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DRUG METABOLISM & DISPOSITION

Special Section – New Models in Drug Metabolism and Transport—Minireview

Perspective on the Application of Microphysiological Systems to Drug Transporter Studies

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ABSTRACT

Transmembrane flux of a drug within a tissue or organ frequently involves a complex system of transporters from multiple families that have redundant and overlapping specificities. Current in vitro systems poorly represent physiology, with reduced expression and activity of drug transporter proteins; therefore, novel models that recapitulate the complexity and interplay among various transporters are needed. The development of microphysiological systems that bring simulated physiologic conditions to in vitro cell culture models has enormous potential to better reproduce the morphology and transport activity across several organ models, especially in tissues such as the liver, kidney, intestine, or the blood-brain barrier, in which drug transporters play a key role. The prospect of improving the in vitro function of organ models highly prolific in drug transporters holds the promise of implementing novel tools to study these mechanisms with far more representative biology than before. In this short review, we exemplify recent developments in the characterization of perfused microphysiological systems involving the activity of drug transporters. Furthermore, we analyze the challenges and opportunities for the implementation of such systems in the study of transporter-mediated drug disposition and the generation of clinically relevant physiology-based in silico models incorporating relevant drug transport activity.

Introduction

Over the course of a century, advances made in mammalian cell culture technology have dramatically broadened the horizon of biomedical research. From primary cell cultures to immortalized cell lines, under controlled conditions, virtually every tissue-specific cell type can be grown in vitro (Hilleman, 1990; Yao and Asayama, 2017). However, the phenotypic properties of cells in culture can differ substantially from those of the parent cells in vivo. Despite profound differences between primary cells and cell lines, inherent plasticity rearranges vital survival pathways and reprograms gene expression, allowing the cultured cells to adapt to a novel and static environment (Harris, 1989; Astashkina et al., 2012). Pathways involved in the absorption, metabolism and elimination of drugs are often poorly retained in vitro, with cell lines and even primary cells displaying progressive loss of gene and protein expression of enzymes and transporters involved in drug uptake, metabolism, and excretion (Tchaparian et al., 2011). Human primary hepatocyte cultures are very representative of these phenomena. Activity of metabolic cytochrome P450 (P450) enzymes has been shown to decrease by 90% after 24 hours in plated hepatocytes compared with freshly isolated cells (Vorrink et al., 2017). Drug transporter–mediated uptake of prototypical substrates (taurocholate, digoxin, pravastatin, or rosuvastatin) was substantially reduced over 4 days in sandwich-cultured hepatocytes (Kotani et al., 2011). Similarly, commonly used renal cell lines, like the proximal tubule human kidney-2 cells, lack the expression of key uptake transport proteins such as organic anion transporters (OATs) and retain limited efflux capability (Jenkinson et al., 2012). Freshly isolated human primary proximal tubule cells show diminished transporter gene expression when in culture as well (Brown et al., 2008). These constraints have thus far limited the use of in vitro models to study the impact of drug transporters in drug disposition across various tissues and their pharmacokinetic (PK) implications. Novel microphysiological systems (MPSs) represent enhanced in vitro models that aim to closely recapitulate in vivo phenotypes, with improved biologic activity. With the adoption of these culture systems rapidly expanding across various research disciplines, this short review provides an overview of recent MPS studies investigating the expression and activity of drug transporters and

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ABBREVIATIONS: AA, aristolochic acid; ABC, ATP binding cassette; ADME, absorption, distribution, metabolism, and excretion; BBB, blood-brain barrier; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; DCF, diclofenac; DMPK, drug metabolism and pharmacokinetics; hRPTEC, human renal proximal tubule epithelial cell; iPSC, induced pluripotent stem cell; ITC, International Transporter Consortium; IVIVE, in vitro–in vivo extrapolation; MATE, multidrug and toxin extrusion transporter; MDCK, Madin-Darby canine kidney; MPCC, micropatterned cocultures; MPS, microphysiological system; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; P-gp, P-glycoprotein; P450, cytochrome P450; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetics; SLC, solute carrier; TEER, transepithelial electrical resistance; TMAO, trimethylamine *N*-oxide.

offers a perspective on the advantages and implications of MPS models for drug transporter studies.

Drug Transporters as Key Players in Drug Distribution

Transporters belonging to two major superfamilies, the solute carrier (SLC) and the ATP binding cassette (ABC) superfamilies, have been extensively characterized for their role in drug uptake, efflux, and disposition. Within these superfamilies, which comprise over 400 members in the human genome, considerable attention has been paid to xenobiotic transporters that are involved in the PK and pharmacodynamics of many drugs (Hillgren et al., 2013). As the understanding of the localization, function, and clinical implications of drug transporters continues to emerge, their importance becomes evident. This includes transporters in organs where barrier function is central to their physiology, such as in the liver and kidney (Giacomini et al., 2010). The International Transporter Consortium (ITC) has identified a substantial number of clinically relevant drug transporters, present in the liver, kidney, intestine, and blood-brain barrier (BBB) (Hillgren et al., 2013). The evaluation of these drug transporters during drug development is now recommended by the ITC and the main regulatory agencies (e.g., the U.S. Food Drug Administration, the European Medicines Agency, and the Japanese Pharmaceuticals and Medical Devices Agency), based on preclinical and clinical observations. Table 1 lists these transporters and their organ localization. In the liver, drug transporters present in the basolateral membrane of hepatocytes handle the uptake of organic anions, cations, prostaglandins, and bile salts, whereas transporter proteins in the canalicular membrane mediate the efflux of drugs, bile salts, and metabolites, often against a steep concentration gradient, from liver to bile. Alongside metabolic enzymes, hepatic transporters play a major role in drug disposition (Faber et al., 2003). Renal transporters localized in the basolateral and apical membrane of the proximal tubule epithelial cells remove drugs and metabolic byproducts (e.g., uremic solutes) from the blood. By concentrating these solutes in the urine (against concentration gradients), proximal tubule drug transporters play a major role in drug excretion (Berkhin and Humphreys, 2001; Masereeuw and Russel, 2010). Along the intestine, drug transporters dictate the absorption of drugs and nutrients and impact the bioavailability of orally administered compounds (Musther et al., 2014). In capillary endothelial cells of the brain, drug transporters maintain the integrity of the BBB and their protective role is responsible for selective drug penetration (Pardridge, 2012).

TABLE 1

Drug transporters recommended by regulatory agencies and the ITC for evaluation in drug development including prospective and retrospective studies and key organ expression

Transporter	Gene	Liver	Kidney	Intestine	BBB
Established					
OAT1	SLC22A6		X		
OAT3	SLC22A8		X		
OCT2	SLC22A2		X		
P-gp	ABCB1	X	X	X	X
BCRP	ABCG2	X	X	X	X
OATP1B1	SLCO1B1	X			
OATP1B3	SLCO1B3	X			
MATE1	SLC47A1	X	X		
MATE2	SLC47A2		X		
Emerging					
OATP2B1	SLCO2B1			X	X
BSEP	ABCB11	X			
MRP2	ABCC2	X	X	X	
MRP3	ABCC3	X	X	X	
MRP4	ABCC4	X	X		
OCT1	SLC22A1	X			

Established transporters are required for evaluation by regulatory agencies (e.g., the U.S. Food Drug Administration, the European Medicines Agency, and the Japanese Pharmaceuticals and Medical Devices Agency). Emerging transporters are recognized as potentially relevant, although their clinical impact is not yet fully understood and established.

In Vitro Systems to Evaluate Drug Transport

Our understanding of the role played by membrane transporters in drug absorption, disposition, and clearance has been an iterative process, built on comprehensive studies, ever since the link between drug resistance and the activity of a membrane transport protein was established. Juliano and Ling (1976) found that mutated Chinese hamster ovary cells expressing a particular surface glycoprotein, now known as P-glycoprotein (P-gp), were resistant to colchicine, in contrast with wild-type cells lacking this protein. Data derived from in vivo models (namely, knockout animals as well as clinical evidence) have been paramount in determining the impact of drug transporters in PK. Although many human transporters have direct orthologs in animal species, with similar ligand profiles, there are notable exceptions, which limit the translation of studies from animals to humans. Furthermore, differences in expression levels and tissue localization of transporters may lead to major differences in tissue-specific drug distribution or accumulation across species (Chu et al., 2013). These considerations are exemplified by the higher expression levels of breast cancer resistance protein (BCRP) and P-gp in the rodent kidney and brain, respectively, compared with humans, as well as the distant homology between human and rodent multidrug and toxin extrusion transporter 2 (MATE2) (Yonezawa and Inui, 2011; Caetano-Pinto et al., 2017b). Undoubtedly, data from clinical studies may be the most relevant in the drug development process. However, human trials are marred by ethical and logistic concerns and are preferentially performed to determine the efficacy and safety of drugs. In vitro models are highly valuable in drug transporter research. For example, vesicle assays and transfected cell lines are very important tools to determine the affinity and specificity of exogenous compounds for drug transporters and to derive kinetic parameters, but these tools are limited in their ability to represent the in vivo situation (Sun et al., 2008; Jenkinson et al., 2012), which consists of a constellation of transporters working together to mediate tissue-specific disposition for a given drug. Thus, new systems to enhance the predictive power of in vitro models to translate to in vivo endpoints are greatly needed, as also highlighted in the recent work by Bajaj et al. (2018) on emerging renal in vitro models.

Cell Lines in the Evaluation of Drug Transport. Conventional models adopted to study drug transporter activity in vitro are widely used as multipurpose tools across biomedical research. These include Madin-Darby canine kidney (MDCK) cells, human embryonic kidney cells, and the human colorectal carcinoma (Caco-2) cell line (Thomas and Smart, 2005; Sun et al., 2008; Dukes et al., 2011). Although these cells offer restricted or poor drug transport activity, they are easy to maintain in culture, can form tight monolayers (e.g., MDCK), and are permissive to the incorporation of foreign genes. The use of cell lines that can overexpress transporter proteins can overcome the loss of activity of a specific drug transporter in cells expanded in culture. Although such systems may be useful in addressing fundamental questions about whether a drug is a substrate or inhibitor of a given transporter, they fail to recapitulate the in vivo situation and are often limited to no more than two transporters of interest. Furthermore, overexpressed transporters are frequently encoded by their respective cDNAs, which have been incorporated in a plasmid downstream of a high-efficiency promoter such as the cytomegalovirus. Thus, cells operate beyond their native physiology, completely obviating transcriptional regulatory pathways.

Primary Cells in the Evaluation of Drug Transport. Freshly isolated primary cells are favored for high-quality predictive drug metabolism and pharmacokinetics (DMPK) studies since they retain more of their physiologic function with transcription occurring in the context of full genes (not cDNAs downstream of high-efficiency promoters), therefore recapitulating native enhancer and promoter activity, and indeed

the in vivo transcriptional milieu. While overexpressing cell lines are a powerful tool to perform multiplex single drug transporter assays (e.g., high-content screening), primary cultures can generate more robust and translatable results for predictive drug development purposes including physiologically based pharmacokinetic (PBPK) modeling (Barton et al., 2013). However, sourcing, growing, and evaluating quality primary cells can be a labor-intensive and costly enterprise, with the cells functionally decaying in culture over a short time and providing narrow assay windows.

The use of micropatterned cocultures (MPCCs) can improve the viability and function of primary cells in vitro. This method, in which a micropattern of adhesion molecules (e.g., collagens) is imprinted onto a culture plate (Ballard et al., 2016), allows the cells to grow in an organized monolayered structure. MPCCs have been effectively used to enhance the life span and maintain the phenotype of cultured primary hepatocytes. MPCC hepatocytes displayed enhanced P-gp and organic cation transporter 1 (OCT1) gene expression and bile canaliculi formation after 1 week, relative to non-MPCCs, as well as increased expression of key P450 enzymes up to 42 days (Khetani and Bhatia, 2008). This approach underscores the impact of adhesion and structural organization on the in vitro phenotype of hepatocytes.

Spheroids and Organoids in the Evaluation of Drug Transport. Also noteworthy are models that enable cells to organize into complex structures that recapitulate tissue architecture and functions, contrasting with typical monolayer cultures. Spheroids are usually generated from immortalized or primary cells which aggregate together to form tissue-like structures. Organoids are typically derived from a stem cell source with the potential to differentiate into a multilineage tissue-like architecture (Fennema et al., 2013; Gunness et al., 2013; Fang and Eglen, 2017).

Liver spheroids, derived from human cryopreserved hepatocytes and maintained up to 21 days, were shown to exhibit relatively stable gene expression of organic anion transporting polypeptide 1B1 (OATP1B1) and increased expression of multidrug resistance-associated protein 2 (MRP2) and the bile salt export pump (BSEP) gene compared with freshly isolated hepatocytes, whereas the profiles of all three genes were upregulated compared with plated hepatocytes (Vorrink et al., 2017). In a follow-up study in the same spheroid model, the protein levels of P-gp, OATP1B1, BSEP, and OCT1 were stable over a 14-day culture period, although it is unclear what changes might occur over the preceding 7-day period where the spheroids form. However, P-gp and OATP1B1 protein levels were decreased relative to sandwich-cultured hepatocytes (Bell et al., 2018). In another model, where spheroids derived from human hepatocytes were cocultured with nonparenchymal cells, relative gene and protein expression levels of P-gp, MRP2, and BCRP (mRNA only) were maintained comparable to liver tissue and freshly thawed hepatocytes, indicating that the profile of apical efflux transporters appeared to be stable up to 35 days in culture. In contrast, mRNA expression of basolateral uptake transporters OATP1B1 and OCT1 was maintained or reduced over time, respectively; but more importantly, relative protein levels for both transporters decreased considerably. In addition, OATP1B3 gene expression was downregulated substantially over time (protein data not available) (Messner et al., 2018). Although the reduction of relative uptake transporter protein levels potentially represents a severe limitation, functional transport activity was not investigated in either model to corroborate the expression profiles, which could clarify the validity and usefulness of liver spheroids for drug transporter studies.

In a renal developmental study, embryonic mouse reaggregated kidney organoids developed a nephron-like organization where the protein expression of both OATs and OCTs was demonstrated. In addition, transport activity was confirmed by the inhibitable uptake of model substrates into proximal tubule–like structures (Lawrence et al., 2015). Human organoids with high levels of kidney structural organization (including defined nephron segmentation) have also been generated from pluripotent stem cells (Takasato et al., 2016). When allowed to vascularize and mature in vivo in rats, kidney organoids derived from murine embryonic kidney cells are capable of glomerular filtration and reabsorption of low molecular weight probes, indicating that aspects of the barrier function of the kidney can be replicated (Xinaris et al., 2012). However, these features are currently not fully replicated or investigated in purely in vitro–based models. Furthermore, organoids typically do not develop a collecting duct system; therefore, the ability to fully replicate the barrier function of the kidney is currently limited. Intestinal-derived murine organoids showed inhibitable glucose, fructose, and peptide transport (Zietek et al., 2015), an indication that these structures have the ability to recapitulate gut physiology.

While the improvement of some phenotypical features observed in such models is promising, the functional characterization regarding drug transport activity is still limited at this stage. In addition, the fact that spheroids and organoids are microstructures that preclude sample collection from specific compartments (e.g., the apical side of cells) limits their usage for in vitro drug transport assays. For example, studies assessing transport of molecules across a cell layer replicating the epithelial barrier function of many absorption, distribution, metabolism, and excretion (ADME) relevant organs such as the intestine or kidney are limited by these practicalities even if spheroids or organoids substantially recapitulate the physiologic drug transport machinery.

MPS models and the Enhancement of In Vitro Phenotypes

Recent developments in microfluidics and microfabrication technologies brought the so-called organs-on-a-chip or MPS models into the spotlight. MPS technology is considered by the World Economic Forum (2016 https://www.weforum.org/agenda/2016/06/top-10-emerging-technologies-2016/) as one of the top 10 recent emerging technologies. The view of the pharmaceutical industry on the application of these technologies was the subject of a recent joint publication coauthored by experts from 11 companies. The implementation of MPS systems was described as having the potential to provide more clinically relevant models, offering improved in vivo predictivity and leading to the reduction in animal experimentation during drug development (Ewart et al., 2017). This perspective is equally shared by the National Institutes of Health National Center for Advancing Translational Sciences (Low and Tagle, 2017). MPS systems are cell culture–based models incorporating flow to allow the formation of tissues that achieve the recapitulation of certain organ functions. With varying levels of complexity, MPS systems aim to create realistic and physiologically relevant organ models with the ambition to represent in vivo biologic responses. In an extensive state-of-the-art review, Donald Ingber, a pioneer in MPS development, identified the incorporation of perfusion over or through cellular structures as a key advantage relative to other three-dimensional in vitro models. Hydrogel cultures, organoids, and spheroids provide higher levels of cellular architecture, but lack microcirculation (Bhatia and Ingber, 2014). Microfluidic channels embedded into chips containing cellular compartments allow continuous perfusion and mimic the flow of physiologic fluids such as blood, bile, and urine. It has been previously documented that altering cell culture dynamics by shaking culture plates or growing cells in extracellular matrix gels benefits the expression and function of drug transporters, and even extends cell viability of both primary cells and cell lines in culture (Dash et al., 2013; Mollet et al., 2017). MPS models combine in a controlled platform a suitable microenvironment that improves cell adhesion with flow, simulating microcirculation, and thus provide a system that can dramatically improve functionality of cells in culture.

Studies conducted in liver, renal, lung, mesenchymal, and endothelial MPS models clearly show that the cellular phenotype and morphology improved, increasingly reflecting the in vivo situation, when cells

transitioned from a flat and static culture to a laminar flow–fed three-dimensional setup. Although the term *organ-on-a-chip* is now in common use, these models usually rely on one or two cell types and only recreate a specific functional unit, rather than a whole organ. This may still fail to recapitulate key cellular functions that depend on cell-cell interactions. For example, liver models make use of hepatocytes, the most relevant functional unit for hepatic metabolic activity, and endothelial cells to recreate the canalicular system; kidney models use proximal tubule epithelial cells as the most relevant cell type for secretory function; gut models use enterocytes for their absorption capabilities; and BBB models use vascular endothelial cells.

In a recent comprehensive review, Ishida (2018) identified the required physiologic profiles of liver, gut, and renal MPS models for DMPK studies. These profiles include the expression of all the specific enzymatic machinery involved in drug metabolism, proper membrane permeability and polarity, and (common to all these systems) the requirement for significant drug transport activity. With increasing recognition of the clinical implications of the role of drug transporters in organ physiology as well as drug disposition (Table 1), it is only sensible that novel MPS models are characterized in terms of transporter expression and activity. MPS platforms are particularly suited to the study of barrier function and cellular interfaces and therefore can provide a far more suitable in vitro environment for drug transporters to operate to their full capacity than conventional models.

MPS platforms are significantly different between organ models and manufacturers, with diverse types of materials, cell sources, and extracellular matrices being used. Standardization guidelines currently do not exist, and different platforms exhibit various data collection endpoints, which makes platform-to-platform comparison a challenging task. As MPS platforms move beyond the prototype stage developed primarily to suit a proof of concept, the growing interest for practical applications is giving rise to scaled-up production and commercially available platforms, which are now finding their niche for both academic and industrial research (Greenman, 2017). Overall, MPS platforms comprise a chip encasing a microcirculation pathway that leads to a growth chamber populated with cells. Perfusion is driven externally by a pump or similar dedicated system, and the flow is usually continuous, with systems providing either a single pass or recirculating flow (Zheng et al., 2016). Figure 1 depicts the most common MPS designs used for liver, intestine, kidney, and BBB models. Depending on the design, a single MPS chip can include microfluidic channels leading to a perfused cellular chamber or comprise multiple paths leading to parallel chambers, providing several cellularized compartments (Ronaldson-Bouchard and Vunjak-Novakovic, 2018). Numerous MPS studies resort to bespoke platforms, developed as prototypes and built by the users, but a variety of commercial platforms are also available. Companies have developed proprietary designs for models including the liver (Emulate, CNBio, Mimetas), kidney (Emulate, Nortis), intestine (Emulate, Mimetas), and BBB (Nortis). Experimental endpoints often include either direct (live) imaging of the chips, immunofluorescence staining to characterize protein expression, effluent collection for bioanalysis of molecules (e.g., biomarkers, drugs, or metabolites), or lysis of cells for RNA analysis (An et al., 2015). These endpoints are common to the majority of examples provided in this review.

Drug Transporter Characterization in MPS Models

Functional units of multiple organs depend on appropriate barrier integrity to support selective permeability of molecules and thus express a plethora of transporter proteins (Table 1). MPS models can provide a compartmentalized platform to study the activity of multiple drug transporters in dynamic, physiologically relevant microenvironments. From a conceptual point of view, MPS models are valuable for drug transport–focused assays because they provide a system that allows correct polarization of cells with a clear basolateral to apical separation, expression of the corresponding native array of transporters, and formation of a highly tight epithelium that can selectively move molecules between compartments. Exposure to laminar flow sustains mechanosensitive responses that reshape the cellular transcriptome and upregulate expression of adhesion molecules (White and Frangos, 2007; Kunnen et al., 2018), and this stimulus has been shown to augment the expression of drug transporters in vitro (Nieskens and Wilmer, 2016).

The expression and activity of drug transporters has been characterized in a limited number of different MPS models. Here we describe

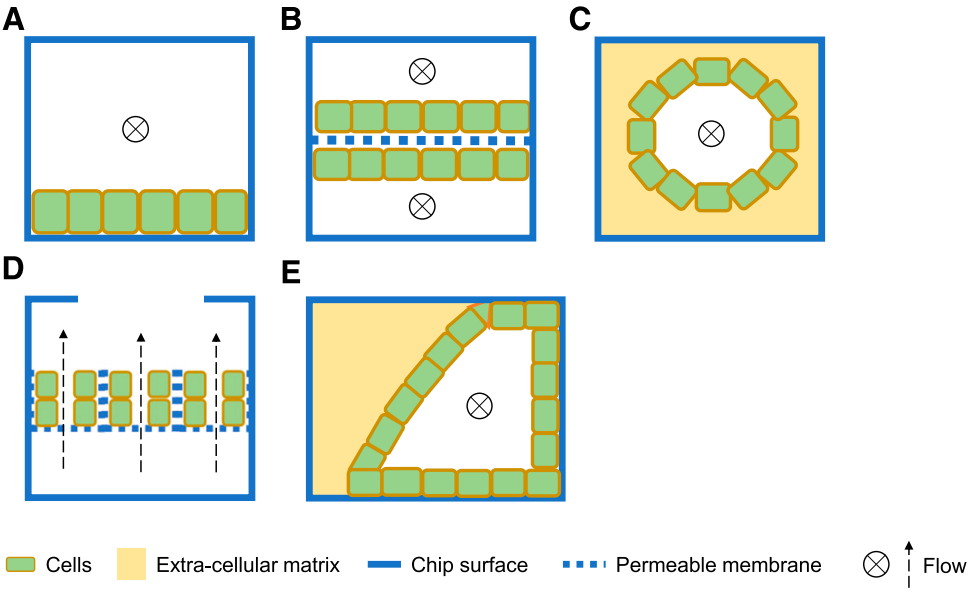

Fig. 1. Cross-section of the most common designs of MPS chips outlining cellular compartments and cell/chip or cell/matrix interfaces. (A) A design where flow is driven above cells adhering to the bottom of a microfabricated channel; this layout is widely used in custom-made chips. (B) A design with two channels separated by a permeable membrane, which can be perfused separately; this layout can be used with cells adhering to only one or both sides of the membrane, and it is a typical choice for coculture applications. (C) A design where cells are entirely adhering to an extracellular matrix, forming a confluent and sealed cell tube; this design is favored by applications involving tubular structures with barrier properties such as vasculature or renal tubules. (D) A design where cells are grown in a permeable and porous surface, such as a scaffold. Cells are perfused through the scaffold, which is coupled to a microfluidic device; this design is used in platforms with recirculating flow. (E) A design where cells are grown into a tube-like structure with an extracellular matrix interface that allows formation of a barrier. Perfusion is gravity driven by passive leveling of the medium resulting from cyclic tilting of the system; cells are therefore exposed to bidirectional flow.

selected examples where drug transporters have been evaluated in organ models that mainly replicate metabolism, secretion, and absorption. Figure 1 details the design of commonly used MPS models. Table 2 lists the cell types used and the parameters investigated for the characterization of drug transporters, including expression and function of specific drug transporter proteins.

Liver. The liver is one of the principal organs of drug metabolism and transport; therefore, availability of physiologically relevant in vitro models is of high importance in drug development. Evaluation of drug metabolism and transport in human in vitro systems is hampered by the short lifespan and dedifferentiation of hepatocytes. MPCC or spheroid cultures have shown promising improvements in the activity of drug transporters. MPS models take hepatocyte cultures a step further with the introduction of flow and have begun to provide a platform to culture hepatocytes over extended periods of time; in doing so, these models demonstrate enhanced viability and activity under dynamic culture conditions. Liver MPS models, alongside other hepatocyte in vitro models, have been the subject of recent reviews summarizing their advantages and giving an overview on current applications (Chang et al., 2016; Hughes et al., 2017; Beckwitt et al., 2018). Here we summarize advances made on the evaluation of hepatic drug transporters.

Urea excretion is higher in cultured human hepatocytes maintained under flow in contrast to stationary cultures. Under flow conditions, cells displayed a well-connected cellular network with bile canaliculi formation, thereby demonstrating higher cellular organization and improved physiologic metabolic activity (Hegde et al., 2014). In two studies using different liver MPS platforms, human hepatocytes under flow conditions were shown to retain the expression and function of MRP2, one of the transporters involved in bile acid secretion, which was demonstrated by the excretion of fluorescent substrates into canalicular structures (Nakao et al., 2011; Vernetti et al., 2016). Bile canaliculi are an environment inherently rich in transporter proteins in vivo, and their recreation in MPS models would be an important feature in liver in vitro models. In terms of gene expression, the mRNA levels of hepatic efflux transporters P-gp, MRP2, and BSEP were shown to be increased 1.5-fold after 6 days in MPS culture compared with the levels in freshly isolated human hepatocytes (Tsamandouras et al., 2017b). In human cryopreserved hepatocytes, a period of 7 days of MPS culture enhanced the gene expression of P-gp, MRP2, and BCRP to about 1.5-fold as well; however, it is unclear whether this translates to a significant change in transport function. In contrast, the expression of BSEP and several uptake transporters, including OCT1, OATP1B1, OATP2B1, and particularly OATP1B3, was reduced compared with freshly thawed hepatocytes (Vivares et al., 2015). These uptake transporter gene expression profiles, maybe with exception of OATP1B1, are broadly in line with data obtained in models such as liver spheroids. However, it is important to emphasize that protein levels show a decay in transporter expression levels in spheroids, which may provide a better indication of the functional capacity (Vorrink et al., 2017; Messner et al., 2018). Of note, comparisons across models can be difficult across studies, as differences in assay conditions (e.g., culture medium), sample preparation, data normalization, and use of reference samples may give rise to varying results. Overall, in the few studies conducted thus far, gene expression of hepatic P450 enzymes and efflux transporters appears to be upregulated in MPS models, whereas basolateral uptake transporters are downregulated. Nonetheless, the focus of most liver MPS studies is usually on general viability, morphology, serum protein secretion, and drug metabolism. Although the expression of drug transporters is assessed, usually by mRNA profiling, the functional characterization is still lagging behind.

Kidney. Drug transporters play an important role in renal drug handling, and active secretion of xenobiotics and their metabolites takes place in the proximal convoluted tubules. Hence, renal MPS models are likely the most well characterized models in terms of drug transporter expression and function. Transcriptomic analysis of MDCK cells, a widely used cell line in transepithelial flux studies, showed that under flow conditions the gene expression of MRP4 was increased 7-fold, relative to static culture. This upregulation was mediated through the induction of the nuclear factor erythroid-derived 2 and the aryl hydrocarbon receptor regulatory pathways. The expression of several SLC transporters and P450 enzymes was also enhanced, revealing a wide-ranging impact of flow on metabolic pathways (Snouber et al., 2012). Human MATE2-K is usually expressed only marginally in static cultures. In cryopreserved human renal proximal tubule epithelial cells (hRPTECs), fluidic culture conditions promoted a 12-fold increase in MATE2-K gene expression (mediated by nuclear factor erythroid-derived 2), which also translated into enhanced functional transport activity, shown by the reduced accumulation of the fluorescent substrate 4′,6-diamidino-2-phenylindole (Fukuda et al., 2017). The lack of OATs in renal in vitro models has long been a bottleneck in DMPK studies (Gozalpour and Fenner, 2018); to date, the actual mechanism by which cells lose these transporters is not fully understood. Human proximal tubule cells grown in an MPS model with continuous flow showed glucose reuptake as well as uptake of the prototypical OAT substrate *para*-aminohippuric acid, a functionality that was absent when the same cells were grown on transwell filters. This recovery of OAT functionality may indicate an active role of shear stress in the regulation of renal uptake transporters (Weber et al., 2016). Coculturing of hRPTECs and endothelial cells was also shown to improve cell viability, mitochondrial activity, and sodium-dependent glucose reabsorption under flow conditions (Vedula et al., 2017). Immortalized human proximal tubule cells showed a high level of membrane polarization in a microplate-format MPS model, while retaining similar levels of P-gp and MRP activity and gene expression (Vriend et al., 2018). In one of the first proof-of-concept studies of renal MPS models, the activity of the efflux transporter P-gp was increased compared with static culture, shown by using the fluorescent probe substrate Calcein-AM, in the presence of the P-gp inhibitor verapamil. Similarly, cytotoxicity of the anticancer agent cisplatin was also increased and could be blocked in the presence of the OCT2 inhibitor cimetidine, indirectly indicating activity of this transporter in the model. Albumin and glucose reuptake was also improved, as well as aquaporin 1 and sodium potassium ATPase protein expression, altogether pointing toward a better renal proximal tubule phenotype in the MPS model (Jang et al., 2013).

Blood-Brain Barrier. Drug permeation across the BBB is critical in the development of drugs to treat neurodegenerative and neuropsychiatric disorders as well as brain metastases. Transporters expressed at the BBB play a critical role in the influx and efflux of drugs into the brain as well as in the integrity of the BBB. Existing in vitro BBB models are usually based solely on endothelial cells and fail to include other cell types needed to sustain a proper barrier function, with mature tight junctions (Wilhelm and Krizbai, 2014). The use of an MPS incorporating separate cellular chambers with a porous membrane interface allowed for the coculture of astrocytes, pericytes, and endothelial cells, thereby recreating a neurovascular unit, by allowing cell-to-cell communication across different compartments (Brown et al., 2015; Phan et al., 2017). To date, BBB research heavily relies on animal models, given the complex nature of the brain microvasculature. Replicating the complexity of cellular interactions and enhancing cell-to-cell adhesion has a stark impact on the formation of the barrier; hence, MPS models provide a significant improvement in in vitro BBB research (van der Helm et al., 2016). Although barrier function was ascertained, the expression and activity of drug transporters, which play a key role in maintaining BBB integrity, has not been the focus of BBB MPS studies

TABLE 2

Selected studies where drug transporter expression and activity were investigated to characterize the MPS models

Organ	Tissue/Functional Unit	Cell Source/Type	MPS Design[a]	Manufacturer	Transporters Investigated	Drug Transporter Characterization			Reference
						Expression	Function	Localization	
Liver	Hepatocyte with bile canaliculus	Freshly isolated rat hepatocytes	Custom design	Custom design	MRP2	ND	CFDA-DA efflux	Immuno-fluorescence	Nakao et al. (2011)
	Hepatocyte with bile canaliculus	Human cryopreserved hepatocytes	C	Nortis	MRP2	ND	CMFDA efflux	ND	Vernetti et al. (2016)
	Hepatocyte with bile canaliculus	Freshly isolated human hepatocytes	D	CNBio	P-gp, MRP2, BSEP	mRNA	ND	ND	Tsamandouras et al. (2017b)
	Hepatocyte with bile canaliculus	Human cryopreserved hepatocytes	D	CNBio	Several, including P-gp, MRP2, BSEP, and hepatic OATPs	mRNA	ND	ND	Vivares et al. (2015)
Kidney	Proximal tubule	MDCK cells	A	Custom design	Several, including MRP4	mRNA	ND	ND	Snouber et al. (2012)
	Proximal tubule	Cryopreserved human proximal tubule cells	A	Custom design	MATE2-K	mRNA	DAPI retention	ND	Fukuda et al. (2017)
	Proximal tubule	Freshly isolated human proximal tubule cells	C	Nortis	OATs	ND	PAH uptake	ND	Weber et al. (2016)
	Proximal tubule	Freshly isolated human proximal tubule cells	C	Nortis	OATs	ND	AA uptake	ND	Chang et al. (2017)
	Proximal tubule	Cryopreserved human proximal tubule cells	B	Emulate	P-gp	ND	Calcein retention	ND	Jang et al. (2013)
	Proximal tubule	Human immortalized proximal tubule cells	E	Mimetas	Several, including P-gp and MRP4	mRNA	Calcein retention, CMFDA retention	ND	Vriend et al. (2018)
BBB	Microvasculature	Human vascular endothelial cells	A	Custom design	P-gp, MRP2	mRNA	ND	ND	Cucullo et al. (2011)
Intestine	Duodenum	Freshly isolated intestinal cells	B	Emulate	Several, including MRP4, MATE1, and OCT2	mRNA	ND	ND	Kasendra et al. (2018)
ND	ND	Human ovarian cancer	A	Custom design	BCRP, P-gp	Protein	ND	ND	Ip et al. (2016)

CFDA-DA, 5-(and-6)-carboxy-2′,7′-dichloro- fluorescein diacetate; CMFDA, 5-Chloromethylfluorescein Diacetate; DAPI, 4′,6-diamidino-2-phenylindole; PAH, *para*-aminohippuric acid; ND, not determined.

[a]The MPS platform design used in these studies is described in Fig. 1. Letters A–E denote the respective figure panel.

thus far. Nonetheless, extended gene expression analysis in vascular endothelial cells revealed that multiple SLC and ABC transporters, including P-gp (2-fold) and MRP2 (3-fold), together with several ion channels and P450 enzymes were consistently upregulated under flow conditions. Furthermore, mRNA levels of adherens junction proteins (cadherins 1, 2, 5, N, and E) and tight junction proteins (occludin and claudin 5) were elevated by 2- to 5-fold, supporting a 7-fold increase in transepithelial electrical resistance (TEER) over time; this indicates that the cells undergo maturation, enhancing their functional properties in fluidic culture. These observations show that cell adhesion, tightness, and polarity are also enhanced by shear stress (Cucullo et al., 2011).

Intestine. Models of the intestine, often referred to as gut MPS models, are critical in assessing and predicting the rate and extent of oral drug absorption that may be limited or enhanced by transporters. Gut MPS models provide a good example of how MPS designs can provide other physiologic stimuli beyond the addition of flow to cell culture, such as cyclic contractions, mimicking peristalsis, an important aspect of intestinal biology (Bein et al., 2018). These conditions facilitated the formation of a tight columnar epithelium by Caco-2 cells, with extensive microvilli. In this environment, bacteria were successfully cocultured on the epithelium surface, recreating the flora that populates the intestinal lumen (Kim et al., 2012). In a further development, a small-intestine MPS generated using human-derived organoids showed extensive brush-border formation, sustained barrier function, elevated levels of polarization, and continuous mucus secretion. Transcriptomic analysis revealed that global gene expression in organoid-derived cells cultured in the MPS was closely aligned with the human duodenum, in contrast with the organoids themselves in static culture and similar gut-MPS models derived from Caco-2 cells. mRNA expression of the MRP4, MATE1, and OCT2 transporters is among those mimicking native expression profiles (Kasendra et al., 2018). In the recently developed gut-MPS models, functional assessment of drug transporters is yet to be conducted and has not been a focus in the characterization efforts of such models.

Cancer. Drug resistance is a major issue in the development of chemotherapeutic drugs and cancer combination therapies and can be a result of regulation and activity of drug transporters. By reducing cellular availability, drug resistance can render cancer treatments ineffective and dramatically limit therapeutic options (Dhandapani and Goldman, 2017). MPS models can be a powerful tool to advance oncology research by better mimicking tumor physiology, an environment rich in transporter proteins. BCRP and P-gp are expressed across different healthy tissues (Table 1) as well as in multiple cancer types, playing a major role in drug resistance (Caetano-Pinto et al., 2017b). Exposing ovarian cancer spheroids to continuous flow recovers the expression of BCRP and P-gp at the protein level, a feature absent in static culture. Furthermore, inhibition of the phosphoinositide 3-kinase/Akt pathway reverses the expression of these transporters, which may be a clear indication that this pathway is involved in shear stress–mediated regulation of drug transport (Ip et al., 2016). Expression, function, and appropriate cellular localization of transporters (features absent in traditional static in vitro models) are augmented and regained in the MPS culture environment. The cellular response to microcirculation positively reprograms signaling pathways involved in differentiation, survival, stress, and metabolism, among others (Desai et al., 2002). The recreation of in vivo–like tumor microenvironments can be a valuable aid in the development of new cancer therapeutics.

Multiorgan Platforms. Building on the physiologic recreation of specific tissue functional units, MPS platforms can also be designed to incorporate multiple cell types to create more organ-like models or to allow interconnectivity between different organ platforms. Through the coupling of multiple organs, such MPS models have the potential to be far more representative of the in vivo situation with regard to their metabolic capacity and their ability to describe PK processes. An opportunity could be to simulate the combined effects of first-pass metabolism and excretion in vitro (Ewart et al., 2017). A few elegant studies utilizing characterized MPS models have demonstrated the possibilities of multiorgan platforms for the study of drug transporter activity across different organ systems.

The metabolism, excretion, and toxicity of aristolochic acid (AA), a potent plant-derived nephrotoxin and carcinogen, was investigated by connecting a kidney MPS model, populated with hRPTECs, downstream of a liver MPS model incorporating hepatocytes. When flow was driven unidirectionally from the liver to the kidney, the nephrotoxic byproduct of AA metabolism (aristolactam) was secreted by hepatic MRPs and actively taken up by renal OATs, leading to cell death in the proximal tubule cells. Hepatic metabolism was required to exert AA nephrotoxicity, as AA administration to the kidney MPS alone did not show an effect. In addition, OAT activity in the kidney MPS was needed because the nephrotoxic effect was abolished in the presence of probenecid, a prototypical OAT inhibitor. This model shows that coupling hepatic metabolism and renal secretion captures a multistep physiologic event otherwise lost in single cell systems (Chang et al., 2017). In a complex collaborative effort involving several laboratories, the coupling of intestine, liver, kidney, BBB, and muscle MPS models was attempted to validate the potential clinical significance of such MPS systems. The uptake, clearance, and distribution of the antihistamine terfenadine and the environmental toxin trimethylamine were studied across the different organs in the model. Results showed that the gut and liver MPSs metabolized terfenadine into fexofenadine, which was subsequently actively secreted by the renal MPS and did not cross the BBB MPS model. In contrast, after absorption in the gut MPS, trimethylamine was metabolized into trimethylamine *N*-oxide (TMAO) in the downstream liver MPS; TMAO was secreted by the renal MPS and crossed the BBB in the brain MPS. These observations are consistent with the clinical data available for the metabolism and transport of both compounds and show, for the first time, TMAO penetration of the BBB (Vernetti et al., 2017), an observation also confirmed by the detection of TMAO in human cerebrospinal fluid (Del Rio et al., 2017).

In a recent tandem approach, it has been shown that up to 10 different MPS models, including key ADME organs such as the liver, kidney, and gut, can be accommodated in a single platform. This concept of an open-system microfluidic platform is compatible with quantitative studies and was described for culture periods spanning 4 weeks studying the in vitro PK of diclofenac (DCF) (Edington et al., 2018). Although drug transporters were not characterized in this study, such models could be useful to study active drug transport across several interconnected organs.

The Relevance of MPS-Derived PK and PBPK Models

MPS platforms incorporating multiple organ models are possibly the most suitable for DMPK applications. The presence and combined function of physiologic mechanisms behind PK (uptake, metabolism, and excretion) makes data generated in MPS platforms valuable for the development of PBPK models. As confidence in the physiologic activity of MPS models increases, the interest in MPS applications to develop PK and PBPK models is on the rise. Traditional PBPK models use in vitro–derived data as input parameters, such as permeability and metabolism and organism features like organ volumes and surface areas to predict human or animal PK, reflecting whole body, viz in vivo, physiology. The same concepts have been used to represent organs within MPS platforms and to simulate the PK of drugs (Watson et al.,

2017). MPS designs can recapitulate organ parameters and systemic circulation, with relative organ volumes reflected in cell compartments and transit and residence times controlled by flow rates (Sung et al., 2014). PBPK-inspired models, incorporating varying levels of complexity such as multicompartmental approaches, can be used to interpret and predict parameters from MPS studies.

Recent studies probing the PK simulation of orally administered drugs using multiorgan MPS models illustrate some of the approaches taken. A mechanistic mathematical model incorporating the operational parameters of an integrated gut-liver MPS (e.g., flow rates, volumes of apical and basolateral gut and liver compartments) was derived using the intestinal permeability and metabolic clearance of DCF and hydrocortisone, after passage through the MPS platforms (Tsamandouras et al., 2017a). Separate and combined PK models of the gut and liver, generated from data collected by operating the gut-liver MPS platform or only the gut or liver components at a time (off-platform) could effectively simulate clearance across the systems. It was determined that drug clearance was not substantially impacted by multiorgan crosstalk. Both DCF and hydrocortisone PK parameters derived from the standalone gut or liver components did not significantly differ from the gut-liver MPS. This study showed the feasibility of an integrative approach for multiorgan MPS PK predictions, accurately describing the DCF experimental results across the multiple platform compartments. The Caco-2 and HT29-MTX intestinal cell lines used in the gut model are known to poorly recapitulate in vivo metabolic function. The authors identified these cell lines as a factor as to why major differences were not observed in the gut-liver model compared with the gut or liver model separately, a limitation that can potentially be resolved by the incorporation of a human tissue–derived gut MPS. In another example of a PK profile derived from an MPS model, the first-pass metabolism of paracetamol was studied using parameters from a gut-liver MPS. Caco-2 cells were used in the gut compartment and, downstream, the immortalized human liver carcinoma cell line HepG2/C3a was used for the liver model. Model predictions determined that the MPS model used would require a larger absorptive surface area and a higher metabolic capacity to reproduce in vivo paracetamol PK (Lee et al., 2017).

Evidence-based PK modeling of MPS parameters relies on the accurate description of the intrinsic parameters of the MPS systems used (e.g., flow rates and volumes). In addition, physiologic parameters are important to reflect specific organ functionalities. Nonetheless, the enhanced metabolic activity observed in the MPS by itself is not sufficient to improve the predictivity of PK models (Choe et al., 2017). Platform and experimental design play an equally important role to derive accurate scaling factors that can potentially be used for in vitro–in vivo extrapolation (IVIVE).

Most studies have focused on metabolic parameters capturing P450 activity and, as multiple-organ MPS models have shown, permeability across organ compartments, which can be a direct result of active drug transport (Chang et al., 2017). Investigating drug transport processes in MPS systems to obtain parameters such as uptake/efflux rates could better reflect the absorption and distribution of drugs and be implemented in PBPK models aimed at IVIVE; therefore, ADME studies in MPS models need to be expanded beyond metabolism studies.

The functional interplay of multiple drug transporters could be determined in MPS platforms that offer activity profiles beyond the level of individual transporters. Parameters describing the action of active uptake/efflux and metabolism can potentially better predict drug interactions and shed light on the physiologic forces driving drug membrane transport. Understanding the expression and localization of transporters is crucial to enable simulation of drug disposition governed by multiple mechanisms. Robust IVIVE models rely on full kinetic characterization of relevant drug transporters (e.g., K_m, V_{max}, efflux ratios), well defined parameters in traditional two-dimensional models that require adaptation to MPS formats. Complex transport and metabolic drug interaction models are limited by our understanding of the true nature of the inhibitory mechanism underlying different pathways and often assume reversible inhibition (Zamek-Gliszczynski et al., 2013). By recapitulating complex interactions, MPS-derived PBPK models could overcome these shortcomings.

The Impact of MPSs Models on Drug Transporter Studies

During drug development, interactions of drugs with transporter proteins are assessed in vitro for several purposes. First, it is important to understand the pathways involved in the transporter-mediated absorption and disposition of a drug candidate. Second, the potential of a drug candidate to cause drug-drug interactions is investigated to assess the impact of comedications on PK and possible effects on pharmacodynamics and toxicity. Third, drug transporter interactions are kinetically characterized to enable IVIVE (that is, to make in vivo predictions about the PK properties and parameters of a drug, such as clearance and bioavailability). Finally, drug transporter interactions are studied to predict drug toxicities. Applying MPS models to investigate drug transport can substantially improve our understanding of their activity during drug development. The power of these platforms relies on the possibility to study the combined function of the multitude of drug transporters present in complex structures, such as the nephron or the bile canaliculi network. Data derived from MPS models can therefore be used to improve the prediction of drug interactions, clearance, and toxicity in vivo in humans. Such data can also be used to improve and refine the design of human clinical trials. For other aspects of drug transporter research (e.g., the identification of substrates and inhibitors or the characterization of genetic polymorphisms), traditional models such as overexpressing cell lines continue to be excellent tools.

Although much has been speculated about the potential uses and applications of MPS models, it needs to be acknowledged that this technology is in its infancy. There are remarkable differences between MPS designs, from bespoke chips to commercially available platforms, and standardization is not on the immediate horizon. Enhancements in physiologic functions and recapitulation of organ-specific features have been demonstrated in a number of exemplar studies. Nonetheless, the number of practical applications harnessing this potential is still limited, and most studies thus far focus on the characterization of models, describing functional readouts of known biomarkers, gene expression patterns, and morphologic features. Unsurprisingly, kidney, liver, and intestine models are at the forefront of the characterization of drug transporters in MPS systems, given the key roles in the absorption and excretion performed by these organs, as well as the regulatory requirements for drug transporter studies in drug development. To improve and widen the use of MPS models, the evaluation of drug transporters needs to be expanded beyond exemplar proteins and include key transporters for each organ studied (e.g., OATPs in the liver, P-gp and BCRP in the gut and brain, OATs in the kidney). Most of the characterization studies summarized in Table 2 have shown that mRNA levels of multiple drug transporters are increased in MPS models compared with traditional culture methods. mRNA analysis is the preferred method to determine expression levels in MPS models. The use of Western blotting or targeted proteomics is still limited by practical issues concerning collection and biologic sample size. Gene expression levels do not necessarily correlate to protein expression and function (Liu et al., 2016; Messner et al., 2018); nonetheless, increases in gene expression have been observed in parallel to functional upregulation of P-gp, MPR4, and MATE1, for example, in renal cell models in

conventional cultures (Caetano-Pinto et al., 2017a; Nieskens et al., 2018). Similarly, in sandwich-cultured mouse hepatocytes, a decrease in MRP4 mRNA expression was accompanied by an intracellular accumulation of taurocholate, indicative of reduced efflux activity (Swift and Brouwer, 2010).

Ultimately, ideal functional characterization should be performed using clinically relevant probe substrates to support results from mRNA and protein expression as well as localization studies. The comparison of MPS to conventional models is an important aspect at this early stage, to support and validate the notion that MPS data are ultimately more physiologically representative through better prediction of in vivo effects.

Limitations, Challenges, and Opportunities of MPS Applications in Drug Transport Studies

Traditional in vitro DMPK studies rely on standardized culture platforms (e.g., multiwell plates and transwell culture inserts) that allow the fast and simple generation and collection of samples. Assays can be multiplexed and automated for a high-throughput data generation, with easy access to incubation supernatants as well as the cells themselves. In most MPS models, cells are encased inside a chip and accessible through microfluidic circuits. This means that the cell culture or incubation solution effluents collected out of the system are dependent on flow. Flow rates are reduced to best mimic organ-like conditions, which dramatically reduces the sample volumes that can be obtained and consequently increases the time intervals at which samples can be taken. To obtain sufficient sample volumes, current studies often rely on sampling regimens over extended periods of time, from a minimum of 1 hour and up to several days (Ishida, 2018). This approach may suit toxicity applications that investigate injury biomarkers as an endpoint, as illustrated by the work conducted over 14 days in a multiorgan MPS in which daily levels of urea and albumin were used to evaluate the cytotoxic effects of doxorubicin, atorvastatin, and valproic acid (Oleaga et al., 2016). Traditional drug transporter assays in vitro are often performed with short incubation times to derive kinetic parameters. Data acquired over longer times will represent drug transport activity at steady state and will not reflect the linear uptake/efflux phase under initial rate conditions. Derivation of parameters from MPS models may be challenging due to these practical limitations, and future MPS systems may need to allow different sample regimens and rely on mathematical modeling to support interpretation of the results obtained (Yu et al., 2015).

Robust PBPK models can capture the PK of a compound accurately, maximizing confidence and identifiability. MPS platforms that provide continuous sampling can therefore be advantageous for these studies. A more elaborate approach involves the combination of mechanistic modeling and defined research questions about MPS biologic functions (e.g., drug absorption and metabolism) in order to design the MPS platform so its features are tailored to suit the application. A model to study gut-liver metabolism enabling TEER measurements, direct effluent sampling from the cell compartments, and a mixing chamber representing systemic circulation was designed using such an approach (Maass et al., 2017; Tsamandouras et al., 2017a). Since MPS platforms comprise intricate compartments and microfluidic circuits, it can be beneficial to understand how the geometry affects volume and compound distribution in the design phase of the system to develop practical applications that will ultimately involve PBPK modeling of physiologic parameters. Furthermore, the compartmental nature of MPS designs is conceivably of benefit for the design of drug distribution studies involving defined organ and body fluid compartments.

As the evaluation of MPS models expands and multiple applications emerge, the challenges are becoming evident. From a technical point of view, issues arise regarding the fact that most available MPS devices are based on a polydimethylsiloxane matrix, a biocompatible organic polymer with adsorptive properties (Regehr et al., 2009; van Meer et al., 2017). This raises the problem of retention of hydrophobic molecules in chips, potentially reducing the concentrations that reach the target cells substantially. The extent of binding is often not investigated across the various models; therefore, the impact on measured endpoints is unclear or just not known (Halldorsson et al., 2015). This can represent a challenge for using data in a quantitative setting, such as IVIVE, and could lead to the underprediction or overestimation of parameters.

Determination of the permeability and integrity of cell monolayers is an important quality control aspect in drug transport studies, and there are examples of MPS prototypes that have been fitted with TEER sensors (Henry et al., 2017). Such platforms may be suitable for DMPK-based applications (e.g., when incorporated into models like the aforementioned gut-liver model) (Tsamandouras et al., 2017a). Many platforms can be easy imaged, allowing the use of fluorescent permeability markers and extensive morphologic characterization using immunofluorescence protocols. Further integration of analytical sensors (e.g., pH and oxygen probes) can potentially enable continuous data collection, overcoming the limitations of extended sampling intervals and reduced volume/effluent collection for bioanalysis (Senutovitch et al., 2015; Zhang et al., 2017). This could represent a major advantage for MPS systems and increase the throughput of these models by expanding the parameters and data points collected per experiment. A chip design incorporating 40 leak-tight Caco-2 gut tubes coupled with automated fluorescent imaging acquisition is an example of how MPS data collection can be multiplexed for toxicity studies (Trietsch et al., 2017). Microsampling, flow rates better aligned with physiologic conditions, and differential oxygenation of multiple compartments are current considerations in the development of novel platforms (Vernetti et al., 2017). MPS-engineered features (e.g., microcirculation) are responsible for enhancing cellular functions; however, key biologic challenges continue to exist.

Since the early days of in vitro cell models, the composition of the culture medium used is an important aspect of maintaining cells with high quality and functionality (Yao and Asayama, 2017). This issue is now translated to MPS models, where different conditions may require modified medium formulations to better maintain augmented cellular features. Multiorgan MPS cultures may require the development of a universal medium, which should be suitable for different cell types but still maintain cell type–specific functionality for the individual organs. Fluidic culture conditions are among the key advantages of MPS systems. However, as models move toward more representative physiologies and multiorgan platforms, the interconnectivity and fluidic circuits that better mimic high levels of vascularization (e.g., the capillary networks surrounding proximal tubules in the kidney or the alveoli in the lungs) will become more of a consideration.

Another recurring challenge is the sourcing of high-quality cells for in vitro studies and MPS applications, as the issues regarding the use of primary cells and cell lines in traditional in vitro models also apply to MPS cultures. Ideally, MPS models would use high-quality primary cells, a source afflicted by high costs and variability from both donors and suppliers. Although prototypical cell lines (e.g., MDCK and Caco-2 cells) can guarantee better reproducibility, they can also have complete transport systems intrinsically abrogated, exemplified by the lack of expression of BCRP and OATs in MDCK cells (Maubon et al., 2007; Quan et al., 2012). Induced pluripotent stem cells (iPSCs) have the potential to fill the gap between primary cells and cell lines, providing an abundant and stable supply of high-quality cells across a range of organ types with native functionalities. Although steps have been taken to create iPSC banks and repositories that could be used as prototypes for different organs as well as disease models, the sourcing of progenitor cells for the generation of iPSC lines is still conducted in the same way as

other cell types and is impaired by donor availability (Stacey et al., 2013; Solomon et al., 2015). Furthermore, despite improvements in differentiation protocols, resulting cells can display immature phenotypes (Okita and Yamanaka, 2011; Osterloh and Mullane, 2018).

Standardization of MPS models would make the applications of these platforms more translatable and concerted efforts of different institutions, such as the National Institutes of Health and regulatory agencies, are considering the feasibility, applications, and prospective uses of MPS platforms in drug development (Marx et al., 2016; Ewart et al., 2017; Greenman, 2017). This could lead to guidelines governing the use of MPS models when this technology is still maturing, and drug transporter studies can positively benefit from such an approach.

Conclusion

As computational models for predicting the PK and pharmacodynamics of candidate drugs are increasingly being developed and used in drug development, parallel in vitro systems are needed to validate, improve, and complement such models. MPS models represent an exciting platform approach that may have the ability to recapitulate in vivo complexities in drug transport mechanisms. The improvements in cellular morphology and function achieved by culturing cells in MPS models can result in enhanced expression of drug transporters and even the recovery of functionality lost in traditional culture. These models can offer new insights into drug transport, metabolism, and secretion and studies have the potential to generate physiologic relevant data of unprecedented quality. As MPS technologies mature and their design and biologic challenges are resolved, drug transport activity representative of the combined effects of multiple drug transporters can now begin to be investigated in detail. Our understanding of the role of drug transporters in drug disposition, clinically relevant drug-drug interactions, and other paradigms in drug development can be dramatically expanded. By incorporating drug clearance parameters reflecting native physiologic drug transport, the clinical predictions of PBPK models can be significantly improved. Where we stand today, MPS models have the potential to substantially impact the way drug transporter function is investigated. As with any technique in its infancy, much needs to be accomplished in terms of assay development and experimental design to push the investigation of drug transporters in MPS models from a morphologic to a more functional characterization.

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