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Organotypic human *ex vivo* models for coronavirus disease 2019 research and drug development

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Abstract

Since the discovery of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in late 2019, intense research efforts on an unprecedented scale have focused on the study of viral entry mechanisms and adaptive immunity. While the identification of angiotensin-converting enzyme 2 (ACE2) and other co-receptors has elucidated the molecular and structural basis for viral entry, the pathobiological mechanisms of SARS-CoV-2 in human tissues are less understood. Recent advances in bioengineering have opened opportunities for the use of organotypic human tissue models to investigate host–virus interactions and test antiviral drug candidates in a physiological context. Although it is too early to accurately quantify the added value of these systems compared with conventional cell systems, it can be assumed that these advanced three-dimensional (3D) models contribute toward improved result translation. This mini-review summarizes recent work to study SARS-CoV-2 infection in human 3D tissue models with an emphasis on the pharmacological tools that have been developed to understand and prevent viral entry and replication.

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic has resulted in an unprecedented shift in research activities aimed at collectively combining efforts to develop treatments for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and minimize its spread in the community. This has led to the uncoordinated use of different cell and tissue models to study SARS-CoV-2 infection and treatment modalities [1]. The choice of model system has important implications in the context of functional pharmacology, where lead compounds encounter a bottleneck in drug discovery at the preclinical or clinical phase because of a lack of therapeutic value. What complicates the matter further is that although COVID-19 is primarily a respiratory disease, it is now clear that it can also result in a multitude of extrapulmonary manifestations, including liver and renal injury, endothelial damage, neurologic symptoms, and beta-cell dysfunction [2].

The expression of SARS-CoV-2 receptors and entry factors has been studied across healthy human tissues [3], but the effects of infection on host cell molecular signatures are limited to the airway epithelium, lung, and intestine and are mostly based on biopsy samples. To allow for longitudinal profiling, the establishment of an accessible panel of human organotypic three-dimensional (3D) tissue models provides a promising tool kit to study the tissue-specific pathobiology of viral infections. Previously, 3D models have been used to study a multitude of enteric infections, including coxsackie B1 virus [4], norovirus [5], rotavirus [6] and enterohemorrhagic *Escherichia coli* [7], hepatitis viruses [8,9], and respiratory viruses, such as respiratory syncytial virus [10], influenza virus [11,12], and parainfluenza virus 3 [13].

The choice of model system depends on several factors, but perhaps the biggest differences are found when investigating the pharmacology of conventional prophylactics versus vaccines. While prophylactics largely depend on the nature of the selected cell system, vaccines rely on an understanding of the virus itself. In the case of COVID-19, which can progress to affect multiple organs, further consideration should be given to the stage of infection (i.e. initial infection vs. multiorgan failure).

To study the complex pathophysiology of SARS-CoV-2 infection in human tissues, cell lines and animal models have important limitations because of species differences in virus tropism and cell line–specific peculiarities of the host cell response. For instance, since

their isolation in the late 1960s, African green monkey kidney cells (Vero) have routinely been used as host cells for virus propagation and the testing of antiviral drug candidates. However, Vero cells lack the capacity to produce interferons, which is an important consideration, particularly for the study of interferon susceptible viruses, such as SARS-CoV-2. Such caveats necessitate the use of other emerging culture models, such as stem cell—derived organoids and 3D primary tissue models, that may better represent the pathophysiology of infection and increase the fidelity of result translation (Table 1). For methodological details, as well as critical discussions of the advantages and limitations of these culture systems, we refer the interested reader to recent comprehensive reviews [14—17].

3D human airway models

Patients with severe COVID-19 can develop acute respiratory distress syndrome characterized by respiratory failure with hypoxemia and acute bilateral pulmonary infiltrates and histopathologic patterns of diffuse alveolar damage and pulmonary microthrombi [18]. Several distal lung epithelial models have been developed that can be infected with SARS-CoV-2 and allow to study viral effects in the distal gas-exchange region of the lungs (Figure 1a).

Human primary alveolar organoids have been generated from cells isolated from normal human distal lung tissue co-cultured with MRC-5 human lung fibroblasts [19]. These organoids were susceptible to SARS-CoV-2 infection and showed robust induction of host response genes, including various cytokines, as well as cell autonomous and non—cell autonomous apoptosis, indicative of alveolar injury. At physiological concentrations, remdesivir significantly reduced viral load, whereas no effects were observed for hydroxychloroquine.

Human alveolar type II (AT2) cells derived from primary human lung tissue have also been used to investigate the host cell response to SARS-CoV-2 infection in long-term 3D culture [20]. These studies revealed rapid viral replication within a few days postinfection paralleled by a strong induction of interferon response gene signatures, indicative of a robust endogenous innate immune response. Furthermore, single-cell sequencing showed that although uninfected AT2 cells were relatively homogeneous, SARS-CoV-2 infection resulted in the subdivision of cells into distinct clusters, of which some were characterized by a loss of AT2 identity and compromised alveolar function. Distal lung organoids have also been generated from adult AT2 or keratin 5—positive (KRT5+) basal cells [21]. Alveolar organoids differentiated into alveolar type I (AT1) cells, whereas basal cell organoids became luminal lined with differentiated club and ciliated cells within bronchiolar structures. Importantly, distal lung organoids with apical-out polarity were susceptible to infection, and club cells were identified as a novel SARS-CoV-2 target population.

Lung organoids generated by differentiation of primary stem cells derived from tissue sections enabled the modeling of both distal and proximal lung epithelium [22]. Notably, proximal epithelial cells were more permissive to SARS-CoV-2 infection, whereas distal epithelial cells showed increased host cell immune response, highlighting the importance of spatially refined tissue modeling.

Alternatively, human pluripotent stem cells (hPSCs) can be used to generate organoids that recapitulate the cellular complexity of the human distal lung, including AT1 and AT2 cells, stromal cells, pulmonary neuroendocrine cells, as well as airway epithelial cells [23]. Notably, this model allowed for sufficient throughput to screen >1200 US Food and Drug Administration FDA)-approved drugs for their ability to inhibit SARS-CoV-2 entry, and the authors identified candidate molecules, including imatinib and chloroquine, which inhibited infectivity at subtoxic concentrations. hPSC-derived AT2 cells can furthermore be cultivated as monolayers with apical-basal polarization and barrier integrity on filters in air—liquid interface (ALI) cultures [24]. The presence of virus particles in the vicinity of lamellar bodies suggests that secretion of lung surfactant may be dysregulated by SARS-CoV-2 infection. As in cultures from primary cells, infection increased inflammatory signaling and loss of mature alveolar phenotypes. It is, however, important to note that these culture models lacked immune cell components.

To overcome this limitation, lung and macrophage co-culture systems were developed based on directed differentiation of hPSCs [25]. Importantly, in this model, both M1 and M2 macrophages inhibited SARS-CoV-2 infection, but only M1 macrophages increased the production of inflammatory cytokines that facilitated lung cell injury. Inhibition of viral entry improved M2-mediated viral clearance and resulted in significant protection of lung cells, suggesting that blockade of viral entry or stimulation of M2 macrophages are potential strategies for therapeutic intervention.

Taken together, both organoids and ALI cultures can be used as tools to study SARS-CoV-2 infection of pulmonary tissues. In particular, human primary models faithfully recapitulate the molecular and cellular phenotypes of distal and proximal lung epithelium. However, they lack the ability to be stably propagated and are consequently more suitable for mechanistic studies or target validations rather than large-scale screens. A major drawback of most current models remains the relatively low cellular complexity, particularly the absence of immune cells, which impairs our

Table 1

Overview of 3D human tissue models for SARS-CoV-2 infection.

Tissue	Cell source	Cell types	Strengths	Limitations	References
Lung	Distal lung tissue co-culture with lung fibroblasts	AT2	Allow for study of proximal–distal axis of the infection	No presence of AT1 cells; long differentiation process (20 days; preprint)	[5]
	Primary human lung tissue	AT1 and AT2	AT1 and AT2 representation; long-term stable 3D culture	Lack of immune cells; tissues derived from heterogeneous clinical samples; limited scalability	[20]
	Primary human AT2	AT1 differentiated from AT2	Long-term feeder-free culture; possibility for short-term propagation	Lack of immune cells; apical-out polarity	[21]
	Primary human Basal KRT5+ cells	Club and ciliated cells differentiated from basal cells			
	Lung tissue adult stem cells	AT1 and AT2	Recapitulation of proximodistal cellularity; scalability	Lack of immune cells (preprint)	[8]
		Basal cells, ciliated cells, club cells, and goblet cells			
	hPSC	AT1- and AT2-like cells	Substantial cellular complexity; cells can be propagated resulting in high scalability	Lack of immune cells; immature phenotype; long differentiation process (50 days)	[23]
	hPSC	AT2-like cells	Self-renewing human lung epithelial lineages; adaptation to 2D ALI cultures mimicking apical viral respiratory infections	Lack of AT1-like cells and immune component	[24]
	hPSC	AT2, AT1, ciliated, basal, stromal, club, neuroendocrine cells, M1 and M2 macrophages	Host cell and macrophages from the same hPSC lines avoiding concern of histocompatibility	Cultures cannot be propagated limiting scalability (preprint)	[11]
Liver	hPSC	Hepatocyte-like cells	The study also investigated the viral tropism in other stem cell–derived tissue models	Lack of nonparenchymal cells; immature liver phenotypes	[27]
	Bile duct–derived progenitor	Cholangiocytes	Allow for the study of SARS-CoV-2 specifically in cholangiocytes	Underrepresentation of liver cell complexity	[28]
	Primary human hepatocytes and nonparenchymal cells	Hepatocytes	Cells retain their mature phenotype on transcriptomic, proteomic, and metabolomic level for many weeks; high-throughput compatible	Use of limited primary material	[29–33]
Intestine	Primary gut epithelial stem cells	Enterocytes, goblet cells, enteroendocrine cells	Mimics the cellular complexity of the human intestine; well-characterized phenotypes; unlimited propagation in 3D culture	Brush borders face the organoid lumen making it cumbersome to study infections of the oral routes	[41,50,51]
	Primary gut epithelial stem cells	Enterocytes	Enteroids can be derived from duodenum, ileum, and colon; compatible with transwell culture	Limited intestinal complexity	[42]
	Primary gut epithelial stem cells	Enterocytes	Robust and long-term stable culture	Limited intestinal complexity	[43]
	Human pluripotent stem cells			Molecular phenotypes not well characterized	[44]

(*continued on next page*)

Table 1. (*continued*)

Tissue	Cell source	Cell types	Strengths	Limitations	References
		Enterocytes, goblet, Paneth, enteroendocrine cells	Mimics the cellular complexity of the human intestine; does not require primary tissue samples		
	Human pluripotent stem cells	Enterocytes, goblet cells, enteroendocrine cells	Mimics the cellular complexity of the human intestine; does not require primary tissue samples	Molecular phenotypes not well characterized; study only uses SARS-CoV-2 spike protein, not live virus	[23]
Kidney	Human pluripotent stem cells	Proximal tubules, loops of Henle, distal tubules, and glomeruli	Mimics the complexity of the human nephron	Complex microenvironment	[47,48]

ALI = air–liquid interface; hPSC = human-induced pluripotent stem cells.

understanding of the pathophysiological mechanisms of SARS-CoV-2 infection of pulmonary tissues.

3D human liver models

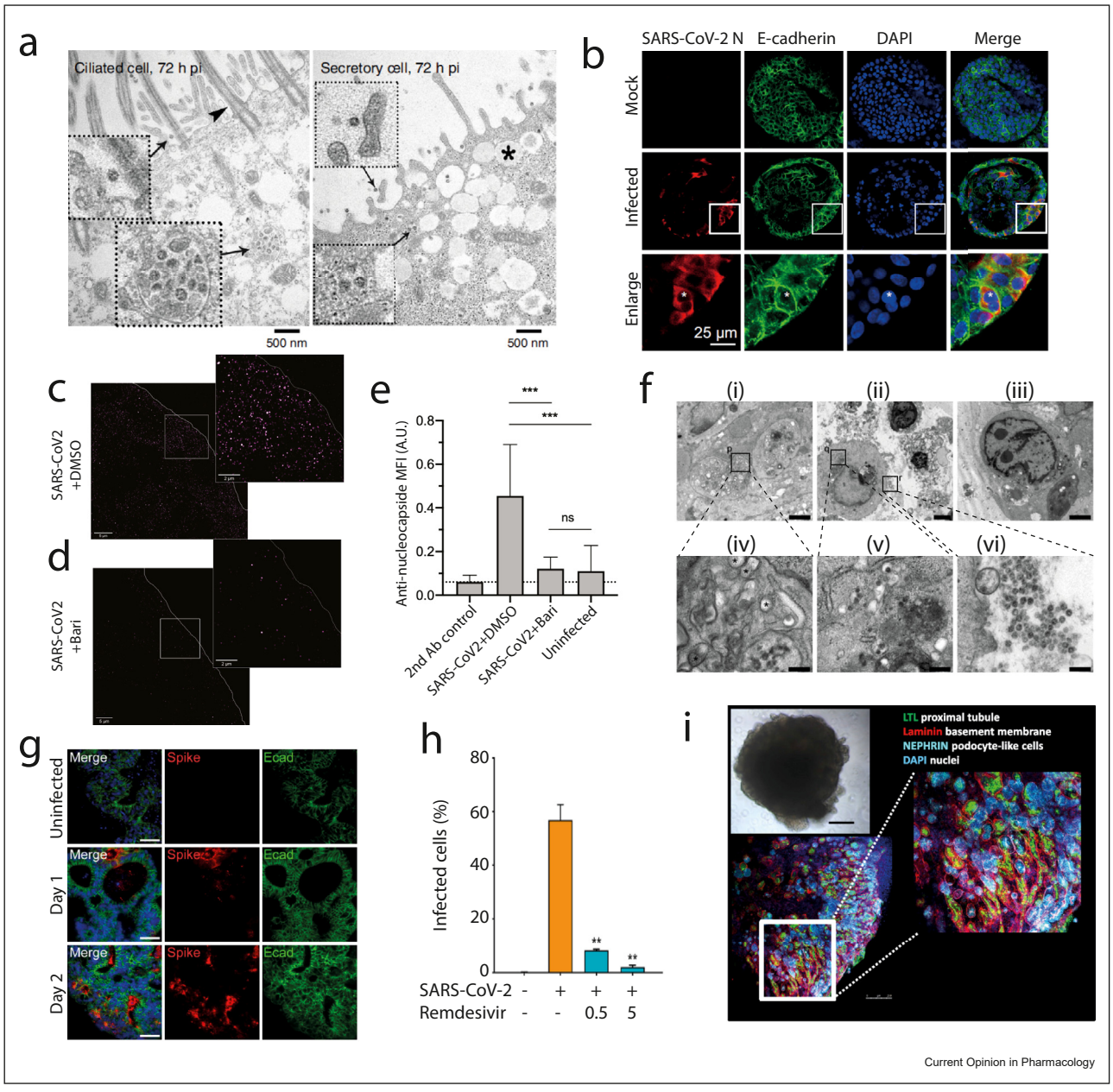
There is considerable interest in organotypic liver models to study virus–liver cell interactions, as well as the pharmacokinetics and toxicity of candidate drugs. This is reinforced by the observation that liver injury has been detected in up to 60% of severely ill COVID-19 patients [26], suggesting that liver models could be useful to study extrapulmonary disease biology *ex vivo*.

Hepatic effects of SARS-CoV-2 have been studied in stem cell–derived organoids and in organotypic 3D cultures of mature human liver cells. In stem cell–derived liver organoids, SARS-CoV-2 infection could be detected in albumin-positive hepatocyte-like cells [27], as well as in cholangiocytes in human liver ductal organoids based on bile duct–derived progenitor cultures [28]. Interestingly, in the latter, the authors demonstrated that infection impaired the barrier and bile acid transporting functions of cholangiocytes by modulating the expression of tight junction and bile acid transport genes, thus providing a possible link between SARS-CoV-2 and cholestatic liver injury (Figure 1b).

Using a well-established primary human hepatocyte 3D spheroid model in which liver cells retain their transcriptomic, proteomic, and metabolomic configuration [29–31], it was shown that mature human hepatocytes are permissive to SARS-CoV-2 infection despite expressing only low levels of angiotensin-converting enzyme 2 (ACE2) [32]. Interestingly, exposure to proinflammatory cytokines, particularly class I interferons, resulted in the induction of ACE2 expression and increased liver cell infectivity [33]. However, whether the full-length functional or truncated nonfunctional ACE2 isoform [34,35] becomes induced in human liver remains to be determined. SARS-CoV-2 infection was moreover found to modulate the host cell response of hepatocytes to inflammatory signaling, specifically altering the expression of coagulation factors and genes involved in platelet activation, which might at least in part explain the coagulation defects observed in COVID-19 patients [33].

The development of a model that recapitulates the pathophysiology of SARS-CoV-2 infection *ex vivo* opened the possibility to test candidate molecules for COVID-19 drug repurposing. Baricitinib, an oral inhibitor of JAK/STAT signaling approved for the treatment of rheumatoid arthritis, had been suggested by artificial intelligence–based knowledge graphs as a candidate therapy for COVID-19 by virtue of inhibiting both excessive cytokine signaling and numb-associated kinases that regulate receptor-mediated endocytosis and viral entry at nanomolar levels [36]. Importantly, baricitinib significantly reversed virus-induced host cell

Figure 1



Organotypic 3D models of pulmonary and extrapulmonary tissues are susceptible to SARS-CoV-2 infection and allow for the study of virus–host cell interactions. (**a**) SARS-CoV-2 infection in human airway epithelia. Both ciliated cells and secretory cells were permissive to SARS-CoV-2 infection. Arrowhead and asterisk indicate cilium and secretory vesicle, respectively. Insets show close-ups of virus particles. Panel reproduced with permission from Ref. [52]. (**b**) Immunofluorescence staining for SARS-CoV-2 N protein and E-cadherin reveals infection of cholangiocyte-like cells in human liver ductal organoids. Panel modified with permission from Ref. [28]. (**c** and **d**) Superresolution dSTORM microscopy of short-term (4 h) infected liver spheroids stained for SARS-CoV-2 nucleocapsid treated with DMSO control (**c**) or baricitinib (100 nM; **d**). (**e**) Baricitinib significantly reduced intracellular viral particle numbers suggesting inhibition of viral entry. Bars indicate means ± SD; ***$P < 0.001$ two-tailed Student's *t* test. Ab = antibody; ns = not significant. Panels **c**–**e** modified with permission from Ref. [33]. (**f**) Transmission electron microscopy images of SARS-CoV-2–infected intestinal organoids. Severely infected and disintegrating cells (i–iii) are shown. Double membrane vesicles (indicated by asterisks) and viral production in the Golgi, characteristic of different stages of the viral lifecycle are shown in (iv) and (v), respectively. (vi) shows extracellular clusters of viruses. Panel modified with permission from Ref. [52]. Scale bars = 2.5 µm in (i) to (iii) and 250 nm in (iv) to (vi). Panel modified with permission from Ref. [41]. (**g**) SARS-CoV-2 staining in human pluripotent stem cell–derived intestinal organoids one and two days postinfection. Viral S protein is shown in red, host E-cadherin in green and nuclei (DAPI) in blue. Scale bar = 50 µm (**h**) Co-treatment of SARS-CoV-2–infected organoids with remdesivir resulted in a significant and dose-dependent decrease of virus-positive cells. Panels **g** and **h** reproduced with permission from Ref. [44]. (**i**) Human kidney organoid at 20 days of differentiation infected with SARS-CoV-2. Note the formation of tubular-like structures (green), podocyte-like cells (turquoise), and organized basement membrane networks (red). Scale bar in light microscopy and confocal microscopy image = 100 µm and 250 µm, respectively. Panel reproduced with permission from Ref. [48].

transcriptomic alterations and reduced infectivity, as well as viral load in 3D liver spheroids at therapeutically relevant concentrations (Figure 1c–e). More importantly and in alignment with these *ex vivo* findings, baricitinb significantly reduced mortality in observational trials of elderly patients in Italy and Spain [33]. The promising clinical effects have since been corroborated by a large randomized controlled trial [37] and have resulted in the FDA emergency use authorization of baricitinib for COVID-19.

Intestinal *ex vivo* models

The manifestation of gastrointestinal (GI) symptoms in a subset of SARS-CoV-2 patients [38,39] and the high expression level of ACE2 in enterocytes [40] have fueled studies into the pathobiology of SARS-CoV-2 in human intestinal tissues. In contrast to lung and liver, *ex vivo* studies of SARS-CoV-2 infection of intestinal cells have only been presented in stem cell–derived organoid cultures. In small intestinal organoids derived from primary gut epithelial stem cells, SARS-CoV-2 productively infected the enterocyte lineage (Figure 1f), whereas enteroendocrine cells and goblet cells were not infected [41]. Infections of ACE2-positive enterocytes were promoted by the mucosa-specific serine proteases TMPRSS2 and TMPRSS4 [42]. Interestingly, TMPRSS2 is expressed on goblet cells, which are themselves resistant to infection but promotes infection of neighboring enterocytes *in trans*. Infectious viral particles were detected in stool samples of a patient with diarrheal COVID-19, and viral replication was found to be higher in human colonoids compared with enteroids [43].

Infection, active replication, and viral spread of SARS-CoV-2 also occurred in intestinal organoids derived from hPSC that recapitulated the cellular heterogeneity of the human intestinal lining (Figure 1g). In this model, the authors observed infection of enterocytes as well as Paneth cells, suggesting potential disruption of hormonal secretion and perturbed local immune defense [44]. The model was successfully used to confirm the antiviral effects of remdesivir (Figure 1h) and the peptidic pan-coronavirus fusion inhibitor EK1, but not of the putative TMPRSS2 inhibitor famotidine. In addition, following a high-throughput screen of FDA-approved drugs for antiviral action, imatinib, mycophenolic acid, and quinacrine dihydrochloride were confirmed to inhibit SARS-CoV-2 infectivity in hPSC-derived colonic organoids [23]. In short, these results suggest that both primary gut stem cell–derived and iPSC-derived organoids provide suitable tools to study SARS-CoV-2 cellular tropism and infection biology and validate antiviral drug effects.

3D renal organoids

Acute kidney injury constitutes a common complication in severely ill COVID-19 patients, occurring in 0.5–7% of cases and in 2.9–23% of intensive care unit patients [45]. The SARS-CoV-2 entry factors ACE2 and TMPRSS2 are expressed in podocytes and proximal tubule cells, suggesting that these are the primary target cell types. Notably, upon *ex vivo* culture, ACE2 expression was twice as high in 3D proximal tubule organoids compared with conventional 2D culture, suggesting that 3D cultured cells might be more readily infectable in organotypic culture configurations [46].

Infectivity with SARS-CoV-2 was also modeled using hPSC-derived kidney organoids that featured clear tubular structures and podocyte-like cells (Figure 1i) with molecular phenotypes resembling second-trimester human fetal kidneys [47,48]. Importantly, viral entry in kidney organoids could be blocked dose dependently by human, but not mouse soluble recombinant ACE2 [48]. Notably, the effect of human soluble recombinant ACE2 was additive with the intracellular viral replication inhibitor remdesivir, suggesting that combinatorial therapies of agents with mechanistically distinct targets might provide promising strategies for COVID-19 [49].

Conclusions

Although conventional monolayer cultures of cell lines were the predominant *in vitro* models to study virus biology and antiviral drug action during previous coronavirus outbreaks of MERS and SARS-CoV-1, these cell systems were mostly superseded by organotypic 3D human culture systems of various pulmonary and extrapulmonary tissues during the COVID-19 pandemic, developments, which were made possible by the rapid advancements in primary tissue and organoid culture methods in the last decade. While the added value of these changes is difficult to accurately quantify, these physiological systems have demonstrated their translational value for the identification of potential targets and the development and testing of antiviral candidates in phenotypically appropriate contexts. On the basis of these experiences, further investments into human tissue engineering, as well as initiatives to improve the benchmarking, standardization, and accessibility to such models thus seem warranted to increase the preparedness for current and future public health emergencies.

Conflict of interest statement

V.M.L. is the CEO and shareholder of HepaPredict AB and co-founder and shareholder of PersoMedix AB. In addition, V.M.L. discloses consultancy work for Enginzyme AB. The other authors declare no conflicts of interest.

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** of outstanding interest

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