Commentary

Organs-on-chips: Progress, challenges, and future directions

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Impact statement

This work is important to the field as it outlines the progress and challenges faced by the NIH Microphysiological Systems program to date, and the future of the program. This is useful information for the field to be aware of, both for current program stakeholders and future awardees and partners.

Abstract

The National Institutes of Health Microphysiological Systems (MPS) program, led by the National Center for Advancing Translational Sciences, is part of a joint effort on MPS development with the Defense Advanced Research Projects Agency and with regulatory guidance from FDA, is now in its final year of funding. The program has produced many tangible outcomes in tissue chip development in terms of stem cell differentiation, microfluidic engineering, platform development, and single and multi-organ systems—and continues to help facilitate the acceptance and use of tissue chips by the wider community. As the first iteration of the program draws to a close, this Commentary will highlight some of the goals met, and lay out some of the challenges uncovered that will remain to be addressed as the field progresses. The future of the program will also be outlined.

Keywords: Bioengineering, microphysiological systems, microfluidics, induced pluripotent stem cells, National Institutes of Health

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The NIH program to date

High attrition rates of promising drugs during the development process are largely due to a lack of safety and efficacy, and it is clear that better predictive tools are needed for the process.[1–3] The National Institutes of Health (NIH) Microphysiological Systems (MPS) program was initiated to help develop alternative tools to aid in this process, supporting the development of MPS platforms that were to be populated with human cells and were to recapitulate functional and physiologically relevant model organ systems *in vitro*, to provide potentially more reliable readouts of toxicity and efficacy than those afforded by current two-dimensional cell culture and animal models.[4] This program, begun in 2012 alongside a parallel program from the Defense Advanced Research Projects Agency (DARPA), and with input and guidance from the Food and Drug Administration (FDA), is now in its final year of funding.

The program consisted of two companion programs, the first of which was a five-year biphasic UH2/UH3 cooperative agreement mechanism with the main goal of representation of multiple organ system platforms, and the other, which was a two-year U18 program focused on the development of renewable cell sources for the platforms, either through use of commercially available primary or stem cell lines, or the development and banking of induced pluripotent stem cell (iPSC) lines. The first two-year UH2 phase of the five-year program aimed to develop and validate microsystems from a variety of organ systems. The second three-year UH3 phase placed an emphasis on integration of the platforms, functionally and/or physically, in order to move towards a goal of a 'human-on-a-chip' that would represent multiple organ systems in concert with each other, and create the most advanced and transformative predictive tools for drug development currently available.

The development and use of iPSCs in the U18 program was encouraged for a number of reasons. Firstly, it removes the need for primary tissue from adult donors, and provides a renewable cell source to populate tissue chip (TC) platforms. Seeding chips with iPSCs from patient-derived sources also allows recreation of organ systems that mimic the *in vivo* biology of that individual or patient population. This possibility could be potentially transformational for screening compounds, testing therapeutics and gene editing techniques, and even clinical trials using TCs in the future. The benefits of using iPSCs also extend to creating better standardization of protocols and outcomes, and introducing the possibility of having a common cell source for differentiation into a variety of tissues. The push for inclusion of iPSCs as renewable cell sources was part of a strategy for enhancing the potential future utility of TC technology, with the anticipation of the use of chips for disease modeling and individualized medicine in the upcoming years.

The management of the NIH program has been innovative in a number of ways. Most importantly, it has focused on fostering partnership and collaboration between research groups and external stakeholders. Biannual in-person meetings alongside DARPA-funded performers from the Wyss Institute and MIT have contributed to advances in this collaborative approach. Unlike most other NIH grants, the MPS program awards are milestone-driven cooperative agreements, in which government officials from a number of Institutes and Centers at the NIH, as part of a trans-NIH working group, are involved with the researchers on each project, receiving regular progress reports and providing feedback to researchers. Milestones and timelines dictate the progress of the projects—failure to meet them, or inadequate progress towards corrective measures to meet them, can be the basis for negotiating changes in direction, or stopping projects.

The in-person meetings of the Tissue Chip Consortium are attended by TC developers, NIH program staff, FDA representatives, biotechnology companies with which Memorandums of Understanding—MOUs—have been signed and, more recently, members of the IQ Consortium to represent the pharmaceutical industry. These meetings serve to update the consortium on progress made to date, as well as to connect subject matter experts. Together with milestone-driven goals of each project being monitored by NIH management, and careful use of administrative supplements to enable different teams to collaborate on common challenges, strong interactions have been formed among consortium members. Additionally, breakout sessions with directed discussion points, Town Halls, and poster sessions help to facilitate progress by addressing current challenges, and allow input from all stakeholders. This type of dialogue and feedback between developers, government agencies, and the private sector is critical for the formation of successful public–private partnerships[5,6] and, ultimately, the goal of making TC technology viable, accessible, and useful to the research and industry communities.

Progress and challenges

Progress

The NIH and DARPA programs commenced after significant groundbreaking work had been accomplished in the field of generation of self-organizing organoids and early microphysiological systems.[7–9] In 2010, the NIH and FDA co-funded the Advancing Regulatory Sciences initiative, a by-product of the joint NIH-FDA Leadership Council. One of the awarded programs from this initiative was the Harvard Wyss group's application to develop a ''Heart-Lung Micromachine'', which led to pioneering work modeling the ''lung on a chip'',[10–12] then a DARPA, FDA, and NIH workshop on the promise of MPS, and later the launch of the NIH and DARPA MPS programs in 2012. Since that time, MPS program awardees have developed an array of diverse platforms that recapitulate physiologically relevant *in vitro* conditions—many of which are detailed in this issue.

Among progress by Consortium members, the field has seen adaptation of human iPSC cardiomyocyte differentiation protocols (hIPSCs)[13] to create an MPS containing a spontaneously beating model of the human myocardium, showing drug responses more similar to *in vivo* responses than traditional 2D models.[14] As detailed in this issue, alternative cardiomyocyte MPS platforms have been bioengineered to measure contractile forces of cardiac tissue on pre-existing scaffolds (Teles *et al.* in this issue) or on so-called ''muscular thin films''.[15] Extending this work, both of these systems have been coupled to other organ systems to monitor drug toxicity,[16,17] and even used to model disease states such as Barth syndrome,[18] drug-induced valvular heart disease,[19] and dilated cardiomyopathy.[20] Additionally, optical signaling, by the inclusion of fluorescent dyes to monitor calcium influx or inclusion of ''sentinel'' cells expressing fluorescent protein biosensors,[21] has allowed real-time readouts on cell activity and health, which when coupled with electrophysiological measures such as transendothelial electrical resistance (TEER)[22] give extensive information on cell and platform functionality.

Other accomplishments of the program include advances in neural tissue engineering. For example, as they will discuss in this issue, the Thomson/Murphy groups in Wisconsin have created groundbreaking human embryonic stem cell-derived neural cell constructs which self-organize into *in vivo*-like neural structures and are proving useful for fast predictive developmental neurotoxicity screening.[23] Creation of a novel blood–brain barrier (BBB) platform which models, for the first time, the interface between vascular and brain tissues,[24] plus its potential for use in drug and therapeutic development, will also be discussed in this issue by Brown et al. Other MPS platforms developed within the program include blood vessel vasculature from iPSC-derived endothelial cells[25] or blood endothelial progenitor cells,[26] as well as vascularized microtumors[27] which recreate physiologically relevant vascularized tumorgenesis *in vitro*[28] and enable dynamic interactions between tissues and tumors, as well as effects of chemotherapeutics on healthy and cancerous tissues, to be probed in ways never before possible. These models of tissue-specific vascularization will also be useful as the importance of the endothelial lining of tissues is further clarified by the scientific community, and microphysiological systems can provide crucial information to pave the way for more complex tissue models. Additionally, Consortium researchers have developed physiologically relevant models of the liver that metabolize drugs, produce albumin and show immune-mediated toxicity,[21] and a kidney proximal tubule model which shows secretory and reabsorption properties as would be seen *in vivo,*[29] both of which offer substantial promise for screening of potential therapeutics in the drug development process.

Supplemental funding provided during the course of the program has been targeted to expand the organ systems represented within the program and address universal challenges facing the field. For example, invitations to model missing organs have led to projects adapting existing platforms to model the testis, subchondral bone, adipose tissue, retina, and bone marrow. Other projects have used CRISPR-Cas9 technology to perform gene editing of the stem cells used to seed devices, to create cell lines with

Table 1 Some key challenges to platform integration

Biological challenges
Appropriate organ scaling
Creation of a universal media
iPSC cell sourcing
Vascularization of tissues
Inclusion of immune components
Consideration of circadian and other cycles on cells
Technical challenges
Drug adsorption and binding to PDMS
Connection of platforms to maintain sterility and avoid bubbles
Flow rate differences between platforms
Inclusion of biosensors
Creating ideal oxygenation and nutrient levels for different organs

iPSC: induced pluripotent stem cells; PDMS: polydimethylsiloxane.

isogenic backgrounds, that are available to all Consortium members.

Challenges

One key focus of the second phase of the program has been integration of organ platforms. This is not a trivial task and requires overcoming many challenges (see Table 1). The most straightforward way to functionally connect multiple organ systems is to sequentially collect media from the output of one platform and feed it into another, i.e. from gut to liver to kidney, to mimic the sequential adsorption, distribution, metabolism, and excretion of compounds and in turn investigate unexpected biological actions of metabolites on other organ systems.

A more elegant and informative solution to functional coupling is physical connection, which is being addressed by MPS researchers and yields promise for drug toxicity screening studies. However, to directly physically couple multiple MPSs, some key biological and technical challenges from Table 1 need to be addressed. Firstly, there is a need for a universal medium for perfusion of different cell types. The MPS program has partnered with ThermoFisher Inc. to work on the challenge of providing nutrients for multiple cell types in a universal media, and supplemental funding for a blood mimetic project has shown that endothelial barriers between tissue types may help regulate molecular transport between a common media and specific tissue populations in linked systems. Additional challenges include the important scaling issue—that correct scaling of organ sizes and cell numbers needs to be taken into account, so that the ratios of active cells between platforms are physiologically relevant and responses to challenges and compounds are accurate. Furthermore, incorporation of an immune component will be a critical addition to the linked systems in the future, as immune responses can shift drug dose responses. Addressing these issues will be important for increasing the validity and utility of TCs for toxicity screening applications.

Technically, a number of challenges continue to be addressed for the physical coupling of organ platforms (see Table 1). NIH-funded teams have demonstrated successful functional integration of a number of systems—from linkage of human fallopian tube and ovarian tissue systems,[30] to development of integrated heart–liver–vasculature systems,[16] to collaborative work addressing the processes and challenges involved in functional and physical linkage of liver, gut, BBB, kidney, and vascular tissue platforms.

The program has seen a diverse range of microfluidic platform designs, but each is different and, like the organ functions they represent, have different flow rates that are appropriate for one organ system but may not be compatible with others. Indeed, hepatic oxygen zonation in the liver can be modeled by alterations in flow rate of the cell medium within that MPS platform alone, so connecting multiple organ systems requires complicated engineering solutions for controlled flow rates. Also, bubbles can easily be introduced when connecting platforms, and on microfluidic devices can not only disrupt cell culture, but cause a loss of sterility within systems. Finally, many platforms use polydimethylsiloxane (PDMS), a clear, flexible, and cheap plastic, for platform fabrication. However, PDMS is highly lipophilic and binds many drugs and compounds such that concentrations introduced to the platforms may have to be hundreds of times higher than that which reaches the tissues eventually. Again, supplemental funding throughout the program has addressed this issue, and investigators continue to work on exploring the modification of PDMS, coating it with inert polymers or investigating treatments such as with gas plasma or UV light, or by investigating the mathematical modeling of adsorption to create algorithms that can account for adsorption and inform experimental design.[31]

Cell sources have been and will continue to be a challenge for these platforms. It is not straightforward to differentiate all tissues from iPSCs, and primary cells from donors or patients can be hard to come by. To help address the challenges of using heterogeneous iPSCs on the platforms, MPS program scientists have created cell lines with isogenic backgrounds, some incorporating fluorescent biosensors, to monitor cell differentiation into specific cells. Additionally, the use of genetic editing technology such as CRISPR Cas9 can be used to generate series of differentiated cells of different tissues, whose lineage is known. In future, this technology can also allow for the possibility of silencing or activating disease-associated genes, allowing remarkable potential for research using these platforms on many disease states and treatments.

Some MPS use primary cells and/or populate their platforms with commercially available cell lines, such as those for kidney proximal tubule[29] and liver.[21,32,33] Others, such as the female reproductive system organ platforms developed by Woodruff and colleagues[34,35] and detailed by Burdette et al in this issue, for now, rely on a mixture of animal and human primary tissues due to difficulties in sourcing the tissues. However in spite of this mixed species approach, these organ platforms have already shown great promise for clinical applicability and a major accomplishment has been reprogramming the mouse ovary to cycle every 28 days as in human, as opposed to every nine days in mice.[30]

Future of the program

Though DARPA will end its support of MPS technology beyond the initial five-year investment, NIH will continue to provide leadership and support in this field. Building upon the outcomes of the original program, the National Center for Advancing Translational Sciences (NCATS) recently announced a second bi-phasic five-year program (RFA-TR-16-017) to invest over $80M in the continued development and application of MPS platforms for disease modeling and efficacy testing. The new program, to begin in mid-2017, consists of partnerships with 10 Institutes and Centers at NIH (National Heart, Lung and Blood Institute, National of Arthritis and Musculoskeletal and Skin Diseases; National Institute of Biomedical Imaging and Bioengineering; the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development; National Institute of Dental and Craniofacial Research; National Institute of Diabetes and Digestive and Kidney Diseases; National Institute of Environmental Health Sciences; National Institute of Neurological Disorders and Stroke; and Office of Research on Women's Health) and reflects the broad range of interest across the biomedical field that TC technology is generating, with calls to represent organ systems including circulatory, endocrine, musculoskeletal, and neural. Additionally, NCATS recently announced a four-year program (RFA-TR-16-019), in collaboration with the Center for Advancement of Science in Space (CASIS), to take advantage of the microgravity environment of the International Space Station National Laboratory (ISS-NL). This program will support the adaptation of existing microfluidic platforms to model disease states and organ pathology at the ISS-NL. Data collected under this program will facilitate the understanding of disease mechanisms, as well as assessment of biomarkers and efficacy and toxicity of therapeutic agents prior to entry into clinical trials. CASIS is the non-profit organization managing the research conducted at the ISS-NL, and is leveraging this unique microgravity environment for a variety of biological, materials and technical research that will produce results with benefits both on Earth and in space. Both of these initiatives will continue the cooperative agreement model utilized by the first iteration of the NIH program. DARPA's program will conclude in mid-2017.

Organ-on-chip technology continues to develop at a rapid pace and the future holds much promise for its continued development. In order to deliver on this promise however, realistic expectations must be discussed and agreed upon within the field. As teams continue to develop and improve platforms, there will be a continued need for feedback from industry partners and the FDA to inform researchers on what is to be expected in terms of the characterization and validation phases necessary before the technology is ready for industrial and therapeutic applications. Functional characterization has been a key focus of the initial TC program, as developers have modeled the appropriate physiological functionality of organ systems, and characterized responses to known pharmacological agents. The next step towards assay validation requires proof of reproducibility between labs and analytical validation. To this end, NCATS recently announced awardees for two independent Tissue Chip Testing Centers and a Tissue Chip Database Center, which are tasked with reproducing platforms and running basic tests for functionality and toxicity as have already been demonstrated in the developers' labs. Over the two-year funding period, the data from these two Testing Centers will be fed to the Database Center, which will help house and organize the large data sets that will be created, and make data available to Consortium members and stakeholders. This is a preliminary step towards assay validation that conforms to guidelines by international and national bodies such as the Organisation for Economic Cooperation and Development (OECD) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), and will aid progress towards eventual therapeutic, commercial and industrial applications. NCATS envisions that in the future, commercial and industrial validation will be in part done by contract research organizations (CROs), providing support for use of TCs via outsourced contracts.

Partnerships and collaborations

One of the defining features of the NIH-funded program has been collaboration from multiple stakeholders. As the MPS program spans a wide range of organs, systems, and associated disorders, the NIH community has been widely involved in contributing expertise and sharing information, with input from over 12 Institutes and Centers. Additionally, partnership with FDA has been, and will continue to be, crucial for regulatory insight and feedback for questions regarding reproducibility, standardization, reliability, and assay validation. As noted, TCs will undergo rigorous testing for all of these, with the goal of validation and acceptance by regulatory agencies, so the FDA's continuous involvement helps investigators define appropriate project milestones and goals to address crucial issues that are required for implementation and acceptance.

Finally, invaluable commercial insight is brought to the consortium by representatives from the IQ consortium—a non-profit panel of pharmaceutical industry representatives whose mission is to aid and augment the capability of member companies to bring transformational solutions to benefit the whole research community, and ultimately, patients and healthcare providers. As the initial application of new MPS technology will likely be in the earlier stages of the drug development pipeline to help identify potential compound toxicities earlier, industry insight is critical for development of marketable and useable platforms. Along with a recognition from academic investigators and investors (including NIH small business programs) of the great potential of MPS platforms, input from industry partners throughout the MPS programs has also helped contribute to the formation of spin-off biotech companies from academia, as well as those offering toxicological and efficacy analytic services to the community using MPS technology in a CRO model. These commercial applications of MPS technology are leading the way towards the program aims of making the technology more widely available to industry, regulators, and academic researchers.

The insight provided by the pharmaceutical and biotechnology communities remains invaluable. Many developers have already established fruitful partnerships with industry, but the public–private partnerships fostered by the TC program help build a common framework for reproducibility and early phases of analytical and assay validation as steps towards commercialization.[5] These relationships between developers, regulatory, and industrial partners will remain critical for the continued development and advancement of MPS technology, as open dialogue and a sense of shared purpose foster strong partnerships. This, in turn, helps accelerate the pace of platform development, refinement, implementation, and regulatory acceptance needed for more widespread commercialization. It also addresses one of the program's key aims: to develop these platforms as useful tools, and encourage the adoption of platform technology for widespread use and translational utility for the understanding and treatment of human disease.

Conclusion

NIH, FDA, and DARPA-funded investigators have made great progress in the development of microphysiological systems over the past decade. Since the inception of the NIH Tissue Chips for Drug Screening program in 2011, MPS developers have successfully demonstrated the feasibility and utility of a multitude of organ systems, of which the list is ever-growing. The challenges of organ integration continue to be addressed, and input from all stakeholders—developers, end-users and regulatory bodies—is critical for the continued development of the technology. In future, NIH investment will focus on the use of TCs for disease modeling, pushing towards MPS use for both individualized precision medicine, and clinical trials on chips for transformative effects on the therapeutic development process.

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