Bioengineering and biophysics of viral hemorrhagic fever

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2

HUMAN ORGANS-ON-CHIPS FOR VIROLOGY

While conventional in vitro culture systems and animal models have been used to study the pathogenesis of viral infections and to facilitate development of vaccines and therapeutics for viral diseases, models that can accurately recapitulate human responses to infection are still lacking. Human organ-on-a-chip (Organ Chip) microfluidic culture devices that recapitulate tissue–tissue interfaces, fluid flows, mechanical cues, and organ-level physiology have been developed to narrow the gap between in vitro experimental models and human pathophysiology. Here, we describe how recent developments in Organ Chips have enabled re-creation of complex pathophysiological features of human viral infections in vitro.

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Advances in Organ Chip Technology

Organ Chips are engineered microfluidic devices which, when populated with human cells, offer the possibility to replicate the complex structures and physiological functions of major functional units of human organs, both alone and when coupled together fluidically to create human body-on-chips systems [1, 2]. They may complement, or even represent alternatives to, some animal models and conventional 2D and 3D culture systems by providing greater physiological relevance and mimicry of human organ-specific responses [3-5]. It has been postulated that Organ Chip technology could help to reduce the high failure rate in current drug development pipelines where many drugs that are found to be safe and effective in laboratory animals fail when they enter human clinical trials [6, 7]. As described by Eroom's law, the number of new drugs approved per billion US dollars spent on R&D has halved roughly every 9 years since 1950. Thus, successful Organ Chips could help to reverse the increasing cost of drug development.

Since 2010, when the first physiological relevant Organ Chip model of the lung alveolus was reported [1, 2], numerous other Organ Chips have been developed and applied to study disease biology and drug analysis. These Organ Chips minimally include one tissue type and generally feature an interface with an extracellular matrix (ECM) and a neighboring vascular and/or connective tissue [2, 8-10]. There are many chip designs available, both commercially and as prototypes from academic institutions, which allow various readouts of cell-, tissue-, and organ-level behaviors. These range from *in situ* imaging (i.e., optical or mass spectrometric) to built-in electrodes for quantification of transepithelial electrical resistance (TEER) and integrated pH and O_2 sensors to sampling liquid flowing through the channels of the devices [11-14]. Recent advances in nano- and micro-technology have extended the types of sensors available for in-chip biological processes in so-called lab-on-chips [15-19]. Chip-based disease models are thus becoming important research tools in numerous applications [10, 20]. Examples include, but are not limited to, diabetic nephropathy and drug toxicities in a kidney glomerulus chip [21, 22], intravascular

thrombosis in a lung alveolus chip [23], Alzheimer's disease in a brain chip [24], and radiation and drug toxicities, as well as modeling a rare genetic disease in a bone marrow chip [25]. As these disease and toxicity models emerge, new opportunities for drug development are created that may contribute to reducing the number of failed clinical trials. Several Organ Chips have been developed that model drug pharmacokinetics and pharmacodynamics across tissues, yet their predictive values largely remain to be validated with clinical data. However, in one recent study, fluidically coupled human Organ Chips have been used to qualitatively predict the drug pharmacokinetic responses in patients [26, 27].

To mimic host–microbe interactions specifically, several Organ Chip platforms have been developed that allow study of both pathogenic and nonpathogenic bacteria, and most recently, viral infections. Among the possibilities being explored are the use of Organ Chip to study infection kinetics, virus–host interactions, viral pathogenesis and evolution, drug responses, and the development of resistance to drugs and vaccines. Here, we provide an overview of Organ Chip technologies described for virology application to date and we discuss opportunities and challenges that lie ahead (**Figure 1**).

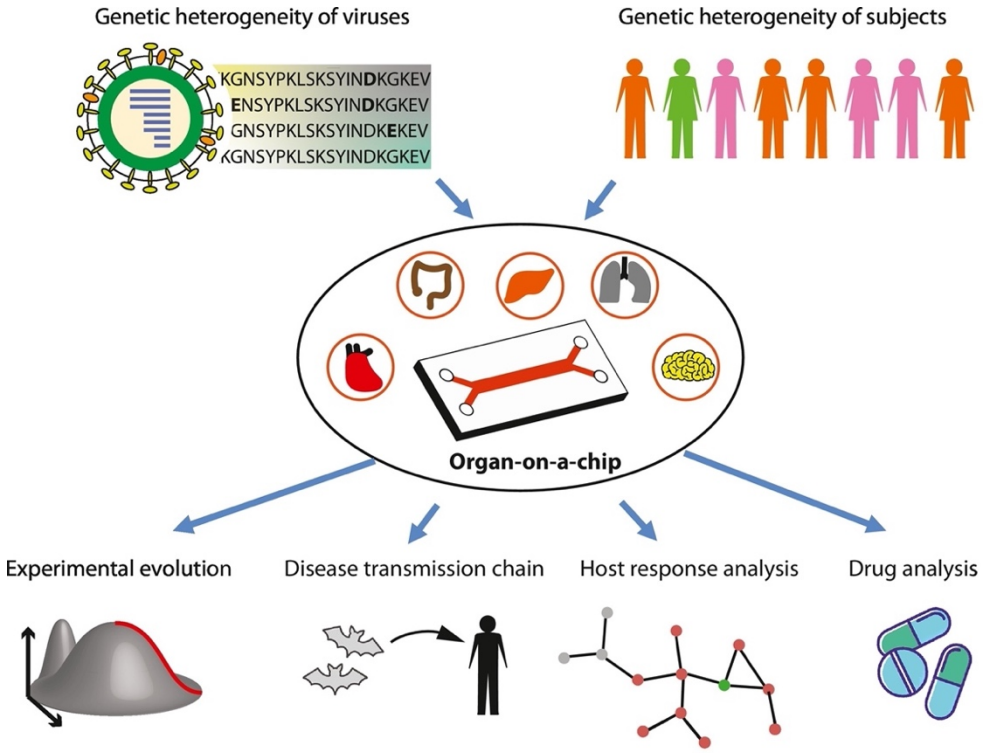

Figure 1. Application of Organ Chips to Virology. Organ Chips are emerging as a platform for viral disease models. It is expected that they could uncover signatures in virus evolution and transmission and provide opportunities for personalized response analysis and novel therapeutic drug development. This is difficult in conventional models or heterogeneous patient populations.

Modeling Viral Diseases in Organ Chips

1 Liver Chip

In vitro liver models are widely used in hepatology and drug development since they provide a reasonable mimic of defective liver function and drug metabolization *in vivo* [28]. A reliable *in vitro* liver model relies on reproducing the key elements of the biological processes and the physiological environment of the liver [29]. Recently, primary human

liver cells, which rapidly lose function in standard culture, have been incorporated into liver chip models to study liver diseases, such as fatty liver [30], metabolic dysfunction [31], and viral hepatitis [32]. In contrast to conventional *in vitro* liver models, typical liver chips can include controlled ratios of various liver cell types in the correct anatomical orientation and they show improved liver-specific functionality when compared to other *in vitro* models. These microfluidic liver chips show stable expression and functionality of Cyp450 family members, asialoglycoprotein receptor (ASGPR) and sodium taurocholate cotransporting polypeptide (NTCP), and also recapitulation of the liver microarchitecture as shown by the presence of hepatic microvilli, tight junctions and functional bile canaliculi. In contrast to other 3D models and spheroids, where limited diffusion of nutrients and oxygen leads to necrotic regions, liver chips show overall improved diffusion and transport properties [29].

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV) that is considered as a major global health problem [33]. HBV is responsible for the most common form of chronic liver infection which may lead to liver fibrosis, cirrhosis, and hepatocellular carcinoma [34]. The inability to reproduce the complexity of the liver environment using conventional *in vitro* models – that usually include only primary human hepatocytes (PHHs) – limits the ability to study host–pathogen interactions of such diseases [28, 35]. In addition, most animal models are not susceptible to HBV infection or cannot be infected by HBV directly due to its narrow host range [35, 36]. However, liver chip platforms have provided new opportunities to study viral hepatitis in a more physiological environment. Even using animal liver cells, valuable data have been obtained on viral infection. A rat liver sinusoid chip system, for example, was applied for the first time in 2015 to study HBV replication [37]. In this system, primary rat hepatocytes (PRHs) and immortalized bovine aortic endothelial cells (BAECs) were cocultured on opposite sides of a microporous membrane in a dual microchannel with continuous perfusion of culture medium. Hepatocytes maintained differentiation status, normal polygonal morphology, hepatocyte-specific function, and the expression of PRH-specific markers (ALB, TFN, HNF-4α, and β-actin) for more than 20 days. PRHs were then successfully infected with

recombinant adenoviruses incorporating the HBV genome. This model was subsequently used to develop a human source model incorporating PHHs [35]. The PHHs retained morphology and viability for 26 days, and expression of the hepatitis B core antigen (HBcAg), and cell secretion of HBV DNA was detected after direct HBV infection.

Later, another human liver model that allowed microfluidic recirculation of nutrients and oxygen through the culture medium was used for the study of HBV (**Figure 2**). In this platform, PHHs were either seeded alone or in combination with Kupffer cells, the resident macrophages in the liver, on collagen-coated polystyrene scaffolds. These liver cultures mimicked the hepatic sinusoid microarchitecture and were functionally stable for up to 40 days, as shown by high albumin expression [32]. This chip model is more susceptible to cell culture- and patient-derived HBV isolates at lower multiplicities of infection (MOI) compared to 2D cultures and liver spheroids. Also, the levels of albumin secretion and activity of Cyp450, phase I, II, and III enzymes were superior to those in other tested liver models. Since hallmarks of host response observed in HBV infection patients were also accurately recapitulated with elevated levels of IL-8, MIP-3α, SerpinE1, and MCP-1, this model can be applied to study host–pathogen interactions and identify biomarkers. Other nonparenchymal cells, such as Kupffer cells, can be added to these cultures to study the contribution of particular liver-resident cell types to antiviral responses against HBV [38]. The continued development of such novel platforms, where a more complex liver microenvironment and architecture can be better mimicked by coculturing different hepatic parenchymal and nonparenchymal cells, is critical to facilitate the study of HBV liver pathogenesis, immune evasion mechanisms, and drug-testing studies in the future.

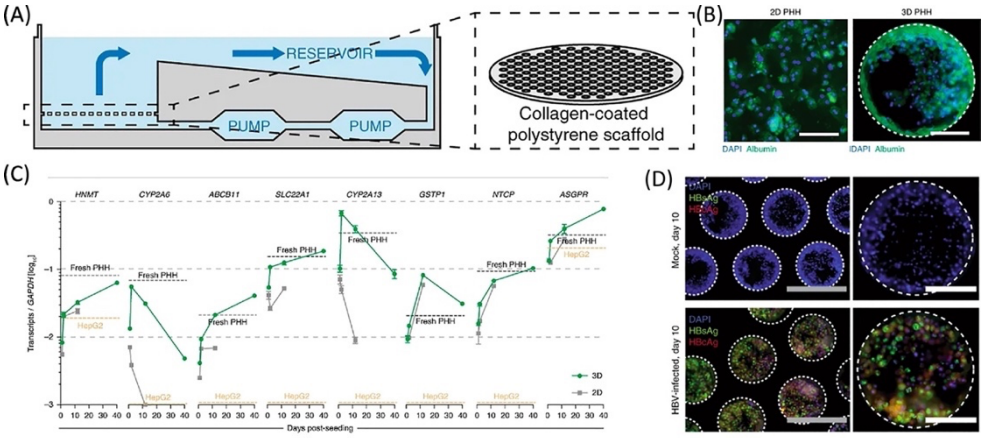

Figure 2. Human Liver Sinusoid Chip for HBV Infection Analysis. (A) Schematic of the human liver sinusoid model, in which the medium is recirculated via a pneumatically driven micro-pump and the collagen-coated scaffold ensures cell adherence. (B, C) The 3D chip model produces higher levels of albumin and Cyp450 compared to 2D culture models. (D) Chip models can be infected with patient-derived HBV. Adapted from [32] with permission. Abbreviations: DAPI, 4',6-diamidino-2-phenylindole dihydrochloride; HBV, hepatitis B virus; PHH, primary human hepatocyte.

2 Gut Chip

The human gastrointestinal (GI) tract is structurally complex and dynamic, thus its *in vitro* simulation is challenging. Animal models for viral infection of the GI tract have different limitations because the inoculated virus bypasses the native initial infection site in human gastrointestinal epithelium and the absence of some virus-associated receptors for infection of polarized epithelial cells [39, 40]. However, models of small-intestine [41] and large-intestine [42, 43] on chips have been successful in studying associated physiological and pathological processes (i.e., metabolism, cancer, host–microbiome interactions, and infection). GI infections are typically caused by bacterial, parasitic, or viral infections that lead to gastroenteritis and inflammation, which can involve the stomach, small intestine,

and/or large intestine. To study such infections, a human gut chip was established [39] which provides a functionally unique platform for the study of enteric virus infection and pathogenesis that is impossible with conventional culture systems [39, 44, 45].

Microfluidic human gut chip models generally contain epithelium-lined intestinal lumen in one channel and a parallel lower 'vascular' channel, separated by a porous matrix-coated membrane that supports highly differentiated human villus intestinal epithelium under continuous perfusion and cyclic mechanical strain which is necessary to mimic the physical microenvironment of human intestine, physiological fluid flow, and peristaltic motion [39]. Undulating villus-like structures spontaneously form in these chips, which display a tight epithelial barrier and villi containing microvilli along their apical surfaces. Coxsackievirus B serotype 1 (CVB1) caused disruption of the villi and integrity of the entire epithelium and passed through the intestinal lumen into the vascular channel. Additionally, active replication and apical release of newly formed infectious virions were found along with detectable cytopathic effects (CPEs) and inflammatory cytokines (IP-10, IL-8) production. This confirmed the ability to mimic secondary infections and disease propagation *in vitro*. This type of model has potential for studying a broad range of enteroviruses. Using state-of-the-art microfluidics technology, engineering models that recreate more realistic and complex host–pathogen interaction for human enteric infection will support further understanding of gut-related infections, as well as the development of vaccines and therapeutics.

3 Nervous System Chip

The nervous system is the most complex system in the human body. It is composed of a collection of different types of nerves and neurons, extensive vasculature and somatic structural support. Our current understanding of human neurobiology and neurological disease is based mainly on animal models, but because of evolutionary diversity, many

cannot faithfully replicate neuron development and disease progression in humans [46]. Furthermore, most *in vitro* studies of neurologic diseases lack cell–cell contact and interstitial fluid flow, which can affect communication between nonsynaptic neurons [47]. Recent innovations in chip-based technologies have made it possible to control neuronal connectivity *in vitro* for deciphering pathological and physiological processes in neuronal networks [48]. Whilst these models will not replace animal models, for example, for behavioral or cognitive studies, they can be useful for examining underlying mechanisms of disease. One widely used chip model is a 3D printed nervous system-on-a-chip that is capable of exhibiting the complexity of some neurological systems and disorders [49].

The device is composed of 3D silicone microchannels printed in parallel on plastic 35 mm dishes for axonal alignment, with a substrate-chamber sealant layer, to prevent fluid exchange, and three individual compartmented chambers on top for isolation and spatial organization of neurons and glial cells (**Figure 3**). This creates a separate fluid environment for neuronal axons and the soma. Therefore, virus can be loaded either to the compartment containing neuronal cell bodies or distal axons [49]. Central nervous system (CNS) neurons, peripheral nervous system (PNS) neurons, and Schwann cells can then be loaded to establish effective biomimicry of the nervous system, creating highly aligned CNS–PNS junction networks with associated Schwann cells. After infusion of the middle chamber containing PNS neurons with pseudorabies virus (PRV), direct infection of PNS neurons, virus replication, and axon-to-cell spread of viral particles to CNS neurons and Schwann cells were observed. However, CNS neurons and Schwann cells appeared refractory to infection, suggesting that there was a bottleneck in virus transmission. This work demonstrated that 3D printing provides one possible solution for the development of a customizable nervous system chip. Further biological relevance can be achieved if mechanical and ECM composition of neural tissues and tissue architectures of brain organization can be matched or reflected [50].

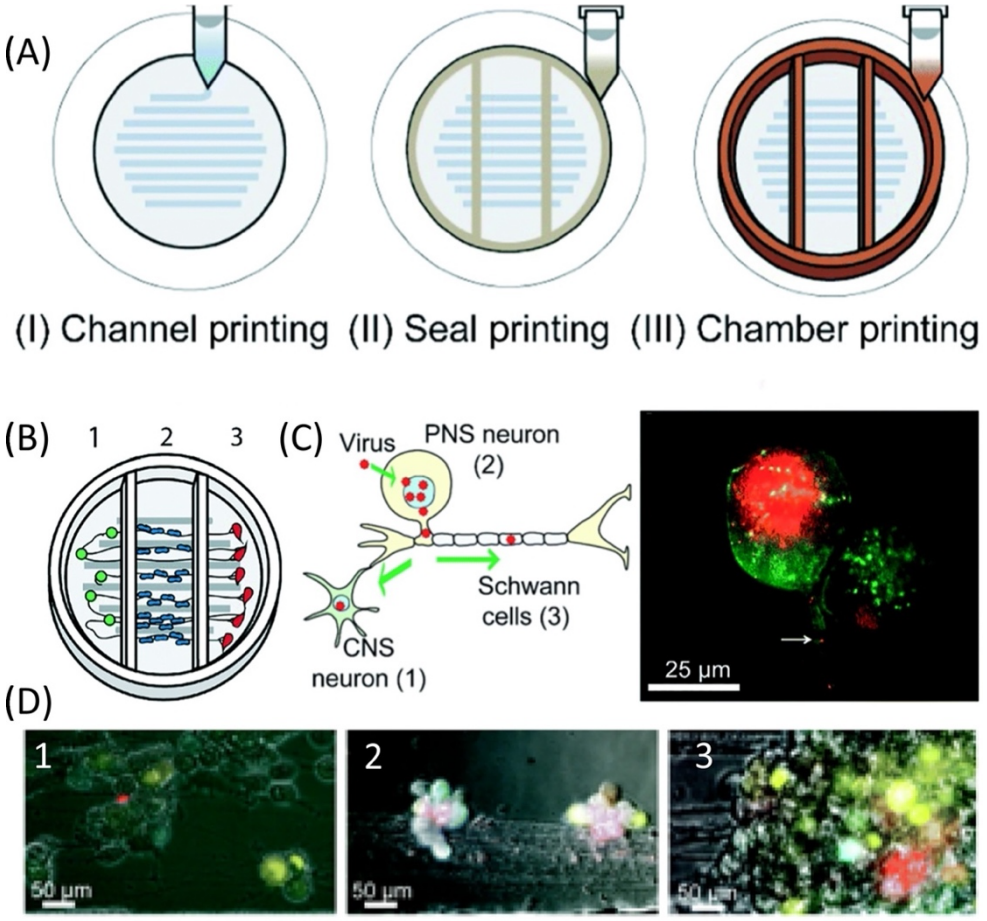

Figure 3. 3D Printed Nervous System on a Chip for Pseudorabies Virus (PRV) Infection and Transmission. (A) Fabrication of the 3D printed model nervous system on a chip. (B) Schematic of central nervous system (CNS), showing CNS neuronal component in chamber 1, peripheral nervous system (PNS) neuronal component in chamber 2, and peripheral nerve component containing axons and Schwann cells in chamber 3. Direct infection of PNS neurons by PRV led to replication as indicated by fluorescence micrograph of immature (red capsid) and mature virus particles (yellow puncta) in the nucleus and axons (C), and spread of viral particles to the CNS neurons and Schwann cells (D). However, CNS neurons (D-1) and Schwann cells (D-3) exhibited only single primary colors, and not a multicolor profile in

PNS neurons (D-2), suggesting a bottleneck in virus transmission. Adapted from [49] with permission from The Royal Society of Chemistry.

4 Kidney Chips

The kidney is a complex and specialized structure containing multiple cell types. This is a major hurdle in its *in vitro* modeling. Conventional *in vitro* kidney models with primary cells have limited growth capacity and, just like liver, tend to lose their phenotype over time [51]. While kidney organoids cultured from human induced pluripotent stem cells (hiPSCs) can contain cell types from different nephron segments, they do not accurately reflect the kidney *in vivo*, in part due to the lack of fluid flow and the correct kidney vasculature [52]. However, advances in kidney chip models are providing new options that overcome many of these hurdles and more closely resemble the physiological environment and anatomy of the human kidney.

A kidney model recently described is composed of a microfluidic channel, a porous membrane, and a static liquid well from top to bottom. It was developed to replicate the microenvironment of distal renal tubules [53]. Distal renal epithelial cells from Madin Darby canine kidney (MDCK) cells were grown on the porous membrane such that there was transcellular transport into the static well, providing an interstitial fluid environment. After 3 days of culture, a confluent monolayer with reabsorption barrier function of the distal renal tubule was formed; continuous injection of culture medium was then applied to imitate the shear force caused by flow of pro-urine along the renal tubules, as occurs *in vivo*. This model was used to study the pathogenesis of PRV-induced kidney dysfunctions. Upon PRV infection, the tight junction of the reabsorption barrier was described as broken, apical microvilli intertwined on the apical membrane and the expression levels of Na^+ transporters (Na^+-Cl^- cotransporter and Na^+,K^+-ATPase) altered. As a result, decreased Na^+ reabsorption was observed, which indicates renal abnormalities in electrolyte regulation. While this

model proved successful in demonstrating virus-related pathogenesis and symptoms, further developments are still needed to better reflect *in vivo* physiological and functional changes in response to infection. Namely, the incorporation of spatial features of kidney, ECM components, heterogeneous composition of (human) cell types and fluid shear stress, as well as the coordination of microvilli, cilia, and glycocalyx with each other in response to flow [52].

5 Lung Chips

Current lung chip models are capable of mimicking the complex microenvironment and function of the lung, including alveolus, alveolar–capillary interface, and small airways [54-58]. They offer novel avenues for studying respiratory diseases, for example, intravascular thrombosis, pulmonary edema, inflammation, and more realistic models for pharmaceutical study [23, 56, 59, 60]. Influenza is a viral infection that attacks the respiratory system. The development of an efficacious antiviral drug against influenza virus is still regarded as a major challenge [61, 62]. This is mainly due to the rapid and continuous influenza virus evolution which leads to the emergence of mutated viruses, making it resistant to existing therapies and drugs [63-65]. Although influenza virus evolution can be investigated in traditional influenza models (e.g., cell lines, chicken eggs, and animals), it has long been known that nonhuman models can introduce false adaption mutations, which has raised a major stumbling block to influenza research in the wider medical community [66-68].

To address this challenge, a human lung airway chip was used to model influenza virus evolution and investigate possible therapeutics (**Figure 4**) [56, 69]. The device contains an 'airway' channel in which primary human lung airway epithelial cells (HLAECs) are grown at an air–liquid interface and human pulmonary microvascular endothelial cells (HPMVECs) are cultured on the 'vascular' channel side in the presence of continuous medium flow to

mimic vascular perfusion. Mimicking *in vivo* morphology, HLAECs differentiated into a mucociliary pseudostratified epithelium and endothelium forming a planar cell monolayer, which resulted in the development of barrier function and mucus secretion. Serine proteases necessary for the activation and propagation of influenza viruses *in vivo* were also detected. When influenza viruses were inoculated through the airway channel to mimic *in vivo* airborne influenza infection, epithelium and endothelium dysfunction occurred and there was a loss of apical cilia, barrier function disruption, and cytokine production. The virulence of different influenza virus strains (H1N1, H3N2, H5N1) could also be recapitulated on these airway chips. In addition, host immune cell recruitment and migration, responding to infection by influenza virus, and viral clearance by immune cells, were reproduced in this device. With this advanced model, patient-to-patient transmission was mimicked by passaging virus from chip to chip to predict influenza virus evolution by mutation under the selection pressure of antiviral drugs (amantadine and oseltamivir). In addition, the device could also support influenza virus evolution through gene reassortment as occurs when different virus strains coinfect the same host by coinfecting with H3N2 and H1N1. Moreover, it provides an alternative preclinical tool for evaluation of new anti-influenza therapeutics (e.g., nafamostat, a serine protease inhibitor).

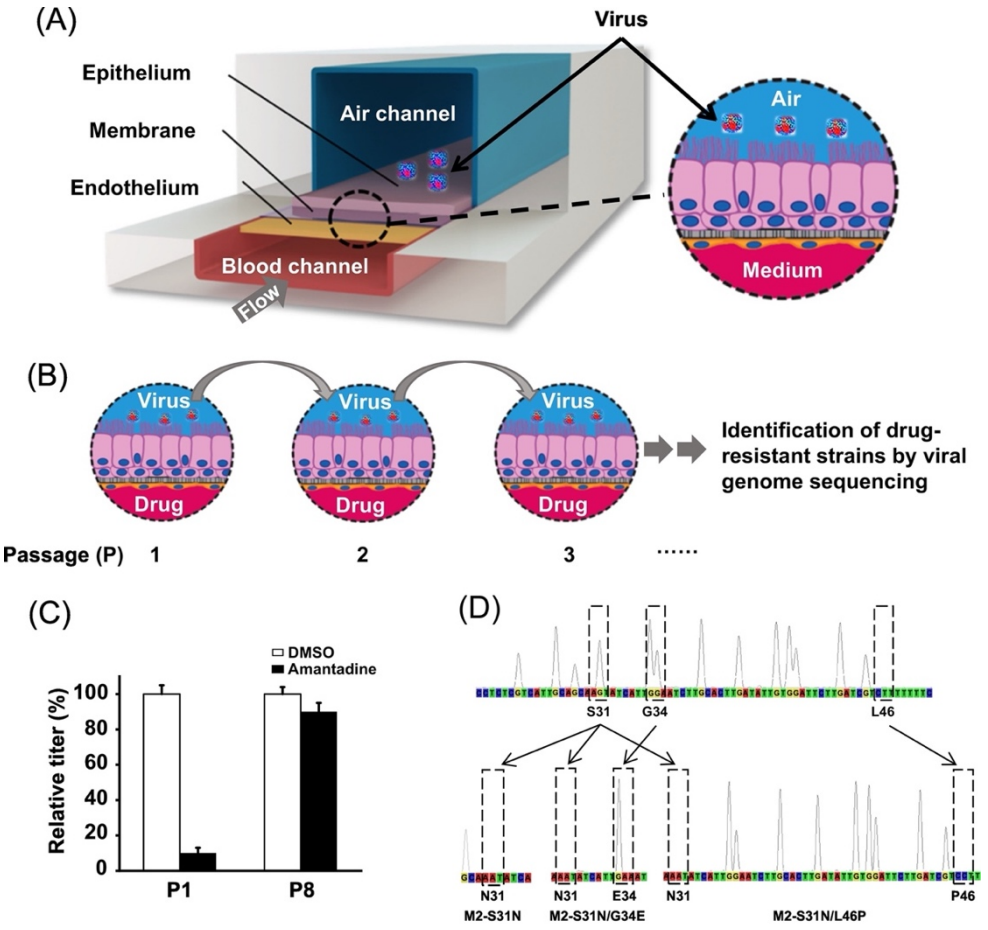

Figure 4. Human Lung Airway Chip Enables Study of Influenza Virus Drug-resistance Evolution. (A) Schematic diagram of a cross-section through the human airway chip. (B) Schematic diagram of the method used to generate and identify drug-resistant virus strains by human chip-to-chip transmission under drug pressure. (C) Titers of progeny viruses at the first and eighth passage (P) in control versus amantadine-treated chips. (D) Sequencing graphs showing three mutants (M2-S31N, M2-S31N/G34E, and M2-S31N/L46P) detected in the amantadine-resistant virus pool. Adapted from [69] with permission from authors.

To confront the current COVID-19 pandemic challenge, the lung airway chip platform was quickly leveraged to repurpose FDA-approved drugs as potential therapeutics against SARS-CoV-2. To mimic human infection by airborne SARS-CoV-2, SARS-CoV-2 pseudoparticles (CoV-2pp) carrying the SARS-CoV-2 spike protein (a key entry factor) were introduced into the air channel and were thus exposed to the human lung epithelial cells that express high levels of ACE2 and TMPRSS2 [70, 71]. With this platform, amodiaquine and toremifene were identified as potential entry inhibitors of SARS-CoV-2. This work suggests that the human lung airway chip could be a particularly useful tool to expedite drug repurposing for pandemic virus-caused crisis situations.

This airway chip platform has also been adapted for the study of virus-induced asthma exacerbation [72]. The migration of immune cells and the production of virus-induced inflammatory infiltrates observed in asthma exacerbations *in vivo*, were reproduced. Infection of the chip with human rhinovirus 16 (HRV16), commonly associated with asthma exacerbation, was showcased by replicating the timeframe of virion release and the hallmarks of the cytopathology observed in humans. Asthma exacerbation was induced by combining IL-13 with HRV16, and the effect of IL-13 on HRV16-induced inflammatory response was confirmed by the altered release of inflammatory markers (IL-6, IFN-λ1, CXCL10). Sole or coinfection of IL-13 and HRV16 resulted in significantly increased adhesion and transendothelial migration of neutrophils under physiological flow and shear stress – while MK-7123, a CXCR2 antagonist, reduced neutrophil adhesion and velocity in all conditions.

Another prevailing lung chip-based approach was used to investigate the complex crosstalk of coinfection with *Staphylococcus aureus* and influenza virus [73]. Alveolar epithelial cells (NCI-H441 cells) and monocyte-derived macrophages were cocultured within the upper chamber of the chip interfacing an air phase, while human umbilical vein endothelial cells (HUVECs) were cultured within the lower chamber, perfused with culture medium. A stable air–liquid interface and biological barrier, a morphological feature of the lung, were formed

after 14 days of cultivation. The model was then coinfected with *S. aureus* and influenza virus to investigate host–pathogen interaction in an immune environment by integrating macrophages and monitoring the resultant immune response. This study demonstrated that coinfection led to overall higher expression of cytokines and chemokines (IL-1β, IL-6, MCP-1, and IFN-γ) when compared to single infection. Moreover, endothelial impairment and subsequent vascular leakage upon coinfection were also demonstrated, which is a hallmark of acute lung injury.

Despite the indisputable utility of these *in vitro* models, which utilize lung-related epithelial and endothelial cells, models incorporating various cell types and tissue components that represent the vascular, muscular, and immune systems, as well as cartilage of human lung, are still lacking. Therefore, further innovations to develop more complex systems capable of simulating a more realistic host–pathogen interaction *in vitro* are needed for the development of therapeutic and vaccination solutions for respiratory disease.

6 Modeling Viral Hemorrhagic Syndrome On-Chip

Impairment of vascular integrity is a hallmark of a number of viral infections in humans, in particular, viral hemorrhagic syndromes (VHSs) caused by viruses such as Ebola (EBOV) and Lassa (LASV) [74, 75]. The integrity impairment is caused directly via infection or indirectly via interactions between vessels and circulating viral materials [76, 77]. Nonhuman primate (NHP) models are used to uncover the pathophysiology associated with VHS and develop corresponding vaccinations and therapeutics [78]. However, NHP models have several limitations. In particular, the need for host-adapted viruses and BSL-4 facilities which are inherently time consuming and extremely costly and are associated with ethical issues [79]. Furthermore, NHP models can also be nonrepresentative of the disease physiology and drug pharmacology observed in patients [26].

The use of Organ Chips in investigating VHS has recently been explored and its applicability to disease modeling and drug analysis has been demonstrated. A high-throughput chip-based model for EBOV-induced vascular integrity loss was developed using phase-guide-based technology (**Figure 5**) [80]. A phase-guide design allows interfacing of two parallel channels without any physical wall in between, while maintaining the ability to grow tissues or form ECM without mixing the channels' contents. To generate a high-throughput VHS model, a total of 96 perfusable and endothelialized microvessels within a fabricated phase-guide channel system was cultured with HUVECs at the interface of a collagen type I network and subjected to continuous perfusion. Vascular barrier function was demonstrated, as was sensitivity to physiological vasoactive compounds such as histamine. Luminal infusion of Ebola virus-like particles (VLPs) led to a dramatic increase in vascular permeability and clear increases in actin stress fiber formation.

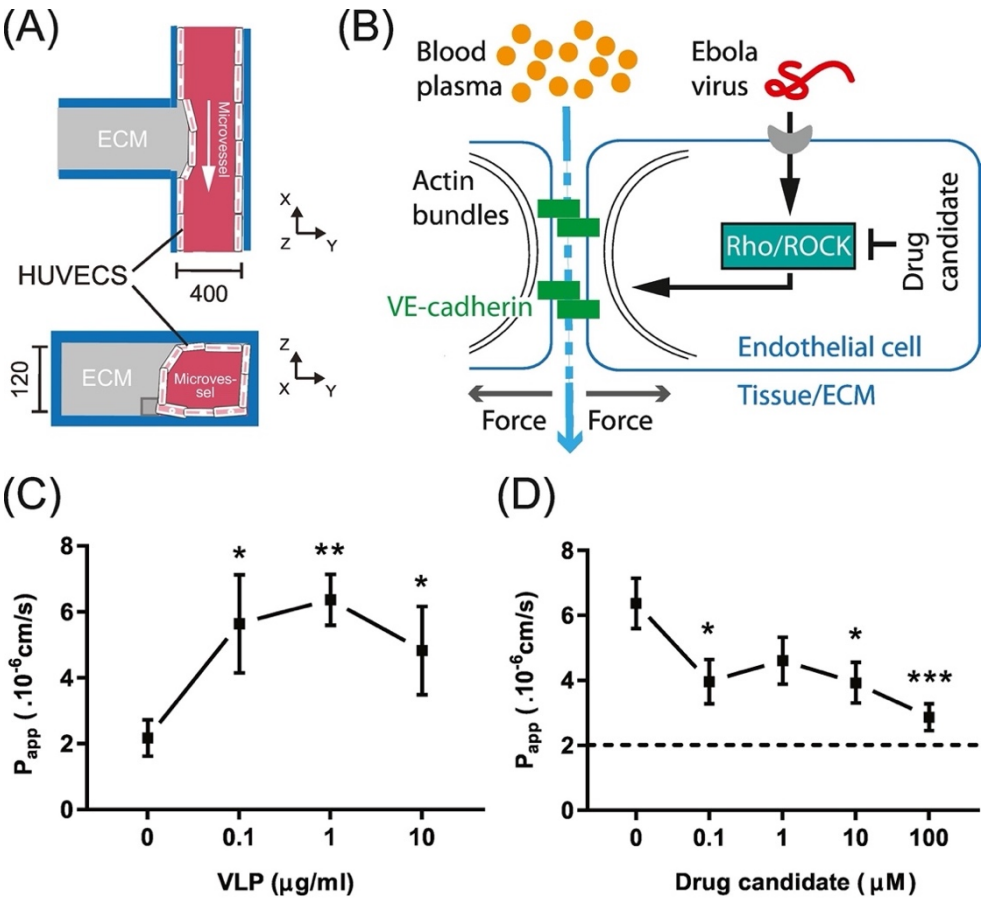

Figure 5. Viral Hemorrhagic Syndrome (VHS) Modeled On-chip to Study Ebola Virus (EBOV)-induced Loss of Vascular Integrity and Efficacy of Drugs. (A) A minimal design for Ebola VHS-on-a-chip. (B) Endothelial processes involved in VHS. (C) Apparent permeability (P_{app}) of microvessels in response to Ebola virus-like particles (VLPs). Microvessels were treated for 2 h with the indicated concentrations of VLPs, followed by a permeability assay. (D) Dose response to a drug candidate (melatonin) in microvessels exposed to 1 µg/ml Ebola VLPs for 2 h, followed by a permeability assay. Abbreviations: ECM, extracellular matrix; HUVECs, human umbilical vein endothelial cells. Adapted from [80] with permission.

This platform proved promising in the assessment of disease severity. In agreement with clinical findings [74, 81], the *in vitro* analysis showed a clear correlation between the integrity loss and viral load as well as the concentration of circulating Ebola glycoprotein ($GP_{1,2}$), a key mediator of vascular cell injury. These results indicated that both VLP- and $GP_{1,2}$-induced vascular impairment can be detected in this chip model. Furthermore, the study demonstrated activation of the Rho/ROCK pathway as necessary and sufficient to produce VHS on the chip. Activation of the Rho/ROCK pathway was seen upon infusion of the VLPs or the purified $GP_{1,2}$, leading to changes in the actin network and the mechanical tension applied to endothelial cell junctions. This raised the question as to whether inhibitors of this pathway, or targeting this mechanical mechanism, can be considered as therapeutic options. As proof-of-concept, the efficacy of two candidate drugs, FX06 [82-84] and melatonin [85-88] to counteract vascular leakage in VLP-treated vessels was demonstrated; these drugs target the mechanical elements VE-cadherin and associated actin bundles and the Rho/ROCK signaling pathway, respectively. This opens new opportunities for developing targeted therapeutics using Organ Chip-based models.

Concluding Remarks

Multiple studies, as described here, have illustrated how viral diseases can be reproduced using Organ Chip technology. Microfluidic Organ Chip platforms can provide accessibility to study virus-induced diseases in (human) organ-relevant settings, in real time and at high resolution, which cannot be achieved by traditional *in vitro* systems or animal models. Additionally, the Organ Chip approach provides flexibility and thus can be adapted to emerging needs, for example, in the case of the current pandemic of SARS-CoV-2 virus that has infected millions and killed thousands of people globally [89, 90]. Although researchers have recapitulated SARS-CoV-2 infection in rhesus macaques [91], ferrets [92], and hamsters [93], none of these animal models faithfully mimics the morbidity or mortality seen in many human patients. Thus, we are still facing many limitations associated with

animal models that may seriously delay or even cause failure in the development of vaccines and therapies. Organ Chip-based models may provide opportunities to overcome at least some of these limitations, now or in the future, and provide a new insight into host response to infection and disease pathogenesis.

Human induced pluripotent stem cells (hiPSCs) and human adult stem cell organoids are now becoming a viable alternative for more widely used primary cells and cell lines to model functional organ or tissue units. In addition, hiPSCs allow many types of cell to be derived from the same genetic background so that fully personalized Organ Chip models are becoming feasible [94, 95]. Significant interindividual variabilities in disease progression have been reported for patients with viral infections. Examples include EBOV and LASV with dead/survivor ratios of 2:1 and about 4:1 (https://apps.who.int/ebola/ebola-situation-reports, https://www.who.int/health-topics/lassa-fever), respectively (similarly for other VHS-causing viruses). What causes this variability is largely unknown. The use of hiPSCs derived from a single patient could enable modeling of disease progression and drug efficacy on individuals or groups of patients as well as targeted drug development [96-98].

Other future possibilities include using 3D printing technology (i.e., customization, rapid prototyping and multimaterial processing capabilities) to create more complicated and rapid fabrication of next-generation Organ Chips. The level of throughput and utility of current Organ Chip analyses is hindered by the commonly used readouts based on high-content microscope images. Combination with other screening assays (in line with metabolomics and sensors, single-cell sequencing etc.) may provide solutions. Finally, viruses such as SARS-CoV-2 or Ebola, may affect several organs in an interconnected manner. Ebola virus, for example, affects vessels directly and indirectly via liver and immune cells [74, 77, 85]. SARS-CoV-2 has been shown to be able to replicate in nasal turbinate, trachea, lung, kidney, and intestine in ferrets as shown by viral RNA quantification and detection of viral antigens [92]. Hence, recapitulating the crosstalk between multiple organs could be the focus of future efforts.

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Disclaimer Statement

D.E.I. is a founder of, and holds equity in, Emulate Inc., and chairs its advisory board. D.E.I. and L.S. are co-inventors on relevant patent applications submitted by Harvard University.

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