Report of the Assay Guidance Workshop on 3-dimensional tissue models for antiviral drug development

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SUPPLEMENT ARTICLE

Report of the Assay Guidance Workshop on 3-Dimensional Tissue Models for Antiviral Drug Development

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The National Center for Advancing Translational Sciences (NCATS) Assay Guidance Manual (AGM) Workshop on 3D Tissue Models for Antiviral Drug Development, held virtually on 7–8 June 2022, provided comprehensive coverage of critical concepts intended to help scientists establish robust, reproducible, and scalable 3D tissue models to study viruses with pandemic potential. This workshop was organized by NCATS, the National Institute of Allergy and Infectious Diseases, and the Bill and Melinda Gates Foundation. During the workshop, scientific experts from academia, industry, and government provided an overview of 3D tissue models' utility and limitations, use of existing 3D tissue models for antiviral drug development, practical advice, best practices, and case studies about the application of available 3D tissue models to infectious disease modeling. This report includes a summary of each workshop session as well as a discussion of perspectives and challenges related to the use of 3D tissues in antiviral drug discovery.

Keywords. 3D tissue models; SARS-CoV-2; antiviral drug discovery; body-on-a-chip; human pluripotent stem cells (hPSCs); induced pluripotent stem cells (iPSCs); microphysiological systems (MPS); organ-on-a-chip; organoid; tissue-derived stem cells.

The National Center for Advancing Translational Sciences (NCATS) Assay Guidance Manual (AGM) program [1] hosted a 2-day virtual workshop on 7–8 June 2022 that covered a broad range of critical concepts, including practical approaches and best practices for developing standardized 3-dimensional (3D) cellular assays with the hope of helping the community to develop antiviral therapeutics for future pandemic threats successfully. This workshop aimed to help scientists establish robust, reproducible, scalable, consistent, advanced 3D tissue models to study pandemic threat viruses. This workshop was jointly organized by NCATS, the National Institute of Allergy and Infectious Diseases (NIAID), and the Bill and Melinda Gates Foundation.

The workshop comprised introductory comments by leadership of the organizing institutions, a keynote presentation, 3 sessions that contained multiple short talks followed by a discussion, and a closing session where discussions were summarized, and future perspectives were discussed (meeting agenda and links to video recordings are shown in Table 1). Introductory comments covered how NCATS is catalyzing translational innovation, Bill and Melinda Gates Foundation's activities around the area of infectious diseases and global preparedness to pandemics, and NIAID's approach to preparing for future pandemics. Principal session topics included an overview of 3D tissue models and their utility and limitations, application of existing 3D tissue models for antiviral drug development, and use of robust and reproducible 3D tissue models from drug discovery through development.

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Table 1. Agenda of the Virtual Assay Guidance Workshop on 3D Tissue Models for Antiviral Drug Development Held 7–8 June 2022

Purpose: This workshop aimed to help scientists establish robust, reproducible, scalable, consistent, advanced 3D tissue models to study pandemic threat viruses.	
Day 1	
Introductory comments	• Joni L. Rutter, National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH) • Robert Jordan, Bill and Melinda Gates Foundation • Emily Erbelding, National Institute of Allergy and Infectious Diseases (NIAID), NIH
Overview of the workshop objectives	• Sarine Markossian, NCATS, NIH
Keynote: SARS-CoV-2, the start of a new scientific renaissance in biological science	• Simon Funnell, UK Health Security Agency (UKHSA)
Session 1: 3D Tissue models: utility and limitations Chair: Marc Ferrer, NCATS, NIH	• 3D cardiovascular disease models Christine Mummery, Leiden University Medical Center • Human tissue stem cell-derived organoids model viral infection Hans Clevers, F. Hoffmann-La Roche Ltd • Deconstructing and reconstructing the patient Linda Griffith, Massachusetts Institute of Technology (MIT) • Body-on-a-chip systems Anthony Atala, Wake Forest School of Medicine • Vasculogenesis and angiogenesis engineering models Zhengpeng (Jason) Wan, MIT • Advances in organ-on-chip platform technologies toward higher throughput, high containment operations, and tools for personalized medicine Jeffrey T. Borenstein, Draper • Discussion and Q&A
Day 2	
Session 2: Utility of the existing 3D tissue models for antiviral drug development Chair: Ann E. Eakin, NIAID, NIH	• Modeling respiratory viral infections in human lung chips Donald E. Ingber, Wyss Institute at Harvard University • Defining viral pathogenesis mechanisms using human cerebral organoids Lee Gehrke, MIT and Harvard Medical School • Human intestinal organoids as a platform for testing antivirals to human norovirus Sashi Ramani, Baylor College of Medicine • Antiviral screening pipeline and lessons learned Sara Cherry, University of Pennsylvania (UPenn) • Developing models for viral infections and disease to incorporate into the drug screening pipeline Emily M. Lee, NCATS, NIH • Using organoid models of tissue resident immunity to study infectious disease Calvin Kuo, Stanford University School of Medicine • Discussion and Q&A
Session 3: Use of robust and reproducible 3D tissue models from drug discovery through development Chair: Robert Jordan, Bill and Melinda Gates Foundation	• Connecting and supporting the tissue modeling community via the MPSCoRe global Working Group Nicole C. Kleinstreuer, National Institute of Environmental Health Sciences (NIEHS), NIH • Towards a microphysiological system (MPS) for evaluating IgG antivirals during pregnancy Evi Struble, US Food and Drug Administration • Modeling viral infections in brain organoids Thomas Hartung, Johns Hopkins University • Standardized human mini-organs on microchips to identify antiviral therapeutics at scale Edwin A. Rosado-Olivieri, RumiViro • Discussion and Q&A
Session 4: Closing session: summary of discussions and perspectives on the challenges ahead	• Simon Funnell, UKHSA • Sara Cherry, UPenn • Nicole C. Kleinstreuer, NIEHS, NIH
Closing statement and adjourn	• Sarine Markossian, NCATS, NIH
Related links	Event information: https://ncats.corsizio.com/c/62153215b176e58b1884dfc5 Day 1 on 7 June 2022: https://videocast.nih.gov/watch=45399 Day 2 on 8 June 2022: https://videocast.nih.gov/watch=45401

Three-dimensional human cell-based tissue models are composed of primary human cells or human pluripotent stem cells (hPSCs) that self-assemble and differentiate to form organ-like structures that mirror the physiology and structure of human organs [2]. Sometimes referred to as microphysiological systems (MPS), organs-on-chips, or in vitro organ constructs, these systems are designed to model complex physiological processes and cell-cell interactions that can be used to probe complex biology associated with disease phenotypes. The use of 3D tissue models for antiviral drug discovery is an active area of research that has yet to be fully harnessed. This report summarizes the proceedings of the meeting, including insights into current research on MPS as well as technical and regulatory challenges associated with the use of MPS in antiviral drug discovery. This report is also timely because it could help scientists generate robust and reproducible data from alternative nonclinical methods that are potentially more predictive for presentation to the Food and Drug Administration (FDA); recently, the FDA Modernization Act

2.0 and the FDA Omnibus Reform Act, which encourage the use of alternative nonclinical studies, were signed into law [3].

SARS-COV-2, AN UNLIKELY DRIVER IN COLLABORATION AND INNOVATION IN DRUG DISCOVERY

The coronavirus disease 2019 (COVID-19) pandemic, resulting from the rapid and ongoing transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), caused global morbidity and mortality, adversely impacting global economies and health care resources. The collaborative environment that materialized as a response to the COVID-19 pandemic, characterized by rapid sharing of data and reagents among investigators, was a testament to the commitment of the scientific and medical community to provide solutions to this deadly pandemic. Interactions between drug manufacturers, academic scientists, physicians, governments, and nongovernmental organizations helped coordinate sharing of data, critical reagents, and assays that led to new advances in antiviral drug discovery. This rapid access to high-quality scientific data for decision making and new tools for antiviral discovery was of clear value to scientific, medical, and nonscientific communities.

This pandemic also exposed a potential gap in the preclinical development of novel treatments for viral diseases due to the limitations of traditional cell culture systems and available animal models [4–6]. The utility of any preclinical model is dictated by its ability to recapitulate the human disease state and response to treatment or preventive interventions. To date, animal models have been the best tools to make such predictions prior to initiating clinical trials, but current scientific achievements have brought forth alternative methods, such as human MPS, that show great promise in complementing and, perhaps even replacing, some animal studies. The quality, reproducibility, and integrity of MPS have improved [7], and new areas of research focused on understanding how best to incorporate humoral as well as T-cell immunity can be assessed in such systems and should be considered. Providing actionable data on antiviral drug efficacy and pharmacokinetics using MPS should also be considered for drug discovery research, which may reduce cost and time of drug discovery, offset the potential for the demand for resource-limited animals such as nonhuman primates, or, in fact, be the only choice for studying both infection and complex biology associated with some infectious diseases. These models may be able to provide necessary results more expediently if they can be developed faster than an animal model for a new pandemic threat virus. They would also be a beneficial alternative in instances where animal housing capacity becomes a rate-limiting issue during a pandemic. In addition, more scientists may be able to perform experiments utilizing a 3D model than an animal study, especially once they become more accessible and standardized.

Consequently, the use of MPS can be a tremendous opportunity to identify therapeutics in a more rapid, accurate, and inexpensive fashion in the future. This workshop showcased many sophisticated methods and platforms currently in use for studying virus replication and pathogenesis that will provide a platform for studying new human viral pandemic threat pathogens that might not easily propagate in animal cell lines or animal models, which would be a barrier to vaccine, drug, and therapeutic development.

3D TISSUE MODELS: UTILITY AND LIMITATIONS

Over the past decade, 3D organotypic cellular models have emerged as promising assay platforms for preclinical drug discovery and development that could help narrow the translation gap (Figure 1). Many reviews and perspectives cover comprehensive listings of models and guidelines for their use in preclinical drug discovery [8–12]. In this section, we provide a broad overview of the available 3D tissue models, the current efforts towards standardization, and their applications in modeling viral infections and antiviral discovery. This section focuses on topics discussed in session 1 of the workshop and is not intended to be a comprehensive review of all current 3D tissue models. We also discuss challenges and opportunities related to the utility of these 3D organotypic models in research and their incorporation into drug discovery and development pipelines.

Overview of 3D Cardiovascular Disease Models and Efforts Towards Standardization

hPSCs are used to build various 3D tissue models, including tissues for modeling cardiovascular disease. hPSCs can differentiate to different cell types, including cardiomyocytes (CMs), but these cells tend to be immature. This is a persistent problem in the field, and it remains crucial to ensure the maturity of the cell types derived from hPSCs prior to their usage in modeling human disease. Work done by Dr Christine Mummery's laboratory at Leiden University Medical Center is addressing this challenge to create physiologically relevant 3D cardiovascular tissue models that mimic the human heart. The Mummery laboratory has developed technologies for scalable and inexpensive production of tissue models to study cardiac disease and toxicity based on mature cardiomyocytes (de Korte, Davis, Mummery, unpublished). Their work has revealed that crosstalk between 3 types of cardiac cells promotes advanced cardiomyocyte maturation [13]. Robust protocols were derived to produce different cardiovascular cell types, including endothelial cells (ECs), CMs, and cardiac fibroblasts; they have all been made available for public use [13, 14]. These cells can be cryopreserved and then be used upon thaw to assemble microtissues in which the CMs mature [15]. Further validation of these tissues shows that they have structural, electrical, mechanical, and metabolic features similar to those of postnatal CMs [13, 14]. These microtissues could be used for multilineage cardiac

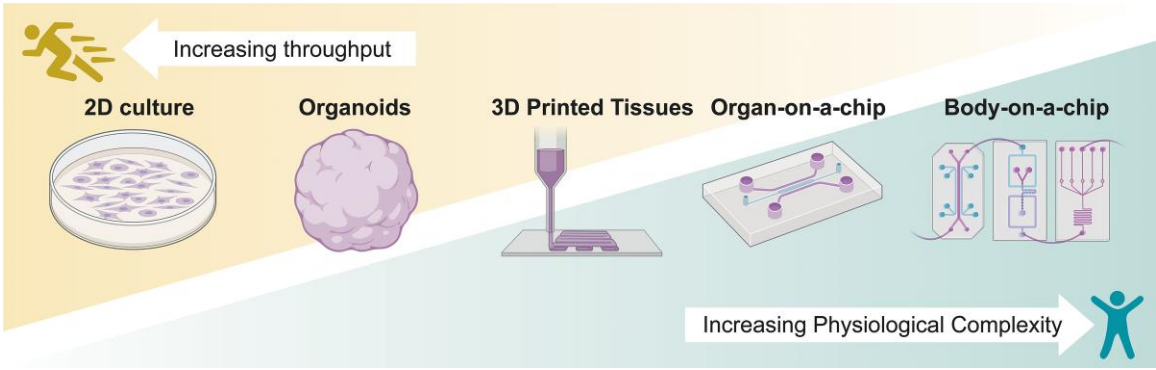

Figure 1. Scheme showing various three-dimensional (3D) tissue models used in preclinical research spanning from low to high complexity. 3D tissue models vary substantially with respect to design, throughput, complexity, and physiological relevance. Physiological complexity and relevance usually contrast assay throughput. Session 1 of this workshop covered a variety of tissue models spanning from organoids to body-on-a-chip systems, and discussed their utility and limitations for drug screening. Figure created with BioRender.com.

disease modeling, cardiotoxicity, and high-throughput screening [13, 14, 16].

Mummery and colleagues have also built 3D organotypic models to study vascular diseases. In an effort towards standardization, they have shown that ECs derived from the same control line of human induced pluripotent stem cells (hiPSCs) show low batch-to-batch variability when measuring their barrier function, but differences are observed when comparing different hiPSC lines. Hence, it is extremely important to establish validation criteria when analyzing differentiation for each cell line. Once validated, these ECs can be added to organ-on-a-chip platforms to build 3D vessels-on-a-chip to study vascular diseases such as hereditary hemorrhagic telangiectasia [17]. Many of the described hiPSC and organoid models can be repurposed for examining effects of viruses, drug repurposing, and screening for antiviral therapeutic targets.

Overview of Organoids Developed at the Hubrecht Institute and Their Use in Modeling Human Viral Infections and Diseases

The group led by Dr Hans Clevers at the Hubrecht Institute in the Netherlands has pioneered the development of human organoids in the past decade [18]. The first organoids developed were intestinal organoids, or human Lgr5 stem cell derived miniguts [19–21]. Similar protocols have been now developed to generate organoids from other epithelial tissues of the human body, including stomach, liver, lung, prostate, mammary glands, and others [22–26]. These organoids produce fully mature cell types specific to the epithelial tissue they are derived from, and these systems have been extremely valuable for disease modeling, including the use of lung organoids to study viral mechanisms of infection, as described in the paragraphs below. Future advancements will improve these systems by including cell types from other organs as well as immune components and/or blood vessels.

Chronic infection by viruses can be studied in these organoids. Human lung airway organoids have been used to study infection by a variety of viruses, including SARS-CoV-2, respiratory syncytial virus, and influenza [26]. Additional work by Clevers and colleagues showed that enterocytes can be readily infected by SARS-CoV and SARS-CoV-2 using human small intestinal organoids (hSIOs). The human intestinal epithelium supports SARS-CoV-2 replication, thus confirming that hSIOs could serve as an experimental model to study coronavirus infection and disease biology [27].

This work highlighted the value of using organoids to assess tropism, study viral mechanisms of infection, and as assay platforms for antiviral testing. They showed that chloroquine did not block viral replication in organoids, despite findings that indicated the drug blocked viral entry into Vero cells [28]. Such differences illustrate important discrepancies in the viral mechanism of infection between cell lines and organoids [4, 6, 29]. Hence, it is important to include organoids in the drug testing pipeline for the development of antivirals to help bridge the translation gap from the bench to the clinic.

Organ-on-a-Chip and Body-on-a-Chip Systems

The Wake Forest Institute for Regenerative Medicine, led by Dr Anthony Atala, has pioneered the production of biofabricated human tissues using bioprinting, organoids, and tissue chip systems. The tissue and organ constructs produced maintain their structural integrity, cell viability, and function long term [30, 31]. Dr Atala and colleagues have been using these biofabricated tissue systems to tackle a major problem in drug development: the lack of translation of preclinical studies into the clinic due to the lack of predictability of cellular and animal preclinical models currently being used. The biofabricated tissue models developed in the Atala laboratory also include the extracellular matrices derived from the specified organs while preserving the tissue's stiffness. This creates structures that have a high fidelity to the human organs they are mimicking [32]. Using their methodologies, they have created many

normal and disease models for many organs, including liver, heart, brain/blood-brain barrier, lung, kidney, and others [33–40].

Dr Atala and colleagues have successfully created multiorgan, humanoid sensor platforms by combining bioprinting, organoids, and tissue-chip technologies in one assay platform, referred to as body-on-a-chip models [41, 42]. These systems are being used to test different drugs and study their toxicity and efficacy on multiple organs at the same time for conditions such as cancer and infectious diseases [41, 43]. In one example, they demonstrated that a drug shown to be nontoxic in an isolated cardiac system caused a severe inflammatory response in a body-on-a-chip model. They have also built in the capability to study the proteome, transcriptome, and metabolome of drug-exposed tissues. Additionally, these systems are being utilized for accelerating gene therapy studies and for small molecule drug screening to identify potential new drugs to treat a variety of disease conditions [44, 45]. These body-on-a-chip models have the potential to help enhance drug discovery and clinical translation. Current research is aimed at standardization for variables such as cell sources, genetic variability, and data analyses systems.

The laboratory of Dr Linda Griffith at Massachusetts Institute of Technology (MIT) has also developed multiple MPS for different organs, connected together physiologically on an open-system microfluidic platform, with flow control, which are compatible with quantitative measurements of tissue functional markers to test the effects of compounds, including lipophilic drugs [46]. This system has been used to study organ-to-organ crosstalk during inflammation [47, 48]. The Griffith laboratory has developed both organoids and MPS to model endometriosis, a complex inflammatory disease characterized by the ectopic growth of the endometrium [49–51]. This work led to the identification of c-Jun N-terminal kinase as a new nonhormonal drug target to treat this disease in a subset of patients [52]. To further their research and test complex disease mechanisms as well as drug interventions, the team successfully built disease models of interactions between patient-derived epithelia, stroma, and immune cells using fully defined synthetic extracellular matrices [53–55]. Focusing on building these complex models of interaction allowed for the creation of tissue banks for highly multiplexed experiments. They also showed that synthetic extracellular matrices better capture in vivo phenotypes and provide more reproducible results than using Matrigel; it is a "low-noise" microenvironment that facilitates crosstalk observations.

Vasculogenesis and Angiogenesis Models

Vasculature provides nutrients and oxygen to the tissues and organs of the body. There is, therefore, a need to incorporate the vascular system in the production of physiologically relevant MPS, with perfusion and long-term viability for better assessment of organ function in normal and disease states. The laboratory of Dr Roger Kamm at MIT has pioneered the engineering of microvasculature in microfluidic systems. They have developed vascularized microfluidic platforms for a variety of applications, including angiogenesis, vasculogenesis, cancer cell extravasation, immune cell migration, vascularized solid tumors, organoids, and models of transport across the blood-brain barrier or other barriers within the body [56–64]. The group has also recently published robust protocols to generate perfusable physiological microvascular networks in microfluidic devices, including using human immortalized endothelial cells as a stable cell source instead of primary cells, a robust 2-step seeding method, and a strategy of applying interstitial flow to promote perfusable microvascular network formation as an excellent effort towards standardization of these models [65–67]. These devices are compatible with quantitative measurements, including imaging, biochemical/omic analysis, as well as assays for functional assessment [68–71].

Advances in MPS Towards Higher-Throughput, High-Containment Operations and Amenability for Personalized Medicine

There is a critical need to increase the robustness, reliability, and sample throughput of the MPS being developed to enhance their utility in drug screening and development of new therapies. Many companies are commercializing devices to address the need for increased throughput by building microfluidic systems in multiwell plate-like platforms, including the multichannel OrganoPlate platform, a microfluidic tissue plate with up to 96 tissue chips per plate by Mimetas (https://www.mimetas.com/en/home/); and the idenTx 40 Plate by AIM Biotech (https://aimbiotech.com/), a system based on microfluidic models developed in Dr Roger Kamm's laboratory; or the PREDICT96, a plate-based 96-device microfluidic platform with integrated pumping in each apical and basal channel in each well, developed by Dr Jeffrey Borenstein and colleagues at Draper (https://www.draper.com/explore-solutions/predict96) [72, 73]. These microfluidic platforms enable the study of complex cellular models with sufficient sample throughput to test many conditions with statistical replicates on a single plate to reduce variability and increase the capacity of these systems while maintaining their fidelity and predictive power. These systems are being used to screen many organ/tissue types, including kidney, liver, lung, blood-brain barrier, and others.

COVID-19 presented an opportunity to address a gap in the preclinical development of therapies, including limitations of both animal models and simplified immortalized cell culture systems. MPS were utilized to test and develop effective therapies for viral infections in a rapid, inexpensive, and accurate manner. As part of the pandemic response, Draper recently generated an airway model, PREDICT96-ALI (Air-Liquid-Interface) [74] for applications in respiratory infectious disease research on influenza

A virus and coronaviruses. Cells were sourced from various regions of the airways of living donors from various demographic populations. These demographics are known to influence the results obtained from infectious disease models. High containment operations (biosafety level 3 [BSL-3] facilities) were accessed, and operations established to utilize the native virus in disease modeling and the therapeutic discovery process. The group has demonstrated robust viral replication of SARS-CoV-2 in the PREDICT96-ALI plates, which were then successfully used to test drugs with different mechanisms of antiviral action. The data generated using antivirals were highly predictive of observed clinical outcomes, paving the way to apply this model in larger, different patient populations in the future as well as to work on emerging variants.

Challenges and Opportunities

MPS can be constructed from patient-derived cells, replicate the physiology of human tissues, and be used for disease modeling and drug screening. Although the technologies to bioengineer these tissues are advancing rapidly, there are still many challenges to make these complex engineered cellular systems available for biologists to validate these models and incorporate them into research. Crosstalk between different disciplines (including biologists, physicists, chemists, and bioengineers) is key for the advancement of these models and their adoption into research, drug discovery/development, and regulatory submissions.

The appropriate 3D tissue model to use depends on the application and question that is being asked, in a "fit-for-purpose" approach. However, regardless of the technology platform used, the biology and physiology of 3D tissue models need to be rigorously tested and validated to the extent possible in vitro, so that they can generate reproducible data, and are scalable for high-throughput screening. Hence, it is critical to keep the balance between innovation and operationalization for their adoption as assay platforms to be used along early discovery, preclinical development, and regulatory filing. Considerations for building robust 3D tissue models include the use of the appropriate cell types (tissue-specific primary human cells, iPSCs, etc.) in appropriate native-like proportions, in conditions where they can interact with each other, and using media that are compatible with all cell types and physiological features like vasculature. In addition, extracellular matrices play a major role in the functionality of the tissues, including their stiffness; thus, it may be critical to incorporate biomimetic extracellular matrices in these tissues. Finally, for tissue-on-a-chip systems there is a need to include proper native-like volume proportions of the organs and assess the proper flow rates.

The rigorous biological validation and assessment of the clinical predictability of 3D tissue models for disease modeling and therapeutics development is challenging because of limited access to in vivo and clinical data to do head-to-head comparisons between MPS and real human data in a quantitative manner. Challenges also remain for the standardization of these 3D tissue models—including reliable cell sourcing, genetic/epigenetic variability with diverse patient cells, and biomarkers of tissue physiology and morphology, in both healthy and disease states. The future of the field relies on the sourcing and scale-up of patient cell production, including cells from diverse populations, standardization, and building cell banks to reduce batch-to-batch variability. The International Society for Stem Cell Research (https://www.isscr.org/) is addressing this issue by working on consensus documents about reagent standardizations, monitoring genetic drifts, and practical advice for their isolation and maintenance that will help scientists standardize stem cells; they will be made available to the public soon. Although standardization is crucial, it should be noted that variability between patients must be accounted for in some situations, and the utility of these models needs to be assessed in a fit-for-purpose manner.

UTILITY OF THE EXISTING 3D TISSUE MODELS FOR ANTIVIRAL DRUG DEVELOPMENT

Three-dimensional tissue models were developed to model a wide variety of tissue types and have proven useful in studying various aspects of viral infection. These models have also been used to evaluate antiviral compounds and have provided rich data sets, exploring the impact of antivirals and virus replication on the host response to infection. Data generated from these physiologically relevant systems can provide antiviral developers with new tools to stratify antiviral compounds based on activity and have a higher probability of impacting viral replication in vivo. In session 2 of the workshop, a summary of the current efforts in the community towards incorporating 3D tissue models in viral research and drug discovery were discussed (Figure 2). In addition, the presenters focused on the challenges and opportunities related to the utility of these models in viral disease modeling and drug development.

Human Organoids for Viral Disease Modeling and Antiviral Discovery

Organoid cultures derived from human embryonic stem cells (cerebral organoids) can be used to study replication of neurotropic viruses and their impact on brain physiology. A team of scientists at MIT and Harvard, including Drs Lee Gehrke and Rudolf Jaenisch, used this system to understand how closely related flaviviruses (like Zika virus and dengue virus) cause differential neuropathology upon infection. Cerebral organoids infected with Zika virus showed infection of neural progenitor cells, astrocytes, and microglia-like cells with virus-induced cell death occurring primarily in neural progenitor cells. Further comparisons between infection of cerebral organoids with Zika virus or dengue virus showed that dengue virus induced a stronger innate immune response in the organoids, which could clear virus infection more efficiently than Zika virus

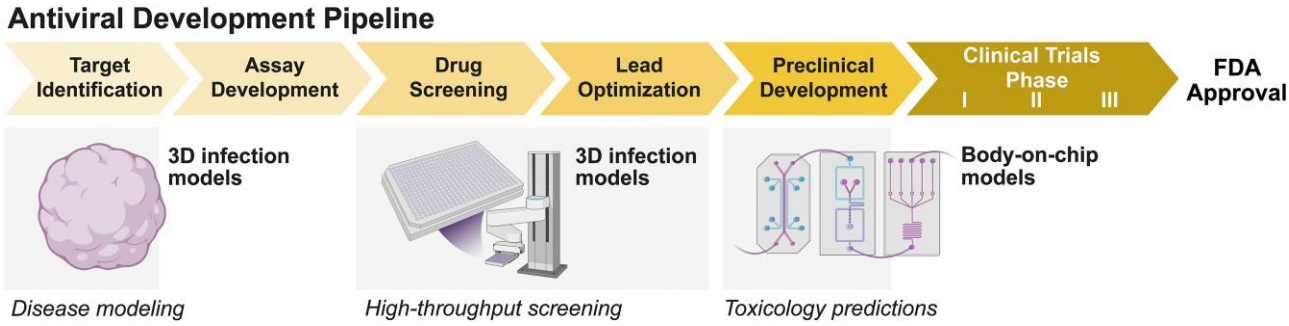

Figure 2. Scheme showing how three-dimensional (3D) tissue models are incorporated into the antiviral development pipeline. Disease modeling with 3D tissue models (organoids, organ-on-a-chip, etc.) is essential for target identification purposes. 3D models of infection can also be incorporated in drug screening and lead optimization stages of the pipeline. Toxicology predictions using body-on-chip models could also be performed. Sessions 1–3 of this workshop presented multiple case studies on how these 3D models of infection have been incorporated into the antiviral drug discovery pipeline. Figure created with BioRender.com.

[75]. This system was used to study Zika virus virulence by infecting cerebral organoid cultures with Zika virus strains that cause mild, moderate, and severe disease. These studies showed that infection of cerebral organoids with the Zika virus strains causing mild disease in humans had little impact on the overall structure of the organoid cultures, while the more virulent strains changed the organization of the ventricles, suggesting there was a different impact on brain pathophysiology.

There are many variables that can affect outcomes in the development and utility of these cerebral organoids. To help the community, this team has devised valuable best practices and tips for developing and working with cerebral organoids. These best practices include the following: to find a good collaborator who is a stem cell biologist for reliable cell sourcing; avoid using hPSCs that show signs of premature differentiation; take precautions to avoid bacterial contamination and add antibiotics if necessary to reduce risk of infection; be gentle when handling and transferring the cultures; culture organoids individually or sparsely (ie, culture them in 24-well plates) to reduce clumping and fusion; if cryosectioning, use embedding substrate (OCT) with blue color to help visualize the organoids; and reduce variability by using batched samples for experiments. Sharing such practical advice is paramount to other research teams—the community can benefit from these teachings, so they produce robust and reproducible data with organoids.

Human intestinal enteroid (HIE) cultures can also be developed to study replication of viruses that infect the gastrointestinal tract. Scientists at Baylor College of Medicine, led by Drs Sasirekha Ramani, Mary Estes, and their team, developed HIEs that support human norovirus replication, providing a system for therapeutic development. Norovirus causes acute gastroenteritis in immunocompetent individuals of all ages and chronic infections in immunocompromised individuals. This virus has been difficult to study for almost 50 years due to the absence of intestinal cell culture models that support robust and reproducible virus replication. The human gastrointestinal tract is composed of a polarized epithelial layer that contains numerous cell types, including enterocytes, enteroendocrine cells, tuft cells, goblet cells, Paneth cells, and stem cells. HIE cultures, which contain these epithelial cells, are established from stem cells isolated from human intestinal biopsies or surgical tissue [76–78]. Other methods using PSCs create intestinal organoid cultures associated with mesenchyme; these cultures do not support human norovirus replication. The development of HIE cultures was a breakthrough for studying previously noncultivable viruses, such as norovirus, and these cultures have been shown to support replication of multiple human norovirus strains representing different genogroups and variants [76, 79]. HIEs also recapitulate genetic susceptibility to norovirus based on the expression of the *Fut2* gene, which is required for infection with most human noroviruses [76, 80]. This model system can be adapted for drug discovery to screen compound libraries for inhibitors of norovirus. Virus replication in HIE monolayers is quantified by real-time quantitative polymerase chain reaction (RT-qPCR), and compound cytotoxicity is measured by release of lactate dehydrogenase or other commercially available assays [81, 82]. The HIE system will greatly facilitate discovery of antivirals targeting norovirus replication and other viruses that replicate in the gastrointestinal tract.

Moreover, organoid cultures derived from tissues in the upper and lower respiratory tracts provide opportunities to study differences in viral replication related to lung physiology. These differences could provide information on how viruses cause pneumonia and other respiratory complications. Lung organoid cultures were derived by Dr Calvin Kuo and colleagues at Stanford University from human alveolar epithelial type II (AT2) or KRT^{+} basal cells from human tissue biopsies representing different areas of the lung [83]. AT2 organoid cultures were developed with lumens lined with differentiated club cells (nonciliated epithelial cells found mainly in bronchioles and in large airways) and ciliated epithelial cells. These distal lung organoid cultures could be inverted to reverse their polarity,

exposing ACE-2 expressing cells on the outer portion of the organoid to enable infection with SARS-CoV-2 and other respiratory viruses. Single-cell analysis of infected cultures demonstrated that SARS-CoV-2 could infect club cells in addition to ACE-2^+ ciliated epithelia. These data suggest that distal lung organoid cultures provide a model for exploring infection phenotypes in the upper and lower respiratory tract.

Conventional organoids typically only include epithelial cells. In general, there is a pressing need for second-generation organoid models that holistically preserve epithelial cells along with stromal components, including immune cells, vasculature, and fibroblasts. Dr Calvin Kuo and colleagues have also been developing such organoids with increased cellular complexity by culturing intact tissue fragments at an air-liquid interface (ALI), including gastrointestinal tissues with stromal components [84, 85]. Culture of cancer biopsies using this method incorporates diverse tumor-resident immune populations without artificial reconstitution including T, B, NK and myeloid cells, and recapitulates anti-PD-1–mediated immune checkpoint inhibition with tumor killing [86]. This method has been recently extended to an adult human lung organoid culture that propagates from intact distal lung fragments with alveoli alongside tissue-resident memory T cells, B cells, and myeloid cells, which has now been applied to SARS-CoV-2 infection [Calvin J. Kuo, unpublished].

Human Organ-on-a-Chip for Viral Disease Modeling and Antiviral Discovery

The organ-on-a-chip technology developed at the Wyss Institute at Harvard University by Dr Donald Ingber and colleagues reconstitutes human organ-level structures and functions by coculturing 2 or more tissue types in adjacent channels of a microfluidic culture device. This technology is designed to replace animal testing in drug development and can also be used to advance the development of personalized medicines. The human lung-on-a-chip technology was initially designed to model alveolar function using human alveolar cells cultured under air within one microfluidic channel on a porous membrane with capillary endothelial cells grown on its underside in a parallel channel that experiences dynamic fluid flow to recreate the alveolar-capillary interface of the lung. The lung alveolus chip also incorporates hollow side chambers to which a vacuum can be applied cyclically to simulate the physical process of breathing and associated tissue deformations [87]. This system has been used to model organ-level responses such as recruitment of circulating white blood cells in response to viral infection, pulmonary edema, mechanical control of innate response to viral infections, and mucus pathophysiology [87–91]. Using this model, physiological breathing motions were discovered to suppress viral infection by stimulating innate immunity and elaboration of protective interferons [90]. This work also led to identification of host genes required for the host inflammatory response to viral infection as well as identification of an existing drug (Azeliragon) that targets this pathway and prevents release of multiple inflammatory cytokines in infected lung chips. These data were included in a submission of an investigational new drug application to the FDA to initiate COVID-19 trials with this compound.

The unique design features of this organ-on-a-chip model have been used to develop models of the lung airway and to model other types of human lung disease. Lung airway chip models of chronic obstructive pulmonary disease (COPD) [92] and other disorders (eg, asthma, cystic fibrosis, cancer) have been developed, and they also have been used to measure the impact of virus infection [92] as well as to evaluate drug toxicity [88]. Infection of COPD lung chip models showed a 10-fold increase in virus replication compared to infection of normal lung tissues, which is consistent with observations from clinical studies (unpublished observations). The lung airway chip was also used to study influenza virus evolution in the presence of exposure to suboptimal drug concentrations, with variants appearing after repeated passage from one chip to another mimicking human-to-human transmission [93]. Using this system, resistance to Tamiflu was generated in 25 chip-to-chip passages. In addition, multiple genetic mutations previously shown to mediate viral resistance to drug therapy in humans were identified in this chip study as well as additional mutations never seen before.

The lung airway chip was useful for identifying existing drugs that may be repurposed as antiviral therapies for SARS-CoV-2, including one (amodiaquine) that moved to human clinical trials for COVID-19 [29]. This was accomplished by testing compounds using pseudotyped virus particles in a BSL-2 laboratory. By using computational approaches to compare transcriptional profiles from uninfected or SARS-CoV-2–infected patients and cells, compounds were identified in public databases that could potentially reverse transcriptional signatures observed during infection. Statins were identified to have the ability to reverse some of these transcriptional signals, suggesting that they may have a positive benefit to patients during SARS-CoV-2 infection. Interestingly, not all statins had equal antiviral effects, and this was confirmed in a retrospective study of more than 4000 COVID-19 patients, indicating off-target effects might contribute to the antiviral activity [94].

Finally, use of a CRISPR screen for potential antiviral long noncoding RNAs in combination with studies in human lung airway and alveolus chips led to the discovery of a new class of immunostimulatory double stranded RNAs (dsRNAs) that act as broad-spectrum antiviral therapeutics [95]. These dsRNAs potently inhibit infection by multiple types of coronaviruses (SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-NL63) and influenza strains (H1N1, H3N2) in lung airway and alveolus chips as well as cell lines in vitro by inducing high levels of expression of protective type I/III interferons. Importantly, they also were shown to potently inhibit infection by native SARS-CoV-2 in a mouse model carried out in a BSL-3 laboratory.

This section focuses on human organ-on-a-chip models for viral disease modeling and therapeutic evaluation discussed and presented in the workshop and is not intended to be a comprehensive review; however, considering its physiological importance, it is imperative to direct the readers to major advancements in the skin-on-a-chip models field for viral disease modeling, such as the vascularized skin-on-a-chip model published by Sun et al in 2022, and other comprehensive summaries found in the cited reviews [96–101].

Incorporation of 3D Tissue Models into the Antiviral Screening Pipeline

Incorporating organoid cultures into high-throughput screening campaigns provides important information regarding antiviral activity in primary cells, especially for compounds that target host proteins required for virus replication. NCATS's intramural 3D Tissue Bioprinting Laboratory, led by Dr Marc Ferrer, is developing physiologically relevant, robust, and ready-to-use in vitro cellular assay platforms to rapidly model the infectivity of emerging viruses and develop new antiviral treatments [9]. They are utilizing primary human airway and alveolar epithelial tissue cultures, which provide an excellent model of respiratory epithelium and can be used to study replication of respiratory viruses [102]. Primary human airway epithelial cells are isolated from human biopsy tissues or derived from iPSCs and cultured at the ALI to induce terminal differentiation into a pseudostratified monolayer culture with mucociliary differentiation, as measured by the presence of functional beating cilia and mucus secretion. They also exhibit characteristic epithelial barrier functions, including expression of cell junctional proteins and the development of robust transepithelial electrical resistance values [103].

The complexity and cost of primary human airway cultures require careful consideration when including this model system into antiviral discovery campaigns. To increase throughput and reduce costs, compounds identified in primary screening assays in transformed cells that have undergone hit confirmation can be evaluated in primary human airway cultures to ensure antiviral activity is retained in primary cells. A screening campaign, led by Dr Sara Cherry and colleagues at University of Pennsylvania, was designed to identify inhibitors of SARS-CoV-2 replication and used this type of screening paradigm. The primary screening assay was conducted on human respiratory epithelial Calu-3 cells to identify compounds that could inhibit SARS-CoV-2 replication. This cell culture model was selected for screening because nonrespiratory cell lines were found to use distinct entry and replication pathways compared to respiratory cell culture models [104]. Compounds active in the primary assay conducted in Calu-3 cells were further profiled in dose-response experiments using the primary human airway epithelial cell cultures generated from bronchial or nasal tissue [105, 106]. Compounds that showed antiviral activity in transformed cultures but were not active in primary human airway epithelial cultures were excluded from further characterization. In addition, the investigators tested libraries of immune agonists and found that activators of the STING pathway could inhibit SARS-CoV-2 infection in ALI models as well as in vivo [105]. Repurposing campaigns of known drugs with human experience also revealed that combinations of the direct acting nucleoside analog molnupiravir with inhibitors of de novo pyrimidine biosynthesis were synergistic in the ALI models as well as in animal models of SARS-CoV-2 infection [106]. By removing candidates that are not active in the ALI model, these data provide a powerful filter for antiviral screening to identify compounds that have a higher likelihood for in vivo antiviral activity.

Dr Emily Lee and colleagues at NCATS have run additional analyses on these primary ALI airway and alveolar epithelial cultures infected with different respiratory viruses using single-cell RNA sequencing. These analyses produced transcription profiles that may help identify potential biomarkers as diagnostic markers of infection [102]. Inclusion of additional organoid cultures that model other organ systems, such as vascular endothelium, brain, heart, kidney, liver, and gut, could be used to expand the repertoire of biomarkers to characterize viruses that infect these organ systems. These data suggest that inclusion of ALI primary airway lung epithelial cells in antiviral discovery programs provides valuable information to help identify antiviral compounds that can be further developed into antiviral drugs.

Challenges and Opportunities

Three-dimensional tissue models, including MPS, are valuable tools for studying virus replication and host responses to infection. These systems are designed to model organ physiology, including unique substructures within organs, like the upper and lower respiratory tracts. 3D tissue models derived from healthy and diseased tissues can be used to understand the host response to infection and impact of virus replication on human disease. Incorporating these models into antiviral screening programs can reduce costs and save time if used as a key secondary assay. These secondary assays can provide important data about the activity of compounds in primary cell systems and help select compounds for further development that are more likely to be active in vivo or in humans. Advances in cryopreservation without losing the unique physiological properties of these models will enable wider use of these systems for drug discovery. MPS can also be used to support regulated studies for preclinical development of antiviral compounds as long as the data generated are predictable and reproducible, and can potentially inform regulatory decision making by providing complimentary information related to the safety or efficacy of an antiviral product. In this regard, proper qualification of these models and assay end points for virus replication, pathogenesis, and cytotoxicity would facilitate broader use of these model systems in drug development.

USE OF ROBUST AND REPRODUCIBLE 3D TISSUE MODELS FROM DRUG DISCOVERY THROUGH DEVELOPMENT

Because of the high cost of drug discovery, industry tends to take a conservative approach to adopting novel technologies for discovery of new drug candidates. "Just because we can doesn't mean we should" is often the default mindset. The practical application of leveraging basic science discoveries from internal and external research programs into developing new drugs is associated with opportunity costs and high attrition rates for new compounds entering the clinic from discovery that must be evaluated in cost-benefit analyses [107]. Return on investment from a commercial perspective drives decision making and motivates organizations to explore novel technologies to reduce costs and increase success rates associated with drug development. Three-dimensional tissue constructs under dynamic conditions, or MPS, could provide value to drug discovery programs by modeling disease phenotypes that are difficult to mimic in cell culture systems or animal models. MPS could also help us understand the impact of new drugs on normal physiological mechanisms and identify potential safety liabilities. MPS mimic unique human organ physiology, so data generated with early drug candidates can impact decisions on whether to advance a new drug candidate, thereby saving time and increasing success rates to provide a better return on investment. In this section, we summarize the cutting-edge applications of 3D tissue models that were discussed in session 3 of this workshop as well as discuss challenges and opportunities, including the use of MPS in regulatory filings.

Use of MPS in Antiviral Drug Screening

Early antiviral drug screening typically involves identifying inhibitors or modulators of well-defined and clinically validated targets using biochemical assays or cell-based tissue models to find new chemical matter that could be developed into novel drugs. The challenge with this process is developing model systems that accurately reflect the disease phenotype where data generated can be translated into humans. MPS provide an opportunity to probe complex biology at an early stage in the drug discovery process to help validate targets and reduce the chance that a drug candidate will fail due to safety or efficacy in later stages in the development process. Thus, model systems that can provide clear go/no-go decision points early in the discovery process save time and resources, increasing success rates while reducing opportunity costs.

To address this challenge, institutions from private and public sectors are moving towards developing MPS that can be adapted for high-throughput screens to screen for antivirals. An example of such cutting-edge work is being done by Rumi Viro (https://rumiviro.science/), where Dr Edwin Rosado-Olivieri and colleagues have developed an MPS model adapted for high-throughput screening in 96- and 384-well plates for early viral drug discovery [108–110]. This high-throughput model is highly robust and sensitive with optimal assay quality metrics such as a Z′ factor >0.5 and coefficient of variation <20%. It also has been extensively validated with neutralizing monoclonal antibodies and direct-acting antivirals that have shown antiviral activity in both preclinical models and clinical studies. Using this technology and an artificial intelligence-based computational biology platform, MPS were developed to define a set of quantitative phenotypic signatures that differentiate between normal healthy tissues and infected tissues. Such models were developed for lung, liver, kidney, and brain and adapted for high-throughput screening of chemical inhibitors that could impact the infectious disease phenotypes. These models can be used to screen up to 20 000 compounds in a single round of high-throughput drug screening. This platform is being used to identify direct acting and host-targeted antivirals for broad-spectrum activity against respiratory virus pathogens. Such systems provide an opportunity to couple screening for molecules that impact host processes important for viral replication while also identifying potential off-target effects to allow for better stratification of hits that could be developed into antiviral drug candidates. These systems offer advantages over traditional drug discovery approaches that typically use transformed cell lines, which often miss drug candidates such as those that are only active in primary cell cultures. They also identify potential safety liabilities that may not manifest in transformed cells. Adapting MPS to high-throughput screening offers a novel system to probe critical virus-host interactions and identify new antivirals for important viral diseases [108].

Use of MPS for Studying Infectious Diseases

The unique nature of MPS for studying complex physiological disease phenotypes is exemplified by work done using brain organoids to study infectious diseases of the central nervous system [111]. One example is work done at the Johns Hopkins University Center for Alternatives to Animal Testing, led by Dr Thomas Hartung, where scientists are using iPSC-derived organotypic brain cultures (BrainSpheres) to study neurological diseases and toxicity [112]. BrainSpheres embody many of the cell types found in human brain tissue, including neurons, astrocytes, and oligodendrocytes with myelinated nerves that are active at an electrophysiological level [112]. The BrainSpheres are permissive to Zika and dengue virus infections, causing a measurable inflammatory response [113]. Infection of BrainSpheres in the presence and absence of microglia with dengue fever virus (DENV-1) or 2 strains of Zika virus (ZIKV-BR, ZIKV-UG) supported low levels of virus replication over a 72-hour period, as measured by RT-qPCR of culture supernatants and immunofluorescence of infected cells. Cytokine levels (TNF-α, CCL2, IL-1B, IL-6) measured by RT-qPCR were elevated in all cultures, but there was notably

more cytokine expression in BrainSpheres cocultured with microglia. This MPS model is also susceptible to SARS-CoV-2 infection, with a small percent of neuronal cells staining positive for spike and M protein by immunofluorescence [114]. These data suggest that BrainSpheres could serve as a model system to study neurotropic virus infection and provide an assay for development of therapeutics to modulate virus-induced neurological disease [115, 116].

Use of MPS for Developing Neutralizing Antibodies for Viral Diseases

Another example of the utility of MPS in therapeutic discovery is work done at the US FDA by Drs Evi Struble and Dayton Petibone for evaluating neutralizing IgG for Zika virus to prevent transplacental virus invasion and infection of the fetus. These polyclonal and monoclonal antibodies could potentially be used to prevent fetal abnormalities associated with Zika virus infection in pregnant women. Although an important product characteristic that should be assessed prior to clinical use, transplacental migration of virus and antibody complexes is difficult to model in cell culture or small animal models. By recapitulating the most salient features of human placenta, an MPS model would provide a tool to study (1) placental infection by Zika virus, (2) placental transfer of this infection to the fetus, and (3) the role of antibodies in blocking or enhancing the infection. For this study, a stepwise approach was adopted for developing the human placenta MPS. Human placental cell cultures or a mammalian cell line expressing the FcRn receptor in a trans-well system were used to measure the migration of the Zika virus glycoprotein (or intact virus) alone or complexed with antibodies from the apical side of the cell monolayer to the basolateral side. The data showed that the Zika virus glycoprotein and intact virus can migrate to the basolateral side of the monolayer and, at the concentration used in the assay, monoclonal antibodies inhibited this process. On the other hand, Zika virus entry in the cell monolayer varied depending on the concentrations of antibody in the assay. At low and high concentrations of antibody, viral entry was inhibited, while intermediate concentrations of antibody either inhibited or enhanced virus entry [117, 118]. Thus, this assay was used to measure the effectiveness of the monoclonal antibody to neutralize virus infection in placental tissue and to generate dose-activity relationships to correlate the dose of antibody with tissue concentration and neutralizing antiviral activity. Ongoing and future work aims to expand the model to include multiple human placenta cells and fetal cells susceptible to Zika virus, aiming to provide a bridge between in vitro and in vivo models to generate data to support the continued evaluation of monoclonal or polyclonal antibody candidates.

The pharmacodynamics data generated from such unique MPS could provide regulators with information needed to support clinical development. Like any conventional assays, it is important that MPS used to support regulatory filings be rooted in strong scientific principles with qualified quantitative end points for activity and toxicological assessments. MPS should be well qualified to address important biological end points that can be translated into clinical outcomes as well as address biological processes that cannot be otherwise studied in traditional model systems. The judicial use of MPS to probe unique aspects of a therapeutic can be highly valuable in preclinical assessment of drug candidates with data generated used to support regulatory filings.

The Microphysiological Systems for COVID Research Working Group

The use of MPS for antiviral drug development has benefited from communication of research methods and sharing reagents between investigators to provide guidance for the development and execution of these models in drug discovery. To facilitate this process, the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research and the US National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) worked with additional partners (including NCATS and NIAID) to establish the Microphysiological Systems for COVID Research (MPSCoRe) global working group [119]. This working group, led by Drs Nicole Kleinstreuer and Anthony Holmes, provides a neutral forum to facilitate interaction and engagement of international research efforts in MPS, raise awareness and facilitate connections with the MPS COVID research community, understand the global regulatory needs for using MPS in drug research, and develop standards for model performance and end points. The MPSCoRe working group has developed several extensions to the open access MPS portal, the BioSystics Analytics Platform supported by NCATS, that provides a resource for information storage, analytics, and modeling (https://mps.csb.pitt.edu). The COVID-19 Disease Model Portal within this platform is a central repository for COVID-19 experimental data that is shared among working group members. The COVID-19 iPSC portal allows users to share information about available iPSC models and cells. The MPSCoRe working group is open to researchers from all sectors. They meet regularly to discuss opportunities and challenges in the field of applying MPS to infectious disease research (https://ntp.niehs.nih.gov/go/mps).

Challenges and Opportunities

The use of MPS for antiviral drug discovery allows for the evaluation of unique aspects of viral-host interactions and novel biology that is difficult to reproduce in standard cell culture models or in vivo systems. MPS may not be a replacement for in vivo preclinical assessment in the near future, but rather complement existing preclinical models to provide detailed mechanistic and toxicological data as well as support product development. While MPS have demonstrated their utility to probe unique biological end points, industry and regulatory

agencies need to better define standardized end points to enable use of these model systems for antiviral drug development. Feedback from European regulators advised researchers to use MPS that are simple, single-organ models and measure pharmacologically relevant end points to establish regulatory feasibility and confidence in the data generated [120, 121]. Industry should work to establish standards and best practices for the use of MPS in product development, as recently proposed for the liver-on-a-chip [7]. Likewise, regulatory agencies need to work with industry to help establish best practices for use of MPS from a regulatory perspective to guide product development.

SUMMARY OF DISCUSSIONS AND PERSPECTIVES ON THE CHALLENGES AHEAD

The COVID-19 pandemic presented a unique opportunity to stress test the available 3D tissue model systems as well as show how impactful these models can be in providing human-relevant insights into pathophysiology and disease mechanisms and as potential platforms for therapeutic development. MPS demonstrate clear advantages over traditional cell culture assays with transformed cell lines for antiviral drug development, and the data produced by these models are not only more predictive of in vivo efficacy but also can be used to support regulatory filings, especially in cases where the gold standard models do not perform well, such as the case of the Omicron variant and its circulating subvariants [5, 122–124]. Recent data from 2 drugs, hydroxychloroquine and imatinib, consolidated MPS as a valuable tool for antiviral development, providing data that were predictive of outcomes in animal models of SARS-CoV-2 infection and in human studies. Hydroxychloroquine effectively inhibited SARS-CoV-2 in Vero E6 cells, but not in MPS nor in humans [4, 29, 125–127]. Imatinib showed a similar pattern to hydroxychloroquine: it inhibited SARS-CoV-2 infection in cell lines but was not effective in preclinical models, including MPS, and it failed to show any effectiveness in humans [6, 128, 129]. These examples emphasize the importance and utility of data generated in MPS for preclinical characterization of drug candidates.

While there are clear advantages to the application of 3D tissue models in antiviral drug development, validation and qualification of these assays to support regulatory filings remains a challenge. For regulatory filings using data generated by MPS, model developers and end users should collaborate with regulators to discuss and develop qualification/validation criteria to ensure data generated in these systems meet expected standards for regulated studies. This type of negotiation with regulatory agencies is exemplified by recent work done by members of the IQMPS consortium (https://www.iqmps.org/), a world leader in this space, which is described in a recent publication [7]. This work focused on qualification criteria and standard compound sets for predicting drug-induced liver injury in a liver chip. This work shows a critical need to use reference compounds for validating these systems and demonstrating that they are reproducible and robust. Although progress has been achieved in validation and qualification of MPS data for regulated studies for individual programs, the quest to achieve a global consensus is still in its infancy. Hence, there is still a pressing need for global interactions and consensus building among different regulatory authorities and developers to establish standard compound sets and qualification criteria that are target-organ specific. Engaging in frequent, iterative cycles of communication with regulatory authorities early in the drug discovery process will help developers design MPS and generate data that will meet the standards required for regulated studies to support development of drug candidates.

Disseminating information about the opportunities to engage with regulatory authorities and harbor advice on developing 3D models is important. To that end, scientists can contact one of the different working groups and centers listed below to start a dialogue and obtain regulatory advice about using these models:

- The Alternative Methods Working Group at FDA (https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda)
- Relevant working groups at European Medicines Agency
    - 3Rs Working Party (https://www.ema.europa.eu/en/committees/working-parties-other-groups/chmp/3rs-working-party)
    - Innovation Task Force (https://www.ema.europa.eu/en/human-regulatory/research-development/innovation-medicines)
- MPSCoRe working group coorganized by NICEATM and others [119].

There is a need to address the gap in accessibility and transferability of these models with guidelines for standardization to leverage them for antiviral drug development. Providing access to these models and proper guidance on their use to virologists who are actively engaged in antiviral drug development is crucial for the advancement of the field of MPS research and for selection of antiviral compounds that can be developed into drugs. One way to achieve this goal is through collaborations between virologists and platform developers across institutions [102, 105, 106]. It is also imperative for the community to work together to create metrics for standardization across platforms and institutions.

This workshop was designed to provide guidelines and considerations for developing robust and reproducible 3D tissue models that can be used for discovery of antiviral therapeutics. As part of this workshop, best practices for MPS were discussed and examples of how these models are used to study

virus replication and biology were presented. Additionally, gaps in research related to the affordability, accessibility, transferability, and reproducibility of these 3D tissue models were identified. These gaps will be addressed in the future with continued research in this area. The benefits of MPS in antiviral research were showcased in this workshop by the many sophisticated methods and platforms already in use in antiviral drug discovery programs. Research will continue to innovate and improve these model systems and address some of the gaps identified, bringing us close towards adoption of MPS as a routine part of antiviral drug discovery and development.

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