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Feature Review

Disease Modeling in Stem Cell-Derived 3D Organoid Systems

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Organoids are 3D *in vitro* culture systems derived from self-organizing stem cells. They can recapitulate the *in vivo* architecture, functionality, and genetic signature of original tissues. Thus, organoid technology has been rapidly applied to understanding stem cell biology, organogenesis, and various human pathologies. The recent development of human patient-derived organoids has enabled disease modeling with precision, highlighting their great potential in biomedical applications, translational medicine, and personalized therapy. In light of recent breakthroughs using organoids, it is only apt that we appreciate the advantages and shortcomings of this technology to exploit its full potential. We discuss recent advances in the application of organoids in studying cancer and hereditary diseases, as well as in the examination of host cell–microorganism interactions.

Organs in a Dish: Organoids Mimic *In Vivo* Tissues

One of the major breakthroughs witnessed by the stem cell field in the past decade includes the development of 'organs' in a dish, also known as organoids. The term 'organoid', which means 'resembling an organ', was used as early as 1946 when Smith and Cochrae used it to describe a case of cystic teratoma [1]. In the years that followed, organotypic structures in culture systems came to be regarded as organoids. Thus, in classical developmental biology studies, 3D cell aggregates such as spheroids were described as organoids, although these did not fully represent *in vivo* tissue structure. Recently, the term 'organoid' has been conferred a somewhat restricted meaning – that of a self-organizing 3D structure grown from stem cells which mimics the *in vivo* architecture and multi-lineage differentiation of the original tissue in mammals. Organoids can be derived from two types of stem cells: (i) **pluripotent stem cells** (PSCs), in other words either **embryonic stem cells** (ESCs) or **induced pluripotent stem cells** (iPSCs), and (ii) organ-specific **adult stem cells** (ASCs), which are tissue-specific resident stem cells (Boxes 1, 2).

Employing defined developmental cues, PSCs can be differentiated into different cell types and grown *ex vivo* as organoid models. Brain, retina, inner ear, stomach, intestine, thyroid, lung, liver, and kidney organoids have been derived from PSCs isolated from mouse and human tissues [2–16] (Table 1). While PSCs have for a long time been available for long-term culture *in vitro* (murine in 1981 and human in 1998), ASCs were originally considered to have limited *in vitro* proliferation potential [17,18]. A key advance in the field came when 3D organoids were generated from adult **Lgr5$^+$ stem cells** from mouse intestine [19–21]. By mimicking the *in vivo* niche environment, ever-expanding organoids were developed which resemble the structure and the functionality of original intestine. Single intestinal stem cells or intestinal **crypts** grown

Trends

Organoids can be developed from pluripotent stem cells and adult stem cells. Organoids have been established for multiple organs including intestine, kidney, brain, liver, stomach, pancreas, ovary, and lung.

Organoids can be used in multiple clinical applications including disease modeling, drug screening, host–microbe interactions, and regenerative therapy. Patient-derived organoids may enable personalized medicine.

Genes can be manipulated within organoids using molecular technologies such as the lentiviral expression system and CRISPR/Cas9; this may enable disease modeling and targeted gene therapy.

The complex interplay between microbes – bacteria, parasites, and viruses – and the host epithelium have been dissected using organoids derived from brain, stomach, and intestine.

Mechanically dynamic designer matrices such as hybrid polyethylene glycol hydrogels might expand the applicability of organoids in the future.

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Table 1. Human and Murine Organoids Derived from ASCs, ESCs, and iPSCs.

Tissue	Type of stem cell	Disease modeled	Living biobank	Refs
Intestine	Human/mouse ASCs	Cancer CF Infectious diseases	CF patients[a]	[21,47,48,76,77,86–88, 92,98,99,101,104]
	Human/mouse ESCs Human iPSCs		No	[15,55,91]
Colon	Human/mouse ASCs	Cancer Ulcerative colitis Crohn's disease	Yes	[22,37,47,50,68, 69,107,108]
Liver	Human/mouse ASCs	Alagille syndrome CF Cancer Lethal liver failure	No	[24,26,40]
	Human iPSCs		No	[8]
Prostate	Mouse/human ASCs	Cancer	No	[28,31,66]
Lung	Human ASCs Mouse fetal cells	Cancer	Yes[a]	[34]
	Human ESCs/iPSCs		No	[6,16,79]
Brain	Human ESCs/iPSCs Mouse ESCs	Autism Microcephaly Infectious diseases Cancer	No	[4,5,43,52–54,56,67, 81–83,106,109,110]
Kidney	Human ESCs/iPSCs	Cancer	Yes[a]	[7,44]
Pancreas	Human/mouse ASCs	Cancer CF	Yes	[25,27,111]
	Human iPSCs			[63,78]
Breast	Human ASCs	Cancer	Yes[a]	[45]
Ovary	Human ASCs		Yes[a]	[30]
Stomach	Human/mouse ASCs	Infectious diseases	No	[23,29,51]
	Human iPSCs Mouse ESCs		No	[94,96]
Esophagus	Human/mouse ASCs	Barrett's esophagus	No	[22]
Lingual	Mouse ASCs	Cancer	Yes[a]	[33]
Taste bud	Mouse ASCs		No	[13,32]
Inner ear	Mouse ESCs		No	[14]
Retina	Mouse ESCs	Retinal degeneration	No	[11,12]

[a]Have been established but are not yet published.

in 3D in Matrigel (an extracellular support matrix secreted by the Engelbreth–Holm–Swarm tumor cell line), in the presence of a defined set of niche factors (epidermal growth factor, EGF; proto-oncogene Wnt-3; Wnt signal amplifier **R-spondin**; and Noggin, a secreted bone morphogenetic protein inhibitor) can form 3D organoids [19]. Since the establishment of the first ASC-derived intestinal organoids, the original culture conditions have been tweaked to develop organoids from various mouse and human tissues including the colon, stomach, liver, lung, prostate, pancreas, ovaries, taste buds, and ligual epithelium [22–34] (Table 1).

The generation of adult tissue organoids requires no genetic transduction with transcription factors, which makes them physiologically compatible with the normal tissue of the host, thus providing an innovative angle to stem cell transplantation [35,36]. To this end, it was demonstrated that mouse colon organoids engrafted into dextran sulfate sodium (DSS) pretreated mice (which induces superficial mucosal lesions) could regenerate histologically and functionally normal crypts containing all differentiated cell types, without inducing any dysplastic

Glossary

Adult stem cells (ASCs): organ-specific stem cells found in adult organisms which have the ability to divide, renew, and regenerate tissue.
Autophagy: a cellular self-destruct mechanism to eliminate dysfunctional components in cytosolic organelles such as the lysozyme.
Biobank: a large collection of comprehensive biological tissue samples or medical records for research purposes.
Crypts: the intestinal epithelium is lined by self-renewing crypt–villus units. Villi are protrusions that include differentiated cells. The crypt is the invaginating region and is composed of proliferating $Lgr5^+$ stem cells and transit-amplifying cells.
Cystic fibrosis (CF): a genetic disorder affecting mostly the lungs, but also the pancreas, liver, kidneys, and intestine. Long-term issues include difficulty breathing and excessive mucus production as well as frequent lung infections.
Embryoid bodies: 3D aggregates of pluripotent stem cells (PSCs), specifically embryonic stem cells (ESCs) from blastocysts of humans, mice, and primates.
Embryonic stem cells (ESCs): embryonic stem cells are PSCs derived from the inner cell mass of the blastocyst of an early-stage preimplantation embryo.
Histone methyltransferase EZH2: a highly conserved methyltransferase that targets lysine 27 of histone H3A and is commonly associated with the silencing of genes involved in differentiation; often overexpressed in prostate and breast cancer.
Hypoxia gradients: brain tumor aggressiveness has been associated with different hypoxia levels in the tumor microenvironment. They can modulate the maturity of the tumor mass.
Induced pluripotent stem cells (iPSCs): pluripotent stem cells derived from adult somatic cells by reprogramming to an embryonic stem cell-like state. The first iPSCs were created from mouse embryonic fibroblasts (MEFs) and skin fibroblasts by the introduction of transcription factors Oct4, Sox2, Klf4, and c-Myc, commonly known as Yamanaka factors. Nanog and Lin28 can be used as replacements for Klf4 and c-Myc to reprogram human cells.

changes for long periods of time [37]. Furthermore, numerous established molecular techniques such as CRISPR/Cas9 technology, mass spectrometry, single-cell RNA sequencing, cryo-electron microscopy (cryo-EM), and high-resolution microscopy can be applied to organoids [38–50]. This enables a more refined understanding of the basic biology underlining tissue homeostasis, stem cell identity, and the pathophysiology of diseases such as cancer, **inflammatory bowel disease** (IBD), and hereditary diseases such as **cystic fibrosis** (CF). Furthermore, by introducing microbes such as *Helicobacter pylori* (*H. pylori*), ***Zika virus*** (ZIKV), and *Norovirus* (NoV) into organoids, various other infectious diseases can also be modeled [29,51–56]. It is therefore justified to claim that organoids hold great potential in expediting clinical applications in regenerative therapy and personalized medicine.

Recent discoveries made using organoids warrant a detailed review of the breakthroughs achieved and the shortcomings of this technology to help to understand the potential that the organoid technology holds for future studies. In this review we elaborate on recent advances regarding the use of organoid systems to study or approximate three specific disease or infection models, namely cancer, cystic fibrosis (CF), and host–microbe interactions (Figure 1, Key Figure).

Modeling Cancer Biology – Tumoroids

Accumulating mutations in normal cells as a result of genetic and environmental factors can lead to cancer development. Cancer cells acquire the capability to invade surrounding tissues and can colonize distant organs via the blood stream or the lymph, a process termed metastasis [57,58]. Surgery, chemotherapy, and radiation therapy have long been employed to treat cancer; however, most regimens have been unsuccessful in eradicating cancer cells completely. One of the main reasons for this is inter and intra-patient tumor heterogeneity. Cancer cells display heterogeneity in terms of their distinct mutational and epigenetic profiles, morphological and phenotypic differences, gene expression, metabolism, proliferation rates, and metastatic potential [59]. To understand cancer biology including tumor heterogeneity, and to translate this knowledge to clinical applications, preclinical cancer models are required [60].

Cancer cell lines and animal models have long been used for cancer modeling. While animal models (e.g., rodents) permit in-depth experimentation, they do not fully reflect the genetic characteristics of human cancers and are unable to truly capture tumor heterogeneity; hence, they are not the ideal systems for studying the oncogenic process. Likewise, cancer cell lines accumulate multiple additional mutations over time in culture conditions. While **patient-derived tumor xenografting** (PDTX) recapitulates the original tumor more closely than do cancer cell lines or animal models, there are several challenges to using PDTX. PDTX is labor-intensive, time-consuming and expensive, and engraftment efficiencies differ among tumors, making it them difficult to use for high-throughput screening. Thus, there are strong needs for a better model system for studying cancer. One of the attractions of the organoid culture system rests in the fact that similar culture techniques can be used for normal tissue and neoplastic tissue: patient biopsies and resections can be developed into organoids which mimic the original cancer tissue. **Tumor organoids** (tumoroids) can be generated very efficiently from tumor tissue and easily propagated under defined conditions. Therefore, tumor organoids can bridge the gap between 2D cancer cell lines and animal-based model systems, including PDTX [61].

Human **pancreatic ductal adenocarcinoma** (PDAC) is characterized by multiple genetic and epigenetic lesions [62]. At present there is no rational system to match patients with drug treatment outcome. Standard chemotherapy also exhibits varying – but low – degrees of effectiveness for PDAC. PDAC treatment lags significantly behind that of other cancers, with no effective targeted drugs in the market, and PDAC has thus become one of the most lethal malignancies today. Various laboratories including ours have established pancreatic

Inflammatory bowel disease (IBD): a group of inflammatory conditions affecting the colon and small intestine. Crohn's disease and ulcerative colitis are the primary types of IBD.
Lgr5$^+$ stem cells: Lgr5 (leucine-rich repeat-containing G protein-coupled receptor 5), also known as G protein-coupled receptor 49 (GPR49), is a receptor for R-Spondin. Lgr5 is a marker of adult stem cells in particular tissues including intestine, stomach, liver, pancreas, hair follicle, kidney, and taste bud; these are known as Lgr5$^+$ stem cells.
Metabolome: the entire content of metabolites present within an organism, cell, or tissue.
Microbiome: the entire collection of microorganisms in a specific niche such as the human gut. The microbiome comprises all the genetic material within a microbiota.
Miniaturized spinning bioreactor: a device in which a biological reaction is carried out under defined conditions. Spinning bioreactors are used for 3D cell cultures by mixing the oxygen and nutrients in the medium using a constant state of motion.
Pancreatic ductal adenocarcinoma (PDAC): a type of exocrine pancreatic cancer originating from cells lining in pancreatic duct.
Paneth cells: located at the base of the crypts of Lieberkühn in the small intestine, they synthesize and secrete substantial quantities of antimicrobial peptides and proteins, thus providing host defense against microbes.
Pattern recognition receptor (PRR): proteins expressed by cells of the innate immune system that can identify two classes of molecules: pathogen-associated molecular patterns (PAMPs), associated with microbial pathogens; and damage-associated molecular patterns (DAMPs), associated with cell components released during cell damage or death. They are classified into four types: Toll-like receptors (TLRs), nucleotide oligomerization domain receptors (NLRs), C-type lectin receptors (CLRs), and RIG-1-like receptors (RLRs).
Patient-derived tumor xenografting (PDTX): transfer of primary tumor tissue from patients into an immunodeficient mouse. PDTX generates preclinical models

Key Figure

Multiple Applications of Organoid Technology

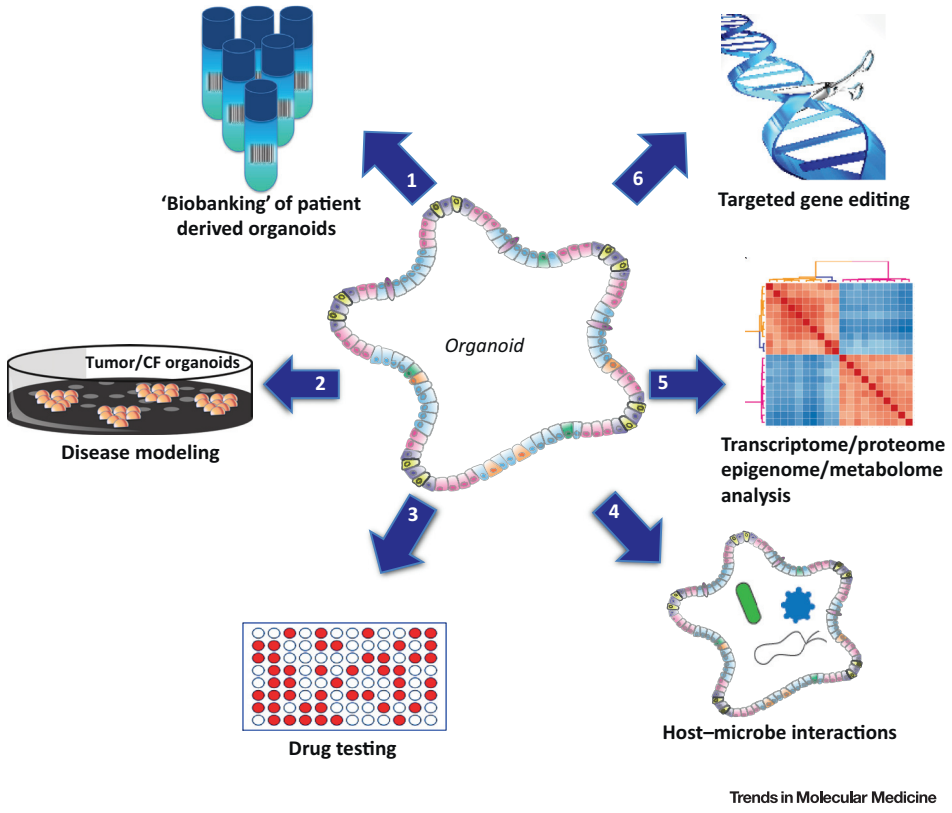


Figure 1. Schematic diagram of how stem cell-derived organoids can be exploited for multiple clinical applications such as organoid biobanking, disease modeling, drug toxicity testing, personalized therapy, host–microbe interaction studies, and omics analysis (transcriptomics, **proteomics**, epigenomics, and **metabolomics**) of healthy and diseased organoids. Moreover, targeted gene therapy using the CRISPR/Cas9 system can be utilized on organoids derived from disease tissue.

tumor organoids derived from resected tumors and biopsies [27]. Tumoroids recapitulate patient-specific histological features and physiological phenotypes. In addition, transplantation of patient-derived organoids can form lesions resembling an original tumor, suggesting that tumoroids can be used for drug screening. As a proof of concept, in one study patient-derived tumor organoids were treated with an epigenetic drug UNC1999 which inhibits **histone methyltransferase EZH2** (a writer for histone H3 lysine 27 trimethylation, H3K27me3). Among five patient tumoroids, the drug significantly reduced the proliferation of only three tumoroid lines. Interestingly, the three tumoroids and their original tumors harbored the H3K27me3 mark, while the other two lines did not, indicating that tumoroids could retain the epigenetic status of the original tumor, suggesting that the epigenetic drug could be used as a potential treatment for a subpopulation of PDAC patients carrying H3K27me3 [63]. Clonally derived organoids from human pancreatic tumor cells may prove to be a valuable tool to predict patient-specific sensitivities and clinical outcomes upon novel therapeutic drug treatment for PDAC in a similar fashion [64,65]. Likewise, human prostate cancer research has long been in search of a good *in vitro* model which is amenable to genetic and therapeutic studies. To address this, researchers have developed culture conditions for human prostate tissues [26].

that facilitate the study of metastasis and drug screening.

Pluripotent stem cells (PSCs): self-renewing stem cells with the ability to differentiate into any type of cell of all three germ layers – ectoderm, endoderm, and mesoderm. PSCs include both embryonic stem cells (ESCs) and induced PSCs (iPSCs).

Proteome: the total spectrum of protein that is expressed by a cell, tissue, or organism.

R-spondin: ligand of the Lgr4, 5, and 6 receptors. The R-spondin–Lgr5 (or Lgr4 and 6) complex inhibits Wnt repressors RNF43 or ZNRF3, resulting in Wnt signaling.

Short-chain fatty acids (SCFAs): end-products of fermentation of dietary fibers by the anaerobic intestinal microbiota in the intestine. They are considered to be the main nutrients for the lining cells of the large intestine and exert beneficial effects on mammalian energy metabolism.

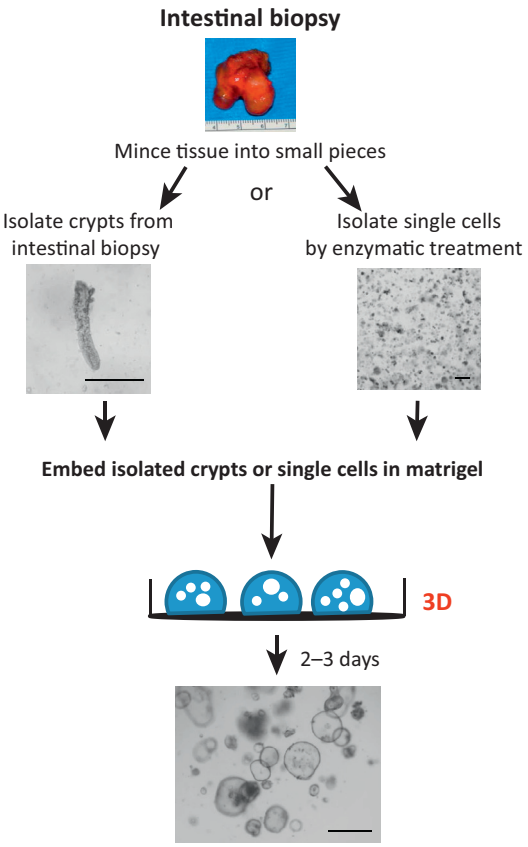
Transcriptome: the sum total of all mRNA molecules expressed by a cell, tissue, or organism.

Tumor organoids (tumoroids): aggregates of cancer cells in the form of spheroids or organoids formed *in vitro*.

Zika virus (ZIKV): a flavivirus transmitted by *Aedes* species mosquitoes. It was first isolated from a rhesus monkey in the Zika forest of Uganda in 1947.

Box 1. Methods for Generating Organoids from ASCs

A tissue biopsy is minced into several pieces using forceps, scissors, and lancets (for intestinal biopsy, crypts can also be isolated by EDTA treatment as an alternative). The small pieces of tissue are then incubated with enzymes such as collagenase, elastase, or dispase for 30–60 minutes at 37 °C to generate a single cell suspension. Single cells are seeded in Matrigel and grown in culture medium (Figure I, left) containing specific tissue growth factors (Figure I, right).



Intestine	Liver	Pancreas	Stomach
Wnt3a	Wnt3a	Wnt3a	Wnt3a
EGF	EGF	EGF	EGF
Noggin	Noggin	Noggin	Noggin
Rspo	Rspo	Rspo	Rspo
Nicotinamide	Nicotinamide	Nicotinamide	Nicotinamide
PGE_2	PGE_2	PGE_2	–
P38 inhibitor	–	–	–
A.83-01	A.83-01	A.83-01	A.83-01
Gastrin	Gastrin	Gastrin	Gastrin
N-acetyl cysteine	*N*-acetyl cysteine	*N*-acetyl cysteine	*N*-acetyl cysteine
Rho-KI	Rho-KI	Rho-KI	Rho-KI
B27	B27	B27	B27 without vitamin A
–	FGF-10	FGF-10	FGF-10
–	HGF	–	–
–	Dexamethasone	–	–

Trends in Molecular Medicine

Figure I. Schematic Diagram Depicting Current Methods for Generating Organoids from ASCs. The table on the right shows the media requirements for respective tissues types [22,26,27,51]. Abbreviations: A.83-01, selective inhibitor of ALK4, 5, 7 (inhibits TGF-β-induced epithelial to mesenchymal transition); B27, optimized serum-free supplement; EGF, epidermal growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; Rho-KI, Rho kinase inhibitor; Rspo, R-spondin.

Subsequently, a catalog of patient-derived prostate cancer cells from biopsy specimens and circulating tumor cells could be developed for long-term cultures of prostate cancer organoids. These organoids recapitulated the essential characteristics and molecular diversity of prostate cancer subtypes, making them a facile model system for pharmacological studies [28,66].

Recently, tumor organoids have been established from human glioblastoma, the most common and aggressive cancer of the brain [67]. Because glioblastoma presents great heterogeneity both at cellular and microenvironmental levels, it has been challenging to generate a good *in vitro* model which recapitulates the *in vivo* situation of this cancer. By modifying a method to develop cerebral organoids, tumoroids could be formed from fresh tumor tissues of glioblastoma patients, from xenografts, and also from brain metastases. These tumoroids displayed **hypoxia gradients** and mimicked cancer stem cell heterogeneity as found in the original

Box 2. Methods for Generating Organoids from PSCs

PSC-derived organoids are generated via multiple steps. Cerebral organoids originate as embryoid bodies (EBs) and grow into neuroepithelial buds. Subsequently, the EBs develop into a forebrain region in the presence of growth factors [4,43,82]. For other organs, PSCs are specified towards an endodermal fate by the addition of activin A. Then, spheroids or aggregates of the cells are formed and committed toward specific tissue types. These cells are further cultured as 3D organoids in Matrigel with medium containing tissue-specific growth factors (Figure I) [6,8,9].

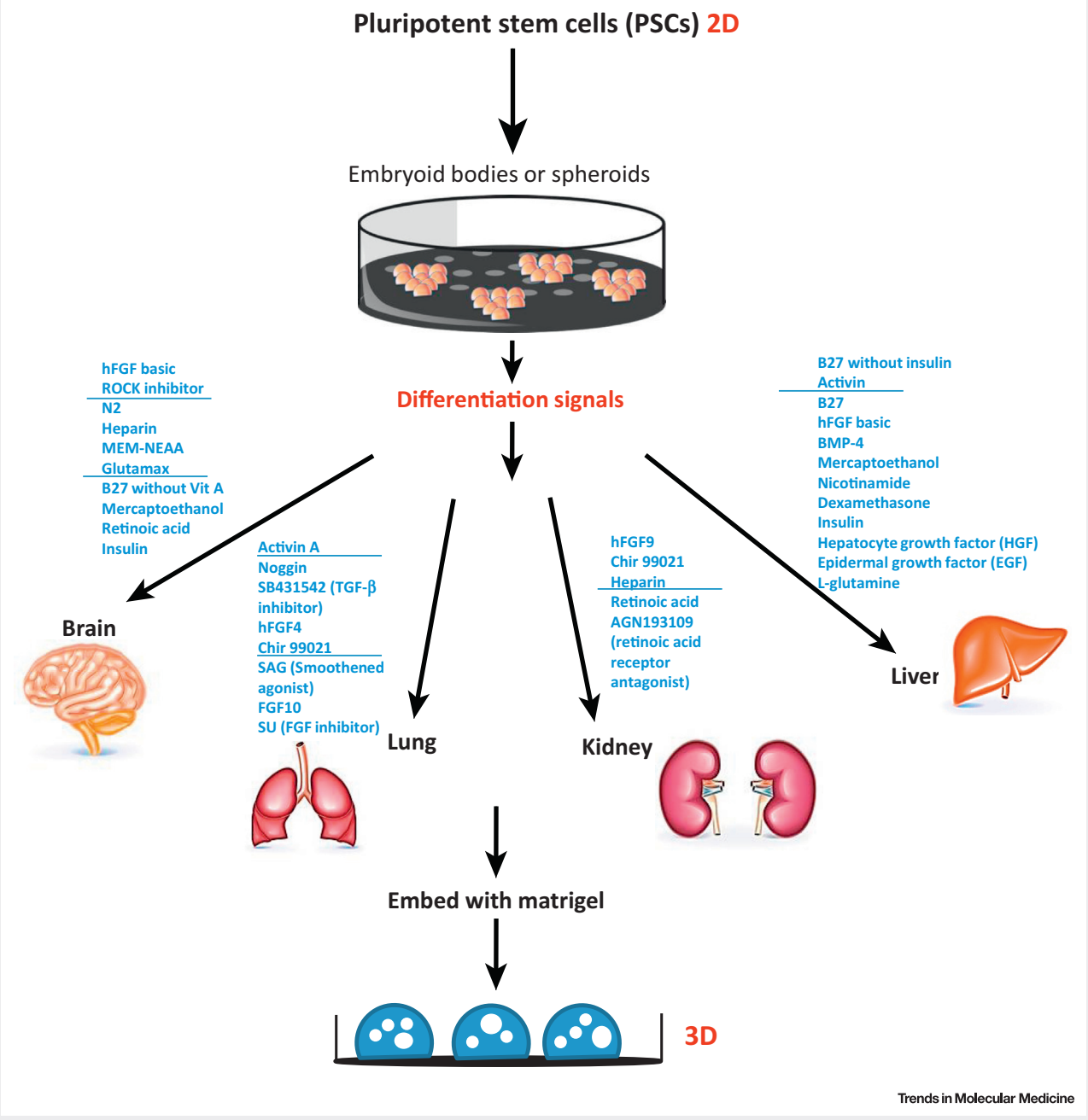


Figure I. Schematic Diagram Depicting Current Methods for Generating Organoids from PSCs. Growth factors required for tissue specification are marked in blue for the respective organs. Horizontal lines separate factors used at different steps of organoid generation.

tumors, thus suggesting that this panel of glioblastoma organoids could be used in diagnostics and therapeutics [67].

Given the urgent need of personalized cancer therapy, we are now developing 'living **biobanks**' of patient-derived organoids. For organoid biobanking, tissue resections are collected after surgery and developed into organoids which can be used immediately as live cultures or frozen in liquid nitrogen and recovered later. Such a pilot bank was established from 20 consecutive colorectal carcinoma (CRC) patients. These tumor organoids represent the various CRC molecular subtypes and allow precise gene–drug association studies and high-throughput drug screens. For instance, of the 20 patient organoids, the only patient organoid line carrying a mutation in the Wnt feedback regulator *RNF43*, instead of mutations in *APC* or *CTNNB1*, was found to be extremely sensitive to a small-molecule inhibitor of Wnt secretion (porcupine) [68]. Tissues of cancer patients are now being collected, developed, and maintained as organoids (http://hub4organoids.eu/living-biobanks/). This collection of organoids derives from patients with various malignancies including colon, prostate, lung, pancreas, breast, and ovarian cancer. Each organoid line is characterized using genome sequencing, expression profiling, and drug testing with known drugs to establish a database linking genetic and transcriptional information to drug responsiveness. The laboratory of Sato has developed a similar colorectal tumor organoid library (CTOL). By using various culture conditions, tumor organoids from a range of grades and subtypes of colorectal tumors including rare subtypes have been generated [69].

Organoids derived from normal tissue can also model cancer. Using gene manipulation technologies in organoids, tumor-related genes can be studied in an isogenic (identical genotype) background. In one study, the expression of mutant *KRAS* and *TP53* was induced in pancreatic organoids derived from human PSCs by using a lentivirus expression system [63]. Transplantation of these organoids into the mammary fat pads of NOD (non-obese diabetic) SCID (severe combined immunodeficiency) mice showed abnormal ductal architecture and nuclear morphology, consistent with neoplastic transformation [63]. Using CRISPR/Cas9-mediated genome editing, different laboratories have independently introduced sequential mutations into normal colon organoids for four or more of the most commonly altered genes in CRC, namely *KRAS*, *APC*, *TP53*, and *SMAD4* and/or *PIK3CA* [47,50]. In the first study, extensive aneuploidy was seen in organoids upon loss of the human APC and TP53 proteins, and xenotransplantation into mouse models showed that quadruple mutant organoids developed adenocarcinomas when xenotransplanted [47]. In the second study, engineered organoids with all five mutations remained genetically stable as euploids and were devoid of aneuploidy or copy-number alterations [50]. When these organoids were transplanted into the kidney capsule of mice, they formed tumors but failed to metastasize to the liver, suggesting that these five mutations led to tumor formation but not to adenocarcinomatous progression [50]. Overall, these models mimic patient tumor formation scenarios much more closely than any other colon cancer model so far. They will undoubtedly be useful for studying colon cancer progression and anti-CRC drug discovery.

Modeling CF – The Hope of Using Personalized Medicine for Hereditary Diseases

Given the fact that organoids faithfully retain the genetic signatures of the original tissue, they can also be used to model hereditary diseases, one of which is CF. CF is an autosomal recessive genetic disorder that affects the epithelial cells of many organs including the lung, pancreas, sweat gland, liver, kidney and intestine [70,71]. CF is one of the most common lethal genetic diseases with a prevalence of approximately 70 000–100 000 people worldwide (www.cfww.org). The disease is characterized by the buildup of viscous, sticky mucus which clogs airways, causing lung infection and subsequent respiratory system damage [72–74]. CF

patients also suffer from chronic digestive system problems leading to diarrhea, weight loss, and CF-related diabetes mellitus (CFRDM). CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene which encodes a chloride channel protein contributing to the regulation of absorption and secretion of salt and water. Over 2000 mutations in the *CFTR* gene have been identified, and are known to affect the quantity and/or function of the CFTR channel in cell membranes, resulting in a wide range of disease severity phenotypes [75].

Classical CF treatments are mainly symptom-based therapies using mucolytics, anti-inflammatory agents, bronchodilators, and antibiotics. However, one major challenge faced by researchers in CF therapy is the considerable heterogeneity in responses between patient groups defined by the same CFTR mutation. In addition, all mutant forms of the CFTR channel cannot be tested in trials against these drugs because of the paucity of the corresponding patients, underscoring the need for a more personalized approach to identify responders to CFTR modulators.

The laboratory of Beekman first reported the use of human intestinal organoids to model CF for drug screening [76]. The CFTR channel is activated by cAMP whose intracellular levels are increased by the adenylyl cyclase activator, forskolin. To test whether the functional activity of CFTR could be measured in organoids, activation of the CFTR channel was induced by adding forskolin to intestinal organoid cultures. Because CFTR is predominantly located at the apical membrane of the intestine (which is equivalent to the luminal side of the organoid), activation of CFTR mediates fluid secretion from cells into the lumen of the organoids, resulting in rapid swelling of the organoids. Such organoid responses can be quantified by automated live-cell imaging analysis.

While healthy organoids responded to forskolin treatment and showed a forskolin-induced swelling (FIS) phenotype, no swelling was observed in organoids derived from *Cftr*^{tm1Cam} knockout (*Cftr*$^{-/-}$) mice or in rectal biopsies taken from CF patients carrying mutations in the *CFTR* gene such as ΔF508 [76]. However, a swelling phenotype of patient organoids was restored in the presence of CFTR-targeting drugs such as VX-809 (also known as lumacafto) and VX-770 (ivacaftor or Kalydeco) (Figure 2). Of note, when five different drugs (CFTR correctors VRT-325, Corr-4a, C8, VX-809, and a CFTR potentiator VX-770) were administered to rectal organoids derived from nine patients harboring various CFTR mutations, the amount of FIS restored by each drug was largely variable between individual organoids from different donors [76]. This indicated that the FIS assay of organoids could accurately represent the functionality of CFTR and that quantification of the CFTR response in organoids might potentially allow drug screening for individual patients. In a remarkable example, rectal organoids derived from a patient carrying extremely rare CFTR mutations (G1249R/ΔF508),responded to one of the drugs, VX-770, in a FIS assay; subsequent drug treatment of the patient showed impressive clinical responses, as evidenced by recovery of pulmonary function and body weight [77].

Living biobanks of organoids derived from CF patients are now being developed, and these might in the future be exploited to prescribe efficient, individually tailored medications of combinations of CFTR modulators to patients (Figure 2A). Furthermore, while human pancreatic and intestinal organoids have been used so far to model CF, organoids derived from lung, kidney, and liver can be established to study and understand the organ-specific pathophysiology of CF, with important implications for potential future therapeutic strategies [77,78] (Figure 2B).

Moreover, patient-derived organoids can be utilized for regenerative medicine in combination with genome-editing technology. In a proof-of-concept study, a ΔF508 mutation at the *CFTR*

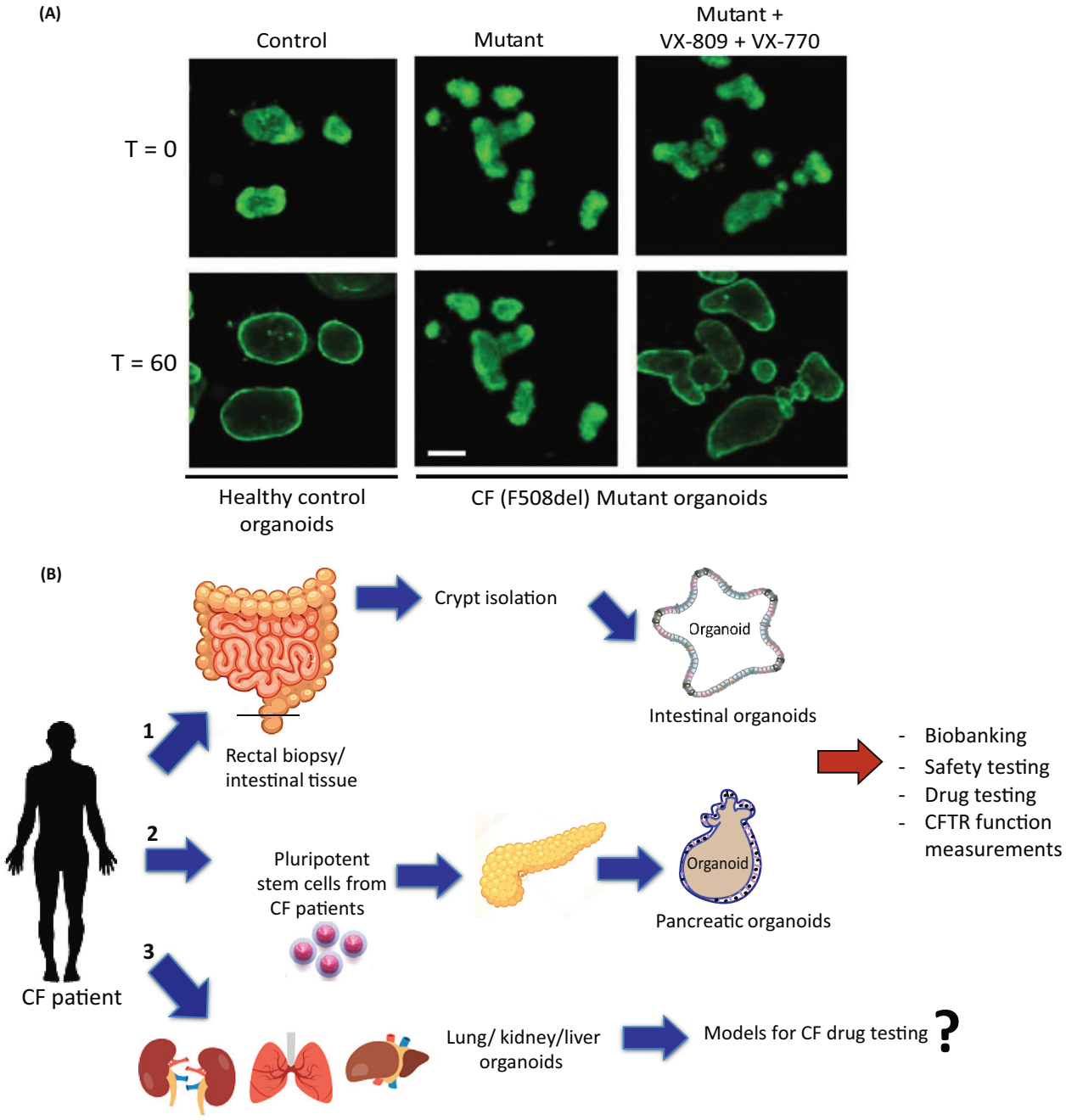


Figure 2. Patient-Derived Organoids for Modeling Cystic Fibrosis (CF) and Personalized Drug Screening. (A) Representative confocal microscopy images of FIS (forskolin-induced swelling) assays in human rectal organoids are shown: the functionality of the cystic fibrosis transmembrane receptor (CFTR) channel is assessed using the FIS assay. In the presence of forskolin, the wild-type CFTR channel in healthy organoids is activated, resulting in rapid swelling of the organoids. FIS is abrogated in organoids derived from CF patients carrying the CFTR mutation ΔF508 or F508del. Treatment with CFTR targeting drugs VX-809 (mutation corrector) and VX-770 (CFTR potentiator) can restore the FIS phenotype of the mutant organoids, thus proving drug efficacy [76]. T = 0 and T = 60 are 0 and 60 minute timepoints; scale bar, 100 μm. Thus, patient-derived organoids can be used in a similar fashion to ascertain the most potent drug for each individual patient. (B) Schematic diagram of multiple applications of organoids derived from CF patients. Tissue-specific pathologies of CF can be studied using organoids generated from distinct tissues. Intestinal organoids derived from the intestine and pancreas of patients, or from patient iPSCs have been reported. In the future, lung, kidney, and liver organoids could be established to study tissue-specific pathologies of CF.

locus was corrected in patient-derived intestinal organoids by homologous recombination using the CRISPR/Cas9 system [48]. Exactly as organoids derived from healthy donors, corrected patient organoids demonstrated a rapid swelling phenotype upon forskolin addition, indicating that the corrected CFTR channel was fully functional [48]. Likewise, other researchers have used CRISPR/Cas9 to correct mutations in iPSCs generated from CF patients. These have been converted into airway epithelial cells displaying normal CFTR function, suggesting that clinical approaches using iPSCs for gene therapy and regenerative medicine might be envisaged [79]. Of relevance, similar approaches might also be applicable to other single-gene hereditary diseases.

Modeling Infectious Diseases – Dissecting the Complex Interplay Between Host and Microbe

The fact that organoids faithfully recapitulate most cell populations found in the organ of origin makes them an ideal model system to study the interaction between a host and infectious organisms [80]. In 2016 the World Health Organization declared the ZIKV outbreak in Central and South America as a Public Health Emergency of International Concern [81]. The evidence suggested that ZIKV infection was strongly associated with microcephaly (abnormally small brain); however, to study ZIKV infection and pathogenesis, a robust model system was required. The laboratory of Knoblich and other researchers have developed brain organoids or 'mini brains' from hPSCs using a multi-step protocol [4,43,82]. These brain organoids originate as **embryoid bodies** and grow into neuroepithelial buds. They subsequently develop into discrete brain regions such as the retina, dorsal cortex, ventral forebrain, midbrain–hindbrain boundary, choroid plexus, and hippocampus, thus recapitulating features of human cortical development. Several groups independently recapitulated brain malformations induced by ZIKV infection using brain organoids generated from human ESCs and iPSCs; studies found that ZIKV infection halts growth, abrogating neurogenesis and causing disruption of cortical layers of human cerebral organoids, suggesting that microcephaly is a consequence of cell death of neural stem cells (NSCs) via apoptosis, **autophagy**, and impaired neurodevelopment in humans [53,54,81]. By analyzing gene expression of organoids upon ZIKV infection, another study reported that the potential mechanism of action of this deadly virus occurred via TRL3-mediated apoptosis [81]. ZIKV activates Toll-like receptor 3 (TRL3) which then leads to the activation of genes that can cause deregulated neurogenesis, an effect seen upon ZIKV infection [81]. To apply this promising organoid system for high-throughput drug screening, another laboratory has engineered unique **miniaturized spinning bioreactors** and developed a method to grow forebrain-specific organoids from human iPSCs in them [56]. This organoid platform is cost-effective and simple to use; the organoids grown in these bioreactors mimic most features of the forebrain. Furthermore, the modular stackable version of this system supports the growth of organoids in a highly reproducible and quantitative manner. Indeed, in proof of principle experiments, exposure of neural progenitor cells (NPCs) to ZIKV led to progressive infection which resulted in more infected cells over time. Furthermore, characteristic features resembling microcephaly (i.e., more cell death and a decrease in NSC proliferation) were reported in these organoids, demonstrating that the organoid platform could be used to model ZIKV infection and might be potentially used for screening therapeutics in the future [56]. Of note, additional studies have made use of organoids to investigate potential mechanisms of ZIKV entry [52,83].

As another example, human *Norovirus* (HuNoV) infections have also been studied using organoids. HuNoV infections are the most common pathogen to induce acute gastroenteritis worldwide [84,85]. HuNoV infection can be lethal for young children, elderly, and immunocompromised patients. Knowledge of the pathophysiology of the virus is still limited and appropriate medicines including vaccines are not available. One of the reasons for this has been the lack of an *in vitro* culture system for growing HuNoV. Recently a study reported for the

first time that HuNoV can successfully infect and replicate in enterocytes in monolayers generated from human intestinal organoids [88]. Bile was found to be required for the replication of particular strains of viruses such as GII.3 HuNoV. In addition, variable infection efficiency between different HuNoV strains was observed in distinct cultures depending on the histo-blood group antigen (HBGA) expression variability in the patients [88]. This recapitulated the infection patterns reported in epidemiological research, indicating that this culture system may be physiologically relevant.

Intestinal organoids have also been applied to study another enteric virus, *Rotavirus*, which is the second most common cause of diarrhea in humans next to HuNoV [87]. Despite the establishment of laboratory-adapted strains of rotavirus, cultivation of clinical strains has been challenging. To obtain clinical rotavirus, it needs to be adapted to a culture system by several passages in primary African green monkey kidney cells. Recently, studies demonstrated that iPSC-derived human intestinal organoids could support the replication of rotavirus from clinical samples [86,87]. These systems have been invaluable in that they may facilitate the development of novel diagnostics, vaccines, and therapeutics against particular viral pathogens.

From another perspective, human protozoan parasites and their interactions with a host remain largely unexplored because most parasites cannot be efficiently cultured *ex vivo* owing to their stringent specificity for human hosts. Organoids however, might hold promise in solving this dilemma [89]. Consequently, one might envisage in the future using human organoids to study host–parasite interactions for highly prevalent – but less well understood – organisms such as *Cyclospora cayetanensis*, *Cryptosporidium* spp., and *Giardia duodenalis*.

Like many viruses, bacterial pathogens such as *Clostridium difficile*, *Shigella flexneri*, and *Salmonella enterica* also cause gastrointestinal diseases and diarrhea. While animal models and established cell lines have been used in the past to gain insights into the pathogenic properties of such bacteria, organoids are now being employed to study the direct interaction and cell type-specificity of such infections [55,90–92]. We and other groups have demonstrated that microbes can be microinjected into the lumen of organoids, which is equivalent to the intestinal lumen *in vivo* [27] (Box 3 Figure I). These models enable studies of the interaction between host epithelial cells and microbes and help to understand the pathophysiology of various disease states (Figure 3A–C). Ongoing studies are currently evaluating whether bacteria such as *Clostridium difficile* or *Shigella flexneri*, when microinjected into the lumen of intestinal organoids derived from iPSCs or ASCs, can massively disrupt organoid architecture and result in a loss of organoid paracellular barrier function (Figure 3D). These studies may help to shed

Box 3. Techniques for Introducing Microorganisms into Organoids

Organoids are closed 3D systems that display the apical side of the epithelium towards the inside (lumen) and the basal membrane towards the outside. The apical membrane is initially targeted by microorganisms *in vivo*. To recapitulate the natural interaction between microbes and a 'host' within organoids, three independent approaches have been established (Figure I).

(i) Infection of Dissociated Cells Before the 3D Structure Is Reformed.

Through mechanical shearing or trypsin treatment, organoids are disrupted into single-cell suspensions in which the apical side is exposed. Mixing dissociated cells with microbes induces infection of the cells. When infected cells are seeded in the 3D matrix they reform organoids and can be used to model infectious disease [81,91,92]. This method is used for manipulating gene expression of organoids through a lentiviral system [38]. A similar approach has been applied to study non-infectious bacteria. When large particles of broken organoids are seeded with microbes in the matrix, non-infectious bacteria enter the lumen of newly formed organoids [98]. The methods are easy to perform and do not need special equipment. However, infection efficiency can vary among different microorganisms. Further, it is impossible to capture the initial interaction between microbes and host cells. Lastly, the methods may induce non-specific responses as a result of the interaction of microbes with the basal side of cells within organoids.

(ii) Microinjection of Microorganisms into the Lumen of Intact Organoids

By modifying a microinjection technology used for ESC injection in mouse genetics, microorganisms can be directly injected into the lumen of the organoid [29,55,96]. Because microbes are introduced into intact organoids, the initial interaction of microbes and early responses of host cells can be detected. The organoids are a closed system, and the effects of either apical or basal interaction can be studied separately. However, this method requires additional equipment such as a microinjector (for injection) and it is difficult to perform precise quantitative experiments owing to the different sizes of organoids that are obtained in culture.

(iii) Addition of Microorganisms to 2D Cultures Derived from Organoids

When cells dissociated from organoids are seeded onto an extracellular matrix (Matrigel or collagen)-coated dish, they grow in 2D with the apical side exposed upwards. Addition of microorganisms directly into the culture media then allows interaction between microbes and the host cell monolayer [88]. The 2D culture consists of distinct differentiated cells such as organoids and allows quantitative introduction of microbes. However, it does not resemble the *in vivo* 3D structure of host cells/tissues.

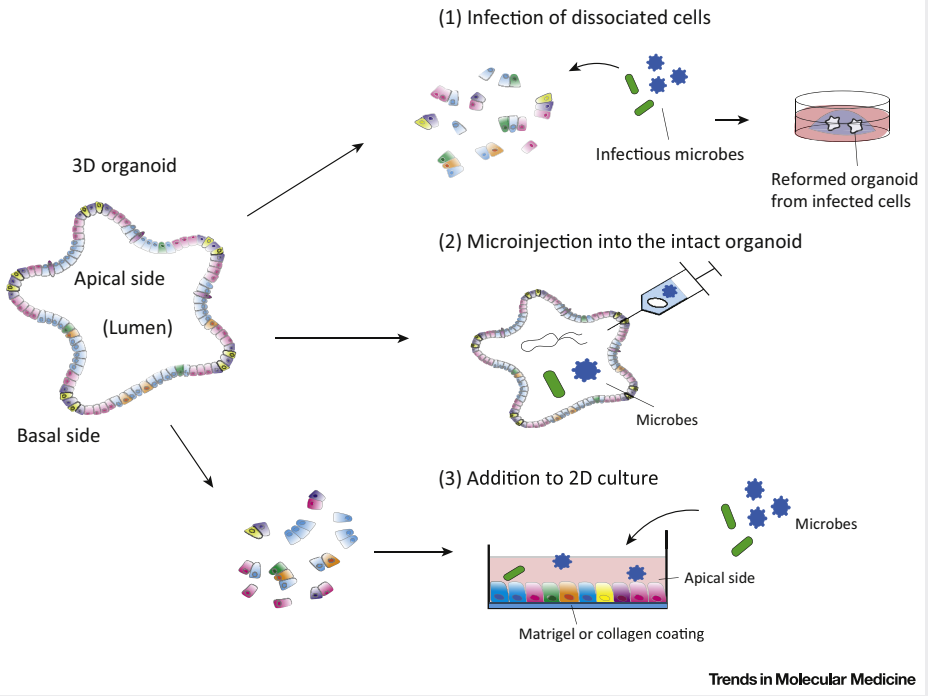


Figure I. Techniques for introducing microorganisms into organoids: (1) infection of dissociated cells, (2) microinjection, and (3) addition to culture.

light into the pathophysiology of infectious diseases and assist the development of better putative therapeutics to counter these infections in the future.

Various epidemiological studies have suggested a contribution of bacterial infections to cancer development. However, the mechanisms behind this association have remained obscure. It has been reported that chronic infection of *Salmonella enterica* serovar Typhimurium is significantly associated with gallbladder carcinoma (GBC) in humans [93]. To understand this association, wild-type (WT) *Salmonella* was infected to pre-transformed mice (Apc^{+}/min), harboring mutations in one allele of the *Apc* gene and overexpressing the *Myc* oncogene [93]. Upon infection with WT *Salmonella*, colorectal adenocarcinomas were observed in the mice whereas infection of mutant *Salmonella* lacking the type 2 secretion system (*ΔprgH*) failed

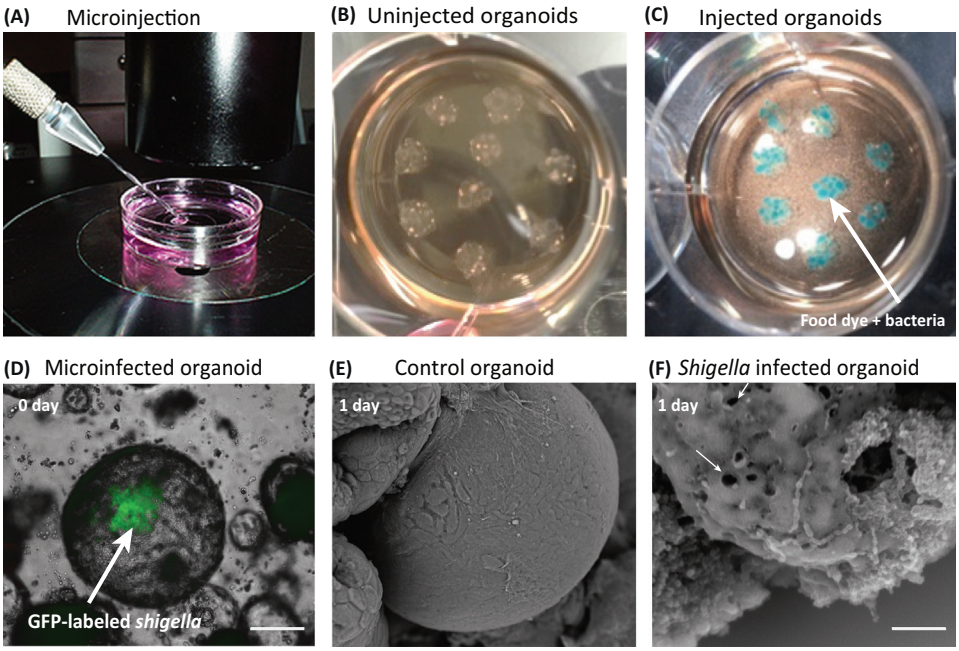


Figure 3. Pathogen Microinjection into Organoids to Study Host–Microbe Interactions. (A) Microinjection of microbes into the lumen of organoids ensures that the apical side of the organoid is exposed to the microbes. (B,C) Injection can be visualized using non-toxic dyes such as food dyes. (D) Microbes expressing fluorescent proteins such as GFP reside in the lumen of the organoid after injection (scale bar, 100 μm). (E,F) Representative Scanning electron microscopy (SEM) images demonstrate that human intestinal organoids can mimic some effects of infection, as seen *in vivo* with *Shigella flexneri*, shown here as an example. These bacteria can cause complete disruption of organoid architecture after 1 day in culture (cell death), a phenomenon commonly seen in many cases of bacterial infection (scale bar, 5 μm).

to develop any colon adenocarcinomas. Mouse gallbladder organoids infected with WT *Salmonella* presented similar transformation features to the GBC mouse model, such as polarity loss and cells bearing large irregular nuclei. In addition, cells from gallbladder organoids pre-exposed to WT *Salmonella* infection showed growth factor independence; specifically, they were able to form new organoids in growth factor-depleted medium, unlike cells which had no previous exposure. Hence, this study exposed a direct association between pathogenic bacteria and cancer, further detailing a mechanism whereby *Salmonella*-mediated activation of Akt and MAPK kinase pathways could drive cellular transformation [93].

From another angle, chronic infection with *H. pylori* can cause gastric ulcer and gastric cancer. When *H. pylori* were injected into the lumen of human gastric organoids derived from ASCs, bacteria were tightly associated with the organoid epithelium and induced transcription of NF-κB target genes including chemokine IL-8 [29]. Inflammation was further promoted by the differentiated cell populations (gland type) in gastric organoids, as assessed by gene expression analysis. Such induced inflammatory responses could represent a mechanistic link to gastric cancer development, at least in this model [29]. In a separate study, human gastric organoids were used to assess how metabolites such as urea – emanating from the organoids – might be chemo-detected by the bacterial chemoreceptor TlpB in *H. pylori* – a receptor locating to the host epithelium and using its urease activity to degrade host urea [94]. Human PSC-derived gastric organoids have also been used to model the pathogenesis of *H. pylori* infection [95,96]. In this system robust epithelial responses including increased cell proliferation

and *H. pylori*-induced activation (tyrosine phosphorylation) of oncogenic c-Met were reported in these organoids. The *H. pylori* virulence factor CagA was shown to form a complex with the c-Met receptor to facilitate infection [95,96]. Collectively, these studies indicate that organoids can be used as robust systems to unravel so far undiscovered roles of specific pathogens and, furthermore, enable precise host–microbe interaction studies which might be impracticable *in vivo*.

The organoid technology can also be applied to study commensal microbiota. While most of the studies on the **microbiome** have uncovered significant correlations between the presence or absence of specific microbial taxa and disease states, the major challenge of discovering a causal link between microbiome fluctuations and significant pathologies remains; however, this is a hurdle that might be overcome using organoids [97]. One of the practical challenges of studying microbiota using *in vitro* organoids is that most microbiota are anaerobic, whereas stem cell-derived organoids are grown under normal oxygen concentrations. Thus, current studies using organoids have been performed mainly with oxygen-tolerant bacteria or bacterial components [98,99].

For instance, a study using murine small intestinal organoids was able to link gut microbiota to epithelial regeneration [98]. The findings demonstrated that when the **pattern recognition receptor** (PRR) NOD2 – that is highly expressed in mouse stem cells – was stimulated by a constituent of bacterial cell walls (peptidoglycan muramyl-dipeptide, MDP), it enhanced the survival of organoids and protected these from oxidative stress-mediated stem cell death. Consequently, the number of organoids formed in culture was increased [98]. Moreover, intestinal epithelial cell turnover was also found to be stimulated by commensal bacteria-derived **short-chain fatty acids** (SCFAs) in mice [100]. Acetate, propionate, and butyrate were added to mouse intestinal organoid cultures, and each significantly promoted organoid development and growth, with an additive effect being shown for a mixture of all three SCFAs [100]. In another study, mouse ileal organoids were exposed to SCFAs and two specific gut bacteria *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* (*F. prausnitzii*) [99]. These organoids displayed fluctuations in the expression of genes involved in host lipid metabolism and epigenetic activation/silencing of transcription, including *Fiaf*, *Gpr43*, *Hdac3*, *and Hdac4* [99]. Of note, bacterium *A. muciniphila* induced stronger gene expression effects relative to *F. prausnitzii* [99]. In organoid cultures, commensals such as *Bacteroides thetaiotaomicron* have also been shown to appropriate the epithelium to advance proliferation and promote niche maintenance [101]. In addition, fluctuations in butyrate (a product of dietary fiber fermentation) were found to potently suppress epithelial stem cell proliferation in mice [102]. However, this suppression was reduced in the presence of colonocytes (epithelial cells lining the colon), suggesting that crypt architecture might potentially play a large role in the response of the intestine to bacterial metabolites in mice [102]. In an organoid **transcriptomic** approach, IL-22RA1 signaling was documented to promote fucosylation (the addition of fucose sugars to glycoproteins/glycolipids) in the intestine via induction of the gene *Fut2* in mice [103]. Fucosylation in turn prevented colonization by the opportunistic bacterial strain *E. faecalis*, restoring anaerobic commensal diversity and thus homeostasis within the gut [103]. Furthermore, previous findings in primary epithelial mouse organoids have demonstrated that the proinflammatory cytokine IFN-γ can trigger **Paneth cell** degranulation and the release of antimicrobial products such as defensins/cryptdins and lysozymes; this may constitute a mechanism by which immune responses contribute to the regulation of epithelial cell barrier formation, homeostasis, and colonization by the gut microbiome [104]. Given that organoid cultures can be generated from human biopsies and efficient microinjection techniques have been established, hopefully it might be possible in the future to further dissect the complex interplay in the intestine between individual resident microorganisms and the epithelium.

Challenges of Organoid Modeling

Organoids however, do not come without shortcomings. They are devoid of the native microenvironment consisting of stromal cells, muscle, blood vessels, and immune cells. The development of coculture conditions of organoids with immune cells or other cells constitutes the next frontier. While *in vivo* tumors harbor a wide range of genetic heterogeneity, it is not clear if tumoroids can capture whole heterogeneous populations stemming from the original tumors. One cannot exclude the possibility that established culture conditions may be suitable for the growth of particular subpopulations of tumor cells. In addition, key components for utilizing 3D culture (e.g., Matrigel and its relative basement membrane extract, BME) are animal-derived matrices which are poorly defined and are not mechanically pliable after plating. Clinical applications will warrant a transition to more-defined and mechanically-dynamic matrices to fully exploit the potential of organoid technologies. Hybrid polyethylene glycol (PEG) hydrogels represent one example of such next-generation 'designer matrices' which might expand the applicability of organoids [105]. Nonetheless, it should be noted that, although unlikely, there is always a possibility that an organoid culture will fail to be established, particularly if derived from diseased tissue, and establishment may be limited by factors such as biopsy size and/or differential growth factor requirements.

Concluding Remarks

In this review we have discussed current advances in organoid technology to study cancer, CF, and infectious diseases. Culture conditions have already been established for generating organoids from tissues of various human organs including lung, liver, kidney, ovary, brain, and colon, and organoid technology is emerging as an application that will enable us to begin to understand multiple facets of diseases such as microcephaly, autism, ulcerative colitis, and Crohn's disease [4,106–108]. As demonstrated in the case of CF, gene-editing techniques such as CRISPR/Cas9 could potentially be applied to organoids derived from patients with monogenic defects, such as Huntington's disease and polycystic kidney disease (PKD), to generate healthy tissues for the purpose of transplantation. Proliferating hepatocyte/liver organoids and pancreatic organoids with functional β-cells may be utilized for the transplantation treatment of patients with hepatic cirrhosis, non-alcoholic fatty liver disease (NAFLD), or diabetes; indeed, the feasibility of transplantation, *in vivo* functional engaftment, and regeneration of liver, colon, and pancreatic organoids have already been demonstrated in various mouse models [24–26,37]. Furthermore, although many questions and hurdles still remain, biobanks of patient-derived organoids should further refine our understanding of inter-patient as well as intra-patient heterogeneity, and hopefully lead to personalized therapies for various diseases (Box 4 and Outstanding Questions). Finally – as demonstrated in the case of ZIKV, organoids may be instrumental not only for understanding diverse mechanisms of action, but its application may also inform drug discovery to combat various pathogenic organisms in the future. In conclusion, stem cell-derived organoid technology holds marked promise for disease modeling, toxicity testing, the development of novel therapeutics, personalized therapy, and regenerative medicine.

Box 4. Clinician's Corner

Organoids can be established from multiple tissues such as colon, intestine, stomach, kidney, pancreas, liver, brain, ovary, and lung (from both mouse and human) and can also be derived from healthy donors and patients.

Because organoids recapitulate the structure and the functionality of their parental/original tissues, they can be an ideal *in vitro* model system to study tissue development as well as the pathology of various human diseases.

Organoids retain the genetic status of the original tissues, and patient-derived organoids enable personalized drug screening for various cancers and other genetic diseases such as CF.

Host–microbe interactions can be precisely studied using organoid models.

Outstanding Questions

Can we generate authentic mini-organs *in vitro*? While current organoids mainly contain epithelial cells, authentic organs consist of many different types of cells including stromal cells, muscle, blood vessels, and immune cells which support the morphology and the functionality of these organs. If we can establish mini-organs that can completely mimic *in vivo* organs in terms of cell composition and shape, it may be possible to study organogenesis in a more complex context.

Can iPSC-derived organoids be used for transplantation to human patients? Several recent studies demonstrated that genetic and epigenetic aberrations observed in early-passage iPSCs were associated with technical limitations, and the genomic stability of iPSCs was comparable to that of ESCs despite reprogramming procedures [112]. However, we cannot rule out the possibility that genetic aberrations might be generated during long-term culture. Thus, there is a need to further improve the protocols for iPSC-derived organoid development to minimize cellular mutations and to be able to use iPSC-derived organoids in transplantation/regenerative medicine.

Can we dissect the interaction of the gut microbiome with the immune system or with the epithelium of the gut using organoid systems? Current approaches to microbiome studies involve multiple different microbes. This makes it virtually impossible to study direct microbe–epithelium interactions. Organoids might potentially allow the dissection of the complex interplay between individual microbes and the human host. Coculture systems (organoids with immune cells and microbiota) will be necessary to gain a better understanding of intestinal dysfunction, host–pathogen interactions, and for developing targeted therapies.

Can synthetic matrices such as hybrid PEG hydrogels be used for the optimal long-term culture of stem cells and organoids of all tissues? While current designer matrices have been customized for growing mouse and human intestinal stem cells, the niche requirements of different tissue organoids must be considered to generate matrices that will facilitate the growth of other tissue organoids.

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