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REVIEW

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Advances in porcine respiratory and intestinal organoids: status and potential application for virus infections

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Abstract

The respiratory tract and digestive tract serve as the gateway between the host and the environment, playing an important role in protecting against viral infections. Diseases caused by viruses that infiltrate the respiratory and gastrointestinal tracts account for the major infectious diseases in pigs, resulting in significant economic losses for the swine industry. However, studies on virus-host interactions are limited due to the lack of suitable research models that can effectively stimulate the highly complex physiological characteristics found in vivo. With the advancement in stem cell technology, organoids that more closely recapitulate the structure, function, and organization of specific organs or tissues in vitro have gradually become a research hotspot. These novel ex vivo models are critical for studying viral infection, investigating viral pathogenesis, elucidating virus-host interactions and developing preventive and therapeutic approaches. Currently, respiratory organoids and intestinal organoids (IOs) have been widely applied in the study of infectious diseases. Therefore, this review primarily summarizes the development of porcine respiratory and intestinal organoids, their applications in studying infection, current limitations, and future perspectives.

Keywords Respiratory tract, Intestine, Organoid, Virus, Pig

Introduction

Respiratory and digestive diseases are common diseases on swine farms. African swine fever, porcine epidemic diarrhea (PED), porcine circovirus disease, porcine enzootic pneumonia and porcine colibacillosis are common diseases on large-scale pig farms [1]. Among them, viruses are one of the primary culprits responsible for the largest number of diseases and the most tremendous economic losses [2]. For example, porcine epidemic diarrhea virus (PEDV) broke out in the United States in 2013, and the virus had impacted approximately 50% of U.S. breeding herds within one year [3]. In 2018, African swine fever virus spread rapidly across China within a year and caused catastrophic damage to the pork industry of China [4]. The respiratory and digestive tracts of the host are dynamic, cellularly diverse and histologically intricate systems under tight regulation. In addition to maintaining normal physiological functions, they must form a protective barrier to resist an entry of the tremendous array of pathogens into the host organism [5–7]. The nasal mucosa, lysozyme, interferon (IFN), SIgA antibodies secreted by the trachea and bronchi, and the alveolar macrophages play important roles in protecting the body against respiratory viruses [8]. The intestinal

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barrier, which consists of the mechanical barrier, chemical barrier, microbial barrier and immune barrier, plays a key role in maintaining intestinal health and preventing the invasion of enteroviruses [9]. Therefore, the respiratory and digestive tracts play an important role in viral infections. Having a suitable model is crucial for elucidating the infection and pathogenic mechanisms of viruses, and animal models and immortalized (cancerous) cell lines are the primary models for studying host–pathogen interactions [10, 11]. Although these model systems have greatly expanded our knowledge of virology, their limitations are also evident. Universally used transformed cell lines are genetically unstable and do not recapitulate the complex composition and microenvironment of untransformed cells, nor the complex interactions between the viruses and the host's immune response [12, 13]. Animal models provide a functional read-out and a more comprehensive model of infection, but they are limited by individual variations, biological differences between species, animal welfare and ethical concerns, and are also hampered by high cost, low throughput, and poor convenience [14]. Successful cultivation of organoids bridges the gap between transformed cell lines and animal models by addressing in vivo complexity to reconcile moderate systemic complexity and reproducibility [15, 16]. Compared to three-dimensional (3D) cultures of tissue explants, organoid systems have better genetic stability and better mimic cell–cell/cell–matrix interactions [13, 17, 18]. Over the past decade, virologists have increasingly turned to organoids as tools to further elucidate virus-host interactions [19–22].

Overview of airway organoids (AOs) and intestinal organoids (IOs)

Organoids, also known as "mini-organs," are 3D structures grown from stem cells. These structures are composed of organ-specific cell types to recapitulate the cellular structure and function of native organs [15, 23]. In recent years, organoids have been widely used in many biological fields, not only for studying the interaction between hosts and pathogenic microorganisms (bacteria, viruses, and parasites) [22, 24–27], but also for immunological research, to understand epithelial cell-immune cell interactions [28–30]. In addition, organoids play an important role in cancer research, tissue regeneration, and clinical drug screening [31, 32]. The starting point for creating an organoid can vary considerably. In the models currently used, the tissue structure is mainly obtained from pluripotent stem cells (PSCs) and adult stem cells (ASCs). Among them, PSC-derived organoids include embryonic stem cells (ESCs)-derived organoids and induced pluripotent stem cells (iPSCs)-derived organoids [12]. In addition to producing epithelial cell types, PSCs can also differentiate into other functional cells, such as fibroblasts and muscle cells. ASCs-derived organoids are generated by directly dissociating intrinsic ASC-containing tissues and exposing them to tissue-specific growth factors [33], followed by tissue-specific enrichment by modulating individual signaling pathways such as bone morphogenic protein (BMP) or Notch [34]. In summary, the growth factors used in the culture of PSCs and ASCs-derived organoids are different because ASCs have undergone a certain degree of differentiation compared with PSCs. PSCs or ASCs grow in various extracellular matrices (such as Matrigel) and self-assemble into 3D structures. Growth factors in the medium can induce further development of organoids from stem cells to differentiated cells that mimic functional epithelium [35]. With the continuous advancement of organoid technology, porcine organoid culture technology has developed rapidly. Porcine organoids derived from ASCs, such as AOs and IOs, have been successfully established in vitro (Fig. 1). They currently play an important role in disease research. In addition, since the size and the composition of the porcine genome and the functional features of porcine organs are similar to humans, porcine organoids are often used to simulate the physiological and pathological functions of humans [36]. As a result, porcine organoids have been widely used in agriculture, veterinary medicine, and biomedicine.

Airway organoids (AOs)

The airway (except the nasal vestibule) to the terminal bronchioles contains five major types of epithelial cells: ciliated cells, goblet cells, small granule cells, brush cells and basal cells [37, 38]. Alveolar sac, as the basic unit of oxygen exchange, is lined with alveolar epithelium composed of flat type I alveolar epithelial (AT1) cells and cuboidal type II alveolar epithelial (AT2) cells [39]. Currently, AOs (respiratory tract and lung) have been widely used to monitor viral infection, explore pathological changes, and identify potential treatments [40–42].

AOs can be obtained from a variety of stem cells, including iPSCs, ESCs, and adult or fetal stem cells from surgical specimens, each of which can differentiate into all cell types [43]. Depending on the type of stem cells, AOs are divided into PSC-derived and ASC-derived AOs. Currently, PSC-derived AOs play an important role in the study of lung developmental biology due to their advantages, such as not being limited by tissue sample scarcity, having stable amplification capacity, being able to differentiate into most cell types, and being accessible for gene editing [44–46]. The culture of PSC-derived AOs involves four essential stages: definitive endoderm, anterior foregut endoderm, lung progenitor cells, and all types of AOs [47–49]. However, only AOs derived from

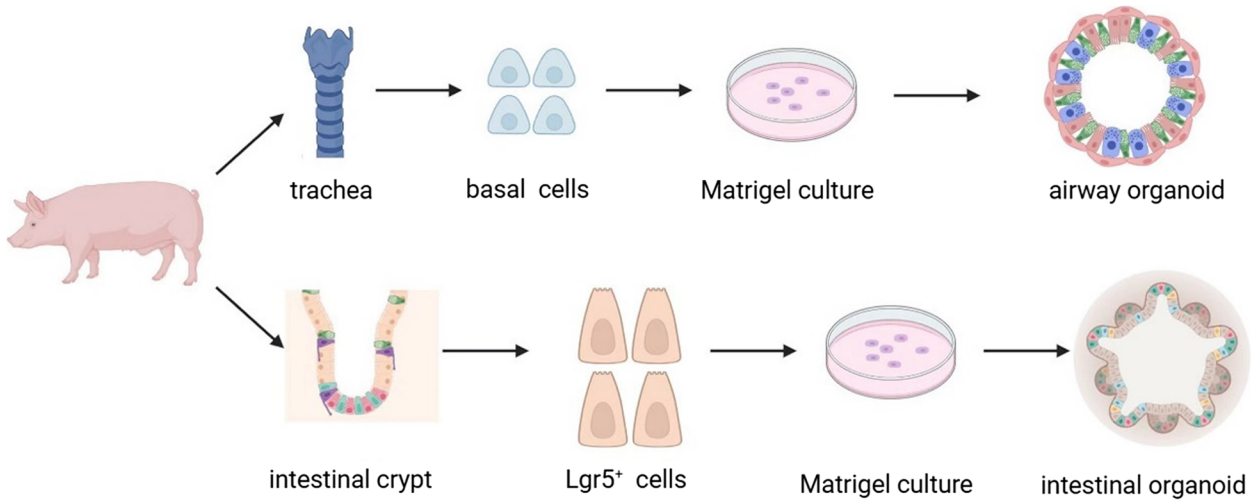

Fig. 1 Schematic diagram of porcine adult stem cells derived respiratory and intestinal organoid culture. Currently, porcine AOs are grown using basal epithelial cells isolated from the trachea. AOs are composed of the four main types of airway epithelial cells, including ciliated cells, goblet cells, basal cells, and club cells. IOs mainly include five main cell types: goblet cells, intestinal epithelial cells, enteroendocrine cells, Paneth cells and intestinal stem cells

human or mouse PSCs have been reported thus far, and AOs from porcine PSCs have not yet been reported.

Although PSC-derived AOs can be differentiated to represent airway and alveolar regions, their differentiation process is complex and tedious. Thus, using PSC-derived AOs as a model remains limited. In contrast, ASC-derived AOs are widely used due to their ability to exhibit complex structures with mature components [50–52]. Unlike intestinal stem cells (ISCs), multiple cell types in the lung are capable of pluripotent differentiation when the lung is injured. In numerous studies researchers have attempted to develop AOs from different progenitor cells from mice and humans, including basal cells, rod cells, secretory cells and AT2 cells [53, 54]. For example, human lung organoids have been developed from normal lung tissue and directed to an immature state in a feeder-free culture system. Organoids in an immature state can faithfully generate epithelial domains that phenocopy the native airway epithelium by adopting a proximal differentiation protocol [55]. Moreover, alveolar organoids composed of AT1 and AT2 cells can be generated by adopting a distal differentiation protocol [56, 57]. Zhou et al. [55] established long-term expanded 3D human AOs with normal lung tissue, and developed an improved two-dimensional (2D) monolayer culture system for the differentiated AOs. Furthermore, due to the basal-out polarity of first-generation organoids, pathogens can rarely attach directly to the apical membrane, making it difficult to initiate infection. Boecking et al. [58] established AOs with externally oriented apical membranes, which is conducive to pathogenic infection of AOs. Before that, most studies of porcine respiratory infections were conducted using immortalized cell lines, primary airway epithelial cells, or porcine lung explants [59–61]. With the development of organoid technology in vitro, our laboratories achieved long-term porcine AOs (Fig. 2), which contain four main airway epithelial cell types: ciliated cells, goblet cells, basal cells and club cells [62]. The results from further experiments indicated that both 3D and 2D AOs can be successfully infected with transmissible gastroenteritis coronavirus (TGEV) and porcine respiratory coronavirus (PRCoV), and can produce significant interferon (IFN) and inflammatory responses, demonstrating that porcine AOs have become a potential universal platform for porcine respiratory infections [62].

Intestinal organoids (IOs)

The intestinal epithelium, which consists of the villus and the crypt, is the fastest self-renewing tissue in mammals, and the ISCs located in the bottom crypt region are an indispensable driving force for its rapid renewal [63]. ISCs undergo asymmetric cell division into new stem cells and committed daughter cells, termed transit-amplifying (TA) cells. TA cells subsequently differentiate into functional cell types, including absorptive enterocytes and secretory cells (Paneth cells, goblet cells, and enteroendocrine cells) [64]. The self-proliferation and differentiation of ISCs are regulated via various signaling pathways. For example, the Wnt signaling pathway plays an important role in promoting cell proliferation and self-renewal [65], the Notch signaling pathway promotes cell differentiation, and the BMP

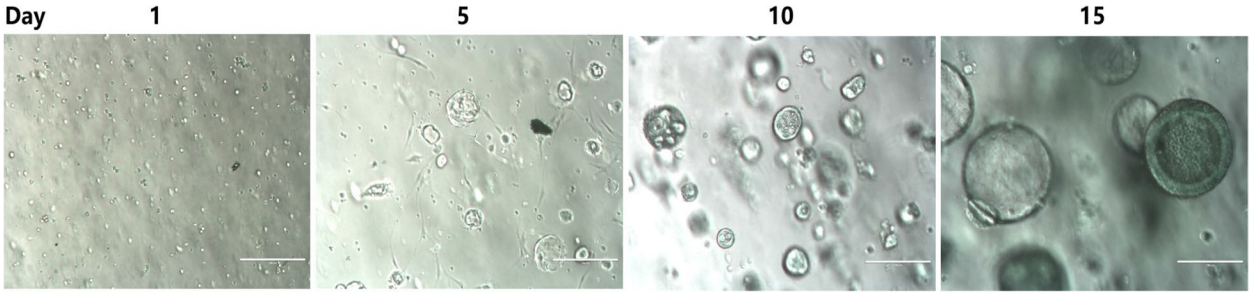

Fig. 2 Porcine ASC-derived airway organoids. Representative images of AO differentiation derived from the trachea of 2-day-old piglets. The tracheal epithelial cells were cultured in a Matrigel matrix, and their daily growth was observed under a microscope [62]. Scale bar = 200 mm

signaling pathway inhibits the activity of the β-catenin protein [66, 67]. In recent years, with the progress of intestinal stem cell isolation technology, it has become possible to achieve long-term culture of intestinal epithelial cells in vitro. IOs can be generated by differentiation from PSCs or by derivation from isolated multipotent stem cells and progenitor cells present in vivo intestinal crypts. Among them, organoids initially created from the small intestinal crypts containing $lgr5^+$ ISCs are called enteroids, those created from the colon crypts containing $lgr5^+$ ISCs are called colonoids [68]. In 2009, IOs were first developed by Sato et al. [69] by culturing mouse intestinal crypts or single ISCs in an extracellular matrix. In 2011, the same research group established crypt-derived human IO cultures by adding nicotinamide and various small molecule inhibitors that are used to promote the growth of mouse organoids [70]. In the same year, Spence et al. [71] established PSC-derived human IOs. Subsequently, other researchers reported the successful culturing and establishment of IOs in cattle, pigs, dogs, cats, chickens, and bats [25, 72–76]. The choice of IO model depends on the purpose of the study [77]. For instance, PSC-derived IOs may be better when studying the intestinal development and differentiation in vivo, while ASC-derived IOs may be preferable for disease research because the characteristics of the tissue of origin are preserved in the organoids. Whichever the IO models, all intestinal epithelial cell types, are polarized toward the lumen, and can be cultured in an in vitro environment for prolonged periods of time. Although recent progress has been made in developing livestock iPSCs and ESC [78], all porcine IO models are developed primarily with tissue-derived intestinal epithelial stem cells (IESCs) (Fig. 1). The first step of porcine IO culture is to obtain the porcine intestinal segments, and then dissociation buffer is used to isolate epithelial crypts that contain IESCs. Next, the isolated crypts were seeded in Matrigel and the growth medium was added [79]. The three proteins Wnt3a, R-spondin1 and Noggin in the growth medium play a key role in culturing porcine IOs [79]. In addition, other supplementary factors in the culture medium, including N2 supplementation, B27 supplementation, nicotinamide, and N-acetylcysteine, ensure the long-term culture of porcine IOs [80]. Matrigel, which is widely used in organoid culture and is prepared from the secretion of EngelbrethHolm-Swarm mouse sarcoma cells [81], has several key limitations, including complex and poorly defined composition, batch-to-batch variability, high cost and safety issues [82]. Therefore, more and more studies are trying to find new organoid culture substrates. Currently, synthetic hydrogel matrices, decellularized extracellular matrices, and natural hydrogels such as type I collagen all have the potential to replace Matrigel [83]. Gonzalez et al. [73] successfully cultured porcine IOs from piglet jejunum for the first time. Since then, porcine IOs from different intestinal segments, such as the duodenum, jejunum, ileum, and colon, have been rapidly established and used as in vitro models in various research fields [22, 84–86] (Fig. 3). Kar et al. [87] found that the transcriptome profiles of different intestinal segments and their derived organoids showed high resemblance, which demonstrates the high complexity of porcine IO and the resemblance to in vivo tissue. Duchesne et al. [88] evaluated the effect of ISC donor age on the growth, morphology, and cellular composition of porcine IOs. This study revealed that IOs derived from young piglets bud and grow faster than those derived from older pigs, but there was no difference in the cellular composition of the organoids. In addition, Li et al. [22] reported that compared with enteroids, the proliferation and differentiation rate of colonoids is significantly slower.

The application of airway organoids (AOs) and intestinal organoids (IOs) in virology research

In the past decade, respiratory viruses, and enteroviruses such as PEDV, TGEV, pseudorabies virus and porcine reproductive and respiratory syndrome virus (PRRSV) have caused serious economic losses in the swine industry [89–91]. Different viruses follow different infection mechanisms in their hosts, leading to different disease

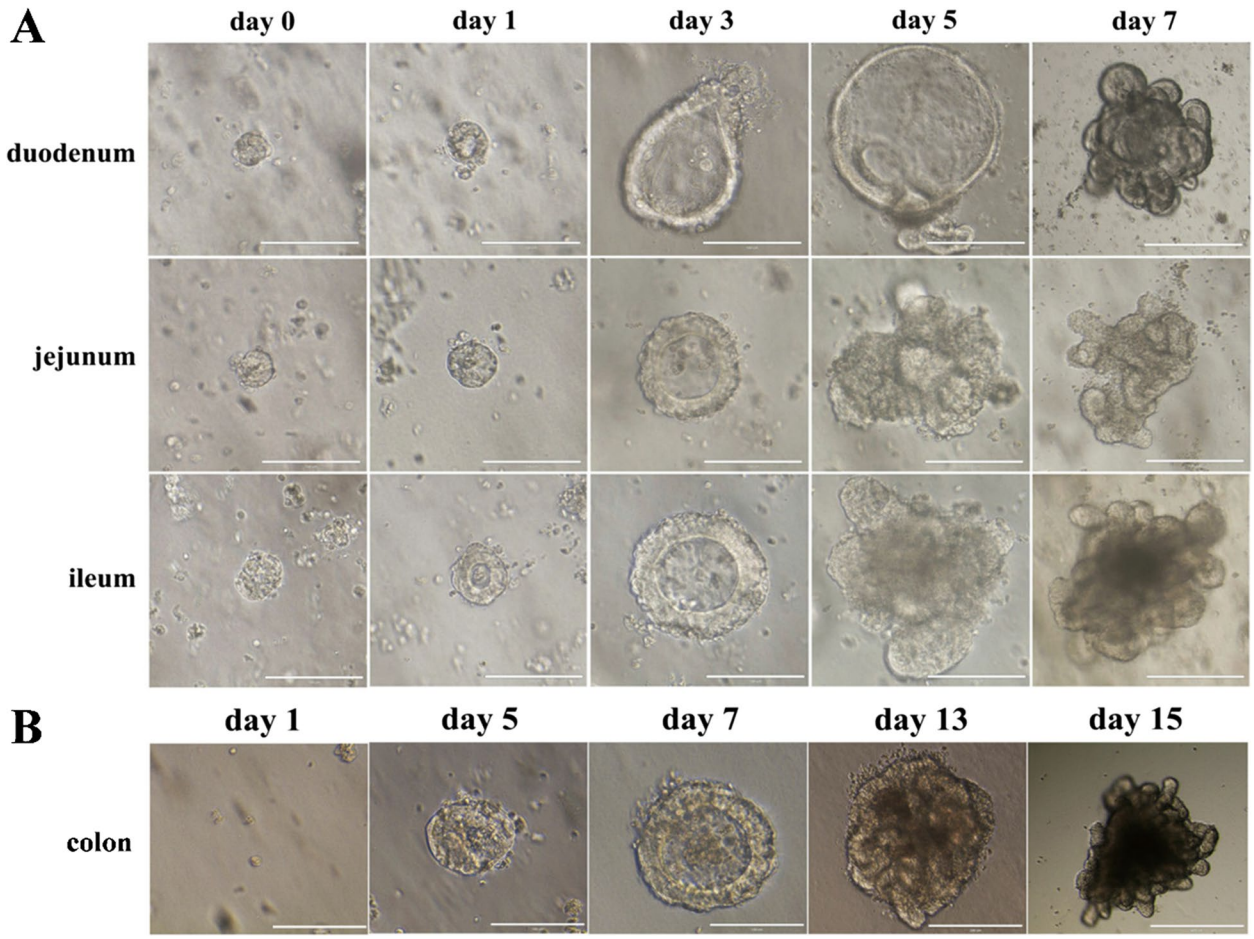

Fig. 3 Porcine ASC-derived intestinal organoids. **A** Representative images of the time course of porcine enteroid differentiation from intestinal crypts. During culture in Matrigel, small spheroids form on day 3 after crypt isolation, gradually mature over time, and form budding-like crypt structures on day 7 [22]. **B** Representative images of the time course of porcine colonoid development. Colonic crypts were isolated and differentiated into budding-like colonoid structures on day 15 [22]

symptoms, so it is important to use the model system that allows for the most accurate recreation of viral infection mechanisms. Cell lines commonly used in previous studies, such as IPEC-J2 and IPI-2I [92], are not susceptible to infection by certain viruses, and there are huge variations in the findings reported from different research groups using the same cell lines [93]. Therefore, there is an urgent need to develop a more physiological culture system for porcine virology research. Currently, porcine organoids, which can simulate better in vivo environments, are widely used in the field.

Airway organoids (AOs) in virology research

AOs provide a reliable platform for studying virus-host interactions in the nasal cavity, proximal lung, and distal lung in vitro. AOs derived from different parts of the respiratory tract provide novel opportunities to study cell tropism of viruses and the immune response of the host. Furthermore, researchers can focus on different cell types or regions in the respiratory tract to target therapeutics of various viruses [94].

AOs have been used extensively for treating respiratory pathogens in humans and rodent species. It has been reported that AOs derived from ASCs can be used to study influenza viruses and respiratory syncytial viruses [55, 95], and airway and lung organoids derived from ASCs and human PSCs have been used in research on severe acute respiratory syndrome coronavirus 2 [20, 96]. Although many viruses, such as PRCoV, swine influenza virus, and PRRSV, can infect respiratory epithelial cells (Table 1), there are few reports on porcine AOs. In 2022, our laboratories successfully generated long-term porcine AOs derived from basal epithelial cells and applied this model to assess the permissiveness of AOs for PRCoV and TGEV infection for the first time [62]. We infected 3D AOs differentiated from the tracheal

epithelium with PRCoV and TGEV and observed that both PRCoV and TGEV could successfully infect pig 3D AOs and mainly infected the secretory cells and ciliated cells. Moreover, the cell-intrinsic response of AOs after viral infection helps to elucidate the pathogenic mechanism of coronavirus, and single-cell RNA sequencing results indicate that PRCoV induces a strong immune response in infected AOs. In most 3D organoid models, the apical side of the cells is facing the inside of the organoid, whereas the apical side of the 2D AO cells is exposed to the air, making it more susceptible to viral infection and therefore more suitable for virologic studies [50]. Likewise, the study found that 2D single-layer AOs were more susceptible to infection than 3D AOs [62]. In addition to 2D AO models, apical-out AO models and organoids seeded on Transwell plates to form organoids and air–liquid interface (ALI) models are helpful for viral infection. The ALI culture system can expose apical cells to the outside environment, so organoids are more susceptible to infection by pathogenic microorganisms than basal-out organoids. However, ALI culture systems rely heavily on the use of tissue culture plate inserts, which are composed of permeable membranes and have limited scalability. As a comparison, apical-out organoids both overcome this limitation and allow the apical cells to be exposed to the outside. However, these culture systems have not yet been applied to porcine AOs [97, 98]. In summary, the porcine AO model can simulate the infection process of the virus in vivo, which lays the foundation for further in-depth exploration of the pathogenic mechanism of respiratory coronavirus.

Intestinal organoids (IOs) in virology research

Diarrhea caused by various intestinal pathogenic microorganisms is a common clinical disease, which seriously endangers human or animal health [106]. In the development of diarrheal diseases, intestinal epithelium is the main target of pathogenic infection [107]. Since traditional in vitro cell models cannot recapitulate the highly complex physiological characteristics of the gastrointestinal tract, IO models that can better simulate the in vivo environment have become important research models. To date, the IOs have been applied for modeling host-bacterial dynamics and interactions between the intestinal epithelium and organisms such as *E. coli*, *Clostridium difficile*, and *Salmonella typhi*. This model has also been utilized to reveal novel and interesting aspects of host-virus interactions as well as features of replication and pathogenesis for enteric viruses [108].

A variety of viruses exist in the porcine intestine, but current studies using IOs primarily focus on porcine enteric coronaviruses, such as PEDV, TGEV, and porcine deltacoronavirus (PDCoV) [109–111] (Table 2). These viruses are the main causes of watery diarrhea in newborn pigs and pose a huge threat to the swine industry and public health [112]. In 2019, our laboratories first studied the susceptibility of porcine intestine to PEDV by establishing porcine IOs from porcine duodenum, jejunum, ileum, and colon. The results showed that PEDV infects multiple types of cells in IOs, including enterocytes, goblet cells and stem cells. Additionally, there are potential differences in the susceptibility of organoids derived from different intestinal segments to PEDV infection; that is, PEDV preferentially infects ileal organoids compared with colon organoids. These findings are consistent with the in vivo results [22]. Furthermore, we found that PDCoV preferably infected the jejunum and ileum, and restricted replication in the duodenum and colon in the established IOs from different intestinal segments in 2020 [21]. Our study provides further evidence that the differences in PDCoV tropism for different intestinal segments are mainly determined by host aminopeptidase N rather than IFN [21]. Additionally, Luo et al. [113] established porcine small IOs to detect the replication of PDCoV in vitro. Double immunofluorescence labeling showed that PDCoV was present in Sox9-positive intestinal cells and Villin1-positive enterocytes. In 2022, our laboratories used IOs to evaluate host epithelial cell responses to infection by three porcine enteric coronaviruses (PEDV, TGEV, and PDCoV), and the results from these studies showed that they act via a contrasting, similar, and unique mechanism to modulate global IFN responses and the expression of antigen-presentation-associated genes [112]. Besides coronaviruses, Lee et al. [114] reported that mammalian orthoreovirus type 3 (MRV3) can infect 2D and 3D porcine jejunal organoids, and that the virus can infect, replicate and activate immune responses in organoids successfully,

Table 1 The respiratory epithelial cells in virus infection

Infected epithelial cells	Virus	Reference
ciliated epithelial cells, secretory cells, type I and II pneumocytes	swine influenza virus	[99, 100]
secretory cells, type I and II pneumocytes	PRCoV	[62, 101, 102]
ciliated epithelial cells, type II pneumocytes	PRRSV	[103–105]

Table 2 Porcine IOs in virus infection

Segment	Model	Pathogen	Reference
duodenum, jejunum, ileum, colon	2D-monolayered organoids	PEDV	[22]
duodenum, jejunum, ileum	3D organoids	PEDV, TGEV	[79]
duodenum, jejunum, ileum	2D-monolayered organoids	PDCoV	[113]
jejunum	apical-out organoids	TGEV	[111]
jejunum	2D-monolayered organoids	PEDV, TGEV, PDCoV	[112]
jejunum	2D-monolayered organoids	MRV3	[114]
jejunum	2D organoids, apical-out organoids	rotavirus	[115]
ileum	3D organoids, 2D-monolayered organoids	TGEV	[110]
ileum	3D orgnoids	rotavirus	[116]
ileum	2D-monolayered organoids	swine acute diarrhea syndrome coronavirus	[117]
ileum	2D-monolayered organoids	rotavirus	[118]

suggesting that 2D and 3D jejunal organoids, as in vitro models, have broader application prospects beyond porcine enteric coronaviruses. Yan et al. [115] reported that apical-out enteroids and 2D filter-grown intestinal epithelial cells but not basolateral-out enteroids were more susceptible to PRV infection. Guo et al. [116] used a 3D IO model to identify the important roles of cell surface glycans in PRV infections.

Because the interior of the IOs is an intestinal cavity and the exterior is wrapped in Matrigel, this structure could limit viral infection. To address this problem, researchers have developed several solutions: (1) Change the 3D structure to a 2D structure. Cell suspension of IOs was resuspended in medium during passage and then plated in Matrigel precoated tissue culture plates or seeded on permeable filter supports using the Transwell system [84, 85, 115, 119]. Compared with plating in tissue culture plates, the Transwell system has advantages in studying cell migration and invasion, and cell–cell interactions [120]. (2) Use microinjection technology to inject microorganisms into the cavities of organoids [121]. (3) Establish the apical-out organoid model. Li et al. [111] have successfully established porcine apical-out IOs, in which TGEV infection can be effective, inducing type I and type III IFN antiviral responses and inflammatory responses.

Limitations of porcine airway organoids (AOs) and intestinal organoids (IOs) in studies with viruses and future perspectives

By recapitulating the intricate cellular organization and microenvironments found in real organs, these organoids are critical for virological research. Although porcine organoids have many advantages, similar to all other model systems, there are important limitations to be considered. Airways and IOs maintain cellular diversity but lack other cell types critical to airway and intestinal function, including immune cells, lymphocytes, endothelial cells, neurons, smooth muscle, and fibroblasts. To address these limitations, many efforts have been made to develop co-cultural organoid models that can be cultured with other cell types [122]. These models allow for a more comprehensive study of interactions between virus-infected epithelial cells and other non-epithelial cells, including monocytes, dendritic cells, lymphocytes, stromal cells, adipocytes, endothelial cells, and neurons. As co-culture systems continue to be refined, we believe that more valuable platforms will be developed for studying pathogenesis beyond the epithelium. Additionally, organoids can be employed in preclinical drug development and toxicology studies, bridging the gap between traditional cell-based assays and animal testing. With the development of this technology, organoids-on-chip will provide a valuable high-throughput screening platform for drug discovery and medical research.

The development of PSC-derived organoids containing mesenchymal cells is also important for increasing organoid complexity. The successful cultivation of porcine PSCs makes it possible to induce the differentiation of stem cells into organoids [78]. In recent years, with the rapid rise of gene editing technology, the potential for generating genetically engineered swine organoids had opened exciting avenues for studying gene function, gene editing, and regenerative medicine applications. At present, studies have successfully transduced lentiviral vectors into porcine IOs, and other researchers have used CRISPR Cas9 technology for gene editing in organoids, but this technology has not been used in porcine organoids [123–126]. The application of bioprinting technology has accelerated the construction process of organoids. Bioprinting can construct 3D living organs and tissues by designing and selectively distributing cells,

bioactive materials, and cytokines to print specific structures of organoids quickly and accurately, which provides a new technology for simulating the in vivo microenvironment to a higher degree [127]. Given that the core and surface of organoids are separated, and the nutrients required for growing cells in the core and the waste produced are limited, microfluidic technologies that can improve material transport and produce more uniform organoids have been developed to overcome these limitations [128]. However, these techniques have not yet been reported in porcine organoids.

Conclusion

Organoids provide an ideal in vitro model for basic research on porcine viral diseases, which is developing rapidly in terms of complexity and standardization. As the field of organoid technology continues to evolve, porcine AOs and IOs are of great value in elucidating the mechanisms of virus recognition by cells, virus entry and replication, and virus-host interactions, and can provide a new theoretical basis for disease treatment and prevention.

Abbreviations

AOs	Airway organoids
IOs	Intestinal organoids
iPSCs	Induced pluripotent stem cells
PSCs	Pluripotent stem cells
ASCs	Adult tissue stem cells
BMP	Bone morphogenic protein
AT1	Type I alveolar epithelial
AT2	Type II alveolar epithelial
PEDV	Porcine epidemic diarrhea virus
PRRSV	Porcine reproductive and respiratory syndrome virus
ESCs	Embryonic stem cells
TGEV	Transmissible gastroenteritis coronavirus
IFN	Interferon
ISCs	Intestinal stem cells
TA	Termed transit-amplifying
IESCs	Intestinal epithelial stem cells
PRV	Porcine rotavirus
PRCoV	Porcine respiratory coronavirus
PDCoV	Porcine deltacoronavirus
ALI	Air–liquid interface
MRV3	Mammalian orthoreovirus type 3
3D	Three-dimensional
2D	Two-dimensional

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Authors' contributions

C.L.: data curation, investigation, and writing. X.D.: data curation and investigation; P.L.: conceptualization, resources, validation, and supervision. X.L.: conceptualization, supervision, edition, and revision. All authors have read and approved the final manuscript.

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Ethics approval and consent to participate

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Consent for publication

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Competing interests

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