1. Title page

Emerging Kidney Models to Investigate Metabolism, Transport and Toxicity of Drugs and Xenobiotics

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2. Running title page

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Nonstandard abbreviations used in the paper:

3,4,5-trichloroaniline (TCA)
American Type Culture Collection (ATCC)
Aminopeptidase N (CD13)
Aquaporin 1 (AQP-1)
Area under the receiver operating characteristic curve (AUC-ROC)
Aristolochic acid (AA)
ATP-binding cassette (ABC)
Breast cancer resistance protein (BCRP)
Chinese Hamster Ovary (CHO)
Conditionally immortalized proximal tubule epithelial cells (ciPTEC)
Conditionally immortalized proximal tubule epithelial cells overexpressing OAT-1 (ciPTEC - OAT1)

Conditionally immortalized proximal tubule epithelial cells overexpressing OAT-3 (ciPTEC - OAT3)

Cyclic guanosine monophosphate (cGMP)

Cytochromes P450 (CYPs)

Drug-drug interactions (DDIs)

European Medicines Agency (EMA)

Extracellular matrix (ECM)

Flavin-containing monooxygenase (FMO)

Food and Drug Administration (FDA)

Gamma glutamyltransferase (GGT)

Glutathione (GSH)

Glutathione disulfide (GSSG)

Glutathione-s-transferase (GST)

Heme oxygenase – 1 (HO-1)

Human embryonic kidney (HEK)

Human primary tubular cells (HPTCs)

Human primary tubular epithelial cells (HPTECs)

Human umbilical cord endothelial cells (HUVECs)

in vitro to in vivo extrapolation (IVIVE)

Induced pluripotent stem cells (iPSC)

Interleukin 6 (IL-6)

Interleukin 8 (IL-8)

International consortium for innovation and quality in pharmaceutical development (IQ)

International Transporter Consortium (ITC)

Investigational new drug (IND)

Kidney injury molecule – 1 (KIM-1)

Lactate dehydrogenase (LDH)

Macrophage colony-stimulating factor (M-CSF)

Madin-Darby Canine Kidney (MDCK)

Metabolites in safety testing (MIST)

Microphysiological systems (MPS)

Monocarboxylate transporter (MCT)

Multidrug and toxin extrusion protein 1 (MATE-1)

Multidrug and toxin extrusion protein 2K (MATE-2K)

Multidrug resistance-associated protein 2 (MRP2)

Multidrug resistance-associated protein 4 (MRP4)

Neutrophil gelatinase-associated lipocalin (NGAL)

New chemical entities (NCE)

Organic anion transporter 1 (OAT1)

Organic anion transporter 2 (OAT2)

Organic anion transporter 3 (OAT3)

Organic anion transporter 4 (OAT4)

Organic cation transporter 2 (OCT2)

organic cation transporter, novel, type 1 (OCTN1)

organic cation transporter, novel, type 2 (OCTN2)

p-aminohippuric acid (PAH)

Peptide transporter 1 (PEPT1)
Peptide transporter 2 (PEPT2)
P-glycoprotein (P-gp)
Pharmaceuticals and medical devices agency (PMDA)
pharmacokinetic/pharmacodynamic (PK/PD)
Pharmacokinetics (PK)
Pig Kidney Epithelial cells (LLC-PK1)
Polycystic kidney disease (PKD)
Polydimethylsiloxane (PDMS)
Proximal tubule cell (PTC)
Renal Proximal Tubule Cells (RPTEC)
Simian virus 40 large T antigen (SV40T)
Sodium-glucose co-transporter-2 (SGLT2)
Solute carrier (SLC)
Sulfotransferases (SULTs)
Telomerase Reverse Transcriptase (TERT1)
Three-dimensional (3D)
Trans-epithelial electrical resistance (TEER)
Two-dimensional (2D)
Urate Anion Exchanger 1 (URAT1)
Uridine-di phosphate (UDP)
Uridine-di phosphate-glucuronosyltransferases (UGTs)
Zonula occludens 1 (ZO-1)
Zonula occludens 3 (ZO-3)

3. Abstract

Kidney is a major clearance organ of the body responsible for the elimination of many xenobiotics and prescription drugs. With its multitude of uptake and efflux transporters, and metabolizing enzymes, the proximal tubule cell (PTC) in the nephron plays a key role in the disposition of xenobiotics and is also a primary site for toxicity. In this mini-review, we first provide an overview of the major transporters and metabolizing enzymes in the PTCs responsible for biotransformation and disposition of drugs. Next, we discuss different cell sources that have been used to model the PTCs in vitro, their pros and cons, and their characterization. As current technology is inadequate to reliably evaluate drug disposition and toxicity in the kidney, the review then discusses recent advancements in kidney microphysiological systems (MPS) and the need to develop robust in vitro platforms that could be routinely used by pharmaceutical companies to screen compounds. Finally, we discuss the new and exciting field of stem cell derived kidney models as potential cell sources for future kidney MPS. Given the push from both regulatory agencies and pharma companies to use more predictive "human-like" in vitro systems in the early stages of drug development to reduce attrition, these emerging models have the potential to be a game changer and may revolutionize how renal disposition and kidney toxicity in drug discovery is evaluated in the future.

4. Introduction

The kidneys perform essential functions in humans by maintaining the composition of blood, its pH, preventing the buildup of waste products, and keeping levels of electrolytes such as sodium, potassium, and phosphate stable. In normal adults, the two kidneys daily filter about 150 - 180 L of blood to produce 1 to 2 L of urine, composed of wastes and extra fluid. The kidneys are also responsible for the elimination of numerous drugs, endogenous metabolites important to maintain physiological homeostasis, exogenous and endogenous toxins, nutrients, etc. The elimination of exogenous and endogenous compounds through kidney occurs as a net result of glomerular filtration, tubular secretion, kidney metabolism, and reabsorption. Evaluation of the mechanisms involved in the elimination of drugs and other exo- and endogenous molecules can provide valuable understanding of their clearance, potential for drug-drug interactions (DDIs), potential for development of kidney and other organ toxicity, and thus on the effect of the elimination of an investigational drug on its pharmacokinetics (PK) in patients with compromised kidney functions.

In the past several decades, there has been a considerable progress made in our understanding of the mechanisms by which the drugs and xenobiotics are eliminated by kidneys. The discovery and identification of several important tubular apical and basolateral transporters and their role in the elimination and reabsorption of xenobiotics and endogenous substrates have spearheaded this renaissance (Morrissey et al., 2013; Nigam et al., 2015; Miners et al., 2017). Furthermore, metabolizing enzymes in kidneys also play an important role in the clearance of xenobiotics and endogenous compounds (Lash, 1994; Lock and Reed, 1998; Lohr et al., 1998; Knights et al., 2013). Therefore, it is important to evaluate early in the development a) the mechanism of clearance of new chemical entities (NCE), b) DDI as a victim if the metabolism and transport is modulated by co-administered drugs, c) DDI as a perpetrator and d) potentials for toxicity. In a recent DDI

guidance, FDA has recommended to perform clinical DDI studies to understand if the NCE could be a victim of kidney transporter inhibition if it undergoes active renal secretion or there are concerns about renal toxicity. The guidance further recommends DDI studies as a perpetrator if *in vitro* studies demonstrated that the NCE has potential for inhibition of cytochrome P450 (CYP) enzymes and transporters including kidney transporters regardless of the investigational drug's route of elimination (FDA 2017a; FDA 2017b).

It should be noted that besides liver, kidney is one of the most frequent targets for drug-induced toxicity. Of the top 200 prescribed drugs, 32% of them undergo renal elimination (Morrissey et al., 2013). About 20 to 30% of intensive care unit patients and ~5% of hospitalized patients develop acute kidney toxicity and nearly 20% of these toxicities were attributed to nephrotoxic drugs (Li et al., 2014). This may be because kidney is an organ that is exposed to a lot of drugs, metabolites and endogenous compounds by being the recipient of the 25% of cardiac output and an organ of elimination of many of these compounds (Tiong et al., 2014). Unfortunately, the nephrotoxicity is identified late in the development programs with only 2% of drug attritions happen in preclinical studies but 19% during phase 3 studies (Redfern et al., 2010). This inability to successfully remove nephrotoxic compounds from development early in the program could be attributed to a lack of appropriate preclinical models to investigate kidney toxicity (Li et al., 2014; Tiong et al., 2014).

Due to accumulating evidences indicating an important role of kidney in the metabolism, transport and clearance of xenobiotics, proteins and hormones, and endogenous compounds, there has been an accelerated growth in the past decade on the development of technologies to investigate the disposition of NCEs targeted to treat human diseases and potential for toxicities. This article highlights the evolution of novel technologies developed to investigate the mechanism of

disposition of drugs by kidneys and potential for toxicities together with an overview of the role of kidney metabolism and transport and their involvement in kidney toxicity.

Overview of metabolism and transport of drugs in kidney and their role in kidney toxicity

The metabolism of drugs in kidney has been described extensively (Lash, 1994; Lock and Reed, 1998; Lohr et al., 1998; Knights et al., 2013; Gundert-Remy et al., 2014; Miners et al., 2017). Numerous enzymes play a role in the metabolism and clearance of endo- and exogenous compounds. These include CYP enzymes, non-CYP enzymes such as uridine-di phosphate-glucuronosyltransferases (UGTs), esterases, glutathione-s-transferases (GSTs), sulfotransferases (SULTs), and some other enzymes highlighted in Table 1. Therefore, kidneys can execute a diverse array of metabolic reactions, such as oxidation, reduction, hydrolysis, and conjugation. Many of these reactions facilitate the elimination of drugs. While these reactions are considered a detoxification mechanism, there are certain metabolism reactions that lead to the formation of reactive species resulting in kidney toxicities. For example, glutathione (GSH) S-conjugates can undergo further metabolism to cysteine S-conjugates that can be eliminated as non-toxic mercapturic acids or catalyzed by β-lyase to unstable thiols. These thiols are highly reactive and can covalently bind to cellular macromolecules causing cytotoxicity and carcinogenicity. In addition to β-lyase, cysteine conjugate S-oxidase and other enzymes can also bioactivate chemicals to produce nephrotoxic species (Lash, 1994).

Nephrotoxicity due to biotransformation in the kidney has been well documented. Chloroanilines are commonly used as chemical intermediates to manufacture dyes, agricultural chemicals, drugs and industrial compounds. 3,4,5-trichloroaniline is the most potent nephrotoxicant among trichloroanilines (TCA). Using isolated renal cortical cells from rats Racine et al. demonstrated that pretreatment with CYP inhibitor piperonyl butoxide, cyclooxygenase inhibitor indomethacin

or peroxidase inhibitor mercaptosuccinate reduced TCA mediated cytotoxicity. However, no effect was seen by the pretreatment with flavin-containing monooxygenase (FMO) inhibitors, indicating that bioactivation of TCA to toxic metabolites by CYPs, cyclooxygenase and peroxidase contributed to the TCA cytotoxicity (Racine et al., 2014). Acyclovir and other antiviral drug induced nephrotoxicities have caused concerns in humans (Izzedine et al., 2005). In vitro studies using cultured human proximal tubular cells (PTCs) suggested that the acyclovir induced toxicity was associated with the formation of acyclovir aldehyde in the kidney by alcohol dehydrogenase (Gunness et al., 2011). Cisplatin causes dose-limiting nephrotoxicity in rodents and humans (Wainford et al., 2008). Inhibition of gamma glutamyltransferase (GGT) prevented cisplatin induced nephrotoxicity in vivo in rats and C57BL6 mice indicating that this enzyme plays a critical role in cisplatin nephrotoxicity. In vitro studies using isolated rat and human renal PTCs demonstrated that amino peptidase-N, renal dipeptidase, and C-S lyase were not involved in cisplatin induced toxicity.

Renal transporters also play an important role in the disposition of drugs and development of kidney toxicity by accumulation of compounds and/or their metabolites. These transporters are part of two super-families of carrier proteins, ATP-binding cassette (ABC) and solute carrier (SLC) (Morrissey et al., 2013; Nigam et al., 2015; Miners et al., 2017). The SLCs generally transport substances either down their concentration gradient or against their concentration gradient coupled with movement of a second substance down its concentration gradient. In the kidney, the most multi-specific SLC transporters appear to be organic anion transporters: OAT1 (SLC22A6) and OAT3 (SLC22A8) and organic cation transporter (OCT2, SLC22A2). A recent publication suggests that another less commonly cited transporter, OAT2, expressed both in the liver and kidney is involved in the elimination of several drugs including creatinine and cGMP in

the kidney (Shen et al., 2017). Accumulating evidence further indicates the importance of several other SLC families of transporters, such as multidrug and toxin extrusion proteins (MATEs, SLC37), peptide transporters (PEPT, SLC15) and organic carnitine/zwitterionic transporters (OCTN, SLC22A4 and SLC22A5). ABC transporters use energy generated by the hydrolysis of ATP to transport molecules across cell membranes. The most commonly linked ABC transporters of significance for pharmaceuticals' transport are P-glycoprotein (P-gp, ABCB1), breast cancer resistance protein (BCRP, ABCG2), and multidrug resistance proteins (MRP, ABCC), such as MRP2 and MRP4. Table 2 shows the nomenclature, common names, and the localization of key transporters located in the PTCs. OAT1, OAT3, OCT2 on the basolateral membrane and P-gp, MATEs, MRP2, MRP4 on the apical membrane are the major PTC transporters known to interact with many renally secreted drugs (Morrissey et al., 2013). Renal transporter mediated elimination of drugs or metabolites has been extensively investigated, and the results indicate that in general, drugs with molecular weight less than 400 Da are substrates of several renal transporters (Varma et al., 2017).

While renal transporters generally enhance renal elimination, certain transporters, such as OAT4, PEPT2, and SGLT2 located on the apical side of the PTCs facilitate reabsorption of their substrates (Burckhardt, 2012; DeFronzo et al., 2017; Tchernitchko et al., 2017). As renal elimination of endogenous and xenobiotic compounds is determined by its net vectorial transport, the PK parameters will be affected when a NCE and/or its metabolites inhibit any of the transporters responsible for the elimination of these molecules. The subsequent imbalance can result in adverse effect on the kidney or any other organ. Because of the significance of the several kidney transporters in regulating drug clearance, regulatory authorities that govern the approval of pharmaceuticals for humans require evaluation of the potential for a NCE to be a perpetrator of

DDI and if renal active secretion is significant (>25% of total clearance), to evaluate the potential to be a victim of DDI (EMA, 2012; FDA, 2017b; FDA, 2017a). Inhibition of these transporters can not only affect the exposure to the co-administered drugs but also affect the elimination of endo- and exogenous toxins, therefore posing significant risks to patients. More recently the International Transporter Consortium (ITC) published recommendations to help guide clinical studies on the currently accepted drug transporter interactions (The International Transporter, 2010). The probe substrates and inhibitors for in vitro and in vivo DDI assessment can be found in FDA and PMDA DDI guidance's (EMA, 2012; FDA, 2017b; FDA, 2017a).

Emerging data associate membrane transporters to kidney disease and toxicity, if a drug is accumulated in the kidney to a certain level (Dresser et al., 2001; Nies et al., 2011; Miners et al., 2017). Accumulation in the kidney can arise when the uptake of the drug into the kidney PTCs is faster than the efflux out of the kidney, a process generally mediated by transporters. Nephrotoxicity occurs when a NCE is a substrate of one or multiple transporters or inhibits the transport of toxicants or co-administered drug(s) resulting in the increase in intracellular and/or circulating concentration of substrate(s) of the transporter involved. For example, the nephrotoxicity of cisplatin has been associated with renal accumulation, determined by the substrate specificity of the OCT2 and MATE families of transporters (Yokoo et al., 2007). OCT2 mediates the transport of cisplatin into PTC from blood; whereas, MATE1/2K transport cisplatin from PTC to urine. Several genetic variants with reduced functionality of OCT2 can lower uptake/accumulation of cisplatin in the PTCs, thus reducing cisplatin induced nephrotoxicity. Tenofovir, is primarily a substrate of OAT1 and to a lesser extent OAT3, which transports the nucleotide antiviral from blood into the PTC. The drug is subsequently transported out of PTC into urine by MRP4. The nephrotoxicity of tenofovir was shown to be due to accumulation of the drug

in the PTC (Moss et al., 2014). Ritonavir, an inhibitor of multiple transporters expressed in the kidney including MRPs, increases the propensity for developing nephrotoxicity in patients who are taking ritonavir in combination with tenofovir compared to patients treated with tenofovir alone (Moss et al., 2014). Cidofovir, a substrate for OATs, has been associated with dose-limiting nephrotoxicity (Ho et al., 2000). Probenecid, an inhibitor of OAT-mediated uptake of cidofovir by PTCs decreases the prevalence of cidofovir-induced nephrotoxicity. OAT1 mediated uptake and accumulation of cephaloridine, a beta-lactam antibiotic has been connected to the cytotoxicity to the PTC. The toxicity could be completely prevented by probenecid (Tune and Fravert, 1980).

While small molecules are excreted via active transport with the aid of transporters, glomerular filtration and passive diffusion, the overall excretion of proteins involves an interplay between excretion and reabsorption. Many large, soluble biomolecules are reabsorbed in the PTCs by endocytosis as part of the renal physiology that deals with the retrieval of filtered proteins, thus preventing them from disappearing from the body through the urine. This is also essential for the recovery of vitamins, hormones, enzymes and drugs. The endocytic receptors megalin and cubilin have been identified as essential receptors responsible for the reabsorption of proteins (Christensen and Gburek, 2004; Nielsen et al., 2016). Megalin is a 600 kDa glycosylated receptor containing a single transmembrane domain (23 amino acids) and an intracellular C-terminal cytoplasmic tail (209 amino acids). It is predominantly expressed in the apical membranes of the PTCs. The cytoplasmic domain of megalin regulates receptor trafficking and endocytosis. Cubilin is a 460 kDa glycosylated extracellular protein that interacts with other membrane proteins for membrane localization and endocytosis. In the PTCs, cubilin is shown to interact with megalin, forming a multi-receptor complex with megalin driving the internalization of the complex and bound ligand. This has been supported by in vitro uptake studies of the cubilin ligands transferrin and

Apolipoprotein A-I showing that the uptake was inhibited by anti-megalin antibodies and megalin anti-sense nucleotides (Kozyraki et al., 2001; Nielsen et al., 2016). It has been further shown that cubilin also depends on a single transmembrane protein, amnionless, for membrane localization and endocytosis. Neilsen et al. extensively reviewed the role of megalin and cubilin in the reabsorption of proteins in the kidney and provided an extensive list of ligands of these receptors (Nielsen et al., 2016). Once the proteins are reabsorbed, they are subsequently degraded in lysosomes. The free amino acids are transported across the basolateral membrane by amino acid transporters.

Since reabsorption of the filtered proteins in the proximal tubule is an important physiological and pathophysiological function by regulating biologically important substances like vitamins, hormones, enzymes, etc., the importance of the proper functioning of megalin and cubilin cannot be overstated. If not duly reabsorbed, the excess proteins in the tubular fluid irrespective of their discrete biological activities are sufficient to initiate a cascade of events leading to tubular injury, interstitial inflammation, fibrosis, and eventual renal scarring (Kozyraki et al., 2001; Christensen and Gburek, 2004).

While all kidney transporters have their own importance, and can play a vital role in the disposition of drugs, an ideal PTC model should at the least demonstrate functionality of OAT1, OAT3, OCT2 on the basolateral side and MATEs, P-gp, BCRP, MRP2, MRP4 on the apical side to be used in assessing the nephrotoxic liabilities of NCEs. In addition, and especially if biologics or large molecules will be assessed, it should also demonstrate megalin and cubilin mediated uptake potential of the NCE. GGT enzyme which is one of PTCs antioxidant defense mechanism should also be present at physiological levels in the model. Finally, many of the metabolic enzymes listed in Table 1 should also be a part of an ideal in vitro PTC model.

Common Sources of Kidney Cells

In the context of drug development, regulatory agencies have specified prominent transport proteins of which assessment of a drug's inhibitory and substrate potentials are required (EMA, 2012; FDA, 2017b; FDA, 2017a). Often, in vitro transporter DDI studies are performed using single transporter transfected cell lines, such as human embryonic kidney (HEK)-293 or Madin-Darby Canine Kidney (MDCK). Singly transfected cells allow for specific interactions to be elucidated, but correlation to in vivo interactions is not always straight forward. Primary renal cell cultures provide a more realistic model, but are limited by donor availability and variability. Recently, the abundances of renal transporters, with the exceptions of BCRP and MATE-2K, within the human kidney cortex have been quantified (Prasad et al., 2016). Although large donor-to-donor and site-specific variabilities were seen, this information helps fill a large gap in the understanding of renal transporters and may be used to better characterize in vitro cell lines and inform more accurate *in vitro* to *in vivo* extrapolation (IVIVE) models. The use of kidney cell lines to evaluate transporter interactions in the context of nephrotoxicity have been previously reviewed (Fisel et al., 2014; George et al., 2017). This section will highlight common sources of renal models with respect to drug transporters, nephrotoxicity, and drug disposition.

Renal Proximal Tubule Cells /Telomerase Reverse Transcriptase (RPTEC/TERT1): One major challenge with human derived cells is their limited capacity to be expanded in culture. Renal Proximal Tubule Epithelial Cells (RPTEs/PTCs) with ectopic expression of Telomerase reverse transcriptase (hTERT) has been shown to immortalize human derived RPTECs for up to 90 population doublings (Wieser et al., 2008). RPTEC/TERT1 cells stably express RNA and protein for OCT2, OCT3, OCTN2, OAT1, OAT3, OAT4, MATE1, and MATE2K. Functional transport of the fluorescent cation 4-Di-1-ASP by OCT/MATE was displayed in RPTEC/TERT1 cells,

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however, OAT function was questionable due to inability to transport p-aminohippuric acid (PAH) (Aschauer et al., 2015a). The lack in OAT function may be overcome by stably transfected lines available through ATCC (ATCC, 2016) though currently limited data is available. RPTEC/TERT1 cells have been utilized for in vitro kidney toxicity assessments via genetic analysis (Aschauer et al., 2015b), and of environmental toxicants (Simon et al., 2014; Simon-Friedt et al., 2015). Recently, highly differentiated 3D tubules of RPTEC/TERT1 cells were developed by sandwiching them between Matrigel layers leading to a branched network of cell free lumen. However, OAT1 expression was negligible even though other characteristics of a well-differentiated epithelium such as polar expression of Na+/K+ ATPase and ZO-3 were present (Secker et al., 2017). Although RPTEC/TERT1 cells provide an advantage of reduced culture time, they are limited by low OAT1 expression/function and require specialized culture media conditions. RPTEC/TERT1 cells can be licensed through Evercyte GmBH.

Conditionally immortalized human PTEC (ciPTEC): Researchers have generated conditionally immortalized PTCs by non-invasively obtaining renal material from the urine and transfecting those cells by using a temperature sensitive mutant U19tsA58 of simian virus 40 large T antigen (SV40T) and hTERT vectors (Wilmer et al., 2010). Transfection with SV40T allows the cells to proliferate at lower temperatures of 33°C and these cells can be cultured for up to 10 days at 37°C. These cells were rigorously characterized and demonstrated multiple characteristics of the kidney epithelium such as presence of tight junction proteins (ZO-1), PTC specific brush border enzyme (aminopeptidase N (CD13)), AQP1, megalin/cubilin endocytic receptors, P-gp, MRP4, and OCT2. However, these cells were lacking the organic anion transporters, OAT1 and OAT3. Later Nieskens et al., generated organic anion transporter overexpressing lines namely, ciPTEC-OAT1 and ciPTEC-OAT3 (Nieskens et al., 2016). These overexpressing ciPTECs demonstrated

functionality of OATs by showing dose dependent cytotoxicity to the antivirals tenofovir, cidofovir, and adefovir and inhibition of fluorescein uptake when the cells were incubated with prototypic substrates and inhibitors of these transporters. Very recently, Fedecostante et al., developed 3D kidney models on decellularized rat scaffolds by re-cellularizing it with ciPTEC-OAT1 overexpressing cells (Fedecostante et al., 2018). These cells showed a strong mRNA expression of a multitude of renal transporters such as OAC2, OAT1, P-gp, BCRP, MATEs, and MRP4. More importantly, the expression levels of the recellularized scaffold was always stronger than its 2D counterpart. When challenged with nephrotoxicants such as cisplatin, tenofovir, and cyclosporine A, the recellularized scaffold showed dose dependent cytotoxicity and for cisplatin the 3D platform showed a higher sensitivity than the 2D monolayer because of higher levels of OCT2 and MATEs in the 3D model. While this is indeed a novel platform, more studies with different types of nephrotoxicants is needed to really show its potential for drug discovery.

Transfected Cell Lines: HEK-293, MDCK, pig kidney-derived cells (LLC-PK1) and Chinese Hamster Ovary (CHO) cell lines are commonly transfected with a single transporter for use in in vitro testing. These cell lines have been transfected with all relevant drug transporters to determine uptake and inhibition parameters of developmental compounds. Transfected cell lines are easily obtained, simple to maintain, and provide relatively reproducible data. These cell lines could be used to characterize substrate selectivity and species differences explaining renal toxicity (Zou et al., 2018). Hence, transfected cell lines are the most common tools used to evaluate transporter interactions in vitro in the kidney. However, lack of physiology limits their utility in toxicity testing or for predictive screens.

Epithelial cell lines from normal adult human kidney (HK-2): In 1994, human renal cortex derived cells have been transfected with the human papilloma virus 16 (HPV-16) E6/E7 genes to

create the immortalized HK-2 cell line (Ryan et al., 1994). HK-2 cells have since been used to show that P-gp is suppressed by vancomycin (Im et al., 2017), elucidate biotransformation and toxicity of acyclovir (Gunness et al., 2011), assess in vitro biomarkers of cisplatin nephrotoxicity (Sohn et al., 2013), and evaluate general nephrotoxicity of compounds (Wu et al., 2009; Li et al., 2017). P-gp (Tramonti et al., 2001) and monocarboxylate transporter (MCT) (Wang et al., 2006) have been fully characterized in HK-2 cells. However, an extensive genetic analysis of HK-2 cells revealed that OAT1, OAT2, OAT3, OCT2, MRP2, and BCRP were not expressed by HK-2 cells, bringing into question their overall utility in assessing renal transporter function or transporter related toxicities (Jenkinson et al., 2012). Thus, HK-2 cells may be limited in future nephrotoxicity assessments as they were shown to be inferior to primary RPTECs/PTCs for in vitro nephrotoxic biomarker (KIM-1, NGAL, and M-CSF) production (Huang et al., 2015). Additionally, the authors are unaware of any reports regarding expression or function of MATEs in HK-2 cells.

RPTEC: RPTECs or PTCs, whether cryopreserved or freshly isolated, are often thought of as the 'gold standard' for in vitro kidney work. Freshly isolated RPTECs have been shown to express mRNA for all the major renal transporters, predominantly in the proximal tubule. Function of P-gp, BCRP, MRP2, OCT2 and OAT1/3 were also established (Brown et al., 2008). It should be noted that determination of specific transporter effects is challenging. For instance, functional studies confirm contributions of both OAT1 and OAT3 mediated transport of substrates such as PAH and statins (Burckhardt and Burckhardt, 2003; Windass et al., 2007; Brown et al., 2008). Commercial entities, such as Solvo Biotechnology, ATCC, etc., offer a wide range of cryopreserved and freshly isolated proximal tubule cells. As large donor-to-donor and site-specific variabilities of renal transporters were shown in isolated healthy kidneys (Prasad et al., 2016) and large differences in native tissue versus cell lines (Hilgendorf et al., 2007), it can be

assumed that large variability in transporter expression and function exists within available RPTECs. This may be further exacerbated by disease states (Motohashi et al., 2002; Habu et al., 2003; Feng et al., 2010). Cryopreserved and freshly isolated RPTECs each come with their own challenges. The availability of primary tissue is limited and many protocols exist for purifying proximal tubule from other renal cells (Elnaz and Katherine, 2018). Cryopreservation can assist with availability issues and cryopreserved RPTECs have been shown to express kidney tubule specific markers (Adler et al., 2016) and are more differentiated than cell lines like HK-2 (David et al., 2004). However, cryopreserved RPTECs lack strong transporter mRNA expression compared to isolated renal tissue (Van der Hauwaert et al., 2014). RPTECs have been used extensively in nephrotoxicity investigations and were reviewed recently (Elnaz and Katherine, 2018). RPTECs were used to describe biotransformation related nephrotoxicity of cisplatin (Wainford et al., 2008) and TCA (Racine et al., 2014). Figure 1 shows phase contrast images of few different cell types discussed to show minor variations in their morphology. Although, both fresh and cryopreserved PTCs shows a higher physiological relevance to the native kidney compared to cell lines in terms of the transporters, their expression can quickly downregulate in conventional static culture models. Hence, advanced dynamic models are needed.

In general, the limitations associated with these in vitro models to predict kidney toxicity are associated with the cell types because not all necessary transporters, metabolizing enzymes or biomarkers are expressed at physiological levels. Therefore, selecting the right end points becomes essential for a particular in vitro model. Many of the available PTC cell types discussed herein have also been utilized for nephrotoxicity prediction, in both 2-D and 3-D systems. Recently, Tiong et. al. reviewed the applicability of PTC cell types and culture systems for nephrotoxicity testing (Tiong et al., 2014). One striking limitation is that few studies include more than 10

compounds. Thus, it becomes extremely hard to generate any reasonable statistics related to the predictive performance of the assay and for researchers to properly assess the utility of the model. To our knowledge, only two groups have looked at a large enough compound set to generate the different parameters related to the predictive performance of the assay such as sensitivity, specificity, area under the receiver operating characteristic curve (AUC-ROC), positive predictive and negative predictive values meaningful. Few different reports were published in evaluating the nephrotoxic potential of over 40 compounds (Li et al., 2013; Li et al., 2014; Kandasamy et al., 2015). The researchers compared the predictive performance of HK-2 cells, LLC-PK1 cells, primary PTCs (HPTCs) and stem cell derived PTCs (HPTC-like). They showed that the primary PTCs cells which retains multiple characteristics of its in vivo counterpart showed the highest accuracy (AUC-ROC − 0.85) while HK-2 cells which have limited transporter expression (Jenkinson et al., 2012) showed the lowest accuracy (AUC-ROC – 0.71). Another report recently compared the nephrotoxic potential of 39 mechanically distinct nephrotoxicants using primary PTCs (HPTECs) and showed that heme oxygenase − 1 (HO-1) combined with cell count yielded the highest predictive performance in their assay (AUC-ROC -0.92) (Adler et al., 2016). However, tenofovir could not be detected as nephrotoxic in their model likely because of the lack of apical to basolateral polarity which was seen when primary PTCs are grown on a flat surface.

Another limitation, particularly of 2D cultures, is the lack of appropriate toxicity biomarkers. Commonly nonspecific cell health markers, such as ATP, apoptosis/necrosis, mitochondrial function, trans-epithelial electrical resistance (TEER), are utilized. 2D cultures of stem cell derived PTCs (HPTC-like) showed that more specific endpoints such as inflammatory cytokines (IL-6) or chemokines (IL-8) demonstrated better predictive performance (AUC-ROC − 0.94) than a

commonly used cytotoxic endpoint such as ATP depletion endpoints (AUC-ROC – 0.65) in primary PTCs (Li et al., 2013; Li et al., 2014).

Importance of fluid shear stress for kidney proximal tubule cells

In vivo, there is a constant flow of glomerular filtrate over the PTCs. In addition to providing several different growth factors and hormones to the PTCs, the filtrate also subjects the PTCs to shear stress which is detected by the mechanosensory cilia located on the apical side of the cells. Although the exact mechanism of this mechanotransduction phenomenon is somewhat controversial (Delling et al., 2016), it has been convincingly shown in numerous studies that fluid induced shear stress leads to enhanced endocytosis in renal cells,(Raghavan et al., 2014; Long et al., 2017) cytoskeletal re-organization (Duan et al., 2008), and presence of continuous tight junction (ZO-1) and adherens junction proteins (E-cadherin) (Duan et al., 2007), characteristics which suggest improved cellular maturity. Hence, over the past decade there has been a great interest to mimic the complex in vivo architecture and microenvironment of the kidney. By using the techniques initially developed for the semiconductor fabrication industry, numerous research groups and startup companies have now developed microfluidic devices using novel materials which can provide biomechanical cues to the kidney cells in vitro. In addition to kidney, numerous other organs such as liver (Domansky et al., 2010), heart (Mathur et al., 2015), brain (Park et al., 2015), lung (Huh et al., 2010), etc. are also being developed.

Kidney microphysiological system (MPS) technologies for drug discovery

Microfluidic kidney MPS

One of the first kidney-on-a-chip models with PTCs was developed by Ingber's group where a polyester membrane coated with Collagen IV was sandwiched between two polydimethylsiloxane

(PDMS) slabs as shown in Figure 2A (Jang et al., 2013). The bottom surface served as a medium or drug reservoir while human primary PTCs were seeded on the top surface. After reaching confluence, a fluidic shear stress of 0.2 dyn/cm^2 was applied to the cells on the top surface which lead to their maturation as demonstrated by increased expression of AQP1 and Na+/K+ ATPase, and increased number of primary cilia compared to the static Transwell model. Furthermore, there was a marked increase in the expression of SGLT2 which lead to increased glucose transport. Enhanced uptake of albumin was also reported possibly by enhanced megalin-cubilin functionality. They were also able to model cisplatin toxicity which is transported to the PTCs via OCT2. PTCs seeded in the microfluidic chips showed increased fidelity to cisplatin by showing reduced LDH release and apoptosis. Additionally, cimetidine, a known inhibitor of OCT2 offered significant protection to the PTCs from cisplatin mediated toxicity in the microfluidic chip while only partial protection was offered in the static model. Increased functionality of P-gp was also noted in the dynamic models compared to the static one. Thus, this work showed that shear stress helped the PTCs achieve a more mature phenotype and a kidney-on-a-chip could better model nephrotoxicity by improved expression of both uptake and efflux transporters. Recently, the development of a glomerulus-on-a-chip from human induced pluripotent stem was also reported by the same group (Musah et al., 2017).

University of Washington researchers collaborated with Nortis Inc. to develop a novel kidney-on-a-chip platform where a single straight microfluidic channel was molded inside a collagen I matrix using microfiber as shown in Figure 2B 1-2 (Weber et al., 2016). Primary human PTCs were seeded inside this collagen-IV coated channel, allowed to adhere for 24 hours and then perfusion of media was initiated inside the lumen. Cells inside the lumen displayed characteristic features of mature PTCs with ZO-1 staining localized to the apical surface and basolateral expression of

Na+/K+ ATPase suggesting polarized epithelia. KIM-1, an important kidney injury biomarker was present at low levels on the kidney-on-a-chip device but is often expressed at high levels in a 2D static model suggesting that the architecture of the device coupled with flow helped with a quiescent phenotype of the cells and prevented epithelial mesenchymal transition. Other characteristics such as strong GGT activity and strong functionality of SGLT2 were also noted in the kidney MPS. The researchers also demonstrated metabolism potential of the MPS by showing bioactivation of vitamin D. Calcifediol, a vitamin D pre-hormone produced in the liver was converted to the active form of vitamin D, calcitriol, through the action of CYP27B1 and to its inactive metabolites via CYP24A1. Finally, the researchers demonstrated that in the MPS device, probenecid, a competitive inhibitor of OAT1/3 and MRP2/4 decreased the apparent permeability of PAH, while no change in the apparent permeability was seen in the static 2D Transwell model. The researchers then coupled the above kidney MPS with a liver MPS to elucidate the nephropathy of a commonly used Chinese herb, aristolochic acid (AA) (Chang et al., 2017). AA is a potent nephrotoxin, however, it often requires bioactivation and formation of reactive metabolites, a process that happens in the liver. AA showed modest cell death and KIM-1 expression when directly added to the kidney MPS before hepatic metabolism. However, when AA was first added to the liver MPS which was coupled to the kidney MPS, increased cell death and KIM-1 expression was seen. Metabolites of AA were actively secreted out of the liver MPS via the action of MRPs and taken up by the PTCs via the action of OAT4 located on the apical side of the kidney MPS to cause nephrotoxicity. This and similar MPS systems could be used to model the toxicity of drugs in the future, develop PK/PD relationships, and be used to investigate organ-organ interactions.

While the above-mentioned kidney MPS platforms show increased maturation of kidney cells compared to their static counterparts, majority of them are currently being fabricated out of PDMS,

a polymer notorious in absorption of small, lipid soluble hydrophobic compounds. Hence, for the wide-spread use of these kidney MPS platforms, alternative materials need to be used for their fabrication which will prevent or at least minimize drug absorption. van Midwound et al., demonstrated that UV-ozone treated polycarbonate and cyclic olefin copolymer showed excellent biocompatibility, ease of device fabrication, and transparency, characteristics required for microfluidic devices, moreover, these thermoplastics significantly minimized adsorption of compounds and thus could be used as alternatives for PDMS in the next generation of kidney MPS (van Midwoud et al., 2012). Furthermore, majority of these kidney MPS platforms currently lack kidney microvasculature. Kidney vasculature plays an extremely important role in vivo by delivering nutrients to the tubular cells and maintenance of a healthy kidney epithelium (Jen et al., 2011). As they also participate in tubular secretion and re-absorption of drugs and xenobiotics they are prone to their insults as well (Basile, 2007). Thus, to accurately model drug clearance and toxicity to the kidney cells, it is vital that the next generation of kidney MPS incorporate kidney vasculature. Some other challenges such as lab-to-lab variability in the fabrication of the device, its robustness, and the need for experienced personnel to assemble the device should also be addressed for the MPS technology to gain a strong foothold in research in industry. Finally, the current throughput of these devices prevents its widespread use, especially in a pharma setting where hundreds of compounds are routinely screened. To increase the throughput of these devices while still allowing flow-based studies for kidney, Mimetas, a Dutch startup company recently launched an SBS compatible, free of PDMS, microtiter 384-well plate MPS called OrganoPlates. These OrganoPlates employ the use of phaseguide technology which enables patterning of liquids and gels (Vulto et al., 2011). Four wells of the plate together form one microfluidic chip thus leading to 96-microfludic chips (2-channel) on a single plate. An extracellular matrix (ECM) is

first pipetted in the gel-channel which gets patterned because of the phaseguide. This matrix serves as the support layer for the cells in the liquid channel where they can adhere and proliferate to form a tube-like structure. The liquid-channel can be perfused using a gravity driven flow eliminating the need for costly pumps. The resulting tubule of cells can be used for several functions such as investigating barrier integrity after a chemical insult, transporter based drug clearance and toxicity, etc. making it an extremely versatile platform. Based on this unique platform, Mimetas along with several collaborators from EU were part of a Crack-iT NephroTube challenge sponsored by GSK, Pfizer, and Roche to develop new models for assessing nephrotoxicity in vitro. Furthermore, by reducing the number of devices in the plate a third channel could be added to grow the kidney endothelial cells thereby mimicking the vasculature for the kidney (Figure 3C 1-2). Although, this new platform could offer a lot of potential and will be useful for testing the nephrotoxic liability of compound, its validation is critical to show its strength over current existing 2D static models. Majority of the above models have focused on characterizing few uptake and efflux transporters. However, a better characterization of the functionality of transporters is needed in each of these models so that the end user knows the strengths and shortcomings of these models in relation to xenobiotic handling and thus can use these platforms accordingly. A large dataset of mechanistically distinct nephrotoxicants also need to be validated in these models. In general, MPS technologies have been shown to exhibit more physiologically relevant phenotypes for many kidney makers compared to their static 2D counterparts, however, currently limited information exists involving the use of kidney specific biomarkers in assessing nephrotoxic liabilities of drugs in MPS to allow for IVIVE. Also, as majority of these MPS are fabricated using some form of lithography or other types of cleanroom based manufacturing processes, inclusion of sensors/biosensors which can allow different readouts

in real-time as per user needs would also be a great addition to the next generation of devices to obtain an efficient in vitro kidney model.

Bioprinted kidney MPS

A new emerging technology of bioprinting is now being increasingly used to develop complex 3D in vitro models (Bajaj et al., 2014; Murphy and Atala, 2014). Recently, Lewis's group demonstrated biofabrication of a 3D human renal PTC model with complex geometry and an open lumen lined by PTCs which allowed active perfusion allowing their maintenance in the construct for over two months (Figure 2D 1-2) (Homan et al., 2016). The PTCs in the 3D chip showed enhanced cell height, microvilli length and density, improved albumin uptake and megalin expression compared to their 2D controls with and without perfusion. Additionally, the researchers showed dose dependent toxicity of a common nephrotoxin, cyclosporine A in their 3D PTC model. As this model was bioprinted, it can be batch produced and can also incorporate user defined size and geometry. Other relevant cell types of the kidney such as endothelial cells can be cultured in conjunction with the PTCs to accurately model the kidney barrier. Thus, this platform has the potential to be developed into a truly unique in vitro system for nephrotoxicity screening and xenobiotic handling. Another example of a 3D bioprinted kidney model was demonstrated by Organovo, a San Diego based company which specializes in the development of 3D printed tissues (King et al., 2017). The unique aspect of their model was the incorporation of kidney interstitial cells such as renal fibroblasts and endothelial cells in addition to PTCs thus allowing development of a diseased model when challenged with TGF-β, a master regulator of fibrosis. In addition, the researchers also demonstrated improved kidney phenotype which could be maintained for up to a month in culture and strong expression of important kidney markers including renal transporters. Importantly, expression of the four transporters most relevant for xenobiotic handling in the

kidney, OAT1/3 (low), OCT2, and P-gp was shown to be maintained over the course of a month and was verified to be similar in comparison to that in the kidney cortex by LC-MS/MS. The ability of this model to be used for nephrotoxicity screening would require its validation with a large library of compounds to demonstrate its potential.

Currently, we believe that there is no "perfect" in vitro kidney model available for investigating nephrotoxicity. However, continued development of these advanced novel in vitro platforms offers a great promise for assessment of nephrotoxicity and understanding of drug clearance for new molecular entities, especially now when more and more research groups in academia and industry have started to work with both small and large molecules many of which cannot be accurately modeled using current in vitro kidney platforms.

Current challenges and future perspectives

In comparison to other tissue/cell types, the use of human renal cells by the pharmaceutical industry is rather limited. In contrast, the use of cryopreserved and/or fresh human hepatocytes is quite common, with multiple companies involved in sourcing and providing hepatocytes. The reasons for this are multiple, but one driving force was the search for alternatives to whole organ liver transplants. While the first successful cadaveric organ transplant occurred in 1950 and the first living twin organ transplant was in 1954, these both involved the kidney (Lawler et al., 1950; Watson and Dark, 2012). In the case of the liver, the first attempted transplant was in 1963 by Dr. Thomas Starzl, but was unsuccessful resulting in death of the patient (Starzl et al., 1964). Thus, greater efforts were expended on isolation and propagation of functional (and transplantable) hepatocytes versus kidney cell types. Using rats, Berry and Friend developed methodologies which resulted in high-yield isolates of liver parenchymal cells using a collagenase perfusion method

(Berry and Friend, 1969). Thus, with decades of experience, robust protocols now exist for isolating human parenchymal (and non-parenchymal) liver cells. The availability of highly characterized hepatocytes from of multiple vendors (e.g. Sekisui Xenotech, TRL/Lonza & ThermoFisher) has created an industry for supplying these cells to the pharmaceutical companies for studying metabolism and safety testing of NCEs. The demand of these cells is driven in part by FDA (and other regulatory agencies) guidance, including the 2016 Safety Testing of Drug Metabolites Guidance for Industry (or MIST), which states "In vitro studies can use liver microsomes, liver slices, or hepatocytes from animals and humans and generally should be conducted before initiation of clinical trials (FDA, 2016)"

The early successes of kidney transplantation likely hindered research on isolation and propagation of cells in vitro. However, it is worth noting that, in comparison to the liver, a relatively simple organ containing only 4 basic cell types (parenchyma, endothelial, stellate and Kupffer cells), the kidney is a very complex organ composed of dozens of specialized cell types (Kriz and Bankir, 1988; Baer et al., 1997). Because of this complexity, attempts to restore organ function in vivo utilizing in vitro isolation techniques for purified cell types are simply not feasible.

In the field of preclinical toxicity, the pharmaceutical industry has historically relied on various proximal tubule cell lines such as LLC-PK1, MDCK and HK2 (Rezzani et al., 2002; Gunness et al., 2010). However, as discussed, these cell lines are not adequate to be used as predictive models. Thus, to address these issues, investigators have turned to isolating primary PTCs (Pizzonia et al., 1991; Baer et al., 1997; Qi et al., 2007). There are now several commercial entities which sell human proximal tubule epithelial cells (e.g. ATCC, Lonza & Biopredic). But the use of these cells is not as widespread or as common as hepatocytes, likely due to many reasons, including lack of specific regulatory guidance on their use, limited characterization, as well as limited availability

of human tissue. In the academic sector, their use is more commonly associated with access to tissue from a linked medical center, given the high costs charged by cell vendors.

To overcome or bypass the hurdle of access to primary kidney cell types, the field has turned to directed differentiation of human induced pluripotent stem cells (iPSCs). However, in comparison to other cells types (e.g. cardiomyocytes, neuronal/oligodendrocytes and hepatocytes) research on the kidney has lagged. This changed in 2012 with the establishment of a differentiation protocol resulting in the derivation of renal podocyte-like cells (Song et al., 2012) with a series of other publications in quick succession (Araoka et al., 2014; Lam et al., 2014; Takasato et al., 2014). Recent research has described generation of kidney "organoids" from iPSCs (Morizane et al., 2015; Takasato et al., 2016; Takasato and Little, 2017) and utilizing organoids to model kidney disease (Freedman et al., 2015; Cruz et al., 2017; Kim et al., 2017). More recently, standardized protocols have been published, detailing the sequential steps and proportions of growth factors and timing for creating kidney organoids. In one protocol, a high efficiency (80-90%) system is described which generates nephron progenitor cells within 9 days of differentiation (Morizane & Bonventre, 2017) and a further 12 days is required to generate kidney organoids with "high reproducibility". This protocol does make use of a 96 well format, lending it to potential utility in high throughput screening systems, but the authors do note that "careful attention to morphological changes indicative of differentiation" is necessary. Another slightly more recent protocol, describes first inducing pluripotent stem cells into a cell type akin to the posterior primitive streak, followed by subsequent programming into cells representing posterior and anterior intermediate mesoderm (Takasato et al., 2017). These two cell types are then aggregated and undergo self-organization into kidney organoids. The resultant organoids are composed of all the cell types of the nephron including the glomerulus, proximal/distal tubules and endothelial network. In a very

recent publication, a system has been described which incorporates a robust differentiation

protocol with little user input (Czerniecki et al., 2018). This system takes human pluripotent stem

cells in 96 or 384 well format plates and completes the entire differentiation process using a robotic

platform. This system yields kidney organoids composed of both glomerular tissue as well as

proximal and distal tubule cells. This has tremendous potential given the relative ease of use, the

high degree of throughput and the efficiency of the differentiation protocol. Potential applications

include preclinical toxicity screening of lead compounds on the resultant organoids as well as

applications in developmental toxicity screening. For the latter, one could envision perturbing the

differentiation process with drugs or chemicals at any point in the protocol. Developmental and

reproductive chemical screening is a critical component of the Tox21 Initiative and this technology

could refine the current animal testing approaches currently used. As the authors also note, this

technology also has application in the drug discovery world when applied to kidney disease e.g.

polycystic kidney disease (PKD). Given there is only one FDA-approved drug for PKD (with ill-

defined mechanism(s) of action), this is clearly an unmet need for a highly prevalent genetic

disorder. With recent advances in single cell transcriptomics it might also be possible to further

characterize these kidney organoids similar to the work done by Park et al. on healthy mouse

kidneys, to discover new cellular targets for kidney disease and to learn more about kidney

development (Park et al., 2018). While great strides are being made in the generation of kidney

organoids, this technology and expertise are still quite limited and currently seen only in a handful

of academic research labs. Before kidney organoids are adopted as part of the preclinical repertoire

on NCE screening, several issues need to be addressed. First, kidney organoids are not a structure

amenable to facilitated transport studies. Given that the primary reason a NCE induces

nephrotoxicity is accumulation in the proximal tubule (Weber et al., 2017), protocols need to be

established for isolation of pure tubule epithelial cells from organoids. Then, these cell isolates could be used to populate 3D MPS as previously described. Second, the differentiation potential towards formation of a kidney organoid will vary depending on the cell source which was used to generate the iPSCs and thus would require some optimization.

The IQ-Pharma consortium, in collaboration with NCATS, has pushed for generation of preclinical species as a cell source for generating MPS for toxicity testing. The reason being the existence of extensive data sets on unanticipated toxicity of lead compounds, with species disparities. This leaves open the question, in the case of "killed" compounds, which species is predictive of clinical trial outcomes? Having available human MPS, in conjunction with appropriate preclinical species MPS, allows one to address this question. The availability of iPSC-derived kidney cells will be a big advance in their adoption in the pharmaceutical industry, as they represent an unlimited cell supply and, with gene editing, will facilitate disease modeling as well as the potential role(s) of polymorphic variants using isogenic control iPSC lines. For drug transport/toxicity, this latter application is particularly useful when applied to the transporters facilitating active uptake and secretion in the proximal tubule. The current caveat is that these cells, much like iPSC-derived cell types for other organs, are immature in phenotype, resembling fetal cells at best.

In the end, what will drive adoption of MPS technologies, including kidney MPS, will be the regulatory agencies world-wide. Given the widespread agreement that animals are not always reliable predictors of clinical trial outcomes, this paradigm shift will occur, it is simply a matter of time. In the United States, the FDA has recognized the potential of this technology and recently signed an agreement with Emulate in 2017 to evaluate this company's MPS technologies at the agency's Center for Food Safety and Applied Nutrition. So, depending on the outcomes of these

efforts, we could see adoption of this new technology as standard practice as part of future IND

filings.

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6. Authorship contributions

Wrote or contributed to the writing of the manuscript: Bajaj, Chowdhury, Yucha, Kelly, Xiao

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8. Footnotes

9. Legend for figures

Figure 1: Morphological comparisons of commonly used kidney cell lines and primary cells derived from induced pluripotent stems cells or human tissue. A) LLC-PK1; B) MDCK; C) HK-2; D) iPSC-derived tubule epithelial cells; E) Proximal tubule epithelial cells grown from human kidney biopsy. (scale bar in box E is 100 μm)

Figure 2: Examples of kidney microfluidic devices (A-C) and bioprinted device (D). (A) Bi-layer PDMS device with a sandwiched porous ECM-coated polyester membrane creating 2 compartments – top compartment for cell seeding with physiological flow and bottom compartment serves as a reservoir. Reproduced with permission from the Royal Society of Chemistry and Jang et al., 2013 (B-1) Single channel 3D MPS platform showing phase contrast and Live/Dead image of PTCs at day 28 within the microfluidic channel. Reproduced with permission from Elsevier and Weber et al., 2016 (B-2) Three channel device of the 3D MPS platfrom shown in B-1. Image kindly provided by Nortis Bio (C-1) Overview of the platform for epithelium and endothelial tubule co-culture. The 3-lane OrganoPlate® contains 40 microfluidic chips in a 384-well plate. Photos show top and bottom of the 3-lane plate with zoom of a single chip. An ECM is patterned by two PhaseGuides. (C-2) A 3D reconstruction of a confocal stack shows the tubular morphology of the RPTEC and HUVEC culture alongside the ECM gel. The HUVEC cells express RFP (purple), the tubes are stained for ZO-1 (green) and ezrin (red). DNA in blue. Scale bars: 100μm. Image kindly provided by Mimetas (D-1) Schematics and images showing the different steps of fabricating a 3D convoluted PTC channel. (D-2) A confocal 3D rendering of PTCs in the channel, actin (red) and nuclei (blue). Reproduced with permission from Homan et al., 2016. The work was published under a CC BY license (Creative Commons

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changes were made to the original figure.

10. Tables

Table 1: A list of drug metabolizing enzymes commonly associated with biotransformation of drugs and xenobiotics in the kidney (Lash, 1994; Lock and Reed, 1998; Lohr et al., 1998; Knights et al., 2013)

Phase I enzymes	Conjugating enzymes	Hydrolytic and other metabolizing enzymes
Cytochrome P450[a] CYP2B6, CYP3A5, CYP4A11, CYP4F2, CYP4F8, CYP4F11, CYP4F12	UDP-Glucuronocyltransferases[b] UGT1A5, UGT1A6, UGT1A7, UGT1A9, UGt2B7	Epoxide hydrolase
Microsomal Flavin adenine dinucleotide-containing monooxygenase	N-acetyl transferase	Catalase
Alcohol and aldehyde dehydrogenase	N-methyl transferase	NADPH-quinone oxidoreductase
Prostaglandin synthetase	Glutathione S-transferase	Superoxide dismutase
Monoamine oxidase		GSSG reductase[c]
Steroid 21 hydroxylase	Sulfotransferases	Esterases, Carboxyesterases
	Thiol S-methyl transferase	
		Cysteine conjugate β-lyase
		GSH peroxidase
		Glutamyl transferase, cysteinylglycinase
a: Major CYP450 enzymes identified in the kidney; b: UGT enzymes expressed in human kidney; c: GSSG- glutathione disulfide		

Table 2: A list of transporters commonly associated with the transport of drugs and xenobiotics

in the kidney proximal tubule.

Common Name (Symbol)	Gene Family-Derived Name	Localization
Organic Cation Transporter 2 (**OCT2**)	SLC22A2	Basolateral
Organic Anion Transporter 1 (**OAT1**)	SLC22A6	Basolateral
Organic Anion Transporter 3 (**OAT3**)	SLC22A8	Basolateral
Organic Anion Transporter 4 (**OAT4**)	SLC22A11	Apical
Urate Anion Exchanger 1 (**URAT1**)	SLC22A12	Apical
Multidrug and Toxin Extrusion Protein 1 (**MATE1**)	SLC47A1	Apical
Multidrug and Toxin Extrusion Protein 2K (**MATE2K**)	SLC47A2	Apical
Oligoeptide Transporter 1 (**PEPT1**)	SLC15A1	Apical
Oligoeptide Transporter 2 (**PEPT2**)	SLC15A2	Apical
Multi-drug Resistance Protein 1 (**P-gp**)	ABCB1	Apical
Breast Cancer Resistance Protein (**BCRP**)	ABCG2	Apical
Multidrug Resistance Associated Protein 2 (**MRP2**)	ABCC2	Apical
Multidrug Resistance Associated Protein 4 (**MRP4**)	ABCC4	Apical

11.Figures

Figure 1

Figure 2