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Circulation Research

COMPENDIUM ON COVID-19 AND CARDIOVASCULAR DISEASE

Microfluidic Organ-Chips and Stem Cell Models in the Fight Against COVID-19

Sandro Satta, Sarah J. Rockwood, Kaidong Wang, Shaolei Wang, Maedeh Mozneb, Madelyn Arzt, Tzung K. Hsiai, Arun Sharma

ABSTRACT: SARS-CoV-2, the virus underlying COVID-19, has now been recognized to cause multiorgan disease with a systemic effect on the host. To effectively combat SARS-CoV-2 and the subsequent development of COVID-19, it is critical to detect, monitor, and model viral pathogenesis. In this review, we discuss recent advancements in microfluidics, organ-on-a-chip, and human stem cell–derived models to study SARS-CoV-2 infection in the physiological organ microenvironment, together with their limitations. Microfluidic-based detection methods have greatly enhanced the rapidity, accessibility, and sensitivity of viral detection from patient samples. Engineered organ-on-a-chip models that recapitulate in vivo physiology have been developed for many organ systems to study viral pathology. Human stem cell–derived models have been utilized not only to model viral tropism and pathogenesis in a physiologically relevant context but also to screen for effective therapeutic compounds. The combination of all these platforms, along with future advancements, may aid to identify potential targets and develop novel strategies to counteract COVID-19 pathogenesis.

Key Words: COVID-19 ■ induced pluripotent stem cell ■ myocyte, cardiac ■ SARS-CoV-2 ■ stem cell

The COVID-19 pandemic, caused by SARS-CoV-2, has led to unprecedented health consequences. SARS-CoV-2 infection induces multisystem dysfunction and vascular disease. Patients with SARS-CoV-2 infection can present with a broad spectrum of clinical manifestations, ranging from completely asymptomatic to multiorgan failure.[1] Furthermore, the rapid rise in SARS-CoV-2 variants poses a new challenge for predicting future viral mutations, highlighting the current gap in knowledge in understanding, diagnosing, and treating patients with COVID-19.

Patients with COVID-19 may present with various cardiovascular complications which include myocardial injury, arrhythmias, heart failure, acute coronary syndrome, and venous thromboembolism.[2] Although many comorbidities contribute to the pathophysiology of COVID-19, damage to the endothelium and altered hemostasis appear to be key drivers and a link between different clinical phenotypes. Endotheliopathy, a concerning feature in patients with COVID-19, has also been observed in other viral illnesses that induce endothelial dysfunction and cardiovascular disease.[3–6] There is also substantial evidence of the association between preexisting cardiovascular disease and increased morbidity and mortality in adults with SARS-CoV-2 infection.[7] High clinical variability may be explained by the expression of binding receptor ACE2 (angiotensin-converting enzyme 2) on the surface of cells in multiple tissues and organs, which SARS-CoV-2 uses as a cellular entry receptor. In response to infection, cells undergo apoptosis and cellular damage.[8] The S protein (spike-binding glycoprotein) also leads to destabilization of ACE2, causing dysregulation of the renin-angiotensin system, and subsequently, cellular dysfunction.[9] Furthermore, unlike SARS-CoV-1, the SARS-CoV-2 S protein exhibits an insertion of 4 amino acids at the S1/S2 junction,[10–12] forming a canonical PRRAR685↓ furin-like cleavage site, which promotes the separation of the S protein Subunit S_1 from the S_2.[13] In addition, analysis of cell-to-cell fusion and S protein processing revealed

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Nonstandard Abbreviations and Acronyms

ACE2	angiotensin-converting enzyme 2
BBB	blood-brain barrier
EC	endothelial cell
hPSC	human pluripotent stem cell
LFIA	Lateral Flow Immunoassay
multi-OoC	multiorgan-on-a-chip
NF-κB	nuclear factor kappa B
OoC	organ-on-a-chip
PCR	polymerase chain reaction
TMPRSS2	transmembrane protease, serine 2

that ACE2 shedding by the TMPRSS2 (transmembrane protease, serine 2) mediates S_2 cleavage and supports viral entry from the plasma membrane.[14] Although ACE2 is considered the main receptor in enabling SARS-CoV-2 viral entry, other entry mechanisms have been proposed. Studies have shown that the human tyrosine-protein kinase receptor UFO specifically interacts with the N-terminal domain of SARS-CoV-2 S, while its knockdown greatly reduced SARS-CoV-2 internalization in low ACE2-expressing cells.[15] Similarly, NRP-1 (neuropilin-1) has been revealed as a potential co-receptor for SARS-CoV-2 entry through the olfactory epithelium of the nasal cavity into central nervous system,[16] and has been observed to bind furin-cleaved substrates, potentiating SARS-CoV-2 infectivity.[17] Other studies showed comparable SARS-CoV-2 particle uptake from human and mouse macrophages, suggesting that additional viral internalization mechanisms may be involved that are not species-specific.[18,19] These studies indicate that other important host receptors or coreceptors might exist that bind to different domain(s) of SARS-CoV-2 and promote the entry of SARS-CoV-2 in different tissues. This could help explain the patient-specific clinical outcome in COVID-19 and the need for the further development of precision medicine against viral illnesses.

Although most people infected by SARS-CoV-2 have temporary mild or moderate symptoms, evidence suggests that some patients may experience persistent symptoms beyond 4 weeks after infection.[20] Long-term manifestations can be respiratory (eg, dyspnea), cardiovascular (eg, heart palpitations), gastrointestinal (eg, diarrhea), and neurological (eg, confusion).[21] Persistent symptoms due to COVID-19 are most commonly referred to as "long-COVID".[22] There is currently no unified agreement in the medical community on how to characterize or treat long-COVID. Therefore, understanding the pathogenesis and mechanisms of long-COVID is paramount. Literature suggests that immune dysregulation, viral persistence,[23] and residual inflammation[24] may contribute to long-COVID.

Treatment for COVID-19 focuses on supporting the patient's ability to fight infection, with multiple strategies available that include antiviral medications and monoclonal antibodies.[1] There are ongoing development efforts for various vaccines and therapeutics, and hundreds of trials are underway to examine the efficacy of different drugs with variable mechanisms of action, ranging from inhibition of viral entry into cells and inhibition of enzymes associated with viral replication, to improving the immune response to the virus.[25] Efforts have focused on developing new and more effective modes of diagnosis, prevention, and therapeutic intervention in the fight against COVID-19.[26] Animal models have shown that the underlying molecular mechanisms can differ greatly compared to humans with the same disease phenotype.[27] This inability to effectively mimic human disease is becoming more evident as the numbers of animal models increase, and it is likely a major reason why many drugs fail in safety and efficacy when they advance from animal studies to human clinical trials.[28]

Organ-on-a-chip (OoC) and human pluripotent stem cell (hPSC)–derived models offer the opportunity to create patient-specific platforms that mimic human biology to aid in development of effective diagnostics, therapeutics, and vaccines for COVID-19 (Figure 1). Advances have been made in microfluidics, stem cell models, and OoC to reconstruct simplified models of human organs to study disease. OoC approaches can recapitulate complex tissue and organ architecture and mimic pathophysiology and host response to viral infection. OoCs can contain engineered, stem cell–derived, or natural miniature tissues grown inside microfluidic chips. To better mimic human physiology, microfluidic chips are designed to control cell microenvironments and maintain tissue-specific functions. Combining advances in stem cell biology, tissue engineering, and microfabrication, OoC has gained interest as an experimental platform to investigate human pathophysiology for tailored disease diagnosis and treatment. Moreover, it has become a powerful tool for detecting SARS-CoV-2 variants and studying COVID-19. Further research will be crucial to elucidate mechanisms of infection, to improve diagnoses, and develop therapies. In this article, we review recent advances in the development of OoC disease models that have been made by leveraging recent advances in tissue engineering and stem cell biology, with a particular focus on their application to elucidate the pathophysiology and infection mechanisms during COVID-19.

COVID-19 DETECTION USING MICROFLUIDIC CHIPS

Advancements in microfluidic technologies have emerged as a tool for individually tailored disease diagnosis and treatment. These are being utilized to develop reliable diagnostic kits for detecting SARS-CoV-2 antibodies, antigens, and nucleic acids. Compared to traditional methods,

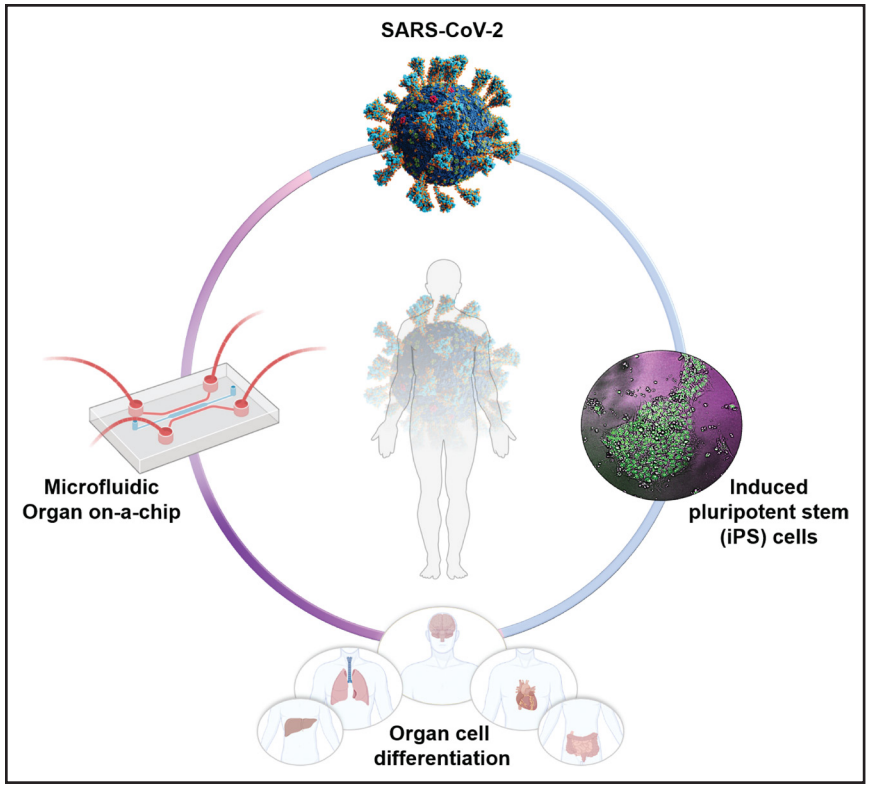

Figure 1. Organ-on-a-chip (OoC) and stem cells as novel methods to study viral infection.
Application of OoC affords an opportunity to uncover virus mutations and transmission in the context of COVID-19, run patient-specific analyses, and identify therapeutic targets. Derivatives of induced pluripotent stem (iPS) cells can mimic tissue- and organ-level structures and functions that are central to explore the host response to SARS-CoV-2 infection.

microfluidic devices analyze small volumes for testing. Furthermore, the use of multichannel detection techniques allows for the analysis of multiple samples simultaneously on a microfluidic device. Microfluidics may create individualized regimens that will enable control of patient-specific drug delivery profiles.[29] Viral detection generally occurs by relying on cell culture, antigen-antibody interactions, or nucleic acids, and they require trained personnel and expensive equipment. Viral particles can be detected through saliva, nasal fluid, and blood. However, detection of antibodies might take days or weeks, hampering the aim of prevention. The SARS-CoV-2 pandemic has highlighted the importance of rapid detection of respiratory viruses. Microfluidic technologies can rapidly and accurately detect respiratory viruses, providing a rapid, affordable, and sensitive analysis of viral particles or genetic material.

Optical Detection Techniques

Microfluidic devices combined with optical detection techniques can provide a powerful tool for high-throughput and multimodal analysis of respiratory viruses. Several detection techniques have been used for SARS-CoV-2 identification including absorbance, fluorescence, colorimetric approaches, and surface plasmon resonance.

Absorbance: A Fiberoptic Biosensor Device for Rapid Detection of COVID-19

This method has been used for direct or indirect molecular detection of SARS-CoV-2.[30] For example, a biosensor matrix has been developed by immobilizing gold nanoparticles on a U-bent fiberoptic probe conjugated to anti–SARS-CoV-2 N protein monoclonal antibodies via covalent bonds.[31] Saliva samples were used to detect SARS-CoV-2 nucleocapsid, and results were obtained in 15 minutes without sample preparation. Viral detection was obtained using loop-mediated isothermal amplification with integrated optical fibers into a chip[32] (Figure 2A).

Lateral Flow Immunoassay Technique

Lateral Flow Immunoassay (LFIA) technique has been widely used for COVID-19 antigen testing due to its low cost, speed, and accessibility compared to molecular tests.[33] Classical LFIA is composed of a sample applicator pad of glass fiber or cellulose, a conjugated pad in which a labeled biorecognition molecule is placed, and a nitrocellulose membrane where the test and control lines are drawn. During testing, the sample binds to the primary biomolecule only if it contains the analyte, which then releases the biomolecule in the conjugated form, binding to a secondary complex to show the positive result. An amplification-free fluorescence detection assay was developed using DNA probes and fluorescent nanoparticle-labeled monoclonal antibodies with 100% sensitivity and 99.5% specificity.[34] A similar approach was used in which 2 test lines for IgG and IgM achieved a multitarget immunoassay.[35] Another device with a dual (colorimetric/fluorescence) functional LFIA biosensor was developed for SARS-CoV-2 spike subunit 1 (S1) protein detection.[36]

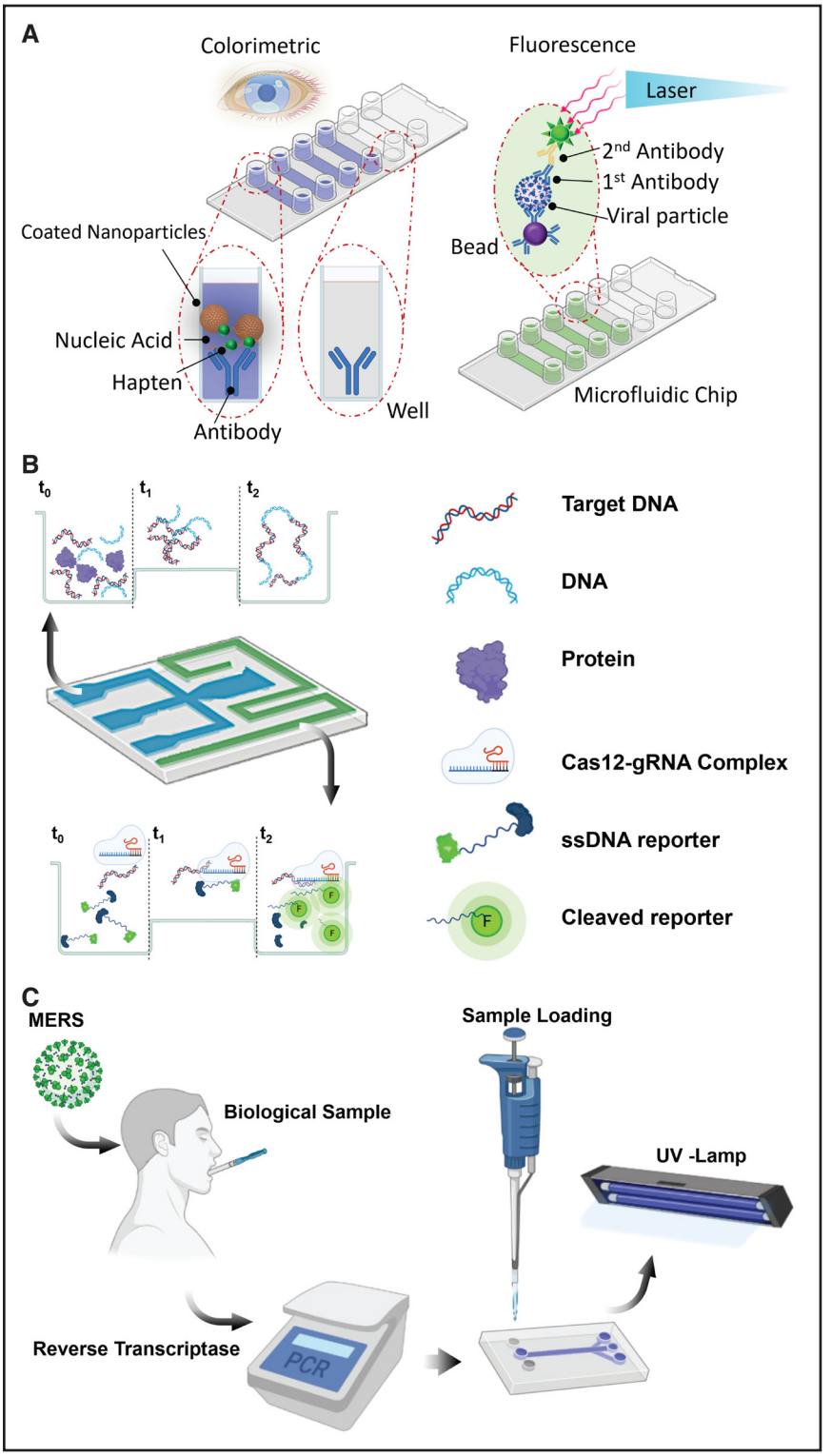

Figure 2. Optical detection techniques using microfluidic chips for COVID-19 and viral testing.
A, Multichannel polydimethylsiloxane-glass hybrid optical-based microfluidic chip using loop-mediated isothermal amplification (LAMP), for detection of respiratory viruses or their products. The sensitivity of the LAMP allows for (1) naked-eye visual analysis through colorimetric detection, and (2) micropore array used for fluorescent detection. **B**, Schematic representation of SARS-CoV-2 detection workflow. A 2-channel microfluidic chip (in green and blue) is used for nucleic acid extraction through isotachophoresis (mode 1—blue channel) and isotachophoresis (ITP)-CRISPR detection (mode 2—green channel). A positive sample shows a strong fluorescent signal compared to the control. **C**, DNA hydrogel formation by isothermal amplification of complementary targets in microfluidic channels (DhITACT-TR) system workflow. Following viral RNA extraction, detection is expressed via fluorescence analysis with SYBR green depending on the concentration of middle east respiratory syndrome (MERS) pathogen strands in a pseudo-serum sample. Rapid diagnosis of MERS viral RNA of DhITACT-TR strongly emitted bright fluorescence. The device allows for optical detection by fluorescence imaging and with the naked eye. gRNA indicates guide RNA; ssDNA, single-stranded DNA; and UV, ultraviolet.

Fluorescence: an Electric Field-Mediated Microfluidic Assay for SARS-CoV-2 RNA Detection Using Isotachophoresis and CRISPR-Cas12

Commonly used methods for detecting SARS-CoV-2 use a fluorescent signal from polymerase chain reaction (PCR) samples. A recent study developed a microfluidic isotachophoresis-CRISPR—based protocol for rapid SARS-CoV-2 detection from nasopharyngeal swab samples.[37] Isotachophoresis application technology separates charged analytes based on their ionic mobility by applying an electric field, achieving automated purification of the extracted nucleic acids. CRISPR-Cas12 enzyme and synthetic guide RNA are complexed, and this complex specifically binds to the viral N and E genes and human

RNase P genes. A similar method enabled the detection of 3 strains of influenza virus (H1N1, H3N2, H9N2) using nucleic acid hybridization with a controllable micromagnetic field.[38] (Figure 2B).

Multiplex Microfluidic Assays for SARS-CoV-2 Antibody Diagnosis

Fluorescence-based detection for antibody diagnosis for SARS-CoV-2 is crucial for retrospective serological-surveillance and the assessment of vaccine efficacy. Microfluidic systems rely on a different approach for SARS-CoV-2 antibody detection. A recent study developed a double-antigen bridging immunoassay, also known as microfluidic DA to D4, which can detect total antibodies including all subclasses and isotypes.[39] Studies have proposed a detection technology based on a sandwich/competitive immunosensor that can run 3 samples per device and can detect up to 24 antibodies and in high-throughput. Another study reported large-scale sample running capability, in which 1024 samples per device can be run in an induced trapping of molecular interactions based on microfluidic nano-immunoassay.[39–42]

Colorimetric

Colorimetric detection of viral particles offers the possibility of visualization with the naked eye and is a preferred method due to its rapid measurement features and low cost. Detection can be achieved in these systems through the aggregation of nanoparticles. Studies have developed a model of DNA hydrogel formation by isothermal amplification of complementary targets in microfluidic channels for in vitro diagnosis of middle east respiratory syndrome coronavirus.[43] This system allows for a quick visual analysis or fluorescence detection under a ultraviolet lamp (Figure 2C).

Machine Learning on-a-Chip for SARS-CoV-2 Detection

Machine learning, a subset of the artificial intelligence field, extracts representative features of datasets and aims to generate potential predictions. Specifically, deep learning using multilayered neural network architectures can be used to discover intricate structures in datasets by the employment of the backpropagation mechanism.[44] Deep learning models have the advantage of learning representative features in a dataset via multilayered neural network training without complicated reasoning. Machine learning could not only improve the performance of on-chip experiments but also extract valuable information from constructing multidimensional SARS-CoV-2 datasets.

Microfluidic devices have many advantages for SARS-CoV-2 detection that could reduce the sample amounts needed and shorten diagnosis time. PCR, regarded as the gold standard for SARS-CoV-2 detection, is time-consuming.[44] One novel microfluidic, paper-based device was designed to detect synthetic RNA of the SARS-CoV-2 genome using deep learning algorithms. In addition, nanopore chips based on machine learning for SARS-CoV-2 detection have been explored,[45] where machine learning algorithms were employed to distinguish nanoparticles and viruses. Detection of a biomarker at low concentrations has emerged as extremely useful in studying human disease. A microfluidic digital immunoassay based on deep learning was reported to detect cytokine storm in patients with COVID-19.[46] A rapid and accurate image processing method for digital signal analysis based on machine learning was developed using a particle agglutination test for SARS-CoV-2 detection.[47] Multidimensional datasets typically generate more accurate results compared to a single indicator and, properly understanding these multidimensional datasets becomes more critical to aiding clinical diagnoses.

There are 2 major classes in machine learning algorithms, supervised learning, and unsupervised learning, distinguished by the existence of labels in the training processes. Supervised machine learning involves predetermined output labels and could enable automatic design of microfluidic flow-focusing droplet generation for COVID-19 detection.[48] By contrast, unsupervised learning involves clustering and pattern recognition without the predetermined output labels.[49] Unsupervised machine learning models of multidimensional datasets have achieved significant headway at disease variant prediction via avoiding learning from labels.[50] It can also classify hospitalized patients with COVID-19 into clinical severity progression groups. Unsupervised learning was adopted to generate COVID-19 patient clusters of 3 distinct immune phenotype groups using serum pro-inflammatory, anti-inflammatory, and antiviral cytokine and anti–SARS-CoV-2 antibody measurements from microfluidic chips of COVID-19 patient samples as input data[51] (Figure 3).

Machine learning can reduce the dimensionality of raw data to an interpretable output via deep neural networks to assist clinical decision-making. The developed machine learning algorithms can extract the valuable information for clinical diagnosis via machine learning algorithms handling the biomarkers of different types and in dynamic changes of a biomarker over time, leading to more accurate performances compared to single biomarkers. The integration of the data from microfluidic arrays and the social determinants of health, such as age, gender, race, and comorbidity including diabetes, hypertension, obesity, hyperlipidemia, and heart disease to construct tensors as input holds the potential to predict which patients with COVID-19 may[52] develop microthrombosis, myocarditis, stoke, or long-COVID. The deep learning training process would allocate the weights of the data from microfluidic arrays and the social determinants of health for disease predictions of patients with COVID-19. From this perspective,

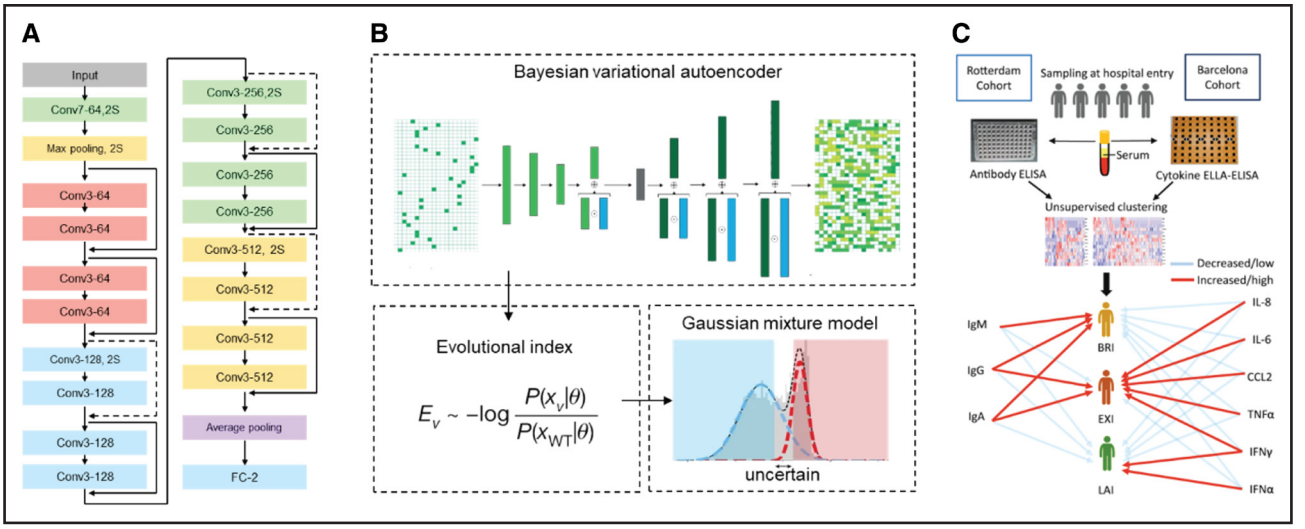

Figure 3. Unsupervised learning pipeline to generate COVID-19 patient clusters.
A, Illustration of the Architecture of ResNet-18 deep learning model. **B**, Representation of algorithms for disease variant prediction with deep generative models of evolutionary data (**C**) Definition of 3 immunotypes at hospital entry that predict clinical outcome via unsupervised clustering. IFN indicates interferon; IL, interleukin; and TNF, tumor necrosis factor.

COVID-19–related data from OoC platforms remains quite valuable, and additional research in this area should be prioritized.[53]

BIOSENSORS FOR LONG-COVID MONITORING ON-A-CHIP

Early diagnosis and monitoring of the several diseases comprising long-COVID would require intensive blood testing together with other biological samples. In addition, factors such as high specificity, sensitivity, and fast processing, would make multiorgan testing an extreme burden for the healthcare system. Biosensors are now being considered for replacing classical screening methods, allowing for a rapid processing of samples with predefined targets of interest for diagnosing disease. The versatility of these platforms makes them well-suited for both standard clinical settings and point-of-care applications for early disease diagnosis and real-time monitoring of disease pathology and therapeutic efficacy. Advancements in nanomedicine and nanotechnology have also increased biosensor sensitivity and reduced analysis time[54] for viral nucleic acids and antibody tests. For this reason and due to their ease of fabrication, functionality, biodegradability, and low-cost, paper-based biosensors have gained attention. The LFIA technique has been widely used in SARS-CoV-2 antigen tests. Specificity for multiple targets can be achieved via the immobilization of biomolecules on the paper substrate through adsorption or covalent binding. Following this principle, a recent study designed a paper-based electrochemical biosensor for diagnosing COVID-19 (Figure 4) and was able to detect SARS-CoV-2 antibodies and antigens.[55] This technology is crucial for outlining the molecular determinants of long-COVID syndrome and for COVID-19 diagnostics in general.

COVID-19: ORGAN DISEASE ON-A-CHIP

Despite their importance, animal models are not always accurate predictors of therapeutic responses in humans, a major limitation of these in vivo models.[28,56] OoC can recapitulate human organ physiology and pathophysiology with high fidelity.[57] To achieve this, OoC technology takes advantage of multiparametric features designed for microfluidic systems.[58–60] Due to the COVID-19 pandemic, OoC models have been developed to study SARS-CoV-2 viral infection and systemic pathophysiology in COVID-19.[61,62] Here, we review how human organ chip systems have been used to model the complex mechanisms of COVID-19.

Lung on-a-Chip

The route of transmission for SARS-CoV-2 is primarily via exposure to respiratory fluids carrying infectious virus.[63] For this reason, the lungs have been recognized as a major target organ for SARS-CoV-2. In the COVID-19 era, one of the most clinically relevant uses of human lung microfluidic chips is in the study of respiratory virus infections (Figure 5A). A biomimetic microsystem lung on-a-chip has been previously developed by Huh et al,[64] to mimic the host response to bacteria and inflammatory cytokines in the alveolar space. To study lung injury in response to SARS-CoV-2, Zhang et al,[65] developed a human alveolar chip in which alveolar epithelial cell lines interfaced with lung ECs were seeded in 2 chambers separated by an ECM (extracellular matrix)-coated

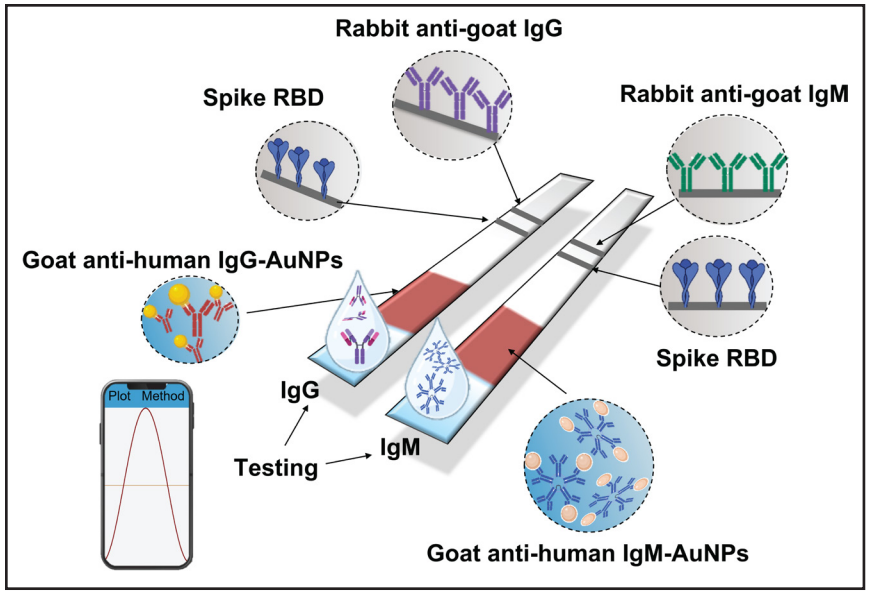

Figure 4. Illustration of the Lateral Flow Immunoassay test strips, colorimetric tests for SARS-CoV-2 IgG and IgM detection.
Multiple target detection such as targeted antibodies or viral proteins can be achieved via immobilization of biomolecules by adsorption or covalent binding onto the paper substrate. Square-wave voltammetry technique is used to monitor the electrochemical signal. AuNPs indicates gold nanoparticles; and RBD, receptor binding domain.

porous membrane, allowing for a coculture of human alveolus epithelial cells, pulmonary microvascular endothelial cells, and immune cells exposed to media flow. Treatment with the antiviral remdesivir inhibited viral replication and cytokine release, along with endothelial detachment. This model was further used by Si et al,[62] to assess the antiviral efficacy of the antimalarial drugs chloroquine or hydroxychloroquine.[66] Microfluidic chips infected with pseudotyped SARS-CoV-2 virus and exposed to clinically relevant doses of chloroquine or hydroxychloroquine were not found to be active in inhibiting SARS-CoV-2 infection and were unlikely to provide clinical benefit against COVID-19, thus predicting the negative results seen in clinical studies.[67] Conversely, amodiaquine, used as a therapy for malaria, successfully inhibited SARS-CoV-2 pseudovirus entry in epithelial cells in the lung on-a-chip and was further confirmed in a hamster model.[62] It was shown that administration of the anticoagulant drug nafamostat together with the antiviral drug oseltamivir significantly reduced the titers of influenza H1N1 in an OoC model.[62] These findings highlight the importance of developing versatile platforms to study viral entry and evaluating drugs for repurposing for future clinical trials.

Gut-on-a-Chip

The human gastrointestinal tract is characterized by a complex and dynamic population of microorganisms. Diverse bacteria populations can be found in the gastrointestinal tract, known as the commensal microbiome in the human intestine, and play a crucial role during homeostasis and disease. A unique advantage of microfluidic chips over organoid and static culture systems is their capability to establish oxygen gradients across the tissue-tissue interface to allow aerobic and anaerobic bacteria to be cultured on the epithelial cell lining in microfluidic channels, which can be sustained for several days.[68] In the context of COVID-19, clinical evidence has shown that the intestine can be infected by SARS-CoV-2.[69] For this reason, biomimetic human gut-on-chip has been used to recapitulate the pathophysiology induced by SARS-CoV-2 at an organ level (Figure 5B). The microengineered gut-on-chip model developed by Guo et al,[70] consisted of 2 chambers, an upper and lower layer. A coculture of intestinal epithelial cells together with mucin-secreting cells was seeded in the upper channel to create the intestinal barrier and was exposed to shear stress to simulate the intraluminal fluid flow. Human endothelial cells (ECs) and immune cells populated the lower chamber under physiological shear stress conditions and were exposed to vascular endothelium under fluid flow. Inoculation of SARS-CoV-2 into the intestinal channel showed infection in most cells within 5 days. At 3 days postinfection, endothelial cells had severe morphological damage and E-cadherin was significantly destroyed. The biomimetic human gut-on-chip provides a scalable, low-cost platform for the study of SARS-CoV-2 viral infection, transmission, and host-virus interactions, recapitulating the disease pathogenesis.

Brain-on-a-Chip

Strong evidence has shown brain-related pathologies in COVID-19 as a consequence of SARS-CoV-2 neurotropism[71] and virus-associated inflammation.[72] Stroke has been reported following SARS-CoV-2 infection.[73] Many studies in humans have presented the incidence and prevalence of acute ischemic stroke, cerebral venous or sinus thrombosis, and intracranial hemorrhage, in patients with COVID-19.[74] However, the mechanisms underlying this cerebrovascular risk in response to SARS-CoV-2 are unknown. In the effort to understand the mechanism leading to the development

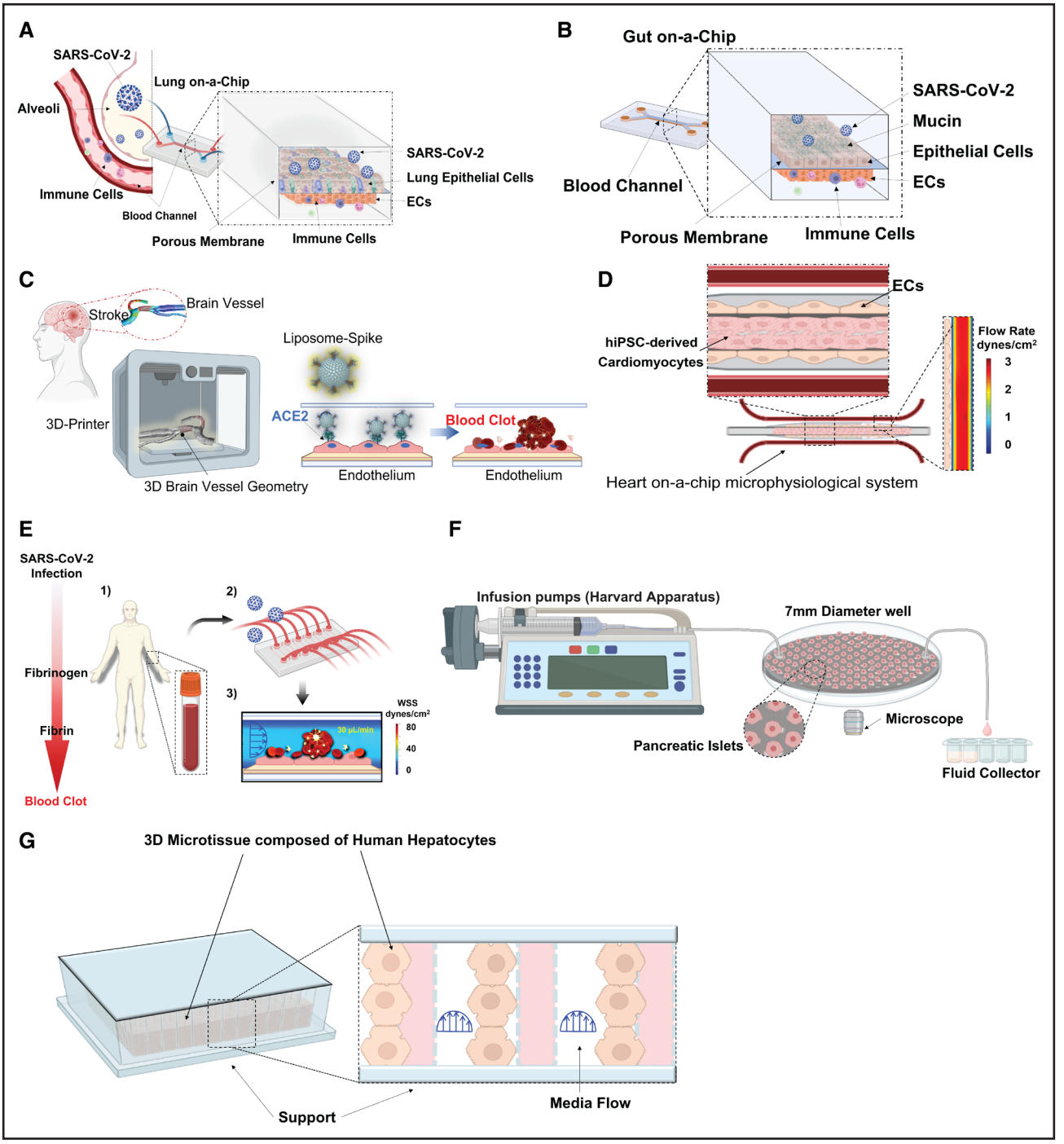

Figure 5. Examples of organ-on-chip approaches used in COVID-19 basic research.
A, Lung on-a-chip. Illustration of 3-dimensional (3D) human alveolus on-a-chip infected by SARS-CoV-2. The 2 channels were separated by a porous polydimethylsiloxane membrane. Upper alveolar epithelial channel interface was formed by coculture of alveolar epithelial cells and the bottom channel was seeded with pulmonary microvascular endothelial exposed flow conditions. The apical layer was exposed to SARS-CoV-2 to achieve epithelial layer infection. To recapitulate physiological immune response, human immune cells were infused into the bottom vascular channel during the progression of virus infection. **B**, Gut-on-a-chip device was achieved by developing an upper and lower channel separated by a porous ECM (extracellular matrix)-coated porous PDMS membrane. Intestinal epithelial barrier was achieved by coculture of Caco-2 cells and intestinal mucin-secreting HT-29 cells in the upper chamber. Human umbilical vein endothelial cells (ECs) were seeded in the lower chamber together with immune cells, under physiological flow conditions. **C**, Brain on-a-chip. Middle cerebral artery model was generated by 3D-printed technology. Monolayer of human brain microvascular ECs were seeded in the degassed polydimethylsiloxane mold after coating with fibronectin. Measurement of ACE2 (angiotensin-converting enzyme 2) together with internalization of liposomes coated with SARS-CoV-2 spike protein fragment S1 was achieved by immunocytochemistry in proximal, stenotic, and distal segments of the endothelialized middle cerebral artery 3D model with computational fluid dynamic modeling of wall shear stress (WSS). **D**, Heart on-a-chip. Representation of microphysiological system. Nutrients were loaded in the red channels through inlet and outlet ports connected to tubes. Endothelial cells were seeded in the (*Continued*)

of a multiplicity of cerebrovascular pathologies associated with severe SARS-CoV-2 infection including ischemic stroke, Kaneko et al,[75] utilized a 3-dimensional (3D)–printed endothelialized model of human-specific intracranial artery stenosis combined with fluorescent nanoliposomes, approximately the size of viral particles, engineered with SARS-CoV-2 spike proteins on their surface. This work suggested that viral SARS-CoV-2 spike proteins that bind to brain microvascular cells are dependent not only on molecular interaction with ACE2 but also require a flow-mediated stimulus.

However, whether SARS-CoV-2 can cross the highly selective blood-brain barrier (BBB) is unknown. Autopsies from patients with COVID-19 have shown cerebral injuries,[76] and animal studies have reported detection of SARS-CoV-2 in the brain after intranasal administration of the virus, suggesting SARS-CoV-2 neuroinvasion.[77] Wang et al[78] developed a multiorgan microfluidic chip to explore the effect of SARS-CoV-2 infection on the human BBB. During this study, a lung on-a-chip and brain-on-a-chip were examined. No infection and apparent changes were detected in the BBB microfluidic chip, suggesting that direct SARS-CoV-2 exposure had no effect on the human BBB. However, when culture medium from the alveolar on-a-chip was used as conditional medium on the BBB on-a-chip, significant increase in BBB permeability was observed, due to the disruption of endothelial tight and adherent junctions (altering ZO-1 and vascular endothelial–cadherin organization), suggesting that SARS-CoV-2 caused BBB injury indirectly. These brain OoC studies can provide useful information about COVID-19 pathology in the nervous system (Figure 5C).

Human Induced PSC–Based Engineered Heart-on-Chip as a Model for COVID-19 Study

Cardiac damage due to SARS-CoV-2 infection was recognized at the early stages of the COVID-19 pandemic. In Wuhan, China, between January 23 and February 23, 2020, 35% of patients with COVID-19 had developed cardiomyopathy, coronary disease, and hypertension, and 28% presented with myocardial injury and elevated levels of troponin T.[79] Further reports showed that 5% to 16% of the patient population developed hypertension, and 15% to 31% developed one form of cardiovascular disease, including coronary artery disease in 11% of patients.[2] Myocardial damage and cardiac and endothelial dysfunction in patients with COVID-19 has been found to occur primarily via the ACE 2 receptor.[80,81] For this reason, there was an urgent need to develop rapid in vitro cardiovascular screening tools for preclinical evaluation of therapeutics for patients with COVID-19. To address this need, Charrez et al,[82] established a screening tool predictive for clinical cardiology. They developed a heart-on-a-chip microphysiological system for drug screening to screen for cardiac symptoms such as arrhythmias, QT prolongation, tachycardia, and Torsades de Pointes potentially leading to cardiac arrest (Figure 5D). Cells exposed to hydroxychloroquine showed a QT increase compatible with arrhythmic events, consistent with previous studies.[83,84] No changes in QT interval were observed upon acute exposure to azithromycin, but a significant increase in arrhythmic events was recorded.[85] Furthermore, a combination of both drugs showed expression of biomarkers directly correlated with cardiotoxicity and cellular damage, together with a synergetic effect in increasing QT intervals compared to the drugs alone, also in agreement with previous studies.[86]

Despite the advancement in recapitulating the physiological microenvironment of the human heart, cardiac manifestations in COVID-19 remain a challenge due to the difficulty in obtaining cardiac tissue from critically ill patients with suspected COVID-19 myocarditis. To address this unique challenge, Bailey et al,[87] developed a human-engineered heart tissue model system for studying COVID-19 cardiac pathology. Among the unique advantages provided, engineered heart tissues can mimic myocardial tissue via self-assembled cellular processes from hPSC-derived cardiomyocytes, generate contractile force, and display electrical coupling.[88] In this study, engineered heart tissues containing hPSC-derived cardiomyocytes and fibroblasts, with or without macrophages, were used to elucidate the mechanism by which SARS-CoV-2 enters cardiomyocytes. This study showed SARS-CoV-2 infection can contribute to cardiomyocyte cell death and myocardial injury and is reflective of the utility of OoCs in understanding COVID-19-associated cardiac symptoms.

Figure 5 Continued. inner chamber for mimicking an endothelial-like barriers connected to the nutrient channel. Human induced pluripotent stem cells (hiPSC)-cardiomyocytes were seeded back-to-back to the endothelial cells allowing cell-cell communication. **E**, Artery-on-a-chip. Schematic of SARS-CoV-2 infection-mediated thrombus formation in the microcirculation. Inflammatory cells are recruited to the site of infection in response to viral infection, activating the extrinsic and intrinsic coagulation pathways, and promoting thrombin production. Increased thrombin levels lead to cleavage of fibrinogen to fibrin, followed by platelet aggregation and fibrin deposition, and ultimately blood clot formation in the microcirculation. **F**, Pancreas-on-a-chip. Representation of the schematic design of the microfluidic device. A single chamber of polydimethylsiloxane, in a 3-layer microfluidic device was developed. For islet immobilization and to allow flow exposure, a 150 µm deep and 500 µm diameter large bottom layer was created in a small circular array. The second layer, 3 mm deep and 7 mm diameter, was created for encompassing the array of wells. Lastly, the topmost layer, a rectangular microchannel of 500 µm deep and 2 mm wide, provides access to these wells. **G**, Liver-on-a-chip. The device was made by creating 12 independent bioreactors for the 3D culture of hepatocytes, to allow the hepatocytes to form 3D microtissue structures in an array of channels coated with collagen I. Media is allowed to flow through every scaffold due to the action of a pneumatically operated pumps in the base of the plate. Direction and flow velocity can be adjusted by an electronic controller.

Artery-on-a-Chip: Modeling of Endotheliopathy and Thrombosis in COVID-19

The endothelium is the largest organ system in the body and is of critical importance for vascular homeostasis and proper cardiovascular function.[89] SARS-CoV-2 has been shown to affect endothelial cells and to promote endothelial disruption, which has been linked to inflammation, thrombosis, altered vascular permeability, loss in contractility, alterations in cellular proliferation, and hypercoagulation in COVID-19.[90] Venous thrombotic events in patients with COVID-19 have also been described[91]; however, the data on arterial thrombosis in patients is limited. Approximately 4% of critically ill patients infected by SARS-CoV-2 developed arterial thrombosis in multiple arteries.[90] Although several sophisticated animal models of thrombosis exist,[92] integrated models for studying platelet-endothelial interactions versus tissue-tissue (eg, epithelial, endothelial) interactions are still underdeveloped and significantly nonpredictive. Recent studies have adopted microfluidic flow chambers to include endothelial lumen in which whole blood can be perfused to visualize platelet-endothelial interactions,[93–95] and the flow rates are controlled using constant-flow pumps to transport blood. While these systems still do not fully recapitulate the blood vessel anatomy, they have been demonstrated to be a useful tool for studying thrombotic events and the effects of shear and recirculating flow[96] on platelet function and coagulation.[97] In the context of COVID-19 Satta et al,[53] used a similar microfluidic platform in which the blood coagulation pattern was recapitulated (Figure 5E). Patient-specific whole blood was used on endothelial cells exposed to SARS-CoV-2 or spike proteins. There remains significant interest and potential in further developing OoC platforms for studying vascular dysfunction in COVID-19.

Pancreas-on-a-Chip: Modeling COVID-19 in the Context of Diabetes

People with diabetes are more likely to have serious complications from COVID-19.[98] Furthermore, it has been suggested that ACE 2–expressing β cells in the pancreas that have undergone cell death post SARS-CoV-2 exposure, result in acute diabetes.[99] Despite the clinical relevance and increasing knowledge that suggests that COVID-19 infection and diabetes exacerbate each other with a feed-forward loop by which COVID-19 infection increases glucose production and insulin resistance, the pathophysiology of COVID-19 in diabetes patients remains unclear. Pancreas-on-a-chip, in the context of SARS-CoV-2 infection, could provide a reliable platform for diabetes research and drug screening. However, the impact of SARS-CoV-2 using an OoC model of the pancreas has not yet been well-assessed (Figure 5F). Conversely from other OoC models that can recapitulate long-term tissue functionality, the progress of creating pancreas-on-a-chip systems is still in its early stage, and most of the developed models focus on short-term culture and functionality screening of pancreatic islets.[100] Mohammed et al[101] developed one of the first microfluidic chip models, using both murine and human islets, where functionality was assessed via fluorescence imaging of intracellular calcium, together with mitochondrial membrane potential and insulin secretion. In this study, a single microfluidic device provided a comprehensive analysis of the functionality of the pancreatic islets which could be used to perform studies in the context of viral infection. Much work remains to be done in this area.

Liver-on-a-Chip

Despite studies being limited to small cohorts, high mortality rates from COVID-19 in patients with chronic liver disease have been previously reported.[102] Thus, it is essential to develop accurate and reproducible models that can replicate liver architecture, regenerative ability, and response to injury (Figure 5G). The development of a chip-based liver is at a very early research stage, and none of the current systems fully match the architectural complexity of a human liver lobule. A liver-on-a-chip system has been recently used by Moravcova et al,[103] to study steatosis in rat hepatocytes via administration of free fatty acids, oleic acid, and palmitic acid, leading to accumulation of intracellular triacylglycerols. In this study, it was observed that PA led to a dose-dependent cytotoxic effects correlated with an increase in reactive oxygen species and hypoalbuminemia. Similarly, in patients with COVID-19, an increase in reactive oxygen species has been reported together with decreased levels of serum albumin in patients with both severe and nonsevere COVID-19.[104] Many commercially available liver-on-a-chip models range from customized 3D liver disease models for in-depth in vitro modeling for drug development such as BioIVT[105] ideal for high-throughput drug screening, to bioprinted human liver tissue models (Organovo),[106] which remain functional and stable for >28 days and are used for predictive toxicology studies, transcriptomic and proteomic analysis, and histological tissue assessment. Despite the great advance in technology and the valuable tools to study the progression of liver disease, this facet of bioengineering is still far from developing a representative model to study liver pathologies, especially in the context of viral infection, which will require real-time monitoring instead of end-point analyses to provide a better understanding of the dynamic changes in the host.

MULTI-OOC

Multi-OoC devices have great potential to advance how biomedical research is conducted. By supporting cross-organ communication, multi-OoC platforms allow the study of multiorgan processes, recapitulating systemic diseases

such as COVID-19 (Figure 6).[107] Reproduction of the organ architecture and function remain one of the main challenges for multi-OoC devices. The 3D bioengineered and bioprinted constructs and recellularized scaffolds have been deployed in combination with microfabricated features for achieving active stimulation through electrical, mechanical, and biochemical properties, aiming to recapitulate human organs.[108,109] The multi-OoC field has been blossoming with biomimetic organs and physiological barriers in the human body,[107] holding great value in reducing animal testing. Nevertheless, several limitations, such as the use of one or few cell types, the lack of cross-organ and cross-chip communication, and the nonphysiological size of multi-OoCs, still need to be overcome.

COVID-19 and post-COVID syndrome or long-COVID have been classified as a multiorgan disease.[110] Thus, multi-OoC approaches can provide complex disease models while revealing key molecular mechanisms.[110,111] Furthermore, the ability to use patient-specific cells and link each mature engineered tissue together enables this model to recapitulate in vitro specific genetic backgrounds at the cellular, tissue, and systemic levels. In the context of COVID-19 and long-COVID, it is difficult to understand systemic damage in the short and long term. Although some of the risk factors for long-COVID have been identified, such as advanced age, elevated body mass index, and comorbidities,[112] the frequency and trigger of symptoms such as anosmia, loss of taste, and dyspnea remain unknown. With recent advances in tissue engineering, biomaterials, and microfluidics, biomimetic organ systems are becoming a fundamental tool for achieving a more realistic goal in understanding complex diseases, drug screening applications, and rapid diagnosis.

MODELING CARDIOVASCULAR SARS-COV-2 INFECTION USING PSCS

Models created from hPSCs provide an effective method for studying viral infection mechanisms in a cell type–specific approach. The COVID-19 pandemic has led to the emergence of various cardiac symptoms in infected patients, prompting an important question that can be answered via human platforms: can SARS-CoV-2 infect human cardiac cells directly? Many groups have attempted to answer this inquiry by generating different types of cardiovascular cell types, such as cardiomyocytes, cardiac fibroblasts, endothelial cells, macrophages, sinoatrial nodal cells, and other vascular cells, from PSCs (Figure 7). Results demonstrate that PSC-cardiomyocytes are highly susceptible to SARS-CoV-2 infection, with quantification of spike RNA or nucleocapsid through qPCR (quantitative polymerase chain reaction) or detection of viral double-stranded RNA via staining.[87,113–117] SARS-CoV-2-infected PSC-cardiomyocyte cultures exhibit cytotoxic effects, including sarcomeric fragmentation, abnormal beating or contraction, and apoptosis.[114,118] Evidence also suggests that sinoatrial nodal cells and organoids can be infected, resulting in ferroptosis and disrupted calcium handling.[119] Nonetheless, macrophages, cardiac fibroblasts, and ECs, despite their vital roles in cardiac viral pathogenesis do not show major indications of direct SARS-CoV-2 infection. More research is necessary in determining how various cell

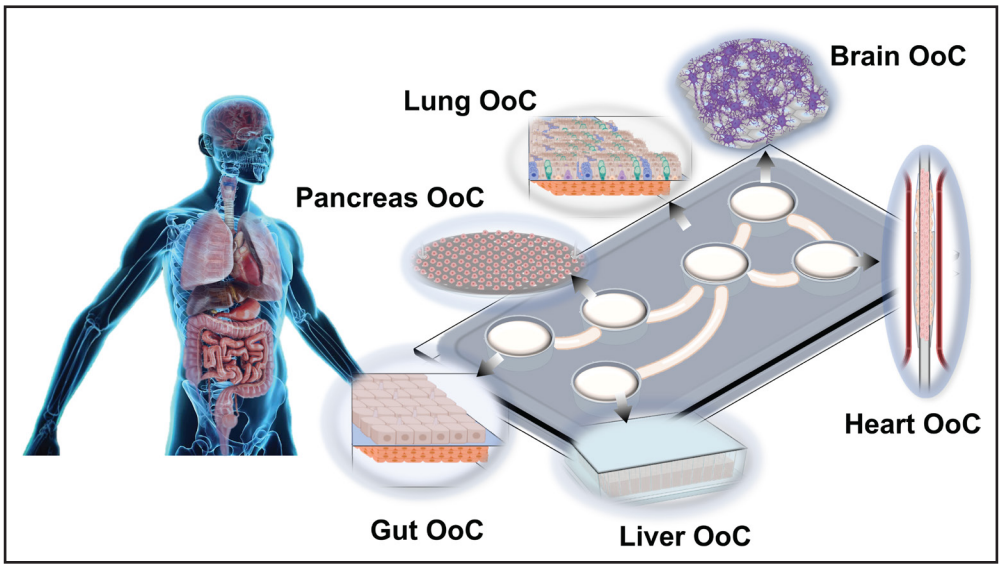

Figure 6. Schematic view of the multiorgan on-a-chip (OoC) approach for COVID-19 studies.
A multibiomimetic-tissue platform allows for integration of a vascular barrier beneath each tissue to create tissue-specific niches in the above chambers, where each engineered organ is connected to enable crosstalk within the system via a vascular network. The presence of a vascular barrier allows for maintenance of each specific media. Multiorgan viral infection studies and drug screening can be achieved, by recapitulating the human physiology in the multiorgan-on-a-chip.

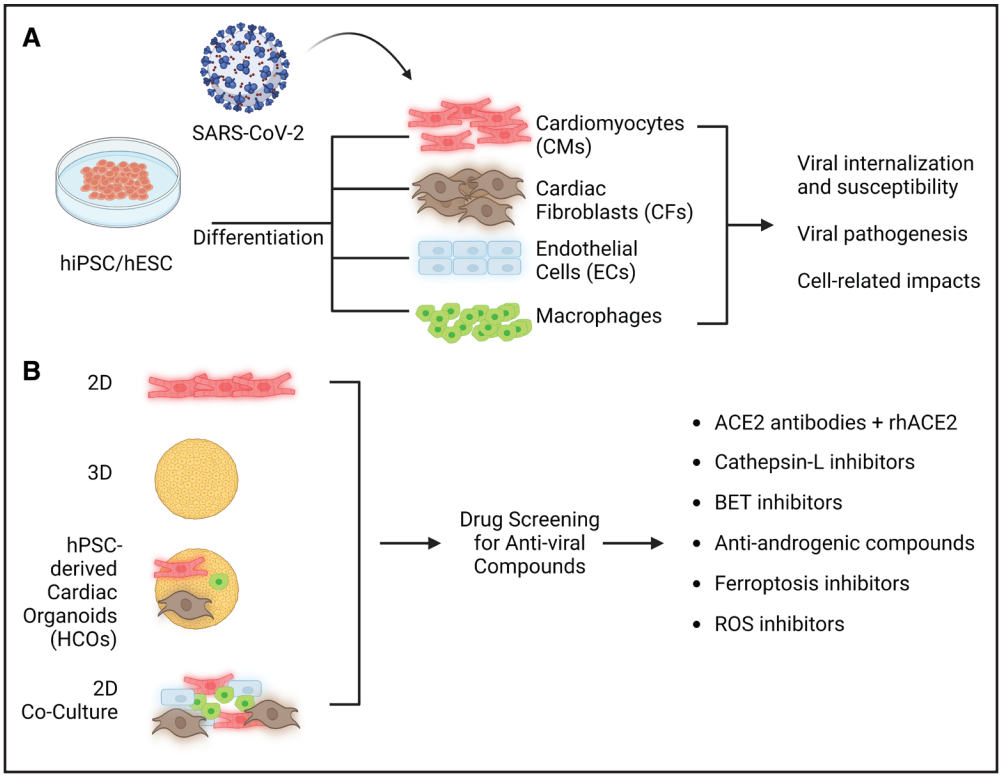

Figure 7. The utilization of cardiac tissue models derived from pluripotent stem cells (PSCs) enables the simulation of SARS-CoV-2 infection effects on the heart, investigation of virus-induced cytotoxicity, and exploration of potential treatments.
A, Cardiac cells produced from PSCs are utilized for establishing SARS-CoV-2 tropism and cellular impact postexposure. **B**, By cultivating stem cell–derived cardiac cells in distinct configurations that better mimic human cardiac functions, more efficient screening of therapeutic solutions becomes viable. 2D indicates 2-dimensional; 3D, 3-dimensional; ACE, angiotensin-converting enzyme 2; BET, Bromodomain and Extra-Terminal motif; CF, cardiac fibroblast; CM, cardiomyocyte; EC, endothelial cells; HCO, hPSC-derived cardiac organoids; hESC, human embryonic stem cells; hiPSC, human induced pluripotent stem cells; hPSC, human PSC; rhACE, recombinant human angiotensin converting enzyme; and ROS, reactive oxygen species.

types respond differently to SARS-CoV-2 infection and influence clinical cardiac symptoms.

The vulnerability of PSC–sinoatrial nodal cells and cardiomyocytes to SARS-CoV-2 presents a useful system for studying the cardiovascular complications of COVID-19. Researchers have consistently noted pathological changes occurring in PSC-cardiomyocytes with 24 hours of SARS-CoV-2 infection, such as disrupted sarcomeres, decreased synchronization in contraction, and a change toward cellular glycolysis.[114,117] These observations indicate the reproducibility and usefulness of these models. Up to 20% of infected PSC-cardiomyocytes showed sarcomere fragmentation following SARS-CoV-2 infection.[114] Additionally, one study demonstrated that papain-like protease can cut bovine myosin heavy chain under laboratory conditions, further enhancing possibilities for investigating viral mechanisms in human systems.[120] Nonetheless, additional research is necessary to confirm whether these same mechanisms manifest in patients.

Infection of PSC-cardiomyocytes led to a cellular increase in inflammatory signaling.[87,121] Genes responsible for inflammation were found to be elevated postinfection, although the interferon response, known for its antiviral properties, was only marginally stimulated.[122] Despite this, pathways involved in antiviral defense, such as protein kinase R and oligoadenylate synthase ribonuclease L were activated in PSC-cardiomyocytes. CCL2, a chemokine that attracts monocytes to fight infection, was produced by infected PSC-cardiomyocytes, while PSC-macrophages released TNF (tumor necrosis factor)-α and IL (interleukin)-6 after SARS-CoV-2 exposure.[121] These molecules are associated with severe COVID-19. SARS-CoV-2 targets and infects sinoatrial nodal cells, which act as cardiac pacemakers, potentially causing arrhythmias. PSC–sinoatrial nodal cells were susceptible to infection with SARS-CoV-2, leading to increased production of chemokines such as CCL2, upregulating ferroptosis, inflammatory signaling, and reactive oxygen species. Interestingly, other cell types did not show this effect, underscoring the necessity of tissue-specific modeling of viral infections.

Investigating Therapeutic Development With Human Induced PSC–Derived Cardiovascular Models

The physiological significance of PSC-cardiomyocytes makes them an excellent tool for assessing potential treatments for cardioprotection and identifying novel antiviral

agents. Research has confirmed that ACE2 is a crucial viral internalization molecule for SARS-CoV-2. Despite its important role in cardiovascular biology, blocking ACE2 using recombinant protein or neutralizing antibody effectively reduced infection rates.[113,114,116,117] Hindering other possible entry receptors TMRPSS2 and furin, however, did not decrease infection in PSC-cardiomyocytes.[87,123] Inhibiting the function of cathepsins, which participate in endolysosomal processes, showed a significant reduction in SARS-CoV-2 internalization.[116,123] Specifically, inhibiting cathepsin L via Z-Phe-Tyr(tBu)-diazomethlyketone prevented infection potentially via regulating a CSTL-dependent endosomal pathway. These findings further underline the utility of assessing antiviral drug efficacy in tissue and cell-specific human systems. To identify compounds that could decrease the expression of ACE2—a crucial SARS-CoV-2 entry point—Food and Drug Administration–approved library screening has been employed. The search yielded hits related to peptidase activity, AR (androgen receptor) signaling, and steroid metabolism.[124] Both dutasteride and finasteride can limit AR signaling by inhibiting 5-alpha reductases, resulting in reduced levels of both TMPRSS2 and ACE2. Surprisingly, such antiandrogenic compounds were able to reduce viral infection rates, despite males and those with prostate cancer being at increased risk for COVID-19. However, the team found no modulation effects on ACE2 in Vero E6 cells in vitro, reinforcing the need for physiologically relevant human systems in future investigations of viral infection.

Through high-throughput screening, various drugs have been repurposed or discovered to effectively combat SARS-CoV-2 infection in vitro as well as in vivo. Postentry viral replication is hindered by the common antiviral small molecule remdesivir, while BET inhibitors like JQ-1 and INCB054329 are proven effective in preventing the onset of SARS-CoV-2-induced cardiac symptoms resulting from cytokine storms.[116,118,123,125] to analyze the infiltration of immune cells in the cardiac tissues of patients afflicted with COVID-19, coculturing PSC-cardiomyocytes alongside PSC-derived monocytes and macrophages has been conducted.[121,126] Berzosertib, an ATR kinase inhibitor, inhibits SARS-CoV-2 postentry, restoring impaired functionality in cardiomyocytes, while proving even more effective when utilized synergistically with remdesivir.[125] Viral-induced ferroptosis, a major cell type–specific toxicity experienced by PSC–sinoatrial nodal cells, is combatted successfully through deferoxamine and the tyrosine kinase inhibitor imatinib.[119] The aforementioned results highlight the significant differences in the way SARS-CoV-2 attacks varying types of cardiac cells, thereby emphasizing the necessity for screening viral-induced toxicity in a cell type–specific fashion for the development of antivirals. Successfully merging multiple antiviral options could offer improved approaches aimed at targeting the entire spectrum of cardiac pathophysiology in COVID-19 more efficiently.

Modeling Multilineage Cardiovascular SARS-CoV-2 Internalization With Organoids

The cardiovascular system presents complex challenges in modeling, with a myriad of cell types including immune cells, working in unison. Every cell is nestled in a 3D niche that affects both structure and function. Thus, to best emulate the physiological environment of the heart, it is critical to represent multiple cell types within a 3D context. Multilineage organoids, where multiple cell types comprise a spontaneously formed or engineered spheroid, have the potential to address some of these modeling gaps.

The earliest studies of SARS-CoV-2 cardiac infection in a 3D context employed cardiospheres comprised of human induced PSC–derived cardiomyocytes.[116] The generated cardiospheres were found to be susceptible to SARS-CoV-2 infection, similar to their 2-dimensional counterparts. A more recent SARS-CoV-2 study employed more complex hPSC-derived cardiac organoids cocultured with endothelial cells.[118] The hPSC-derived cardiac organoids comprised a self-organized mixture of cardiomyocytes, epicardial cells, fibroblasts, and ECs, which formed branched structures surrounded by pericytes. hPSC-derived cardiac organoids modeled the effect of the cytokine storm on cardiac function, a key pathological element observed in COVID-19. Importantly, they found that cytokine receptor genes were expressed at a similar level in the hPSC-derived cardiac organoids compared to the adult human heart, but most of this expression is enriched in nonmyocytes. Although SARS-CoV-2 RNA is not frequently detected in blood, viremia, and infection of blood vessels is an avenue through which SARS-CoV-2 could spread. To determine if SARS-CoV-2 can infect capillaries, one group employed hPSC-derived capillary organoids comprised of a lumen surrounded by CD31+ endothelial lining, PDGFR+ pericyte coverage, and basal membrane.[127] After infection with SARS-CoV-2, viral RNA was detected in the capillary organoids by qPCR. The supernatant of infected organoids could infect Vero E6 cells, suggesting the production of live virus. Simultaneous incubation with human recombinant ACE2 antibody reduced infection by over 10-fold.

Although most of these models included multiple cell types, many of them focused on bulk analysis of viral infection without researching how the virus and possible therapeutics impact specific cell types or cell-cell interactions. Additionally, there was little analysis or discussion of the spatial localization and penetrance of the virus. Also, there is not always a direct correlation between required drug concentrations and dose-response curves in vitro and in vivo. More complex cardiac organoid systems have recently been developed, such as gastruloid models encompassing the early development of the heart.[128,129] They offer a pioneering physiological complexity that would be interesting to study following infection with

SARS-CoV-2. Finally, organoid models are currently unable to assess the involvement of other organ systems or the systemic environment as potential contributors to cardiac damage. The interaction of immune cells with cardiac cells in the context of COVID-19 will be essential to elucidate mechanisms of viral-induced damage in the heart in a physiological context that more closely mimics that of patients.

Modeling Cardiac-Immune Interactions in COVID-19

The cardiac system does not function in isolation, and there have been multiple clinical reports suggesting the involvement of the immune system in COVID-19 cardiac damage. The cytokine storm, such as elevated levels of IL-1B and IL-6, is strongly correlated with morbidity and mortality. Postmortem analyses of cardiac tissue from patients with COVID-19 have identified the infiltration of CD11b+ macrophages,[130] CD68+ macrophages,[131] and T cells,[132] suggesting that immune infiltration may underlie some of the cardiac damage observed in patients.

Multiple 2-dimensional studies have incorporated the elements of the immune system (Figure 8). One study found that infection of hESC (human embryonic stem cell)-derived cardiomyocytes induced the expression of chemokines, such as CCL2, as well as inflammatory and immune response pathways, such as signaling pathways involving TNF, cytokines, NF-κB (nuclear factor kappa B), and IL-17.[121] This mirrors what has been observed in postmortem patient heart samples. Additionally, significantly higher levels of CCL2 were detected in the media of infected hESC-derived cardiomyocytes, suggesting active production of the signaling molecule. CCL2 is a chemoattractant for circulating monocytes, which then differentiate into macrophages at the chemokine-secreting site. To determine if infection by SARS-CoV-2 induces monocyte recruitment via the secretion of CCL2, hESC-derived cardiomyocytes, and monocytes were tested in a transwell migration assay. Twenty-four hours after infection, 400-fold more monocytes had migrated across the well in the infected wells compared with the control. CCL2 incubation alone was sufficient to recruit monocytes across the well. Additionally, blocking CCL2 from the hESC-derived cardiomyocytes with neutralizing antibodies blocked monocyte recruitment, suggesting that SARS-CoV-2 induces the secretion of CCL2 by cardiomyocytes, which then recruits circulating monocytes. To determine the effect of recruited macrophages on the trajectory of infection, a novel immunocardiac platform was employed where hESC-derived cardiomyocytes were cocultured with hESC-derived macrophages.[126] Although macrophages themselves are not infected, the presence of macrophages reduced infection in cardiomyocytes in a dose-dependent manner by over 2-fold, which was confirmed by qPCR and immunostaining.

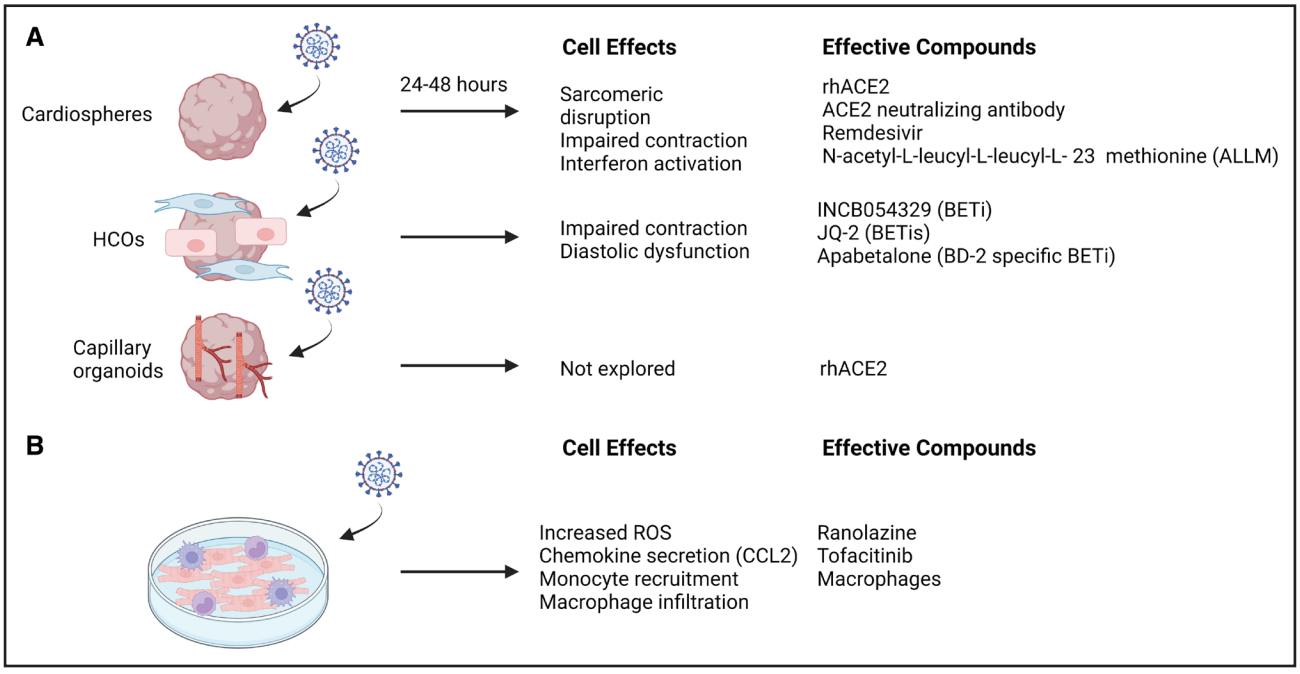

Figure 8. Multilineage organoids and cardiac-immune coculture systems for studying SARS-CoV-2 infection.
A, Three-dimensional models comprised of pluripotent stem cell (PSC)-derived cardiomyocytes (CMs), endothelial cells, fibroblasts, and pericytes can model cell type–specific pathologies and screen for effective compounds in a manner more closely simulating the in vivo heart. **B**, Cocultures of PSC-derived CMs with various immune cells, such as macrophages and monocytes, have revealed the key involvement of the immune system in various pathological effect, as well as immune-specific therapeutic targets. ACE, angiotensin-converting enzyme 2; ALLM, calpain inhibitor; BETi, bromodomain and extraterminal protein inhibitor; CCL2, chemokine (C-C motif) ligand 2; HCO, human pluripotent stem cell–derived cardiac organoids; rhACE, recombinant human recombinant human angiotensin converting enzyme; and ROS, reactive oxygen species.

Although these immunocardiac models are novel, they are still missing some key elements of the in vivo physiological milieu. Although monocytes and macrophages are critical responders of the innate immune system, they are accompanied by the adaptive immune system, such as B and T cells. The infiltration of B and T cells leads to the clearance of virus and the release of inflammation-inducing cytokines. Immunocardiac models will need to incorporate the adaptive immune system to better capture the response in patients. Additionally, these models have only incorporated the use of cardiomyocytes and not any of the other cardiac cell types. Although fibroblasts and ECs are not highly susceptible to viral infection, they still show an immune response to infection and thus may be key mediators of the downstream damage. Models involving multiple cardiac cell types already exist and could be adapted to involve the immune system too, potentially even in a 3D context. With the addition of the adaptive immune system and more complex cardiac systems, immunocardiac models could enable interrogation of multiple cardiac diseases such as myocarditis and heart failure.

CONCLUSIONS

Recent advancements in microfluidics, biomimetic OoC, and human stem cell–derived models have enabled the rapid enhancement of detection, pathogenic modeling, and therapeutic development in the context of COVID-19. SARS-CoV-2, as a highly infectious virus with broad impacts on multiple cell types and organ systems, requires physiologically relevant human platforms to interrogate its pathogenesis in both acute and long-COVID phase, as well as to develop novel treatment strategies and effective therapeutics.

The first step in fighting COVID-19 is the rapid detection of SARS-CoV-2 in patients, which has been aided by the advancement of microfluidic technologies. Multiplexed chambers and flow channels have enabled the simultaneous detection of multiple viral analytes, leading to more specific and sensitive tests. Optical detection techniques, such as absorbance-based fiberoptic biosensor devices and LFIA techniques have enabled a fast, reproducible, and cost-efficient detection of viral genetic material or protein. LFIA has also been used to develop biosensors for SARS-CoV-2. Additionally, fluorescence-based methods utilizing isotachophoresis and the binding of CRISPR-Cas12 have been developed to rapidly detect viral genes. Colorimetric assays enable detection with the naked eye and have been previously used to detect other coronavirus disease, such as middle east respiratory syndrome–CoV. Machine learning techniques have been used to design and optimize microchannels, distinguish the biomarkers and background in terms of performing on-chip experiments, and improve the chip-based SARS-CoV-2 detection techniques by utilizing the vast amounts of data generated to predict optimal adjustments and clinical outcomes in terms of multidimensional SARS-CoV-2 datasets processing.

Once infected, SARS-CoV-2 causes broad systemic dysfunction throughout the body, including severe impacts on the lungs, heart, brain, liver, and gut. Thus, capturing the effects of viral infection on each organ system is paramount for widely effective therapies. Multiple OoC models have been developed to identify cellular tropism and cellular responses. Although these OoC models offer deeper interrogation into organ pathophysiology, and many are highly engineered, they still lack the complexity of in vivo organ systems and are not always comprised of human cells. Multi-OoC systems have also been developed to model the interaction between various organ systems in the context of disease. Although still limited, these systems incorporate different organ tissues into various chambers that are then connected through vascular flow. These systems more faithfully recapitulate the complexity of the human body and may emulate the systemic microenvironment that is key to disease trajectory.

Human stem cell–based model systems are emerging as largely spontaneous-forming physiologically relevant platforms to study disease. These models have been largely utilized in the cardiac space, where a variety of simple and complex platforms can the help elucidate the pathophysiology of COVID-19 and to screen for effective therapeutic compounds. Many model systems incorporate multiple cell types, such as the cardiac stroma, to better emulate cardiac physiology. Multilineage organoids offer an exciting avenue to explore viral pathogenesis in a more physiological context and have been used to identify molecular mechanisms of viral pathogenesis. Novel systems are starting to incorporate immune cells, which have been largely missing in cardiac stem cell models. Currently, this has been achieved by coculturing PSC-derived monocytes and macrophages with cardiomyocytes, but future systems could incorporate the adaptive immune system or a 3D context. These studies have revealed the pivotal role of the immune system in regulating cardiac response to infection, as well as capturing the stimulation and effects of the cytokine storm. Future models incorporating more complex 3D gastruloids and further components of the immune system could better capture the patient milieu and even be utilized to study other cardiac diseases with an immune component, such as myocarditis. Overall, these complex physiological systems, which are consistently advancing, have the broad potential to elucidate the molecular mechanisms leading to the severe effects of COVID-19 and provide insights into the future development of novel therapeutic strategies.

OoC and PSC technologies are of pivotal importance for applications in disease modeling, including for

COVID-19. Advances made in intertwining these fields can be used to address many questions regarding human biology and medicine, which cannot be addressed using simple cell culture models alone. 3D organoid cultures have been pivotal in providing patient-specific mechanistic insights and they are ideal for high-throughput studies; however, they do not recapitulate the physiological microenvironment achieved with the OoC (ie, shear stress, coculture, barrier function, or the physiological recruitment of circulating immune cells). The combination of these technologies would provide a powerful in vitro preclinical model, incorporating the human pathophysiological microenvironment together with the patient-specific iPSCs and organoids from different donors, and from defined genetic subpopulations or patients with different comorbidities.

For these reasons, the use of healthy and diseased human OoC models represent a major advantage for testing drug efficacy and toxicity compared to the use of animal models. The current COVID-19 pandemic showed the need for faster methods to accelerate drug repurposing, drug discovery, and precision medicine. In addition, the ability to model virus evolution and compare viral variants will allow for the prediction of new emerging strains and potential threats to the host[133] and would set the basis for the development of novel drugs and seasonal vaccines. Progress in the field of iPS cells has made it possible to manufacture single or multiorgan chip systems using organotypic cells from the same donor for personalized medicine, to improve therapeutic approaches, and decrease drug toxicity. As a result, patient-specific OoC could decrease cost and time, and increase the effectiveness of the therapy and the success of the drug development process. However, there are challenges and difficulties in accepting and trusting these alternative approaches that need to be overcome before animal models can be replaced. Furthermore, although powerful, OoC are low throughput and would be more valuable at the last stage of the drug development process rather than at the early screening stage. High-throughput OoC needs to be developed, refining the devices currently available with many parallel culture chambers, or developing automated instruments that are able to analyze multiple lower-throughput OoC simultaneously. Further limitations in the use of iPS cells in OoC is their frequent inability to exhibit a fully mature phenotype, which would require corroborating studies using fully mature cell lines.

Despite the enhancements in the biomimetic OoC field and the capability of accurately recapitulating human physiology, the biggest challenge that OoC face is to convince the scientific community, as well as pharmaceutical, regulatory, and academic researchers of their advantages over animal models. As this field continues to grow and more laboratories are adopting this tool, we are now slowly moving towards the possibility of a real reduction of animals for more efficient and effective basic science and personalized medicine applications, including in the fight against viral illnesses such as COVID-19.

ARTICLE INFORMATION

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Disclosures

None.

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