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REVIEW

Mini-guts, macro-insights: unravelling the complexities of cell death and wound healing in intestinal organoids

Mini bağırsaklar, büyük keşifler: bağırsak organoidlerinde hücre ölümü ve yara iyileşmesinin sırlarını çözmek

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Abstract

Intestinal organoids represent three-dimensional *in vitro* models that closely mimic the structure and physiological functions of the human gut. These "mini-guts" are generated from two primary sources: adult intestinal stem cells (ISCs) or pluripotent stem cells (PSCs). While ISC-derived organoids maintain the specific genetic identity of the donor, making them valuable for personalized medicine, PSC-derived models are particularly useful for studying developmental biology and genetic engineering. This review examines the current methods for generating these organoids and explores their growing role in biomedical research. Specifically, we focus on their application in modeling epithelial injury and wound healing processes. Unlike traditional two-dimensional cell cultures, organoids provide a more accurate environment to observe how the intestinal lining repairs itself after mechanical or chemical damage. Furthermore, these systems have become essential for investigating different modes of cell death including apoptosis, necroptosis, and pyroptosis in the context of infections and chronic inflammatory diseases. Current organoid models have limitations, mainly because they lack vascular, neural, and immune components. We discuss how integrating these models with microfluidic organ-on-chip technologies and co-culture systems can address these challenges. Improving the physiological complexity of intestinal organoids will be critical for translating laboratory findings into effective clinical therapies for gastrointestinal disorders.

Keywords:. Intestinal organoids; pluripotent stem cells; wound healing; cell death; inflammatory bowel diseases.

Öz

Bağırsak organoidleri, insan bağırsağının yapısını ve fizyolojik işlevlerini yakından taklit eden üç boyutlu in vitro modellerdir. Bu "mini bağırsaklar", iki ana kaynaktan üretilir: yetişkin bağırsak kök hücreleri (ISC'ler) veya pluripotent kök hücreler (PSC'ler). ISC'lerden türetilen organoidler, donörün spesifik genetik kimliğini koruduğu için kişiselleştirilmiş tıp için değerlidir; PSC'lerden türetilen modeller ise özellikle gelişim biyolojisi ve genetik mühendisliği çalışmalarında yararlıdır. Bu derleme, bu organoidleri üretmek için mevcut yöntemleri incelemekte ve biyomedikal araştırmalardaki artan rollerini araştırmaktadır. Özellikle, epitel hasarı ve yara iyileşme süreçlerini modellemede kullanımlarına odaklanıyoruz. Geleneksel iki boyutlu hücre kültürlerinden farklı olarak, organoidler, mekanik veya kimyasal hasar sonrası bağırsak zarının kendini nasıl onardığını gözlemlemek için daha doğru bir ortam sağlar. Ayrıca, bu sistemler enfeksiyonlar ve kronik enflamatuar hastalıklar bağlamında apoptoz, nekroptoz ve piroptoz dahil olmak üzere farklı hücre ölümü türlerini araştırmak için vazgeçilmez hale gelmiştir. Mevcut organoid modellerinin, esas olarak vasküler, nöral ve immün bileşenlerden yoksun olmaları nedeniyle sınırlamaları bulunmaktadır. Bu modellerin mikroakışkan organ-on-chip teknolojileri ve ko-kültür sistemleriyle entegre edilmesinin bu zorlukları nasıl aşabileceğini tartışıyoruz. Bağırsak organoidlerinin fizyolojik karmaşıklığını artırmak, laboratuvar bulgularını gastrointestinal bozukluklar için etkili klinik tedavilere dönüştürmek açısından kritik öneme sahip olacaktır.

Anahtar kelimeler: Bağırsak organoidleri; pluripotent kök hücreler; yara iyileşmesi; hücre ölümü; inflamatuar bağırsak hastalıkları.

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INTRODUCTION

The intestine is one of the most complex structures in humans, playing a central role in fundamental physiological processes such as nutrient metabolism, regulation of immune responses, maintenance of epithelial barrier integrity, and combating pathogens[1]. As research into the intestinal immune system and pathogen interaction has advanced, it has been revealed that intestinal metabolism and environmental interactions affect physiological processes not only locally but also systemically through the gut–liver, gut–lung, gut–brain, and gut–endocrine axes [1]. The disruption of intestinal epithelial integrity, immunological stress responses, and the fight against pathogens contribute to the pathogenesis of many gastrointestinal diseases[1].

Traditional intestinal research has long been based on cell lines and animal models. Both of these models present various limitations for translational research. Cell lines cannot fully represent human physiology as they lack cell–cell and cell–matrix interactions. Animal models, on the other hand, are restrictive due to high costs, long experimental durations, and interspecies biological differences[2].

Organoid technology marked a significant turning point in 2009 when Hans Clevers and his colleagues first generated small intestinal organoids *in vitro* from Leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5)-positive intestinal stem cells (ISCs)[3]. Selected as one of the year's most innovative technologies by Science in 2013 and designated as "Method of the Year" by Nature in 2017, organoids have become a revolutionary tool in biomedical research[4]. Due to their human origin and their ability to mimic physiological conditions with high fidelity, organoids offer significant advantages in creating *in vitro* models for various diseases, including tumor biology, primary sclerosing cholangitis, and IBD[5].

Intestinal organoids possess the potential to reduce failure rates in clinical development processes by elucidating disease mechanisms, evaluating treatment strategies, and predicting potential off-target effects[3]. These characteristics have also established organoids as a crucial tool in personalized medicine applications. With interdisciplinary integration, organoid technology has rapidly diversified; advanced models such as patient-derived organoids, tumor organoids, microfluidic organ-on-chip systems hydrogel-based engineered organoids have emerged[3,6]. Today, intestinal organoids are utilized in a wide spectrum of applications, ranging from modeling Crohn's disease and colorectal cancer to drug screening, microbiota studies, and biomolecular transport research. Moreover, in recent years, the therapeutic potential of organoids in regenerative medicine applications for ulcerative colitis and short bowel syndrome has become a major area of research[6].

This review discusses intestinal organoids and their types, *in vitro* generation methods and their use in cell death and epithelial wound healing, in light of current knowledge, along with their advantages and disadvantages.

Unlike previous reviews that primarily focus on either organoid generation techniques or their general applications, this review uniquely integrates the mechanistic interplay between epithelial wound healing and diverse cell death pathways within intestinal organoid systems. By consolidating recent findings on apoptosis, necroptosis, and pyroptosis in the context of epithelial injury, this work provides a comprehensive and mechanistically oriented perspective. Furthermore, it highlights emerging bioengineering approaches, including organ-on-chip and co-culture systems, as critical tools for overcoming current limitations and enhancing the translational relevance of organoid models.

'MINI-GUT' MODEL: RECAPITULATING INTESTINAL EPITHELIUM *IN VITRO*

Intestinal organoids are three-dimensional (3D) cultures grown *in vitro* that partially mimic the structure, cellular diversity, and functions of intestinal tissue[3,4]. Often referred to as "mini-guts," these self-organizing structures can be derived from ISCs or pluripotent stem cells (PSCs)[3]. They offer a powerful and physiologically more relevant model system for studying human intestinal biology, development, and diseases, overcoming the limitations of conventional 2D cell cultures or animal models[4].

Intestinal organoids possess a polarized epithelial layer surrounding a functional lumen, containing cell types found in proportions and relative spatial organization similar to those observed in the *in vivo* intestinal epithelium[7]. These structures demonstrate a high degree of cellular diversity, closely mirroring the *in vivo* epithelium[7]. They comprise various cell

types, including active Lgr5+ stem cells, Paneth cells, enterocytes, goblet cells, tuft cells, and enteroendocrine cells, alongside transit-amplifying (TA) cells (Figure 1). Morphologically, they organize into crypt-villus-like domains where budding regions mimic the intestinal crypts[8]. While stem cells are localized within these crypt analogues, differentiation takes place as cells migrate upward[8]. Furthermore, the epithelium maintains a distinct polarity; generally, the apical side faces the internal lumen, whereas the basolateral side is oriented outward[8].

In the context of intestinal organoids, epithelial renewal is driven by a population of Lgr5+ stem cells nestled at the base of the crypts[9]. These cells undergo differentiation into TA cells, establishing a proliferative flow that moves along the crypt-villus axis[9]. This hierarchical differentiation is strictly governed by the synergy of multiple signaling cascades, with the Wingless/Integrated (Wnt) pathway playing a central regulatory role[3].

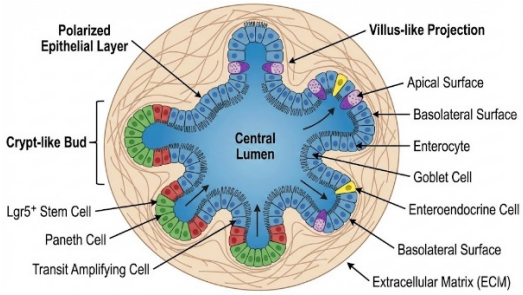


Figure 1. Schematic representation of cellular architecture in an intestinal organoid.

The illustration depicts a polarized epithelial monolayer enclosing a central lumen, embedded within the extracellular matrix (ECM). Recapitulating *in vivo* tissue organization, the organoid comprises crypt-like buds (stem cell niche) and villus-like projections (differentiated zone). The crypt base harbors Lgr5+ stem cells and Paneth cells, whereas the progression toward villus domains reveals transit-amplifying cells alongside differentiated lineages, including enterocytes, goblet cells, and enteroendocrine cells. Cell polarity is distinct, with apical surfaces facing the lumen and basolateral membranes contacting the ECM. Created by the author.

Specifically, Lgr5+ crypt basal columnar (CBC) cells divide continuously to generate progenitors that migrate upward[10]. As these progenitors transition into TA cells, they engage in rapid mitotic cycles dividing four to five times every ~12 hours as they advance toward the crypt-villus junction[10]. Upon reaching the crypt exit, TA cells arrest their mitotic activity and enter a post-mitotic state[10]. At this critical juncture, they commit to specific cell lineages, differentiating into enterocytes, goblet cells, enteroendocrine cells, or tuft cells[10].

Following lineage commitment, these cells continue their upward migration along the villus for a duration of 3 to 5 days[11]. Throughout this journey, the cells mature by losing their Lgr5 phenotype and upregulating Ezrin, Radixin, and Moesin (ERM) proteins, which are vital for the structural integrity of microvilli in enterocytes[11]. The cycle concludes when senescent cells at the villus tip are extruded from the epithelium, thereby ensuring constant tissue turnover[11].

Generally, intestinal organoids are categorized into two primary classes based on their origin: those derived from Adult ISCs and those generated from PSCs[12].

ADULT INTESTINAL STEM CELL-DERIVED ORGANOIDS (ISC-DERIVED)

The utilization of adult tissue stem cells to generate intestinal organoids has marked a paradigm shift in the study of gastrointestinal biology and pathology[3,12]. Unlike other models, these systems offer distinct advantages by faithfully recapitulating the physiological characteristics of the source tissue[3,12].

The foundational methodology involves the direct isolation of intestinal crypts the invaginated niches located at the base of the villi from adult biopsies or surgical resections[3,12]. These structures contain the essential cellular machinery for epithelial homeostasis: ISCs[12]. Specifically, the population marked by $Lgr5^+$ serves as the primary engine for this renewal. Residing at the crypt base, these Wnt-dependent cells retain the multipotency required to regenerate the entire epithelial lineage[3,12].

Successfully culturing the intestinal organoids requires the precise reconstruction of the *in vivo* stem cell niche[3,12]. Within the isolated crypts, endogenous Paneth cells play a symbiotic role, acting as a physiological source of essential ligands, including Epidermal Growth Factor (EGF), Wnt3, and Notch ligands. To sustain this environment *in vitro,* the culture medium acts as a signaling cocktail: Wnt ligands (specifically Wnt3 and R-spondin-1) are critical for driving self-renewal and maintaining crypt architecture, while EGF drives cellular proliferation. Furthermore, the inclusion of Noggin serves to

inhibit Bone Morphogenetic Protein (BMP) signaling, thereby preserving the undifferentiated crypt phenotype, while Notch activation regulates the delicate equilibrium between TA cells and secretory lineages[3,13].

Physically, the establishment of this model relies on a specialized 3D framework. By embedding the isolated crypts and their resident $Lgr5^+$ cells within a supportive ECM, researchers create a scaffold that facilitates cell survival and spontaneous self-organization, allowing the stem cells to proliferate and differentiate into functional organoid structures[3,13].

1. Crypt isolation

The initial phase of organoid establishment involves the precise liberation of crypts from the intestinal epithelium, a process achieved through mechanical dissociation or enzymatic digestion. While various reagents exist for this purpose, protocols typically favor the use of specific proteolytic enzymes to maintain structural integrity while ensuring efficient separation[14].

Collagenase (specifically types I, II, or IV) is frequently employed to disrupt the surrounding stromal architecture, thereby exposing the underlying epithelial structures[14]. Complementing this, Dispase targets the ECM, facilitating the detachment of the epithelial layer from the basement membrane[14]. In scenarios involving larger tissue fragments, these enzymes are often utilized in a synergistic combination; this dual-enzyme approach effectively maximizes the yield of viable crypts by simultaneously degrading the connective tissue and isolating the epithelial units[14].

2. Extracellular matrix embedding and microenvironmental simulation

Following isolation, the establishment of a viable 3D architecture necessitates the encapsulation of crypts within a specialized hydrogel, such as Matrigel, Cultrex, or Geltrex. These matrices are employed to faithfully recapitulate the native cellular microenvironment, serving as a structural scaffold that mimics the *in vivo* basement membrane[15].

These hydrogels are replete with essential matrix constituents notably laminin, collagen IV, entactin/nidogen, and heparan sulfate proteoglycans which function as critical ligands for cell-surface integrin receptors. The engagement of these receptors acts as a primary signaling mechanism, regulating fundamental cellular behaviors including adhesion, polarization, proliferation, and lineage specification[15].

Beyond providing physical support, these matrices act as a reservoir for a diverse array of mitogenic growth factors, such as EGF, Transforming Growth Factor-beta (TGF-β), Fibroblast Growth Factor (FGF), Insulin-like Growth Factor (IGF), and Platelet-Derived Growth Factor (PDGF)[16]. This biochemical cocktail synergistically promotes stem cell self-renewal and drives epithelial expansion[16]. Ultimately, by emulating tissue-specific physicochemical cues, the matrix directs complex morphogenetic events specifically apical-basal polarization, lumenogenesis, and the budding of crypt-like domains thereby ensuring the successful development of a physiological organoid topology[16].

3. Optimization of culture conditions and translational utility

The maintenance of stem cell pluripotency and self-renewal capacities within the organoid system is strictly governed by a defined cocktail of growth factors [16]. Central to this formulation is R-spondin, which functions as a potent agonist of the Wnt signaling pathway, thereby driving stem cell expansion[16]. Concurrently, EGF is supplied to induce general epithelial mitogenesis, while the addition of Noggin is essential for antagonizing BMP signaling; this inhibition prevents premature differentiation and preserves the stem cell niche. Supplementation with Nicotinamide is also frequently employed to further stabilize stem cell function. Under these optimized biochemical conditions, embedded crypts undergo rapid self-organization, maturing within days from simple structures into complex, cystic organoids characterized by distinct crypt-like budding[3].

The translational significance of ISC-derived organoids, whether of small intestinal or colonic origin, lies in their ability to faithfully recapitulate the *in vivo* physiology of the source tissue [12]. Unlike transformed cell lines, these models exhibit remarkable genetic stability and phenotypic fidelity. They retain the specific regional characteristics, genetic signature, and disease traits of the original donor. This preservation of donor identity renders them an indispensable tool for the development of patient-specific disease models and the advancement of personalized medicine strategies[3,12].

GENERATION OF PLURIPOTENT STEM CELL-DERIVED INTESTINAL ORGANOIDS (PSC-DERIVED)

The derivation of organoids from PSCs encompassing both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) represents a distinct paradigm in tissue engineering[17]. Unlike adult stem cell models, this approach exploits the intrinsic pluripotency of PSCs to reconstruct the intestinal architecture de novo [17]. This process relies on a stepwise, directed differentiation protocol that recapitulates the temporal and spatial signaling cues of *in vivo* embryogenesis[17].

1. Induction of definitive endoderm

The initial phase involves the lineage restriction of human PSCs into Definitive Endoderm (DE), the germ layer precursor to the gastrointestinal tract[18]. This specification is primarily driven by the modulation of the Nodal/Activin pathway [18]. Experimentally, PSC colonies at high confluence (approximately 85–90%) are transitioned from maintenance media (e.g., mTeSR1) to a differentiation medium supplemented with Activin A, a potent TGF-β superfamily ligand[18,19].

Over several days, the concentration of Activin A is escalated within a basal medium characterized by low serum (0.2%) and elevated glucose levels[20,21]. This biochemical environment suppresses alternate fates and promotes the expression of key endodermal transcription factors, specifically *FOXA2* and *SOX17* [20,21].

Quality control via flow cytometry is standard at this stage to confirm a high purity population (>85%) expressing Chemokine CXCR Receptor 4 (CXCR4) and c-Kit alongside the nuclear markers[20,21].

2. Hindgut specification and spheroid formation

Following the successful establishment of the DE monolayer, the protocol shifts toward posteriorization to specify the hindgut lineage[22,23]. This transition is mediated by exposing the cells to a synergistic signaling cocktail comprising high concentrations of FGF4 and Wnt3a (typically 500 ng/mL)[22,23].

Under these conditions, the cells upregulate *CDX2*, a critical homeobox protein marking the mid- and hindgut identity[22,23]. Physically, this stage is characterized by a significant morphological event: the formation of 3D structures known as hindgut spheroids[22,23]. These spheroids spontaneously detach from the monolayer and become free-floating[22]. This morphogenetic movement signals the successful specification of precursors destined to form the small intestine and colon[22].

3. 3D culture and self-organization

To facilitate structural maturation, the free-floating spheroids are harvested and embedded within a supportive ECM, such as Matrigel [15]. This hydrogel serves as a surrogate basement membrane, providing the necessary physical support for crypt development[15].

The culture medium is supplemented with a defined set of growth factors—including EGF, Noggin, and Retinoic Acid alongside Wnt agonists. This precise environment triggers spontaneous self-organization[22,24].

1. Wnt signaling drives the proliferation of $Lgr5^+$ stem cells[24].
2. EGF stimulates general epithelial mitosis[25].
3. Noggin acts as a BMP antagonist, preventing premature differentiation and expanding the stem cell pool[26].

Through these mechanisms, the spheroids undergo symmetry breaking and morphogenesis, evolving into complex structures that mirror the native villus-crypt architecture.

4. Terminal maturation

The final phase of the protocol extends over several weeks, during which the organoids achieve full cellular complexity[27]. The epithelium differentiates into the complete repertoire of functional cell types, including absorptive enterocytes, mucus-secreting goblet cells, and antimicrobial Paneth cells[3,27].

Structurally, these mature organoids exhibit a polarized epithelium with a functional lumen, capable of mimicking physiological barrier functions and transport mechanisms[3,27]. This high degree of biological fidelity makes them an invaluable platform for disease modeling, toxicology screening, and patient-specific therapeutic testing[27].

COMPARATIVE ANALYSIS OF INTESTINAL ORGANOIDS

Structural and functional implications of stem cell source

Intestinal organoids derived from adult ISCs and PSCs represent distinct yet complementary paradigms in biomedical research[3,22]. While both models successfully recapitulate the 3D architecture, cellular heterogeneity, and physiological organization of the intestinal epithelium, their specific applications diverge based on their biological origin and developmental potential (Table 1)[3,22].

ISC-derived organoids act as a robust platform for personalized medicine, primarily due to their ability to retain the epigenetic and genetic landscape of the donor tissue[28]. By reconstructing patient-specific epithelial barriers *in vitro*, these models offer superior biological fidelity compared to traditional 2D cultures[29]. In the clinical management of IBD, ISC-derived models facilitate the investigation of barrier dysfunction and pharmacological efficacy such as the modulation of E-cadherin distribution by 5-aminosalicylic acid or azathioprine in a patient-specific context[30]. Similarly, in colorectal oncology, organoids established from metastatic lesions demonstrate high predictive validity (approximately 80%) for chemotherapeutic sensitivity[30]. By preserving tumor heterogeneity and key histopathological markers (e.g., Ki67, CK20), these models enable precise screening of agents like 5-FU and oxaliplatin, thereby minimizing ineffective interventions[31]. However, the predominantly epithelial nature of ISC-organoids limits the study of complex interactions involving immune or mesenchymal components[4]. Furthermore, the enclosed apical lumen inherent to the 3D structure necessitates the development of 2D monolayers or organ-on-chip systems to effectively study apical-interface phenomena, such as drug absorption and microbiome interactions[32].

Conversely, PSC-derived organoids provide a scalable platform ideal for high-throughput screening and developmental biology[33]. The indefinite self-renewal capacity of pluripotent cells allows for large-scale production, while their differentiation protocols mimic embryonic organogenesis[34]. This makes them uniquely suited for modeling congenital disorders like Hirschsprung disease and for utilizing Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas gene editing to correct hereditary defects[35]. Unlike ISC models, PSC protocols offer the flexibility to co-differentiate mesenchymal and neural lineages, enabling the engineering of complex tissues that incorporate vascular-like structures or enteric nervous system components[35]. Despite these advantages, PSC models face challenges regarding maturation; they often exhibit a fetal-like phenotype rather than fully adult physiology[36]. Additionally, the complexity and cost of differentiation protocols introduce variability that hampers standardization across laboratories[37].

Table 1. Comparison of ISC-derived and PSC-derived intestinal organoids

Feature	ISC-Derived Organoids	PSC-Derived Organoids	Reference
Source Material	Adult Tissue: Crypts isolated from biopsies or surgical resections (Lgr5+ stem cells).	Pluripotent Cells: iPSCs or ESCs.	3,22
Generation Speed	Fast: Established in days; expandable within 1 week.	Slow: Requires step-wise differentiation (Endoderm—Hindgut Organoid), taking 4–8+ weeks.	25,37
Developmental Stage	Adult: Maintains the epigenetic signature and maturity of the donor tissue.	Fetal: Physiologically immature; resembles the fetal intestine unless transplanted or matured.	36,57
Cellular Complexity	Epithelium Only: Contains epithelial lineage (Paneth, Goblet, Enterocytes) but lacks stroma.	Complex Tissue: Contains epithelium plus mesenchyme (stroma), and potential for smooth muscle/neural cells.	35,58
Primary Application	Precision Medicine: Patient-specific drug screening, Cystic Fibrosis, CRC.	Developmental Biology: Modeling organogenesis, genetic defects, and tissue engineering.	12

Lgr5+: Leucine-rich repeat-containing G-protein-coupled receptor 5 positive cells; iPSCs: Induced pluripotent stem cells; ESCs: Embryonic stem cells; CRC: Colorectal cancer

Both systems are instrumental in toxicological assessments and pharmacokinetic profiling, including the analysis of transporter proteins and metabolic enzymes[38]. Ultimately, the selection of stem cell source is dictated by the specific research objective: ISC-derived models are the gold standard for predicting individual clinical outcomes and preserving disease-specific traits, whereas PSC-derived systems offer unparalleled opportunities for studying developmental mechanisms and genetic modulation[39]. Future advancements will likely focus on overcoming current limitations regarding tissue maturity and microenvironmental complexity to further enhance the translational value of these models[40].

USE OF INTESTINAL ORGANOIDS IN EPITHELIAL WOUND HEALING STUDIES

The utilization of intestinal organoids has emerged as a pivotal approach for modeling the complex dynamics of damage and restitution within the intestinal epithelium[3]. Unlike conventional two-dimensional cell culture systems, organoids provide a physiologically relevant 3D context that more accurately recapitulates the intricate architecture and functional stages of tissue repair[41]. This capability addresses significant limitations inherent in traditional models, offering a superior platform for investigating the cellular mechanisms underlying wound healing[41]. Intestinal epithelial wound healing is a multistep process classically divided into three sequential phases: restitution, in which surviving epithelial cells rapidly migrate to cover the denuded area; proliferation, in which stem and progenitor cells expand to replenish lost cells; and remodeling, in which tissue architecture and barrier function are fully restored[42]. Intestinal organoid systems are particularly well-suited to recapitulate the restitution and proliferative phases, enabling real-time observation of stem cell-driven re-epithelialization in a physiologically relevant context[42].

Methodological approaches to simulating injury in these systems generally fall into distinct categories, primarily distinguishing between mechanical and chemical induction (Figure 2)[43]. Mechanical damage models often adapt the principles of the traditional "scratch assay" to the organoid context[43]. Since organoids typically develop as 3D cysts within matrices such as Matrigel, mechanical injury can be studied by physically disrupting these structures to observe their subsequent reassembly[15]. Alternatively, organoids may be dissociated to establish 2D monolayers where the apical surfaces are exposed. In this configuration, a physical gap is introduced using specialized tools, allowing researchers to monitor the collective migration and proliferation of epithelial cells as they attempt to restore continuity, a process that mirrors the early phases of *in vivo* wound closure[43].

Complementing physical injury models, chemical damage protocols are essential for replicating the pathological conditions associated with inflammatory disorders, such as IBD or severe infections[44]. Researchers can simulate the inflammatory milieu by introducing specific cytokines like Tumor Necrosis Factor-alpha (TNF-α) and Interferon-gamma (IFN-γ), or by applying chemical irritants such as Dextran Sulfate Sodium (DSS), which are standard agents in colitis research[45]. These models are particularly instrumental in assessing the breakdown of barrier function, mechanisms of cell death, and the subsequent activation of surviving stem cells required to re-epithelialize the compromised tissue[46].

Building upon these foundational models, recent investigations have significantly expanded our understanding of the epithelial microenvironment's role in regeneration. Contemporary studies utilizing advanced co-culture systems have demonstrated that epithelial restitution is not an autonomous process but relies heavily on crosstalk with the stromal and immune compartments[47]. For instance, recent findings from organoid-macrophage co-cultures have revealed that specific signaling molecules secreted by activated immune cells are requisite triggers for the Wnt signaling pathway, which drives the regenerative capacity of Lgr5+ stem cells following injury[48]. Furthermore, the integration of microbiome research has uncovered that metabolites derived from commensal bacteria, such as short-chain fatty acids, directly influence epithelial barrier integrity and accelerate wound closure rates[49]. Parallel advances in bioengineering have allowed researchers to manipulate the stiffness of the ECM, leading to the identification of mechanotransduction pathways, particularly YAP/TAZ signaling, as critical regulators of cellular reprogramming during the repair phase[50].

The distinct advantage of using organoid systems lies in their high structural fidelity. They maintain the critical apical-basolateral polarity and robust intercellular junctions that are often lost in standard

cell lines, thereby enabling a more precise analysis of barrier integrity[3]. Crucially, these systems allow for the direct observation of stem cell dynamics, tracking their proliferation and differentiation as they repopulate crypt domains—the primary engine of intestinal regeneration[41]. Furthermore, the capacity to derive organoids from patient-specific iPSCs or biopsies facilitates a personalized research approach, permitting the comparative analysis of healing kinetics across diverse genetic backgrounds and disease phenotypes[22].

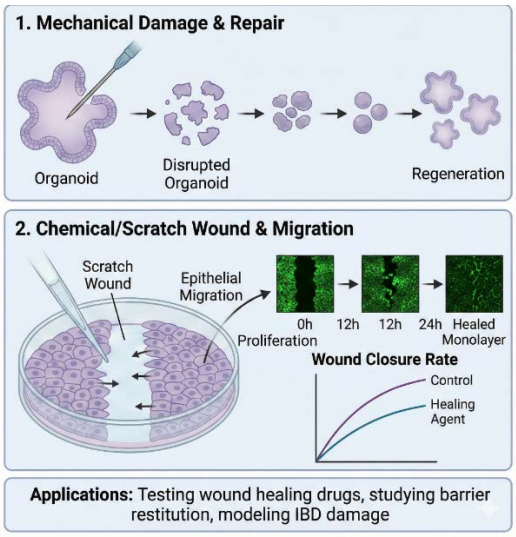


Figure 2. Schematic representation of *in vitro* injury and repair assays.

The illustration delineates two complementary models for studying intestinal epithelial regeneration. (1) The upper panel demonstrates the mechanical disruption of 3D organoids, where fragmented structures undergo self-organization to regenerate intact organoids. (2) The lower panel depicts a 2D scratch wound assay on an epithelial monolayer. Following mechanical injury, the restitution of the barrier is driven by cell migration and proliferation, visualized here through time-lapse imaging (0–24 h). The accompanying graph illustrates the quantification of wound closure kinetics, a metric utilized to evaluate the efficacy of healing agents, model IBD damage, and study barrier restoration. Created by the author.

Consequently, these sophisticated models serve a broad spectrum of clinical and research applications. They provide a controlled environment for high-throughput screening of therapeutic compounds capable of accelerating tissue recovery or mitigating inflammation[41]. Beyond drug discovery, they are indispensable for dissecting the molecular pathways that are dysregulated in chronic non-healing states and for evaluating how extrinsic variables, such as microbial metabolites and growth factors, influence the quality of repair. Ultimately, by offering a window into the complex signaling interplay and cellular heterogeneity of the human gut, intestinal organoids represent a critical tool for elucidating disease pathogenesis and developing novel regenerative therapies that traditional cultures cannot support[41].

APPLICATIONS OF INTESTINAL ORGANOIDS IN CELL DEATH PATHOGENESIS

Intestinal organoids have established themselves as robust, physiologically relevant platforms for dissecting the multifaceted mechanisms of cell death, ranging from programmed apoptosis to unregulated necrosis[30]. Unlike traditional immortalized cell lines, which often exhibit aberrant survival signaling, organoid systems preserve the native 3D architecture and signaling heterogeneity of the gut epithelium[30]. This structural fidelity allows for the precise quantification of cell viability using standard nuclear exclusion dyes, such as DNA stains like Propidium Iodide (PI), alongside the functional assessment of caspase activity[30]. Consequently, these models have become indispensable for evaluating pharmaceutical toxicity and modeling genetic susceptibilities, offering a level of predictive accuracy that closely mirrors *in vivo* tissue responses[30].

A primary application of this technology lies in the modeling of homeostatic epithelial turnover[3,30]. In the healthy gut, the rapid renewal of the lining necessitates the continuous elimination of senescent cells through a process known as apical extrusion[30]. Organoids successfully recapitulate this delicate balance between proliferation and shedding. Recent investigations into mechanobiology using these systems have further elucidated the underlying physics of this process; specifically, studies have demonstrated that mechanical crowding within the epithelial layer triggers the activation of the Piezo1 channel, which subsequently induces the extrusion of live cells to maintain barrier integrity[30]. This offers a dynamic window into how Wnt and B-cell lymphoma 2 (Bcl-2) signaling pathways coordinate to prevent hyperplasia or atrophy[30].

Beyond homeostasis, organoids serve as sophisticated host-pathogen interaction models (Figure 3)[1,3,51]. They permit the controlled infection of human epithelium with enteric bacteria such as Salmonella or Shigella, enabling the study of pathogen-induced cytotoxicity in a species-specific

context[51]. While traditional models focused on simple apoptosis, contemporary organoid research has shifted focus toward inflammatory cell death modes like pyroptosis. For instance, recent findings have highlighted how specific bacterial toxins trigger inflammasome activation (e.g., NLR Family Pyrin Domain Containing 3 (NLRP3)) within the organoid epithelium, leading to rapid cell lysis and the release of alarmins[52]. These studies provide critical insights into how the host defense machinery sacrifices infected cells to contain microbial dissemination, a process often dysregulated in severe infections[1,52].

In the context of chronic inflammatory disorders, such as IBD, organoids allow for the simulation of the "leaky gut" phenotype. By exposing healthy organoids to pro-inflammatory cytokines like TNF-α and IFN-γ, researchers can mimic the barrier collapse seen in patients. Crucially, recent literature utilizing these models has identified that this cytokine-induced damage is not merely apoptotic but often involves necroptosis a regulated form of necrosis mediated by the Receptor-Interacting Protein Kinase (RIPK)-1/RIPK-3 kinase complex[53].

Blocking this pathway in organoid models has been shown to restore barrier function, identifying potential therapeutic targets that were previously obscured in less complex systems[53]. Similarly, ischemia-reperfusion injury models have been developed by subjecting organoids to hypoxia followed by re-oxygenation, mimicking the cellular stress encountered during intestinal surgeries[53].

Furthermore, these systems are revolutionizing toxicology and chemotherapeutic screening. Organoids are extensively used to assess the off-target cytotoxicity of antineoplastic agents like 5-Fluorouracil (5-FU) on healthy stem cells[30,54]. A striking development in this field involves the study of circadian rhythms; recent work has shown that intestinal organoids retain an intrinsic circadian clock, and the timing of drug administration significantly impacts toxicity levels. This finding suggests that organoids can be used to optimize "chronotherapy" regimens, minimizing damage to healthy tissue while maximizing efficacy[30,54]. The ability to perform such high-content analyses distinguishing between the sensitivity of Lgr5+ stem cells and differentiated enterocytes via live-cell imaging underscores the transformative potential of organoids in developing safer therapeutic strategies[30].

PARADIGM SHIFTS IN GASTROINTESTINAL MODELING AND PHYSIOLOGICAL ANALYSIS

The emergence of 3D organoid architectures has fundamentally altered the investigative landscape of the intestinal epithelium, offering a level of structural fidelity that transcends traditional *in vitro* limitations. By strategically harnessing the distinct capabilities of Adult ISC and PSC-derived systems, researchers can now navigate between the granular precision required for personalized medicine and the scalability essential for developmental biology. These platforms have proven indispensable not merely for static disease modeling, but for interrogating dynamic mechanistic processes ranging from the modulation of the Lgr5+ stem cell niche to the intricate orchestration of epithelial restitution encompassing programmed cell death pathways such as apoptosis and necroptosis.

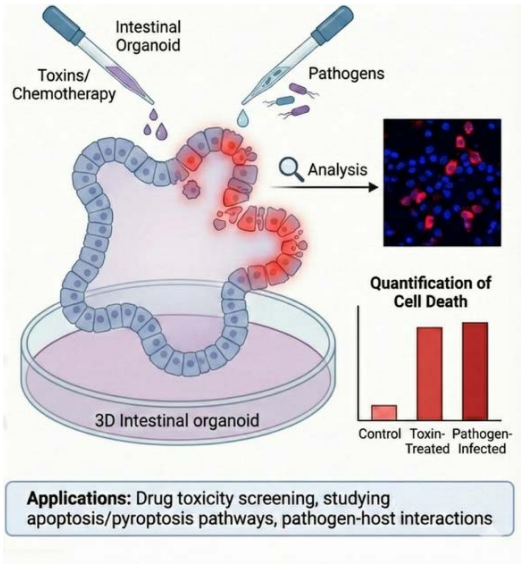


Figure 3. Modeling epithelial injury and cell death mechanisms *in vitro*.

The diagram demonstrates the susceptibility of intestinal organoids to chemical injury (toxins/chemotherapy) and bacterial infection. The resulting cellular damage is characterized by the loss of epithelial integrity and the induction of cell death, visualized here via fluorescent staining of compromised nuclei. Quantitative analysis confirms increased mortality rates in treated groups compared to controls, validating the model's efficacy for studying pathogen-host dynamics and screening pharmaceutical safety profiles. Created by the author.

Consequently, these systems serve as robust biological proxies, enabling the rigorous assessment

of chemotherapeutic toxicity and the dissection of pathophysiology in chronic inflammatory disorders, thereby providing a window into *in vivo* biological complexity that was previously inaccessible.

TECHNOLOGICAL CONVERGENCE AND TRANSLATIONAL HORIZONS

Despite their transformative potential, current organoid iterations remain reductive, primarily restricted by the absence of a systemic microenvironment encompassing vascular, neural, and immune elements. Organ-on-chip systems are microfluidic devices in which living cells are cultured within microscale channels, enabling the simultaneous recapitulation of hemodynamic shear forces, tissue–tissue interfaces, and mechanical deformation that are absent in static organoid cultures [55]. Co-culture systems, on the other hand, involve the combined cultivation of organoids with immune cells (such as macrophages or T lymphocytes), mesenchymal stromal cells, or microbial communities, thereby enabling the study of multicellular crosstalk in a controlled *in vitro* environment [56]. The integration of these complementary technologies with organoid platforms represents a critical step toward achieving the physiological completeness required for translational applications.

The trajectory of the field is therefore defined by the pursuit of physiological completeness; this necessitates the interdisciplinary convergence of microfluidic organ-on-chip technologies with advanced co-culture protocols to reintroduce hemodynamic forces and multicellular interactions. As these bioengineering challenges are addressed to enhance tissue maturation and functional complexity, the focus increasingly shifts toward translational applications. Ultimately, the evolution of these models holds the promise of bridging the gap between bench-side molecular discovery and clinical reality, particularly in developing regenerative therapies for debilitating conditions such as short bowel syndrome and unlocking true personalized therapeutic strategies.

CONCLUSION

Intestinal organoids have emerged as transformative *in vitro* platforms that bridge the gap between conventional cell culture systems and *in vivo* physiology. Their ability to recapitulate epithelial architecture, cellular heterogeneity, and stem cell dynamics provides an unprecedented opportunity to investigate complex biological processes such as wound healing and regulated cell death mechanisms.

This review highlights that intestinal organoids are not only valuable for modeling epithelial restitution and regeneration but also serve as powerful systems to dissect the mechanistic basis of apoptosis, necroptosis, and pyroptosis under both physiological and pathological conditions. The integration of these pathways within a single experimental framework represents a significant advancement over traditional reductive models.

Despite these advantages, current organoid systems remain limited by the lack of vascularization, immune components, and neural inputs. Emerging technologies such as organ-on-chip platforms and advanced co-culture systems are expected to overcome these barriers by introducing dynamic microenvironmental cues and multicellular interactions.

Ultimately, the continued evolution of intestinal organoid technology will enhance its translational impact, enabling more accurate disease modeling, personalized therapeutic strategies, and the development of regenerative approaches for gastrointestinal disorders.

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