ANNUAL REVIEWS

Annual Review of Pharmacology and Toxicology

Application of Microphysiological Systems to Enhance Safety Assessment in Drug Discovery

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Annu. Rev. Pharmacol. Toxicol. 2018. 58:65–82

First published as a Review in Advance on October 13, 2017

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

https://doi.org/10.1146/annurev-pharmtox-010617-052722

Keywords

microphysiological systems, toxicology, drug discovery

Abstract

Enhancing the early detection of new therapies that are likely to carry a safety liability in the context of the intended patient population would provide a major advance in drug discovery. Microphysiological systems (MPS) technology offers an opportunity to support enhanced preclinical to clinical translation through the generation of higher-quality preclinical physiological data. In this review, we highlight this technological opportunity by focusing on key target organs associated with drug safety and metabolism. By focusing on MPS models that have been developed for these organs, alongside other relevant in vitro models, we review the current state of the art and the challenges that still need to be overcome to ensure application of this technology in enhancing drug discovery.

INTRODUCTION

Attrition of promising drug candidates as a consequence of unacceptable toxicity is a major barrier to drug research and development (R&D) productivity (1, 2). Although the root causes are complex and wide ranging, the need for more predictive toxicology models and earlier testing to increase future clinical success is recognized (3). Microphysiological systems (MPS) are miniaturized models that combine bioengineering and biology to generate organ function in vitro (4). In addition to modeling healthy organ function, patient-centric MPS models that address the translational assumptions from bench to bedside are also now emerging. Consequently, these models are rapidly gaining credibility as a potential solution to the R&D productivity challenge.

Discovery-phase toxicology goals are to identify the most promising drug candidates and eliminate those with unacceptable toxicity as early as possible. However, it is not practical to generate an exhaustive hazard and translational risk profile during the early stages of the drug discovery process, nor is it possible to accurately predict risks for all potential clinical toxicities. Consequently, drug developers take a tiered approach to balance the screening volume needs with high translational confidence to select small numbers of potential candidates. Thus, throughput and rapid data generation that can influence early-stage drug design are key considerations for any in vitro model deployed at this stage. Evaluating drug target liabilities (on- as well as off-target effects) involves a complex cascade of screens and assays to build understanding of drug pharmacology and safety in healthy and disease scenarios. To generate this level of understanding, scientists are increasingly turning to complex human and animal three-dimensional (3-D) models involving microfluidics and stem cells to assess organ-specific and interorgan toxicity profiles. These models promise to enhance biological understanding during drug discovery as well as increase confidence in cross-species translation, a key gap in humanizing drug discovery (**Figure 1**).

Because MPS hold promise, global government funding and drug regulatory agencies are making a substantial effort to support the development and advancement of the technology. The Advancing Regulatory Sciences Initiative, jointly funded in 2010 by the US National Institutes of Health (NIH) and Food and Drug Administration (FDA), was key in providing momentum to the field. Further funding was realized in 2012 by a partnership between the NIH, FDA, and Defense Advanced Research Projects Agency (DARPA), and in Europe, Horizon 2020, the open

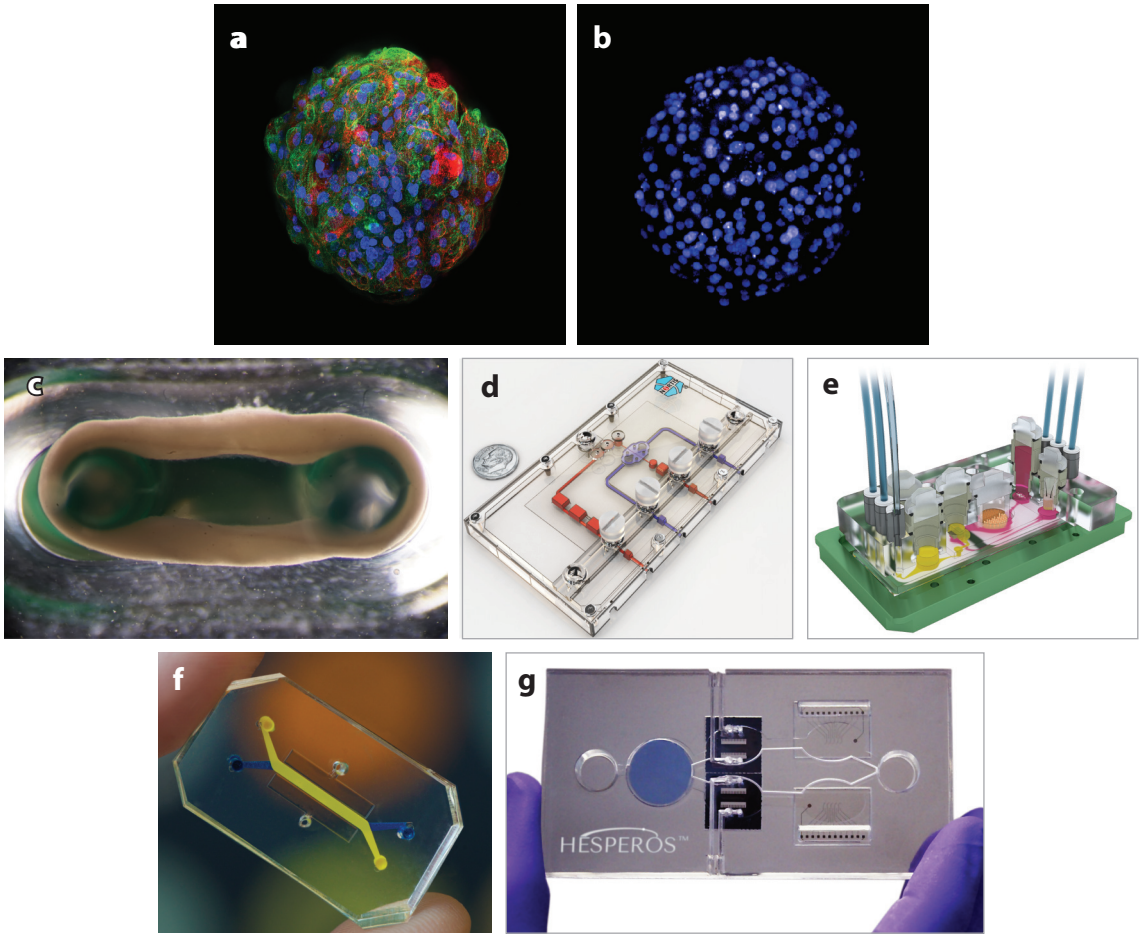

Figure 1

Diversity of complex in vitro and microphysiological system (MPS) models. (*a*) Cardiac microtissues staining includes cardiomyocytes (actin; *green*), fibroblasts (collagen; *orange*), and endothelial cells (CD31; *red*) with nuclei (*blue*) (46). (*b*) Hepatic spheroid with primary human hepatocytes stained with Hoechst H33342 (*blue*) (18). (*c*) The induced pluripotent stem cell–derived cardiomyocytes in a collagen-containing extracellular matrix are aligned using flexible posts and mechanically stimulated to create a physiological model of engineered heart muscle (61). (*d*) The renal proximal tubule MPS is tubular in design and includes fluid flow (122). (*e*) Three-dimensional engineered human tissues are supported in a matrix chamber with perfusion. The design supports micro-organ tissues (i.e. liver, gastrointestinal tract, skin) that can be physically linked (123). (*f*) The lung alveolar MPS uses microchannels for air and media perfusion and vacuum channels to mimic breathing (124). (*g*) A pumpless four-organ body-on-a-chip system with on-device measurement. Two reservoirs on either side provide a defined volume of recirculating medium. The ability to disassemble the system enables further viability and biomarker measurement as endpoint assays (112).

innovation platform CRACK IT, and Innovate UK's Non-Animal Technologies platform are also supporting the field's development. Pharmaceutical industry representatives have established partnerships with government agencies and academic innovators to support the need for rigorous testing of organ platforms and establish a path for successful adoption (5).

Here we explore the state of the art with respect to advanced organ model MPS, focusing on the liver and the heart (as organs frequently associated with clinical toxicity). In addition, we assess other key organ MPS models, including the kidney, gastrointestinal tract, lung, and skin,

as they are associated with compound attrition as a consequence of the route of drug delivery, the pharmacokinetic (PK) profile, or both (6, 7).

TARGET ORGAN: LIVER

The liver is of central importance for drug metabolism. Culture systems using hepatic cell lines constitute an integral part of most drug development pipelines, but these transformed cells poorly represent primary human hepatocytes (PHH) both functionally as well as in transcriptomic and proteomic phenotypes (8, 9). Consequently, they possess limited power to predict human drug metabolism and toxicity in humans (10). For a long time, PHH in 2-D monolayers have been considered as the in vitro model of choice for metabolite profiling, PK analyses, and the prediction of adverse hepatic effects (11). Yet PHH dedifferentiate rapidly and lose hepatic functionality in 2-D culture within hours (12, 13), which severely impairs their predictive power in longer-term (>24-h) experimental evaluations of drug metabolism and hepatotoxicity.

Thus, to enable longer-term studies in which PHH phenotypically and functionally resemble hepatic in vivo conditions more closely, an arsenal of novel, advanced, 3-D in vitro systems have been developed (14). The simplest of these are sandwich cultures in which hepatocytes are seeded between two layers of gelled collagen, resulting in preserved cell polarity (15, 16). However, implementation for other purposes is limited, as dedifferentiation is substantially slowed but not prevented (17).

PHH spheroid cultures represent an emerging cell culture model in which hepatic cells adhere to each other instead of an artificial substrate, which minimizes problems with drug adsorption to culture scaffolds. In spheroids, hepatic cells remain viable and functional for multiple weeks and maintain their transcriptomic and proteomic signatures (18–21). The phenotypic and functional stability of these 3-D platforms is facilitated by cell-cell communication and maintenance of cell polarity in combination with extracellular matrix composition that better mimics the intact organ (22, 23). PHH spheroids exhibit superior sensitivity, especially to drugs that require metabolic activation, act via reactive oxygen species, or inhibit bile flow. They can indicate hepatotoxicity at concentrations that approximate clinically relevant serum levels after multiple weeks of exposure, mimicking the delayed manifestation of drug-induced liver injury (DILI) in humans (20, 24).

The use of perfused systems such as hollow-fiber bioreactor systems allows scientists to mimic microenvironmental factors in the intact liver, such as hemodynamics and shear stress, which have been shown to impact hepatocyte morphology and functional activity (25). Hepatocytes in these reactors can be functionally maintained for multiple weeks, which enables predictive analyses of drug metabolism (26, 27). However, this technology is not suitable for large-scale analyses owing to the large number of cells needed and the limited accessibility of cells, which impedes repeated sampling.

A plethora of different approaches have been developed to support hepatocyte functionality in plate or chip formats (14). The micropatterned coculture system, in which hepatocytes are cocultured with fibroblasts, is possibly the most extensively characterized of such platforms, and hepatocytes in such systems maintain expression of relevant enzymes for multiple weeks (28). This setup has been used to detect hepatic liabilities via a panel of 35 DILI-positive and -negative compounds (29). The culture system can be expanded to support cocultures with Kupffer cells to assess the effect of hepatic inflammation on drug response (30). However, the relatively high exposure levels necessary to evoke toxicity and the use of supporting mouse fibroblasts raises questions about the physiological relevance of this setup.

Advances in 3-D culture systems have resulted in MPS models in which the phenotypes of cultured cells closely resemble their in vivo counterparts (31–33) and have the potential to enable

a significant leap forward in predicting drug metabolism and toxicity in humans. Thus, owing to their physiological, molecular, and histological phenotypes, coupled with their long-term stability, these models are receiving growing attention from academia and industry.

Responses to most medications are highly variable between patients, causing lack of efficacy or adverse reactions to pharmacological interventions (34). In addition to genetic contributions, a variety of other responsible factors, including gender, age, diet, concomitant diseases, or coadministered medications, have been described that contribute to this interindividual variability (35). Novel hepatic 3-D in vitro systems offer the possibility to model these genetic, physiological, and environmental predisposing factors and to reproduce the spectrum of responses across individuals. In such systems, hepatocytes from different donors retain their interindividual variability on the proteomic level in an in vitro setting (20). Functional differences in drug metabolism due to genetic predispositions are maintained, as evidenced by differential metabolic fluxes of dextromethorphan between extensive and poor CYP2D6 metabolizers after multiple weeks in culture (36).

Hepatic 3-D cultures represent versatile tools to model differences in drug response due to various morbidities. Cholestatic disease can be mimicked by repeated exposure to bile acids that sensitizes hepatocytes to compounds with cholestatic liabilities (24), and modulation of glucose can be used to stimulate the hepatic manifestations of metabolic syndrome (37). In some patients, steatosis progresses to nonalcoholic steatohepatitis (NASH), an inflammatory condition mediated at least in part by proinflammatory cytokines secreted by Kupffer cells (38). The option to establish hepatocyte nonparenchymal cell cocultures allows researchers to mimic these complex events and thereby establish pathophysiologically relevant in vitro models. These systems will be useful for assessing drug PK and toxicity in diseased livers as well as for the screening of anti-NASH and antifibrotic compounds. Recent data indicate the utility of such coculture systems as in vitro models for hepatic fibrosis (39).

TARGET ORGAN: HEART

Cardiomyocytes constitute the main functional unit of the heart and are embedded in a stroma of fibroblasts, endothelial cells, extracellular matrix, and vasculature. These cells are highly ordered, allowing an anisotropic spread of excitation through the heart, facilitating ventricular contraction and relaxation in a synchronized manner. Perturbation of this tightly controlled process can result in changes in cardiac function and structure. To minimize cardiac safety liabilities, in vitro screening strategies assessing these perturbations are now an integral part of drug development. To date, this has focused on overexpressing cell lines; rat myoblast H9c2 cells that exhibit a skeletal muscle phenotype (40); mouse HL-1 cells (atrial phenotype) (41), which lack the mature cardiomyocyte phenotype; and isolated cardiomyocytes from preclinical species, which undergo dedifferentiation. The advancements in human stem cell–derived cardiomyocytes (SCDCs) have offered an opportunity to develop improved models that more closely resemble target patient conditions and are amenable to long-term culture. However, although SCDCs in monolayer cultures beat spontaneously, they have an embryonic phenotype, have a resting membrane potential that is nonphysiological, and contain disorganized sarcomeres in monolayer (42–44).

Aggregated SCDC models represent an emerging approach in which cardiomyocytes adhere to each other instead of an artificial substrate. In this configuration, cardiomyocytes remain viable and functional for weeks (45–47). They respond to both electrical and pharmacological stimulation and display typical contraction and calcium transients, but morphologically they represent the embryonic heart. One advantage of aggregate models is the ability of cardiomyocytes to be placed in coculture with nonmyocytes to emulate the complex interplay between cells in the

heart (48), resulting in superior sensitivity and pharmacological relevance to drugs that modulate cardiac contractility (46).

To improve on these models, researchers have defined and validated several fundamental design principles: for example, dynamic mechanical loading to facilitate auxotonic contractions (49), electrical stimulation (50), and inclusion of nonmyocytes for maturation (51). The ideal model would incorporate all these features to ensure (*a*) appropriate tissue-specific architecture, biochemistry, and molecular profiles; (*b*) synchronized and rhythmic contractions originating from a functional syncytium with defined pacemaker cells; (*c*) classical responses to physical and pharmacological stimulation, such as a positive force frequency relationship and an inotropic response to beta-adrenergic stimulation; and (*d*) physiologically relevant electrophysiology.

Numerous MPS models have been developed that display some of these characteristics (52–59). These models represent a significant advance because they have the potential to predict all aspects of cardiotoxicity. The major difference between microtissue aggregate approaches (SCDCs) and MPS models is their morphological maturity; specifically aligned sarcomeres; increasingly physiologically relevant resting membrane potentials; and synchronized, rhythmic contractions. Despite this improved physiology and the fact that functional and structural integrity is maintained for weeks, the pharmacological benefit still requires further characterization, thus highlighting the need for industry and academia to work together to allow the potential of these models to reach fruition.

The use of perfusion systems allows the microenvironment of the heart to be mimicked in terms of hemodynamics (55, 59, 60). Cardiomyocytes in these conditions display typical contraction transients and key cardiac proteins (e.g., α-actinin). In these approaches, cardiomyocytes are also subjected to alignment, and consequently, it is unclear whether the improvements in cardiac physiology result from perfusion or other physical cues, although such models do emphasize the importance of the microenvironment.

The development of customized matrixes, electromechanical stimulation, and chemically defined protocols are enabling further refinement and are moving the field forward (53, 54, 56, 61). Cardiomyocytes that undergo these protocols develop M-bands and display t-tubulations, stable resting membrane potentials, and maturing calcium handling with a positive force frequency relationship demonstrating improved excitation coupling properties. These advancements are a significant advance in terms of replicating the function and morphology of the healthy and diseased heart. Improvements to further capture the biological complexity of the heart incorporating noncardiomyocyte interactions (e.g., endothelial cells, fibroblasts, progenitor cells, and pericytes) and control of the heart via other organ systems (e.g., autonomic nervous system and renal control) offer the potential to build on these approaches. These additional features, as well as methods to enhance throughput, need to become a priority to enable the development of an in vitro model capable of recapitulating all cardiac biology, thus allowing robust detection, mitigation, and investigation of cardiovascular liabilities.

OTHER TARGET ORGANS

Kidney

The kidney is a complex organ, composed of >30 distinct cell types that combine to form the nephron, that actively secretes waste and toxins and reabsorbs water and xenobiotics. These actions are, in part, regulated by the mechanical microenvironment that includes tubular fluid flow, blood pressure, and osmotic gradients. Kidney glomerular and tubular epithelial cells are especially sensitive to these mechanical stimuli, with fluid flow being a key modulator of cellular signal

transduction, cytoskeleton organization, cell differentiation, and gene and protein expression for both active transporters and Phase I/II enzymes in the kidney (62–64). Consequently, the kidney is highly susceptible to drug-induced injury. Twenty percent of adult kidney disease cases are iatrogenic (65), and patients with chronic kidney disease have greater susceptibility to adverse drug reactions (66).

The cells in the proximal tubule are highly metabolically active and play a key role in the active transport and reabsorption of solutes. Additionally, the proximal tubule is exposed to filtered or secreted drugs and toxic metabolites from the glomerulus and peritubular capillaries (67, 68). Thus, recapitulation of solute and drug transport into proximal cells is a crucial requirement for accurate kidney toxicity models. But kidney cell lines have poor transport properties (69), and primary human proximal tubule cells quickly lose their physiological properties in culture (70).

MPS models that incorporate physiologically scaled apical fluid shear to the proximal tubular epithelial monolayer result in enhanced cell polarization, differentiation, alkaline phosphatase activity, albumin transport, and glucose reabsorption compared to cells in traditional Transwell culture (71). This MPS model also responds in the expected manner to toxic insult, including improved transporter functionality (cation transporter 2 and P-glycoprotein). In vivo–like pathophysiology observed in this system suggests that it can serve as a useful tool for evaluating human-relevant nephrotoxicity and could offer a significant improvement in not only predicting toxicity but also understanding the mechanism of toxicity.

More recently, a renal cell line MPS model was exposed to gentamicin using two different regimens that mimic the PK associated with bolus injection or continuous infusion (72). The bolus-type injection regimen showed lower cytotoxicity and nephrotoxicity based on kidney injury marker 1 levels. Permeability of the epithelial layer was also improved when intravenous bolus was compared to continuous infusion. A similar protocol in Transwell culture showed inconclusive results owing to lack of cell polarization. The flow dynamics in MPS can therefore be used not only to create appropriate microenvironments but also to leverage better compound PK when evaluating toxicity.

MPS models hold great potential to reproduce physiology and characterize absorption, distribution, metabolism, and excretion (ADME) and drug toxicity in the kidney, but to accurately capture its biological complexity, these models need to incorporate cell-matrix, cell-cell (e.g., podocyte–glomerular endothelial cells–mesangial cells, tubular epithelial cell–endothelial cells, tubular epithelial cell–stromal cells), tubule-tubule (e.g., proximal tubule–distal tubule, glomeruli–proximal tubule), and lumen-interstitium interactions (e.g., luminar flow to interstitial flow via efflux or influx, osmotic gradient via countercurrent flow). These models also need to respond to specific mechanical stimuli (e.g., flow shear stress, peristaltic motion). More research is required to demonstrate the value of these systems in predicting the clearance of drugs via the specific pathways associated with the kidney (e.g., excretion via glomerular filtration, active transport, passive transport) as well as drug metabolism in the kidney.

Lung

The respiratory system can be divided into conducting (trachea, bronchi, and terminal bronchioles) and respiratory zones (bronchioles, alveolar ducts, and alveolar sacs). The respiratory region is covered by a layer of epithelial cells, which form an efficient barrier to various insults and are highly important in processes that involve the interplay with immune cells (73). Because of this complex nature of the respiratory tract, there are multiple sites and cell types that could be targets for toxicity after drug treatment, especially via inhalation.

In one of the first MPS models described, the alveolar region of the lung was created with epithelial cells that were seeded on one side of a membrane with endothelial cells on the other. A vacuum was applied to emulate inspiration and expiration. By introducing stretch, researchers demonstrated in vivo–like properties (74). In a further study, the investigators also recapitulated IL-2–induced pulmonary edema (75). The importance of mimicking breathing movements was also demonstrated in a different study in which patient-derived pulmonary alveolar epithelial cells were shown to have different permeability properties, metabolic activity, and cytokine release profiles when exposed to mechanical strain (76).

Researchers have described a growing number of 3-D air-liquid interface epithelial models for drug efficacy or toxicity testing (77). Historically, most of these models represented the bronchial epithelium that, owing to its role in barrier function, is a frequent site of drug-induced pathology (78). However, because of the lack of flow in these models, they are not able to mimic the shear stress influence on cell phenotype or replicate the circulation of nutrients or the transport of waste products and mediators. The importance of flow was illustrated in a microfluidics study showing human airway epithelial cells had increased sensitivity to pollen when compared to the equivalent static setup (79). Similarly, in a bronchial epithelium model with a microvascular endothelium exposed to fluid flow, there was a differential response between cells from healthy individuals and those from chronic obstructive pulmonary disease patients (80).

Researchers have also developed a static human airway smooth muscle model (81). In this model, the muscle layer contracts in response to a cholinergic agonist, and by inducing an asthmatic state with IL-13, hypercontractility and altered relaxation could be observed.

Gastrointestinal Tract

The gastrointestinal tract is not only a target for toxicity after drug treatment but is also highly important for bioavailability and activity of drug substances owing to its absorption and metabolic functions. The intestinal epithelium forms a brush border to transport nutrients and also acts as a barrier against pathogens. There is a complex interaction between the intestinal mucosa, gut microbiota, and resident immune components (82, 83). Several complex human intestinal models have been developed, including organoid models, which have been used successfully to determine risk assessment profiles (84, 85). However, the drawbacks with these models are that they lack the mechanical deformation or the luminal flow occurring in vivo and do not mimic the important and complex host-microbe cross talk. Recently, multiple studies have demonstrated the impact of microfluidics on human intestinal epithelial Caco-2 3-D Transwell cell cultures. One study showed that perfusion flow enhanced barrier function of Caco-2 cells (86), whereas others have shown the effects of coculturing human intestinal cells with microbial cells to study host-microbe interactions (87). As with the lung, cells in the gastrointestinal tract are subject to mechanical deformation during peristalsis, which has been modeled in an MPS device (88). Cells in this model show enhanced barrier function, cytochrome P450 activity, and apical mucus secretion. Recently, this model was extended to include the microbiome and show its contribution to human pathophysiology (89).

Skin

The skin is the largest organ in our body and functions as the major barrier between the external environment and our internal organs. Many variations of simple human in vitro skin models now are available (90–92). These models display increasing complexity (93, 94), with the most recent model including a perfusion component that supplies nutrition (95). Such models can also be used to study systemic absorption, by measuring test molecule permeation from the epidermis to the

vascular channels (96, 97). Although very early in development, these models offer the potential to investigate systemic effects of drug application in multiorgan models. Further development of skin disease models will require two-way migration of immune cells into and out of the skin via the microfluidic vasculature (98).

MULTIORGAN MICROPHYSIOLOGICAL SYSTEMS

The improvements in physiological relevance of single-organ models have raised the possibility that a combination of these models in a common media circuit might transform drug discovery. Multiorgan MPS models mimic the physiological interaction of various interconnected organ models, thus emulating whole-organism functionality and response to xenobiotics.

The value of interconnecting these single-organ models in a combined media circuit was first reported by Viravaidya and colleagues (99), who showed that metabolites of naphthalene generated by the liver led to lung toxicity in a multiorgan MPS. A recent study interconnecting liver and colorectal tumor microtissues treated with the prodrug cyclophosphamide further highlighted that only a perfused and interconnected coculture impacts tumor growth significantly (100). The discontinuous transfer of supernatant via pipetting from static liver microtissues treated with cyclophosphamide did not affect the tumor.

Because multiorgan MPS show broad possibilities for application, the systems that have been reported in the literature have a similarly broad technological and biological background. The mode of pumping [external peristaltic pumps (101); on-chip micropumps (102, 103); programmable electromagnetic micropumps (104); or passive, gravity-driven flow (100, 105, 106)] and the format of the device (microtiter plate–based or proprietary design) are prominent examples.

To allow for interpretation of pharmacodynamic (PD) data obtained using multiorgan systems, time-dependent concentration profiles of the drugs in the circulation and tissue models should simulate those in the human body. The use of physiologically based MPS and the introduction of relevant barrier tissues, such as skin, lung, and gastrointestinal tract, are needed to model the bioavailability of a drug accurately. The rate of first-pass and systemic metabolism in the liver and excretion by the kidney are key determinants in PK. A study describing a multiorgan device connecting a human primary intestinal model and a skin biopsy in a common media circulation with liver spheroids and a kidney model was recently published (103). The kidney proximal tubule segregated the media flow through the organs from fluids excreted by the kidney. All four-organ models were viable over 28 days in coculture, and a reproducible homeostasis among the cocultures was generated, representing a step toward an in vitro ADME assay platform with high in vivo relevance.

Another barrier tissue limiting drug distribution from the blood to tissues is the endothelial lining of the microvasculature. Several studies have been performed integrating a vascular network (107–109). These platforms exploit their highly adjustable flow rates and shear stresses. This is essential when working with cells used to constant physical stimuli, such as endothelial cells (110). The integration of a fully closed microvascular network, covering all channel surfaces and penetrating organ models, is required for the integration of an immune system (111). The compatibility among different cell types represents another major issue, although the exclusive use of induced pluripotent stem cells from a single donor is a practical solution when attempting to develop multiorgan devices comprising parts of the immune system.

A further challenge is the choice of media. A common medium supporting the function of the entire microorganism that balances critical media components for each organ model in the interacting system has yet to be found. However, the inclusion of serum or undefined serum substitutes might limit the predictive capacity of MPS in drug development. Recently, a four-organ MPS cocultivating heart, skeletal muscle, neuronal liver, and liver models in a serum-free medium over 14 days reproduced the toxicity of five drugs (112).

Although current multiorgan MPS show great potential in the early elimination of toxic drug candidates, these models answer only specialized questions concerning drug distribution, metabolism, and effects on predefined organ systems. A systemic model that recapitulates the intact organism is required for a full testing paradigm that will eventually eliminate the need for animal testing in drug discovery. This topic has been reviewed by members of academia, industry, and regulatory bodies (111).

MATHEMATICAL MODELING AND SIMULATION

Effective safety assessment of drug candidates requires incorporating multiple pieces of quantitative data, including exposure, time, and complex biological endpoints, which can benefit greatly from iterative computational and experimental analyses. In particular, the design of MPS devices and interpretation of readouts are often complicated by the need to account for differences in features such as geometry, flow, or timescales. This is often necessary because when organ systems are reduced to MPS scale, differences in geometry may follow different scaling relations (113, 114). As these considerations are inherently quantitative decisions, a mathematical modeling and simulation (M&S) approach can be used to tune MPS as well as translate readouts to the in vivo context (115).

A variety of computational techniques can be applied to design, analysis, and translation of MPS devices, including modeling of convection and diffusion (116), physiologically based PK (PBPK) models (117), exposure-response (i.e., PK/PD) models (105), and quantitative systems pharmacology (QSP) models (118, 119). One common use of these modeling approaches has been to describe the function of the device itself. By modeling the flow, volume, and mixing rates of the MPS, for example, it becomes easier to quantify the kinetics of the drug and metabolite in vitro (118, 120, 121). Additionally, these M&S approaches can help translate MPS endpoints to an in vivo context. For example, in vivo drug candidate ADME predictions can be based on MPS data. Specifically, measurement of drug clearance in MPS liver models can be converted to an intrinsic clearance rate and then scaled to predict human PK by using predefined PBPK models of human physiology (117).

M&S is also being applied to analyze the biological PD readouts of MPS through PK/PD and QSP techniques. In these applications, a PK model describing drug and metabolite concentration in the MPS is combined with a mathematical model of the PD endpoint. Such PK/PD models have already been used to analyze metabolic activation in a liver-tumor model (120), in which the effect of active metabolites on tumor cells was quantified with a traditional cell-kill PD model driven by a PK model for the metabolite. More complex models of PD endpoints can also be accomplished. For example, a liver-immune model has been developed (121) in which a mathematical model of lipopolysaccharide-receptor binding and internalization was used to drive turnover models of both tumor necrosis factor-α and IL-6. The mechanistic nature of this model illustrated a potential mechanism for reduced response to repeated stimuli and provided a path to simulate responses that were not tested directly in the MPS.

As more complex cellular data become available from high-throughput and omics approaches, QSP models will likely be increasingly used to model MPS devices. An early example has recently been applied to a model of renal toxicity (118) in which an existing systems biology model of nuclear factor-like 2 signaling, glutathione, and reactive oxygen species was used to simultaneously analyze multiple transcriptional and biochemical cellular responses in kidney cells to xenobiotic challenge.

As MPS models evolve in complexity, M&S will be a vital tool to maximize the value of the data being generated. In particular, the importance of M&S will increase as multiorgan MPS devices become more common. M&S will be key in the quantification of interactions between various subunits through signaling molecules to understand how each tissue influences the others and in translating these effects to reflect the in vivo scenario.

REGULATORY PERSPECTIVE

The failure of new chemical entities to progress through preclinical and clinical development to marketing authorizations is also a concern for regulatory authorities. The potential to improve preclinical testing with innovative MPS is poised to accelerate the preclinical development of new drugs with a better safety and efficacy profile and will help scientists understand how genetic variations may affect responses to these drugs. European Union regulators and the US FDA are already of the opinion that the potential of these systems cannot be ignored, particularly as an alternative to conventional cell culture and animal models. Regulators are keen to support companies and investigators in developing and applying new nonanimal approaches that can improve drug development and should never be seen as barriers to this technology. It is the responsibility of the MPS developers to demonstrate their capabilities to the regulators and to engage with them at an early stage so they can provide guidance as data emerge and the technology grows. Given the enormous potential these models hold, many regulators believe it is vital that they work alongside investigators and industry in the development and validation processes, as this presents a new challenge to regulators and industry alike. One such example is being led by the American Institute for Medical and Biological Engineering in partnership with the National Institute of Biomedical Imaging and Bioengineering and the National Center for Advancing Translational Sciences.

Given the complexities of organ function and regulatory requirements, it is unlikely that MPS will replace the current animal-based pivotal safety assessment testing paradigm anytime soon. Data from MPS can, however, inform and add to information gained from these pivotal safety studies, especially in investigating unexpected effects seen either in animals or in the clinic (D.R. Jones, personal communication). As individual systems are improved, it should be possible to progressively replace one animal-based assay at a time, particularly in PK/PD investigations but also in safety pharmacology and, ultimately, repeat-dose toxicology studies.

FUTURE PERSPECTIVES

Complex organoid and MPS models of both human and animal systems will play a critical role in optimizing discovery-phase toxicology. Together, their goal is to (*a*) improve the prediction of organ toxicity liabilities, (*b*) support mechanistic understanding via biomarker investigation (e.g., targeted or untargeted omics analysis) in a more physiologically relevant test system, (*c*) provide a basis for comparative toxicology between preclinical test species and patients, and (*d*) inform future designs of higher-throughput experimentation to design out undesired effects that could impede exploration of the clinical hypothesis or result in an unacceptable therapeutic index (TI). Understanding the TI of lead compounds is of fundamental importance and, alongside the translational relevance of advanced models, is critical to the assessment of candidate drug safety. In addition to improving recapitulation of organ biology, minimally quantitative human-relevant translational understanding is required that compensates for MPS and species differences and accounts for PK and toxicokinetics. M&S methods based on human systems pharmacology models offer a viable solution to providing a translational framework and critically quantifying toxicological risk. The development of such systems pharmacology and toxicology models for even the major toxicities is a daunting task that will likely require precompetitive data sharing and the standardization of test models.

Key challenges still need to be overcome. The small scale of MPS models enables a more precise control of environmental stimuli but may hinder detection of analytes owing to the small number of cells and subsequent small volume available for analysis. Collaborative efforts from various fields such as cell biology, pharmacology, toxicology, chemical engineering, sensors, microfluidics, and

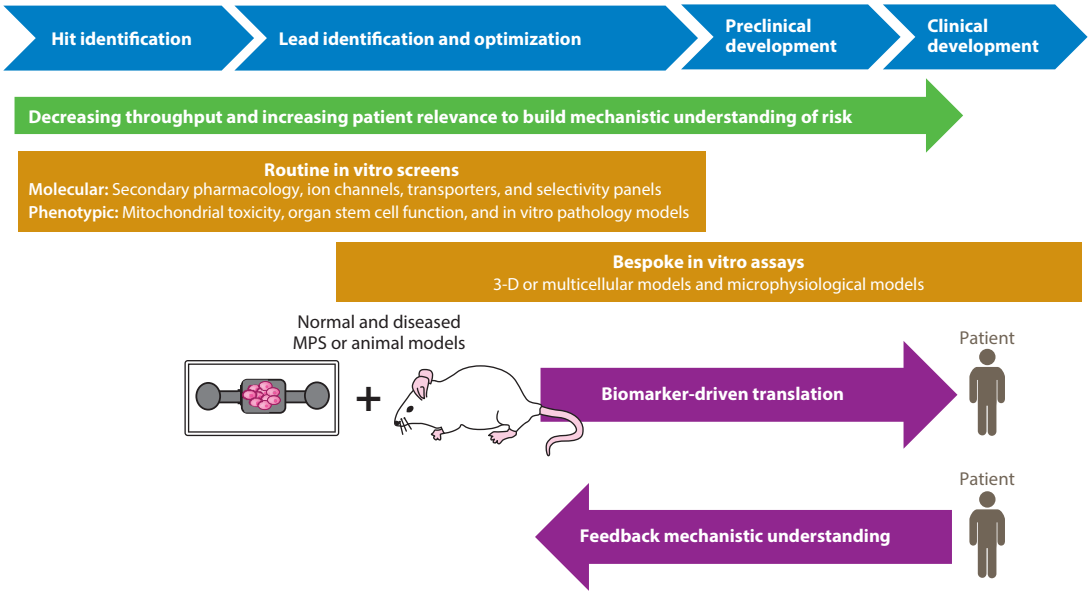

Figure 2

A typical drug discovery operating model, including potential deployment of screening assays and microphysiological systems (MPS). Small-molecule drug discovery typically involves three key phases: hit identification, lead identification and optimization, and clinical development. Initially, large numbers of hits are screened and used to design and select the most promising drug-like candidates. Establishing chemical liabilities involves the use of chemical structure–based computational in silico screens, selectivity and off-target profiling, general cytotoxicity, genetic toxicology, and organ-specific toxicity assessments. However, increasingly complex, bespoke, lower-throughput screens and assays are deployed as drug leads mature during the discovery phase. To improve translational understanding of drug safety and efficacy in the context of disease, complex human and animal three-dimensional and MPS models are now starting to facilitate selection of the best drug candidates and identify safety and efficacy biomarkers. Similarly, MPS can be used to gain mechanistic insight into emerging clinical toxicology (back translation). In the future, inclusion of multiple patient samples will enable insights into the variation of drug responses within disease populations.

engineering will be needed. Proper implementation must be encouraged, and potential end users should understand the intent of MPS technology and set realistic expectations in study design. These systems are not intended to fully recreate the entire organ but rather focus on specific regions of the interest, often those that are more susceptible to compound-related toxicity. Effective MPS study designs include a good understanding of the research question and what gaps remain, as well as the MPS organ models' context of use (5). The ultimate goal is to model human organs and the whole body in health and disease. However, to validate the translational relevance, investment in preclinical variants of MPS models will be critical. As these issues come to mind, it remains evident that industry collaborations with MPS developers are essential. Building a valid MPS model requires not only a precise cellular manipulation but also a detailed understanding of the human body's intricate response to xenobiotic insult. As such, today's systems struggle to recreate aspects of functioning that are governed by complex signals from the endocrine and immune systems.

In conclusion, we see a role for MPS models to assist in the goals of early drug discovery (**Figure 2**). With the advent of MPS that preserve in vivo phenotypic characteristics as well as the interindividual variability of sample donors, it will start to become possible to mimic in vivo biological variability in an in vitro setting and thus to predict an increasing fraction of the interindividual differences in drug efficacy, metabolism, and drug toxicity. In turn, this will improve the probability of success of innovative medicines.

DISCLOSURE STATEMENT

Any opinions expressed by D.R.J. in this review are his own, are not necessarily shared by other assessors at the Medicines & Healthcare Products Regulatory Agency (MHRA), and cannot be considered to be MHRA policy. V.M.L. and M.I.-S. are cofounders and owners of HepaPredict AB. W.-H.Z. is cofounder and scientific advisor of Myriamed GmbH. U.M. is a shareholder of TissUse GmbH, a company commercializing microfluidic systems. J.H. has ownership in Hesperos Inc., which offers services to the pharmaceutical industry for human-on-a-chip systems. S.G. receives funding from the Dutch government [ZonMw (MKMD) project number 40-42600-98-010], the Netherlands Initiative for Regenerative Medicine, and EUROSTARS project 8855.

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