


Functional Drug Screening using Kidney Cells On-A-Chip: Advances in Disease Modeling and Development of Biomarkers

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Introduction

Kidney disease affects >10% of the population in the United States and leads to a high risk of mortality and morbidity. The kidney has immense potential for regeneration in the appropriate environment. Providing an appropriate environment has been a very active area of research in nephrology for the development of biomarker and disease modeling. Cell-based screens rely on the function of these kidney cells in culture systems. This often leads to the failure of screening hits translating into mouse models for testing its efficacy in disease. Identification of nephrotoxic therapeutic agents before the clinical stage would allow modifications to maintain efficacy with lower kidney toxicity (1). Early identification will reduce nearly 20% of the drugs that fail in phase 3 clinical trials, improving the drug development pipeline (2). Thus, better biomarkers and disease modeling systems are needed for proficient drug discovery.

Cell Culture Systems and Cell Function

The apical surface of kidney proximal tubular epithelial cells (PTECs) is exposed to constant glomerular flow, which promotes cytoskeletal rearrangements, alters the expression of apical and basolateral transporters, and modulates sodium transport *in vivo* (3–5). At the same time, the basolateral surface of kidney PTECs is exposed to the interstitial space, promoting the movement of amino acids, glucose, active transport of ions, and drugs across the epithelium. In cell culture systems, both the above-mentioned components are lacking. The Transwell culture system mimics crossepithelial transport (6–8), but lacks fluid flow and the apical function of *in vivo*-like kidney functions. Kidney-on-a-chip is a fluid-flowing device consisting of a structural component, often made of polydimethyl siloxane, and a microfluidic component either attached to a pump or syringe with constant flow pressure (9–11). Several investigators, including those in our group, have examined the microfluidic kidney-on-a-chip platform, which provides the fluid flow component to PTECs and glomerular cells, and shows the functional and structural improvements

of these two cell types (12–19). Kidney-on-a-chip promotes actin cytoskeleton rearrangement and tight junction protein expression, and improves cellular transport functions compared with static cultures (15,17,20,21).

Biomarkers in Cell Culture Systems

Biomarkers play a critical role in the assessment of cellular function, particularly in the culture system. Many biomarkers that show sensitivity in *in vivo* settings fail to show sensitivity *in vitro*. This limits assessment of the cell function. Two main cell types that are well studied in the kidney are renal PTECs and podocytes that are damaged in response to various stimuli, including many investigational drugs. PTECs play an important role in active clearance, intracellular concentration, reabsorption, and interstitial accumulation of drugs, thus making them more susceptible to injury (22,23).

Another prominent segment of the kidney that is prone to frequent injuries is the glomerulus, which filters electrolytes and fluids from the blood, retaining plasma proteins (24,25). The two specialized cell types in the glomerulus that show injury are the glomerular endothelial cells and podocytes.

We found kidney-on-a-chip using PTECs show a significant increase in biomarkers such as kidney injury molecule-1 (KIM-1) and heme oxygenase-1, compared with static culture after injury (20). In this issue, Vormann *et al.* modeled ischemia reperfusion injury using a vessel-on-a-chip platform to assess the functionality of PTECs in a combination of human endothelial cells. The authors found increased sensitivity of cisplatin, tobramycin, and cyclosporin A to PTECs. Moreover, adenosine treatment rescued PTECs from ischemia reperfusion injury. Thus, kidney-on-a-chip shows promise for the development of predictive biomarkers for the preclinical screening of nephrotoxic compounds.

Disease Modeling

Disease modeling is a critical parameter for screening compounds to identify a drug for preclinical studies.

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Table 1. Kidney-/organ-on-a-chip platforms and cell types tested

Cells	Segment	Author/Reference
RPTECs	Proximal tubule	Adler <i>et al.</i> (2016) (20)
RPTECs	Proximal tubule	Jang <i>et al.</i> (2013) (17)
RPTECs	Proximal tubule	Ferrell <i>et al.</i> (2018) (37)
RPTECs/NIH3T3	Proximal tubule/fibroblast	Oo <i>et al.</i> (2011) (38)
HK-2	Proximal tubule	Zhou <i>et al.</i> (2015) (39)
RPTECs, HK2	Proximal tubule	Frohlich <i>et al.</i> (2012) (15)
MDCK, GC-ASC	Proximal/distal tubule	Huang <i>et al.</i> (2012) (40)
ARPCs	Proximal tubule	Sciancalepore <i>et al.</i> (2014) (41)
MDCK, IMCD	Proximal tubule/collecting duct	Jang <i>et al.</i> (2010) (42)
IMCD	Collecting duct	Jang <i>et al.</i> (2011) (43)
Mouse glomerular endothelial cells (GEnCs) and mouse podocytes (MPC-5)	Glomerulus	Zhou <i>et al.</i> (2016) (19)
Rat primary glomerular endothelial cells	Glomerulus	Wang <i>et al.</i> (2017) (44)
iPSC derived	Glomerulus	Musah <i>et al.</i> (2017) (45)
Ci-PTEC	Proximal tubule	Vriend <i>et al.</i> (2018) (46)
Human and rat PTECs and hepatocytes	Hepatocytes/proximal tubule	Chang <i>et al.</i> (2017) (12)
Human primary kidney cells, HK-2	Kidney/proximal tubule	Hoppensack <i>et al.</i> (2014) (47)
iPSC derived	Glomerulus/proximal tubule/vascular	Homan <i>et al.</i> (2019) (35)
Human primary podocytes/glomerular endothelial cells and immortalized podocytes	Glomerulus	Petrosyan <i>et al.</i> (2019) (30)
OK	Renal epithelium	Ferrell <i>et al.</i> (2013) (48)
HREC and MDCK	Renal epithelium	Ferrell <i>et al.</i> (2010) (49)
MDCK	Renal epithelium	Jayagopal <i>et al.</i> (2019) (50)
PTEC-TERT1	Proximal tubule	Homan <i>et al.</i> (2016) (10)
LLC-PK1	Proximal tubule	Essig <i>et al.</i> (2001) (14)
OK, LLC-PK1, and MDCK	Proximal tubule	Raghavan <i>et al.</i> (2014) (51)

RPTECs, renal proximal tubule epithelial cells; iPSC, induced pluripotent stem cell; Ci-PTEC, conditionally immortalized proximal tubular epithelial cell line; OK, opossum kidney.

Modeling Tubulointerstitial Disease

Kidney-on-a-chip shows promise for disease modeling, owing to its closer resemblance to *in vivo* conditions. Investigators, including us, have shown that kidney-on-a-chip shows a better response to injury markers in response to nephrotoxic compounds (20,26,27). We found that expression of KIM-1 after nephrotoxic compound treatment shows a significant increase compared with static culture (20,26). Many compounds are biotransformed by the liver, producing nephrotoxic products (28,29). Chang *et al.* modeled human hepatocytes and PTECs to investigate the biotransformation of aristolochic acid–on-a-chip, and found that biotransformation of aristolochic acid by hepatocytes increases the toxicity toward kidney cells, as shown by increased cell injury markers, including KIM-1 expression (12).

Modeling Glomerular Disease

Coculture of podocin and glomerular endothelial cells–on-a-chip showed a better injury response to puromycin as shown by albumin leakage; the serum from patients with membranous nephropathy (MN) showed increased albumin leakage (30). In addition, mechanistically, podocytes showed activation of signaling similar to MN, such as mislocalized nephrin, due to the activation of complement signaling molecule, C3d (31), leading to the loss of a slit diaphragm structure (32). Further, it was found that treatment with a therapeutic agent, α -melanocortin stimulating hormone, known to ameliorate MN, reduces albumin leakage

in the kidney-on-a-chip platform (30). Finally, kidney-on-a-chip shows promising results for modeling podocytes from genetic kidney disease (30). The summary of cells used to model segments of nephron is shown in Table 1.

The development of kidney organoids provides additional benefits, where kidney tubular and glomerular-like structures develop in the dish (33,34). The addition of a microfluidic component to the kidney organoids showed vascularization and early maturation, indicating the addition of a flow system mimics *in vivo*-like conditions,

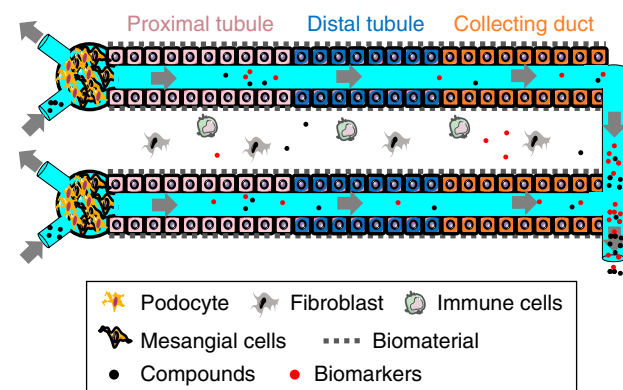


Figure 1. | Schematic for functional kidney-on-a-chip for compound screening and evaluation of biomarkers.

compared with static organoid culture (35). A schematic of kidney-on-a-chip for compound screening and biomarker evaluation is shown in Figure 1.

Future Challenges

Although kidney-on-a-chip systems show promise for biomarker and nephrotoxicity assessment, they need optimization for high throughput screening. The optimization of a new cell culture system is often challenging and requires optimization for seeding density and modulation of flow. There is an immense need for improving the imaging of the kidney-on-a-chip using high magnification microscopes to understand the biologic process. Coculture of different cells from the kidney improves cellular functions (36), but lacks circulatory and immune cells. Kidney-on-a-chip provides an opportunity to add circulatory immune cells (T cells, B cells, macrophages, and neutrophils) and nonimmune (fibrocyte) cells into the circulation. The addition of these cells will mimic *in vivo*-like signaling crosstalk among these cells and creates a microenvironment with secretory proteins, such as TGF- β (fibrosis), TNF- α (acute kidney injury and fibrosis), KIM-1 (proximal tubular injury), and neutrophil gelatinase-associated lipocalin (tubular injury marker) in the culture medium, and are crucial for disease modeling. Thus, efforts are being made to create a complete kidney-on-a-chip with circulatory cells and kidney cells for disease modeling. Developing a complete nephron by combining cells from the different segments of kidney such as the glomerulus, PTECs, distal tubules, and collecting ducts will allow us to investigate segment-specific nephrotoxic preclinical compounds. With the combined efforts of bioengineers developing new biomaterials, and cell biologists using them to create the *in vivo*-like microenvironment, we anticipate great advances in drug screening and disease modeling in the coming years.

Disclosures

A.K. Ajay reports being a founder of CompEdge and Sense Research Foundation; this conflict of interest is managed by the Mass General Brigham office of external collaboration.

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Author Contributions

A.K. Ajay conceptualized the study, was responsible for the formal analysis, funding acquisition, investigation, project administration, resources, and software, provided supervision, wrote the original draft, and reviewed and edited the manuscript.

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