Farm and Companion Animal Organoid Models in Translational Research: A Powerful Tool to Bridge the Gap Between Mice and Humans

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Animal organoid models derived from farm and companion animals have great potential to contribute to human health as a One Health initiative, which recognize a close inter-relationship among humans, animals and their shared environment and adopt multi-and trans-disciplinary approaches to optimize health outcomes. With recent advances in organoid technology, studies on farm and companion animal organoids have gained more attention in various fields including veterinary medicine, translational medicine and biomedical research. Not only is this because three-dimensional organoids possess unique characteristics from traditional two-dimensional cell cultures including their self-organizing and self-renewing properties and high structural and functional similarities to the originating tissue, but also because relative to conventional genetically modified or artificially induced murine models, companion animal organoids can provide an excellent model for spontaneously occurring diseases which resemble human diseases. These features of companion animal organoids offer a paradigm-shifting approach in biomedical research and improve translatability of *in vitro* studies to subsequent *in vivo* studies with spontaneously diseased animals while reducing the use of conventional animal models prior to human clinical trials. Farm animal organoids also could play an important role in investigations of the pathophysiology of zoonotic and reproductive diseases by contributing to public health and improving agricultural production. Here, we discuss a brief history of organoids and the most recent updates on farm and companion animal organoids, followed by discussion on their potential in public health, food security, and comparative medicine as One Health initiatives. We highlight recent evolution in the culturing of organoids and their integration with organ-on-a-chip systems to overcome current limitations in *in vitro* studies. We envision multidisciplinary work integrating organoid culture and organ-on-a-chip technology can contribute to improving both human and animal health.

Keywords: animal organoid, comparative medicine, One Health, public health, translational research

INTRODUCTION

Organoids are three-dimensional (3D) cellular structures that possess self-organizing and self-renewing properties, resembling the structures and functions of the originating donor organs (1, 2). Organoids can be generated from adult stem cells (ASCs) (3, 4) or pluripotent stem cells (PSCs) such as embryonic stem cells (ESCs) (5, 6) and induced pluripotent stem cells (iPSCs) (7, 8). Both ASCs and ESCs are undifferentiated cells that are either present among differentiated cells in a tissue or organ or derived from the inner cell mass of blastocysts, respectively (9). iPSCs are derived from differentiated somatic cells which are converted to regain pluripotency (9). While ASCs can only differentiate into distinct cell types of their original tissue, ESCs and iPSCs can differentiate into all cell types within the body (9). Further, organoids can be generated from tissue samples from both healthy and diseased animals. Thus, they can effectively model both normal and pathological conditions in *in vitro* environment (10–12). At present, development of farm and companion animal organoids has only been reported using ASCs (2). In this paper, the term farm animals refers to common production animals in agriculture such as pig, cattle, sheep, horses and chickens. The term companion animals refers to common domesticated animal species that have close daily relationship with humans (13) such as dogs, cats, and rabbits.

Although many studies have been conducted on organoids in humans and mice (5, 14, 15), studies focusing on organoids in farm and companion animal species have been gaining attention in various fields including veterinary and translational medicine as well as biomedical research. This is because companion animals that develop spontaneous chronic disorders similar to those of humans have been suggested to serve as better models of human patients than traditional murine models where the disorders are usually genetically or chemically induced (16, 17). This is a paradigm-shifting approach in biomedical research following on sometimes poor predictive values of the preclinical murine disease models for human clinical trials (16). Natural disease models of companion animal organoids possess great potential in *in vitro* to *in vivo* translatability, which can complement human organoid studies through their application in comparative medicine. Studying zoonotic diseases in farm animals have also gained promise in contributing to human health because many of them serve as key reservoirs for infectious diseases which result in significant morbidity and mortality in humans (1). Farm animal organoids can offer a useful model to investigate mechanisms of disease development and host defense mechanisms, which are relevant for achieving improved management strategies against pathogens with public health concerns. Additionally, relevance of farm animal organoids to human health is extended to the field of agricultural productivity through improved animal health, feed efficiency and reproductive success, which can contribute to food security to support an ever growing world population.

This multi- and trans-disciplinary approach to achieve optimum health outcomes in both humans and animals has become more popularly referred to as One Health, which recognizes that the health of humans, animals and the environment are closely linked to each other (18–20). While One Health is not a new concept for zoonotic diseases and food security, application of this initiative to naturally occurring chronic conditions between animals and humans has gained more attentions in order to achieve better predictive values during preclinical studies (16). One Health initiatives focusing on research that could benefit both veterinary and human medicine could greatly improve relevance and efficacy of *in vitro* studies in translational and biomedical research in humans (18, 19). Studying farm and companion animal organoid models enables detailed mechanistic investigation of normal physiology and development of organs and tissues (11, 21, 22), mechanisms of diseases (23–25), toxicity and drug efficacy testing (26–28), and investigation of novel diagnostics and therapeutics (3, 29–31), which all could play an important role in bridging a gap between conventional murine models and humans. Animal organoids derived from different species would provide an effective tool to evaluate cross-species variations in disease susceptibility or species-specific infectivity of zoonotic and zooanthroponotic pathogens, providing a great alternative to animal models for infection studies.

In this paper, we review a brief history of organoids and recent advances in organoid culture systems in different animal species, focusing mainly on farm animals which carry significant economic and public health impacts and companion animals which naturally develop spontaneous diseases similar to humans. We also describe key roles that farm and companion animal organoids can play in the context of One Health initiatives with a particular focus in their potential applications to comparative medicine as well as in their relevance to public health and food security in agricultural production animals. We also describe advantages and limitations of 3D organoid culture systems and different culture platforms to overcome some of these limitations such as organoid-derived two-dimensional (2D) monolayer culture systems and organ-on-a-chip technology. Finally, we discuss future perspectives of multidisciplinary work integrating organoid culture and organ-on-a-chip technology in veterinary and translational medicine.

DEVELOPMENT OF FARM AND COMPANION ANIMAL ORGANOID MODELS: HISTORY AND RECENT UPDATES

A Brief History From Cell Aggregates to 3D Models

While studies of organoids have grown dramatically during the past decade, the history of 3D organoid cultures started as early as 1907 when self-organizing and regenerating capacity of dissociated sponge cells were demonstrated for the first time (32). Later, successful generation of different types of organs from dissociated amphibian pronephros (33) and chick embryos (34) were described in 1944 and 1960, respectively. These results highlighted the importance of internal self-organization rather than external induction in the process of organogenesis. In 1981, isolation and establishment of PSCs from mouse embryos were

reported for the first time (35, 36). Following this study, isolation and culturing of human PSCs were first achieved from blastocysts in 1998 (37). These studies pioneered stem cell research, together with subsequent studies which reported establishment of iPSCs from reprogrammed mouse and human fibroblasts (38, 39) and human somatic cells (40) in mid-2000s.

As described earlier, organoids can be generated from ASCs (3, 4), ESCs (5, 6), or iPSCs (7, 8). Development of 3D organoids began to increase in the late 2000s and early 2020s. Two landmark studies reported generation of cerebral cortex tissue from mouse and human ESCs (41) and intestinal organoids from a single intestinal mouse ASC (42). Although murine and human organoids remain to be the most extensively investigated models, organoids of other animal species have been gaining more attention in recent years including farm and companion animals. **Supplementary Table 1** provides a summary of previous studies related to 3D organoids in humans and various animal species.

Farm and companion animal organoids developed to date are summarized in **Table 1**. Early studies include the successful generation and transplantation of canine intestinal organoids (3) and the creation of chicken embryo-derived intestinal organoids (80). Since then, successful development of organoids and mechanistic studies utilizing organoids have been reported on a variety of tissues in various farm and companion animal species. At the same time, inter-species variations in optimum culture conditions have been noted in some tissues (44). Knowledge of such variations is important when considering translational applications of animal organoids. In the following subsections, we will provide the most recent updates on farm and companion animal organoids organized by organ systems and discuss their potential as One Health initiatives and in translational medicine, i.e., linking *in vitro* data to *in vivo* studies.

Organoids of Digestive Systems

Farm and companion animal organoid models of digestive systems that have been described to date include those derived from tissues of esophagus, small intestine, and the colon. Successful development of intestinal organoids, referred to as enteroids or colonoids depending on the origin of the tissue, have been reported in various animal species.

In farm animals, porcine intestinal organoid model was first reported using distal duodenum and proximal jejunum of neonates (45). Since then porcine organoid models have been successfully developed from various sections of gastrointestinal tract (49, 50) such as esophageal submucosal gland (43), duodenum (23, 51–54), jejunum (23, 46, 51, 53–63, 66), ileum (23, 44, 47, 51, 53, 54, 64, 65), and the colon (48, 51, 53, 67). Several studies reported bovine organoids derived from jejunum (10, 60), ileum (44, 71, 73, 74), and the colon (72). Intestinal organoids of these species have been used in applied research such as investigation of epithelium-microbe interactions and modeling of bacterial, viral and parasitic infections (23, 51, 60, 74), some of which have important public health implications due to the risk of zoonotic infections. On the other hand, only a few studies can be found on equine and ovine

TABLE 1 | Summary of normal, diseased, and applied organoid models described in selected farm and companion animal species to date.

Animals	Models				
	Organs	Normal	Diseased	Applied	References
Farm animals					
Pig	Esophagus	✓			(43)
	Intestine	✓			(44–48)
		✓	✓		(49)
		✓		✓	(23, 50–65)
			✓		(66, 67)
	Gallbladder	✓	✓		(68)
	Testis	✓			(69, 70)
Cattle	Intestine	✓			(10, 44, 71, 72)
		✓		✓	(60, 73, 74)
	Mammary gland	✓			(75)
	Oviduct	✓			(76)
Sheep	Intestine	✓			(44)
	Pancreas	✓		✓	(77)
Horse	Intestine	✓			(44, 78)
	Uterus	✓		✓	(79)
Chicken	Intestine	✓			(44, 80–83)
		✓		✓	(84, 85)
Companion animals					
Dog	Intestine	✓			(11, 44, 86)
		✓	✓		(87)
		✓		✓	(3)
	Liver		✓	✓	(88, 89)
	Kidney	✓			(90)
	Bladder		✓	✓	(91, 92)
	Prostate		✓	✓	(12)
	Skin	✓			(93, 94)
	Thyroid		✓		(95)
Cat	Intestine	✓			(44)
		✓		✓	(96)
	Liver	✓		✓	(4, 29)
Rabbit	Intestine	✓			(97)
		✓		✓	(98)

Normal = Organoids derived from tissues/cells of normal animals.
Diseased = Organoids derived from tissues/cells of animals which developed spontaneous diseases.
Applied = Normal or disease model organoids that are used for applied research such as toxicity and efficacy testing of chemicals, drugs, and radiations, investigation of host-microbe interactions through bacterial co-culture, and validation studies as a source of cell transplantations and gene therapy.

organoids. Horse intestinal organoids were successfully cultured and expanded into 3D structure from jejunum (78) and ileum (44). Sheep intestinal organoids have been generated from ileum (44). Chicken intestinal organoids have been developed from small intestine (84), and more specifically from the sections of duodenum (85), jejunum (81), and cecum (44) in other studies. Furthermore, embryonic small intestines have been used to develop intestinal organoids in chickens (80, 82, 83, 85).

In companion animals, canine intestinal organoids have been successfully generated from duodenum, jejunum, ileum, and the colon using not only whole intestinal tissue sections but also much smaller endoscopic biopsy samples (3, 11, 44, 86, 87). The successful generation of disease model organoids has also been described using tissues derived from dogs with spontaneous gastrointestinal diseases such as inflammatory bowel disease and colorectal adenocarcinoma (87). Since these diseases have similar presentation and treatment options to those in humans, studies on canine enteroids and colonoids have potential application as a useful model for chronic gastrointestinal diseases (11). Cat ileum- and colon-derived organoids have been published with a potential to be used as a feline coronavirus infection model (44, 96). The establishment of rabbit intestinal organoid models have been reported using duodenal and cecal tissues (97, 98). These models provide a useful tool in biomedical research such as studies of human bacillary dysentery, which have been facing a major challenge due to a lack of representative animal models until recent development of infant rabbit infection models (99).

Conditioned medium containing "WRN factors" have been used as an effective growth medium in development and long-term maintenance of intestinal organoids in both humans and a variety of animals including pigs, cattle, sheep, horses, chickens, dogs, and rabbits (44, 100). The term WRN factors refers to Wnt3a, R-spondin and Noggin (44). Both Wnt3a and R-spondin are Wnt signaling pathway activators, which are essential for crypt proliferation, whereas Noggin is a bone morphogenetic protein (BMP) signaling pathway inhibitor which induces expansion of crypt numbers (101). Supplementation of these factors to the organoid culture media is considered critical in both human and animal organoids (100). However, relatively short-term maintenance, i.e., low passage number, of intestinal organoids was reported in cats (44). Optimization of culture conditions has been attempted to enhance productivity. For instance, addition of glycogen synthase kinase 3 (GSK-3) and rho-associated kinase (ROCK) inhibitors to the conditioned media containing WRN factors have been described in horses and dogs to improve intestinal stem cell survival (78, 87).

Organoids of Hepatobiliary Systems

Besides in mice (102) and humans (103), the establishment of liver organoids has only been reported in dogs (88, 89) and cats (4, 29). A long-term hepatic organoid culture in companion animals was first reported in dogs using biopsy-derived tissue (88). Following this study, the establishment of a long-term feline hepatic organoid culture was described using ASCs derived from post-mortem tissue samples (4). In these studies, fresh or frozen liver tissues were cultured in Matrigel droplets and R-spondin-1-based culture medium, and they were successfully cryopreserved. It is important to note that the study demonstrated the importance of Wnt3a supplementation to the culture media to achieve long-term stable proliferation of canine hepatic organoids (88), This is in contrast to murine, human and feline hepatic organoids where the addition of Wnt3a is not needed for long-term culture (4, 102, 103).

Dogs with copper toxicity due to the COMMD-1 gene deficiency could provide the best preclinical model for investigation of inherited copper toxicosis in humans, such as Wilson's disease. Hepatic organoids from these dogs can be used to investigate the disease pathophysiology and new treatment strategies in copper toxicosis in both dogs and humans (88, 89). Canine liver organoids could also serve as a useful tool for investigation of infectious diseases such as viral hepatitis, which is also seen in humans (104). A greater predisposition for lipid accumulation in feline liver compared to humans would make feline hepatic organoids an excellent model for investigation of human lipid-storage diseases such as hepatic steatosis (4, 29).

Porcine gallbladder organoids are the only animal model that have been described concerning the biliary system (68). The study developed both normal and disease model organoids using tissues of gallbladder and cystic ducts and evaluated their functions such as anion and fluid secretions and mucus production. Since some pigs are born with naturally occurring cystic fibrosis, which results in a life-shortening multi-organ disorders including gallbladder due to genetic mutations and causes significant morbidity in humans, affected pigs present a good model for investigation of disease pathogenesis.

Organoids of Pancreas

Sheep pancreatic duct organoids were generated using pancreatic duct cells from healthy fetus (77). The organoid was cultured in Matrigel containing EGF, R-Spondin-1, FGF10, and Noggin in Wnt3a-conditioned medium (77), which is a similar system described in mouse (105). Furthermore, this study demonstrated an importance of an appropriate amount of copper supplementation to the medium in order to achieve formation and growth of sheep pancreatic duct organoids (77). It is known that copper plays a pivotal role in maintaining normal physiology in mammalian cells, yet its optimal concentrations for tissue or organ development have not been determined. The finding in this study may provide a useful *in vitro* model to investigate the role of copper and its effective concentration in the context of organ development and physiology.

Organoids of Urinary Systems

In a dog kidney organoid, budding tubule-like structures were formed from small pieces of renal tissues obtained from a euthanized dog (90). The study demonstrated that stem cell self-renewal and differentiation into tubular cells are promoted by high cell density, which induces signal transducer and activator of transcription-3 (STAT3) expression. Further, it was shown that treatment with either the STAT3 inhibitor AG490 or the STAT3 activator lipopolysaccharide reduced or increased colony forming efficiency in a dose-dependent manner, respectively. The findings would be useful for the development of normal human kidney stem cells considering their clinical applications and research such as investigations of nephrogenesis (106), nephrotoxicity screening (26), disease modeling, and regenerative medicine applications (107).

Bladder organoids were initially studied as an *ex vivo* model using mucosal biopsies obtained from porcine bladder (108). It was developed for preclinical experimental research such

as pharmacology and toxicology screening, with the potential to replace animal models. More recently, neoplastic bladder organoid models have been described in dogs (91, 92). In these studies, bladder organoids were derived from the cells taken from urine samples. The disease model organoids were used to test effects of various anti-cancer drugs, demonstrating their use as a preclinical model for drug efficacy testing (91). Although normal bladder organoids have not been reported in animals other than mouse (109), such models in large animals would be useful for studies on normal physiology and host responses to external stimuli such as exposure to pathogens and antimicrobial drugs (110). Moreover, canine organoid models of bladder cancer would provide an important research tool for translational study including diagnostic biomarkers and optimization of therapeutic options as it resembles muscle-invasive bladder cancer in humans (91).

Organoids of Male Reproductive Systems

Farm and companion animal organoid models that have been described to date concerning male reproductive systems include those of prostate and testis. For prostate, prostate cancer organoids have been reported using cancer cells in urine samples obtained from prostatic cancer bearing dogs (12). Similarly to bladder cancer organoids (91), the organoids were used to test effects of anti-cancer drugs and irradiation (12). Prostate cancer also occurs in humans and it bears poor prognosis in dogs (12). Therefore, the canine prostate cancer organoids would provide a useful translational model for advanced prostate cancer in humans, providing fresh insights into cancer treatment.

Testicular organoids have been successfully established from testicular tissues of pig, mouse, macaque (monkey), and human, where cells were harvested using a two-step enzymatic digestion process (69). Testis-specific cell types such as germ cells, Sertoli cells, Leydig cells, and peritubular myoid cells, were identified in these organoids. More recently, porcine organoids with vascular structures was reported for the first time allowing the coexistence of various cell types in organoid culture system (70).

In general, pigs are considered as superior animal models to mice due to their physiological and anatomical similarities to humans when considering biomedical and pharmaceutical studies (70). Therefore, studies on porcine testicular organoids possess wide application potentials for a variety of clinical and preclinical research including investigation of causes and treatment of male infertility and strategies to preserve fertility potentials in patients undertaking chemotherapy. Although testicular organoids have not been developed in other large animals, such studies would attract strong interests in the field of breeding industries which deal with high-value specimens such as horses and cattle (76). Moreover, farm animal organoid models provide useful tools to investigate reprotoxic effects of environmental stimuli or exogenous substances such as high ambient temperature, nutrition and water quality, and various chemicals such as antibiotics and food preservatives, which in turn would affect reproductive performance of economically important animals.

Organoids of Female Reproductive Systems and Accessory Organs

Organoids of female reproductive systems that have been described to date concerning farm and companion animals are limited to those of endometrium in horses (79) and oviduct in cattle (76). The latter was only shown as a preliminary unpublished data (76). Equine endometrial organoids have been generated under similar conditions to those described for mice and humans (111, 112). However, the study demonstrated that R-spondin-1 and hepatocyte growth factor were not essential in growth of equine endometrial organoids, which was different from murine and human organoids. The horse endometrial organoids were established from both fresh and frozen-thawed endometrial biopsy samples. They retained the ability to maintain 3D structures and responded to hormonal stimuli, providing a novel *in vitro* culture model for evaluation of endometrial physiology, pathophysiology, and potential therapeutics for uterine diseases.

As for accessory organs of the female reproductive systems, development of mammary organoids have been reported using udder tissues in cattle (75). Another approach to establish a 3D culture model of primary bovine mammary epithelial cells has been described using cells obtained from milk (113). This promising model presents a great advantage of using non-invasively collected cells unlike the other studies. The technique will greatly contribute to preservation of animal welfare. Besides these studies, there is a single study which reported the development of a 3D culture model from caprine mammary gland tissue as a part of developing *in vitro* viral infection models (114).

Few studies have been reported on 3D culture of mammary tissue in pigs, although lactation of sows presents critical issue for survival of piglets. While further studies are needed to improve organoid technology in these species, the establishment of a robust technique for these species would provide a great insight into tissue biology such as development of mammary gland, mechanisms of lactation, and mechanisms of and host responses to bacterial and viral infections. Advances in the technology would not only contribute to welfare of these animals but also have economic impacts on farm animal industry by improving milk production.

In companion animals, dogs has been suggested as an excellent animal model for human breast cancer due to many clinical and molecular similarities such as spontaneous development of the disease with an intact immune system, age of onset, hormonal etiology, disease progression and outcomes and gene expressions (115). Organoids of spontaneously occurring canine mammary tumors could present great potential for cancer research, drug development, and treatment trials by serving as a good preclinical model.

Organoids of Integumentary System

Studies to develop 3D skin models in animals originally started for use in the pharmaceutical and cosmetic industries (116). Initially, pig skin was considered a good model to test drugs of topical formulation (117). At present, availability of animal

skin models are very limited due to the development of human 3D skin models and most of them are developed for use in veterinary medicine or comparative biology research (116). Nonetheless, investigation of animal skin models possesses some values in studies on comparative biology and skin diseases such as bacterial and atopic dermatitis in dogs, which hold similar clinical course and presentation to those in humans (118). Additionally, skin models of farm animals, especially those of which skin components may be included for human consumption such as poultry, may serve as a good tool to evaluate impacts of toxic substances on skin.

The studies on canine keratinocyte and epidermal organoid culture systems have been reported (93, 94). In the study, keratinocyte organoids from both microdissected interfollicular epidermis and hair follicle tissues were successfully established using culture medium containing Noggin, R-spondin-1, and Rho kinase inhibitor (93). The authors reported that hair follicle-derived organoids grew best when the medium was supplemented with fibroblast growth factor (FGF) 2, and FGF10, whereas interfollicular epidermis derived organoids grew best when the medium was supplemented with epidermal growth factor (EGF), FGF10, Forskolin and transforming growth factor (TGF) β inhibitor. The study also demonstrated that Wnt3a was not essential to grow canine keratinocyte organoids, thus was removed from the expansion media. Furthermore, an attempt to optimize the canine keratinocyte organoid system and develop an epidermal organoid revealed that supplementation of Wnt3a and other factors to differentiation medium did not have beneficial effects on inducing differentiation of the interfollicular epidermis-derived organoids closer to normal epidermis (94).

Organoids of Endocrine Systems

Thyroid organoids have been successfully generated from canine follicular cell thyroid carcinomas (FTCs) (95). Canine FTC-derived organoids conserved the expression of proteins involved in iodine uptake, and hence opened new research possibilities as an *in vitro* model to modulate iodine uptake and improve radioiodine therapy for thyroid cancer. While development of thyroid cancer following transplantation of genetically engineered oncogenic thyroid organoids has been described in mouse (119), this is the first organoid model which is derived from spontaneous thyroid cancer in any species (95). Since FTCs, although rare, do occurs in humans (120), canine spontaneous cancer organoids would serve as a useful model for preclinical studies on human FTCs.

ROLES OF FARM AND COMPANION ANIMAL ORGANOIDS IN ONE HEALTH INITIATIVE

Public Health: Farm Animal Organoids as a Tool for Studying Zoonotic Infections

There are many shared health threats between humans and animals. They include not only zoonotic infectious diseases but also non-infectious diseases such as metabolic disorders, chronic inflammatory disorders, and neoplastic diseases, antibiotic resistance, and environmental contamination such as toxic agents in the air, soil and drinking water. Among these, zoonotic infectious diseases are particularly important in the context of public health.

With growing global human populations and advancement in transportation systems, points of contact between humans and animals increase (18). It is now known that many animals including both wildlife and domestic species play an important role in the spread of zoonotic diseases as potential reservoirs. For instance, severe acute respiratory syndrome (SARS), which resulted in a major outbreak worldwide in early 2000s, and the current ongoing pandemic of the new coronavirus 2019 disease (COVID-19) are caused by previously unrecognized coronaviruses which are most likely evolved from bats as a natural reservoir (SARS-CoV and SARS-CoV-2, respectively) (18, 121). A recent study demonstrated susceptibility of bat intestinal organoids to SARS-CoV-2 and sustained viral replications in organoids, which could not have been achieved using bat cell lines, by establishing an *in vitro* infection model (122). This study demonstrated previously unculturable novel viruses in established cell lines could be cultured in organoids. Additionally, SARS-CoV-2 has been shown to infect various domestic and wildlife species other than humans including dogs, cats, minks, ferrets, hamsters, lions and tigers (121). Animal organoids derived from different species would provide an effective tool to evaluate cross-species variations in viral susceptibility or species-specific infectivity of viruses with zoonotic and zooanthroponotic potentials, providing a great alternative to animal models for infection studies.

Besides wildlife, domestic animal species also play an important role in public health. Some pathogens that infect livestock, causing clinical or subclinical diseases, also infect humans and cause clinical diseases in susceptible individuals or in a group of people, leading to epidemics, e.g., *Salmonella typhimurium*, *Escherichia coli*, *Toxoplasma gondii,* and *Giardia duodenalis*. To investigate host-pathogen interactions and disease responses upon exposure to potential pathogens, infectious disease models have been created by co-culturing healthy intestinal organoids from livestock with pathogens or bacterial toxins. The reported models include those infected with *Salmonella typhimurium* and *Toxoplasma gondii* in pigs and cattle (60) and Enterotoxigenic *Escherichia coli* (ETEC) in pigs (23), and those exposed to Shiga toxins produced by Enterohemorrhagic *Escherichia coli* (EHEC) in cattle (73). These models offered a useful alternative to animal models, which require a large amount of resources such as money, labor and housing facilities. Besides enabling significant cost reduction, they provided a new insight for mechanisms of disease development and effects of virulence factors on host epithelium through creating a model closely mimicking the *in vivo* tissue. Also, since many pathogens have host specificity, farm animal organoids could serve as a good model to study host-pathogen interactions and protective mechanisms of hosts when organoids of asymptomatic carrier species are used. Furthermore, infection models could serve as useful tools for screening efficacy and adverse events of vaccines and antibiotics against infectious diseases, thus helping to improve public health.

Food Security: Farm Animal Organoids as a Tool for Improving Agricultural Production

Humans are always in danger of falling into a severe food shortage due to the increase in the world population, climate change caused by global warming, and the spread of infectious diseases [20, 123]. It has been proposed that more than 50% increase in food production compared to the production levels in 2012 will be necessary to fulfill the needs of global population by 2050 [20].

To date, organoids of digestive systems have been developed in multiple farm animal species. They have been used for studies concerning nutrition, toxins and infectious diseases with a great expectation to improve productivity in one way or another. For example, porcine intestinal organoids have been used to evaluate effects of different nutrients such as dietary fiber [65], glutamate [59], and vitamin A [63] as well as fungal-derived toxins such as Deoxynivalenol (DON) [55] on development and homeostasis of intestinal epithelial cells. Organoids of ovine pancreatic duct have been used to investigate the effect of dietary copper on organ growth [77]. Organoids of chicken intestine have been used to assess how different chemicals such as growth factors, hormones, vitamins, pesticides, enterotoxins, endotoxins, mycotoxins and *Lactobacillus acidophilus* bacteria affect the cells [84, 85]. All these studies have important implications for improving feed efficiency by providing potentially useful information to formulate a superior dietary composition to achieve enhanced gut health and tissue development, hence helping to achieve improved animal growth.

Moreover, studies on infectious diseases using organoids would not only contribute to improving production efficiency of farm animals through decreased morbidity and mortality but also minimize economical damage for infectious disease management. In addition to the bacterial and parasitic infection models described in the previous section, several viral infection models have been established using porcine intestinal organoids. They include infections with enteric coronavirus (ECoV) such as porcine epidemic diarrhea virus (PEDV) [50, 51], porcine deltacoronavirus (PDCoV) [53], and transmissible gastroenteritis virus (TGEV) [62]. Although these viruses have significant economic impact in the pig industry due to high morbidity and mortality in piglets [124], the lack of effective *in vitro* models has been a limiting factor for in depth studies on these diseases. This was mainly due to poor infectivity and replicability of viruses in *in vitro* culture systems [62]. However, with the emergence of organoid technology and with the development of effective *in vitro* infection models, it is expected that these models would greatly help scientists to understand pathogenesis of these important diseases. Knowledge obtained through organoid studies would offer new insights to improve herd health management strategies in the long run, contributing to the reduction of economic losses as well as the increase in agricultural production.

Although organoids of reproductive systems and applications thereof in farm animals remain very limited, their potential for improving reproductive success are unlimited as discussed in the earlier sections. Improved milk production would not only have great economic benefit to dairy industry, but also to other industries dealing with food production animals as both quality and quantity of lactation determine survival of offspring, which is critical for sustainability of the industry. Similarly, studies relating to male and female fertility and maintenance of pregnancy to term also comprise important aspects of reproductive success. Therefore, further studies must be conducted to develop robust organoid culture techniques in these tissues in major food production animals.

Comparative Medicine: Companion Animal Organoids as a Tool for Improving Human Health

Organoids derived from human patients would be a superior tool to those of animal counterparts when applications for personalized medicine such as patient-specific drug efficacy testing and cell transplantation to individual patient are set as the primary goal of organoid studies [125]. However, there will still be a gap in translatability between *in vitro* findings and *in vivo* application even using human samples. Companion animals which develop diseases similar to those in humans can play a pivotal role in fundamental studies on pathophysiology or preclinical studies on safety and efficacy of new therapeutics [16]. There would be a value in investigating cross-species similarities and differences in drug safety and efficacy to better predict their performance in human clinical trials as the Food and Drug Administration (FDA) requires preclinical safety and efficacy studies from various animal species in addition to mice prior to human trials [126]. Therefore, using organoids from companion animals with naturally occurring diseases can not only model diseases of humans *in vitro* but select the most promising drug candidates for such diseases. Subsequently, those drug candidates can be tested in companion animals with the disease prior to human clinical trials. This pipeline of preclinical studies using animal patients which suffer from naturally occurring diseases will also allow such veterinary patients to receive benefits of new potential therapeutics while they provide the data which bridge between preclinical and human clinical trials. Moreover, studies using organoids from various animal species (i.e., cross-species examination) would contribute to reducing failure rates in human clinical study, thus accelerating approval of new drugs. This approach could also contribute to achieving strong ethical benefits according to 3R principle (reduce, refine, replace) by avoiding, or reducing if not complete, the use of laboratory animals for the purpose of *in vivo* preclinical trials in new drug development.

A large number of case-derived tissue samples are necessary to create various types of disease model organoids and use them for research with reasonable reliability. With the recent increase in the number of companion animals who receive life-long veterinary care, reports on animal diseases which have similarities to those seen in humans have been growing [16, 127]. Therefore, strategic collaboration with veterinary medicine would accelerate accumulation of disease samples which are common between humans and animals, helping to develop a solid base, or so called disease organoid biobanks, for translational research.

EVOLUTION OF ORGANOID CULTURE SYSTEMS

Farm and companion animal organoids have a great potential for contributing to human health based on One Health initiatives. Organoids would continue to strengthen their presence in the fields of biology and life science as the technology becomes more robust and efficient in the next decades. The 3D organoid culture system is a relatively new technique and possesses unique characteristics from those of traditional 2D cell culture system. Other advanced culture systems such as organoid-derived 2D monolayer and organ-on-a-chip systems have been emerging to overcome the weakness in current organoid systems (**Figure 1**). These advanced culture platforms offer great potential to widen the scope of research through dissociation of 3D organoids into single cells and their integration into the advanced culture systems.

Traditional 2D Cell Culture System

2D cell culture systems including primary cells and cell lines have been widely used as *in vitro* models since the early 1900s (128). Primary cell culture uses the cells which are freshly isolated from a donor. Although these cells are considered to behave similarly to those in the living organism due to the fact that the cells are cultured shortly after they are harvested from a donor, they have limited proliferation potential and tend to lose their phenotypic characteristics over time (26). On the other hand, immortalized cell lines can be stably cultured for long time and have homogenous traits. Cell lines are usually derived from tumor tissues or created via *in vitro* viral transformation to become artificially cancerous (2). Although these cell lines can grow and divide continuously, tumor-derived cell lines usually have chromosomal mutations which affect cellular metabolism and physiology (129, 130). Such abnormalities also evolve during passage, leading to problems with reproducibility (130). Additionally, immortalized cell lines may not fully represent normal traits of the original tissue because the process of immortalization, or changes in genomic contents, may alter cellular characteristics and functions (26). Hence, an alternative *in vitro* model that can sustain multiple passages over prolonged periods while maintaining normal cellular functions and characteristics of the original tissue and possibly a living organism has been sought after.

3D Organoid Culture System

3D organoid culture system is a newly developed technique which provides more physiologic cell populations and microenvironment to perform biomedical research. 3D organoid culture relies on proliferation and differentiation of stem cells (131); therefore, organoids retain "stemness" as the original organ (2). They contain multiple cell types which collectively exhibit organ-like phenotypes, architectures, and functions including cell-cell interactions similar to those observed in the original tissue (131). For instance, intestinal organoids have multicellular composition with intestinal stem cells, enterocytes, Goblet cells, Paneth cells, and enteroendocrine cells (101), expressing polarization as an intestinal epithelium (5). These cells are well differentiated and can reproduce the fine structures of intestinal lumen consisting of crypts and villi (5, 42) and cell junctions, which are common to *in vivo* tissues, enabling effective cell-cell communication through the exchange of ions, secretion of vesicles, and transduction of electrical stimulations (128, 132). Moreover, these organoids show the biological functions of gut, such as absorption and secretion (5, 42). These features make organoids very useful for specialized experiments such as swelling assays that simulate fluid secretion in the body (133).
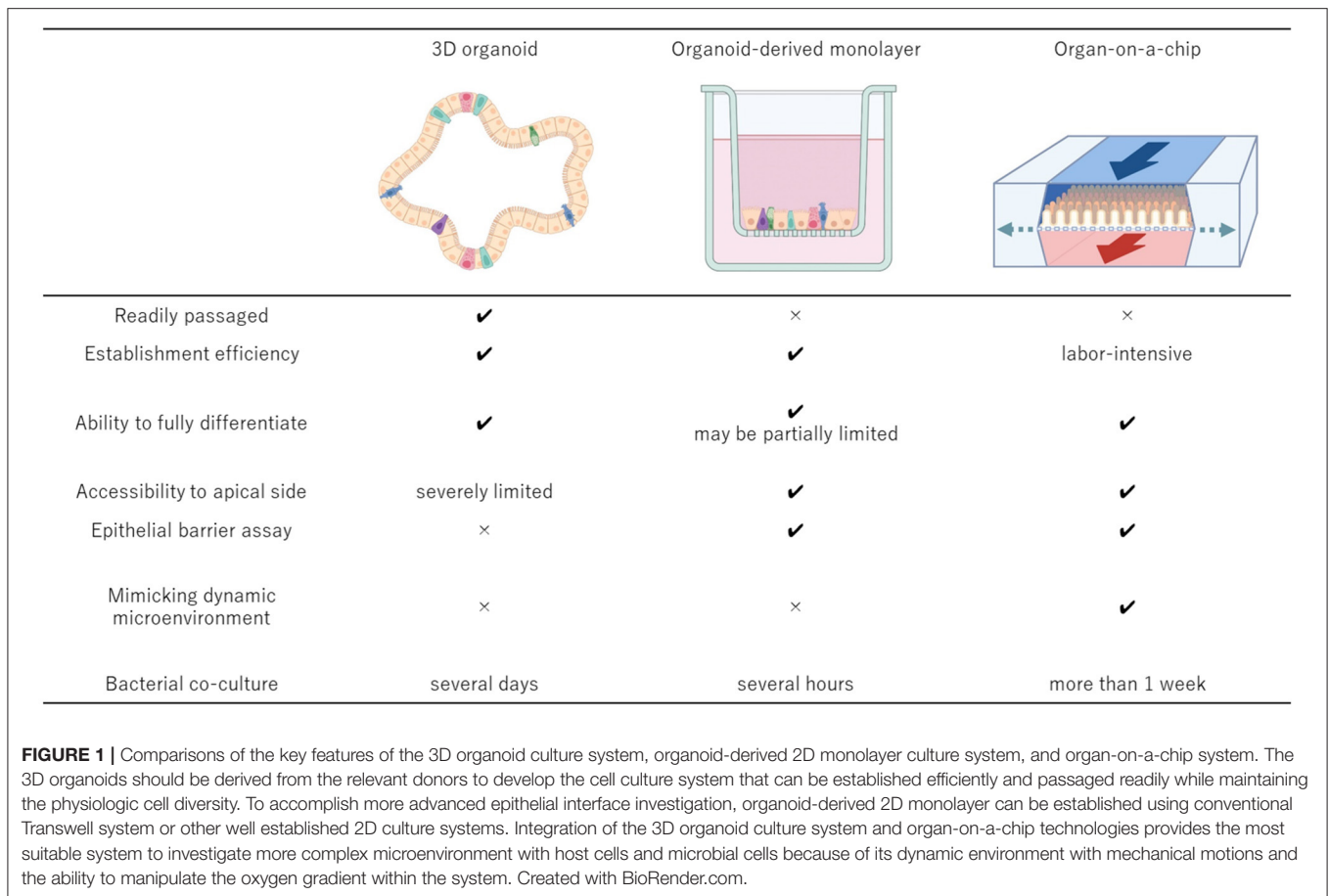
Another benefit of 3D organoid culture is the fact that they can be isolated from both normal and diseased donors. Since organoid cultures maintain the traits of the original tissue, organoids derived from tissues of clinical cases can recapitulate the pathology of disease (133–136). Therefore, patient-derived organoids are very useful natural models for studying pathophysiology of the disease (135, 136) and establishing disease-specific living biobanks for large scale genetic analyses such as in cases of neoplasia (137–140). Patient-derived organoid models also present great potential for precision medicine such as preclinical drug efficacy testing and pharmacodynamics studies (141, 142).

The 3D organoid culture can be expanded for long time on the scale of months or even 1 year or longer, which has been reported in multiple studies involving various tissues such as gastrointestinal tract, liver and pancreas taken from both humans and animals (42, 44, 101–103, 105, 143). Additionally, organoids can be cryopreserved and re-cultured from cryopreserved samples, maintaining cellular characteristics similar to that of the original organoids (144). Successful recovery of cryopreserved organoids (44, 145) can provide a banked resource for future use. Furthermore, organoids are useful for standard biological analyses such as live cell imaging using inverted phase-contrast and confocal microscopies (101), gene expression analysis (14), genetic modification (146), and enzyme activity assay (14).

Although organoids are a promising model with many advantages as described above, their self-organizing capacity could become a limitation when studies that necessitate apical access to the cells are considered. 3D organoids form hollow cystic structures as they grow up in extracellular matrix such as Matrigel (147, 148). This structure makes it very difficult not only to study interactions between epithelial cells and exogenous substances such as drugs, nutrients, toxins and microbial pathogens, but also to investigate functions and cell-cell interactions of epithelium such as intracellular transport, secretion, and absorption. To overcome this limitation, microinjection technique has been used to introduce enteric pathogens or other compounds into organoid lumen (149, 150). However, microinjection is labor-intensive and has low success rates without special equipment and experienced technicians (151), making the technique unsuitable for high-throughput applications. Moreover, an invasive process of this technique has a possibility to compromise the epithelial barrier function, resulting in unintended leakage.

Organoid-Derived 2D Monolayer Culture System

More recently, organoid-derived 2D monolayer culture models have become increasingly described in multiple animal species including pigs (23, 46, 47, 49–51, 53, 54, 58, 62, 64), rabbits

FIGURE 1 | Comparisons of the key features of the 3D organoid culture system, organoid-derived 2D monolayer culture system, and organ-on-a-chip system. The 3D organoids should be derived from the relevant donors to develop the cell culture system that can be established efficiently and passaged readily while maintaining the physiologic cell diversity. To accomplish more advanced epithelial interface investigation, organoid-derived 2D monolayer can be established using conventional Transwell system or other well established 2D culture systems. Integration of the 3D organoid culture system and organ-on-a-chip technologies provides the most suitable system to investigate more complex microenvironment with host cells and microbial cells because of its dynamic environment with mechanical motions and the ability to manipulate the oxygen gradient within the system. Created with BioRender.com.
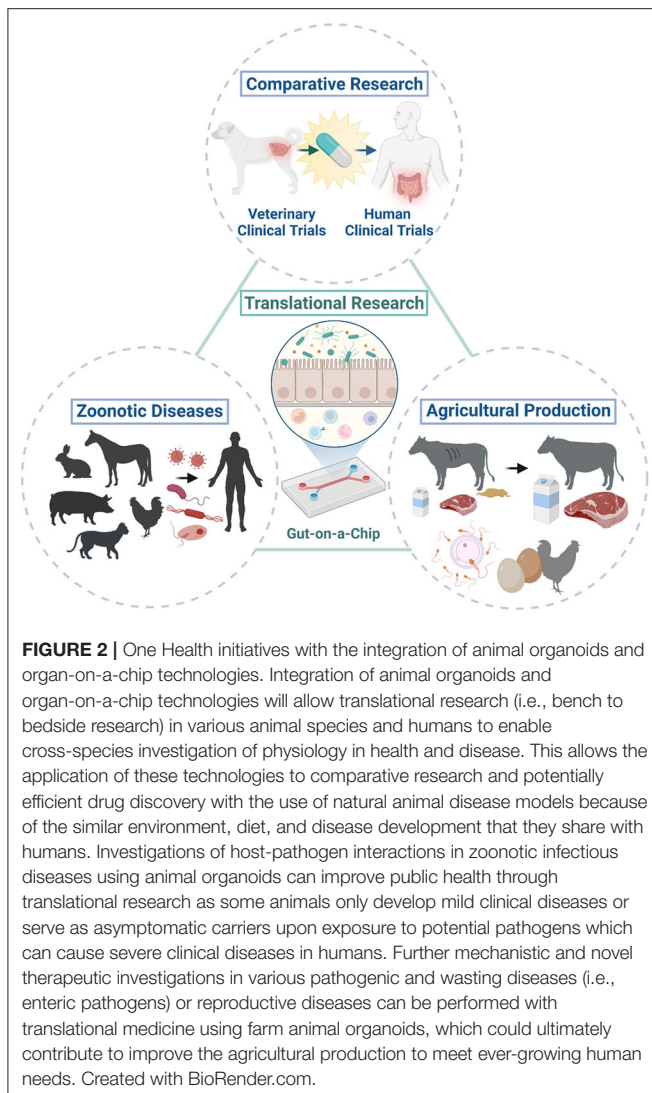
(97, 98), cattle (72), and dogs (11). This culture system was developed to get over the limitation of 3D culture system which arose when intestinal organoids are to be used in studies. The major advantage of the organoid-derived 2D monolayer system is that it provides much easier access to the luminal surface compared to the conventional 3D organoid culture, and thus serving as a better *in vitro* model to study epithelial interaction to luminal microenvironment for preclinical drug testing and infection modeling (23, 50, 51, 53, 54, 62, 64, 98). Comparing to the traditional 2D cell culture system which includes only one cell type, the organoid-derived nature of this system provides a physiologically more representative model to *in vivo* tissue due to greater cellular diversity. Additionally, by culturing the cells on a Transwell insert, basolateral access to the cells in addition to normal apical access becomes feasible, enabling studies involving transport assays.

In this system, intestinal organoids are enzymatically dissociated into single cells, then seeded on top of nanoporous Transwell inserts pre-coated with Matrigel and collagen (11). Unlike other technique involving physical disruption of 3D organoids into fragments, which is used in a study to establish enteric infection models (60), cell polarization is preserved and accessibility to the apical side is much improved, making this model better suited for studies to investigate epithelial-luminal interactions with a high-throughput procedure. Furthermore, this model can also be used to evaluate integrity of epithelial

tight junction barriers by measuring transepithelial electrical resistance (TEER) and apparent paracellular permeability (Papp) values (11).

However, the organoid-derived 2D monolayer system also has drawbacks in comparison to the 3D organoid system. For example, the Paneth cell marker lysozyme positive cells that were present in the original murine 3D organoids were not detected in the 2D monolayer culture (152). Although this was not the case when porcine intestinal organoids were dissociated and seeded to 2D monolayer culture (47), the result raises a concern as to whether this 2D monolayer culture system could reproduce the pathophysiological features of the *in vivo* intestine due to the lack of an important cell type. Moreover, organoid-derived 2D monolayer cultures cannot be easily passaged and propagated (153) as opposed to the 3D organoid cultures. In addition, 2D monolayer cultures on Transwell inserts cannot be co-cultured with bacteria for a long time as microbial overgrowth tends to occur under static culture conditions due to accumulation of the waste products and consumption of nutrients (100, 154). Therefore, host-pathogen interactions can only be evaluated for a short period of time, usually from half an hour to several hours (154). Since crosstalk between epithelial cells and commensal bacterial flora occurs constantly in tissues *in vivo*, it is important to establish a stable co-culture system that can be maintained for a prolonged period in order to improve our understanding of host-microbe interactions (155).

FIGURE 2 | One Health initiatives with the integration of animal organoids and organ-on-a-chip technologies. Integration of animal organoids and organ-on-a-chip technologies will allow translational research (i.e., bench to bedside research) in various animal species and humans to enable cross-species investigation of physiology in health and disease. This allows the application of these technologies to comparative research and potentially efficient drug discovery with the use of natural animal disease models because of the similar environment, diet, and disease development that they share with humans. Investigations of host-pathogen interactions in zoonotic infectious diseases using animal organoids can improve public health through translational research as some animals only develop mild clinical diseases or serve as asymptomatic carriers upon exposure to potential pathogens which can cause severe clinical diseases in humans. Further mechanistic and novel therapeutic investigations in various pathogenic and wasting diseases (i.e., enteric pathogens) or reproductive diseases can be performed with translational medicine using farm animal organoids, which could ultimately contribute to improve the agricultural production to meet ever-growing human needs. Created with BioRender.com.

Microfluidic Organ-on-a-Chip Technology

While 3D organoid and organoid-derived 2D monolayer cultures are powerful tools for both fundamental and applied research, it has been a big challenge to develop more complex systems which better represent dynamic tissue-tissue interactions occurring *in vivo* organs. A major difference from the 3D organoid and organoid-derived 2D monolayer cultures is that the organ-on-a-chip system cultures cells in a continuously perfused chamber to remove the waste product and supply continuous nutrients. It allows *in vitro* organ models to recapitulate mechanical movement of tissue, air, and fluid and their interactions within a defined culture system which contains minimum functional units of an organ (156). The system also allows stable bacterial co-culture for a relatively long period without causing bacterial overgrowth (155, 157), unlike the other static culture systems. This is crucial benefit that microfluidic technology can offer especially when investigating host-microbe interactions that occur in healthy tissue as well as in the face of challenge with

bacterial pathogens. Greater control over various parameters within the culture system compared with the other static cultures facilitates more advanced studies dealing with various physiological functions and responses to external stimuli.

Various organ-on-a-chip systems consist of two closely apposed microchannels which are separated by a thin, porous membrane. While the system allows culture conditions of the two channels to be controlled separately from each other, cells on each side are capable of communicating with each other through the microporous membrane (158). This unique structure of the system has made it possible to create more relevant organ-specific microenvironment *in vitro*. Some of microfluidic devices allow the application of vacuum to the chambers and create pressure-driven deformation to the chamber wall, imitating dynamic stretching associated with breathing or peristaltic movement of the gut. In a gut-on-a-chip system, vascular endothelial cells and intestinal epithelial cells are cultured on each side to create lumen-capillary interface. Dynamic condition mimicking the *in vivo* intestine is created through introduction of luminal flow with varying nutrient gradients and bacterial contents, inflammatory cytokines as well as peristalsis-like mechanical movement (157). Anoxic-oxic interface can also be created by flowing culture medium with controlled oxygen gradient into each compartment (155). The system has been applied to investigate effects of microbiome, inflammatory cells, and mechanical movement on intestinal health and cellular differentiation under physiologically more relevant conditions (157, 159).

To date, several different organ-on-a-chip devices have been successfully developed in humans, which include, liver (160), brain (161, 162), heart (163, 164), kidney (165), and retina (166, 167) besides lung (158) and intestine (168). They have been used in studies on physiology (167), pathology (169), toxicology (161, 165, 170), and pharmacology (164, 171–173). On the other hand, there are only two reports regarding application of the organ-on-a-chip technology to farm and companion animals, which described canine and bovine oviduct-on-a-chip systems, respectively (174, 175). Each of these studies described potential of animal derived-organ-on-a-chip models as a disease model relevant to humans and *in vitro* fertilization studies. Organ-on-a-chip systems derived from farm and companion animals would contribute to fill the gap that organoid cultures and static monolayer systems could not support adequately. Further advances in the organ-on-a-chip technology and its application would greatly improve our understanding of fundamental biology and pathology, thus enhancing health care management of both animals and humans.

CONCLUSIONS AND FUTURE PERSPECTIVE

Animal organoids from farm and companion animals have made and can make significant contributions to human health as One Health initiatives (**Figure 2**). Farm and companion animal organoid models can offer a useful tool to investigate host-pathogen interactions and host defense mechanisms against

zoonotic infectious diseases efficiently especially when the species are affected only mildly or sub-clinically. Other infectious or non-infectious disease organoid models of farm animals would provide new insights for improving heard health and agricultural productivity through improved disease management and reproductive success, leading to sustainable food production. Furthermore, disease organoids from companion animals affected with naturally occurring diseases can not only serve as a useful model for human diseases but also provide a good candidate for preclinical drug screening for the development of effective treatment.

There are various new culture platforms established to further strengthen what organoids can offer in biomedical research including organoid-derived 2D monolayer and organ-on-a-chip technology. Improvement in the complexity of the experimental designs can provide an essential *in vitro* platform to support the 3R principles (reduce, refine, replace the use of laboratory animals in new drug development) and contribute to the health and welfare of animals as well as to enhance the health of humans through translational research. Finally, we envision that the establishment of organ models integrating 3D organoid culture and organ-on-a-chip systems could lead to deeper insights in a range of different pathological conditions and broaden opportunities as One Health initiatives.

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MK, TG, YT, and IN wrote the original manuscript. MK and YA edited the manuscript. MK, TG, YT, and YA designed and wrote the tables and figures. All authors contributed to the article and approved the submitted version.

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