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## Title

# Perspective on the application of microphysiological systems to drug transporter studies

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Microphysiological systems in drug transporter studies

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**Abbreviations**

AA, aristolochic acid

ABC, ATP-Binding Cassette

AHR, Aryl Hydrocarbon Receptor

AQP1, Aquaporin 1

BBB, Blood brain barrier

BCRP, Breast Cancer Resistance Protein

BSEP, Bile Salt Export Pump

Caco-2, human Colorectal Carcinoma cell line

CMV, cytomegalovirus

CYP, Cytochrome P450

DAPI, 4',6-diamidino-2-phenylindole

DCF, Diclofenac

EMA, European Medicines Agency

FDA, Federal Drug Administration

HC, Hydrocortisone

HEK, Human Embryonic Kidney cells

hRPTEC, human renal proximal tubule epithelial cells

iPSC, Induced pluripotent stem cells

ITC, International Transporter Consortium

IVIVE, *in vitro in vivo* extrapolation

MATE, Multidrug And Toxin Extrusion Transporter

MDCK, Madin-Darby Canine Kidney cells

MPS, Microphysiological System

MRP, Multidrug Resistance-associated Protein

$Na^+K^+$-ATPase, Sodium Potassium ATP-ase

NrF2, Nuclear Factor Erythroid-Derived 2

OAT, Organic Anion Transporter

OATP, Organic Anion Transporting Polypeptides

OCT2, Organic Cation Transporter 2

PAH, *para*-aminohippuric acid

PBPK, Physiologically Based Pharmacokinetic

P-gp, P-glycoprotein

PK, Pharmacokinetic

PMDA, Japanese Pharmaceuticals and Medical Devices Agency

SLC, Solute Carrier

TEER, Transepithelial Electrical Resistance

TMA, Trimethylamine

TMAO, Trimethylamine N-oxide

**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 physiological conditions to *in vitro* cell culture models have enormous potential to better reproduce the morphology and transport activity across several organ models especially in tissues like the liver, kidney, intestine or the blood-brain-barrier where 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. Further, we analyse 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 re-arranges vital survival pathways and re-programs 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 (CYP) enzymes have been shown to decrease by 90% after 24 hours in plated hepatocytes, compared to 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 (HK-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 dimished transporter gene expression when in culture as well (Brown et al., 2008). These constraints have so far limited the use of *in vitro* models to study the

impact of drug transporters in drug disposition across various tissues and their pharmacokinetic implications. Novel microphysiological systems (MPS) 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 offers a perspective on the advantages and implications of MPS for drug transporter studies.

**Drug transporters as key players in drug distribution**

Transporters belonging to two major superfamilies, the SoLute Carrier Superfamily (SLC) and the ATP-Binding Cassette Superfamily (ABC), 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 pharmacokinetics 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 (International Transporter 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 (Hillgren et al., 2013). The evaluation of these drug transporters during drug development is now recommended by the ITC and the main regulatory agencies (the US Federal Drug Administration – FDA, the European Medicines Agency – EMA, and

the Japanese Pharmaceuticals and Medical Devices Agency – PMDA), based on pre-clinical 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, while 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 bi-products, such as 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 on the bioavailability of orally administered compounds (Musther et al., 2014). In capillary endothelial cells of the brain, drug transporters maintain the integrity of the blood-brain-barrier (BBB) and their protective role is responsible for selective drug penetration (Pardridge, 2012).

### *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 by Juliano and Ling in 1976, where mutated Chinese hamster ovary cells expressing a particular surface glycoprotein (nowadays known as P-glycoprotein (P-gp)) were resistant to colchicine, in contrast to

wildtype cells lacking this protein (Juliano and Ling, 1976). 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 pharmacokinetics. Though 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. Further, 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 rodent kidney and brain, respectively, compared to 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 preferentially performed to determine the efficacy and safety of drugs. *In vitro* models are highly valuable in drug transporter research, where 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.* on emerging renal *in vitro* models (Bajaj et al., 2018).

***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 the Madin-Darby Canine Kidney (MDCK) cells, the Human Embryonic Kidney (HEK) cells and the human colorectal carcinoma (Caco-2) cell line (Thomas and Smart, 2005; Sun et al., 2008; Dukes et al., 2011). Although offering restricted or poor drug transport activity, these cells 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 or not a drug is a substrate or inhibitor of a given transporter, they fail to recapitulate the *in vivo* system and are often limited to no more than two transporters of interest. Further, 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 (CMV). 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 favoured for high quality predictive 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 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) modelling (Barton et al., 2013). However, sourcing, growing and evaluating quality primary cells can be a labour intensive and costly enterprise, with the cells functionally decaying in culture over a short time and providing narrow assay windows.

The use of micropatterned co-cultures (MPCCs) can improve the viability and function of primary cells *in vitro*. This method, where 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 OCT1 gene expression and bile-canaliculi formation after 1 week, relative to non-MPCC, as well as increased expression of key CYP 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*

Noteworthy are also models that enable cells to organize into complex structures that recapitulate tissue architecture and functions, contrasting with typical monolayers cultures. Spheroids are usually generated from cell lines, immortalized or primary cells which aggregate together to form these tissue-like structures. Organoids are typically derived from a stem cell source with the potential to differentiate into a multi-lineage 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 MRP2 and BSEP compared to freshly isolated hepatocytes whereas the profiles of all three genes were upregulated compared to 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 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 co-cultured 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). Whilst 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 organic anion and organic cation transporters (OATs and OCTs, respectively) 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. Further, organoids typically do not develop a collecting duct system and 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.

Whilst 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 micro-structures 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 ADME relevant organs such as the intestine or kidney are limited by these practicalities even if spheroids or organoids substantially recapitulate the physiological drug transport machinery.

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**Microphysiological systems and the enhancement of *in vitro* phenotypes**

Recent developments in microfluidics and microfabrication technologies brought the so-called organs-on-a-chip or microphysiological systems (MPS) into the spotlight. MPS technology is considered by the World Economic Forum one of the top ten recent emerging technologies (Forum, 2016). The view of the pharmaceutical industry on the application of these technologies was the subject of a recent joint publication co-authored by experts from eleven companies. The implementation of MPS 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 Center for Advancing Translational Sciences (Low and Tagle, 2017). MPS are cell culture based models incorporating flow to allow the formation of tissues which achieve the recapitulation of certain organ functions. With varying levels of complexity, MPS aim to create realistic and physiologically relevant organ models with the ambition to represent *in vivo* biological 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 physiological 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 so 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 transition from a flat and static culture to a laminar-flow fed three-dimensional set-up. Although the terminology 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 which depend on cell-cell interactions. 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 blood-brain-barrier models use vascular endothelial cells.

In a recent comprehensive review, Seiichi Ishida (Ishida, 2018) identified the required physiological profiles of liver, gut and renal MPS 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, 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 blood-brain-barrier 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 liver (Emulate, CNBio, Mimetas), kidney (Emulate, Nortis), intestine (Emulate, Mimetas), and blood brain barrier (Nortis). Experimental endpoints often include either direct (live) imaging of the chips, immunofluorescent staining to characterize protein expression, effluent collection for bioanalysis of

molecules such as 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 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 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, and 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 de-differentiation 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 and in doing so, these models demonstrate enhanced viability and activity under dynamic culture conditions. Liver MPS, 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 the Multidrug Resistance-associated Protein 2 (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 its recreation in MPS models would be an important feature in liver *in vitro* models. In terms of gene

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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 to the levels in freshly isolated human hepatocytes (Tsamandouras et al., 2017b). In human cryopreserved hepatocytes, a period of 7 days MPS culture enhanced the gene expression of P-gp and MRP2 and BCRP to about 1.5-fold as well, however, it is unclear if this translates to a significant change in transport function. In contrast, the expression of BSEP and several uptake transporters including OCT1, OATP1B1, OATP2B1 and in particular OATP1B3 were all reduced compared to 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 normalisation and use of reference samples may give rise to varying results. Overall, in the few studies conducted so far, gene expression of hepatic CYP 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 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 Multidrug Resistance-associated Protein 4 (MRP4) was increased 7-fold, relative to static culture. This upregulation was mediated through the induction of the nuclear factor erythroid-derived 2 (NrF2) and Aryl hydrocarbon receptor (AHR) regulatory pathways. The expression of several SLC transporters and CYP enzymes was also enhanced, revealing a wide-ranging impact of flow on metabolic pathways (Snouber et al., 2012). The human MATE2-K is usually expressed only marginally in static cultures. In cryopreserved human renal proximal tubule epithelial cells (hRPTEC), fluidic culture conditions promoted a 12-fold increase in MATE2-K gene expression, mediated by NrF2, which also translated into enhanced functional transport activity, shown by the reduced accumulation of the fluorescent substrate 4',6-diamidino-2-phenylindole (DAPI) (Fukuda et al., 2017). The lack of Organic Anion Transporters (OATs) in renal *in vitro* models has been a bottleneck in DMPK studies for a long time (Gozalpour and Fenner, 2018), and 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 (PAH), a functionality which was absent when the same cells were grown on trans-well 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). Co-culturing

of hRPTEC 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 to 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 presence of the Organic Cation Transporter 2 (OCT2) inhibitor cimetidine, indirectly indicating activity of this transporter in the model. Albumin and glucose re-uptake was also improved, as well as Aquaporin 1 (AQP1) and sodium potassium ATP-ase ($Na^+K^+$-ATPase) protein expression, altogether pointing towards a better renal proximal tubule phenotype in the MPS model (Jang et al., 2013).

***Blood Brain Barrier***

Drug permeation across the blood brain barrier (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 co-culture 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 and 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 so 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 CYP 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, an indication 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, are critical in assessing and predicting the rate and extent of oral drug absorption which may be limited or enhanced by transporters. Gut MPS provide a good example of how MPS designs can provide other physiological 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 where successfully co-cultured 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 human duodenum, in contrast to 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 can be a powerful tool to advance oncology research by better mimicking tumor physiology, an environment rich in transporter proteins. The Breast Cancer Resistance Protein (BCRP) and P-gp are expressed across different healthy tissues (Table1) 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 PI3K/Akt pathway reverses the expression of these transporters, maybe 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 re-programs signaling pathways involved in differentiation, survival, stress and metabolism amongst 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.

***Multi-organ platforms***

Building on the physiological recreation of specific tissue functional units, MPS 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 regards to their metabolic capacity and their ability to describe pharmacokinetic (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 multi-organ 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 hRPTEC, downstream of a liver MPS model incorporating

hepatocytes. When flow was driven unidirectionally from the liver to the kidney, the nephrotoxic bi-product 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 as the nephrotoxic effect was abolished in presence of probenecid, a prototypical OAT inhibitor. This model shows that coupling hepatic metabolism and renal secretion captures a multi-step physiological 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, blood-brain-barrier and muscle MPS was attempted to validate the potential clinical significance of such MPS models. The uptake, clearance and distribution of the antihistamine terfenadine and the environmental toxin trimethylamine TMA was studied across the different organs in the model. Results showed that the gut and liver MPS 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, TMA is metabolized into trimethylamine N-oxide (TMAO) in the liver MPS, downstream, 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 confirm 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 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 cultures periods spanning four weeks studying the *in vitro* PK of DCF (Edington et al., 2018). Whilst 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 physiological mechanisms behind PK - uptake, metabolism and excretion - makes data generated in MPS platforms valuable for the development of physiologically based pharmacokinetic models (PBPK). As confidence in the physiological activity of MPS 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, incorporated varying levels of complexity such as multi-compartmental approaches, can be used to interpret and predict parameters from studies in MPS systems.

Recent studies probing the PK simulation of orally administered drugs using multi-organ 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 diclofenac (DCF) and hydrocortisone (HC), 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 multi-organ cross talk. Both DCF and HC PK parameters derived the from standalone gut or liver components did not significantly differ from the gut-liver MPS. This study showed the feasibility of an integrative approach for multi-organ 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, and were identified by the authors as a factor to why no major differences were observed in the gut-liver model against gut or liver 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 modelling of MPS parameters rely on the accurate description of the intrinsic parameters of the MPS systems used (e.g. flow rates and

volumes). In addition, physiological parameters are important to reflect specific organ functionalities. Nonetheless, the enhanced metabolic activity observed in 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 CYP activity and, as multiple organ MPS 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 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, and therefore ADME studies in MPS 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 physiological 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. Km, Vmax, 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 MPS 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 co-medications 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 pharmacokinetic 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 human. 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 physiological functions and recapitulation of organ-specific features has been demonstrated in a number of exemplar studies. Nonetheless, the number of practical applications harnessing this potential is still limited, and most studies so far focus on the characterization of models, describing functional readouts of known biomarkers, gene expression patterns and morphological features. Unsurprisingly, kidney, liver and intestine models are at the forefront of the characterization of drug transporters in MPS, given the key roles in the absorption and excretion performed by these organs, as well as the regulatory requirements for drug transporters in drug development. To improve and expand 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 liver, P-gp and BCRP in gut and brain, OATs in kidney). Most of the characterization studies summarized in Table 2 have shown that mRNA levels of multiple drug transporters are increased in MPS models when compared to traditional culture methods. mRNA analysis is the preferred method to determine expression levels in MPS. The use of western blotting or targeted proteomics is still limited by practical issues concerning collection and biological 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 through better prediction of *in vivo* effects MPS data are ultimately more physiologically representative.

**Limitations, challenges and opportunities of MPS applications in drug transport studies**

Traditional *in vitro* DMPK studies rely on standardized culture platforms, such as multi-well 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 which 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 regimen over extended periods of time, from 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 multi-organ MPS where 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 not reflect the linear uptake/efflux phase under initial rate conditions. Derivation of parameters from MPS may face challenges due to these practical limitations and future MPS models maybe need to allow different sample regimen and rely on mathematical modelling 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 modelling and defined research questions about MPS biological functions (e.g. drug absorption and metabolism), to design the MPS platforms 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 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 physiological parameters. Further, 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 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 (PDMS) 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 extend 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, as such IVIVE leading 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 have been fitted with trans-epithelial electrical resistance (TEER) sensors (Henry et al., 2017). Such platforms may be suitable for DMPK based applications, for example 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 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 how MPS data collection can be multiplexed for toxicity studies (Trietsch et al., 2017). Micro-sampling, flow rates better aligned with physiological 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 biological 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 systems, where different conditions may require modified medium formulations to better maintain augmented cellular features. Multi-organ MPS cultures may require the development of a universal medium, 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. However, as models move towards more representative physiologies and multi-organ platforms, the interconnectivity and fluidic circuits that better mimic high levels of vascularization, such as 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. Ideally, MPS models would use high quality primary cells, a source afflicted by high costs and variability from both donors and suppliers. Whilst prototypical cell lines, such as 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 (iPSC) 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). Further, 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 when this technology is still maturing, and drug transporter studies can positively benefit from such an approach.

## Conclusion

As computational models for predicting the pharmacokinetics 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 physiological relevant data of unprecedent quality. As MPS technologies mature and their design and biological 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 physiological drug transport, the clinical predictions of PBPK models can be significantly improved. Where we stand today, MPS 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 morphological to a more functional characterization.

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## Authorship Contributions

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**Figure Legends**

Figure 1: Cross section of the most common designs of MPS chips outlining cellular compartments and cell/chip or cell/matrix interfaces. **A** represents 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** shows a design with two channels separated by a permeable membrane, that 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 co-culture applications. **C** demonstrates a design where cells are entirely adhering to an extra cellular matrix, forming a confluent and sealed cell tube; this design is favoured by applications involving tubular structures with barrier properties such as vasculature or renal tubules. **D** illustrates 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 re-circulating flow. **E** shows a design were 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 levelling of the medium resulting from cyclic tilting of the system, cells are therefore exposed to bidirectional flow.

Table 1: Drug transporters recommended by the regulatory agencies and ITC for evaluation in drug development including prospective and retrospective studies and key organ expression. Established transporters are required for evaluation by the regulatory agencies. Emerging transporters are recognized as potentially relevant, although clinical impact is not yet fully understood and established.

| Transporter | | Gene | Liver | Kidney | Intestine | Blood brain barrier |
|---|---|---|---|---|---|---|
| *Established* | | | | | | |
| **Organic Anion Transporter 1** | OAT1 | SLC22A6 | | X | | |
| **Organic Anion Transporter 3** | OAT3 | SLC22A8 | | X | | |
| **Organic Cation Transporter 2** | OCT2 | SLC22A2 | | X | | |
| **P-glycoprotein** | P-gp | ABCB1 | X | X | X | X |
| **Breast cancer resistance protein** | BCRP | ABCG2 | X | X | X | X |
| **Organic Anion Transporting Polypeptide 1B1** | OATP1B1 | SLCO1B1 | X | | | |
| **Organic Anion Transporting Polypeptide 1B3** | OATP1B3 | SLCO1B3 | X | | | |
| **Multidrug And Toxin Extrusion transporter 1** | MATE1 | SLC47A1 | X | X | | |
| **Multidrug And Toxin Extrusion transporter 2** | MATE2 | SLC47A2 | | X | | |
| *Emerging* | | | | | | |
| **Organic Anion Transporting Polypeptide 2B1** | OATP2B1 | SLCO2B1 | | | X | X |
| **Bile Salt Export Pump** | BSEP | ABCB11 | X | | | |
| **Multidrug Resistance-associated Protein 2** | MRP2 | ABCC2 | X | X | X | |
| **Multidrug Resistance-associated Protein 3** | MRP3 | ABCC3 | X | X | X | |
| **Multidrug Resistance-associated Protein 4** | MRP4 | ABCC4 | X | X | | |
| **Organic Cation Transporter 1** | OCT1 | SLC22A1 | X | | | |

Table 2: Selected studies where drug transporter expression and activity were investigated to characterize the MPS models. The MPS platform design used in these studies is described in Figure 1.

| Organ | Tissue/Functional unit | Cell source/type | MPS design | Manufacturer | Transporters investigated | Drug transporter characterization | | | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Expression | Function | Localization | |
| Liver | Hepatocyte with bile canaliculus | Freshly isolated rat hepatocytes | Custom design | n/d | MRP2 | n/d | CFDA-SE efflux | Immuno-fluorescence | (Nakao et al., 2011) |
| Liver | Hepatocyte with bile canaliculus | Human cryopreserved hepatocytes | C | Nortis | MRP2 | n/d | CMFDA efflux | n/d | (Vernetti et al., 2016) |
| Liver | Hepatocyte with bile canaliculus | Freshly isolated human hepatocytes | D | CNBio | P-gp, MRP2, BSEP | mRNA | n/d | n/d | (Tsamandouras et al., 2017b) |
| Liver | Hepatocyte with bile canaliculus | Human cryopreserved hepatocytes | D | CNBio | Several including P-gp, MRP2, BSEP and hepatic OATPs | mRNA | n/d | n/d | (Vivares et al., 2015) |
| Kidney | Proximal tubule | MDCK cells | A | n/d | Several, including MRP4 | mRNA | n/d | n/d | (Snouber et al., 2012) |
| Kidney | Proximal tubule | Cryopreserved human proximal tubule cells | A | n/d | MATE2-K | mRNA | DAPI retention | n/d | (Fukuda et al., 2017) |
| Kidney | Proximal tubule | Freshly isolated human proximal tubule cells | C | Nortis | OATs | n/d | PAH uptake | n/d | (Weber et al., 2016) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Kidney | Proximal tubule | Freshly isolated human proximal tubule cells | C | Nortis | OATs | n/d | AA uptake | n/d | (Chang et al., 2017) |
| Kidney | Proximal tubule | Cryopreserved human proximal tubule cells | B | Emulate | P-gp | n/d | Calcein retention | n/d | (Jang et al., 2013) |
| Kidney | Proximal tubule | Human immortalized proximal tubule cells | E | Mimetas | Several including P-gp, MRP4 | mRNA | Calcein retention<br>CMFDA retention | n/d | (Vriend et al., 2018) |
| Blood brain barrier | Microvasculature | Human vascular endothelial cells | A | n/s | P-gp, MRP2 | mRNA | n/d | n/d | (Cucullo et al., 2011) |
| Intestine | Duodenum | Freshly isolated intestinal cells | B | Emulate | Several, including MRP4, MATE1, OCT2 | mRNA | n/d | n/d | (Kasendra et al., 2018) |
| n/d | n/d | Human ovarian cancer | A | n/d | BCRP, P-gp | Protein | n/d | n/d | (Ip et al., 2016) |

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Figure 1