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Feature

Harnessing the power of microphysiological systems for COVID-19 research

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The pharmaceutical industry is constantly striving for innovative ways to bridge the translational gap between preclinical and clinical drug development to reduce attrition. Substantial effort has focused on the preclinical application of human-based microphysiological systems (MPS) to better identify compounds not likely to be safe or efficacious in the clinic. The Coronavirus 2019 (COVID-19) pandemic provides a clear opportunity for assessing the utility of MPS models of the lungs and other organ systems affected by the disease in understanding the pathophysiology of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and in the development of effective therapeutics. Here, we review progress and describe the establishment of a global working group to coordinate activities around MPS and COVID-19 and to maximize their scientific, human health, and animal welfare impacts.

Keywords: Microphysiological systems; MPS; COVID-19; SARS-CoV-2; 3Rs

Introduction

Human health has been challenged by previously unknown emergent infectious diseases since the dawn of humanity. However, the increasing incidence of potentially pandemic diseases in the 21st century, including influenza, Middle East Respiratory Syndrome (MERS), and SARS, has highlighted how difficult these diseases are to prevent and contain. In December 2019, SARS-CoV-2 and the associated COVID-19, emerged and spread rapidly around the world. By May 2021, over 163 million cases had been confirmed in more than 220 countries, with nearly 3.4 million deaths reported.[1]

Developing novel and effective diagnostics, therapeutics, and vaccines requires a clear understanding of the characteristics of the virus, including mechanisms of cell entry, replication, and transmission. The response of the global biosciences research community in meeting this challenge has been unprecedented. Researchers put aside competition and came together across sectors and borders to identify, within months, essential features of the SARS-CoV-2 virus, including angiotensin-converting enzyme II (ACE2) dependency for cellular entry, the structure of the spike protein, and patterns of viral replication. Animal models, including mice, hamsters, ferrets, and nonhuman primates,[2] contributed to this understanding, but they have limitations. Their varied susceptibility to disease and symptoms that can differ

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substantially from the human condition emphasizes the need to concurrently develop and apply models based on human biology.

The role of microphysiological systems

Until recently, studies of infectious diseases were limited by models that failed to sufficiently mimic certain aspects of host–virus interactions and organ-level functions. The past decade has seen rapid advances in bioengineering and the emergence of a new field of research focused on the development and application of MPS, *in vitro* platforms of complex cell models in a microenvironment that mimics biochemical, electrical, and mechanical responses to model organ-level function.[3–5] These systems include organ-on-a-chip, organoid, and bioreactor models, with multiple human cell types under physical forces, such as fluid flow, interacting to create complex structures and reproduce biological activities, such as barrier function,[6] membrane transport,[7] neuronal network signaling,[8,9] thrombosis,[10] and metabolism.[11,12]

Originally developed to bridge the gap between monolayer cultures of immortal or primary cell lines and animal models in recapitulating human biology in drug development, MPS are now applied across the biosciences. The diversity of MPS available provides researchers with a plethora of models and tools to address their specific research needs. However, as with any model system, they have their limitations and, thus, appropriate application of different MPS relies on their context of use and the scientific question driving the study. Here, we briefly describe some of the main advantages and disadvantages of two of the most widely used MPS approaches, organoids and organs on chips. More detailed reviews of the utility of organoids,[13] organs on chips,[14] and the design, fabrication, and application of MPS more broadly[15] have been provided by others.

Organoids

Organoids are 3D organotypic multicellular *in vitro* tissue constructs usually derived from adult or pluripotent stem cells. Methods for generating organoids vary, but often cells are provided with minimal differentiation information, relying instead on intrinsic self-organisation to shape the tissue. Although this method has been applied widely because of its simplicity, it also results in random configuration, making the production of these biomimetics variable from batch to batch, between multiple organoids within a culture, and between areas of a single organoid itself. They also lack vasculature, representative microenvironments, and immune cells, limiting their size and utility in studying processes that require these components. Despite this, their ability to mimic functional characteristics of native organs make them attractive tools for modeling various aspects of basic biology and in safety and efficacy testing early in drug development.

Organs on chips

Organs on chips are microfabricated cell culture devices that recapitulate the functional units of organs *in vitro*. The hollow microfluidic channels within the organ chips are lined with organ-specific cells on one surface and vascular endothelial cells on the other. Media flow rates and mechanical forces can be applied and exquisitely controlled to mimic the physical microenvironment of the organ being studied. For example, lung chip models comprise alveolar epithelial cells cultured in one compartment and pulmonary vascular endothelial cells cultured in another compartment separated by a thin flexible membrane. Each channel can be exposed to different media (e.g., air in the epithelial channel and a blood mimetic in the endothelial channel) and, all the while, the chip is cyclically mechanically stretched to simulate breathing.[16] The manner in which organ-on-a-chip models are fabricated allows for precise control of cellular and tissue architecture, resulting in consistent products with relatively low variability between chips. It is also possible to incorporate tissue function sensors to measure different endpoints in real-time, such as transepithelial electrical resistance to determine barrier function and fluorescent biomarkers for concurrent imaging.

MPS have great potential to reproduce some human responses more faithfully than currently used animal models (Fig. 1).[17] For example, they could replicate interactions between SARS-CoV-2 and various organs (e.g., kidney, lung, or blood vessels) and related immune reactions, as well as rapidly assess the effectiveness of existing and new therapies.[18,19] They also offer significant opportunities to reduce the reliance on animal models and make

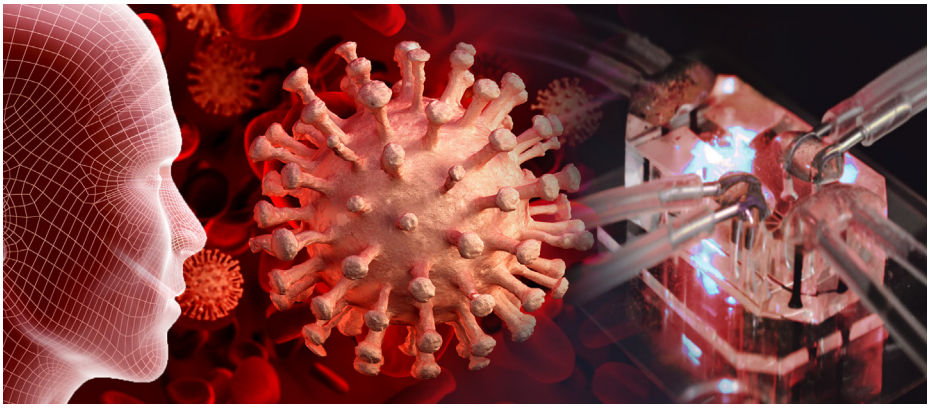

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FIGURE 1

Organ-on-chip approaches are human-relevant systems to understand infectious disease mechanisms and design effective treatment and prevention strategies.

substantial cost savings across the Big Pharma R&D pipeline.[20–22]

Their potential utility is reflected in the significant number of new models for assessing the impact of SARS-CoV-2 on the lungs and other organ systems affected by the disease. The opportunities afforded by MPS in this regard have been reviewed by others,[23–25] but the recent literature is full of examples of MPS being applied to specific research questions related to understanding the pathophysiology of the disease and its treatment. Much of this research focuses on the lungs for obvious reasons. Lung-on-a-chip and lung organoid models infected with native virus reveal important aspects of both infection response and viral replication. Primary human lung alveolar cells cultured as organoids have been used to corroborate the dependence of SARS-CoV-2 on ACE2 for cell entry, the role of interferon-associated and proinflammatory genes in the robust innate immune response to virus, and to better understand the kinetics of viral replication.[26,27] These findings have been further supported by studies using lung chip models, which include a vascular compartment maintained under flow and which enable infection at the air–liquid interface, physiological features that are lacking in organoid models. Furthermore, incorporating circulating immune cells in these models has helped to elucidate the interplay between viral infection and immune cell recruitment and its role in barrier injury and exacerbated inflammation.[28] They have also been used to demonstrate that damage to the vasculature within the lungs caused by SARS-CoV-2 is a direct consequence of endothelial infection, rather than an indirect consequence of immune cell-mediated cytokine storm and the formation of a pro-coagulatory environment, which has also been reported in animal and clinical studies.[29] However, it is now well recognized that COVID-19 is not just a disease of the lungs, with significant clinical evidence of the virus infecting organs in which ACE2 receptors are prevalent, such as brain, kidneys, and gut. MPS have a significant role in understanding the impacts of SARS-CoV-2 in these organs. Examples include the use of brain organoids to examine SARS-CoV-2 neurotropism,[30] inhibition of infection by human recombinant soluble ACE2 of human blood vessel and kidney organoids,[18] and the use of gut organoids to demonstrate infection and replication of virus within the small intestine.[31] These studies highlight the potential utility of MPS as experimental models that will aid our understanding of the viral life cycle, replicate the mechanisms leading to disease, and allow preclinical evaluation of molecule-level therapies, such as repurposing existing drugs for COVID-19 treatment.

Such a response to a singular focus on a global scale is laudable, but risks being fragmented, increasing the chances of duplicating effort and already overstretched resources. Significant challenges exist for model developers, drug and vaccine manufacturers, and international regulatory authorities to coordinate their efforts and understand the use, validity, and context of use of the many systems being developed. Given the rapidly increasing number of available MPS models for SARS-CoV-2 research, how do potential end users navigate through this sea of models to identify the right ones for their needs? When you include the abundance of already available models that could be used for COVID-19 research applications, the task can be compared to looking for a needle in a haystack. Given how much is riding on these choices, it is important that the right ones are made.

Coordinating global efforts

This very question was posed by Ewart and Roth.[32] They recommended that, to accelerate MPS model adoption and application, an inclusive strategy is needed in which industry, academics, government, and model developers work closely together and communicate frequently. This is not an unexpected solution to the problem but achieving such a vision is not a straightforward task because it requires significant resources and commitment to establish a framework to globally connect such diverse stakeholder groups. The UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) and the US National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) have joined forces to provide leadership in this space. Working in partnership with the US National Institute of Allergy and Infectious Diseases, Division of Microbiology and Infectious Diseases, US Army DEVCOM Chemical Biological Center, and US National Center for Advancing Translational Sciences (NCATS), the MPS for COVID Research (MPSCoRe) working group has been established to coordinate global tissue-chip and other MPS efforts to study COVID-19 and future infectious disease applications. The aim is to connect researchers, methods developers, drug and vaccine manufacturers, and regulators to collectively maximize the scientific, animal reduction, and public health impacts that tissue-chips offer in better understanding and treating COVID-19, and to support their adoption to prepare for future diseases with epidemic potential. Open communication among key stakeholders is crucial to understand the various needs and applications in infectious disease research and therapeutic development, such that MPS can be designed and selected to be 'fit-for-use' to address specific questions.

The working group was conceived to deliver six key objectives: (i) provide a centralized, neutral forum to enable interaction and engagement between international collaborative research efforts; (ii) connect technology developers and end users in raising awareness of available COVID-19 and MPS technologies to support their application in assessing the safety and efficacy of potential novel therapeutics; (iii) work with regulatory authorities to communicate global regulatory needs and decision contexts to identify crucial model variables and end points that can contribute to regulatory guidelines and aid product development; (iv) provide cross-discipline and -sector expertise in characterizing model performance and readiness criteria; (v) support the assessment of these novel *in vitro* model systems against gold-standard preclinical (*in vivo*) and human clinical data that are concurrently generated; and (vi) ensure the animal reduction opportunities these model platforms offer are recognized.

The project is in the early stages, but it is already supporting the expansion of the Microphysiology Systems Database (MPS-Db)[33] to include a COVID-19 disease portal (https://mps.csb.pitt.edu/diseases/covid-19/). To accelerate the development and adoption of human MPS for testing therapies and improve disease understanding,

researchers are able to share experimental data, analytic tools, model designs, and study components through the online portal. The portal went live in April 2021 and has collated a significant number of links to further information and resources to support the development and application of MPS better able to recapitulate the pathophysiology of COVID-19 in various organ systems. Details of commercially available MPS, as well as components used in designing and implementing SARS-CoV-2/COVID-19 studies, have been uploaded to the platform to support access to existing MPS and the development of new models. The next phase of platform development will see MPSCoRe members upload and share their own COVID-19 MPS models and study data generated by them. Model details and data collected in the portal include model schematics, cell sources/types, key references, model variations, study designs, and data generated in response to various stimuli (both assay data and associated metadata). Depending on the permissions structure assigned by the primary user, other users of the database can access these data and use the inbuilt modeling capabilities to reanalyze them and, therefore, maximise the potential impact of each individual study. The development of the COVID-19 disease portal and the creation of a comprehensive centralized hub for COVID-19 infection and pathogenesis in the MPS-Db will significantly improve the speed and efficiency with which researchers obtain the information required to inform the design, development, and application of human MPS experimental models for therapeutic development.

Additional programs of work are under development and will focus on addressing the key challenges affecting the uptake of MPS models for SARS-CoV-2 research, as identified through a survey of the MPSCoRe working group membership. The survey was completed by a diverse group of individuals largely representing academia (46%), industry (21%), and different government agencies (21%), who work in a range of areas spanning method and therapeutic development, research applications, and regulatory decision-making. The

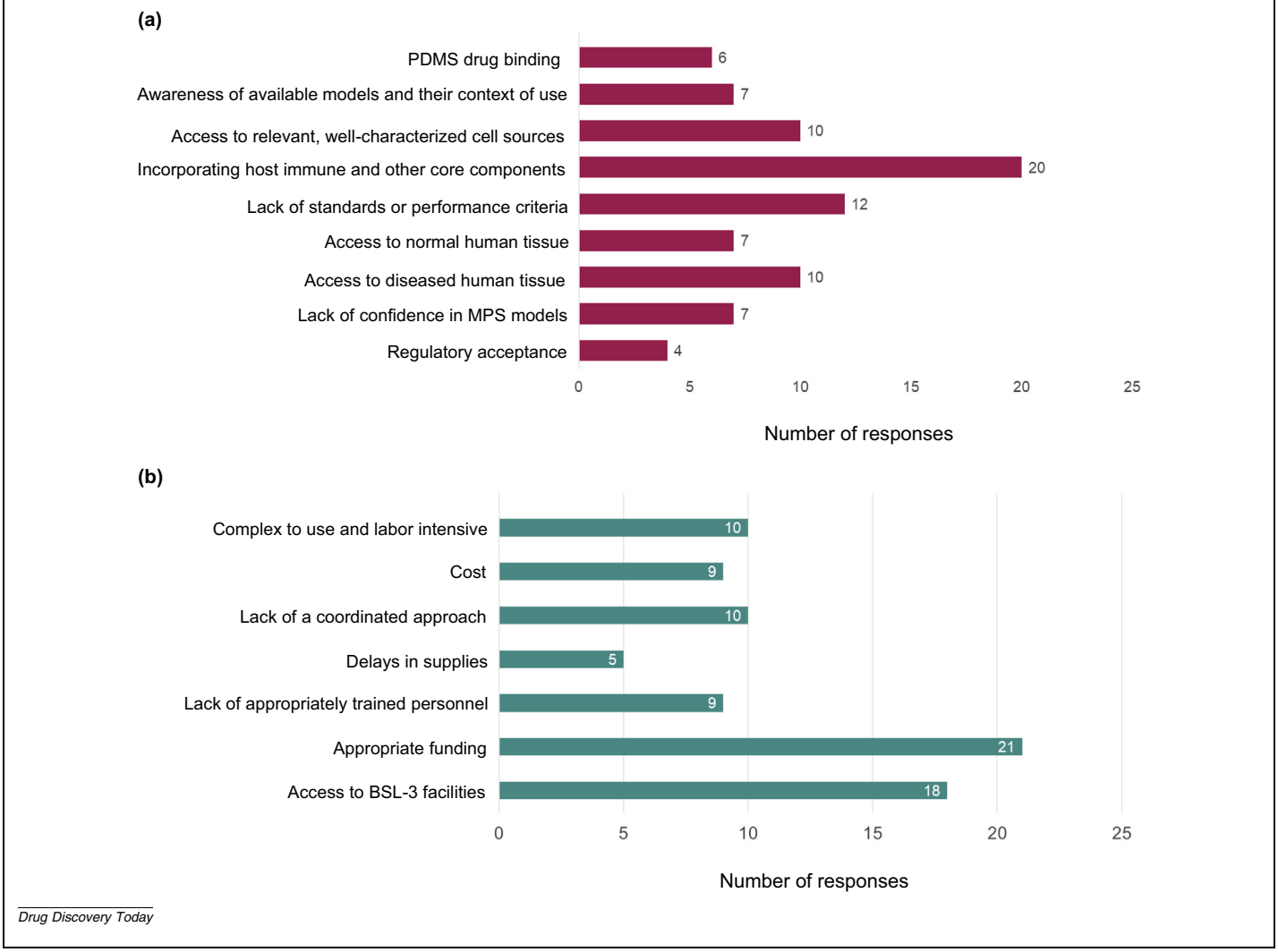

FIGURE 2

Challenges affecting the adoption of microphysiological systems (MPS) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) research and drug development. MPS for COVID Research (MPSCoRe) working group members were asked to select their top three scientific **(a)** and practical **(b)** challenges/barriers affecting the use of MPS for SARS-CoV-2 research.

remaining 12% of respondents included regulators and those that selected 'Other' because they do not fit within the specified categories. Respondents were asked to select their top three scientific and practical challenges to the wider adoption of MPS for SARS-CoV-2 research (Fig. 2). The MPSCoRe working group identified a variety of scientific challenges, including model complexity and incorporation of innate and adaptive immune responses, and practical challenges, such as the need for model standardization, interlaboratory characterization, and defined performance criteria, and access to appropriate biosafety-level facilities, funding streams, and repositories of human tissues and cell sources. The responses suggest that multiple factors contribute to the problem. On their own, each might be resolvable but, when combined, might prove to be an overwhelming challenge.

The experience and expertise within the working group will be instrumental in developing strategies to address the challenges highlighted as barriers to the more widespread adoption of MPS as integrated approaches in the researchers' armory of tools to understand and treat COVID-19 and other infectious diseases. The next stage of the project will be identifying the key priorities and developing distinct, collaborative programs of work aimed at expediting MPS development and adoption. A central tenet of the MPSCoRe group is to ensure opportunities exist for MPS model and therapeutics developers to share their models, data, and research needs and so it will continue to deliver regular workshops and webinars (recordings available online at https://ntp.niehs.nih.gov/go/mps) that will be accessible to the wider research community to inform progress and facilitate wider engagement. It is hoped that, through the MPSCoRe working group, it is possible to support the global research community in fully realizing the scientific, human health, and animal welfare impacts that MPS offer for infectious disease research. The rapidly expanding body of work within the MPS and COVID-19 research fields demonstrates the enormous potential of these physiologically relevant systems in recapitulating human disease attributes, providing mechanistic insights, and supporting the design of effective therapeutic strategies.

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