Introduction

The relevance and potential roles of microphysiological systems in biology and medicine

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Abstract

Microphysiological systems (MPS), consisting of interacting organs-on-chips or tissue-engineered, 3D organ constructs that use human cells, present an opportunity to bring new tools to biology, medicine, pharmacology, physiology, and toxicology. This issue of *Experimental Biology and Medicine* describes the ongoing development of MPS that can serve as *in-vitro* models for bone and cartilage, brain, gastrointestinal tract, lung, liver, microvasculature, reproductive tract, skeletal muscle, and skin. Related topics addressed here are the interconnection of organs-on-chips to support physiologically based pharmacokinetics and drug discovery and screening, and the microscale technologies that regulate stem cell differentiation. The initial motivation for creating MPS was to increase the speed, efficiency, and safety of pharmaceutical development and testing, paying particular regard to the fact that neither monolayer monocultures of immortal or primary cell lines nor animal studies can adequately recapitulate the dynamics of drug–organ, drug–drug, and drug–organ–organ interactions in humans. Other applications include studies of the effect of environmental toxins on humans, identification, characterization, and neutralization of chemical and biological weapons, controlled studies of the microbiome and infectious disease that cannot be conducted in humans, controlled differentiation of induced pluripotent stem cells into specific adult cellular phenotypes, and studies of the dynamics of metabolism and signaling within and between human organs. The technical challenges are being addressed by many investigators, and in the process, it seems highly likely that significant progress will be made toward providing more physiologically realistic alternatives to monolayer monocultures or whole animal studies. The effectiveness of this effort will be determined in part by how easy the constructs are to use, how well they function, how accurately they recapitulate and report human pharmacology and toxicology, whether they can be generated in large numbers to enable parallel studies, and if their use can be standardized consistent with the practices of regulatory science.

Keywords: Organs on chips, tissue-engineered organ constructs, microphysiological systems, drug discovery and development, drug safety and toxicity, drug–organ interactions, systems biology, quantitative systems pharmacology, environmental toxicology, induced pluripotent stem cells

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Introduction

Two thousand years ago, the formal study of anatomy and physiology began with the work of Galen of Pergamon, representing the beginning of two millennia of top-down, reductionist research seeking the fundamental principles and processes of biology. As shown in Figure 1, biological science fully spans the many different scales from the animal to the molecule. The past 60 years have witnessed remarkable progress in biology and medicine, starting with major advances at the organ level following the expanded use of animal studies and the development of isolated organ preparations. Given the speed with which the global knowledge of biology is progressing, only a very small number of the biologists, engineers, physicists, and physicians developing organs-on-chips have had firsthand experience with the isolated animal organ systems shown in Figure 2, and hence may not appreciate how much was learned from the 1880s through the 1960s about regulatory biology and biochemistry, particularly with regard to hormones and metabolism, from experiments on isolated organs.[1–7] This type of research was followed by sweeping discoveries at the cellular level resulting from the development of the cell culture techniques that are so prevalent today. Concurrently, at the molecular level we have seen a triumph in reductionist science with our newfound ability to understand and control much of the information contained in DNA and RNA and the consequent manifestations in molecular

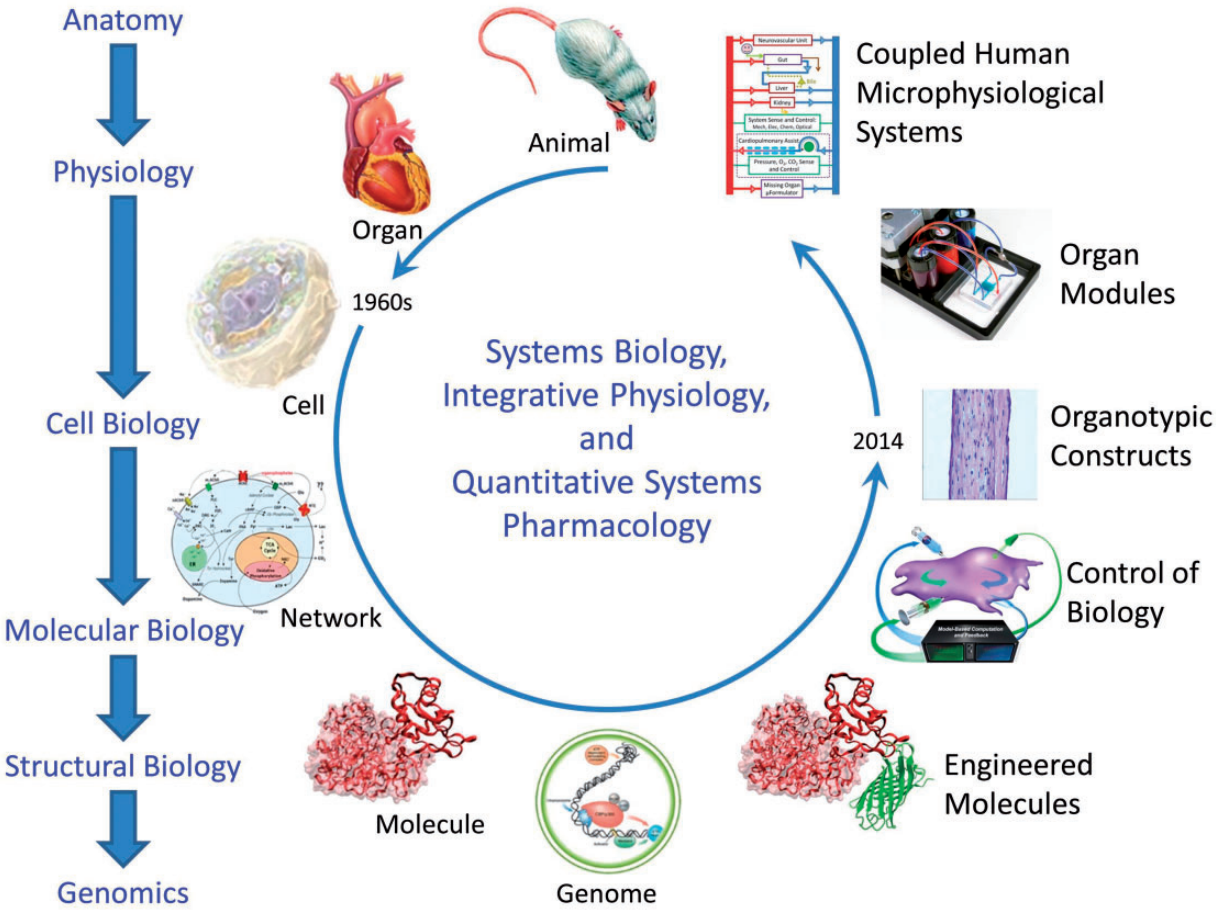

Figure 1 The hermeneutic circle of biology and microphysiological systems

structures and interactions. One might have hoped that once we reached the reductionist limit of biology, i.e. full knowledge of the genome and its epigenetic regulation, we would be able to promptly return full circle to understanding, from first principles, complete physiological systems and whole organisms – a bottoms-up approach to anatomy and physiology. However, the process of discovery in biology is actually governed by the universal hermeneutic rule "that we must understand the whole in terms of the detail and the detail in terms of the whole."[8] Such a circle can be viewed as expanding as the body of knowledge increases, in what is often termed a hermeneutic spiral. It is important to appreciate that "the circle of understanding is not a 'methodological' circle, but describes an element of the ontological structure of understanding."[8] While individual investigations have circumscribed smaller circles (from a whole to details and back to the whole), biology in its entirety is making its first complete cycle, as illustrated in Figure 1 and evidenced by the growing level of integration of physiology, systems biology, and quantitative systems pharmacology.[9] Ideally, microphysiological systems (MPS) will help drive our explorations along the spiral trajectory that bounds our growing knowledge of biology.

Like language, biology is highly contextual. While reductionist studies can be readily performed on isolated biochemical reactions and cellular organelles, such as mitochondria, biology distinguishes itself from the physical sciences by the breadth and depth of the spatial and temporal scales over which biological systems are connected. Biology is possibly the most integrative of all the sciences – transient metabolic and signaling phenomena at the molecular scale can influence cellular behaviors, tissue function, and organism behavior over long periods of time, and vice versa. Epigenetic studies are demonstrating that signaling states can be inherited across several generations. Biology spans multiple dimensions of complexity[10] (molecular, structural, temporal, and algorithmic, etc.) that support emergent phenomena observable at various levels of abstraction. This span of dimensions is extraordinarily challenging and exciting for some, and intimidating to others.

The intimate connections across the spatiotemporal scales of biology in turn have major implications in the conduct and interpretation of biological experiments. Unless the experiments are performed in the proper context, the results may be self-consistent, reproducible, and informative of subcellular processes, yet misleading or irrelevant to human systems physiology or medicine, as are some toxicology experiments conducted with immortal cells confined to live as a static monolayer on hard plastic (or matrix protein surfaces). The rapidly developing field of systems biology attempts to span scales with integrative,

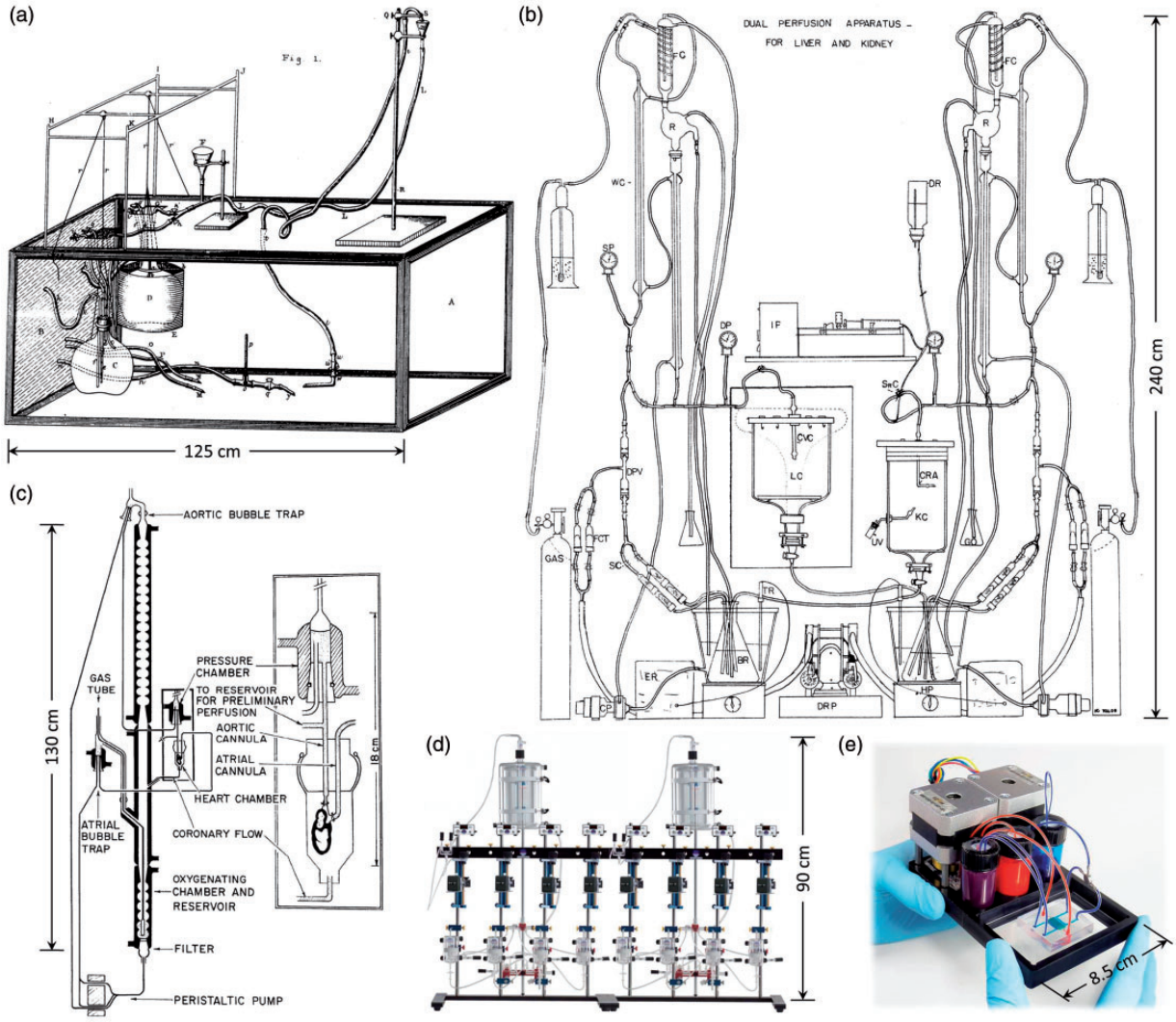

Figure 2 Organ baths for physiological research. (a) 1883: The first system for experiments on the isolated, blood-perfused heart and lungs from a dog.[1] The large frame, glazed on three sides and the top, was supported by a water-filled iron trough heated by Bunsen burners. The exposed heart and lungs, still in the animal, were isolated from the systemic circulation by cannulation of the aorta and the superior vena cava and perfused with defibrinated calf's blood. (Reproduced with the permission of the Royal Society of London) (b) 1961: A system for investigating the effects of anticancer drugs on bovine blood, kidney, and liver (LC: liver chamber, CVC: inferior vena cava cannula, KC: kidney chamber, CRA: renal artery cannula, UV: urine vial with ureteral cannula, BR: common blood reservoir, DRP: dual respiratory pump, CP: circulating pump for blood, IP: drug infusion pump, DR: drug reservoir).[3] (Reproduced with the permission of the National Science Teachers Association) (c) 1967: The first working heart perfusion system used to study the role of pressure on glucose and oxygen consumption in the rat.[4] (Reproduced with the permission of the American Physiological Society) (d) 2014: A modern, commercially produced eight-organ bath system for physiological and pharmacological research. (Courtesy of Desmond Radnoti) (e) 2014: A demonstration model of a perfusion controller for a brain neurovascular unit on a chip. (Courtesy of Virginia Pensabene, Frank E Block III, Philip Samson, David K Schaffer, and Dmitry Markov)

bottoms-up modeling coupled with massively parallel measurements, but the extrapolation will be compromised if the foundational data are not consistent with the context of the integrated biological system being modeled. Studies of protein–protein interactions and gene and metabolic regulatory networks inform the development of mathematical models of these phenomena, but ultimately, the challenge is to obtain biological data that fully span the spatiotemporal scales of complete biological systems operating in a realistic context.

Today, we have an excellent foundation in cell biology and genetics. Unfortunately, neither genetics nor current studies of static monolayer monocultures of cell lines with periodic media replacement fully inform us as to how cells will interact with a three-dimensional (3D), perfused microenvironment that includes cells of other types.[11,12] Vascular perfusion and its shear forces polarize cells and regulate barrier and transport functions. Interstitial flows and diffusion produce concentration gradients that guide cell migration and differentiation. A heterogeneous and chemically complex matrix with tissue-specific mechanical properties and electrical and mechanical activity can affect cellular signaling, metabolism, and disease. Continuing counterclockwise in Figure 1 to close the circle, we need integrative tools to help us progress from the reductionist view of molecules towards a modern understanding of tissues, organ–organ interactions, and the breadth of developmental and regulatory controls of complete physiological systems that

emerge from these genetic and regulatory networks. Nowhere would such predictions be more important than in support of the development of new drugs, in particular the anticipation of the efficacy, toxicity, and safety of a drug in humans.[13,14] Although there is a growing suite of techniques, primarily through optogenetics,[15,16] that enable interrogation of intact biological systems, there are few techniques that span the distances as we transition from cellular monolayers to complete organisms. We need to expand our ability to manipulate the genome and engineer molecules to a new level of biological control at all levels of the system,[17] which will allow us to ask very particular questions of biological systems. For this, MPS offer great promise.

The biology and medicine of MPS

For the uninitiated, the theme of this issue immediately raises two questions – what are MPS and why are they of interest?

What are MPS?

An MPS is an interconnected set of two- or three-dimensional cellular constructs that are frequently referred to as organs-on-chips or *in-vitro* organ constructs. The constructs are made with immortalized cell lines, primary cells from animals or humans, or, more recently, organ-specific cells derived from naïve cells, human embryonic stem cells, and induced pluripotent stem cells (iPSCs). Individually, each construct is designed to recapitulate the structure and function of a human organ or organ region, paying particular attention to the cellular microenvironment and cellular heterogeneity. When coupled together to create an MPS, these constructs offer the possibility of providing, *in vitro,* an unprecedented physiological accuracy for the study of cell–cell, drug–cell, drug–drug, and organ–drug interactions, if drug delivery can be properly modeled. Ultimately, they could be used to create, with iPSC-derived cells, a homunculus-on-a-chip tailored to a single patient for use in a personalized or precision medicine scenario.

A convenient measure of the size of an MPS is how it relates in either structure or function to the human organ it is mimicking.[18] Organ constructs scaled to a millihuman (mHu) most often resemble engineered tissues fabricated with macroscopic features, whereas organ-on-chips at the scale of tens of a nanohuman (nHu) to a fraction of a microhuman (μH) are most often created using microfabricated devices, and may involve different monolayers of human cells growing on opposite sides of a thin, permeable planar scaffold, either in a transwell geometry[19] or between microfluidic channels.[20–22] The criteria for the relative scaling of each organ in a multiple organ system have yet to be fully explored, but it is viewed that functional or metabolic scaling is more appropriate than geometric or allometric scaling.[18,23] Once individual organs are fabricated and tested, the next step is to connect several organs together into a multi-organ MPS.[24–27] Depending upon the application, both individual and coupled organs would need to be validated, possibly in the regulatory sense, with functional assays and test compounds with known pharmacology or toxicology. Ideally, a human MPS, such as that illustrated in Figure 3, will provide a context for the study of biology, pharmacology, and toxicology that is much closer to human physiology than monolayer monocultures of immortal cells. This issue does not attempt to review the entire field of organs-on-chips and 3D cell culture, for which there are excellent resources,[14,26–35] but instead explores a small number of systems in detail.

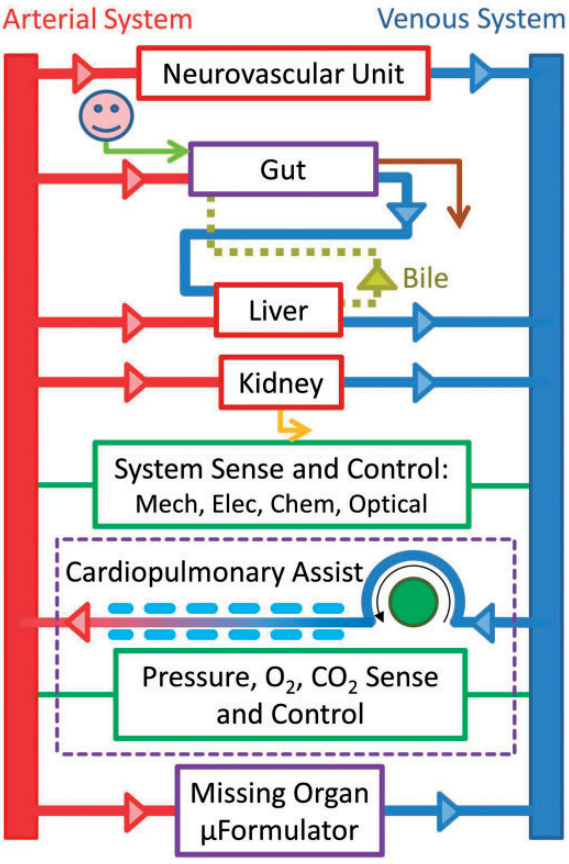

Figure 3 A schematic representation of the components of a hypothetical integrated microphysiological system (MPS) containing a neurovascular unit, a gut, a liver, and a kidney, to recapitulate the organs responsible for absorbing and metabolizing drugs that should or should not be transported across the blood–brain barrier. The requisite support functions to keep the organs alive would be provided by a cardiopulmonary support unit that would deliver O_2 and nutrients, remove CO_2 and wastes, and sense and control pressure, flows, and dissolved gases. Sensing and control of organ function would include mechanical, electrical, and chemical control of the organs, sensors for metabolic and signaling activity, and a missing-organ microformulator to provide the hormonal, nutrient, and metabolite profile of organs that are not included in the system. Ideally, bile would be collected and returned to the gut. This drawing is oversimplified, since the neurovascular unit, the gut, and possibly the kidney will have two or more compartments (blood/cerebral spinal fluid/neuronal; vascular/luminal; and vascular/tubular, respectively) and hence may each have a separate perfusion system for the cerebral spinal fluid, gut luminal flow, and urinary filtrate, respectively

Why are MPS of interest?

As I said above, the idea is simple: the study of monolayer monocultures of cells grown on plastic and/or matrix proteins has provided remarkable insights into biological processes operating on intracellular scales, but this type of experiment fails to recreate the proper context to

Table 1 Shortcomings of static, one-dimensional cell culture, particularly in well plates

Nutrient and metabolite transport is limited by diffusion.
It is difficult to create and maintain controlled concentration gradients.
Extracellular concentrations *in vitro* mimic neither extracellular concentrations *in vivo* nor the relationship of these latter concentrations to intravascular concentrations.
Open-surface cultures may not have significant interstitial flow and the associated signaling.
It is hard to reverse experiments, *i.e.* achieve rapid washout without disrupting the cells.
Daily or less-frequent media changes result in significant cyclic changes in nutrients, metabolites, and pH.
Paracrine and autocrine factors may be diluted to 100th to 1000th of their physiological concentrations by the media above cells.
It is not possible to provide shear forces to maintain endothelial and epithelial polarization.
It is difficult to provide mechanical forces to cells without the use of cumbersome, vacuum-actuated, flexible-bottom chambers.
Small-volume wells with a supposedly homogeneous cellular phenotype do not recapitulate the heterogeneous tissue microenvironment.
The microenvironment in the corners at the outer circumference of a well in a plate may not reflect that at the center of the well.
Wells near the outside of a plate may have different gas environment than those at the center.
The Young's modulus, Y, describing the stiffness of plastic may be 10^4 to 10^5 that of Y for tissue.
It is difficult, but not impossible, to create well-to-well connections with controlled flow that can model organ–organ interactions.
Centralized fluid handler and plate reader hardware are not well suited for:
Simultaneous dynamic experiments on a large number of different wells;
Fast, real-time, closed-loop control of the chemical and mechanical microenvironment;
Complex exposure protocols.

recapitulate cell–matrix, cell–cell, cell–tissue, and cell–organism interactions.[11,12] Nowhere is this more obvious than in pharmacological research to discover and develop new drugs and assess their safety and toxicology.[14] The extrapolation from cells-on-plastic to animals to humans fails in part because of the spatiotemporal scales that need to be spanned in this process, but also because there are vast differences between the planar, homogeneous cellular microenvironments in immortal cell cultures with periodic media changes and those in living tissues with continuous perfusion and cyclic hormonal regulation. Some of the shortcomings of static, one-dimensional cell culture are summarized in Table 1. These would suggest the need for realistic heterogeneous cells growing in 3D extracellular matrices with tissue-like perfusion, stiffness, and proper dynamic mechanical, chemical, and electrical cues – exactly what is offered by organs-on-chips and 3D tissue constructs.

And there are significant differences in the metabolic and signaling mechanisms between humans and lower animals, such that studies of drug responses in animals are often not predictive of human responses. As will be discussed in the accompanying articles, there are also pressing ethical concerns regarding experiments on both animals and people, and for rare disease conditions there may be neither a suitable animal model nor the sufficient number of affected humans for clinical studies. MPS offer us an opportunity to return to the physiology of the whole organs of Figure 2 or specific organ regions, albeit on the mHu or μHu scale, with the advantages of all the physical, chemical, and biological technologies and reduction in the size of experimental models developed over the past 60 years, and with a much more detailed understanding of the parts to inform explorations into the whole.

The articles in this thematic issue provide an overview of the state-of-the-art in the biology and medicine of MPS. All of the minireviews and brief communications are authored by researchers participating in the Microphysiological Systems Program directed by the National Center for Advancing Translational Sciences (NCATS) of the National Institutes of Health (NIH)[36] and funded in part by the NIH Common Fund. This program represents a collaboration between NIH and the Defense Advanced Research Projects Administration (DARPA)[37] and the Food and Drug Administration (FDA).[38] Most articles will expand upon the rationale for the development of organs-on-chips and human organ constructs in support of the ultimate goal to create and apply MPS to drug development and testing. The article following my introduction provides the historical perspective from NIH for the NCATS MPS program and presents expectations for the future.[39] Next is a perspective on the scientific, practical, and governmental regulatory expectations for such efforts.[40] The body of the issue comprises 14 articles describing constructs that can serve as *in-vitro* models for bone and cartilage,[41] brain,[42] gastrointestinal tract,[43,44] lung,[45] liver,[46,47] microvasculature,[48] reproductive tract,[49] skeletal muscle,[50] and skin,[51] as well as the interconnection of organs-on-chips to support physiologically based pharmacokinetics[52] and anticancer drug screening.[53] These research areas will eventually benefit from microscale technologies that regulate stem cell differentiation.[54] As a whole, this issue should provide a useful overview into the biology and medicine of MPS.

Addressing challenges

The articles in this issue present significant recent progress in development of organs-on-chips and 3D organ constructs. They help set the stage for research forthcoming in 2014–2017 under the NCATS MPS program.[39] In addition to targeting improvements in the efficiency and accuracy of studies of drug toxicity, safety, and efficacy in humans, this research should, as pointed out by Slikker,[40] result in important advances in our understanding of fundamental biology and physiology. A large number of investigators are rapidly implementing a completely new set of tools that

Table 2 Technical challenges being addressed for microphysiological systems

Building the MPS models
Capturing an appropriate level of biological complexity and accuracy
Specifying the size and fluid volume of each organ
Creation and maintenance of cellular heterogeneity
Optimization of cellular matrix composition, stiffness, and topography
Control of fluids within the scaled volume budgets (4.5 mL for mHu, 4.5 μL for μH)
Elimination of bubbles and reduction of the rate at which they are created
Development of a universal medium or blood surrogate
Perfusing, superfusing, or vascularizing organs
Obtaining sufficient human cells
Controlling MPS models
Connecting the organs together and controlling each and all of them
Accounting for missing organs
Developing numerical models of organs and multi-organ MPS for design, analysis, and control
Using MPS models
Delivering and distributing the drug
Minimizing non-specific analyte binding
Targeted and untargeted analytical chemistry in nL bioreactors
Integration, mining, and interpretation of omni-omic data
Diagnosing organ and MPS health vs. disease
Disseminating MPS models
Matching the cost of mHu or μHu MPS to the pharmaceutical, toxicological, and basic science marketplaces
Validating individual organs and MPS from the perspective of regulatory science

should accelerate the completion of the first cycle of the entire hermeneutic circle of biology shown in Figure 1.

Whether organs-on-chips and 3D organ constructs are used for pharmacology, toxicology, physiology, or systems biology, there are a number of challenges that must be kept in mind,[28,55–57] particularly when multiple organs are coupled together to create MPS to model drug–organ–organ interactions and organ–organ regulation. As listed in Table 2, these include the complexity of biology; the need for small and controlled fluid volumes; the requirement to perform analytical chemistry in nL volumes; the need to create and maintain heterogeneous 3D tissue constructs; determining the proper functional scaling of organ sizes, perfusion media volume, and the minimum number of cells and topography required to create the desired organ functions; controlling the integrated organs; accounting for the contributions of missing organs; obtaining sufficient human cells; organ vascularization; and minimizing cost. I will now address several of these in detail.

The volume problem

Foremost is the question of volumes and the challenge of solving complex engineering problems associated with the creation, maintenance, and analysis of small microfabricated bioreactors.[56,58] In regard to conventional cell culture, media is typically changed every day or two, depending upon the metabolic activity of the cells and the extent of media buffering. For physiological responses that occur with one- or two-day time constants, analysis of conditioned media every day or two may detect the steady depletion of nutrients, secretion of metabolites, and production of paracrine and autocrine signals. For metabolic and signaling events that are faster, transient chemical signals will be diluted, as shown in Figure 4. This dilution may lead one to miss key dynamic signaling events that can be detected only when the extracellular media is reduced to physiologically appropriate volumes, as was done with the demonstration that dendritic cells secrete factors that can stimulate naïve CD4+ primary human T cells without the formation of an immune synapse.[59] One of the great advantages of microfluidic systems is that they can support media-to-cell ratios that are much closer to physiological values than can be achieved in a culture flask, Petri dish, or well plate, thereby avoiding a thousand-fold dilution of paracrine, autocrine, and other signaling molecules and metabolites.

The scaling of the volume that should be associated with each organ is harsh: an adult male human has a blood volume of ~4.5 L, implying that the fluid budget for a mHu MPS is 4.5 mL and only 4.5 μL for a μHu.[18] The daily intake of fluid is 2.5 L/day for an adult human,[60] which suggests that one could withdraw and replace only 2.5 mL or 2.5 μL of water each day from a mHu or μH, respectively, without affecting the concentration of metabolites and signaling molecules. While it is straightforward to adjust the dose of a drug or toxin to account for any excess fluid volume in an MPS, it is much harder to adjust for dilution of drug or toxin metabolites whose rate of production is determined by number of cells present. Dilution of these products into an excessive volume of media could seriously affect dose–response studies in any case where the active compound is not the drug or toxin but a product of cell metabolism or signaling.[23,61] How does one minimize reservoir, pump, tubing, and interconnect dead volume? How does one add fluid to compensate for sample withdrawal, evaporation, or transfer of a sample to another organ? The small dimensions and hence volumes of microfluidic channels create problems with bubbles that need to be eliminated before they are swept past cells, and ideally their generation will be understood and minimized. If pipettes are used to deliver drugs or transfer small volumes between organs, one must pay attention to the effect of evaporation, media density, surface tension, and viscosity on the volume transferred, as well as fluid and selective drug retention within or on the pipette tip. There may be comparable problems if microfluidics are used to transfer the fluids from organ-to-organ or MPS to the analytical instruments. In small volumes, surface binding of drugs and metabolites to a microfluidic device or analytical instrument can affect concentrations, both in their measurement and the exposure to cells.

Delivering and distributing the drugs and toxins

While MPS models should immediately be applicable to modeling human physiology, their ultimate utility as a general tool for pharmacology and toxicology is predicated on being able to deliver the drug or toxin in a physiologically realistic manner. The MPS obviously needs to include the

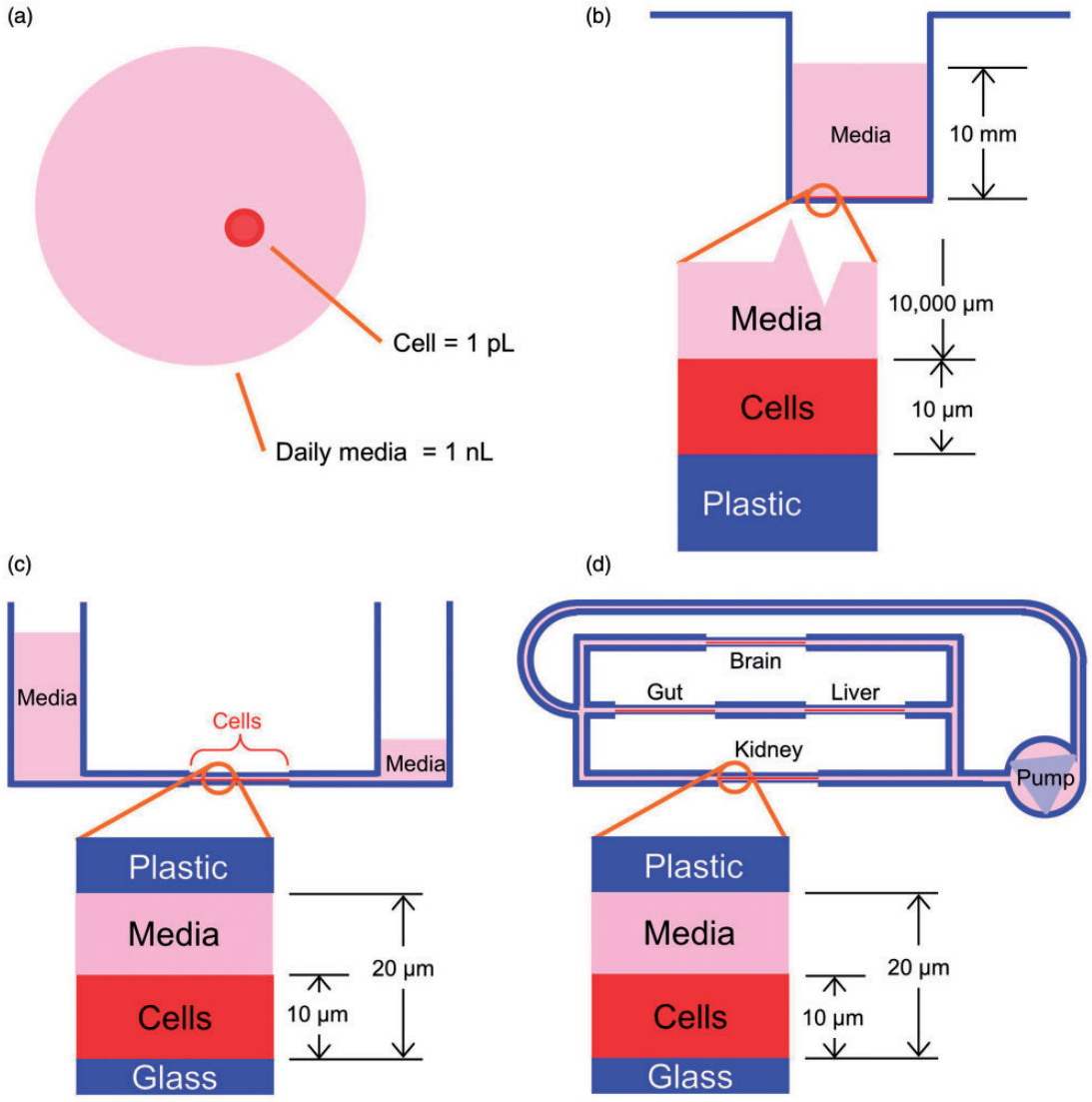

Figure 4 Media volumes in cell culture and organ constructs. (a) A representation of the typical spherical volumes occupied by a single cell (~1 pL) and the media that is required to keep that cell viable for one day is 1000 times larger (~1 nL). (b) Cells cultured in a well plate with daily media changes, wherein the volume of media is 1000 times the cell volume and hence the media height is approximately 10 mm, which results in a dilution of dynamic metabolites and paracrine and autocrine signals by a factor of 1000. (c) An organ construct grown in a microfluidic device with reservoirs that use gravity and height differences to superfuse the construct. The total system volume may lead to significant dilution of transient signaling molecules and metabolites. (d) Coupled microphysiological systems in which each organ and the system's fluid volume are scaled to the same functional size, and a low-volume, on-chip pump is used to recirculate the media and provide appropriate shear forces. The volume of the tubing and pump is comparable to the scaled, total human blood volume that includes both the vascular system and the missing organs. The mechanisms for delivery of O_2 and nutrients and removal of CO_2 and wastes are not shown. In (c) and (d), the cells will ideally be supported by an extracellular matrix that contains appropriate cell types to reflect the organ microenvironment and cellular heterogeneity

target organ, but also the means by which the drug or toxin enters the system. Thus it will be necessary to consider the vehicle of the drug under investigation, the distribution from the site of administration, both the specific (hormone-carrying) and general (albumin) carrier proteins, and efflux/influx membrane "pumps," all of which will influence drug disposition.

Analytical chemistry in small volumes

The volume problem extends to the analytical chemistry to quantify the metabolic response of cells to drugs and toxins – the means by which we interrogate an MPS. A reasonable estimate is that a daily withdrawal and replacement of 10% of the MPS fluid volume might not adversely affect organ–organ communication through conditioned media, but that places an upper limit of 450 μL and 450 nL for daily analysis from a mHu and a μH MPS, respectively.

How does one best select from modalities such as electrochemical sensing of pH, glucose, lactate, oxygen and neurotransmitters; optical monitoring of intracellular $[Ca^{2+}]$ signaling; fluorescent reporters of the cellular microenvironment (pH, $[O_2]$, reactive oxygen species, mitochondrial membrane potential, intracellular pH, transmembrane potential, receptor occupation, etc.); and affinity binding probes such as surface plasmon resonance, ELISA, and microbead-based assays? There is clearly a need for analytical instrumentation whose sensor size or sensing volume is

scaled to μL and nL bioreactors.[58] These instruments need to support a wide dynamic range of some analytes, such as glucose and lactate, whose concentrations differ widely between different cell culture media and may need to be calibrated specifically for the perfusion media. Multianalyte microphysiometry has been shown to have the sensitivity and response time to study the metabolic dynamics of the exposure of small numbers of cells to drugs and toxins,[62–70] and there are ongoing efforts to fully integrate this approach into the MPS program. Fluorescent dyes could enable readouts of key process variables, but fluorescent dyes can have unintended effects on cellular metabolism,[63] only a limited number of dyes can be used at one time without overlap or the need for spectral deconvolution, and sequential measurements may lead to photobleaching and phototoxicity. Untargeted searches for drug and toxin metabolites with volumes as low as 100 nL can be accomplished using ion mobility-mass spectrometry (IM-MS).[56,71,72] It is interesting to note that point-of-care diagnostic devices that can utilize a single droplet of blood may be adaptable to an MPS, particularly at the mHu scale,[73] since the small size of the MPS models may preclude conventional clinical laboratory assays that are designed to utilize milliliters of blood.[74,75]

There is obviously a tradeoff between sampling frequency, sampling volume, and the number of analytes that can be quantified in the sample, whether with optical interrogation or analytical chemistry. The analytical requirements for assessing cellular toxicity may be vastly less than those required to discern the mechanism of action of a drug or toxin, with the latter possibly benefitting from untargeted searches using both ultraperformance liquid chromatography (UPLC) IM-MS of cellular supernatant and cytosol, and subcellular matrix-assisted laser desorption ionization (MALDI) MS imaging of intact cellular monolayers. Targeted searches, for example to monitor organ function or search for a specific toxic metabolite, can be conducted using either affinity binding or MS platforms that are tuned to specific molecules. Untargeted searches present major challenges in bioinformatics and identification of molecular species of particular interest. There is an as-yet unanswered question of how one diagnoses the ''health'' of an individual organ construct or an entire MPS. What should an experimentalist infer and what is the correct response were an MPS to fail to thrive or to encounter massive organ failure – two clinical conditions that present challenging diagnoses?

Universal media

It is widely recognized in the MPS community that there is a need for a blood surrogate that can serve as a universal media without serum; deliver sufficient O_2 and remove CO_2 through either a hemoglobin or a perfluorocarbon O_2 carrier (or possibly another O_2 carrier) or by scaling perfusion channel diameters and flow rates; provide transport proteins required for organ–organ communication; and maintain proper osmolarity, pH, and salinity. The surrogate must also promote the maintenance of ''normal'' intracellular ions and transport proteins. Today, individual cell types require customized media, yet this problem may be minimized by the use of endothelial and epithelial barriers to isolate the cellular microenvironment from surrounding fluids.[20,21,31] The isolated organ studies in Figure 2a and b used bovine blood, but the investigators had already noticed differences between the age of the calf from which the blood was drawn or the manner in which the blood was collected,[1,3] and the MPS community may have comparable discoveries awaiting. It may also be possible to use blood or blood components to support MPS.

Accounting for missing organs

What are the criteria for delivering soluble effector molecules, such as morphogens, growth factors, hormones, metabolites, and cytokines, that would be produced by organs not included in the MPS? How does one remove compounds that would be metabolized by missing organs? What does one add to media to account for evaporative losses through the devices and tubing, and should an evaporative interface be included to mimic human lungs and skin? To what extent can the missing organ problem be solved with a computerized, integrative physiological systems model[76,77] to drive a missing-organ microformulator? How do we simulate the effect of exercise on a homunculus-on-a-chip? With a microfluidic stress test? A treadmill-on-a-chip? How do we regulate metabolic activity at the organ level? Metabolic scaling is worthy of consideration, but metabolic activity can be tightly controlled through chemical, mechanical, and electrical stimuli, the environmental temperature, and by deliberately limiting the availability of oxygen and nutrients. How many variables can be changed in a single experiment, and can Fisher randomized multiparametric questionnaires be used to guide the design of experiments?[78] Will the organs in an MPS self-regulate to homeostasis, or will investigator intervention be required to ensure MPS metabolic and functional stability?

Developing numerical models of organs and multi-organ MPS

The design of a properly scaled MPS will require attention to the relative size of each organ and its perfusion channels, and an understanding of the pharmacokinetics and pharmacodynamics of the drugs being studied and their metabolites. It will be necessary to control a missing-organ microformulator. These tasks may be accomplished best using physiologically based numerical simulations. A computational model may guide data interpretation through seeking a solution to the appropriate inverse problem. Model-based control may be the best way to maintain MPS over long-term studies.[17] What can we learn about an MPS by studying the noise in the intrinsic and extrinsic regulatory systems as we attempt to maintain or challenge homeostasis in a homunculus-on-a-chip? Can we prevent, induce, control or utilize biological oscillations in the homunculus?

Costs

There are two different scales by which costs can be examined – global investment and the cost of the research instrumentation. At the scale of nations, in the United States, programs supporting MPS development include ones funded by the FDA,[38] DARPA,[37] NIH,[36] and the Defense Threat Reduction Agency (DTRA).[79] The Environmental Protection Agency (EPA) has solicited proposals for organotypic culture models for predictive toxicology,[80] but awards have yet to be announced. The U.S. commitment to date is approaching $200 million. There are significant efforts in Europe as a result of legislation to replace toxicity testing in animals.[81] There is a high level of interest in developing new tools for the assessment of the toxicity of engineered nanomaterials and the identification of the mechanism of action of any observed toxic effects,[82] and improving the efficiency of predictive human toxicological testing, developing an understanding of complex pathways of toxicological relevance, identifying early, predictive markers of toxicological relevance, reducing the use of laboratory animals in safety testing, and meeting regulatory requirements.[83] The European Commission is funding a Body on a Chip project as a collaboration between multiple European academic and industrial partners, with the goal of developing a comprehensive *in-vitro* model that allows identification of multi-organ toxicity and/or decreased efficacy due to metabolic activity.[84] New startup companies have already been created in the U.S. and Europe to develop organ-on-chip technology, and there is obvious interest by major pharmaceutical companies. As MPS research advances the development of physiologically realistic 3D tissue constructs, the breadth of both application and investment should expand.

At the scale of the individual system, it is interesting to note that the apparatus in Figure 2b was reported to have cost $1500 in 1961.[3] It is unclear how many of these a research laboratory or pharmaceutical company might have been able to afford or physically accommodate at the time (the authors refer to a simple, single-organ system that could be built for one-tenth of the cost by using, for example, a modified door closer instead of a ventilator[85]), but as shown in Figure 2d, compact isolated tissue baths are still in widespread use. While one might argue that the price of a single MPS instrument would be irrelevant should it provide a significant advance in rapid screening or predictive capability, particularly as related to chemical and biological defense, my personal belief is that the adoption of a technology such as organs-on-chips will reflect a balance between information gained, ease of use, physical size, reliability, and the cost of each instrument, particularly when large numbers of instruments would be required for massively parallel experiments to increase throughput and decrease time-to-decision. I also predict that there will be a major shift in the topology of biological experimental apparatus when the size and portability of modular analytical instruments and system controllers reach that of a well plate and their cost approaches $100: Instruments will be consumables; each experiment will have dedicated yet disposable hardware; and massively parallel, closed-loop, automated 3D tissue experiments can be made at a realistic cost. This low-cost experimental setup should rapidly advance pharmacology, personalized medicine, toxicology studies, and systems biology. Hence there is a strong motivation to reduce the size and cost of each organ construct and its controller, so that more complicated systems could be assembled and studied in detail. Finally, the return on the investment at all scales will have wider ramifications for the costs of evaluating new drugs, the identification of unrecognized environmental toxins, and the ability to model human disease *in vitro.*

While these goals and challenges may appear daunting, it is reassuring to realize that multiple groups are already tackling them. By addressing them and answering the associated questions in the course of the DARPA, NIH-NCATS, and other MPS programs, the biological community should produce integrated organ microfluidics modules that are compact, low-cost, and easy-to use, and that allow investigators to control and assess individual and interconnected human organs-on-chips and organotypic tissue constructs in large-scale screens of thousands of chemicals.

Opportunities

We have already discussed the intended utility of MPS – they may lead to improvements in the development of new drugs and optimization and repurposing of existing ones, and in the process minimize the risk of adverse and even fatal drug interactions. A reduction in the number of false-positives and false-negatives in drug discovery and development would improve accuracy and reduce cost as the pharmaceutical industry determines the toxicity, safety, and efficacy of drugs in humans. Just as tumor explants are being considered as a means to screen drugs for cancer therapy, ultimately it may be possible, through iPSC technology, to create an MPS that is derived from a patient's own cells and could provide the most accurate determination, for example, of which anticancer drug might have the greatest efficacy with the fewest adverse side effects. Beyond the scope of this thematic issue is the potential of small-volume organ-on-chip technologies to contribute to the optimization of the protocols for stem cell differentiation for repair and replacement of human organs, including the study of the maturation of stem cells and identification of sex-related differences.

But the uses of MPS go far beyond pharmacology. The general application of this technology will support a deep understanding of biology and complex systems and a more complete assessment of yet-unknown effects of environmental toxins. Organs-on-chips can serve, in Don Ingber's words, as ''living histological sections'' that may provide much more information than fixed histological sections from an animal. It may be possible to observe these cell cultures continuously under the microscope. Organ-on-chip systems may enable rapid detection of the mode of action of chemical and biological warfare agents, particularly pathogens maliciously engineered to avoid detection or confuse the response to an attack. The simultaneous electrochemical, mass spectrometric, and optical measurement of the dynamics of tens to hundreds or even thousands of cellular variables will allow an unprecedented advance in

our understanding of living cells and how they respond to pharmaceuticals, cellular or environmental toxins, and chemical/biological/nuclear agents; our recognition of toxin–toxin adverse synergisms; and our ability to develop drugs that are used for toxin prophylaxis and treatment.

The fundamental challenge to any attempt to model a biological system *in vitro* is the complexity of biology, as best exemplified by the hundreds of thousands of different chemicals that are active in any large biological organism. An MPS should be viewed as an approximation of reality, not as an accurate reconstruction. We should not fall into the trap of unrelenting detail and accuracy, lest we fail to recognize that ''[t]he best material model for a cat is another, or preferably the same cat.''[86] It is important to recognize that, following Einstein's apocryphal advice to ''make one's theory as simple as possible but not too simple,'' we should make our organ constructs and integrated MPS as simple as possible but not too simple. Were we to succeed in fully recapitulating a μH on a chip, it would be too complicated for us to understand! So the role of these systems is to provide us readily fabricated and easily studied model systems to test hypotheses. Were a test to fail, it would be necessary to determine whether the hypothesis or the model upon which it was tested was at fault. But we already know that we can learn a great deal from an imperfect model, just as was shown 60 years ago using the isolated organ systems in Figure 2. Thinking of biology as a hermeneutic circle (Figure 1), we will not be able to understand the whole until we understand the parts, but we will not be able to understand the parts until we understand the whole. I expect that MPS will play a key role in the next of what may be many cycles.

While a skeptical reader may wonder whether integrated MPS are either too complicated to understand or too simple to be realistic, one must recognize that new understandings of biology and medicine can come from unusual sources – we simply need to remember Dr William Beaumont and the gastric fistula of Alexis St Martin[87,88] that taught us much about the stomach and digestion. Possibly integrated MPS, their glass windows into tissue-engineered constructs, and the ability to control both the intracellular microenvironment and intra-organ communication will provide a new vista not only into drug toxicity, safety, and efficacy and environmental toxicology, but also into systems biology, integrative physiology, and quantitative systems pharmacology – overlapping, expanding areas of excitement in biology and medicine. MPS can now help us return to the concept of organ interactions on a scale that maintains relevant tissue–volume relationships with high analytical sensitivity.

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