



Human organoid models to study SARS-CoV-2 infection

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Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is one of the deadliest pandemics in history. SARS-CoV-2 not only infects the respiratory tract, but also causes damage to many organs. Organoids, which can self-renew and recapitulate the various physiology of different organs, serve as powerful platforms to model COVID-19. In this Perspective, we overview the current effort to apply both human pluripotent stem cell-derived organoids and adult organoids to study SARS-CoV-2 tropism, host response and immune cell-mediated host damage, and perform drug discovery and vaccine development. We summarize the technologies used in organoid-based COVID-19 research, discuss the remaining challenges and provide future perspectives in the application of organoid models to study SARS-CoV-2 and future emerging viruses.

Origin and history of organoid models

Organoids are miniaturized in vitro organ models that are derived from stem cells or adult tissues extracted from patients in a specific three-dimensional (3D) microenvironment. They can self-organize and differentiate into functional cell types, which highly mimic the characteristics of the organs in vivo. Analyzing the formation of organoids can deepen the understanding of the mechanisms of human development and tissue/organ regeneration. In addition, organoids provide powerful platforms for disease modeling and drug discovery.

The origin of organoids can be traced back to 1907 when H. V. Wilson tried to regenerate organisms in vitro and showed that the mechanically separated sponge cells can reassemble and self-organize into new sponge organisms with normal functions¹. In early studies of 3D cell culture from 1965, organoids were defined as abnormal cellular growths or intracellular structures², and it was 1975 when Rheinwald and Green first described the method of long-term culturing human cells³. As 3D culture methods progressed, Emerman and Pitelka cultured mammary epithelial cells by floating collagen gels, with expression of milk protein for a month in 1977 (ref. ⁴). In 1981, for the first time, embryonic stem cells (ESCs) were isolated from mouse embryos, and were cultured in vitro⁵⁻⁷ and from human blastocysts in 1998 (ref. ⁸).

These advances fueled further developments in the field of stem cell biology and organoids. For example, in 2006, pluripotent stem cells (PSCs) were induced from mouse embryonic or adult fibroblasts by introducing four transcription factors⁹. In 2008, Eiraku and colleagues were able to generate cortical tissues that displayed apicobasal polarity from ESCs using the 3D aggregation culture system¹⁰ and, in 2009, Sato et al. demonstrated that the presence of leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5)-positive adult intestinal stem cells can induce the formation of 3D structures in Matrigel, which can self-organize and differentiate into crypt-villus organoids in the absence of a non-epithelial niche¹¹. Many 3D-cultured organoid systems have since been successfully generated, including gut¹², retinal¹³, brain¹⁴, liver¹⁵, kidney¹⁶, stomach¹⁷, lung¹⁸⁻²⁰, pancreas²¹, colon²², cardiac²³⁻²⁵ and blood vessel²⁶ organoids derived from human pluripotent stem cells (hPSC-derived organoids) and stomach²⁷, liver²⁸, pancreas²⁹, placenta³⁰ and lung³¹ organoids derived from adult tissues (adult organoids; Fig. 1).

Organoids to study infectious diseases

Organoids have been applied to model human diseases, including infectious diseases. This animal-free system is easy to use and provides new insight into human physiology and pathology. Early work started during the Zika virus (ZIKV) outbreak. In 2016, using brain organoid (BO) models, ZIKV was found to infect neural progenitor cells resulting in increased cell death and reduced proliferation^{32,33}. Subsequently, ZIKV-infected BOs were used to study viral tropism and strain pathogenicity and in whole-genome analyses^{34,35}. In addition, BOs were used in chemical screening studies, which identified several potential anti-ZIKV drug candidates³⁶. A recent study demonstrated both ZIKV and herpes simplex virus type 1 impaired the growth of BOs, induced distinct morphological defects and transcriptional changes, and eventually led to microcephaly, providing key insights into the pathophysiology of the disease³⁷. Before the COVID-19 pandemic, several studies applied human lung organoids to study respiratory diseases such as respiratory syncytial virus³¹ and influenza virus³⁸. These studies stimulated the field to apply the organoid platform to study newly emerging viruses, such as SARS-CoV-2.

COVID-19 and SARS-CoV-2

COVID-19 is an infectious disease caused by SARS-CoV-2 that leads to a severe acute respiratory syndrome, a hyperinflammatory response and widespread multi-organ damage (Fig. 2). Common symptoms of COVID-19 include fever, cough, fatigue, shortness of breath and loss of taste and smell.

Many studies have reported the entry factors for SARS-CoV-2. The first-identified and predominant SARS-CoV-2 receptor is angiotensin-converting enzyme 2 (ACE2) present on the cell surface of diverse human cell types³⁹. Viral entry into the cell is further facilitated by the cleavage of the spike protein to promote viral membrane fusion, which can be mediated by co-receptors transmembrane serine protease 2 (TMPRSS2)³⁹ and cathepsin L (CTSL)⁴⁰. The FURIN cleavage site also plays an important role in SARS-CoV-2 entry^{41,42}. Recent studies identified neuropilin-1 (NRP1) as another factor that can facilitate SARS-CoV-2 entry⁴³⁻⁴⁵.

As ACE2 is widely distributed in mammalian cells, SARS-CoV-2 can infect the human body through the respiratory tract and spread quickly toward other organ systems. As a result, COVID-19 not only causes severe respiratory diseases, but also induces damage in

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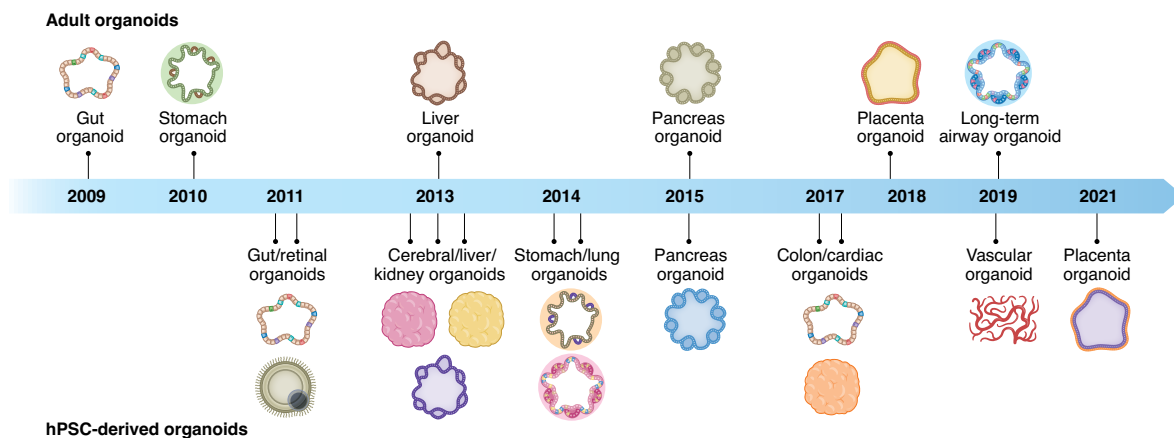


Fig. 1 | A summary of studies leading to the establishment of different organoid types. Schematic showing the timeline of the generation of different hPSC-derived and adult 3D organoids.

other organs, including brain, heart, liver, kidney, intestinal tract and pancreas. These complications include mental, cognitive or physical disorders, venous thromboembolism, myocarditis, heart failure, arrhythmia, cardiac arrest, acute kidney injury, liver injury, hyperglycemia and ketosis, acute cerebrovascular disease, and gastrointestinal complications.

Several monolayer culture systems, such as Vero E6 cells or ACE2-overexpressing cancer cells, have been utilized for therapeutic screening and mechanism studies of SARS-CoV-2 infection. But two-dimensional (2D) cell culture models cannot simulate the complexity of the host organisms and often have a different phenotype from the natural tissue. A platform with higher similarity to in vivo physiological and pathological characteristics of human tissues/organs, such as 3D organoids, can serve as an advanced model to study SARS-CoV-2 infection, mimic pathophysiology of injured organs and perform drug discovery.

Organoid models for COVID-19 research

Respiratory organoids. During embryonic development, respiratory epithelial cells are derived from the endodermal layer, followed by a complex series of signal transduction events to generate multiple compartments, including alveoli, proximal and intermediate airways, and respiratory bronchioles⁴⁶. SARS-CoV-2 mainly targets epithelial cells in the respiratory system and causes severe cough, excessive mucus production, and shortness of breath. In severe COVID-19 cases, individuals develop pneumonia, progress to acute respiratory distress syndrome, and finally develop respiratory failure. Therefore, lung alveolar, lung airway and bronchial organoids have been widely applied to monitor virus infection, explore pathological changes and identify potential therapeutics (Table 1).

Alveolar lung organoids. Alveolar lung organoids (ALOs) contain type 1 and 2 alveolar epithelial (AT1 and AT2) cells and mesenchymal cells. ACE2 is mainly expressed in a subpopulation of AT2 cells, while the co-receptor TMPRSS2 is widely distributed⁴⁷. hPSC-derived ALOs have been applied to model SARS-CoV-2 infection in alveoli, which demonstrated AT2 cells are permissive to SARS-CoV-2 infection^{47,48}. Furthermore, SARS-CoV-2-infected hPSC-AT2 cells were able to recapitulate host immune responses⁴⁹, leading to an inflammatory phenotype, induction of nuclear factor κ B (NF- κ B) signaling, and loss of the mature alveolar population⁵⁰.

hPSC-derived ALOs have also been used to identify drug candidates and antibodies blocking SARS-CoV-2 entry or replication. For example, a high-throughput screen of drugs approved by the US Food and Drug Administration (FDA) found three

drug candidates that block SARS-CoV-2 entry⁴⁷. Another chemical screening identified drugs that reduce ACE2 levels and inhibit SARS-CoV-2 in hPSC-derived cardiac cells and ALOs⁵¹. In addition, EK1 peptide blocking of spike protein and TMPRSS2 inhibitors were found to be effective against viral entry in hPSC-ALOs⁴⁸. Finally, a human neutralizing antibody was found to inhibit SARS-CoV-2 infection in hPSC-ALOs⁵². In addition to viral entry, several drug candidates were validated to block SARS-CoV-2 replication in hPSC-ALOs, including remdesivir⁵⁰, a class of 2-phenyl-1,2-benzoselenazol-3-one compounds targeting SARS-CoV-2 main protease (M^{pro})⁵³. Functional studies found that 25-hydroxycholesterol (25HC), an interferon-stimulated gene (ISG) and broad viral inhibitor, could inhibit viral entry in hPSC-ALOs. Mechanistic studies found 25HC can activate acyl-CoA: cholesterol acyltransferases and induce the depletion of accessible cholesterol from the plasma membrane⁵⁴.

Single adult human AT2 or keratin 5 (KRT5)-positive basal cells can generate adult ALOs, which contain AT1 and AT2 cells⁵⁵ with apical-out polarity of ACE2 expression. Similar to hPSC-ALOs, the major target of SARS-CoV-2 in adult ALOs is AT2 cells. Infected AT2 cells showed similar features of COVID-19 lungs, including type I/III interferon response^{55,56}, interferon-mediated inflammatory responses, loss of surfactant proteins and apoptosis⁵⁷. Furthermore, mutation analysis calculated that a single SARS-CoV-2 particle was responsible for viral infection in most of the AT2 cells⁵⁷. Several drugs, including remdesivir and the combination of ciclesonide, nelfinavir and camostat with remdesivir, have demonstrated anti-SARS-CoV-2 activity in adult alveolospheres⁵⁸. In addition, interferon was also shown to block viral replication in adult alveolospheres⁵⁹.

Lung airway organoids. hPSC-derived airway organoids (AWOs) consist of functional multiciliated cells, basal cells, mucus-producing secretory cells, CC10-secreting club cells, and so on. Upon SARS-CoV-2 infection, hPSC-AWOs undergo notable metabolic changes, including downregulation of lipid metabolism⁵² and upregulation of glycolysis⁶⁰. A high-content chemical screen using hPSC-AWOs identified that GW6471 can block SARS-CoV-2 infection by inhibiting the hypoxia-inducible factor 1- α (HIF1 α) pathway and glycolysis rates. Several inhibitors of fatty acid biosynthesis, such as xanthohumol, 5-(tetradecyloxy)-2-furoic acid and ND-646, have also been found to block SARS-CoV-2 infection⁶⁰.

The adult AWOs, which have similar cellular components as hPSC-AWOs³¹, also support active viral replication⁴⁹. Hence adult AWOs have been used to study replication dynamics of SARS-CoV-2

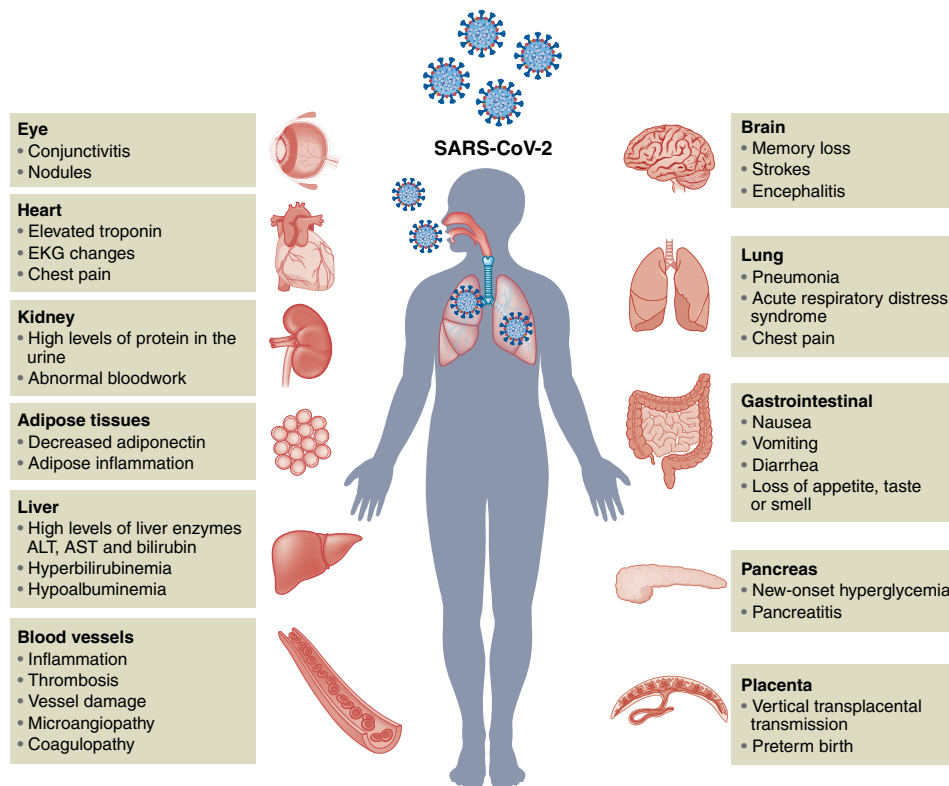


Fig. 2 | Complications of different organ systems in individuals with COVID-19. COVID-19 is caused by SARS-CoV-2 virus. Although SARS-CoV-2 primarily target lungs, individuals with COVID-19 develop symptoms in many organs. ALT, alanine transaminase; AST, aspartate aminotransferase; EKG, electrocardiogram.

variants, including B.1.1.7, B.1.427, B.1.429 and B.1.617.2. In a live virus system, SARS-CoV-2 variant B.1.1.7 showed higher replication efficiency than an ancestral isolate B.1.1.7, and a subsequent study demonstrated variant B.1.617.2 exhibited higher replication efficiency than B.1.1.7. In a pseudovirus system, variants B.1.427 and B.1.429 carrying the mutation p.Leu452Arg in the spike protein, demonstrated a higher entry activity than variants carrying the p.Asp614Gly mutation⁶¹. Transcriptomics and biomarker-based analyses identified the upregulation of ISGs⁶², as well as cytokines and chemokines, after viral infection. Moreover, a humanized decoy antibody (ACE2-Fc) was used to block viral infection through disruption of the binding of ACE2 and viral spike protein, thus blocking entry in adult AWOs⁶³.

Bronchial organoids. In addition to lung ALOs and AWOs, adult bronchial organoids (BCOs) have been used in SARS-CoV-2 studies. Microarray assays identified the change of cytokine/chemokines upon SARS-CoV-2 infection⁶⁴. By comparing high-throughput expression matrix data in SARS-CoV-2-infected BCOs with those of other cell types, colony stimulating factor 3 (CSF3) was identified as a potential drug target⁶⁵. Another study found that the microRNA hsa-MIR-5004-3p targets the leader sequence of SARS and SARS-CoV-2, the expression of which decreases after SARS-CoV-2 infection in BCOs⁶⁶.

In summary, respiratory organoid models have demonstrated that ciliated cells, club cells and a subpopulation of AT2 cells, which are arranged from the proximal to distal ends in the airway and terminal alveoli, are permissive to SARS-CoV-2 infection. These findings are consistent with data from autopsies of individuals with COVID-19 (ref. ⁶⁷), indicating that respiratory organoid models can recapitulate in vivo SARS-CoV-2 infection.

Intestinal organoids. Individuals with COVID-19 commonly exhibit gastrointestinal symptoms, such as diarrhea, vomiting or belly pain⁶⁸, and human intestinal organoids (IOs) have been identified as a physiologically relevant model to study intestinal pathophysiology in vitro. IOs contain several cell types including enterocytes, goblet cells, enteroendocrine cells, transit-amplifying cells and stem cells⁶⁹. hPSC-derived and adult IOs, including small intestinal organoids (SIOs), colonic organoids (COs) and ileal organoids (ILOs), have been used to study SARS-CoV-2 infection.

Both hPSC-derived SIOs and COs have been shown to be permissive to SARS-CoV-2 (refs. ^{47,70,71}). SARS-CoV-2 infection was accompanied by ultrastructural changes in hPSC-SIOs, and induction of a robust transcriptional response, including ISGs⁷¹. SARS-CoV-2 induced organoid deterioration, which could be rescued by remdesivir⁷¹. In addition, SARS-CoV-2 inhibitors identified using hPSC-ALOs⁴⁷ and hPSC-AWOs⁶⁰ were further confirmed using hPSC-COs. One study also found peptides or antibodies against interferon-induced transmembrane proteins (IFITMs), cofactors for efficient SARS-CoV-2 infection, could inhibit SARS-CoV-2 entry and replication in hPSC-SIOs⁷².

Adult SIOs and COs have also been used to evaluate SARS-CoV-2 infection in the gastrointestinal tract. Studies in adult SIOs containing mutations of 19 host factors previously implicated in coronavirus infection, demonstrated that ACE2, dipeptidyl-peptidase 4 and TMPRSS2 could regulate SARS-CoV-2 entry in SIOs⁷³. Another study showed TMPRSS2 and TMPRSS4 could facilitate SARS-CoV-2 spike fusogenic activity and promote virus entry into SIOs⁷⁴. In SIOs, enterocytes were demonstrated to be permissive to SARS-CoV-2 infection with an induction of a generic viral response program⁷⁵. Direct comparison of SARS-CoV-2 and SARS-CoV on SIOs showed the two viruses exhibit distinct dynamics of virus–host

Table 1 | Summary of the application of organoids to study SARS-CoV-2

			Entry factor expression	Tropism	Host response	Therapeutic discovery		
						Small molecules	Antibodies/peptides	Vaccines
Respiratory organoids	ALOs	hPSC-ALOs	ACE2 in AT2 cells; TMPRSS2 and FURIN widely expressed ^{47,48,52,70}	AT2 cells ^{47,48}	Apoptosis ⁵² ; upregulation of inflammatory, NF- κ B signaling, and loss of the mature alveolar population ⁵⁰ ; induction of chemokine, TNF signaling, IL-17 signaling and cytokine-cytokine receptor interaction pathways ⁴⁷	Remdesivir ^{50,52} ; antiandrogenic drugs ⁵¹ ; imatinib, mycophenolic acid and quinacrine dihydrochloride ⁴⁷ ; camostat/nafamostat ⁴⁸ ; 25HC ⁵⁴	Human neutralizing antibody ⁵² ; EK1 peptide ⁴⁸	
		Adult ALOs	ACE2 in AT2 cells ⁵⁹ ; TMPRSS2 widely expressed ⁵⁵	AT2 and club cells ^{55,56,59}	Apoptosis ⁵⁹ ; upregulation of type I/III interferon response ^{55,56} ; interferon-mediated inflammatory responses, loss of surfactant proteins, apoptosis ^{57,67} ; upregulation of epithelial cell-autonomous pro-inflammatory response ⁶⁷	Remdesivir ^{58,67}		
	AWOs	hPSC-AWOs	ACE2, NRP1 and TMPRSS2 in ciliated cells; FURIN and CTSL widely expressed ^{52,60}	Ciliated cells ⁶⁰ and club cells ⁵²	Apoptosis ⁵² ; downregulation of lipid metabolism ⁵² ; upregulation of glycolysis ⁵⁰	Remdesivir ⁵² ; GW6471, xanthohumol, 5-(tetradecyloxy)-2-furoic acid and ND-646 (ref. ⁶⁰)	Human neutralizing antibody ⁵²	
		Adult AWOs	ACE2 in ciliated cells ⁶⁰		Induction of ISGs, cytokines and chemokines ⁶² ;		Humanized decoy antibody (ACE2-Fc) ⁶³	
IOs	SIOs	hPSC-SIOs	ACE2 and TMPRSS2 in the gastrointestinal tract ⁷¹	Enteroendocrine and paneth cells ⁷¹	Apoptosis and a robust transcriptomic response, including ISGs ⁷¹	Remdesivir ⁷¹	Peptides or antibodies toward IFITM ⁷²	
		Adult SIOs	ACE2 in enterocytes ⁷⁵	Enterocytes ⁷⁵	Induction of cytokines and ISGs attributed to type I and III interferon responses ⁷⁵			
	COs	hPSC-COs	ACE2 in enterocytes ⁴⁷	Enterocytes ⁴⁷	Induction of cytokines and chemokines ⁴⁷	Imatinib, mycophenolic acid and quinacrine dihydrochloride ⁴⁷		
		Adult COs	ACE2 in enterocytes; TMPRSS2, FURIN and CTSL in COs ⁷⁷	Enterocytes ⁷⁵	Induction of pro-inflammatory pathway and interferon-mediated signaling pathway ⁷⁷			
	ILOs	Adult ILOs	ACE2 in enterocytes ^{74,75} ; TMPRSS2, FURIN and CTSL in SLOs ⁷⁷	Enterocytes ⁷⁵	Induction of pro-inflammatory pathway and interferon-mediated signaling pathway ⁷⁷			
KOs		hPSC-KOs	ACE2 and TMPRSS2 in kidney tubules ^{82,116}			MEDS433 (ref. ⁸³)	Human soluble ACE2 (ref. ⁸²) hrsACE2 (ref. ⁸¹)	

Continued

Table 1 | Summary of the application of organoids to study SARS-CoV-2 (continued)

		Entry factor expression	Tropism	Host response	Therapeutic discovery		
					Small molecules	Antibodies/peptides	Vaccines
LOs	hPSC-LOs	ACE2 in hepatocytes ⁸⁴	Hepatocytes ⁸⁴	Upregulation of cytokine-cytokine receptor interaction, IL-17 signaling, chemokine signaling pathway, TNF signaling and NF- κ B signaling pathway; downregulation of cellular metabolism ⁸⁴			
	Adult LOs	ACE2 in cholangiocytes ⁸⁵ ; TMPRSS2 is broadly expressed ⁸⁵	Hepatocytes ⁸⁴ and a subset of cholangiocytes ⁸⁵	Induction of cytokine-cytokine receptor interaction, IL-17 signaling, chemokine signaling pathway, TNF signaling and NF- κ B signaling pathway ⁸⁴	Remdesivir ⁶³	rsACE2 (ref. ⁶³)	
BOs	hPSC-BOs	ACE2 is expressed, but low levels of TMPRSS2 were detected ⁴⁸	Limited infection in cortical astrocytes ⁸⁸	Innate immunity and neurodegeneration genes ⁴⁸ ; distribution of Tau from axons to soma, and hyperphosphorylation and neuronal death ⁹⁰ ; metabolic changes ⁹¹		ACE2 antibodies ⁹¹	
	hPSC-CPOs	ACE2 in mature choroid plexus cells ⁹⁴ ; TMPRSS2 and NRP1 in CPOs ⁸⁸	Choroid plexus, astrocytes and radial glial progenitors ⁹³	Induction of type I interferon astrocytic response ⁹⁵ ; cell death, inflammatory response and cellular function deficits ⁸⁸ ; epithelial damage, leakage of blood-brain barrier ⁹⁴			
TOs	Adult TOs						SARS-CoV-2 vaccine
ROs	hPSC-ROs	ACE2 and TMPRSS2 in hPSC-ROs ⁹⁸					
	Adult ROs	ACE2 and TMPRSS2 in cornea, limbus, sclera and retinal pigment epithelium ⁹⁹	Cornea, limbus, sclera and retinal pigment epithelium ⁹⁹	Host immune response, including NF- κ B signaling pathway ⁹⁹			
CDOs	hPSC-CDOs			Upregulation of pro-inflammatory factors, including IFN- γ , IL-1 β and poly(I:C), drive systolic and diastolic cardiac dysfunction ¹⁰¹	INC054329 (ref. ¹⁰¹)		
CAPOs	hPSC-CAPOs					hrsACE2 (ref. ⁸¹)	

interaction. For instance, more robust propagation of SARS-CoV triggers minimal cellular responses, whereas SARS-CoV-2 elicits a stronger cellular response with lower replication capacity⁷⁶. Single-cell RNA-sequencing (scRNA-seq) analysis of SARS-CoV-2-infected SIOs revealed an induction of pro-inflammatory pathways and the interferon-mediated signaling pathway⁷⁷. Interestingly, in adult

COs, interferon (IFN)- γ treatment promoted the differentiation of mature keratin 20 (KRT20)-positive enterocytes, which showed high expression levels of ACE2, and promoted SARS-CoV-2 infection and production⁷⁸. Finally, IFN- β 1 and IFN- λ were reported to inhibit SARS-CoV-2 replication in SIOs⁶⁹. Several drugs have also been tested in IOs, such as the heat shock protein 90 inhibitor

17-allylamino-17-demethoxygeldanamycin (17-AAG), which suppresses Middle East respiratory syndrome-related coronavirus and propagation of SARS-CoV-2 in SIOs. It was also found that polyamine supplementation and autophagy induction modulated cellular metabolism and limited SARS-CoV-2 growth in SIOs⁷⁹.

Kidney organoids. Kidney damage such as acute kidney injury is common in individuals with COVID-19. Thus, hPSC-derived kidney organoids (KOs) have been applied to study SARS-CoV-2 infection in kidney. A recent study comparing ACE2 expression levels in 2D and 3D kidney proximal tubule epithelial cells found twofold higher expression of ACE2 in 3D KOs compared to 2D culture conditions, highlighting the significance of applying 3D organoids for COVID-19 disease modeling⁸⁰. scRNA-seq of hPSC-derived KOs in 3D suspension culture further revealed ACE2 expression in solute carrier family 3 member 1 (SLC3A1)-positive solute carrier family 27 member 2 (SLC27A2)-positive proximal tubule cells and podocalyxin like (PODXL)-positive nephrin (NPHS1)-positive podocin (NPHS2)-positive podocyte cells⁸¹. Accordingly, hPSC-KOs were shown to be permissive to SARS-CoV-2 infection and support viral replication⁸¹. Studies also found that both a variant of human soluble ACE2, consisting of 1–618 amino acids fused with an albumin-binding domain⁸², and clinical-grade human recombinant soluble ACE2 (hrsACE2)⁸¹, can neutralize SARS-CoV-2 infectivity in hPSC-KOs. Finally, a human dihydroorotate dehydrogenase inhibitor, MEDS433, was also found to inhibit SARS-CoV-2 infection in hPSC-KOs, highlighting the use of organoids in drug discovery applications⁸³.

Liver organoids. A high prevalence of liver test abnormalities and liver injury have been reported in individuals with COVID-19. The liver-related complications include elevation of total bilirubin and alanine aminotransferase abnormalities. Because hepatocytes and cholangiocytes are two major functional cell types in the liver, both hepatocyte and cholangiocyte organoids containing these cell types have been used to study SARS-CoV-2 infection.

In hPSC-derived liver organoids (hPSC-LOs), ACE2 expression is detected in most albumin-positive hepatocytes. Both SARS-CoV-2 pseudo-entry virus and live SARS-CoV-2 were shown to infect hepatocytes⁸⁴. This finding was further validated in adult hepatocyte and cholangiocyte organoids. Transcript profile analysis of SARS-CoV-2-infected human LOs revealed several upregulated pathways, including cytokine–cytokine receptor interaction, interleukin (IL)-17 signaling, chemokine signaling, tumor necrosis factor (TNF) signaling and NF- κ B signaling pathways, while cellular metabolism was largely downregulated⁸⁴, which is similar to the transcriptional changes in COVID-19 lung autopsy samples¹⁷. In liver ductal organoid models, a subset of cholangiocytes express ACE2, which has been shown as a target of SARS-CoV-2 (ref. ⁸⁵). In addition, liver ductal organoids generated from individuals with nonalcoholic steatohepatitis showed strongly increased permissiveness to SARS-CoV-2 pseudo-entry virus infection, suggesting that nonalcoholic steatohepatitis may be a risk factor for SARS-CoV-2 infection⁸⁶. Adult LOs have also been used for drug testing. A combination therapy method using remdesivir and rsACE2 synergistically improved the therapeutic effects against SARS-CoV-2 in primary human liver spheroids⁶³.

Brain organoids. Individuals with COVID-19 experience an array of neurological symptoms, ranging in severity from loss of smell and taste, to memory loss, to life-threatening strokes. hPSC-BOs, including both whole-brain and regional brain-specific BOs⁸⁷, have been applied in SARS-CoV-2 studies. In hPSC-BOs, SARS-CoV-2 infection was identified in cortical, hippocampal, hypothalamic and midbrain organoids by immunostaining, with limited detection in neurons and astrocytes⁸⁸. Astrocytes were found to boost SARS-CoV-2 infection in BOs⁸⁹. A study focused on

SARS-CoV-2-infected cerebral organoids found an induction of innate immunity and neurodegeneration genes⁴⁸. Distribution of the protein Tau from axons to soma and hyperphosphorylation were also detected in infected neuron cells⁹⁰. In addition, scRNA-seq of infected hPSC-derived BOs showed enriched expression of genes associated with metabolic processes in neurons, including electron transport–coupled proton transport, and mitochondrial electron transport of cytochrome c to oxygen and NADH to ubiquinone⁹¹. SARS-CoV-2 infection in BOs can be inhibited by ACE2-blocking antibodies or by administering cerebrospinal fluid from individuals with COVID-19 disease⁹¹. Further, DICER1, an isoform of DICER (a protein that cleaves double-stranded RNA), protects BOs from SARS-CoV-2 infection⁹².

In addition to neuronal lineage, hPSC-derived choroid plexus organoids (CPOs) have been used to study brain damage in individuals with COVID-19 disease⁹³. scRNA-seq revealed ACE2 expression in mature choroid plexus cells, but not in neurons or other cell types in choroid plexus and telencephalic organoids⁹⁴. In CPOs, few neurons and neural progenitors can be infected⁹³, which is consistent with the studies using 2D hPSC-derived cortical neurons⁸⁴. Interestingly, if pericyte-like cells (PLCs) are integrated into hPSC-cortical organoids, the organoids form a PLC–basement membrane–astrocyte structure and showed an increased proportion of neuronal cell population. SARS-CoV-2 was able to infect PLCs in integrated organoids, with spreading to astrocytes, which induced type I interferon astrocytic response⁹⁵. In addition, viral infection induced an inflammatory response and cellular function deficits, followed by cell death, in hPSC-CPOs⁸⁸. Furthermore, SARS-CoV-2 has been shown to cause damage of the tight junctions between the epithelial cells of the barrier and caused leakage of cerebrospinal fluid in a CPO model⁹⁴.

Tonsil organoids. Many of current efforts on organoids focus on host response and drug discovery. Recently, human tonsil organoids (TOs) were developed to evaluate humoral immune responses to SARS-CoV-2 vaccine⁹⁶. The interaction between follicular helper T cells and B cells in specialized germinal centers (GCs) plays a key role in the development of antigen-specific humoral responses. TOs, derived by reaggregating the dissociated primary human tonsils, maintained the tonsil cellular components and supported antigen-specific somatic hypermutation, affinity maturation and class switching of human B cells⁹⁶. Upon immunization with a live attenuated influenza vaccine, TOs developed GCs with clear light zones and dark zones, a distinction that is of key importance for GC selection. And B cells migrated from the dark zones to the light zones and interacted with follicular dendritic cells and follicular helper T cells to regulate the GC reaction. This facilitated the development of long-lived antibody-secreting plasma cells and high-affinity memory B cells. Finally, TOs derived from different donors were applied to evaluate an adenovirus-based SARS-CoV-2 vaccine. CD8⁺ T cell activation and specific IgG and IgA antibodies were notably increased in TOs from several donors at day 14 after stimulation⁹⁶. This highlights the potential to use these TOs to determine the personalized response to vaccine candidates.

Other organoids. About one-third of hospitalized individuals with COVID-19 develop ocular abnormalities, including conjunctivitis⁹⁷. ACE2 and TMPRSS2 were found to be expressed in hPSC-derived retinal organoids (ROs)⁹⁸. SARS-CoV-2 pseudovirus⁹⁸ and live SARS-CoV-2 (ref. ⁹⁹) can infect hPSC-derived ROs. The infected hPSC-derived ROs and adult human ocular cells showed robust SARS-CoV-2 replication and host immune response, including the NF- κ B signaling pathway⁹⁹.

COVID-19 causes cardiovascular disorders, including acute myocardial injury, myocarditis and arrhythmias by infection, immune cell-mediated damage and the cytokine storm¹⁰⁰. Recently,

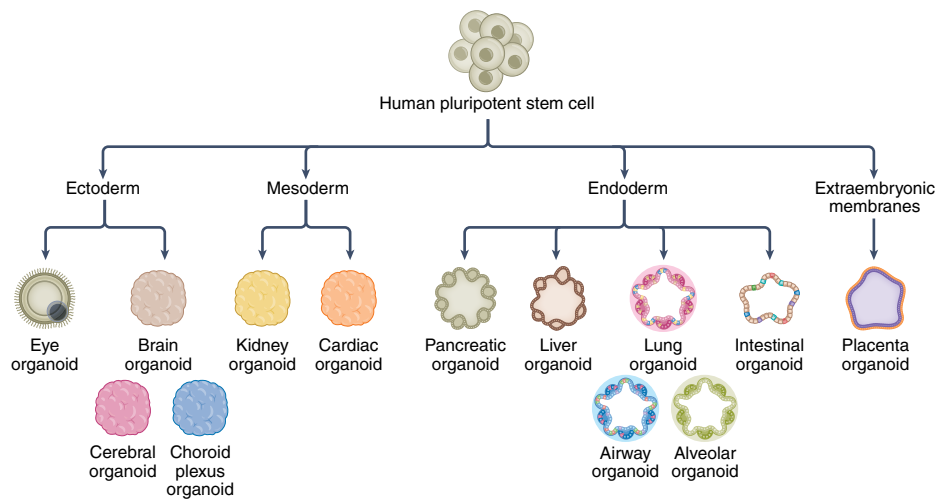


Fig. 3 | Directed differentiation of hPSCs to organoids in three germ layers. hPSCs have unlimited proliferation capacity and developmental potentials to produce organoids in all three germ layers.

hPSC-derived cardiac organoids (CDOs) co-cultured with endothelial cells were applied to mimic the cytokine storm observed in the hearts of individuals with COVID-19. Pro-inflammatory factors, including IFN- γ , IL-1 β and poly(I:C) drove systolic and diastolic cardiac dysfunction, which could be rescued by INCB054329, a bromodomain and extraterminal domain inhibitor¹⁰¹.

Studies have shown that 5 of 29 proteins of SARS-CoV-2 can damage blood vessels by changing the endothelial permeability¹⁰². Human capillary organoids (CAPOs) resemble human capillary structure with a lumen, endothelial cells, pericyte coverage and a basal membrane. SARS-CoV-2 was detected by quantitative PCR with reverse transcription in CAPOs and can be blocked by hrsACE2 with no obvious toxicity⁸¹.

Organoids to study host genetic variants, viral variants and species differences. Organoids provide a physiologically relevant platform to study SARS-CoV-2 infection. In addition to viral tropism, host response and drug discovery as described above, organoids have also been applied to study the impact of host genetic variants, to examine species differences and to evaluate the properties of different SARS-CoV-2 variants. For example, a single-nucleotide polymorphism (rs4702; AA to GG allele) located in the 3' untranslated region of *FURIN* was shown to decrease *FURIN* expression and further influence SARS-CoV-2 infection in hPSC-derived ALOs⁷⁰. In addition, organoids from different species can be generated to study the species differences in their permissiveness and immune responses to viral infections. For example, SARS-CoV-2 was also shown to infect and replicate in bat SIOs¹⁰³. Studies have also used organoid models to compare the infection and host response against different SARS-CoV-2 variants using ALOs and SIOs⁶¹.

Differences between hPSC-derived and adult organoids. In organoid-based COVID-19 studies, both hPSC-derived and adult organoids have been used. There are pros and cons for hPSC-derived and adult organoids regarding the availability, editability, maturity and diversity. hPSCs, in theory, have unlimited proliferation capacity and developmental potential to produce organoids in all three germ layers¹⁰⁴ (Fig. 3). hPSC-derived organoids can be easily scaled up, which allows large-scale studies, such as metabolic profiling^{52,60} and drug screening^{47,51}. Some organoids, such as brain and cardiac organoids, are only available from hPSCs. In comparison, adult organoids derived from adult tissues, have limited self-renewal capacity for a prolonged period of time, which limits their

application for scalable research. Gene-editing technology has been applied to both hPSC-derived and adult organoids to study the role of genes/variants in SARS-CoV-2 infection^{70,72,73}. Compared to adult organoids, the gene knockout and precise gene-editing techniques are better established in hPSCs, which makes it feasible to explore the biological function of single variants in viral infection. One major advantage of adult organoids is the maturity. Different from adult organoids, most hPSC-derived organoids still present fetal or neonatal identities. Additional work still needs to be done to further improve the maturation status of hPSC-derived organoids. Finally, adult organoids can be easily derived from donors from diverse backgrounds. Substantial efforts have been applied to develop induced pluripotent stem cells (iPSCs) from different genetic and disease backgrounds¹⁰⁵. Together, organoids with diverse genetic backgrounds can be applied to explore the impact of genetic background on disease progression.

Future perspectives and methodologies. In the last 2 years, important progress has been made to apply organoids to COVID-19 disease modeling. However, additional investigations should be done to further optimize the organoid models for infectious diseases, such as improving organoid complexity by adding niche/immune cells, combining 3D printing and organ-on-a-chip technology to develop platforms that closely resemble the physiology and pathology of human systems, applying single-cell technology to perform in-depth studies of virus–host interactions at high resolution, as well as applying genomic sequencing and gene editing to explore genotype–phenotype correlations during viral infection (Fig. 4). These technologies will strengthen organoid culture methods and accelerate the research process of new antiviral therapeutics and vaccines, which may help investigate other viral outbreaks in the future.

Immune vascular organoids. Most organoid platforms used for COVID-19 studies only contain the cells of the host tissue/organ, which lacks other niche components, such as the immune cells and cells of the vascular system. Immune cells are the key to many aspects of COVID-19 pathophysiology and disease progression. Immune-mediated damage might play an equally or even more important role in host damage than direct infection. Thus, in vitro co-cultured organoids with immune cells can be used to study the pathophysiology of COVID-19 caused by immune cells, in addition to the consequences of direct infection. Furthermore, organoids

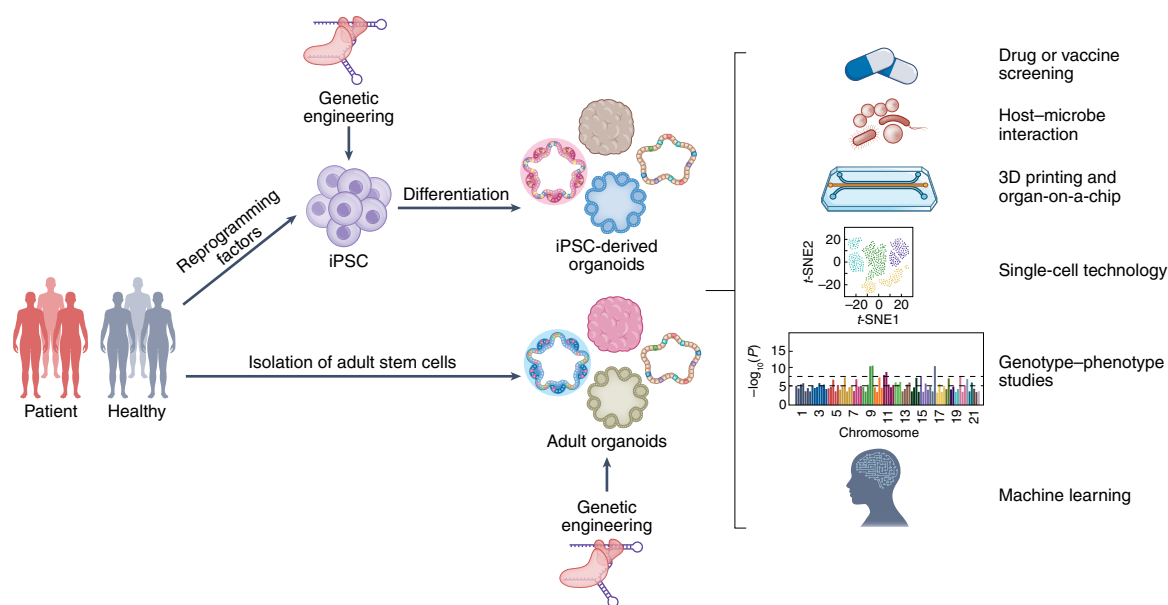


Fig. 4 | Future applications of organoid models for infectious disease research. Organoids have been and will be used in a variety of applications, including drug or vaccine screening, host-microbe interaction, 3D printing and organ-on-a-chip technology, scRNA-seq, genome-wide association studies and machine learning.

with immune cells allow a better understanding of the interaction between infected host cells and immune cells, how immunomodulatory molecules released from infected cells affect immune components, and how immune responses in turn affect infected host tissues. Recently, an immune-host co-culture system containing hPSC-derived cardiomyocytes and macrophages showed that, upon SARS-CoV-2 infection, hPSC-derived cardiomyocytes secrete CC motif chemokine ligand 2 (CCL2)¹⁰⁶, which is a chemokine that recruits monocytes. The monocytes differentiate into macrophages that damage the cardiac tissues by secreting the pro-inflammatory cytokines TNF and IL-6 (ref. ¹⁰⁷). Furthermore, a high-content chemical screen using the immune-host co-culture system identified a janus kinase inhibitor, which blocks macrophage-mediated cardiac cell damage¹⁰⁷. Coincidentally, baricitinib, a janus kinase inhibitor, has received emergency-use authorization from the FDA for the treatment of individuals hospitalized with COVID-19. Together, these examples highlight the importance of the immune components in organoid-based models to study viral infection.

Another limitation of many organoid platforms is the lack of blood vessels. Thrombotic complications are frequent in COVID-19 and contribute to mortality and morbidity. Co-culture of organoids with vascular endothelial cells and pericytes to form a blood vessel-containing organoid in a suitable spatial structure provides promise in further developing organoid model systems¹⁰⁸. An immune-vascular-organoid model, containing host organoids, immune cells and vascular cells, will advance organoid technology and provide a next-generation model to study the infection of emerging viruses (Fig. 5).

Organoids and bioprinting system. In addition to including different cell types in organoids, it is also important to create organoids with appropriate spatial cellular location and interorgan interactions. 3D bioprinting technologies use tissue-specific cell types laden in bioink and reconstruct human organ-like structures by layered printing technology¹⁰⁹. The organ simulation system technology generated by 3D bioprinting has great prospects in the study of human virus infection. For example, 3D-bioprinted lung-like structures can be used to study SARS-CoV-2 infections as they

provide an air-cell interface in a complex hollow structure composed of multiple layers and cell types, which cannot be achieved with traditional tissue engineering methods. Considering the many advantages of the 3D-bioprinted human in vitro system in simulating viral infections, efforts should be made to generate more complex 3D lung models that support SARS-CoV-2 infection¹¹⁰.

Organoids on microfluidics system. Another innovative technology merging with organoids is organs-on-chips, which are microfluidic devices for cell and tissue culturing in continuously perfused small chambers. The organ-on-a-chip system allows for the creation of dynamic and controllable microenvironments suitable for studying the virus-host interactions, the evolution of resistance to viral therapy, the development of new antiviral therapies and the underlying viral pathogenesis. Using a vascularized lung-on-a-chip model to study SARS-CoV-2 infection, alveolar epithelial cells showed susceptibility to infection and decreased expression of platelet endothelial cell adhesion molecule (PECAM-1), resulting in the disruption of the barrier and establishment of a pro-coagulatory microenvironment¹¹¹. Organoids cultured on a chip provide new possibilities to recapitulate the physiological functions at the organ level, which will further enhance the understanding of COVID-19 pathogenesis.

Organoids and single-cell multi-omics analysis. One major advantage of organoids is that they contain multiple types of cells, which can be utilized to explore the interaction between the virus and different host cells, as well as the interaction among host cells upon viral infection. In the early stages of COVID-19 studies, scRNA-seq had been extensively applied to determine the expression of SARS-CoV-2 entry factors in different types of cells within organoids. However, overall ACE2 expression based on scRNA-seq analyses is low. As an example, the percentage of ACE2⁺ cells in lung AT2 cells varies between 0.3% and 2.4%¹¹², depending on the individual, which reflects the low detection sensitivity of scRNA-seq platforms¹¹². Thus, there is strong need for careful interpretation of scRNA-seq data of SARS-CoV-2 entry factor expression. The scRNA-seq data need to be further validated with independent approaches, such as immunostaining or flow cytometry analyses.

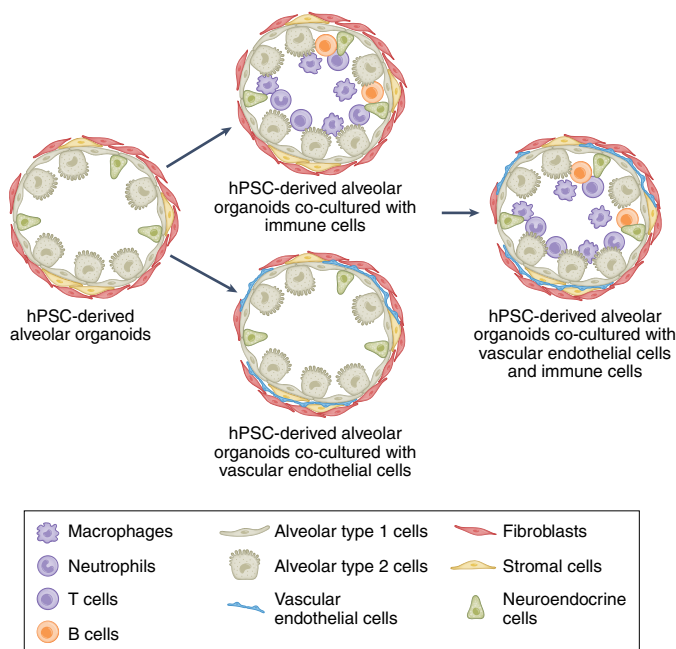


Fig. 5 | Development of vascular-immune-alveolar organoids. The immune vascular organoids, containing host organoids, immune cells and vascular cells, provide a physiologically and pathologically relevant model for human disease modeling.

Although technically challenging, scRNA-seq started being applied to directly analyze the SARS-CoV-2-infected tissues/organoids in a BSL-3 setting¹². These types of studies are expected to provide in-depth understanding of host cell responses and host cell interactions upon SARS-CoV-2 infection at single-cell resolution.

Organoids and genome-wide association studies. Both hPSC and adult organoids can be derived from individuals with diverse genetic backgrounds, which provide a useful model to explore the impact of genetic variants on disease progression, including viral infection. In one way, CRISPR-based gene-editing approaches, which can efficiently knock out a single gene/locus or knock in a single variant¹³, provide a high-throughput platform for generating isogenic hPSCs/organoids. These isogenic hPSCs/organoids can be used to determine the precise role of a single gene/genetic locus, or even a single-nucleotide polymorphism, in viral infection and host damages. On the other hand, by combining whole-genome sequencing and screening of organoids from different donors, scientists can perform genotype–phenotype studies to identify novel genes/loci/variants associated with viral infection. The identified genes/loci/variants can be further validated using isogenic organoid models developed by genetic editing tools. In addition, these hPSC/organoids derived from different donors can also be applied to investigate individual responses to antiviral drugs, which will facilitate the development of personalized treatment for individuals with COVID-19.

Organoids and deep machine learning. Deep machine learning has been effectively used in molecular and cellular biology, drug discovery, protein structure prediction and translational biomedicine. The utilization of deep machine learning in the field of viral genetics has mainly focused on the prediction of viral mutations related to drug resistance. Recently, DeepNEU, an unsupervised deep machine learning technology based on a fully connected recurrent neural network architecture with a network processing layer for each input, used a set of defined reprogramming transcription factors to

simulate the iPSC system. In DeepNEU, AT2 cells can be simulated, and were shown to be permissive to simulated SARS-CoV-2 virus infection¹⁴. Infected lung cells and organoids can be used to stimulate and assess the impact of potential functional mutations in the SARS-CoV-2 genome¹⁵. These data were consistent with recently published data and provided a new approach to identifying potentially effective anti-SARS-CoV-2 drug combinations.

Conclusions

Studies using organoid platforms have contributed substantially to COVID-19 disease modeling and drug discovery. Compared with animal models, organoid platforms still have some limitations due to the lack of vasculature, immune cells, and interorgan communication. At the current stage, animal models are still required for vaccine and drug development. In the future, these organoid platforms will be used to simulate more complex organs, mimic the intra-organ interaction, and to explore pathogenic mechanisms. Multiple tissues and organoids from healthy or COVID-19 donors can provide reliable molecular assessments of viral susceptibility in individuals of different age, sex or race groups, and provide personalized treatment strategies for current and future pandemics.

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Author contributions

S.C., Y.H., L.Y. and L.A.L. prepared the manuscript. Y.H. and L.Y. prepared the figures.

Competing interests

S.C. is the cofounder of OncoBeat. The other authors declare no competing interests.

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