Nutritional and host environments determine community ecology and keystone species in a synthetic gut bacterial community. Nat Commun 14:4780.
https://doi.org/10.1038/s41467-023-40372-0

163. Donia MS, Fischbach MA (2015) Small molecules from the human microbiota. Science 349(6246):1254766. https://doi.org/10.1126/science.1254766
164. Donaldson GP, Lee SM, Mazmanian SK (2016) Gut biogeography of the bacterial microbiota. Nat Rev Microbiol 14(1):20–32. https://doi.org/10.1038/nrmicro3552
165. Kim R, Wang YL, Sims CE et al (2022) A platform for coculture of primary human colonic epithelium with anaerobic probiotic bacteria. Front Bioeng Biotechnol 10:890396. https://doi.org/10.3389/fbioe.2022.890396
166. Lee HN, Jung KB, Kwon O et al (2022) *Limosilactobacillus reuteri* DS0384 promotes intestinal epithelial maturation via the postbiotic effect in human intestinal organoids and infant mice. Gut Microbes 14(1):2121580. https://doi.org/10.1080/19490976.2022.2121580
167. Bertaux-Skeirik N, Feng R, Schumacher MA et al (2015) CD44 plays a functional role in *Helicobacter pylori*-induced epithelial cell proliferation. PLoS Pathog 11(2):e1004663. https://doi.org/10.1371/journal.ppat.1004663
168. Williamson IA, Arnold JW, Samsa LA et al (2018) A high-throughput organoid microinjection platform to study gastrointestinal microbiota and luminal physiology. Cell Mol Gastroenterol Hepatol 6(3):301–319. https://doi.org/10.1016/j.jcmgh.2018.05.004
169. Shin YC, Than N, Min S et al (2024) Modelling host–microbiome interactions in organ-on-a-chip platforms. Nat Rev Bioeng 2(2):175–191. https://doi.org/10.1038/s44222-023-00130-9
170. Liu J, Lu RH, Zheng XL et al (2023) Establishment of a gut-on-a-chip device with controllable oxygen gradients to study the contribution of *Bifidobacterium bifidum* to inflammatory bowel disease. Biomater Sci 11(7):2504–2517. https://doi.org/10.1039/D2BM01490D
171. Abdollahi S, Zarin B, Vatani M et al (2025) Biomimetic culture substrates for modelling homeostatic intestinal epithelium in vitro. Nat Commun 16:4120. https://doi.org/10.1038/s41467-025-59459-x
172. Chen Y, Rudolph SE, Longo BN et al (2022) Bioengineered 3D tissue model of intestine epithelium with oxygen gradients to sustain human gut microbiome. Adv Healthc Mater 11(16): 2200447. https://doi.org/10.1002/adhm.202200447
173. Chen JY, Yuan ZY, Tu YF et al (2023) Experimental and computational models to investigate intestinal drug permeability and metabolism. Xenobiotica 53(1):25–45. https://doi.org/10.1080/00498254.2023.2180454
174. Cameron O, Neves JF, Gentleman E (2024) Listen to your gut: key concepts for bioengineering advanced models of the intestine. Adv Sci 11(5):2302165. https://doi.org/10.1002/advs.202302165
175. Zeggini E, Gloyn AL, Barton AC et al (2019) Translational genomics and precision medicine: moving from the lab to the clinic. Science 365(6460):1409–1413. https://doi.org/10.1126/science.aax4588
176. Collier CA, Mendiondo C, Raghavan S (2022) Tissue engineering of the gastrointestinal tract: the historic path to translation. J Biol Eng 16(1):9. https://doi.org/10.1186/s13036-022-00289-6
177. Donkers JM, van der Vaart JI, van de Steeg E (2023) Gut-on-a-chip research for drug development: implications of chip design on preclinical oral bioavailability or intestinal disease studies. Biomimetics 8(2):226. https://doi.org/10.3390/biomimetics8020226
178. Sauer AK, Pfaender S, Hagmeyer S et al (2017) Characterization of zinc amino acid complexes for zinc delivery in vitro using Caco-2 cells and enterocytes from hiPSC. BioMetals 30(5): 643–661. https://doi.org/10.1007/s10534-017-0033-y
179. Kramer JA, Sagartz JE, Morris DL (2007) The application of discovery toxicology and pathology towards the design of safer pharmaceutical lead candidates. Nat Rev Drug Discov 6(8): 636–649. https://doi.org/10.1038/nrd2378
180. Calatayud M, Gimeno J, Vélez D et al (2010) Characterization of the intestinal absorption of arsenate, monomethylarsonic acid, and dimethylarsinic acid using the Caco-2 cell line. Chem Res Toxicol 23(3):547–556. https://doi.org/10.1021/tx900279e
181. Onozato D, Yamashita M, Nakanishi A et al (2018) Generation of intestinal organoids suitable for pharmacokinetic studies from human induced pluripotent stem cells. Drug Metab Dispos 46(11):1572–1580. https://doi.org/10.1124/dmd.118.080374
182. Kwon O, Jung KB, Lee KR et al (2021) The development of a functional human small intestinal epithelium model for drug absorption. Sci Adv 7(23):eabh1586. https://doi.org/10.1126/sciadv.abh1586
183. DeSesso JM, Williams AL (2008) Contrasting the gastrointestinal tracts of mammals: factors that influence absorption. In: Macor JE (Ed.), Annual Reports in Medicinal Chemistry. Sciencedirect, p.353–371
184. Vergères G, Bogicevic B, Buri C et al (2012) The NutriChip project–translating technology into nutritional knowledge. Br J Nutr 108(5):762–768. https://doi.org/10.1017/s0007114512002693
185. Wang YP, Gao YF, Pan YC et al (2023) Emerging trends in organ-on-a-chip systems for drug screening. Acta Pharm Sin B 13(6):2483–2509. https://doi.org/10.1016/j.apsb.2023.02.006
186. Ma C, Peng YS, Li HT et al (2021) Organ-on-a-chip: a new paradigm for drug development. Trends Pharmacol Sci 42(2): 119–133. https://doi.org/10.1016/j.tips.2020.11.009
187. Lin X, Yu ZX, Liu Y et al (2025) Gut-X axis. Imeta 4(1):e270. https://doi.org/10.1002/imt2.270
188. Kim J, Hyun J (2021) Soft magnetostrictive actuator string with cellulose nanofiber skin. ACS Appl Mater Interfaces 13(37): 43904–43913. https://doi.org/10.1021/acsami.1c09410
189. Aburto MR, Cryan JF (2024) Gastrointestinal and brain barriers: unlocking gates of communication across the microbiota–gut–brain axis. Nat Rev Gastroenterol Hepatol 21(4):222–247. https://doi.org/10.1038/s41575-023-00890-0
190. Jalili-Firoozinezhad S, Miranda CC, Cabral JMS (2021) Modeling the human body on microfluidic chips. Trends Biotechnol 39(8):838–852. https://doi.org/10.1016/j.tibtech.2021.01.004
191. Kim MH, Kim D, Sung JH (2021) A gut-brain axis-on-a-chip for studying transport across epithelial and endothelial barriers. J Ind Eng Chem 101:126–134. https://doi.org/10.1016/j.jiec.2021.06.021
192. Moysidou CM, Owens RM (2021) Advances in modelling the human microbiome–gut–brain axis *in vitro*. Biochem Soc Trans 49(1):187–201. https://doi.org/10.1042/bst20200338
193. Huang JJ, Xu ZY, Jiao J et al (2023) Microfluidic intestinal organoid-on-a-chip uncovers therapeutic targets by recapitulating oxygen dynamics of intestinal IR injury. Bioact Mater 30: 1–14. https://doi.org/10.1016/j.bioactmat.2023.07.001

194. Tsamandouras N, Chen WLK, Edington CD et al (2017) Integrated gut and liver microphysiological systems for quantitative *in vitro* pharmacokinetic studies. AAPS J 19(5):1499–1512. https://doi.org/10.1208/s12248-017-0122-4
195. Trapecar M, Communal C, Velazquez J et al (2020) Gut-liver physiomimetics reveal paradoxical modulation of IBD-related inflammation by short-chain fatty acids. Cell Syst 10(3): 223–239.e9. https://doi.org/10.1016/j.cels.2020.02.008
196. Ogunrinola GA, Oyewale JO, Oshamika OO et al (2020) The human microbiome and its impacts on health. Int J Microbiol 2020:8045646. https://doi.org/10.1155/2020/8045646
197. Hobby GP, Karaduta O, Dusio GF et al (2019) Chronic kidney disease and the gut microbiome. Am J Physiol Ren Physiol 316(6):F1211–F1217. https://doi.org/10.1152/ajprenal.00298.2018
198. Lee Y, Kim MH, Alves DR et al (2021) Gut-kidney axis on chip for studying effects of antibiotics on risk of hemolytic uremic syndrome by Shiga toxin-producing *Escherichia coli*. Toxins 13(11):775. https://doi.org/10.3390/toxins13110775
199. Marsland BJ, Trompette A, Gollwitzer ES (2015) The gut–lung axis in respiratory disease. Annals ATS 12(Supplement 2): S150–S156. https://doi.org/10.1513/annalsats.201503-133aw
200. Lyu M, Fan GW, Xiao GX et al (2021) Traditional Chinese medicine in COVID-19. Acta Pharm Sin B 11(11):3337–3363. https://doi.org/10.1016/j.apsb.2021.09.008
201. Guo YQ, Luo RH, Wang YQ et al (2021) SARS-CoV-2 induced intestinal responses with a biomimetic human gut-on-chip. Sci Bull 66(8):783–793. https://doi.org/10.1016/j.scib.2020.11.015
202. Tordesillas L, Goswami R, Benedé S et al (2014) Skin exposure promotes a Th2-dependent sensitization to peanut allergens. J Clin Invest 124(11):4965–4975. https://doi.org/10.1172/jci75660
203. Lee HR, Sung JH (2022) Multiorgan-on-a-chip for realization of gut-skin axis. Biotechnol Bioeng 119(9):2590–2601. https://doi.org/10.1002/bit.28164
204. Vernetti L, Gough A, Baetz N et al (2017) Functional coupling of human microphysiology systems: intestine, liver, kidney proximal tubule, blood-brain barrier and skeletal muscle. Sci Rep 7:42296. https://doi.org/10.1038/srep42296
205. Janssen R, de Kleer JWM, Bo HM et al (2024) Food allergen sensitization on a chip: the gut–immune–skin axis. Trends Biotechnol 42(1):119–134. https://doi.org/10.1016/j.tibtech.2023.07.005
206. Choe A, Ha SK, Choi I et al (2017) Microfluidic gut-liver chip for reproducing the first pass metabolism. Biomed Microdevices 19(1):4. https://doi.org/10.1007/s10544-016-0143-2
207. Herland A, Maoz BM, Das D et al (2020) Quantitative prediction of human pharmacokinetic responses to drugs via fluidically coupled vascularized organ chips. Nat Biomed Eng 4(4):421–436. https://doi.org/10.1038/s41551-019-0498-9
208. Maschmeyer I, Lorenz AK, Schimek K et al (2015) A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. Lab Chip 15(12): 2688–2699. https://doi.org/10.1039/c5lc00392j
209. Davoudi Z, Atherly T, Borcherding DC et al (2023) Study transportation of drugs within newly established murine colon organoid systems. Adv Biol 7(12):2300103. https://doi.org/10.1002/adbi.202300103
210. Kim R, Hwan S (2024) Recent advances in gut- and gut–organ-axis-on-a-chip models. Adv Healthc Mater 13(21):2302777. https://doi.org/10.1002/adhm.202302777
211. He CF, He JK, Wu CT et al (2025) 3D printing for tissue/organ regeneration in China. Bio-Des Manuf 8(2):169–242. https://doi.org/10.1631/bdm.2400309
212. Murphy JF, Lavelle M, Asciak L et al (2024) Biofabrication and biomanufacturing in Ireland and the UK. Bio-Des Manuf 7(6): 825–856. https://doi.org/10.1007/s42242-024-00316-z
213. Cao Q, Zhang YQH, Deng RY et al (2023) Biomanufacturing in Japan: frontier research from 2018 to 2023. Bio-Des Manuf 6(6):617–645. https://doi.org/10.1007/s42242-023-00261-3
214. Gao LJ, Liu ZX, Dikovsky D et al (2024) Innovation leading development: a glimpse into three-dimensional bioprinting in Israel. Bio-Des Manuf 7(3):358–382. https://doi.org/10.1007/s42242-024-00275-5