Commentary

Fitting tissue chips and microphysiological systems into the grand scheme of medicine, biology, pharmacology, and toxicology

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Impact statement

Microphysiological systems (MPS), which include engineered organoids and both individual and coupled organs-on-chips and tissue chips, are a rapidly growing topic of research that addresses the known limitations of conventional cellular monoculture on flat plastic – a well-perfected set of techniques that produces reliable, statistically significant results that may not adequately represent human biology and disease. As reviewed in this article and the others in this thematic issue, MPS research has made notable progress in the past three years in both cell sourcing and characterization. As the field matures, currently identified challenges are being addressed, and new ones are being recognized. Building upon investments by the Defense Advanced Research Projects Agency, National Institutes of Health, Food and Drug Administration, Defense Threat Reduction Agency, and Environmental Protection Agency of more than $200 million since 2012 and sizable corporate spending, academic and commercial players in the MPS community are demonstrating their ability to meet the translational challenges required to apply MPS technologies to accelerate drug development and advance toxicology.

Abstract

Microphysiological systems (MPS), which include engineered organoids (EOs), single organ/tissue chips (TCs), and multiple organs interconnected to create miniature *in vitro* models of human physiological systems, are rapidly becoming effective tools for drug development and the mechanistic understanding of tissue physiology and pathophysiology. The second MPS thematic issue of *Experimental Biology and Medicine* comprises 15 articles by scientists and engineers from the National Institutes of Health, the IQ Consortium, the Food and Drug Administration, and Environmental Protection Agency, an MPS company, and academia. Topics include the progress, challenges, and future of organs-on-chips, dissemination of TCs into Pharma, children's health protection, liver zonation, liver chips and their coupling to interconnected systems, gastrointestinal MPS, maturation of immature cardiomyocytes in a heart-on-a-chip, coculture of multiple cell types in a human skin construct, use of synthetic hydrogels to create EOs that form neural tissue models, the blood–brain barrier-on-a-chip, MPS models of coupled female reproductive organs, coupling MPS devices to create a body-on-a-chip, and the use of a microformulator to recapitulate endocrine circadian rhythms. While MPS hardware has been relatively stable since the last MPS thematic issue, there have been significant advances in cell sourcing, with increased reliance on human-induced pluripotent stem cells, and in characterization of the genetic and functional cell state in MPS bioreactors. There is growing appreciation of the need to minimize perfusate-to-cell-volume ratios and respect physiological scaling of coupled TCs. Questions asked by drug developers are followed by an analysis of the potential value, costs, and needs of Pharma. Of highest value and lowest switching costs may be the development of MPS disease models to aid in the discovery of disease mechanisms; novel compounds including probes, leads, and clinical candidates; and mechanism of action of drug candidates.

Keywords: Organs-on-chips, engineered organoids, drug development, homunculi, disease models, toxicology

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Introduction

This article serves as an introduction to the second thematic issue in *Experimental Biology and Medicine (EBM)* dedicated to the rapidly growing field of microphysiological systems (MPS), which encompass organs-on-chips (OoCs), tissue chips (TCs), and engineered organoids (EOs). As we introduce the 14 other papers in this issue, we first offer our observations about how these devices, either operating individually or coupled to create MPS homunculi, relate to the established fields of medicine, biology, pharmacology, and toxicology. An understanding of this relationship is critical for a proper assessment of the strengths, weaknesses, opportunities, and threats associated with MPS research and development, and hence the appropriate integration of MPS research into the grand scheme of biomedical science. This introduction of the commentaries, reviews, and original research reported in this thematic issue will analyze several different areas of MPS research and place them in the larger historical and scientific context. In particular, we will discuss medical and biological problems for which conventional cell culture is inadequate and MPS approaches might provide solutions.

We begin with an admittedly cynical view of two-dimensional (2D) biology on plastic, the mainstay of biomedical research. Much of what we know about cellular biology is based upon 70 years of research into the *in vitro* culture of cells. Yet, one could make a reasoned argument that cell biology using immortalized cells cultured as monolayers on stiff plastic in high glucose media is in fact studying cancerous, inbred, fat, lazy, and diabetic cells that gorge themselves on sugar once a day, don't exercise, don't sleep, and do not experience fluctuations in thyroid, stress, sex, or other hormones. They talk only to cells of like mind, live in the dark and in their own excrement, and don't bury their dead. At the end of every day, they may be starving and slowly suffocating in an increasingly acidic environment. Most important, cells growing on hard plastic provide biologists and tissue engineers with reproducible, statistically significant results that describe 2D life on plastic in quantitative detail. Such "flat" biology, however, may not in general be relevant to human physiology in particular, or to the *in vitro* optimization of three-dimensional (3D) tissues. Indeed, the addition of the third dimension leads to drastic changes.[1–10] Although this description hyperbolizes the situation for effect, it emphasizes the need for physiologically relevant models of human tissue function. Whether tissue models need to be 2D, 2.5D (i.e. planar barriers such as the blood–brain barrier or the vascular–air interface in pulmonary alveoli), 3D, or even 4D (incorporating time as a variable) will be determined by the complexity of the physiological function being recapitulated and the accuracy required to answer a specific question. What is beyond dispute, however, is the need for validated, reliable, user-friendly models that provide the appropriate cell–cell and/or cell–matrix interface to ask key questions about the responses of human tissues and compartmental interfaces to a wide array of stimuli.

The articles in this thematic issue provide several additional examples of how more physiologically relevant responses are obtained from cells grown in a semisolid medium that allows them to associate in more than two dimensions. Experimental evidence for the role of the extracellular environment on the development and maintenance of cell phenotype dates back to Mina Bissell's pioneering work on culturing breast cancer cells and acinar tissues (reviewed in Schmeichel and Bissell[11] and McMillin et al.[12]). Despite an increased appreciation that "form and function interact dynamically and reciprocally" and that "we know everything about the sequence of the gene, the language of the gene, the alphabet of the gene, . . . we know nothing, but nothing, about the language and alphabet of form."[13] The ability of MPS to recapitulate tissue microenvironments for long periods of time while supporting various high-content imaging and analysis techniques is allowing researchers to study both form and function at a physical scale and a level of complexity between that of 2D biology on plastic and the *in vivo* or *in vitro* animal organ, creating cellular real estate that by definition occupies three dimensions.

Real estate is, of course, important for cell and tissue identity, but diversity in the neighborhood can also be a key factor. Tissues may be composed of one cell type, but organs are invariably made of a variety of cells with mutually supportive roles. As a general area of research, MPS enable the study of collections of cells in several forms: engineered tissues-on-chips, engineered biological-interfaces-on-chips, self-assembling printed or EOs, and even implantable micro-organs. (While one might argue that OoCs and organoids are separate fields of inquiry, the two approaches are moving closer together, and hence we endorse the most general definition of MPS.) Several of the papers in this thematic issue address the long-term support of cellular heterogeneity possible with the MPS approach, for example in the liver,[14–16] the gastrointestinal system,[17] the brain,[18,19] skin,[20] and the female reproductive tract.[21] We will discuss the cellular neighborhood later in the context of MPS immunology.

The entry of MPS onto the biomedical scene

Fully *in vitro*, complex, appropriately scaled TC models began to emerge with the turn of the century.[22,23] Decades of work on synthetic scaffolds that would sustain functional cells for long periods, bioreactors to tightly control environmental conditions, and improved microfabrication and microfluidic techniques all combined to provide accessible systems to mimic tissue functions in isolation, outside the body. TCs truly captured the attention of mainstream biology with the publication of two papers demonstrating the essential elements both necessary and sufficient for lung organotypic function[24] and pathology.[25] Ingber and colleagues constructed the basic functional unit of the human lung by assembling the essential elements of the alveolar–capillary interface. A microfluidic device contained two channels separated by a thin, porous polydimethylsiloxane (PDMS) membrane coated with extracellular matrix protein to encourage cell growth. Lung epithelial cells were grown to confluence on top, and pulmonary endothelial cells formed a monolayer from below the

membrane. Air was introduced over the epithelial layer, and blood-simulating media flowed over the endothelial cells. This arrangement allowed independent circulation of nutrients, cells, waste, etc. to the two channels. The stretch experienced in breathing was simulated by applying vacuum to separate flexible lateral channels running parallel to the cell layers. The group made a pivotal observation in showing that physiological functions like nanoparticle uptake from the air interface only occurred with the application of cyclic stretch. They also showed that neutrophils injected into the blood channel could wiggle through the membrane pores into the epithelial layer. In a further study, the group reproduced the pulmonary edema that ensues with IL-2 treatment in cancer patients. These two lung-on-chip papers were also remarkable in that they triggered excitement that led to the substantial investment by the Defense Advanced Research Projects Agency (DARPA) and the National Institutes of Health (NIH) in MPS and tissue-chip development.

The MPS community that has emerged since this landmark work has attempted to address for a multitude of primary organs the same important questions about how to enable physiologically relevant responses. What constitutes the minimum unit that demonstrates organ-specific responses to stimuli? Can different interfaces within an organ be represented in different chips that can be linked to give meaningful aggregated responses? Can such units that give organotypic responses be linked to show whole organism effects? What are the most effective "stress tests" to apply to reveal the strengths and weaknesses of TCs? Are the results generalizable or are the systems only appropriate in a very narrow "fit for purpose" exercise? Can the platforms be "one size fits all," or perhaps made in a few "plug and play" type configurations, or must each tissue be designed "from the ground up"? Can individual organ chips or MPS homunculi created by interconnecting multiple TCs recreate a specific patient's illness, or at least that of a particular genomic disease group? In short, can engineering create microenvironments that permit cells to adequately replicate *in vitro* the structure and function observed *in vivo*?

DARPA and the NIH (with significant critical input from the Food and Drug Administration (FDA)) set out to address such questions with complementary programs focused upon the design and development of TCs for toxicity screening. In many ways, this was a chance to harvest the low-hanging fruit of this emerging field. A fundamental principle in engineering research is to design to a definite goal. To that end, there is a wealth of Absorption/Distribution/Metabolism/Excretion Toxicology (ADME-Tox) data available (if not assembled in a single, publically available repository) on many hundreds of compounds that have been examined as drug candidates. Detailed information exists on dosing, metabolism, persistence, physiological effects, efficacy, complications, and other outcomes from animal and human testing. Such data can be used to establish compound training sets to adjust the parameters of a given tissue platform, followed by arrays of test compounds (ideally both known and unknown) to assess performance. Demonstrating that TCs can predict ADME-Tox for a single organ or a complex of linked organs would go a long way in establishing the feasibility of this general approach for drug development.

The DARPA program laid out a very challenging goal to be achieved within five years: develop a system containing 10 different chips (each representing different organ functions) that are linked in such a way as to predict the whole human response to a drug candidate. The NIH solicitations also asked for chips that would predict human response to drug challenges and that, in the later stages of the work, the cultured tissues would be functionally linked. But there was no specific expectation for 10 organ mimics to be fully integrated. NIH also made provisions to address cell sourcing issues, particularly the development of the specific phenotype of cells using induced pluripotent stem cells as the starting point. As the program matured, NIH expressed an interest in on-chip disease models, particularly for rare diseases that are difficult or impossible to study in humans and for which there may be no realistic animal model.

The DARPA and NIH programs operated independently but synergistically. The previous MPS thematic issue in *EBM* published manuscripts highlighting early progress from both of these efforts.[26–42] It was recognized that MPS might help close the hermeneutic circle of biology, which describes how it is impossible to understand the whole (the compete organism) without understanding the parts (the genome, proteome, metabolome, etc.), and that understanding the parts requires an understanding of the whole.[26,43] As we will see in the present issue, challenges outlined in 2014 are being recognized by multiple groups, most notably the "volume problem" that is associated with the dilution of signaling molecules into large volumes of perfusate.[26] Commentaries helped define possible roles for MPS in drug development and toxicology.[27,28] Organ systems that were reviewed included bones and joints in health and disease,[29] a brain-on-a-chip,[30] the gastrointestinal system,[31,32] the lung,[33] the liver,[34,35] the reproductive tract,[36] skeletal muscle,[37] skin,[38] and vasculature.[42] The importance of applying physiologically based pharmacokinetic (PK) models to a body-on-a-chip was clearly delineated.[39] A review of microscale technologies for regulating human stem cell differentiation introduced the complexities of directed stem cells toward the desired phenotype,[41] something that is proving critically important as MPS researchers wean themselves from cell lines and primary cells. These articles provide a detailed view of the state of the art in MPS research in 2014.

The DARPA MPS program and the first round of the NIH National Center for Advancing Translational Sciences (NIH/NCATS) TC funding are now completed and are generally considered to have exceeded expectations. Indeed, the original goals of generating tools that could augment technologies for predictive toxicology in drug development have led to explorations into efficacy and mechanisms of action for drug candidates. Novel research is yielding insights into normal and abnormal tissue and organ function and is becoming especially useful in revealing disease processes. In addition, other Federal efforts have embraced the MPS approach to

address their own mission. A prime example that is well under way is the Environmental Protection Agency (EPA) Science to Achieve Results program to develop organotypic culture models for predictive toxicology. Another is the Defense Threat Reduction Agency's X vivo Capability for Evaluation and Licensure Program. With all of the activities and accomplishments over the past four years, it is an appropriate time for a second *EBM* MPS thematic issue. This reflects both ambitious goals and remarkable achievements in a short time. We now have a collection of relatively basic (compared to the complex organs they represent) reproducible tools that are the building blocks that can begin to address questions about organ (and perhaps organismal) physiology, metabolism, and disease initiation/progression (among other aspects) as a function of controlled inputs. The early stages of linking these tissues are also being established. However, fundamental operational issues remain and must be addressed if these systems are to advance to regular use in research laboratories and as part of the pipeline in drug development. One way that the operational issues will be addressed is through the commercialization of MPS technologies. Zhang and Radisic[44] discuss the emergence of about 20 companies in this sector, and undoubtedly the customer service, engineering, and marketing departments of each of these companies, no matter how small, will quickly realize what the customer needs and would like to have. Similarly, it is heartening to see TCs programs springing up within pharmaceutical companies and at the larger biotechnology companies, as well as the emergence of many small start-ups and contract research organizations, based on the intellectual property that is being developed in this arena. A number of these are drawing from academia, and in this translation the differences will become quickly apparent between a PhD student or postdoc running a self-developed assay and Pharma technician operating a productized version of that assay. As all of these commercial organizations focus on turning the benchtop systems into serious tools for drug development, progress into the testing laboratory and the clinic should be accelerated.

The success of the first efforts from the DARPA and NIH MicroPhysiological Systems grants also encouraged questions of a more complex nature, resulting in NIH issuing follow-on Funding Opportunity Announcements to supplement the first round of MPS awards, specifically to build chips representing organs missing from the consortium efforts (PA-16-178) and to address rare diseases (PA-16-173). Additionally, an open competition for the "next generation" of MPS focused on disease modeling and efficacy testing (RFA-TR-16-17) was announced by NCATS in partnership with several other NIH Institutes. The rapid evolution of the field was reflected in that the proposal call articulated that "an essential feature" would be integration of "bioengineering, microfluidics, material science, 'omic' sciences, computational biology, disease biology, pathology, electrophysiology, pharmacology, biostatistics and clinical science." The success of the DARPA and NIH programs is manifest in the diversity of organ and TCs and interconnection schemes developed and evaluated and the excitement engendered, but it is beyond the scope of this introduction and both the first and second *EBM* thematic issues to review all of this work. We are able to present only a subset of this work and offer our apologies to those whose research could not be included.

Fitting into the "Grand Scheme"

The field of MPS/TCs is still emerging: serious efforts are about a decade old. Yet advances have been rapid, both within the individual laboratories, in the cooperation between groups, across the silos of different US government agencies, and with the pharmaceutical company customer base who will be the first movers of this technology for practical application. We are beginning to see the application of MPS to problems in medicine, biology, pharmacology, and toxicology, and another *EBM* MPS thematic issue is in order. For this issue, the grantees currently supported by the NIH and DARPA programs; NIH program officials; and leaders from Pharma, the FDA, and the EPA have contributed updates on progress in the field over the last three years. This issue does not represent an exhaustive review of the state of the art in MPS, but instead offers perspectives, reviews, and original research that reflect insights gained primarily from the NIH/NCATS program. We begin with three commentaries and a review presenting the perspectives of the NIH,[45] the pharmaceutical industry,[46] developmental toxicologists,[47] and a small MPS company.[14] Each provides us with updated lists of challenges. A comparison with the 2014 papers indicates that progress is being made on many fronts, particularly with cell sourcing, maintaining cells in organ constructs for long periods of time without dedifferentiation, synthetic extracellular matrices, organ scaling, vascularization, tissue characterization, and addressing the volume problem. These papers discuss challenges that are coming to the fore, including the need to incorporate both the innate and acquired immune systems and hierarchical vascularization into MPS homunculi. More groups need to address the *in vivo* to *in vitro* extrapolation from drug studies in an MPS homunculus to a human in the clinic. Opportunities include the use of CRISPR cas9 genome editing to convert normal MPS into disease models. Knudsen *et al.*[47] point out the power of combined *in vitro* MPS and *in silico* computational modeling, particularly in the context of understanding developmental and reproductive toxicology. Hughes *et al.*[14] provide a pragmatic review of the state of the art and challenges in both liver and coupled-organ MPS studies and express justifiable optimism for the continued development of MPS technologies and applications.

It is reasonable to assume, given the breadth of talent and institutions either in the field or just now entering it, that each of the challenges facing full development and deployment of MPS will be addressed in due time. Obviously the translation of TCs into mainstream medicine, biology, and toxicology is requiring a remarkable range of science, engineering, and medicine. Rather than view the papers in this thematic issue from those three perspectives, we examine them using what could be considered as a principal component analysis, in the mathematical sense. We identify the first three principal

components of MPS efforts as (1) technological refinements of the hardware; (2) how the cells are grown, i.e. perfecting body-on-a-chip (BoC) and MPS homunculi components; and (3) the application of analytical techniques to evaluate how well the cells in the devices recapitulate the requisite biology and drug responses. Together, these three components span much of what is needed to apply functioning MPS devices to problems in medicine, biology, pharmacology, and toxicology.

The articles in this issue do not provide any in-depth examination of the first principal component, MPS hardware development, which is relatively stable and now being commercialized,[44] but there are several notable advances reported since 2014. Hughes *et al.*[14] discuss hardware issues in their review of the liver and interconnected systems. Wang *et al.*[48] review progress toward self-contained BoC systems. The device developed by Nortis, described in 2014,[42] is now in widespread use and is part of the Pittsburgh liver described in this issue,[15,16] albeit not using the pulled-fiber vascularization. MPS approaches to the study of female reproductive biology, reviewed by Young *et al.*,[21] have utilized a multiorgan platform recently announced by Draper Laboratory.[49,50] Cyr *et al.*[51] discuss the application of microfluidic microformulators for replicating hormonal circadian rhythms in individual or coupled organ MPS studies. The introduction of low-cost, multiport microformulators should simplify the customization and temporal control of TC perfusion media and accommodate the secretions of organs missing from an MPS homunculus.[51,52] The greatest change in MPS bioreactor design will occur when organ chips are perfused by hierarchical vascular systems, as discussed by Phan *et al.*[19]

The hardware challenges that must be addressed include the balancing of the flow between different organs in an MPS homunculus; expanding the capabilities of openable bioreactors that allow access to the cells for proteomic and transcriptomic analysis while minimizing fluid volumes and supporting required shear forces; recirculation[48,53–58] and timed media replacement without the introduction of bubbles; the expanded use of endothelial barriers to minimize problems with media incompatibility between different coupled organs; automation of drug delivery (including simulation of the PKs of drug delivery) and sample acquisition; and the minimization of tubing, wires, and PDMS. Although not as convenient as PDMS, the introduction of styrene–ethylene–butylene–ethylene formulations may prove to be a significant advance in elastomeric devices.[59–63] The use of gradients in organ-on-chip devices will prove to be a powerful technique to recapitulate certain aspects of physiology.[16,64–66]

As the hardware is becoming more stable, the second principal component, cell sourcing, will continue to be of critical importance and represents a major emphasis in this thematic issue. Knudsen *et al.*[47] address differences between animal and human cells and issues regarding how sex differences in cultured cells might affect toxicology. Hughes *et al.*[14] analyze the problems of cell sourcing and common media. As evidence of the MPS community moving far past monocultures on 2D plastic, there are a growing number of testimonials regarding the advantages of combining multiple cell types in a single organ and linking multiple organs.[15–21,48] As multiple organs are being combined to produce MPS homunculi, the "volume problem" is becoming better appreciated,[26,52] and efforts are under way by a number of groups to minimize the ratio of perfusion volume to cell volume lest drug metabolites and cellular signaling molecules be diluted below the threshold of physiological effect.

One of the reasons that hardware issues are not at the fore is that attention is being directed toward using the existing hardware to explore the diversity of cells that can be studied with these platforms – primary, progenitor, precursor, stem cell-derived, etc. While primary cells may be the most physiologically relevant, they may be terminally differentiated and not divide once seeded in a bioreactor, they cannot be standardized or obtained reproducibly, banked cells may be pooled from multiple individuals, and they can be quite expensive. There is a possibility that well-established tumor lines may adopt a "normal" phenotype when supplied with the right extracellular cues.[67] Human induced pluripotent stem cells (hiPSCs) would allow development of multiple tissues from the same "patient" and thus offer the option for personalized/precision medicine. Engineering of these cells would allow incorporation of optically marked cells as sentinels for tissue maturation, etc.[68] Original research from the Murphy group reported in this issue is representative of an important trend toward replacing the vagaries of Matrigel™ with much more controlled hydrogels that can support much better control of iPSC differentiation.[18] The ability to expand iPSCs with adequate yield might in the future enable "*in vitro* Phase IV" studies.

As the hardware and cell culture techniques are being refined, we are seeing an increased sophistication in our third principal component – the analytical techniques that are being applied to MPS experiments. To date, most TC studies have reported morphological features; the expression of small sets of genes; or the secretion of a few, organ-specific compounds. A much more comprehensive battery of techniques is already in regular use in the pharmaceutical industry, including genomics, transcriptomics, and proteomics. In recent MPS studies, we are seeing functional measurements such as contractility, calcium signaling, and electrophysiological responses of cardiac and skeletal muscle,[37,69–75] electrical resistance and molecular permeability of barriers,[76–82] and metabolomic responses to inflammatory challenges.[78] In this issue, the Parker group uses a variety of measures, including gene expression profiling, to quantify factors that affect maturation of neonatal rat cardiomyocytes into a more mature phenotype.[83] As TCs continue to recapitulate more tissue-level functions, we will see more functional measurements that could not be possible with monolayer monocultures.

The MPS field needs to conduct comprehensive, quantitative comparisons between *in vitro* and *in vivo* studies, as has been recently demonstrated with a weighted gene coexpression network analysis that compares rat liver *in vivo* with both mouse liver *in vitro* and rat primary hepatocytes growing in a dish.[84] The analysis showed that a mouse liver was a better model of the rat liver than the primary rat

hepatocytes in a dish, which more closely resembled a rat liver exposed to a significant toxic load. The MPS community should consider comparing, for example, a mouse with a mouse-on-a-chip to confirm that the appropriate physiology is being recapitulated and to inform the extrapolation from existing animal assays to *in vitro* and *in vivo* human studies, as discussed by Ewart *et al.*[46] Knudsen *et al.*[47] point out the serious implications of the discordance between animal and human responses. A major effort to create mouse OoCs could also examine the effects of genetic variations by creating mouse chips from the ~150 isogenic, fully sequenced cohort of recombinant inbred murine strains, which exhibit over five million common variants and ~12,000 missense mutations, that have been developed and characterized at the University of Tennessee Health Sciences Center.[85]

Metabolomics is rapidly moving into prominence as the instrumentation improves and the databases expand, but the annotation of the databases and the interpretation of the resulting metabolic and signaling networks have not yet proven useful to many potential end users. Metabolomic analysis techniques are just now being applied to OoCs,[78] and ultimately the MPS effort could benefit from untargeted multiomics studies of mechanism of action.[86]

As the analytical approaches are broadened, it will be important to develop mathematical methodologies to interpret data from TCs and MPS homunculi. Foremost is the use of PK and pharmacodynamic (PD) approaches,[87–91] which have long been advocated by the Shuler group for TC and body-on-a-chip assays,[39,48,53,92–96] and are proving valuable in liver-on-chip studies.[97,98] Ultimately, these types of analyses and their extension to physiologically based pharmacokinetics (PBPK) will be critical for the *in vitro* to *in vivo* extrapolation that will be necessary to relate TC and MPS homunculi studies to clinical trials in humans.[90,91,93,99–104] These models may help address the volume problem in coupled-organ MPS. MPS/PKPD models will also be useful for predicting human response to multiple drugs, as this is difficult to do with animal experiments only. By combining MPS and PKPD (or PBPK) models, it should be much easier to predict response to various scenarios using multiple drugs. The experimental space is large, particularly since the order in which drugs are given, the timing between administration, and the amounts given can all be important. A PKPD model based on a MPS could provide important insights for the design of clinical trials, and it could be validated with *in vitro* MPS experiments using the microformulator technology[51,52] that is discussed by Cyr *et al.*[51] in this issue.

MPS immunology

There is increased recognition that the addition of immune function to MPS should be a priority, as discussed in this issue by Blutt *et al.*[17] and Abaci *et al.*[20] and elsewhere.[105,106]

An excellent place to start the discussion of the future of MPS immunology is with the lymph node. An early *in vitro* model of the immune response by specific individuals to vaccines involved two transwell stages, one that served as a peripheral tissue equivalent and another for the lymphoid tissue equivalent, with manual transfer of cells between the two stages.[107] This approach might be implemented in microfluidic bioreactors, but there does not yet appear to be a pressing need to do so. The spatial complexity of the lymph node has been examined with a microfluidic device that can separately stimulate B-cell and T-cell zones within an isolated slice of a mouse lymph node,[108] but such zones have yet to be reconstructed with a tissue-engineered human lymph node equivalent. (The authors note that the use of microfluidics to perfuse a lymph node slice has similarities to the use of microfluidic devices to perfuse brain slices.[109–117] Liver and intestinal slices have also been used in microfluidic devices.[118] In each case, the complexity of the neighborhood is recreated using sliced tissues.) While it is tempting to add a single lymph node to a multiorgan MPS homunculus, it would be more realistic to have a lymph node that was specialized to each organ, as occurs *in vivo.* More practical in the near term would be to create a single lymph node, study the behavior of that node in isolation, and then consider adding one lymph node to a single organ, such as the liver or brain. One might even consider activating lymphangiogenesis in a single, solid organ and building an integral lymph node to study, for example, cancer metastasis.[119] To recreate a distributed lymph system with its multitude of varied functions is beyond the state of the art.

But there is more to immunity than the lymph node. Giese and Marx[120] provide an excellent review that discusses how to emulate *in vitro* innate immunity in non-lymphoidal tissue and adaptive immune responses in lymphoidal tissue. At the end of their review, they succinctly assess the status of *in vitro* immune models:

> Unfortunately, none of the existing immunocompetent nonlymphoid or professional lymphoid 3D *in vitro* systems reviewed here provides a translational alternative to recapitulate the entire adverse immunogenicity pathway in man; neither do the human *in silico* immunity models and the 'humanisation' of the immune system of laboratory mice.

But there is movement in the correct direction. We have already seen an elegant proof-of-concept demonstration of a leukocyte crossing the planar endothelial–epithelial alveolar interface.[24] One concern regarding MPS immunology might be the storage, pumping, renewal, and disposal of leukocytes circulating through a distributed, interconnected MPS homunculus – a human has approximately 5×10^{11} leukocytes, with a daily vascular distribution of 4×10^{11} leukocytes and a daily lymph node return of 3×10^{10} leukocytes from the tissue to the blood.[120] Another separate and essential question is whether the leukocytes, particularly those returning from an organ, will function in a manner that replicates their behavior *in vivo.*

A classic heterogeneous neighborhood problem is the interaction of resident macrophages and dendritic cells (and their equivalents in particular organs), and other resident immune cells within non-lymphoidal tissue. Research reports in this thematic issue that address MPS immunology describe the inclusion of microglia in the brain[18] and

Kupffer cells in the liver.[15,16] The gut, lung, and skin serve as immune barriers to external pathogens, and the gut has a key role in initiating an adaptive immune response to pathogens.[120] Leukocyte migration and other immune interactions in these organs have been studied using *in vitro* MPS approaches,[24,25,67] as reviewed elsewhere[120] and in this issue for skin[20] and gut.[17] Multichamber MPS devices have been developed[121] specifically to study the activation of immune cells by the transfer of nutrients across the gastrointestinal epithelium.[122]

The ability to study intestinal flora in MPS has already been demonstrated.[67] But the GI tract is also an entry point for pathogens. Mills and Estes[123] discuss the spatial complexity of the cellular niche formed by infections and the immune cells that fight them, and the need to vascularize models and innervate non-neuronal models for which immune activity can be neuronally modulated. Given a recent, elegant demonstration that hiPSCs can be used to create intestinal organoids with a functional enteric nervous system,[124] the growth of neural networks within 3D, perfused MPS organs is closer than one might guess.

There are other components of the immune system that might be incorporated into MPS homunculi. The ability of a microfluidic spleen to cleanse blood of pathogens has been demonstrated,[125] but the role of the spleen in synthesizing antibodies has not. The neighborhood problem has also been addressed in the development of a bone marrow bioreactor that is first implanted in a mouse and then harvested for *in vitro* studies in an MPS bioreactor.[126]

A central problem limiting the current procedures of testing immunotherapies is the phylogenetic distance between laboratory animals and humans, such that some pathogens can affect one species but not another, and there are significant differences in innate and adaptive immune cells between species.[120] But there are differences between people. We have already seen the inclusion of macrophages, glial cells, and Kupffer cells in MPS studies, but these cells are part of the innate immune system, which does not separate "self" from "not self." The transplantation of solid organs and blood components and cancer immunotherapy clearly face the challenges of major histocompatibility complex compatibility, as characterized by the large number of different human leukocyte antigen classes and types.[121] Just as one does not want to trigger organ rejection or host-versus-graft disease in a human, the addition of adaptive immune cells in an MPS homunculus runs the same risk. Ultimately, it should be possible to start with a patient's tumor, fibroblasts, and leukocytes (or possibly just the tumor and a patient's stem cells) and build a patient-specific homunculus that could be used to study cancer immunotherapy. This would represent the ultimate diagnostic for precision medicine[127]: patient-specific MPS devices.

Fitting MPS devices into the core activities of pharmaceutical R&D

Drug developers must address a multitude of questions in the course of bringing a drug to market. The majority of these questions relate to pharmacology and efficacy, and it is here that MPS systems can have the greatest benefit to drug developers. Some of these questions, in approximately chronological order, are: Is there a useful experimental model for the human disease of interest? Are the drug targets of interest functionally relevant in this experimental disease model? Do compounds that bind these drug targets produce meaningful PD at the biochemical, cellular, and/or physiological level? Can these PD responses serve as clinical biomarkers of pharmacological effect in patients? Are there adverse effects produced by my compounds and are these effects relevant to the intended patient population? What are the exposure–response relationships associated with pharmacology and toxicity? What is the metabolism and disposition of my compounds? Are there genetic and environmental contributors to pharmacology and toxicity that are relevant to patients?

The biochemical, cellular, and organotypic functions demonstrated by human cells in MPS devices suggest that these devices could become useful tools for drug developers trying to answer questions such as those presented above. MPS devices might be preferable to existing approaches and identifying those useful applications would allow greater operational integration between the MPS community and the drug developers. Areas of focus that we considered include human disease biology/pharmacology, ADME-PK-clinical pharmacology, and toxicology.

An MPS application that has been prioritized by the NIH and Pharma is to improve drug toxicity evaluations. Each year the development of thousands of compounds is terminated due to adverse findings in non-clinical animal toxicology studies, and dozens more are terminated due to adverse events in the clinic. Avoiding such failures would benefit patients and drug developers alike. In addition, animal toxicology studies are standardized tests with endpoints that have not changed in decades, they have been applied to thousands of compounds, and much of the historical data and compounds themselves can be made available to robustly characterize the predictive value of MPS models. Furthermore, use of human cells allows assessment of the hypothesis that MPS devices could more accurately predict safe compounds, safe doses and exposures, and types of toxicities than do animals. According to some visionaries, we might one day replace animal tests with MPS that are superior at protecting human safety, are higher throughput, and less expensive than current approaches. Toxicology is understandably a sensible early application of MPS technologies (and hence the interest of the EPA in applying MPS to developmental toxicology).

Are any MPS ready to fill such an ambitious role in drug development? Early and encouraging data support the use of human cell MPS for drug toxicity testing. Structural injury and modulation of organotypic function in response to compound treatment have been demonstrated with MPS devices for many of the major target organs of toxicity, including heart, liver, kidney, and GI tract,[17] using compounds with known toxicity to these organs. Cardiac safety warrants particular attention, both for its importance as a target organ for toxicity and for the diverse modes of cardiac toxicity. MPS devices for the heart are capable of

detecting transient alterations in calcium flux, direct measures of force, electrophysiology, heart rate, and conduction velocities in muscle assemblies that resemble syncytia (reported in this issue[83] and elsewhere[69,71,73]). This appears to improve upon existing non-clinical tools used by Pharma and the gold standard of instrumented animals that have been administered drugs *in vivo*. The value of these alternative MPS for not only cardiac but many other organ systems is in the richness of data that can inform our understanding of the modes of toxicity of our compounds, to better model *in vivo* exposure–response relationships, and to do so at a throughput and cost that will help us identify alternative compounds with less severe toxicological liabilities during preclinical drug development. It is also recognized that the source of human cells in these systems can support an unprecedented level of translational value, including non-clinical assessments of presumed high-risk patients using cardiomyocytes from stem cells taken from patients known to have experienced cardiovascular disease.[128]

ADME-PK-clinical pharmacology is a second area where one might expect a significant contribution to Pharma from MPS approaches. PD/PK analysis, already being applied to MPS studies as discussed above and elsewhere in this issue, is critical in multiple aspects of drug development. For example, MPS may help us better predict human drug clearance mechanisms and rates. Clearance is the key parameter needed to evaluate drug PK properties. This parameter is not definitively known until a radiolabeled ADME mass balance study is conducted in humans. Standard non-clinical practices of allometric scaling of *in vivo* PK data from animals can be unacceptably inaccurate, and use of PK parameters from cultured human 2D cell systems fails to capture the anatomic and physiologic complexity of the processes that determine hepatic and renal drug clearance in humans. For example, experimental determination of efflux over the sinusoidal membrane from hepatocytes back into the blood is difficult to measure experimentally and is frequently assumed to occur via passive diffusion only. This ignores the role of drug transporters that could be accurately represented in MPS. Other mechanistic advances to better model hepatic and renal clearance are needed because early and accurate prediction of clearance mechanisms helps drug development teams assess the clinical risks of low exposure, high PK variability, drug–drug interactions, and pharmacogenomics liabilities at a point in drug development at which lower risk compounds can be prioritized. A second valuable application of MPS to drug development is in the evaluation and identification of compounds with suitable predicted human PK properties, including improved assessment of the mechanisms and rates of human drug clearance.

A third area of focus for MPS in drug development is the improvement of evaluations of compound pharmacology and efficacy as related to normal and disease biology. MPS disease models are a primary area of focus for the next wave of funding by NIH/NCATS. One example of the value in this space is worthwhile. For years, oncology models have relied upon immune-deficient (nude) mice carrying tumor xenografts of human cancer cell lines. The positive predictive value of these models has been hugely disappointing because so many compounds that are efficacious in mouse xenograft experiments fail at great expense in human clinical trials. Yet Pharma continues to rely upon them heavily. Again, institutional familiarity with the strengths and weaknesses of these models promotes their continued use despite their demonstrated limitations. This makes them ripe for displacement. Whereas the nude mouse endpoints consist primarily of measurements of tumor volume and a hoped-for PD endpoint connected to the mechanism of action of the drug, MPS offer an actual window into the cellular behavior of an anatomically and physiologically more relevant model based on human components, including patient-derived cells. Exposure–response for PD endpoints, along with toxicology endpoints, has the potential to replace animal cancer models that we know are poor predictors of the activity and efficacy of anticancer agents in humans. The microformulators being created using MPS pump and valve technologies[51,52] may prove quite useful in this setting by simulating a range of different PK profiles to determine the most suitable PD and toxicity profile without requiring medicinal chemists to produce unique drugs for such evaluations.

A skeptic would justifiably point out that these examples skim the opportunities from the surface of a much deeper challenge associated with the discordance between *in vitro*, animal, and human studies,[129] and therefore do not contribute all that is necessary for tools to supplant the state of the art. The heart is exceptional for having a breadth of cell and tissue-based endpoints that are directly relevant to human drug safety and that can be altered pharmacologically and instantaneously using compounds with well-defined properties, including concentration–response. Still, we cannot measure blood pressure in these *in vitro* cardiac model systems, and this is a commonly measured clinical parameter that is a frequently encountered cardiovascular liability. Contemporary MPS models of the liver, another major organ of toxicity, lack bile ducts, which are a crucial toxicological target. Drugs withdrawn from the market for hepatic toxicity usually involve adverse effects on the biliary system. Similarly, a proximal tubule is not, nor has it been represented as, a model for the nephron, let alone a kidney. It is one part of a highly complex tissue that will require multiple connected MPS to model *in vivo*. These remarks are relevant to the intended context of use of MPS for toxicology testing in Pharma. The variety and number of toxicological endpoints currently interpreted in drug safety assessments are large, and many will not be reproducible in early MPS. The numbers of tissues that experience compound-related toxicity are also large, even without considering interactions between tissues. On top of this, the mechanisms by which compounds cause toxicity are often unknown, and the translational relevance of findings is challenging, not only from animal to man, but in the case of MPS, *in vitro* human to *in vivo* human.

This list of concerns is relevant at the local level for individual drug development teams with compounds causing organ toxicities, and these issues should also affect MPS

developers. A toxicologist might look to MPS devices and ask

> Is there a device that can detect the toxicity that my team is concerned about, will it work for my novel compounds with unknown mechanism of toxicity, and most important, if I get no toxicological response for a new compound, will I be able to trust its negative predictive value?

If the answer is that the first set of compounds that tested "not toxic" in human MPS devices turn out to be toxic in animals *in vivo*, at least two things will occur. First, the uncertainty about how these discrepant data should influence the fate of this particular compound will be very difficult to resolve, not only for the company but also for a regulatory agency. And second, the institutional memory of the discrepant data between human MPS and animals *in vivo* will be long lived. This is not entirely fair, because compound toxicities in rats, dogs, and monkeys often are not observed in humans. But this example is certain to arise, and for this reason toxicologist drug developers look not only for valid, fully qualified tests, but also for sufficient evidence to support regulatory acceptance of the meaning of data from human MPS.

Disrupting the field of drug toxicity evaluations, or any other part of drug development, requires overcoming many types of barriers. Drug developers and regulators have great familiarity with the strengths and weaknesses of existing methods. Toxicology study findings for nearly any compound have precedent in animals and the translational value to humans at some level, such that interpretation of new data is supported by a wealth of historical experience. What is most important is that the results of these animal toxicology studies establish No Effect Levels that are very good predictors of the safety of starting doses in First in Human Phase 1 studies. While there are notable exceptions, the frequency at which serious adverse events occur in Phase 1 studies is actually very low. Safe administration of novel drugs is a track record everyone wants to maintain. Switching to alternatives will only occur when we have great confidence in the value of a negative finding. Most work to date has been focused on demonstrating positive predictive value: that a system can respond to a toxic compound. What if we don't see an effect? Does that mean that the dose is safe?

Another thought experiment is instructive. Let us suppose we all reach agreement to replace animal tests with a set of MPS devices that use human tissues to detect all the major modes of toxicity for all the major target organs. Surely Pharma R&D would be interested in such a system? Let us further assume that there are only 10 major organs that we must be concerned about, and that each organ can be modeled with just two MPS devices (e.g. pancreas-on-a-chip has both endocrine and exocrine pancreas; kidney-on-a-chip has both glomerulus and tubules and ducts, etc.). And finally, let us say that each of these devices is extremely predictive of *in vivo* toxicity, with a false positive rate of just 5% each. The probability of a non-toxic compound coming through this battery with a result of "non-toxic" in all 20 tests is mathematically 36%. In the other 64% of cases, one would expect at least one positive result. This is true of any battery of tests and is a familiar hindrance to proponents of screening assays for early detection of drug safety risks. The more MPS devices required for safety evaluations, the greater the risk of terminating the development of a safe compound. Conversely, decreasing the number of tests will increase the risk of a patient receiving a drug that is toxic, but that tested "not toxic" in MPS devices (i.e. a false negative). This reflects the well-known trade-off between sensitivity and specificity of diagnostic tests.

There is an additional serious issue beyond the multiplicative probability of success that accompanies the coupling of multiple TCs, each with a non-zero probability of spontaneous failure, which suggests that it may be better to grow organs separately and connect the best to create an MPS homunculus. One advantage with mouse models is that if an engineered mouse dies or looks ill, the researcher is immediately informed that the mouse in its entirety is not functioning properly. Criteria for acceptable systems performance will be needed for MPS homunculi.

These hypothetical examples help explain why toxicologists may be reluctant to switch to new tests when existing tests are widely understood and have been used successfully to protect patient safety for decades. Consideration of the use of MPS devices for pharmacology and efficacy testing might lead one to a different conclusion. Biological models do not exist for many human diseases, or have weak mechanistic relevance to human disease, and/or are known to be poorly predictive of efficacy in human clinical trials. This is true not only for rare diseases but also for major diseases. Xenografts in mice have long been a staple of cancer research, in spite of decades of overly optimistic results in non-clinical efficacy studies. Contemporary MPS devices have shown interesting vascular biology that supports growth of human-derived tumor cells and delivery of drugs that inhibit tumor growth.[40] Combined with genetic tools and pharmacological treatments, MPS devices offer opportunities to address high value objectives such as prioritizing drug targets, screening compounds, and exploring concentration–response.

These considerations may best be appreciated by comparing the benefits and drawbacks of applying MPS to discrete places along the drug development pipeline. Abaci and Shuler[99] have reviewed the insertion points for MPS assays in the drug development pipeline, as do Ewart *et al.*[46] in this issue. To provide a complementary perspective, our Table 1 clusters the relevant issues into three groups: Disease Biology/Pharmacology, ADME-PK-Clinical Pharmacology, and Toxicology. According to this presentation, the value of MPS to drug developers is greater for Disease Biology/Pharmacology than for Toxicology or ADME-PK-Clinical Pharmacology. Nevertheless, all three areas of application make sense, and indeed the opportunity to combine PD/efficacy, ADME, and toxicology into a single system is likely to ultimately represent the most compelling scientific and business case.

Table 1. Value, costs, and needs in applying microphysiological systems (MPS) to three components of drug development.

Area of focus	Test systems	Value	Barriers/switching costs	Needs
Disease biology/ pharmacology	– Human only – Healthy and diseased tissues – Biochemical, cellular, physiologic responses	– Identify novel drug targets – Identify novel disease mechanisms – Develop pharmacodynamic (PD) biomarkers – Establish exposure–response relationships for pharmacokinetic (PK)/PD modeling – Screen compounds for pharmacology properties	– Lack of personnel and organizational experience in generating and using MPS data – Timing of investments is difficult because the MPS field is fast-moving	– More human disease models (partially addressed by NIH/NCATS Tissue Chips 2.0) – More scientists/technologists with MPS training – Evidence of MPS model robustness – Commercial suppliers of human primary cells and differentiated cells derived from induced pluripotent stem cells (iPSCs) with reliable function and confirmed genotype
ADME-PK-clinical pharmacology	– Animals and human – Healthy and diseased tissues – Drug metabolism, disposition, and pharmacokinetics	– Predict clearance mechanisms, rates, and dose–exposure relationships for all routes of administration – Predict *in vivo* compound disposition – Robust evaluations of drug–drug interactions – Role of genetic variation on drug clearance, metabolism, and disposition	– Companies/regulators are comfortable with existing, embedded *in vitro* metabolism and transporter assays – Poor exposure and PK in humans are no longer common causes of drug termination thanks to existing *in vitro* assays	– Primary cells with drug metabolizing enzymes (DMEs) and transporter activity representative of humans – Cell banks with diversity of haplotypes for DMEs and drug transporters – Methods to create mature, differentiated human iPS-derived cells – Assessment of predictive value of MPS (both animal and human) for *in vivo* ADME/PK properties
Toxicology	– Animals and human – Healthy and diseased tissues – Biochemical, cellular, physiologic responses	– *In vitro* detection of toxicities that today require *in vivo* studies – Avoid inappropriate drug terminations due to toxicities unique to animals – Earlier termination of drugs unacceptably toxic to humans – Decreased animal usage – Investigate toxicology mechanism of action (MOA) and drug combination studies	– Existing methods are embedded in drug development in spite of recognized limitations – Data interpretation is in its infancy. Equivalency of human MPS to whole humans must be established. – Can we use “not toxic in human MPS” to set safe starting doses in humans as effectively as we can with animals?	– Assessment of predictive value of MPS (both animal and human) for *in vivo* toxicology – Incorporation of important cell types for toxicity to major organs: ○ Liver – bile ducts and sinusoids ○ Kidney – glomeruli – Reliable commercial sources of primary and iPSC-derived cells

Areas of focus - These describe functional areas in large pharmaceutical companies. They also correspond to training at the PhD level. Non-clinical absorption/distribution/metabolism/excretion (ADME) – PK is combined with clinical pharmacology because the core competencies to address them overlap.
Test systems - General description of what is being done in this area of focus.
Value - Specific ways that Pharma can use MPS systems to advance drug development.
Barriers/switching costs - Pharma already has tools for every Area of Focus. This column identifies challenges that MPS developers face selling devices to Pharma.
Needs - Additional variables that will impact the value and timing of implementation of MPS systems by drug developers in Pharma.
NIH/NCATS: National Institutes of Health/National Center for Advancing Translational Sciences.

From the perspective of providing long-lasting *in vitro* 3D tissue neighborhoods, MPS created entirely with human cells have a great deal to offer. As evidenced by the articles in this thematic issue and the explosion of articles elsewhere, the MPS/organ-on-chip communities are bringing together advanced technologies whose development and integration Pharma may not be willing to fund alone, including 3D bioreactors, stem cell differentiation, microfluidic controls, analytical chemistry, and multiomics. The remarkable and timely investment by DARPA and NIH has demonstrated the promise of great returns in the future! The translation from the bench to bedside requires collaboration and expertise in cell biology, biomedical engineering, physiology, pharmacology, toxicology, bioinformatics and biostatistics, and computer science. Academic and commercial players in the MPS/OoC community have demonstrated their willingness to address the translational challenges required to apply MPS/OoC technologies to accelerate drug development. A closing word of caution is that we will never create a perfect microHuman BoC or MPS homunculus, but analyses such as the ones we present in this review should help guide the compromises we make as we build useful models, even toy models, of human physiology.

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