Chapter

# Organoid Technology and the COVID Pandemic

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## Abstract

COVID-19 is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and has emerged as a devastating pandemic. SARS-CoV-2 not only causes respiratory illness but also leads to impairment of multi-organ function. Scientists are racing to evaluate a range of experimental therapeutics to target COVID-19 systemically. The World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) are accelerating global research priorities to mobilize innovation towards diagnostics, treatments, and vaccines against COVID-19. In this scenario, information about appropriate organ-specific physiologically relevant models is critical to generate knowledge about the pathophysiology and therapeutic targeting of COVID-19. Human and animal organoids are providing a unique platform, demonstrating their applicability for experimental virology. This review provides a brief analysis of the available organoid models used to study and device strategies to combat COVID-19.

**Keywords:** COVID-19, Organoids, Infection, ACE2, Challenges, Gut, Lung, Brain

## 1. Introduction

It is quite well-known that classical 2D cell lines and *in vivo* models have been used near universally to investigate biological mechanisms and assess novel therapies across a large range of clinical problems [1]. Nevertheless, the results from these experiments are critically limited by a systemic lack of translational power for the response, efficacy, safety, and toxicity in humans despite its primary benefits in clinical research [2, 3]. Cell lines generically display insufficiency and inaccuracy in modeling the immune system, stromal components, and organ-specific functions after multiple passages [4]. Leaving aside animal welfare arguments, species-specific variations in organ development and pathogenesis are a long-standing bottleneck due to which animal models cannot mimic a given human disease that is polymorphic, to begin with [5]. Therefore, to define and treat disease pathology seamlessly, biologists exploited the critical features of stem-cell and came up with three-dimensional (3-D) or organotypic cultures or organoids from human samples that could successfully phenocopy cell-type composition, architecture, and to some extent, functionality (e.g., contraction, filtration, excretion, neural activity, etc.) of their natural counterparts [6–8].

Organoids, a term coined for referring to 'mini organs', [9] are best described as *in vitro* three dimensional (3D) cellular clusters exclusively derived from healthy cells – like primary tissue, embryonic stem cells, or induced pluripotent stem cells (iPSCs) [10] or even tumor cells [11]. Since these cells are capable of self-renewal

and self-organization, organoids portray outstanding similarity to organ functionality as the tissue of origin compared to other conventional routes [2, 12]. The sole purpose of developing organoids is to recreate and miniaturize the multicellular structure of organs while retaining the 3-dimensional construct indefinitely.

It can now be commented that the development of organoid technology has generated a robust new methodology to zoom into the physiological events *ex vivo*, and this fact can be explained. Firstly, scientists have a wider domain of cell types to choose from, some of which were historically hard-to-access; secondly, organoids contain multiple differentiated cell types; and thirdly, organoids are genetically stable [13]. The intrinsic nature of this innovative near-physiological technology has created a paradigm shift in our understanding of basic developmental biology or stem cell research directed to a host-pathogen relationship in infectious diseases, degenerative conditions, genetic disorders, oncology, genome engineering, biobanking, and regenerative and personalized medicine [14, 15]. Through a complete visualization of spatiotemporal cellular interactions, organoid modeling reflects the predominant structural and functional properties of essential organs like kidneys [16], lungs [17–20], gut [21], brain [22], prostate [23], heart [24] and retina [25].

Human organoids are intrinsically human-derived, rapid-to-set-up, robust in scaling up, and ideal for genetic manipulation and personalization [26]. In simple terms, the organoid is an attractive strategy for clinical applications and bridges the gap between basic research and clinical practice. Along these lines, biomedical and pharmaceutical investigations on particularly relevant, rigorously designed, well-characterized, and controlled organotypic models will travel a long way in redefining fundamental discoveries, testing novel hypotheses at the 3D level and for the validation of critical data without sacrificing the integrity of any living being in the name of science. It should also be kept in mind that this technology is still in its infancy; much of the current hype originates from its enormous potential rather than a finite number of real-life scientific advancements. Hence, COVID-19 researchers use bronchial, respiratory, liver, kidney, intestine, and brain organoids to study the pathogenesis of SARS-CoV-2 and virus-specific cellular reaction on various organ systems.

In this chapter, we aim to answer a plethora of scientific questions related to the situation around the SARS-CoV-2 battle in the light of organoid technology, emphasizing key findings in therapeutic interventions meant to prevent and cure the serious medical threats imposed by SARS-CoV-2. We will highlight the state-of-the-art tools and methodologies available for human organoid lines and deep-dive into the case studies of fantastic *in vitro* organ models that well-known research groups have employed for understanding the root cause of COVID-19 devastation.

## 2. Virology and organoid

It is well-known that immortalized cell lines and animal models have paved the way for identifying the pathobiology of obligate intracellular parasites or viruses. Despite their paramount role in this field, none can adequately reproduce human disease pathology or exactly recapitulate the homeostatic functions of a normal cell. Therefore, virologists have moved on from carrying out investigations on non-natural hosts to patient-derived organoid models to address the unmet need for human model systems in studying virology and its therapeutic interventions [27]. Organoid technology, a human-based model technique, has broadened the scope for studying viral infections by enhancing the translatability of results from *in vitro* cell cultures or *ex vivo* animal systems to a more human *in vivo* mimicking condition. Since the route of host-pathogen interactions largely varies based on virus nature

and its host type, including age, sex demographic profile, and genetic constitution of the hosts, it is crucial to have an accurate prototype of its natural host to conduct the experiments.

### 2.1 Culturing the unculturable

At almost all stages of replication, viruses associate closely with the host cell, and therefore the cell model used to research virus infection is crucial. Primary cells better represent the phenotype of healthy cells *in vivo* but have a short lifetime, are difficult to culture, and are heterogeneous and thereby renders manipulating them difficult. The widespread use of immortalized cell lines for culturing diverse virus strains is a common practice, but the induction of interferon-stimulated genes and other antiviral defenses is defective in many tumor-derived and artificially immortalized cell lines. These flaws can interfere with virus replication, particularly when cells are infected at lower, more physiologically important multiplicities. Moreover, there are some challenging cases where the virus fails to adapt in man-made culture conditions, like, norovirus or other enteric viruses, which remain unculturable to date in any kind of cell line system. Luckily for us, stem-cell-derived human intestinal organoids have successfully grown and studied these viral cultures up to one round of infection [28]. Similarly, respiratory viruses which are challenging to grow in cell lines like human coronavirus HKU1, human bocavirus, and human rhinovirus C could be successfully isolated from clinical specimens using Human airway epithelial (HAE) cultures [29–31]. These data prove that there is room for discovering unknown viruses and their mechanism of infection, pathogenesis, and immune escape through the fine-tuning of crucial features of the organoid platform [32].

### 2.2 Reproducing the natural virus host environment

Viruses isolated from patient samples like feces, blood, or nasopharyngeal swabs infested with a particular infection, can be grown on organoids without any imposed mutation or adaption. These cultures will now exactly recapitulate the fundamental features and infectivity profiles of the natural host cell [33, 34]. Therefore, conclusions drawn on the various aspects of organotropism, receptor usage, innate immunity induction, etc., is now even more reliable than laboratory-adapted or ATCC strains. The readouts used for post-infection analysis may differ in cell lines vs. organoids based on the culture environment and discussed in the following sections.

### 2.3 Provide new insights

Data from cell lines have earlier shown that the small open reading frame upstream of the main polyprotein ORF which is also present in the 5'UTR genomic region in enteroviruses, cannot be utilized for the initiation of translation [35]. Lulla et al. had reported for the first time that the small protein encoded by this uORF is crucial for virus release in human intestinal organoids [36]. The viruses lacking this uORF are therefore attenuated in this model. Later on, other publications on intestinal organoids have reiterated that different enteroviruses infect different cell types and induce an antiviral response characteristic of a particular cell type [37, 38].

To assess the influence of host conditions such as age and comorbidities on the progress and severity of viral infections, cross-interactions between co-detected pathogens in a single host can be studied closely with organoids. This was never feasible with cell lines because different viruses are often not culturable on the

same cell line. For example, respiratory viruses are well-known for causing asthma and pathologies like cystic fibrosis or chronic obstructive pulmonary disease. HAE infection samples collected from healthy and asthmatic donors with rhinovirus have shown a unique airway epithelial structure with inflammatory signaling in asthmatic patients [39, 40].

### 2.4 Utilization in fighting the SARS-CoV-2 pandemic

Multiple types of organoid models were used to study the detrimental effect of SARS-CoV-2 infection on human hosts and its potential therapeutic interventions [41]. To begin with, HAE cultures served as faithful models for the lungs where efficient replication occurred through the infection of ciliated cells in the airway [42]. Therapeutic investigations on organoid models showed the repurposed drug remdesivir and remdesivir–diltiazem to be functional in resisting further SARS-CoV-2 infection [43]. Lamers et al. had proved for the first time that the human gut epithelium is the second major replication site of the virus [44]. Combined with the novel insights from other organoid research groups, it was proved that the SARS-CoV-2 genome is detectable in feces even after the virus is absent from oropharyngeal swabs, which explains the outcome of intestinal infection and potential fecal transmission [45].

These findings were closely followed by the observation of increased efficiency to infect secondary tissue by the virus. In terms of relative importance, the next area of investigation using organoids has been establishing the neuro-invasive aspect of SARS-CoV-2 by using brain organoid models [46]. Epidemiological studies showed the direct contribution of SARS-CoV-2 infection to neurological complications like headaches, ischemic stroke, and encephalitis, including cranial nerve-related complications such as anosmia and hyposmia, and ageusia [47, 48]. Recently, Pellegrini et al. utilized choroid plexus organoids to demonstrate the potential viral tropism for choroid plexus epithelial cells that affect the epithelium [49]. Damage to this barrier is suspected as a possible entry route for the virus into the cerebrospinal fluid and the brain.

### 2.5 Extensive research in Zika virus pandemic

Zika virus, a mosquito-borne flavivirus, is reportedly the causative agent for the infection known as ZIKV. Although adult victims show mild symptoms, newborns are marked with microcephaly, a condition in which infants are born with an abnormally small head. Being spread in over 70 countries and territories globally, [50] ZIKV is declared a global health emergency by WHO whereby microcephalic fetal tissues have shown traces of ZIKV in damaged fetal brains [51]. Due to accessibility challenges with live infected human fetal samples and postmortem tissues showing a diverse range of quality and genetic history, clinical examinations are replaced for good by brain organoid model studies. These focus on cellular tropism and pathogenesis mechanisms of ZIKV in controlled settings [52].

In 2016, the first study on brain organoid models was published by Tang et al., where they used monolayer cultures of forebrain-specific neural progenitor cell (NPCs) to model ZIKV infection during human brain development [53]. These were the initial results towards projecting that ZIKV more efficiently infects NPCs layers over human pluripotent stem cells (hPSCs) or immature neurons. Infection of cerebral organoids and human neurospheres with ZIKV and dengue virus 2 (DENV2) has proved that only ZIKV attenuates NPC growth, suggesting that the extreme aftereffect of ZIKV infection as an exceptional feature of the flavivirus family [54]. Later on, studies using brain organoids derived from hPSCs have also

led to a significant understanding of various other aspects of ZIKV infection on fetal brain development [52].

Due to the limited accessibility of organoid methodologies to virology research groups and the delay in the pace of commercialization of this technology, the majority of the published work so far has been a result of cross-functional collaborative efforts [55]. This challenge is closely followed by complications arising from heterogeneity inherent to the structural complexity and cell-type diversity in brain organoid models compared to simpler analogs such as neurospheres [56]. Moreover, the low-throughput nature of culturing and analyzing organoids creates a significant obstacle in drug screening which usually needs a high-throughput styled experimental protocol. We anticipate the evolution of more sophisticated brain organoids in the future that involves the co-culturing of endothelial cells or microglial cells to enhance the physiological relevance of modeling ZIKV infection during fetal human brain development.

### 2.6 Technical challenges

The classical nature of 3D organoid models was closed round structures embedded in Matrigel, challenging to infect with viruses as receptors needed for infection are always located deep inside. This shortcoming was overcome in HAE cultures where cells are grown on a Transwell. Therefore, round gut organoids can be easily transformed into an open organoid model where they are accessible from the upper and lower sides simultaneously to establish the desired infection [28, 57, 58]. This model system is technically advantageous for infectious disease studies and drug-testing in antimicrobial therapy.

The next significant challenge worth consideration is readouts used for analysis after infection. Due to the release of viral particles in a nonlytic manner, virus cultures in primary cellular models do not result in plaque-like cytopathic effect (CPE) most of the time, for example, in the case of enterovirus A71. Huang et al. have shown using human intestinal organoids that are infected with enterovirus A71 that viral release happens through exosomes instead of a lytic process characteristic of a classical RD cell line [59]. This production is quantified through back titration or plaque assays using cell lines. The aforementioned protocol of measurement of the number of viral particles is a matter of concern in the case of primary cultures, which calls for more suitable evaluation methods.

## 3. COVID-19 and organoid

The severe acute respiratory syndrome coronavirus (SARS-CoV) first emerged in the human population in November 2002. Phylogenetic analysis of this viral isolate indicated that it has a zoonotic origin, and horseshoe bats (*R. sinicus*) seem to be its natural reservoir. With local travel restrictions and a wildlife trade ban, there were no further naturally acquired human cases of SARS in Guangdong, China. In late 2019, a novel coronavirus, SARS-CoV-2, again crossed the animal-to-human interspecies barrier to infect humans [60]. Palm civets and other mammals acted as their amplification hosts, which resulted in a super-transmissible form that could effectively spread from human to human at an unprecedented rate. This rapid propagation happened by the deposition of infected droplets or aerosols on the respiratory epithelium. This led to a pneumonia outbreak in Wuhan, China [61] which causes coronavirus disease-19 (COVID-19) marked by symptoms like fever, cough, shortness of breath, myalgia, fatigue, and sometimes gastrointestinal symptoms such as nausea, vomiting, and diarrhea [62]. Viral RNA was detected

in patients' respiratory, stool and urine specimens. This condition can extend to severe lung injury and multi-organ failure, eventually leading to death in senile and comorbid patients.

In a few months, the virus had disseminated globally and sustained its pathogenicity irrespective of external factors. After WHO declared this a public health emergency of pandemic proportions, there were several lockdowns, social distancing protocols, hygienic measures, strict travel bans, strategic medical care, and vaccination programs to control the obnoxity of this outbreak. Even after one year of a relentless pandemic situation, the world is trying hard to combat the collateral damage to the global economy, public health, and civil life.

Genomic analyses of SARS-CoV-2 prove ~96% identity to the bat coronavirus BatCoV RaTG13 and 88% identity to two other bats SARSr-CoVs [61, 63, 64]. Sharing multiple similarities with SARS-CoV [65], phylogenetic analysis of SARS-CoV-2 shows that it belongs to lineage B of the beta-coronavirus genus in the family Coronaviridae [63] and has a possible common host cell receptor due to similarity in the receptor-binding domain. Animal model studies further confirmed that Angiotensin-converting enzyme 2 (ACE2)-dependent viral entry into cells is a critical step [66]. The evolution of different mutants is another concern, and quick studies can help understand the infectivity, pathogenesis, and targeting better. The B.1.1.7 variants in England, B.1.351 mutant in South Africa, P.1 in Brazil, B.1.427 in California, and now B.1.617, a "double mutant" common in India, have caused havoc on life and the economy.

Like SARS and MERS, pathobiology of the recently emerged COVID-19 is not limited to the respiratory tract because the damage has been observed and confirmed repeatedly in multiple organs [65], albeit the lungs are the main site of the infection. To investigate the rationale behind the organotropism of SARS-CoV-2, we need 3D model systems that mimic the physiological conditions at their best. Herein, organoid technology comes in as the basic framework of COVID-19 research with a much higher impact than animal models and cellular studies. Fortunately, the past decade has witnessed a revolutionary breakthrough in the generation of organoids for almost every human organ, including intricate systems like the heart, intestine, brain, and lung organoids. In the following sub-sections, we will discuss the constitution, contribution, limitations, and future applications of organoid technology in understanding the mechanism of organotropism by SARS-CoV-2 (Section 3.1-3.4), which influences and, in most cases, aggravates comorbid conditions in COVID-19 patients.

The first step in the pipeline of using 2D and 3D models for COVID-19 studies *in vitro* starts with tissue dissociation from different organs and is followed by stem/progenitor cell isolation using popular sorting methods like fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) (**Figure 1**). Sorted stem/progenitor cells are cultured in a 3D organoid culture system and subjected to SARS-CoV-2, which mimics the organ-specific infection. Different aspects of the post-infection studies like infection rate, gene expression analysis, infection mechanism, immune-response, inflammatory response, and histology can be studied. The 3D-organoid models can then be subjected to drug screening, drug repurposing, and vaccine development-related studies (**Figure 1**). **Figure 1** provides a layout of the COVID-19 research platform.

### 3.1 Lung organoid

Dan et al. described an approach to synthesizing patient-specific lung tissue in a modular method to model relevant human lung disease, as well as for high-throughput drug screening to detect targeted therapies [67]. The first development of long-term differentiated human airway organoid cultures, which can morphologically and functionally simulate human airway epithelium, was done by Z. Zhou

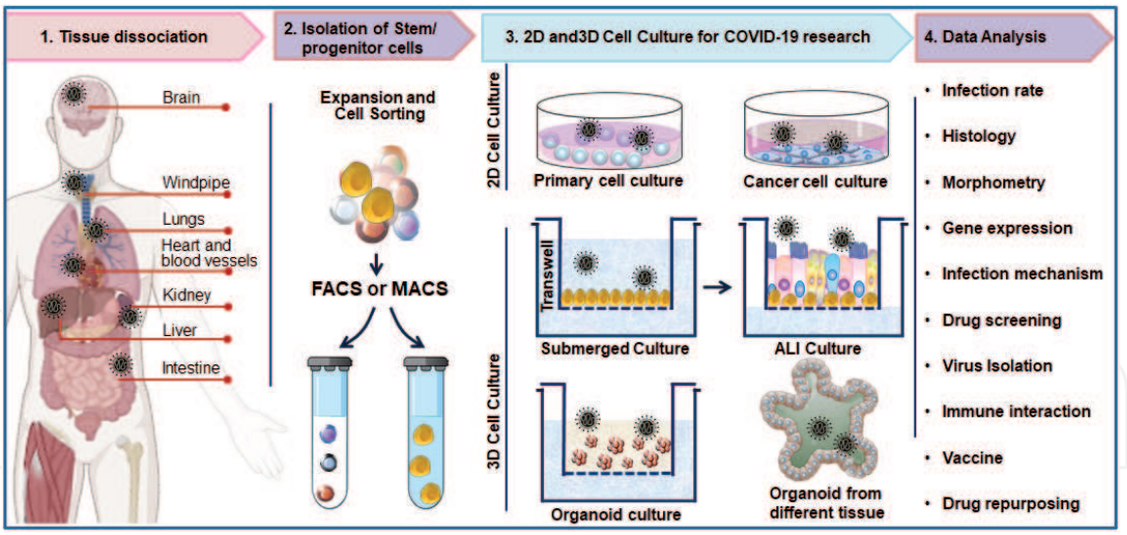


**Figure 1.**
*The COVID-19 research platform's layout using 3D organoids. Tissue dissociation from various organs is the first step in the pipeline for using 2D and 3D models for Covid-19 studies* in vitro. *Isolated stem/progenitor cells by fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) cells are grown in 3D using extracellular matrix mimetics and nourished with niche-specific culture medium. Stem/progenitor cells derived from various tissues self-organize into tissue-specific organoids. 3D organoid cultures are infected with SARS-CoV-2. Various aspects of post-infection studies can be conducted as shown.*

et al. to predict the infectivity of influenza viruses [68]. Optimized to contain the four major airway epithelial cell types- ciliated, goblet, club, and basal cells, these organoids were exposed to two 'pairs' of already studied viruses. Resultantly, the pair of humans-infective virus replicated more robustly than the pair of matched viruses poorly infective in humans.

Several *in vitro* models, such as Vero cells, Huh7 cells, and human airway epithelial cells, have been used early on in the COVID-19 pandemic to isolate and study the SARS-CoV-2 virus. These studies took a notch up when SARS-CoV-2 was isolated and propagated in TMPRSS2-expressing Vero E6 cells, thereby proving the indispensable role of TMPRSS2 serine protease in viral replication. Nevertheless, these models are limited by their poor representation of the histology, physiology, and pathology of the events occurring in our respiratory tract [69]. Y. Han et al. have developed a lung organoid model of alveolar type II cells using human pluripotent stem cells (hPSCs) that could be adapted for drug screens [70]. This organotypic culture was able to express ACE2 and are permissive to SARS-CoV-2 infection. High throughput screening experiments identified FDA-approved drug candidates, ima-tinib and mycophenolic acid, which are efficient inhibitors of SARS-CoV-2 entry. Pre- or post-treatment with these drugs at physiologically relevant levels decreased SARS-CoV-2 infection of hPSC-derived lung organoids.

To test the validation of Remdesivir, a Covid-19 drug candidate, A. Mulay et al. successfully developed and infected differentiated air-liquid interface cultures of proximal airway epithelium and organoid cultures of alveolar epithelium by SARS-CoV-2 [71]. They displayed an epithelial cell-autonomous proinflammatory response that proved the relevance of this platform for studying COVID-19 pathobiology and rapid drug screening against SARS-CoV-2.

### 3.2 Brain organoid

While the Coronavirus disease 2019 manifests clinically acute respiratory symptoms along with fever [72], a large subset of patients, especially younger victims, develop complete or partial olfactory dysfunction (anosmia/hyposmia)

during the course of infection [73]. This loss in olfaction occurred without (83%) associated rhinorrhea or nasal congestion at a median of 0.5 days after symptom onset [74]. While the majority of patients recovered within a couple of weeks from the onset of olfactory symptoms, few continued to have refractory and disabling anosmia [75]. Neurological symptoms like headache, dysgeusia, confusion, seizure, and viral encephalitis have been reported in 36.4% of 214 COVID-19 patients in Wuhan, China, where 45.5% of patients had severe SARS-CoV-2 infections [47, 76]. Similarly, France and Germany reported neurologic findings in 84.5% (49/58) and 36.4% (8/22) of COVID-19 patients, respectively, of which the latter studies had detected viral RNA in brain biopsies of patients who succumbed to the disease [77].

In 2016, D. Pamies et al. had put forward human mini-brains or BrainSpheres- an organotypic brain model derived from iPSC for the first time [78], comprising of different types of neurons, astrocytes, and oligodendrocytes. After its application on Zika, Dengue, HIV, and John Cunningham (JC) virus, they used this model to understand the extent of SARS-CoV-2 virus infection in human brain cells. Their results demonstrated that SARS-CoV-2 could infect and replicate in cells of neuronal origin, thereby proving the critically potential neurotropism of SARS-CoV-2. In yet another study, the same group had shown that SARS-CoV-2 could directly infect and effectively damage the olfactory sensory neurons of golden Syrian hamsters [75]. The entry receptor of the spike protein in SARS-CoV-2, ACE2, is widely detected in the brain, especially in the substantia nigra, middle temporal gyrus, and posterior cingulate cortex [79, 80]. Interestingly, serine protease TMPRSS2 expression was undetectable in the BrainSpheres, which suggests an alternative mechanism for spike (S) protein priming during viral entry. Together, these findings indicate that the human brain might be an extra-pulmonary target of SARS-CoV-2 infection.

Initially, it was proposed that anosmia and ageusia happen due to infection of non-neuronal cells in the olfactory system [81], which was busted by reports supporting the presence of viral particles in the CNS biofluid [82] and signs of neural damage biomarkers in the plasma of COVID-19 patients [83]. Taken together, a direct infection rather than a secondary immune response seems more accountable for neurological outcomes and predicted future neurodevelopmental disorders. Given that the human brain is arguably an extra-pulmonary target of SARS-CoV-2 infection, biologists and neuroscientists also need to figure out the impact of SARS-CoV-2 on a prototypical developing brain. Brain organoid research or the BrainSphere model is also limited by the absence of microglia or brain immune cells since they originate from the mesoderm germ layer and invade the developing brain from the blood, unlike neural precursor cells [84].

### 3.3 Gut organoid

While most COVID-19 patients suffer from mild to severe respiratory illnesses, >50% of patients manifest gastrointestinal disorders with prolonged symptoms like diarrhea, nausea, etc., which becomes severe to fatal when left unattended [85]. Although the virus has been detected in the upper respiratory tract of humans, proving the nasopharynx as a prominent site of replication, the highest expression of ACE2 occurs in the brush border of intestinal enterocytes [86]. Interestingly, when 53% of a cohort of 73 hospitalized patients had SARS-CoV-2 RNA in stool specimens, viral RNA was found in rectal swabs of 23% of patients even after negative nasopharyngeal testing, which implied fecal-oral transmission route leading to gastrointestinal infection or vice-versa [87, 88]. Of note, viral nucleoprotein-positive cells were found in the gastrointestinal epithelial cells from biopsy specimens [89] and pediatric patients [90]. Also, the SARS-CoV-2 receptor ACE2 is highly

expressed on differentiated enterocytes suggesting that the intestine is a vital target organ for the pathogen. Therefore, models to understand the mechanism of SARS-CoV-2 and validate drug efficiency in the gut for COVID-19 patients are the need of the hour.

Based on the high homology of SARS-CoV-2 to SARS-related coronaviruses isolated from horseshoe bats, J. Zhou et al. established and characterized expandable intestinal organoids derived from Chinese horseshoe bats of the *Rhinolophus sinicus* species that can recapitulate bat intestinal epithelium [41]. These bat enteroids were readily infectable and could sustain SARS-CoV-2 replication. They also demonstrated active replication of SARS-CoV-2 in human intestinal organoids along with isolation of infectious virus from the stool specimen of diarrheal COVID-19 patients [91]. This again confirmed that the established culture conditions for human intestinal organoids could be extended to other members of the mammalian species.

This report, along with the work done by M. M. Lamers et al. [44] and R. Zang et al., unanimously reported that the intestine is a potential site of SARS-CoV-2 replication since enterocytes, the most common cell type of the intestinal epithelium, get readily infected [92]. M. M. Lamers et al. established human small intestinal organoids (hSIOs) from primary gut epithelial stem cells containing all proliferative and differentiated cell types of the *in vivo* epithelium [44]. Of note, hSIOs have been utilized for *in vitro* culturing of norovirus for the first time. The authors used confocal and electron microscopy to show that SARS-CoV and SARS-CoV-2 infect enterocyte lineage cells in an hSIO model. They reported similar infection rates of enterocyte precursors and enterocytes, whereas ACE2 expression increased ~1000-fold upon differentiation at the mRNA level. Therefore, while the infected enterocytes upregulated the viral response genes through cytoplasmic sensing of the viral RNA genome, the host-cell membrane-bound serine proteases TMPRSS2 and TMPRSS4 were found to cleave the SARS-CoV-2 spike protein to facilitate viral entry. They conclude the following facts from this study: (a) intestinal epithelium supports SARS-CoV-2 replication, (b) hSIOs serve as a faithful biological model for coronavirus infection, and (c) viral entry is supported even at low ACE2 concentrations.

Since organotypic cultures are derived from pluripotent or organ restricted stem cells having the ability to mimic a natural 3D environment, they need a cell source with excellent self-renewal ability. The gut is one such source that allows unlimited replenishments of a particular cell type or tissue. Single-layered human intestinal organoids (HIOs) derived from human adult gut stem cells contain only epithelial cell types [93]. Pluripotent stem cells derived from HIOs (PSC-HIOs) made of endodermal/mesodermal progeny [94], resembling epithelium and fibroblasts or gut capillaries, respectively [95]. While PSC-HIOs are not 100% mature, HIOs are architecturally too simple, resulting in lower *in vivo* transplantability and analytical access to intermediate developmental stages. Until further modifications are done on them, both models are comparable and complementary to each other with model-specific pros and cons. As per previous reports, HIOs express ACE2 and are susceptible to SARS-CoV-2 [44, 92].

Inspired by the prior human intestinal organoids derived from pluripotent stem cells (PSC-HIOs) for modeling of gastrointestinal infections, J. Kruger et al. used this organoid model to dissect SARS-CoV-2 pathogenesis and then study its inhibition by remdesivir and famotidine (histamine-2-blocker), a potential drug candidate for COVID-19 treatment [96]. Immunostaining for ACE2 and TMPRSS2 showed large expression in the gastrointestinal tract with maxima in the intestine. This ready infection of organoids with SARS-CoV-2 followed by the viral spread across entire PSC-HIOs subsequently led to organoid deterioration except goblet

cells lacking ACE2 expression. The drug testing data showed that remdesivir and EK1 (but not famotidine) effectively inhibited SARS-CoV-2 infection in a dose-dependent manner at a low micromolar concentration which rescued the morphology of PSC-HIOs. This is a benchmark study that has established the applicability of PSC-HIOs in the field of organ-specific drug testing related to gut infection, like SARS-CoV-2, rotavirus, norovirus, enterovirus 71, and human adenovirus.

### 3.4 Human capillary organoids

Since ACE2 is the SARS-Cov-2 receptor, clinical-grade human recombinant soluble ACE2 (hrsACE2) has already undergone clinical phase 1 and phase 2 testing. hrsACE2 slowed or even stopped the virus's systemic dissemination from the lungs to other tissues, including potentially reducing SARS-CoV-2 attacks on the endothelial cells of the blood vessel linings. hrsACE2 has shown promising therapeutic efficacy in treating severe COVID-19 [97]. To this end, V. Monteil et al. pursued the development of engineered human blood vessel organoids and human kidney organoids to get confirmatory evidence on the effect of hrsACE2 on SARS-CoV-2 infection in multiple human organoid models [98].

To begin with, they first isolated the SARS-CoV-2 from a nasopharyngeal sample of a patient in Sweden with confirmed COVID-19, cultured it on Vero E6 cells, and successfully isolated the virus for characterization by next-generation sequencing and electron microscopy. The cellular studies showed that hrsACE2 can reduce viral growth in Vero E6 cells by a factor of 1,000–5,000. Their data demonstrated that hrsACE-2 can inhibit *in vitro* SARS-CoV-2 infection in a dose-dependent manner, unlike mouse rsACE2 highlighting the specificity of hsrACE2 in blocking SARS-CoV-2 entry. With the *in vitro* evidence at hand, they moved on to the organoid model studies.

Before getting into the deeper details, let us have a look at the background for capillary organoid research in the light of SARS-CoV-2. It was already well-known during that time that viremia initiates during the course of COVID-19 despite the irregular observation of viral RNA in blood [88]. However, a viral size of 80–100 nm is suggestive of the fact that local tissue infections can only occur through the viremic invasion into vascular endothelial cells unless there is preexisting tissue damage. This hypothesis was tested by infecting iPSC-derived human capillary organoids, which resemble human capillaries with clear lumen, lined by CD31+ endothelial cells and PDGFR+ pericyte cells and basal membrane [99]. A qRT-PCR analysis of these organoids for the presence of viral RNA indicates a gradual rise in the levels of viral RNA from day 3 to 6 of infection, proving active replication and production of progeny virus. This was followed by a marked decrease in replication without any associated toxicity on adding hrsACE2 to the capillary organoid culture.

SARS-CoV-2 can directly infect blood vessel cells which can also shed progeny viruses. Most importantly, this can be significantly inhibited by hrsACE2 at the early stages of the infection. This is the underlying rationale behind the hope of using soluble ACE2 for protecting the host body from lung injury and block the virus from entering target cells. Having said that, no data on its impact during the advanced stage of COVID-19 is currently accessible [98].

### 3.5 Kidney organoids

Since renal organotropism was becoming increasingly prominent in SARS-CoV-2, M. Glatzel et al. did an *in silico* data analysis of single-cell RNA sequencing that was available in the public datasets. Their calculations revealed that RNA of

genes (ACE2, TMPRSS2, CTSL) that help to promote the viral infection is enriched in multiple kidney-cell types from fetal development through adulthood. This corroborates previous reports stating that enrichment may facilitate SARS-CoV-2–associated kidney injury [77]. They also quantified the SARS-CoV-2 viral load in precisely defined kidney compartments obtained with the use of tissue microdissection from the samples of patients who underwent autopsy. The findings revealed that 50 percent of patients had observable SARS-CoV-2 viral loads in all kidney compartments tested, with glomerular cells being the most often infected [100].

V. Monteil et al. adapted their previously published procedure [97] to produce kidney organoids from human embryonic stem cells into 3D suspension culture to assess if SARS-CoV-2 would directly invade human tubular kidney cells [101]. Kidney organoids showed conspicuous tubular-like shapes, as observed by Lotus tetraglobus lectin (LTL), a standard marker of proximal tubular epithelial cells. Similar to their human capillary organoid study, infections of kidney organoids were monitored for at least six days post-infection, and their qRT-PCR data were analyzed for the presence of viral RNA. The team used Vero E6 cells to determine the virus's progeny. SARS-CoV-2 reproduced in kidney organoids, as predicted in cells and tissues that express ACE2. The engineered kidney organoids developed infectious progeny virus, as shown by the ability of supernatant from infected kidney organoids to infect Vero E6 cells on day six post-infection. hrsACE2 significantly decreased SARS-CoV-2 infections in a dose-dependent way in the human kidney organoids, with no evidence of toxicity. In summary, engineered human kidney organoids can also be infected with SARS-CoV-2, and this infection can be inhibited by hrsACE2, similar to blood vessel organoids.

Taken together, renal tropism explains the major clinical signs of kidney injury in patients with COVID-19 having mild or severe symptoms. These studies also predict that SARS-CoV-2 infection can potentially aggravate any preexisting renal conditions. The coronavirus receptor ACE2 is expressed in kidney organoids, which may help researchers further understand the disease's systemic effects, and multiple questions regarding the pathogenesis can be answered. Thus, the development of multi-organ organoids can address the multi-organ dysfunction, a symptom of COVID-19 illness.

## 4. Future directions and conclusion

After SARS-CoV and MERS-CoV, SARS-CoV-2 is the third coronavirus in terms of pathogenicity to jump to humans within two decades. This suggests that similar zoonotic coronavirus spillovers can happen again in the near future. Nevertheless, the events relating to coronavirus pathogenesis and transmission are not completely known yet. There is a lack of efficient *in vitro* systems to accurately model host tissues. As conventional animal models, like mice, are not natural hosts to SARS-CoV-2 infection, there is a surge in the development of alternate pre-clinical models to recapitulate the targeted human organs.

Herein, organoid technology used to model human organ development and various human pathologies in a petri dish has played a significant role in understanding SARS-CoV-2 infection and replication. For drug response studies, drug screening, and repurposing, organoids, especially patient-derived organoids, have become popular. Organoid-based studies are leading to personalizing drugs, formulating regenerative medicine, and establishing gene therapy. In comparison to age-old animal models and cell lines, there has been a noticeable improvement in the reproducibility of results and statistical power of experiments. From all previous data, human organoids of lung, gut, kidney, brain, and blood vessels represent excellent experimental models to study the biology of SARS-CoV-2 [44].

Having said that, researchers working in this field are still trying to identify and troubleshoot the inherent challenges in various aspects of handling organoids, including the maintenance costs, cross-technique artifacts, and interpretation of data [26]. It is now well known that the generation and handling of organoids are way more tedious than two-dimensional cell culture protocols. Moreover, the essential growth factors being more expensive and not explicitly tested for applications in the organoid system, one has to prepare them in-house. With the emergence of various commercial sources for reagents tailored to the organoid culture, there is reason to believe that this problem will be fixed quickly. Moreover, the range of cellular heterogeneity for a particular organoid system needs to be improved. Also, mimicking the native micro-and matrix-environment encountered by cells within organoids remains a challenge. Reverse engineering methodologies are only in their infancy as it comes to developing rigorous protocols for the *in vitro* maturation of organoids that are largely fetal-like in cultures [102]. Advances in stem cell, progenitor cell, and pluripotent stem cell handling and directed differentiation techniques will soon help create more physiologically relevant organoids.

In combination with genome editing techniques for manipulating 3D models, organoid technology will be implemented at a large scale in basic and clinical research in the forthcoming era [14]. Progress with other technologies, such as microRNA switches and potentially CRISPR–Cas9, 3D bioprinting, and 3D organoids, will further advance the fast-developing multi-organ disease modeling COVID-19 and its associated therapeutic build-up. Though organoid technology suffers from multiple lacunae but COVID-19 has shown the feasibility and practicality of the organoid platform, suggesting further investment to create an *in vitro* organ mimicking reliable system for successful and effective discovery of therapeutics.

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## References

[1] Hoarau-Véchot, J., et al., *Halfway between 2D and Animal Models: Are 3D Cultures the Ideal Tool to Study Cancer-Microenvironment Interactions?* International Journal of Molecular Sciences, 2018. **19**(1):181.

[2] Leach, T.S., et al., *Tissue organoid models and applications*, in *Principles of Tissue Engineering*. 2020. p. 1537-1549.

[3] McGonigle, P. and B. Ruggeri, *Animal models of human disease: Challenges in enabling translation.* Biochemical Pharmacology, 2014. **87**(1): p. 162-171.

[4] Zhou, J., et al., *Microfluidic device for primary tumor spheroid isolation.* Experimental Hematology & Oncology, 2017. **6**(1).

[5] Ben-David, U., et al., *Patient-derived xenografts undergo mouse-specific tumor evolution.* Nature Genetics, 2017. **49**(11): p. 1567-1575.

[6] Rossi, G., A. Manfrin, and M.P. Lutolf, *Progress and potential in organoid research.* Nature Reviews Genetics, 2018. **19**(11): p. 671-687.

[7] Clevers, H., *Modeling Development and Disease with Organoids.* Cell, 2016. **165**(7): p. 1586-1597.

[8] Fatehullah, A., S.H. Tan, and N. Barker, *Organoids as an in vitro model of human development and disease.* Nature Cell Biology, 2016. **18**(3): p. 246-254.

[9] Kelava, I. and M.A. Lancaster, *Dishing out mini-brains: Current progress and future prospects in brain organoid research.* Developmental Biology, 2016. **420**(2): p. 199-209.

[10] Shi, Y., et al., *Induced pluripotent stem cell technology: a decade of progress.* Nature Reviews Drug Discovery, 2016. **16**(2): p. 115-130.

[11] Vlachogiannis, G., et al., *Patient-derived organoids model treatment response of metastatic gastrointestinal cancers.* Science, 2018. **359**(6378): p. 920-926.

[12] Simian, M. and M.J. Bissell, *Organoids: A historical perspective of thinking in three dimensions.* Journal of Cell Biology, 2017. **216**(1): p. 31-40.

[13] Schutgens, F. and H. Clevers, *Human Organoids: Tools for Understanding Biology and Treating Diseases.* Annual Review of Pathology: Mechanisms of Disease, 2020. **15**(1): p. 211-234.

[14] Artegiani, B. and H. Clevers, *Use and application of 3D-organoid technology.* Human Molecular Genetics, 2018. **27**(R2): p. R99-R107.

[15] Xu, H., et al., *Organoid technology in disease modelling, drug development, personalized treatment and regeneration medicine.* Experimental Hematology & Oncology, 2018. **7**: 30.

[16] Takasato, M., et al., *Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis.* Nature, 2015. **526**(7574): p. 564-568.

[17] Zacharias, W.J., et al., *Regeneration of the lung alveolus by an evolutionarily conserved epithelial progenitor.* Nature, 2018. **555**(7695): p. 251-255.

[18] Chen, Y.W., et al., *A three-dimensional model of human lung development and disease from pluripotent stem cells.* Nature Cell Biology, 2017. **19**(5): p. 542-549.

[19] Dye, B.R., et al., *In vitro generation of human pluripotent stem cell derived lung organoids.* eLife, 2015. **4**.

[20] Sachs, N., et al., *Long-term expanding human airway organoids for*

*disease modeling.* The EMBO Journal, 2019. **38**(4).

[21] Fujii, M., et al., *Human Intestinal Organoids Maintain Self-Renewal Capacity and Cellular Diversity in Niche-Inspired Culture Condition.* Cell Stem Cell, 2018. **23**(6): p. 787-793.e6.

[22] Lancaster, M.A., et al., *Cerebral organoids model human brain development and microcephaly.* Nature, 2013. **501**(7467): p. 373-379.

[23] Drost, J., et al., *Organoid culture systems for prostate epithelial and cancer tissue.* Nature Protocols, 2016. **11**(2): p. 347-358.

[24] Devarasetty, M., et al., *Optical Tracking and Digital Quantification of Beating Behavior in Bioengineered Human Cardiac Organoids.* Biosensors, 2017. **7**(3): 24.

[25] Nakano, T., et al., *Self-Formation of Optic Cups and Storable Stratified Neural Retina from Human ESCs.* Cell Stem Cell, 2012. **10**(6): p. 771-785.

[26] Kim, J., B.-K. Koo, and J.A. Knoblich, *Human organoids: model systems for human biology and medicine.* Nature Reviews Molecular Cell Biology, 2020. **21**(10): p. 571-584.

[27] Sridhar, A., et al., *A Perspective on Organoids for Virology Research.* Viruses, 2020. **12**(11).

[28] Ettayebi, K., et al., *Replication of human noroviruses in stem cell-derived human enteroids.* Science, 2016. **353**(6306): p. 1387-1393.

[29] Pyrc, K., et al., *Culturing the Unculturable: Human Coronavirus HKU1 Infects, Replicates, and Produces Progeny Virions in Human Ciliated Airway Epithelial Cell Cultures.* Journal of Virology, 2010. **84**(21): p. 11255-11263.

[30] Dijkman, R., et al., *Human Bocavirus Can Be Cultured in Differentiated Human Airway Epithelial Cells.* Journal of Virology, 2009. **83**(15): p. 7739-7748.

[31] Hao, W., et al., *Infection and Propagation of Human Rhinovirus C in Human Airway Epithelial Cells.* Journal of Virology, 2012. **86**(24): p. 13524-13532.

[32] Jazaeri Farsani, S.M., et al., *Culturing of respiratory viruses in well-differentiated pseudostratified human airway epithelium as a tool to detect unknown viruses.* Influenza and Other Respiratory Viruses, 2015. **9**(1): p. 51-57.

[33] Tapparel, C., et al., *Growth and characterization of different human rhinovirus C types in three-dimensional human airway epithelia reconstituted in vitro.* Virology, 2013. **446**(1-2): p. 1-8.

[34] Bochkov, Y.A., et al., *Mutations in VP1 and 3A proteins improve binding and replication of rhinovirus C15 in HeLa-E8 cells.* Virology, 2016. **499**: p. 350-360.

[35] Meerovitch, K., R. Nicholson, and N. Sonenberg, *In vitro mutational analysis of cis-acting RNA translational elements within the poliovirus type 2 5′ untranslated region.* Journal of Virology, 1991. **65**(11): p. 5895-5901.

[36] Lulla, V., et al., *An upstream protein-coding region in enteroviruses modulates virus infection in gut epithelial cells.* Nature Microbiology, 2018. **4**(2): p. 280-292.

[37] Drummond, C.G., et al., *Enteroviruses infect human enteroids and induce antiviral signaling in a cell lineage-specific manner.* Proceedings of the National Academy of Sciences, 2017. **114**(7): p. 1672-1677.

[38] Essaidi-Laziosi, M., et al., *Propagation of respiratory viruses in human airway epithelia reveals persistent virus-specific signatures.* Journal of

Allergy and Clinical Immunology, 2018. **141**(6): p. 2074-2084.

[39] Bai, J., et al., *Phenotypic Responses of Differentiated Asthmatic Human Airway Epithelial Cultures to Rhinovirus.* Plos One, 2015. **10**(2).

[40] Kessler, M., et al., *Chronic Chlamydia infection in human organoids increases stemness and promotes age-dependent CpG methylation.* Nature Communications, 2019. **10**(1).

[41] Clevers, H., *COVID-19: organoids go viral.* Nature Reviews Molecular Cell Biology, 2020. **21**(7): p. 355-356.

[42] Milewska, A., et al., *Replication of Severe Acute Respiratory Syndrome Coronavirus 2 in Human Respiratory Epithelium.* Journal of Virology, 2020. **94**(15).

[43] Pizzorno, A., et al., *Characterization and Treatment of SARS-CoV-2 in Nasal and Bronchial Human Airway Epithelia.* Cell Reports Medicine, 2020. **1**(4).

[44] Lamers, M.M., et al., *SARS-CoV-2 productively infects human gut enterocytes.* Science, 2020. **369**(6499): p. 50-54.

[45] Wu, Y., et al., *Prolonged presence of SARS-CoV-2 viral RNA in faecal samples.* The Lancet Gastroenterology & Hepatology, 2020. **5**(5): p. 434-435.

[46] DosSantos, M.F., et al., *Neuromechanisms of SARS-CoV-2: A Review.* Frontiers in Neuroanatomy, 2020. **14**.

[47] Zhang, B.-Z., et al., *SARS-CoV-2 infects human neural progenitor cells and brain organoids.* Cell Research, 2020. **30**(10): p. 928-931.

[48] Ramani, A., et al., *SARS-CoV-2 targets neurons of 3D human brain organoids.* The EMBO Journal, 2020. **39**(20).

[49] Pellegrini, L., et al., *Human CNS barrier-forming organoids with cerebrospinal fluid production.* Science, 2020. **369**(6500).

[50] Heukelbach, J., et al., *Zika virus outbreak in Brazil.* The Journal of Infection in Developing Countries, 2016. **10**(02): p. 116-120.

[51] Heymann, D.L., et al., *Zika virus and microcephaly: why is this situation a PHEIC?* The Lancet, 2016. **387**(10020): p. 719-721.

[52] Ming, G.-l., H. Tang, and H. Song, *Advances in Zika Virus Research: Stem Cell Models, Challenges, and Opportunities.* Cell Stem Cell, 2016. **19**(6): p. 690-702.

[53] Tang, H., et al., *Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth.* Cell Stem Cell, 2016. **18**(5): p. 587-590.

[54] Garcez, P.P., et al., *Zika virus impairs growth in human neurospheres and brain organoids.* Science, 2016. **352**(6287): p. 816-818.

[55] Qian, X., et al., *Using brain organoids to understand Zika virus-induced microcephaly.* Development, 2017. **144**(6): p. 952-957.

[56] Kelava, I. and Madeline A. Lancaster, *Stem Cell Models of Human Brain Development.* Cell Stem Cell, 2016. **18**(6): p. 736-748.

[57] Stanifer, M.L., et al., *Asymmetric distribution of TLR3 leads to a polarized immune response in human intestinal epithelial cells.* Nature Microbiology, 2019. **5**(1): p. 181-191.

[58] Roodsant, T., et al., *A Human 2D Primary Organoid-Derived Epithelial Monolayer Model to Study Host-Pathogen Interaction in the Small Intestine.* Frontiers in Cellular and Infection Microbiology, 2020. **10**.

[59] Shih, S.R., et al., *Exosomes Facilitate Transmission of Enterovirus A71 From*

*Human Intestinal Epithelial Cells.* The Journal of Infectious Diseases, 2020. **222**(3): p. 456-469.

[60] Zhu, N., et al., *A Novel Coronavirus from Patients with Pneumonia in China, 2019.* N Engl J Med, 2020. **382**(8): p. 727-733.

[61] Zhou, P., et al., *A pneumonia outbreak associated with a new coronavirus of probable bat origin.* Nature, 2020. **579**(7798): p. 270-273.

[62] Huang, C., et al., *Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China.* Lancet, 2020. **395**(10223): p. 497-506.

[63] Chan, J.F., et al., *Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan.* Emerg Microbes Infect, 2020. **9**(1): p. 221-236.

[64] Lu, R., et al., *Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding.* Lancet, 2020. **395**(10224): p. 565-574.

[65] Zhu, Z., et al., *From SARS and MERS to COVID-19: a brief summary and comparison of severe acute respiratory infections caused by three highly pathogenic human coronaviruses.* Respiratory Research, 2020. **21**(1).

[66] Zamorano Cuervo, N. and N. Grandvaux, *ACE2: Evidence of role as entry receptor for SARS-CoV-2 and implications in comorbidities.* eLife, 2020. **9**.

[67] Wilkinson, D.C., et al., *Development of a Three-Dimensional Bioengineering Technology to Generate Lung Tissue for Personalized Disease Modeling.* STEM CELLS Translational Medicine, 2017. **6**(2): p. 622-633.

[68] Zhou, J., et al., *Differentiated human airway organoids to assess infectivity of emerging influenza virus.* Proceedings of the National Academy of Sciences, 2018. **115**(26): p. 6822-6827.

[69] Elbadawi, M. and T. Efferth, *Organoids of human airways to study infectivity and cytopathy of SARS-CoV-2.* The Lancet Respiratory Medicine, 2020. **8**(7): p. e55-e56.

[70] Han, Y., et al., Identification of Candidate COVID-19 Therapeutics using hPSC-derived Lung Organoids, 2020.

[71] Mulay, A., et al., SARS-CoV-2 infection of primary human lung epithelium for COVID-19 modeling and drug discovery, Cell Rep, 2021. **35**(5): p. 109055

[72] Guan, W.J., et al., *Clinical Characteristics of Coronavirus Disease 2019 in China.* N Engl J Med, 2020. **382**(18): p. 1708-1720.

[73] Lechien, J.R., et al., *Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study.* European Archives of Oto-Rhino-Laryngology, 2020. **277**(8): p. 2251-2261.

[74] Yuen, K.-Y., et al., *Olfactory Dysfunction in Coronavirus Disease 2019 Patients: Observational Cohort Study and Systematic Review.* Open Forum Infectious Diseases, 2020. **7**(6).

[75] Zhang, A.J., et al., *Severe Acute Respiratory Syndrome Coronavirus 2 Infects and Damages the Mature and Immature Olfactory Sensory Neurons of Hamsters.* Clinical Infectious Diseases, 2020. ciaa995

[76] Helms, J., et al., *Neurologic Features in Severe SARS-CoV-2 Infection.* New England Journal of Medicine, 2020. **382**(23): p. 2268-2270.

[77] Puelles, V.G., et al., *Multiorgan and Renal Tropism of SARS-CoV-2.* New England Journal of Medicine, 2020. **383**(6): p. 590-592.

[78] Pamies, D., *A human brain microphysiological system derived from induced pluripotent stem cells to study neurological diseases and toxicity.* Altex, 2017: p. 362-376.

[79] Chen, R., et al., 2020.

[80] Hoffmann, M., et al., *SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor.* Cell, 2020. **181**(2): p. 271-280.e8.

[81] Brann, D.H., et al., *Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia.* Science Advances, 2020. **6**(31).

[82] Zhou, H., et al., *A Novel Bat Coronavirus Closely Related to SARS-CoV-2 Contains Natural Insertions at the S1/S2 Cleavage Site of the Spike Protein.* Current Biology, 2020. **30**(11): p. 2196-2203.e3.

[83] Kanberg, N., et al., *Neurochemical evidence of astrocytic and neuronal injury commonly found in COVID-19.* Neurology, 2020. **95**(12): p. e1754-e1759.

[84] Bullen, C.K., *Infectability of Human BrainSphere Neurons Suggests Neurotropism of SARS-CoV-2*.* Altex, 2020.

[85] Wei, X.-S., et al., *Diarrhea Is Associated With Prolonged Symptoms and Viral Carriage in Corona Virus Disease 2019.* Clinical Gastroenterology and Hepatology, 2020. **18**(8): p. 1753-1759.e2.

[86] Hikmet, F., et al., *The protein expression profile of ACE2 in human tissues.* Molecular Systems Biology, 2020. **16**(7).

[87] Xiao, F., et al., *Evidence for Gastrointestinal Infection of SARS-CoV-2.* Gastroenterology, 2020. **158**(6): p. 1831-1833.e3.

[88] Wang, W., et al., *Detection of SARS-CoV-2 in Different Types of Clinical Specimens.* Jama, 2020.

[89] Xiao, F., et al., *Evidence for Gastrointestinal Infection of SARS-CoV-2.* Gastroenterology, 2020. **158**(6): p. 1831-1833 e3.

[90] Xu, Y., et al., *Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding.* Nat Med, 2020. **26**(4): p. 502-505.

[91] Zhou, J., et al., *Infection of bat and human intestinal organoids by SARS-CoV-2.* Nature Medicine, 2020. **26**(7): p. 1077-1083.

[92] Zang, R., et al., *TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes.* Sci Immunol, 2020. **5**(47).

[93] Sato, T., et al., *Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche.* Nature, 2009. **459**(7244): p. 262-5.

[94] Spence, J.R., et al., *Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro.* Nature, 2010. **470**(7332): p. 105-109.

[95] Holloway, E.M., et al., *Differentiation of Human Intestinal Organoids with Endogenous Vascular Endothelial Cells.* Developmental Cell, 2020. **54**(4): p. 516-528.e7.

[96] Krüger, J., et al., *Drug Inhibition of SARS-CoV-2 Replication in Human Pluripotent Stem Cell–Derived Intestinal Organoids.* Cellular and Molecular Gastroenterology and Hepatology, 2021. **11**(4): p. 935-948.

[97] Zhang, H., et al., *Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target.* Intensive Care Medicine, 2020. **46**(4): p. 586-590.

[98] Monteil, V., et al., *Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2.* Cell, 2020. **181**(4): p. 905-913.e7.

[99] Wimmer, R.A., et al., *Human blood vessel organoids as a model of diabetic vasculopathy.* Nature, 2019. **565**(7740): p. 505-510.

[100] Pei, G., et al., *Renal Involvement and Early Prognosis in Patients with COVID-19 Pneumonia.* J Am Soc Nephrol, 2020. **31**(6): p. 1157-1165.

[101] Garreta, E., et al., *Fine tuning the extracellular environment accelerates the derivation of kidney organoids from human pluripotent stem cells.* Nature Materials, 2019. **18**(4): p. 397-405.

[102] Lou, Y.-R. and A.W. Leung, *Next generation organoids for biomedical research and applications.* Biotechnology Advances, 2018. **36**(1): p. 132-149.