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Review

Comparative gastrointestinal organoid models across species: A Zoobiquity approach for precision medicine

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ABSTRACT

Gastrointestinal (GI) health underpins systemic well-being, yet the complexity of gut physiology poses significant challenges to understanding disease mechanisms and developing effective, personalized therapies. Traditional models often fail to capture the intricate interplay between epithelial, mesenchymal, immune, and neuronal cells that govern gut homeostasis and disease. Over the past five years, advances in organoid technology have created physiologically relevant, three-dimensional GI models that replicate native tissue architecture and function. These models have revolutionized the study of autoimmune disorders, homeostatic dysfunction, and pathogen infections, such as norovirus and *Salmonella*, which affect millions of humans and animals globally. In this review, we explore how organoids, derived from intestinal and pluripotent stem cells, are transforming our understanding of GI development, disease etiology, and therapeutic innovation. Through the "Zoobiquity" paradigm and "One Health" framework, we highlight the integration of companion animal organoids, which provide invaluable insights into shared disease mechanisms and preclinical therapeutic development. Despite their promise, challenges remain in achieving organoid maturation, expanding immune and neuronal integration, and bridging the gap between organoid responses and *in vivo* outcomes. By refining these cutting-edge platforms, we can advance human and veterinary medicine alike, fostering a holistic approach to health and disease.

Contents

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1. Introduction

The gastrointestinal (GI) tract is a complex ecosystem where multiple cell types work together to perform essential physiological functions. This epithelial barrier, along with diverse cell populations such as smooth muscle cells, mesenchymal cells, endothelial cells, intestinal neuronal cells, and immune cells, orchestrates processes ranging from digestion to immune defense. The intricate interactions among these components are crucial for maintaining gut homeostasis, and thus their dysfunction can result in various pathological conditions in both humans and animals [1,2].

Recent advancements in organoid technology have facilitated the creation of increasingly sophisticated systems that incorporate multiple cell types, including immune components and elements of the enteric nervous system [3–5]. By mimicking the three-dimensional architecture and physiological functions of organs, organoids offer unparalleled insights into human organ development, disease mechanisms, and therapeutic strategies [6,7]. These advanced models faithfully replicate the physiological complexity of the GI tract more accurately than traditional two-dimensional monolayer cell culture models, providing powerful platforms for disease modeling and drug development across species.

The “Zoobiquity paradigm”, which explores the fundamental biological connections between human and animal diseases, offers a novel framework for organoid research [8,9]. This comparative biological approach is particularly valuable in GI research, as digestive disorders demonstrate striking similarities across species in terms of genetic susceptibility and therapeutic responses [9–11]. For example, spontaneous cancers in canine populations have provided critical insights into the genetic and environmental factors driving tumor development, often yielding more physiologically relevant findings than conventional rodent models with artificially induced tumor [12]. By leveraging comparative biology, the Zoobiquity approach accelerates cross-species discoveries, enhancing our understanding and treatment of human diseases.

Integrating organoid technology with comparative medicine principles enables the development of sophisticated translational models that bridge species-specific biological insights.

This review explores the intersection of intestinal organoid technology and comparative medicine within the Zoobiquity framework, highlighting how species-specific organoid models can improve our understanding of shared disease mechanisms and expedite therapeutic development for both human and veterinary medicine. This integrated approach holds great potential for advancing targeted treatments across species and deepening our knowledge of evolutionarily conserved disease mechanisms.

2. Gastrointestinal organoids from intestinal stem cells (ISCs)

The development of GI organoids marked a pivotal advancement in GI research, exemplified by the generation of the first small intestinal organoids (enteroids) from *Lgr5*+ intestinal stem cells (ISCs). Originally described in 2009 [13], these ISCs, embedded in Matrigel—a complex extracellular matrix (ECM)—and supplemented with a defined growth factor cocktail comprising R-spondin, Noggin, and epidermal growth factor (EGF), demonstrate a remarkable capacity for self-renewal and organoid formation [13]. This breakthrough subsequently facilitated the establishment of colonoids, achieved by incorporating Wnt3A into the enteroid culture conditions, reflecting the distinct molecular requirements of colonic epithelium [14].

Human enteroid and colonoid cultures were further optimized by incorporating additional factors, including gastrin, nicotinamide, a p38 inhibitor, and a transforming growth factor-beta (TGF-β) inhibitor [14]. Maintained within a three-dimensional Matrigel matrix, these organoids recapitulate the architectural and functional hierarchy of the intestinal epithelium, particularly the crypt domain, which is characterized by proliferative undifferentiated cells. The withdrawal of specific factors—notably the p38 inhibitor and nicotinamide—induces cellular differentiation, leading to the formation of specialized cell types, including enterocytes, goblet cells, and enterochromaffin cells, thereby mimicking the villus domain organization [14,15].

A notable feature of these enteroid models is their retention of region-specific gene expression patterns, corresponding to their site of origin—whether derived from the duodenum, jejunum, or ileum. This spatial memory phenomenon, maintained through distinctive transcriptional profiles, enables the study of segment-specific intestinal functions and pathologies [16]. However, ISCs-derived intestinal organoids possess an important limitation: they are composed exclusively of epithelial cell populations, lacking mesenchymal components. While this characteristic is advantageous for studying epithelial-specific processes, it presents a significant constraint for investigations requiring epithelial–mesenchymal interactions or stromal signaling networks (Fig. 1). As discussed in the following section, GI organoids derived from pluripotent stem cells (PSCs) have overcome this limitation by incorporating both epithelial and mesenchymal components.

3. Gastrointestinal organoids from pluripotent stem cells (PSCs): advanced models with multi-lineage complexity

The development of GI organoids from PSCs represents a pivotal advancement in modeling complex intestinal tissue architecture. PSCs, which are characterized by their capacity for self-renewal and

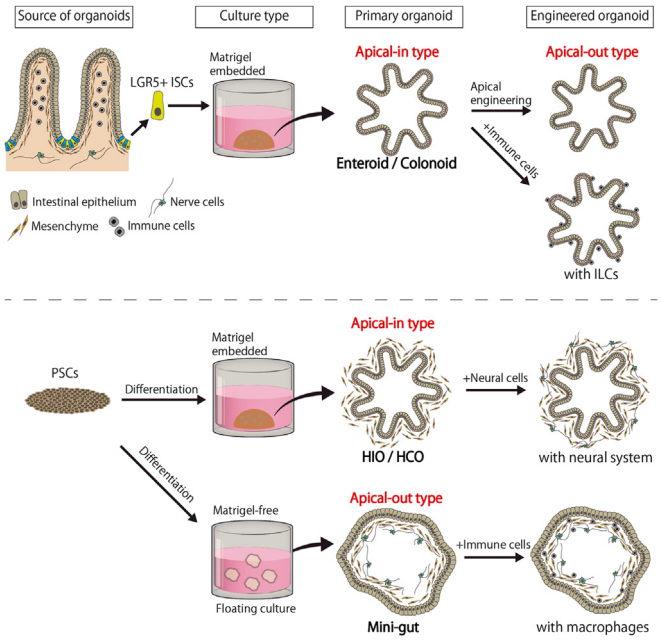

Fig. 1. Summary of intestinal organoid development from intestinal stem cells (ISCs) or pluripotent stem cells (PSCs). Primary organoids are first established as enteroids or colonoids from ISCs, embedded in Matrigel, and as Matrigel-free mini-gut structures from PSCs. Advanced “engineered organoids” are generated through modifications such as apical polarity engineering (e.g., apical-in or apical-out orientations) and the incorporation of additional cell types, including immune cells (e.g., intraepithelial lymphocytes and macrophages) and neural components. These engineered models enable the study of complex interactions between the intestinal epithelium, immune system, and neural system, simulating *in vivo*-like microenvironments.

pluripotent differentiation, can be directed toward an intestinal fate by precisely recapitulating embryonic developmental signals. This approach has facilitated the establishment of human intestinal organoids (HIOs) that mimic small intestinal architecture [17]. By modifying differentiation protocols, particularly through the temporal activation of bone morphogenetic protein (BMP) signaling, human colonic organoids (HCOs) have been generated, thereby expanding the repertoire of intestinal models [18]. A defining feature of PSCs-derived intestinal organoids is their incorporation of both epithelial and mesenchymal components, creating a more physiologically relevant tissue architecture (Fig. 1). Initial HIOs exhibit characteristics of fetal intestinal tissue, lacking mature markers such as sucrose-isomaltase and DEFA5 [19]. Various strategies have been developed to enhance HIOs maturation, including kidney capsule engraftment [20] and interleukin-2 (IL-2) supplementation [21], which promote the acquisition of adult intestinal characteristics.

Advances in organoid complexity have also been achieved by integrating neural components. For example, the incorporation of neural crest cells during *in vitro* organogenesis has enabled the development of HIOs with functional enteric nervous system elements [4]. Additionally, the advent of "mini-guts" marks a significant milestone in organoid technology [5]. These advanced structures incorporate cellular components from all three germ layers: epithelial populations (including enterocytes, enterochromaffin cells, goblet cells, and intestinal stem cells), mesenchymal derivatives (such as smooth muscle cells and interstitial cells of Cajal), and neuronal elements. A notable innovation involves the manipulation of epithelial polarity. Mini-guts naturally exhibit an apical-out morphology and can be maintained in suspension culture without exogenous extracellular matrix (ECM) support, facilitating direct access to the luminal surface [5] (Fig. 1). Complementary techniques have been developed to reverse the polarity of conventional enteroids from apical-in to apical-out orientations, enhancing experimental accessibility to the intestinal apical surface [22] (Fig. 1). These structural advances have significantly broadened the applications of organoid models in studying intestinal physiology and pathology. For instance, mini-guts have been used to examine the absorption of glucose, dipeptides, and cholesterol, as well as the inhibitory effects of natural ingredients on nutrient absorption, by simply adding these substances to the culture medium [23].

4. Integration of immune components in gastrointestinal organoids: advancing physiological complexity

While conventional ISCs- and PSCs-derived intestinal organoids have provided valuable insights into epithelial biology, they lack the immune components that are integral to intestinal homeostasis and disease pathogenesis. Recent technological innovations have enabled the development of increasingly sophisticated immunocompetent organoid systems (Fig. 1).

A pivotal breakthrough in immune–epithelial modeling was achieved with the integration of intraepithelial lymphocytes (IELs) into mouse enteroid cultures [24]. This co-culture system uncovered previously uncharacterized aspects of IEL dynamics within the intestinal epithelium, offering new insights into immune surveillance mechanisms. Further advancements included human-specific models, where macrophages derived from peripheral blood were successfully incorporated into monolayer cultures of human enteroids on Transwell platforms [25]. These macrophage-containing models have revealed multiple roles of tissue macrophages in the intestinal environment: maintaining epithelial barrier integrity, modulating inflammatory responses, coordinating host-microbiome interactions, and contributing to intestinal development and differentiation, thereby providing deeper insights into immune-epithelial interactions in controlled and physiologically relevant contexts.

Patient-derived organoid models have revolutionized our understanding of individual disease pathogenesis and enabled the development of personalized treatment strategies. However, the requirement for multiple tissue samples from the same patient has posed significant limitations to this approach. A persistent challenge in the field has been the limited availability of matched epithelial and immune cells from individual donors, crucial for studying personalized immune responses. Recent innovations in PSC technology have addressed this limitation: Tsuruta and colleagues demonstrated the feasibility of generating both intestinal tissue and monocytes from an isogenic PSCs line source, with subsequent differentiation of monocytes into tissue-resident macrophages within mini-gut structures [26]. This isogenic approach not only circumvents tissue accessibility constraints but also provides a coherent model for studying cell–cell interactions.

Another significant advancement involved the discovery that BMP signaling during HCO development can induce the formation of hemogenic endothelium-like cells. This effect enables the co-development of colonic organoids and tissue-resident macrophages without explicit co-culture procedures [27], representing a substantial step toward recapitulating the developmental processes that establish tissue-resident immune populations *in vitro*.

5. Applications in human disease etiology: from pathogen interactions to therapeutic development

5.1. Advanced modeling of host-pathogen interactions

GI infectious diseases impose a substantial global health burden, with a wide range of pathogens—including viruses, bacteria, and parasites—affecting populations worldwide. For instance, norovirus impacts approximately 21 million individuals annually in the United States [28], while *Salmonella* species infect an estimated 93.8 million people globally [29]. The burden of intestinal parasitic infections is particularly alarming, affecting 1.5 billion individuals worldwide [30]. Despite their prevalence, understanding the molecular mechanisms of these infections and the complex dynamics of host-pathogen interactions has been challenging due to the species-specific nature of many pathogens and limitations in traditional model systems.

The advent of human intestinal organoids has transformed the study of pathogenic interactions by providing a physiologically relevant context (Fig. 2). A pivotal achievement was the cultivation of human norovirus—a pathogen previously resistant to *in vitro* culture—within human enteroids [31,32]. This breakthrough enabled detailed investigations into host defense mechanisms, revealing critical insights into interferon-mediated responses [33]. Beyond norovirus, human intestinal organoids have facilitated studies of diverse viral pathogens, including rotavirus [34], enterovirus [35], and SARS-CoV-2 [35–37]. These studies have revealed key mechanisms of viral entry and replication, leading to the identification of potential therapeutic targets and novel antiviral strategies.

Organoid models have also advanced the understanding of bacterial pathogenesis, particularly in relation to clinically relevant pathogens such as *Salmonella* species [38,39] and *Clostridioides difficile* [40]. The conventional architecture of enteroids and HIOs, where the apical epithelial surface is oriented inward, however, presents technical challenges for infection studies, as pathogens must be microinjected into the organoid lumen. The development of apical-out organoid systems has addressed this limitation, enabling more efficient and scalable approaches for studying

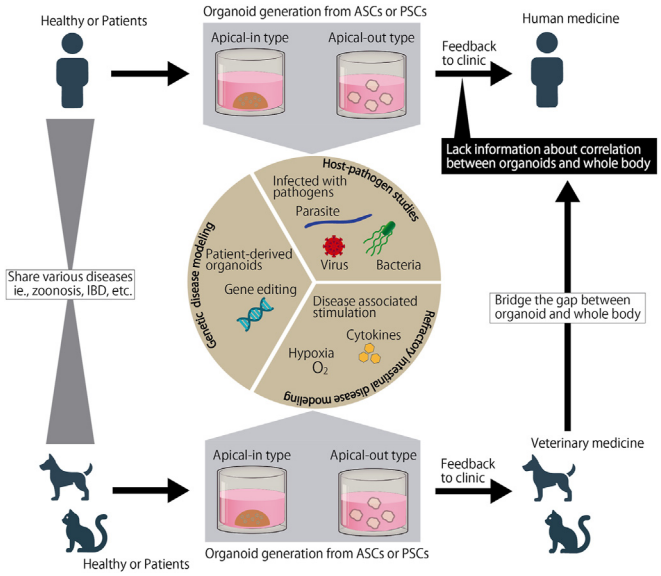

Fig. 2. Overview of intestinal organoid applications and the Zoobiquity approach in organoid studies. Organoids derived from adult stem cells (ASCs) or PSCs can be used for modeling host-pathogen interactions, refractory intestinal diseases, and genetic disorders. Pathogens such as viruses, bacteria, parasites, and disease-related factors (e.g., cytokines and hypoxia) can be applied to these systems for in-depth analyses. Organoids also provide platforms for gene editing, patient-derived models, and translational feedback to clinical settings. The Zoobiquity framework bridges human and veterinary medicine, emphasizing shared diseases (e.g., zoonoses, IBD) and facilitating comparative studies integrating organoid-based insights with whole-body systems.

host–pathogen interactions and expanding their utility for high-throughput screening applications in therapeutic development.

Translating research findings into therapeutic strategies requires comprehensive analyses of host response mechanisms, including epithelial barrier function, innate immune responses, and pathogen-specific cellular tropism. Investigations into pathogen behavior—such as mechanisms of cellular entry and replication, virulence factor expression, and host cell manipulation strategies—have provided valuable insights by establishing physiologically relevant human infection models, creating new opportunities for therapeutic development. These mechanistic studies using human intestinal organoids lay the groundwork for identifying novel drug targets and designing targeted interventions, offering unparalleled insights into disease mechanisms previously inaccessible with traditional model systems.

5.2. Mechanistic insights into refractory intestinal diseases

Refractory intestinal diseases, such as inflammatory bowel disease (IBD) and necrotizing enterocolitis (NEC), remain complex challenges in gastroenterology. These disorders result from intricate interactions among microbial dysbiosis, innate immune dysregulation, altered immune cell function, and environmental factors, including dietary influences and smoking [41,42]. GI organoid technology provides a powerful platform to dissect these mechanisms under controlled experimental conditions (Fig. 2).

Studies using patient-derived organoids have provided insights into potential cellular mechanisms of IBD pathogenesis. Enteroids generated from patients with active IBD show differences in intestinal stem cell expression profiles compared to those derived from healthy donors or patients in remission [43]. Enteroids from severely inflamed tissues maintain structural abnormalities in tight junctions and desmosomes, even in the absence of inflammatory stimuli [44], suggesting possible epigenetic or cellular memory mechanisms. While these findings demonstrate the ability of organoids to preserve certain disease-specific traits *in vitro*, further investigation is needed to determine their clinical relevance and utility in understanding the complex pathogenesis of IBD.

Organoid studies have also elucidated the role of inflammatory mediators in IBD. Investigations into IL-17, a key Th17-associated cytokine implicated in IBD pathogenesis [45], have demonstrated its direct impact on intestinal epithelial cells, including reduced proliferation and viability via caspase-1 (CASP1)-dependent pyroptosis [46]. These results suggest that targeting IL-17 signaling pathways could offer promising therapeutic opportunities. Additionally, chronic inflammation in IBD often results in intestinal fibrosis, characterized by wall thickening, stricture formation, and impaired motility [47]. Human PSC-derived intestinal organoids, which incorporate both epithelial and mesenchymal components, have been instrumental in studying this fibrotic process. Inflammatory conditions in these organoids induce extracellular matrix (ECM) production [48], while TGF-β stimulation upregulates pro-fibrotic genes, such as *COL1A1* and *FN1*, in myofibroblasts [49]. Importantly, spironolactone has been identified as a potential therapeutic agent capable of attenuating TGF-β-mediated fibrotic responses in these models [49].

NEC research has similarly benefited from organoid technology. For example, organoids derived from NEC patients display compromised Wnt signaling and reduced proliferative capacity, deficits that can be ameliorated with Wnt7b supplementation [50]. Mouse intestinal organoids exposed to NEC-associated conditions, including hypoxia and pathogenic bacteria, revealed villus-region-specific vulnerability to necroptosis [51]. These findings highlight the significance of spatial differences in cell death signaling pathways. Furthermore, the therapeutic potential of human breast milk and its oligosaccharide components—established clinical interventions for NEC—has been validated in NEC-in-a-dish models through their demonstrated anti-necroptotic effects [52].

While intestinal organoids replicate only a subset of gut functions and cannot fully capture the multifactorial nature of refractory intestinal diseases, they remain invaluable tools for studying specific disease mechanisms. By focusing on epithelial cell-autonomous responses and innate immune functions, organoids enable the identification of key pathogenic signals and therapeutic targets, driving progress in understanding these complex diseases and developing innovative treatments.

5.3. Genetic disease modeling: advancing precision medicine through patient-derived intestinal organoids

The integration of organoid technology into genetic disease research has established an advanced platform for investigating hereditary gastrointestinal disorders. Patient-derived intestinal organoids, generated from ISCs or PSCs offer physiologically relevant models that replicate key cellular and molecular features of genetic diseases. By incorporating modern gene-editing technologies, these organoid systems facilitate both mechanistic exploration and therapeutic development (Fig. 2).

Cystic fibrosis (CF), a monogenic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, primarily disrupts epithelial fluid transport, impacting multiple organ systems. Intestinal organoids have provided a robust platform for studying CF through the widely adopted forskolin-induced swelling assay [53]. This functional test exploits the fluid movement facilitated by *CFTR* activity: normal organoids swell upon forskolin stimulation, reflecting fluid movement from the basolateral to the apical side, whereas organoids derived from CF patients fail to exhibit this response due to defective *CFTR* function

[53]. The therapeutic potential of genetic correction has been demonstrated through CRISPR-Cas9 editing of patient-derived organoids, where restoration of *CFTR* function resulted in the recovery of forskolin-induced swelling [54]. This accomplishment underscores the potential of genetic correction as a therapeutic strategy and positions organoid platforms as pivotal tools for evaluating novel treatments.

Beyond CF, organoid technology holds significant potential for other hereditary gastrointestinal disorders. For instance, organoids derived from patients with familial adenomatous polyposis (FAP) recapitulate APC mutation-driven tumor formation [55], while organoids from patients with congenital diarrheal disorders such as microvillus inclusion disease have helped elucidate cellular defects in intestinal absorption [56]. Patient-derived organoids preserve the full genetic context of the disease, including modifier genes that may influence severity. This capability, coupled with the ability to derive multiple organoid lines from a single patient, supports robust experimental designs with well-matched controls. Furthermore, the combination of organoid technology with advanced gene-editing tools enables the generation of isogenic organoids—models that differ only in the disease-causing mutation—enhancing precision in experimental studies [57].

Organoid platforms facilitate systematic characterization of both well-established genetic conditions and variants of uncertain significance through controlled genetic manipulation. Their capacity to replicate disease-specific phenotypes, coupled with the precision of genetic editing, positions organoids as foundational tools for advancing precision medicine in genetic disorders. Additionally, the sustained viability of organoid cultures allows for detailed temporal analyses of disease progression and therapeutic responses, providing critical insights into the dynamic nature of hereditary disorders.

The synergy between organoid systems and emerging molecular technologies continues to expand the scope of disease modeling and therapeutic screening. By enabling high-resolution studies of inherited intestinal pathologies, organoid technology enhances our understanding of their mechanisms and paves the way for innovative therapeutic interventions, reinforcing its role as a cornerstone in genetic disease research and treatment development.

6. Integration of Zoobiquity and stem cell technology: a novel paradigm for translational medicine

6.1. Scientific rationale for the Zoobiquity approach

Recent regulatory advancements, including the December 2022 legislation by the U.S. Food and Drug Administration (FDA), have removed the requirement for animal testing in drug development [58]. While this shift reflects, in part, the significant progress made in organoid technology, it highlights a pressing need for validating the translational relevance of organoid systems to *in vivo* physiology. Current limitations in correlating organoid responses with organismal outcomes underscore the necessity for complementary validation strategies. The Zoobiquity framework, which leverages shared biological principles across species, provides a robust foundation for addressing these translational challenges [59] (Fig. 2).

Companion animals such as dogs and cats, which naturally develop spontaneous diseases with notable parallels to human conditions, represent a vital resource in this framework. For example, IBD in these species closely mirrors the pathological features of human IBD [11,60]. Additionally, companion animals are susceptible to zoonotic pathogens, including *Salmonella* [61], *Campylobacter* [62], and various parasitic organisms (*Giardia*, *Cryptosporidium*, intestinal helminths) [63], alongside viral pathogens like norovirus and rotaviruses [64,65]. These infections pose significant health burdens in both veterinary and human medicine, often requiring prolonged antibiotic treatment and supportive care, with potential long-term complications including chronic inflammation and gut dysfunction. Current therapeutic approaches are limited by increasing antimicrobial resistance and incomplete understanding of host-pathogen interactions. We expect that investigating these naturally occurring diseases through the lens of intestinal organoids derived from companion animals will provide novel insights into disease mechanisms and therapeutic responses relevant to both human and veterinary medicine, potentially leading to more effective treatments and preventive strategies.

Furthermore, companion animal clinical trials will help bridge the gap between preclinical organoid studies and human clinical applications. Evaluating novel therapeutics in these trials will provide intermediate data that may predict translational outcomes more reliably than traditional animal models or human organoid systems alone, and will have implications for both veterinary and human medicine.

6.2. Advancement of veterinary stem cell technology

The establishment of intestinal organoids in companion animals has closely mirrored the methodological advances achieved in human systems. Researchers have successfully derived intestinal organoids from canine and feline ISCs isolated from biopsy specimens [66,67]. Notably, feline ISCs-derived organoids have shown susceptibility to feline coronavirus infection. These studies revealed the essential role of specific viral receptors and cellular proteases in viral entry, providing insights into potential therapeutic targets and underscoring their utility for studying infectious diseases [66]. While intestinal organoids derived from PSCs have not yet been developed in companion animals, significant progress has been achieved in establishing embryonic stem cells and induced pluripotent stem cells (iPSCs) in canine [68–72] and feline [73,74] species. Moreover, the successful differentiation of canine PSCs into definitive endoderm [75] provides a foundation for generating PSCs-derived intestinal organoids in these species. We anticipate that these models will facilitate in-depth investigations into developmental abnormalities, breed-specific genetic disorders, and host–pathogen interactions, all within a physiologically relevant, species-specific framework.

Recent technological advances in companion animal intestinal organoid systems have established robust platforms that support both fundamental research and therapeutic development. Building on improved ISCs isolation methods and progress in PSCs differentiation, these innovations enable a more precise study of species-specific disease mechanisms. As these techniques, particularly those related to PSCs-derived organoid generation and genetic modification, continue to improve, researchers will be better equipped to model complex diseases and test innovative treatment strategies. Collectively, these achievements represent a significant step forward in veterinary regenerative medicine and lay a critical foundation for future comparative investigations and One Health initiatives, ultimately fostering a more holistic understanding of health across diverse species.

7. Conclusion and future perspectives

The development of intestinal organoid technology marks a transformative advancement in GI research, offering unprecedented insights into fundamental biological processes and disease mechanisms. These intricate three-dimensional culture systems have proven invaluable across diverse research domains, including

the study of infectious disease pathogenesis, refractory intestinal disorders, and hereditary conditions. The progressive integration of immune components has elevated the physiological relevance of these models, enabling sophisticated investigations into intestinal immune responses within controlled microenvironments. However, the increasing complexity of organoid systems introduces analytical challenges, underscoring the importance of meticulous experimental design and the careful selection of methodologies tailored to specific research objectives.

Simultaneously, the growing recognition of the synergy between human and veterinary medicine presents new opportunities for advancing global health. Companion animals, particularly dogs and cats, serve as invaluable models for naturally occurring diseases that exhibit remarkable homology to human conditions. Applying organoid technology to these species not only enhances our understanding of companion animal pathologies but also provides translational insights with direct relevance to human medicine. This bidirectional exchange of knowledge exemplifies the potential of the Zoobiquity framework, which highlights the shared biological foundations across species.

Looking ahead, the integration of comparative biology principles with advanced organoid technologies holds the promise of establishing increasingly sophisticated platforms for addressing complex biological questions and driving the development of innovative therapeutic strategies. As these experimental systems evolve, tackling existing technical challenges and broadening their applications across diverse species and disease contexts will be essential. This integrated approach, leveraging the strengths of both human and veterinary research, represents a pivotal step toward achieving transformative therapeutic solutions that advance health for all species within the framework of the One Health paradigm.

Declaration of competing interest

The authors have no relevant conflicts of interest to declare.

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