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Teaser The importance of mini-guts for drug development to combat cystic fibrosis, cancer and infectious disease, in addition to their use in drug screening, personalized medicine and developing new medical tools for drug development are highlighted.

Mini-gut: a promising model for drug development

Yue-Bang Yin[1,3], Hugo R. de Jonge[3], Xin Wu[1,2] and Yu-Long Yin[1]

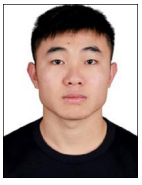
[1] Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences; National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production; Hunan Provincial Engineering Research Center for Healthy Livestock and Poultry Production, Changsha, Hunan, 410125, China
[2] Institute of Biological Resources, Jiangxi Academy of Sciences, Nanchang 330096, China
[3] Department of Gastroenterology and Hepatology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

Until recently, major advances in drug development have been hampered by a lack of proper cell and tissue models; but the introduction of organoid technology has revolutionized this field. At the level of the gastrointestinal tract, the so-called mini-gut comprises all major cell types of native intestine and recapitulates the composition and function of native intestinal epithelium. The mini-gut can be classified as an intestinal organoid (IO), derived from pluripotent stem cells, or as an enteroid, consisting only of epithelial cells and generated from adult stem cells. Both classifications have been used as models to develop drugs against cystic fibrosis, cancer and infectious disease, as well as for drug screening, personalized medicine and the development of new medical tools. In this review, we highlight and discuss the importance of mini-guts for drug development and point out their limitations and future prospects.

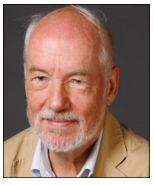
Introduction

The gastrointestinal (GI) tract, in which multiple transporters and enzymes are involved in drug absorption, transport and metabolism, is one of the most active, complex and dynamic organs in the body [1]. The intestinal epithelium consists of a single cell layer comprising proliferative crypts and differentiated villi and includes various cell types including secretory cells (e.g., goblet cells and enteroendocrine cells), absorptive cells (e.g., enterocytes), Paneth cells and stem cells [2,3]. As the most abundant cell type in the epithelium, enterocytes contain a large amount of drug-metabolizing enzymes and xenobiotic processing proteins that play a vital part in the metabolism of drugs and xenobiotics, digestion, nutrient absorption and waste elimination [4]. Goblet cells are filled with mucin granules for the synthesis and secretion of mucus [5].

Corresponding authors: Wu, X. (wuxin@isa.ac.cn),



Dr Yue-Bang Yin was born in 1989, he is currently an Assistant Professor at the Institute of Subtropical Agriculture, the Chinese Academy of Sciences, and visiting postdoctoral fellow of Erasmus MC University Medical Center. During his PhD studies, he used enteroids to model rotavirus infection and to test antiviral therapies. He is now continuing his work on intestinal organoids to develop novel antiviral drugs and feed additives improving intestinal health and unravel the underlying mechanism of action. He has published 24 articles in peer-reviewed journals with an H index of 9.



Prof. Dr Hugo R. de Jonge is a Professor at the Erasmus MC University Medical Center in Rotterdam, The Netherlands. He is an expert in the field of cyclic nucleotide (in particular cGMP) signaling in epithelial cells and develops new therapies to combat CFTR-opathies. His research group uses human intestinal and biliary organoids to tailor therapies to individual patients. His expertise in organoid technology is apparent from his directorship of a high-level course on this topic at the MDI-Biological Laboratory in Maine, USA. So far, he has published >230 papers in peer-reviewed journals with an H index of 55.

Prof. Dr Xin Wu is a Professor in Institute of Subtropical Agriculture, the Chinese Academy of Sciences. His research interests focus on intestinal physiology, development and function. Currently, he is working on the effects of different diabetes drugs on stemness and differentiation of intestinal stem cells. He is also developing placenta organoids. So far, he has published >52 full papers in peer-reviewed journals with an H index of 19.

Prof. Dr Yu-Long Yin is a Professor at the Institute of Subtropical Agriculture, the Chinese Academy of Sciences and a academician of the Chinese Academy of Engineering. He has achieved many significant results in nutrition, botanical drug development, intestinal health and physiology, among others. He has been awarded as 'Thomson Reuters Highly Cited Researcher' award several times. His research interests focus on intestinal physiology, development and function. He has published >300 peer-reviewed articles in the field with an H index of 52.

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Enteroendocrine cells secrete hormones to modulate a variety of physiological processes [6]. Paneth cells serve as a niche to support stem cells by secreting growth factors and also produce bactericidal products (e.g., defensins) to protect against pathogenic invasion [7]. Intestinal stem cells (ISCs) express high levels of leucine-rich-repeat-containing G-protein-coupled receptor 5 (Lgr5), and are rapidly dividing every 24 h to support the extremely high intestinal self-renewal rates and are capable of differentiating into all other intestinal cell types [3]. The intestinal epithelium has been referred to as the front-line for the metabolism of drugs, because it expresses a wealth of drug-metabolizing enzymes [e.g., cytochrome P450 (CYP)3A4] [8]. Accordingly, intestinal epithelium models are indispensable for pharmacologic investigations and drug development.

As an emerging model, the mini-gut (also known as an intestinal organoid) model has gained broad attention because these structures comprise most if not all cell types of the intestinal epithelium [3]. Moreover, single-cell sequencing revealed that the mini-gut contains subtypes of cells that were not identified earlier [9,10]. Thus, this system recapitulates the major features of native tissues, which makes this model highly valuable for investigating the complexities of interplay between cell types during homeostasis and disease states [11]. The mini-gut model was demonstrated to be suitable for multiple applications such as investigating infectious diseases [12], regenerative medicine [13], oncology [14], pharmacology [15] and nutrition [16]. In this review, we systematically describe the roles of the mini-gut in drug development, as well as limitations and future prospects for the field.

Previous models for drug development

The development of a new drug requires high costs (typically US$1 billion), which urges pharmaceutical investigators to carefully select the most suitable preclinical model for drug development [17]. To date, >4000 cell lines from >150 different species have been established all over the world [18]. Before the advent of mini-guts, 2D monolayer cultures of human colorectal adenocarcinoma cell lines such as Caco-2, HT-29 and DLD-1, among others, were broadly used as intestinal epithelial models for drug development by academic groups and in the pharmaceutical industry for decades [19,20]. Yet, these cell models have significant shortcomings: (i) cell lines are often grown in a monolayer format, which does not necessarily reflect the *in vivo* epithelium and tissue morphology that are inherently present in a physiological 3D structure [19]; (ii) cell lines only consist of a single cell type and fail to mimic cell–matrix interactions that involve multiple intestinal cell types [21]; (iii) cell lines often harbor mutations or chromosomal abnormalities, which render the results obtained difficult to interpret or to reproduce [3]; (iv) cell lines do not faithfully reproduce the multiple functions of a differentiated intestinal epithelium including mucus production, villi and crypt formation and CYP-based drug metabolism, among others [19]. Accordingly, the drawbacks of intestinal cell monolayer cultures limit their applications for drug development and disease modeling [2]. 3D culturing methods and co-culture methodology with different cell types can overcome some of the limitations of 2D cell cultures, such as a lack of appropriate cell–cell and cell–extracellular-matrix signaling, which are essential for cellular functions *in vivo* [22,23]. For example a 3D co-culture system of Caco2 cells with mucus-producing HT29-MTX cells was capable of better-recapitulating intestinal mucus secretion and barrier function than 2D monolayers of Caco2 cells [23]. In another study, it was found that 3D cultures of HT29-MTX cells in l-pNIPAM hydrogel scaffolds formed multilayer and villus-like structures [24]. Interestingly, Caco2 cells co-cultured with immune cell lines including human macrophages (THP-1) and human dendritic cells (MUTZ-3) in a 3D format offered a powerful platform for evaluation of the safety of nanomaterials in the context of inflammation [25]. However, a caveat in the use of cell lines is the dramatic heterogeneity in gene expression and phenotype that can exist between samples of the same cell line in different laboratories, as exemplified by analysis of HeLa cells using multi-omic methods [26]. Therefore, the response of cell lines to environmental cues, including drugs or microbial infection, can differ considerably between different laboratories, hampering their use as a preclinical model for drug discovery.

Primary intestinal cells, *ex vivo* xenografts and animal models are often used in pharmaceutical studies and drug development because they have been suggested to represent *in vivo* physiology more closely [21]. Primary cells merely comprise a single cell type and often cannot be passaged long-term, which hampers their applications [3]. Similarly, *ex vivo* xenografts have a short lifespan (<8 h), which results in a significant roadblock for applications in normal intestinal physiology [19]. Animal models especially mice have been beneficial platforms for understanding human physiology and novel drug investigation for a long time. Yet, mouse models very often do not recapitulate the full spectrum of human phenotypes, and large animal models are generally time consuming and expensive [27]. Moreover, animal models commonly fail to offer experimental precision when determining the effects of candidate drugs [21], and stricter ethical constraints hamper their broad utilization as experimental models at this point in time [22]. Thus, a more physiological preclinical model is still needed.

Definition of the mini-gut

The mini-gut is also named an intestinal organoid. The term 'organoid' denotes a group of cells 'resembling an organ', and the three most important characteristics that define these structures include self-organization, multicellularity and functionality [28]. Although the definition and nomenclature associated with a mini-gut vary and remain unclear [29], the current consensus is that two types of mini-gut models have been developed: those derived from pluripotent stem cells (PSCs) [30] and those generated from adult stem cells (ASCs) present in an intestinal crypt niche [31]. The mini-gut model derived from PSCs was first reported by Spence *et al.* [30]. This type of mini-gut was associated with the transition from a simple cuboidal epithelium to a highly convoluted pseudostratified epithelium surrounded by mesenchymal cells within 14 days. This ultimately matured into a columnar epithelium with villus-like involutions that protruded into the lumen of the mini-gut, which was surrounded by a mesenchymal layer (Fig. 1a) [30]. The mini-gut model derived from primary single stem cells or isolated intestinal crypts was initially developed by Hans Clevers and Toshiro Sato (at present working in Keio University School of Medicine, Japan) and colleagues from the Hubrecht Institute [31]. These mini-guts grew faster than PSC-derived mini-guts, and the upper opening of the crypt rapidly

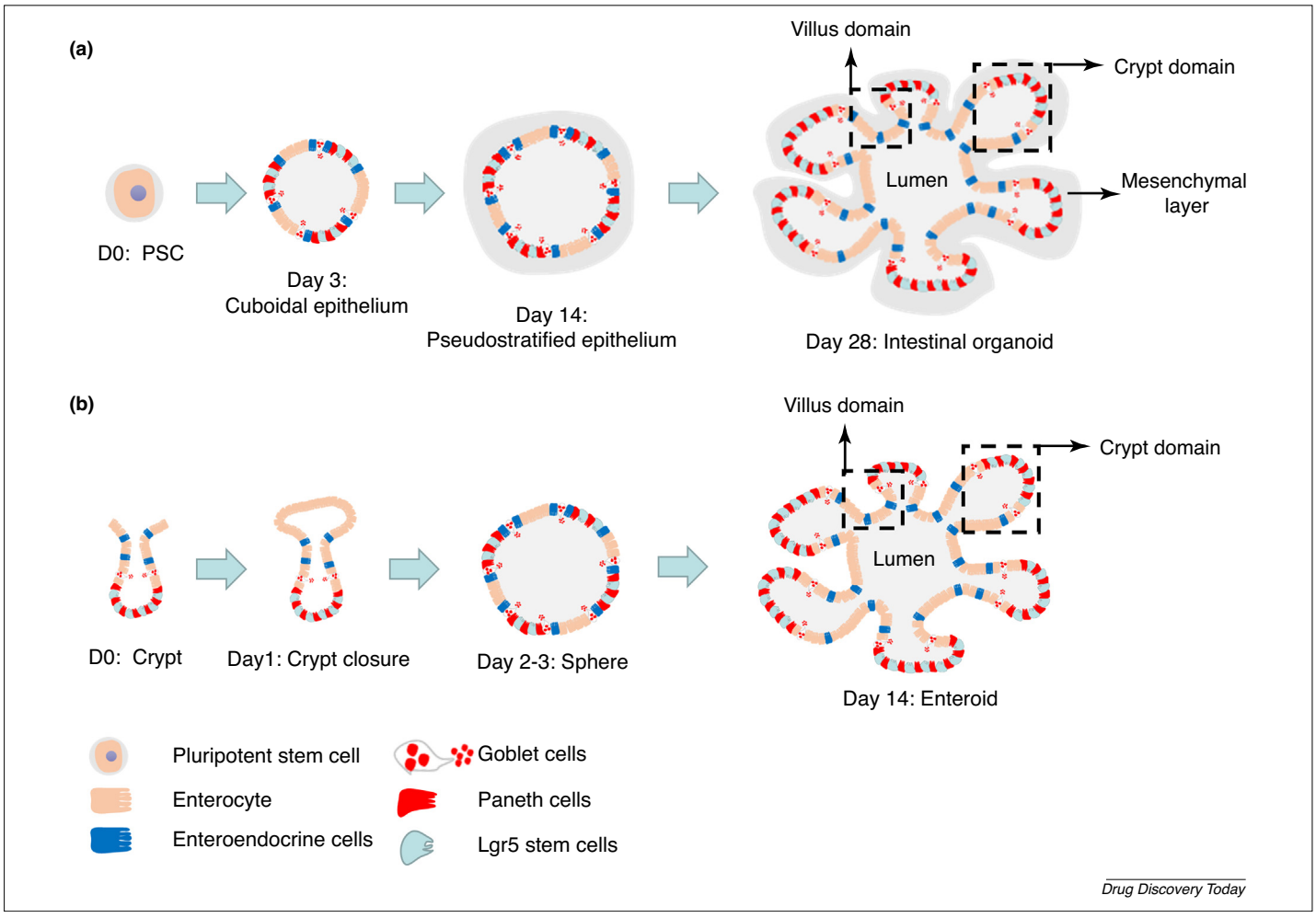

FIGURE 1

Schematic of an intestinal organoid (IO) and enteroid morphology and growth process. **(a)** IOs cultured from pluripotent stem cells (PSCs) initially formed a simple cuboidal epithelium and then grew out into a highly convoluted pseudostratified epithelium surrounded by mesenchymal cells within a period of 14 days. **(b)** Growing enteroids from intestinal biopsies is a relatively fast process (1 week) and involves multiple steps (i.e., sealing of the upper opening of the crypt followed by the forming of a closed sphere containing various types of differentiated functional intestinal cells and eventually the generation of a mature organoid consisting of >40 crypt domains surrounding a central lumen).

became sealed, which was followed by the formation of a closed sphere containing various types of differentiated functional intestinal cells; this eventually developed into a mature mini-gut consisting of >40 crypt domains surrounding a central lumen (Fig. 1b) [31].

Mature PSC-derived mini-guts contain functional enterocytes, goblet cells, Paneth cells, enteroendocrine cells and nascent ISCs, which support long-term culture of at least nine passages comprising >140 days without any growth failure [30]. Similarly, intestinal stem-cell- or crypt-derived mini-guts are reminiscent of the normal gut consisting of fully polarized enterocytes, goblet cells, enteroendocrine cells, Paneth cells and stem cells [31]. These types of mini-guts can be passaged weekly at a 1:5 ratio and can be continuously cultured for periods longer than 1.5 years without genetic or physiological changes [3]. Both types of mini-guts faithfully reproduce key functions of the *in vivo* intestinal epithelium, including secretion, absorption and cell renewal, suggesting that they are superior to 2D cell line culture models [3,30]. Importantly, adult stem-cell-derived mini-guts maintain their regional identity after many passages, implying that they can be used to study segment-specific functions [32]. Herein, the mini-gut might be defined as organ-like tissues derived from PSCs [denoted as an intestinal organoid (IO)] or as enteroids and colonoids, existing merely of epithelial cells and generated from primary stem cells or from the intestinal crypt niche. These structures are grown on an artificial scaffold (usually Matrigel®) that, together with a cocktail of stem cell growth factors, supports the self-renewal of organ-specific stem or progenitor cell populations and their differentiation into a variety of functional cell types typical of the *in vivo* intestine. Mini-guts are associated with promising applications and can lead to new insights into the development of novel drugs.

Mini-guts can model drug or therapy development for various diseases

The intestine plays a vital part in digestion, absorption, secretion and establishing a protective epithelial barrier between the digestive environment and the body [19]. As a site of metabolism and absorption, the intestine is often utilized for assessing the

pharmacokinetics of orally administered drugs, drug-metabolizing enzymes and the expression of uptake and efflux transporters [8]. 2D cultures of intestinal cell lines have been broadly used to develop new drugs and explore pharmacokinetics in experimental systems; however, these approaches cannot be used to accurately evaluate human intestinal pharmacokinetics owing to their inherent shortcomings [31]. It was stated that 90% of experimental drugs failed to move forward to the clinical phase when tested using conventional cell lines and animal models [33]. Mini-gut models have been suggested to better reflect the biology of the *in vivo* intestinal epithelium that can circumvent the limitations of cell lines and animal models, and thus these models have the potential to model and develop new drugs or therapies against various diseases including cystic fibrosis (CF), tumors and infectious diseases.

Modeling and developing therapeutics against CF using mini-gut cultures

CF is a rare disease characterized by mutations in an epithelial chloride channel, named cystic fibrosis transmembrane conductance regulator (CFTR). Approximately 85 000 people are affected by CF worldwide [15]. CF patients suffer from viscous mucus in the pulmonary and GI tract, and are thus susceptible to bacterial infections, aberrant inflammation and malnutrition [34]. CFTR mutations result in impaired function of this cyclic AMP (cAMP)- and cyclic GMP (cGMP)-regulated ion channel [35]. Various CFTR mutations (up to 2000) are implicated in a broad spectrum of phenotypes [15], which limits the use of conventional cell lines and animals to model self-renewal of CF. Moreover, owing to the absence of a proper *in vitro* model, effective drugs or therapies are lacking, except for the relief of bacterial pressure and inflammation and the tight regulation of nutrient uptake and physical growth [34].

Mini-gut models form the basis for the development of new therapies to combat this complicated disease and are associated with several merits: (i) mini-guts can be cultured long-term and maintain genetic and/or epigenetic stability; (ii) they can be cryopreserved in liquid nitrogen, which enables the establishment of mini-gut biobanks from individuals with CF for subsequent drug screening, gene analysis and correction, and precision personalized therapy [21]; (iii) mini-guts are ideally suited to identify drug-responsive individuals with rare CFTR genotypes [15].

Dekkers *et al*. developed a sophisticated assay to model CF using mini-guts, named forskolin-induced swelling (FIS) [34]. As an anion channel, CFTR is involved in fluid and electrolyte homeostasis at epithelial surfaces, and forskolin activates fluid secretion into the mini-gut lumen in a CFTR-dependent manner, which results in rapid volumetric expansion or swelling of the mini-guts [34]. This effect is significantly attenuated in mini-guts derived from CF patients or mice carrying the most common CFTR mutation: F508del, and is completely lost in *CFTR*-deleted mini-guts [34]. For FIS, 7-day-old mini-guts (with or without CFTR correctors) are subcultured in 96-well plates, and labeled with calcein green after 24 h; this is followed by stimulation with forskolin and direct imaging by confocal microscopy (Fig. 2a) [34]. FIS systems are characterized by ease-of-use, speed and simple visual analysis, which provides a robust avenue to develop effective treatments against CF. This assay can be scaled up to a 384-well format, allowing medium-throughput screening (MTS) of candidate CFTR correctors in mini-guts derived from a single individual.

Using mini-gut technology and FIS assays, several groups have tried to develop new strategies to combat CF (Table 1). Dekkers *et al*. isolated mini-guts from the rectal epithelia of 71 individuals expressing 28 different CFTR genotypes; using this approach, they found that VX-770 and VX-809 co-treatment was superior in restoring CFTR-F508del function compared with treatment with a single drug [15]. CRISPR/Cas9 genome editing has also been used to correct the CFTR locus via homologous recombination in cultured ISCs from CF patients [36]. Moreover, a panel of G-protein-coupled receptor (GPCR)-modulating compounds was screened using FIS assays, indicating that β_2-adrenergic receptor agonists robustly induce CFTR function [37]. This was confirmed in CF patients who exhibited a significant improvement in the baseline potential difference of the nasal mucosa, illustrating the power of mini-guts as preclinical models to develop and test effective therapies against CF [37]. The FIS assay could also be used as a bioassay to measure CFTR modulators in plasma of CF patients [38]. Collectively, mini-guts can be used as important tools for the preclinical selection of effective CFTR-modulating drugs for CF patients, the testing of future drugs and the discovery of therapeutic combinations. Further, this can be accomplished in a cost-effective manner by building up mini-gut biobanks and identifying effective CFTR modulators via MTS.

Mini-guts as a tool for developing anti-colorectal-cancer therapies

As the third-most-common cancer worldwide, colorectal cancer (CRC) is associated with significant morbidity and mortality [39]; however, the effects of current anti-CRC therapies are still unsatisfactory owing to the frequent occurrence of tumor recurrence and metastasis [40]. CRC often exhibits high genetic and epigenetic diversity, which means that it is extremely difficult to predict the effects of particular antitumor therapies [41]. Moreover, the cellular diversity and plasticity of cancer cells often result in tumor resistance to chemotherapeutics [42]. The development of molecular targeted cancer therapies and drug-screening models to stratify patients and achieve maximum benefit for expensive treatments is therefore important [40]. This might provide a promising avenue to identify optimal treatments against particular tumors by tailoring therapies to individual patient tumors, because conventional immortalized cell lines, xenografts established from mice or genetically engineered mouse models fail to faithfully represent human cancers [43].

Recently, a highly efficient and tractable mini-gut culture system was developed for colorectal tumors; this made it possible to monitor cancer growth and drug responses using the patient's own tumors [41]. As such, patient-derived mini-guts display close similarity to original patient tumors based on phenotypic and genotypic profiling [40,44]. Another essential advantage of tumor mini-guts is that they can be cultured from frozen tissues and the characteristics of these mini-guts are similar to those derived from fresh tissue, which circumvents difficulties associated with the accessibility of fresh primary tumors [43]. Tumor mini-guts have been utilized to identify oncogenes and to develop new therapies and personalized medicines (Table 1, Fig. 2b). As an example, the deficiency of *FBXW7*, a tumor suppressor gene that is commonly

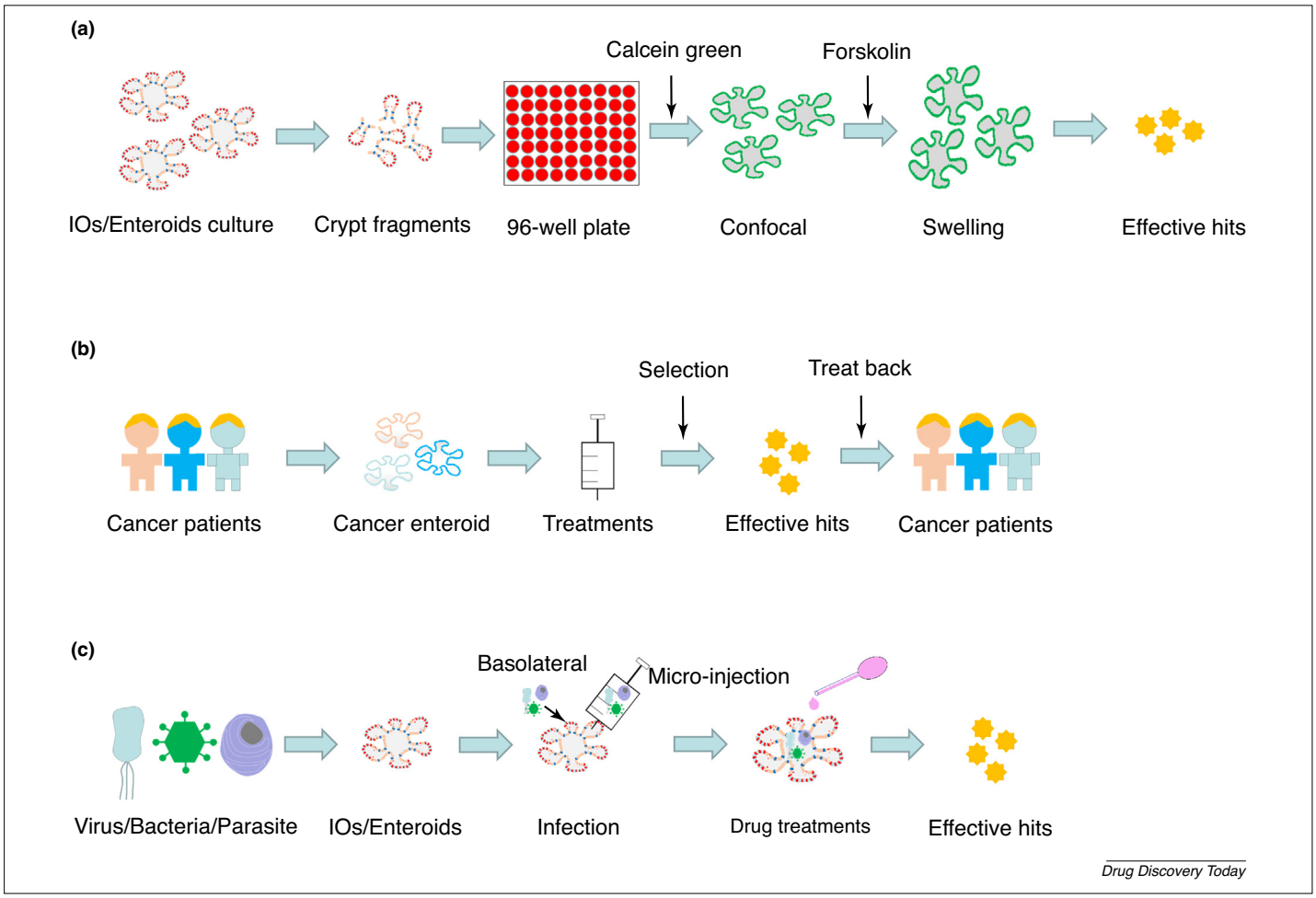

FIGURE 2

Mini-guts are used for drug development in cystic fibrosis (CF), cancer and infectious disease. **(a)** Forskolin-induced swelling (FIS) was developed for diagnosis and drug screening of CF patients at an individual level: in this example, 7-day-old mini-guts [with or without cystic fibrosis transmembrane conductance regulator (CFTR) correctors] are subcultured in 96-well plates and labeled with calcein green after 24 h, followed by stimulation with forskolin and direct imaging by confocal microscopy. **(b)** Tumor mini-guts were isolated from patients, followed by *in vitro* treatment with candidate anticancer drugs, and effective drugs were used to treat patients back in the clinic. **(c)** Mini-guts were infected with viruses, bacteria or parasites using basolateral supplementation or microinjection, followed by treatment of candidate drugs to develop antipathogen drugs.

mutated in CRC and various other epithelial cancers, is closely related to resistance to several types of chemotherapies [e.g., 5-fluorouracil (5-FU)] [40]. Rapid budding events in the crypt region were observed in *fbxw7*$^{\Delta G}$ (mutated) mini-guts, and 5-FU rescued the drug-resistant phenotype of these mini-guts through the induction of terminal differentiation [40].

Further, the human Tacc3 protein is involved in mitotic spindle assembly and is aberrantly expressed in a variety of cancers. Yao *et al.* established mini-guts from the ileum of genetically modified mice carrying conditional *Tacc3* alleles (*Tacc3*$^{fl/fl}$) to confirm that the disruption of this gene remarkably attenuates the expansion of the crypt domain in mini-guts [42]. This result was consistent with *in vivo* results showing that Tacc3 disruption results in a significant decrease in tumor number and prolonged survival in corresponding mutant mice, indicating that this protein might be a promising target for chemotherapy against intestinal tumors [42].

As another example, inositol-requiring transmembrane kinase/endonuclease 1α (IRE1α) is an endoplasmic reticulum (ER) stress sensor, and its ablation represses the growth of mini-guts; moreover, knockdown of IRE1α suppresses the proliferation of colon cancer cells *in vitro* and xenograft growth *ex vivo*, indicating that it plays a pivotal part in colonic tumorigenesis and the development of treatments against CRC [45]. In addition, Tp53 exerts a tumor-suppressive effect, and its deficiency helps tetraploid colonocytes form morphologically near-to-normal colonoids. Injection of tetraploid Tp53$^{-/-}$ colonoids into immunodeficient mice generated proliferating tumors, which was not observed in immunocompetent mice, suggesting that tetraploidy was particularly oncogenic in the context of deficient immunosurveillance [46].

In another study, CRISPR/Cas9 genome editing was used to introduce multiple driver pathway mutations into human normal or adenoma-derived mini-guts to explore the roles of these mutations in CRC tumorigenesis and metastasis [47]. Oligomeric proanthocyanidins were confirmed to exert effective antitumorigenic effects in patient-derived colorectal tumor mini-guts by targeting cancer stem-like cells [48]. Xu *et al.* demonstrated that one of the most common flavonoids: apigenin, significantly suppressed the proliferation of CRC cells and the growth of mini-guts, indicating that it is a potential anti-CRC drug [49]. Importantly, van de Wetering *et al.* demonstrated that organoid technology supported the establishment of a living biobank of tumor mini-gut cultures from 20 CRC patients; these mini-guts closely mimicked

TABLE 1

Intestinal organoids (IOs) or enteroids are used for modeling diseases and drug development

Author	Year	Type of organoid	Findings and innovation
CF			
Dekkers *et al.* [34]	2013	Enteroids	Developed a forskolin induced swelling (FIS) with enteroids
Dekkers *et al.* [38]	2015	Enteroids	VX-770 in plasma from different donors induced variable CFTR function in enteroids
Dekkers *et al.* [15]	2016	Rectal enteroids	VX-770 and VX-809 co-treatment was better at restoring CFTR-F508del function than treatment with a single drug
Schwank *et al.* [36]	2013	Enteroids	CRISPR/Cas9 genome editing system was used to correct the CFTR locus
Vijftigschild *et al.* [37]	2016	Enteroids	β_2-adrenergic receptor agonists robustly induced CRTR function
Cancer			
Boileve *et al.* [46]	2013	Colon enteroids	Tetraploidy was particularly oncogenic in the context of deficient immunosurveillance
Li *et al.* [45]	2017	Enteroids	The ablation of IRE1α repressed the growth of enteroids, indicating that IRE1α played a pivotal part in colonic tumorigenesis and developing treatments against CRC
Lorenzi *et al.* [40]	2016	Enteroids	5-FU rescued the drug-resistance phenotype of fbxw7$^{\Delta G}$ through the induction of terminal differentiation in fbxw7$^{\Delta G}$ enteroids
Matano *et al.* [47]	2015	Enteroids	CRISPR/Cas9 genome editing was used to introduced multiple driver pathway mutations into human normal or adenoma-derived intestinal organoids to explore the roles of these mutations in tumorigenesis and metastases of CRC
Ohta *et al.* [41]	2014	Enteroids	Colorectal tumor enteroids were cultured
Toden *et al.* [48]	2018	Enteroids	Oligomeric proanthocyanidins (OPCs) exerted effective antitumorigenic effects in patient-derived colorectal tumor organoids
Van de Wetering *et al.* [50]	2015	Tumor enteroids	A living biobank was built by establishing tumor organoid cultures from 20 consecutive colorectal carcinoma (CRC) patients, and enteroids support proof-of-concept drug screening with a library containing 83 bespoke compounds
Xu *et al.* [49]	2016	Enteroids	Apigenin significantly suppressed the proliferation of colorectal cancer cells and the growth of intestinal organoids
Yao *et al.* [42]	2016	Enteroids	The disruption of Tacc3 remarkably attenuated the expansion of the crypt domain in enteroids, manifesting that Tacc3 could serve as a promising target for chemotherapy against intestinal tumors
Infectious disease			
Drummond *et al.* [59]	2017	Enteroid	Enteroviruses could infect human enteroids, and enteroids induced antiviral and inflammatory signaling pathways in a virus-specific manner
Engevik *et al.* [64]	2015	IOs	*Clostridium difficile* infected IOs via microinfection challenge, which reduce MUC2 production and NHE3 mRNA and protein expression
Ettayebi *et al.* [52]	2016	Enteroids	Human enteroids were able to model norovirus infection, which also assessed virus neutralization and inactivation
Finkbeiner *et al.* [55]	2012	IOs	IO was susceptible to laboratory strain of rotavirus (SA11) and clinical rotavirus isolates
Fischer *et al.* [65]	2016	Enteroids	Expression of antimicrobial peptides was significantly induced by treatment with 5 μg/ml flagellin
Heo *et al.* [66]	2018	Enteroids	*Cryptosporidium* was able to infect enteroids, secondary to inducing type I interferon immunity
Hill *et al.* [63]	2017	IOs	IOs can be stably associated with nonpathogenic *Escherichia coli*
Huang *et al.* [60]	2017	Enteroid	AIV infected chicken enteroids, which rendered enteroid damage, and suppressed intestinal stem cell proliferation and differentiation
In *et al.* [62]	2016	Colon enteroids	EHEC infected colon enteroids
Yin *et al.* [12]	2015	Enteroids	Mouse and human primary enteroids were liable to SA11 rotavirus strain and clinical rotavirus isolates
Yin *et al.* [56]	2016	Enteroids	A panel of immunosuppressants was screened by using human enteroids to confirm that mycophenolic acid (MPA) potently inhibited rotavirus infection
Yin *et al.* [57]	2018	Enteroids	PI3K/Akt/mTOR pathway was demonstrated to promote rotavirus infection, and inhibiting of the pathway by different chemical inhibitors potently suppressed rotavirus infection in enteroids
Yin *et al.* [58]	2018	Enteroids	Six thioguanine (6-TG) remarkably inhibited rotavirus infection in *in vitro* cells and enteroids
Zhang *et al.* [61]	2014	Enteroid	*Salmonella* infected enteroids to disrupt epithelial tight junctions, stimulate NF-κB signaling to induce inflammatory responses, and remarkably reduce the expression of stem cell markers (Lgr5 and Bmi1)
Drug screening			
Gracz *et al.* [67]	2015	Enteroids	A modified microraft array (MRA) was developed, which supported assaying stem-cell–niche interactions
Van de Wetering *et al.* [50]	2015	Colon enteroids	A living enteroid biobank of colorectal cancer patients was used to screen antitumor drugs
Vijftigschild *et al.* [37]	2016	Enteroids	GPCR-modulating compound library was screened using enteroids, found that β_2-adrenergic receptor agonists were the most potent inducers of CFTR function
Yin *et al.* [56]	2016	Enteroids	A panel of immunosuppressants were screened using enteroids

TABLE 1 (*Continued*)

Author	Year	Type of organoid	Findings and innovation
Personalized medicine			
Dekkers *et al.* [15]	2015	Enteroids	Enteroid derived from a specific disease or a specific individual was used to develop particular therapy treating CF
Kasendra *et al.* [71]	2018	Enteroids	Intestinal chip established using enteroids could be useful as a research tool for studies of metabolism, nutrition, infection and drug pharmacokinetics, as well as personalized medicine

the characteristics of the original tumor [50]. This biobank comprised a broad spectrum of genetic changes and thus supported large-scale mutational analyses of CRC [50]. Notably, this study also supported proof-of-concept for the use of mini-guts in anticancer drug screening: a library of 83 chemostatic compounds of different chemical classes was successfully screened to identify optimal drugs that target CRC [50]. Taken together, the mini-gut model can provide insights into carcinogenesis and can be used for the identification of new oncogenes and therapeutic targets, gene analysis and correction, and personalized medicine based on drug screening.

Mini-guts as a platform for modeling infectious diseases and corresponding therapies

The GI tract is one of primary sites for many infectious diseases including viral, bacterial and parasitic infections [42]. Traditionally used intestinal cell lines and animal models fail to mimic the complexity of *in vivo* normal human physiology and pathophysiology, and thus they might be not sufficient to model infectious diseases [12]. By contrast, mini-guts have many features that are similar to the original epithelia, representing a potentially desirable platform to investigate pathogenesis and therapies for infectious diseases including viruses, bacteria and parasites (Fig. 2c, Table 1).

Norovirus is an important enteric virus that causes acute gastroenteritis regardless of age [51]. No effective antivirals or vaccines are currently available owing to the lack of suitable *in vitro* culture models [52]. Ettayebi and colleagues were the first to confirm that human mini-guts could also model norovirus infection [52]. Monolayers of human mini-guts were inoculated with human noroviruses (HuNoVs), and robust increases in the genomic RNA of HuNoVs were observed 96 h post-infection [52]. Interestingly, bile was found to help some HuNoV strains to replicate in mini-guts [52]. Importantly, mini-guts were found to support the assessment of virus neutralization and inactivation, suggesting that they might be used to detect the efficacy of antivirals against norovirus [52]. Similarly, IOs were found to support the replication of clinical HuNoV isolates [53]. In mice, Wilen *et al.* recently confirmed that mouse norovirus has tropism for tuft cells (CD300lf receptor) [54], whereas HuNoVs have tropism for enterocytes in human mini-guts, which is closer to the scenario in humans [52]. Hence, mini-guts are promising models for norovirus-related studies and the development of new strategies to treat this infection.

As a major enteric virus that causes severe gastroenteritis, in particular in infants below 5 years of age, rotavirus has been demonstrated to readily infect mini-guts. Finkbeiner *et al.* first reported that IOs were susceptible to a laboratory strain of rotavirus (simian SA11) and clinical rotavirus isolates [55]. Subsequently, Yin and colleagues demonstrated that mouse and human primary mini-guts were susceptible to the SA11 rotavirus strain and clinical rotavirus isolates [12]. Interestingly, compared with Caco2 cells, mini-guts responded differently to rotavirus infection as judged by the expression pattern of a panel of interferon-stimulated genes, and the widely used antiviral interferon (IFN) was less effective against rotavirus infection in mini-guts than in Caco2 cells and showed diverse antiviral effects toward different clinical rotavirus isolates from individual patients in mini-guts [12]. Further, a panel of immunosuppressants was screened using human mini-guts to confirm that mycophenolic acid potently inhibits rotavirus infection by inhibiting inosine-5′-monophosphate dehydrogenase (IMPDH) in the host, indicating its possibly dual benefits for preventing organ rejection in transplantation patients and combatting rotavirus infection [56]. The phosphoinositide 3 kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway was shown to promote rotavirus infection, and inhibiting this pathway using different chemical inhibitors was found to potently suppress rotavirus infection in mini-guts, indicating a potential system for the development of antivirals to combat infection [57]. Additionally, a drug commonly used to treat inflammatory bowel disease (IBD), namely 6-thioguanine (6-TG), remarkably inhibited rotavirus infection in *in vitro* cell lines and mini-guts by dynamically inhibiting its cellular target: Rac1 [58]. Thus, primary screening or testing in cultured cell-line models could be followed by the use of mini-guts to further validate promising targets, which could provide a proof-of-principle approach to develop new medicines to combat infectious pathogens.

Although enteroviruses belong to a class of enteric viruses, the events involved in the infectious process in the GI tract remain largely unclear. Drummond *et al.* found that other enteroviruses including echovirus 11 (E11), coxsackievirus B (CVB) and enterovirus 71 (EV71) are also capable of infecting human mini-guts [59]. Upon enterovirus infection, mini-guts induced antiviral and inflammatory signaling pathways in a virus-specific manner, and enteroviruses were found to infect particular cell populations in human mini-guts [59]. These findings highlight the advantages of such models, in comparison with conventional cell lines, in investigating these types of viruses, suggesting that mini-guts might form the basis for the identification of novel therapeutic targets and for the development of strategies to treat enteroviruses.

In addition to enteric viruses, mini-guts have been used to model infection by avian influenza virus (AIV), bacteria including *Salmonella*, Enterohemorrhagic *Escherichia coli* (EHEC), *Clostridium difficile* and *Cryptosporidium* (Table 1, Fig. 2). AIV infection induces mini-gut damage and suppresses ISC proliferation and differentiation [60]. In *Salmonella*-infected mini-guts, which result in the

disruption of epithelial tight junctions, nuclear factor (NF)-κB signaling is stimulated to induce inflammatory responses, remarkably reducing the expression of stem-cell markers (Lgr5 and Bmi1) [61]. Further, EHEC was found to infect mini-guts from the luminal side following microinjection and luminal exposure of mini-gut monolayers, respectively [62,63]. *C. difficile* was also found to infect IOs following microinjection, which resulted in a reduction in mucin 2 (MUC2) production and Na^+/H^+ exchanger 3 (NHE3) mRNA and protein expression [64]. Notably, the expression of antimicrobial peptides (defense factors against pathogens) was significantly induced when bacterial challenge was mimicked via treatment with flagellin [65]. As a protozoan parasite, *Cryptosporidium* was able to infect mini-guts, which resulted in the induction of type I interferon immunity [66]. Remarkably, by combined use of single-cell sequencing and mini-guts, Haber *et al.* showed that pathogenic infections caused the changes of the proportions of intestinal cell types in mini-guts, indicating that *Salmonella* infection elevated the abundance of absorptive cell types including Paneth cells and enterocytes whereas *Heligmosomoides polygyrus* increased the abundance of secretory cell types including goblet and tuft cells [9]. Taken together, mini-guts can be used to gain insights into infection and pathogenesis and to develop novel strategies to combat infectious pathogens.

Mini-guts as an avenue for drug screening

As a 'casting a wide net' approach, screening is commonly used to develop novel drugs and precision depends mostly on the use of preclinical models. Mini-guts recapitulate most if not all physiological features of the *in vivo* intestine, and therefore might be better models for drug screening compared with cell lines [37]. Mini-guts have been applied to screen effective drugs to treat CF [37], antivirals [56] and antitumor drugs [50]. Gracz and colleagues developed a modified microraft array (MRA) that allowed the assessment of stem-cell–niche interactions and mini-gut development, as well as the interrogation of gene expression during the early stages of stem-cell-fate decisions [67]. This might serve as a robust avenue for MTS or even HTS to develop new drugs. Another advantage of mini-guts is that they could be isolated and cultured from distinct intestinal sections, which might be a benefit for drug screening assays specific to particular regions of the GI tract [68]. Thus, mini-guts could have great potential for screening to advance the drug discovery process.

Mini-guts as a platform for personalized medicine

The efficacies of drugs often rely on patient-specific pharmacokinetic and pharmacodynamic properties [38], which means that a preclinical model representing patient-specific features will aid in identifying optimal treatment strategies for particular diseases. Progress in culturing mini-guts has advanced the development of personalized medicine. A prime example is that mini-guts derived from an individual patient have been used to develop personalized therapeutics for CF [38]. Cristobal *et al.* performed a quantitative mass-spectrometry-based proteomic analysis and a comparative transcriptomic analysis of human colorectal tumor mini-guts, and identified different characteristics exhibited by mini-guts derived from individual patients at the proteomic level, which should result in the development of personalized strategies to treat tumors [69]. Patient-derived mini-guts are also able to recapitulate patient responses in the clinic, which further enhances their potential for use in personalized medicine [44]. Recently, de Winter-de Groot *et al.* demonstrated that a correlation exists between CFTR modulator effects in rectal organoids and *in vivo* responses in CF patients [70]. Therefore, mini-gut technology provides important insights into personalized medicine, increasing drug efficacy and safety, and coping with the problem of patient-to-patient variations in disease.

Novel mini-gut-based approaches for drug development

Intestinal chip

Kasendra *et al.* developed an intestinal chip (gut-on-a-chip) using primary 3D mini-guts, in which dissociated mini-guts were cultured on a polydimethylsiloxane (PDMS) chip [71] (Fig. 3a). The chip contained an upper epithelial channel 1 mm high ×1 mm wide and a lower vascular channel 0.2 mm high ×1 mm wide. In between was a thin (50 μm) flexible PDMS membrane containing multiple pores with a 7 μm diameter; 40 μm spacing separated the upper and lower channel and the extracellular matrix was coated on the PDMS membrane for the growth of mini-guts [71]. On this chip, the epithelium formed villi-like projections lined by polarized epithelial cells undergoing multi-lineage differentiation. Further, the microfluidic mini-gut-on-chip has several advantages compared to Matrigel®-based mini-guts: (i) the chip more closely resembles the human gut *in vivo* with respect to its gene expression profile; (ii) the upper epithelial channel and lower vascular channel of the chip are surrounded on either side by two hollow chambers of 1 mm high ×300 μm wide, which allow cyclic suction to mechanically stretch and relax the sidewalls, thus, the attached flexible PDMS membrane and adherent tissues in the central channel mimic peristaltic motions of the *in vivo* intestine; (iii) the mini-gut chip permits fluids to flow across the epithelial layer, and subsequently the fluids can be continuously collected to analyze drug metabolism, nutrient digestion, mucus secretion and the establishment of intestinal barrier function over a period of multiple days *in vitro* [71]. Furthermore, microfluidic mini-gut-on-chip supports personalized medicine including the exploration of patient-specific disease mechanisms and corresponding therapies through the establishment of mini-gut-on-chips from particular patients [71]. The intestinal chip can also be built using IOs, which likewise yield all intestinal epithelial subtypes representing an amenable avenue to support personalized medicine [71,72]. Therefore, the intestinal chip using mini-guts has great potential for understanding and modeling the development, homeostasis and diseases of the human intestine, in addition to the generation of new drugs and personalized medicine [71].

Hanging drop culture system

Recently, a hanging drop culture system based on chicken embryo mini-guts was developed by Panek *et al.* [73]. In this system, the cell pellet was resuspended in DMEM/F12 culture medium supplemented with 5% BD Matrigel® and other mini-gut growth factors including insulin–transferrin–selenium premix, epidermal growth factor (EGF), prostaglandin E_2 and Wnt3a; subsequently, the cell suspension aliquots were applied to a 96-well Perfecta3D® hanging drop plate, and cultured in an incubator (Fig. 3b) [73]. It was found that the intestinal chicken mini-guts could easily grow in

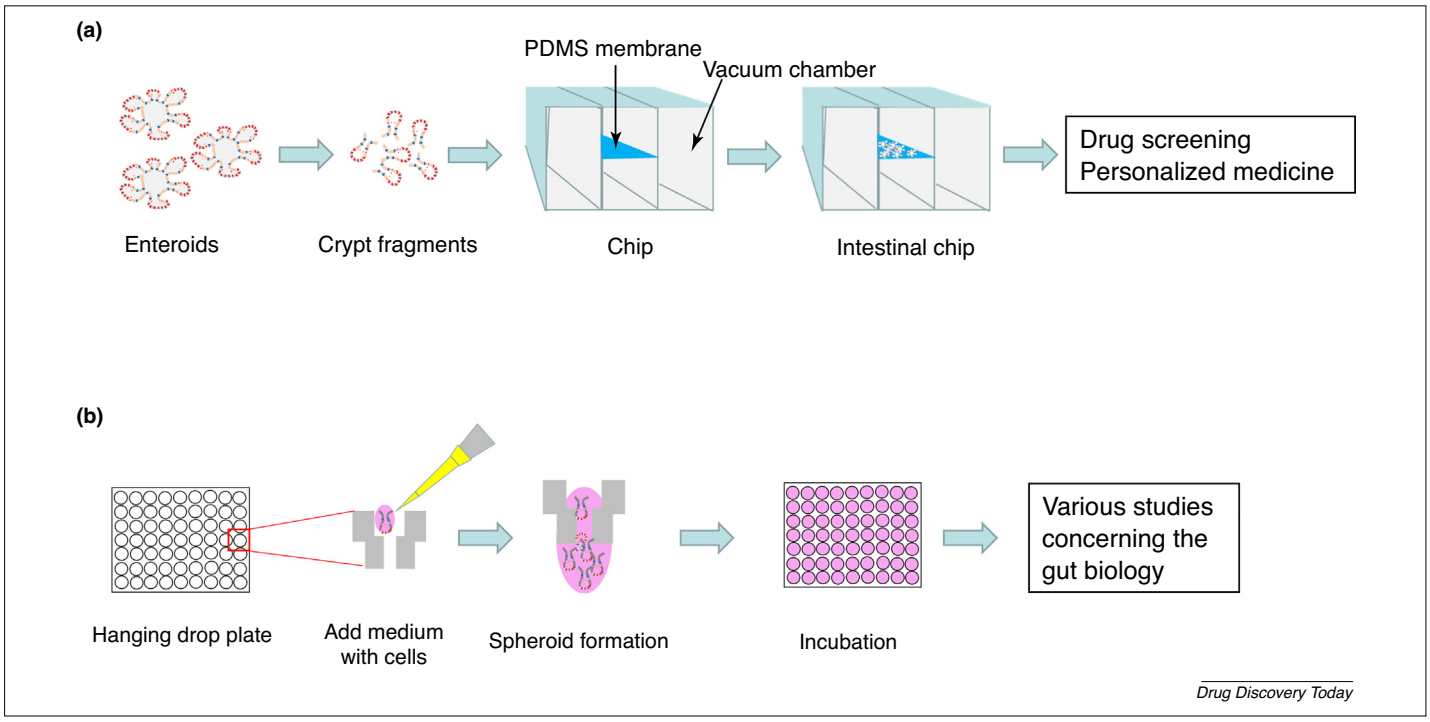

FIGURE 3

New approaches developed using mini-guts include the intestinal chip and the hanging drop culture system. **(a)** The intestinal chip contains an upper epithelial channel, a lower vascular channel and a thin flexible polydimethylsiloxane (PDMS) membrane containing multiple pores between the upper and lower channel; dissociated mini-guts can be cultured as monolayers on a PDMS membrane and these 2D cultures can be used for drug screening and personalized medicine. **(b)** In the hanging drop culture system, the cell pellet was resuspended in mini-gut culture medium, then the cell suspension aliquots were mounted into a 96-well Perfecta3D® hanging drop plate and cultured in an incubator.

the hanging drop and that these mini-guts presented a similar morphology to mini-guts grown in 3D Matrigel® [73]. Furthermore, the hanging drop system was associated with several advantages compared with a 3D Matrigel®-based system: (i) much less culture media and Matrigel® were required, which reduced culture cost considerably; (ii) there was no need to wait for Matrigel® solidification, which accelerated the cell-seeding procedure; (iii) the ability to further dilute Matrigel® medium using this system can circumvent experimental bias caused by the heterogeneity of different batches of Matrigel® [73]. The hanging drop culture system thus has the potential to be applied for the culture of mini-guts derived from adult intestinal tissue, which makes it possible to use this method for various studies concerning gut biology and drug development.

Cloning 'ground state' intestinal stem cells

Wang *et al.* developed a novel method for cloning 'ground state' intestinal stem cells, which could have great potential for regenerative medicine [68]. In the method, the human intestinal stem cells are capable of being maintained in a highly clonogenic, 'ground state' by treatment with a special media consisting of novel combinations of growth factors and regulators of transforming growth factor-b/bone morphogenetic protein (TGF-b/BMP), Wnt/β-catenin, EGF, insulin-like growth factor (IGF) and Notch pathways. The intestinal stem cells can be generated from different regions of the intestine including duodenum, jejunum, ileum and ascending, transverse and descending colon, and grown into mini-guts that present a region-specific signature and genomic stability. It was demonstrated that ISC-derived colonoids could be used for modeling *C. difficile* infections. With this method, it could also be possible to clone patient-specific, 'ground state' stem cells from endoscopic biopsies, which might provide a window for regenerative medicine, preclinical trials and disease modeling.

Microraft arrays

Gracz *et al.* and Samsa *et al.* described the MRA based on a cell raft array (CRA) system (Qiagen, Cell Microsystems, LLC) and mini-gut culture technique [74]. In this assay, a four-reservoir (quad-well) CRA system is used, which traps cells within microwells, and the four-reservoir cassette provides separate media environments to enable performing different treatments or replicates [74]. Notably, MRA enables the long-term 3D culture of mini-guts and traps mini-guts within spatially defined microwells, which facilitates identification, tracking and retrieval of individual mini-guts. MRA also bring insights into studying stem-cell–niche interactions and mini-gut development, and provides a high-throughput culture platform to question gene expression over the differentiation of ISCs [67].

Concluding remarks and future perspectives

As superior models, mini-guts can mirror the *bona fide* features of the *in vivo* intestinal epithelium, consisting of various functional intestinal cells including enterocytes, enteroendocrine cells, goblet cells, Paneth cells and ISCs. Emerging studies have used mini-guts to investigate pharmacology and develop new drugs, indicating their powerful applications in these fields. We highlight the importance of mini-guts for drug development to combat CF, cancer and infectious disease, in addition to drug screening, personalized medicine and developing new medical tools for drug development. Compared with IOs, considerably more studies (32

out of 36 articles) used enteroids and colonoids as models for drug development at this point in time.

Although mini-guts have numerous advantages compared with conventional cell lines and animal models, they still have several limitations: (i) they lack epithelial–immune-cell communication; (ii) nervous system regulation is absent in mini-guts; (iii) circulation is lacking in these systems; (iv) they do not possess tissue–tissue interfaces and mechanical forces (fluid flow and peristalsis-like motions); (v) the Matrigel®-based artificial niche is expensive and batches can different in quality; (vi) the cyst-like structure with tight junctions in Matrigel® prevents lumen penetration of drugs and washout; (vii) last but not least, different culture conditions could result in large shifts in cell composition and differentiation in mini-guts, which might affect the reproducibility of experimental results among different laboratories or even among different researchers in the same laboratory, especially when conditioned media are used. So, a detailed standard protocol describing organoid culture methodology and composition of media is desperately needed [75]. These limitations might impede the broad applications of mini-guts for drug development. Therefore, further studies are needed to mitigate these limitations. In fact, efforts have been made to mimic the complicated *in vivo* scenario, such as the co-culture of mini-guts with immune cells (e.g., intraepithelial lymphocytes) [76] and intestinal microflora [47]. Interestingly, a hanging drop culture system makes it possible to reduce the quantity of Matrigel®, thus providing the opportunity to reduce costs associated with mini-gut culture. Whereas the broad expansion of this method has not been reported, applications using this approach need to be further described, and increased efforts need to be devoted to reduce the costs of culturing mini-guts [73]. Sachs and colleagues reported that mini-guts fuse to form self-organizing tubes in floating collagen gels [77], providing insights into the generation of an artificial intestine. Mini-gut-based intestinal chips make it possible to mimic peristalsis-like motions [19]. The monolayer culture of mini-guts might circumvent the problem of lumen penetration of drugs and washout. Recently, Zafra *et al.* developed an optimized constitutive and inducible base-editing vector system to elevate the efficiency of gene editing in organoids [78]. Thus, the combination of CRISPR/Cas9-based gene editing technology and organoids might open a new window for exploiting diseases of the digestive system and developing relevant novel drugs [79]. However, data concerning the utilization of mini-guts as preclinical models still need to be expanded, and further efforts are required to continuously improve the mini-gut models to make them more-accurate replicates of native epithelium and to accelerate drug development.

Conflicts of interest

The authors have no conflicts of interest to declare.

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