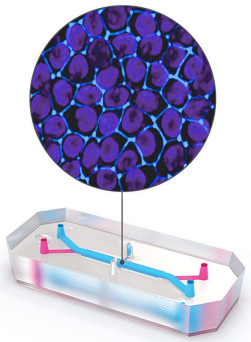
Proximal Tubule Kidney-Chip for Modeling Human Physiology



Introduction

The kidney is a complex organ composed of over 20 different cell types and approximately one million nephrons.[1] Primary functions of the kidneys are to eliminate waste from the body and to maintain homeostasis through the regulation of fluid volume and composition through urine production. In an average human adult, the kidneys filter 150-180 liters of blood, resulting in one to two liters of urine per day.[2]

The formation of urine occurs in three stages: glomerular filtration, tubular reabsorption, and tubular secretion.[1] The proximal tubule plays a significant role in these processes through modulating glomerular filtrate concentrations, drug transport, and metabolism, which makes it susceptible to damage by nephrotoxic compounds.[3] Understanding how kidneys eliminate waste/toxins is critical in investigating renal physiology and assessing nephrotoxicity of novel compounds in drug development.

Due to the complex physiology of the kidney—including specialized structures and the dynamic microenvironment—there has been an increasing need to find a more reliable and physiologically relevant model system to study renal physiology and diseases.[1] Traditionally, the study of kidney pathology and toxicity has been accomplished using two-dimensional (2D) cell cultures of established kidney cell lines or primary human proximal tubule cells.[4,5] These conventional models lack physiologically-relevant cytoarchitecture and a dynamic microenvironment[1,6] and are poor predictors of nephrotoxicity and drug behavior in the kidneys with respect to translation into the clinic.

To circumvent the challenges of 2D cell cultures, organoid cultures have been developed to recreate the 3D cytoarchitecture of the human kidney.[7] Organoids increase cellular heterogeneity and demonstrate some characteristic functions of mature tubules.[4] However, these have limitations such as lack of full maturation and vascularization, hypoxic and metabolic deficits, off-target cell populations (presence of non-renal cells), and issues with scalability and reproducibility.[7,8] While animal models have been used as the standard for nephrotoxicity and safety assessments in drug development and discovery, there are limitations such as high cost, low throughput, and ethical concerns.[1] More importantly, animal models are poor predictors of clinical outcomes.[9] For example, while 2-8% of drug candidates are rejected at the preclinical level due to nephrotoxicity, this number increases to upwards of 20% in human clinical trials and use following approval due to species

Key Highlights

- The Proximal Tubule Kidney-Chip represents a more physiologically relevant system for drug discovery and development applications
- Other potential applications include investigating human physiology and pathology
- Testing across a broad range of applications, including general toxicity, mechanistic toxicity, and nutrient metabolism studies
- Models diseases and predicts not only drug toxicity, but also treatment efficacy

differences.[3] Therefore, there is a need for improved preclinical models that better emulate kidney physiology and build an enhanced drug safety profile that successfully translates into the clinic and reduces animal burden.

Organs-on-Chips technology addresses some of these challenges by creating a physiologically relevant microenvironment providing 3D cytoarchitecture, extracellular matrix (ECM) components, fluid flow, and the inclusion of the different cell types found in the organ system of interest.[1,6] The Proximal Tubule Kidney-Chip could provide a platform to assess structural, mechanical, transport and absorptive properties aligned with human physiology in order to better predict clinical responses.

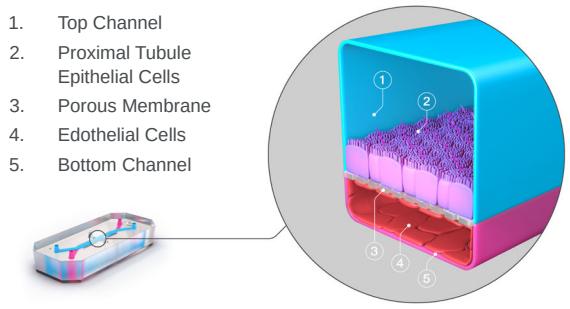


Figure 1. Proximal Tubule Kidney-Chip: Design and architecture of the chip.

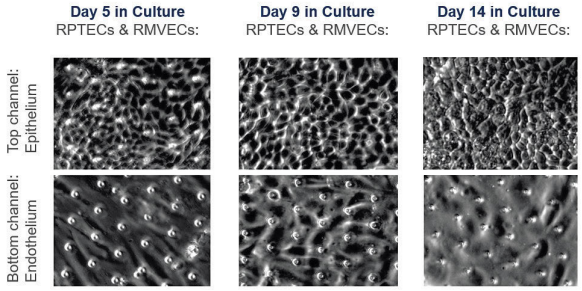


Figure 2. Morphology of cells in the Proximal Tubule Kidney-Chip. *Renal proximal tubule epithelial cells (RPTECs) and renal microvascular endothelial cells (RMVECs) cultured in flow on the Proximal Tubule Kidney-Chip for 5, 9, and 14 days.*

Goal

To develop and characterize a Proximal Tubule Kidney-Chip that emulates *in vivo* physiology by recreating the tissue-tissue interface of the kidney proximal tubule and peritubular capillary in the Kidney-Chip, that can be utilized to study renal physiology and pathophysiology, evaluate drug clearance and drug-drug interactions, and assess the safety and efficacy of therapeutics.

Results

To create the Proximal Tubule Kidney-Chip, Emulate's Chip-S1® stretchable chip was coated with ECM protein, collagen type IV and Matrigel, to generate a luminal (top) and a vascular (bottom) channel (**Figure 1**). Primary renal proximal tubule epithelial cells (RPTECs) isolated from the human proximal tubule were seeded on the luminal channel and primary human renal microvascular endothelial cells (RMVECs) were seeded on the vascular channel, then these cells were cultured under the flow rate of 60 µL/hour within the Zöe-CM1® Culture Module.

To confirm the morphology of RPTECs and RMVECs after culturing with flow for five, nine, and 14 days, micrographs were obtained of both the top and bottom channels (**Figure 2**). Under fluid conditions, the characteristic morphologies of the cells are observed as well as the retention of the polarized cytoarchitecture. Further imaging using immunofluorescence (**Figure 3**) confirmed the presence of specific cell markers including apical tight junction protein zonula occludens 1 (ZO-1) and the endothelial adherent protein VE-Cadherin. Staining and imaging were also performed for specific markers known to be abundant along the proximal tubule including beta-catenin, occludin, aquaporin 1 (AQP1), and Na/K-ATPase, as well as acetylated tubulin staining demonstrating the presence of cilia and scanning electron microscope of cilia and brush borders. Renal transporter function was assessed in RPTECs in monoculture or co-cultured with generic endothelial cells, human umbilical vein endothelial cells (HU-

VECs) or with kidney-specific RMVECs. Each of the three cultures were stained for sodium/phosphate (Na/Pi) co-transporters and demonstrated higher expression of this transporter only in co-culture with kidney-specific RMVECs compared to monoculture and co-culture with HUVECs (**Figure 4A**). Quantitative analysis of relative fluorescence signal intensity of the three cultures (**Figure 4B**) revealed statistically significant differences in Na/Pi cotransporter expression between monoculture and co-cultures.

To further evaluate physiological transport function of the Proximal Tubule Kidney-Chip, albumin uptake was measured using two different methods. Fluorescein isothiocyanate (FITC)-labeled human albumin was added in the luminal channel and uptake of albumin was assessed by fluorescence microscope (**Figure 5A**). Additionally, untagged albumin was added in the luminal channel and uptake of albumin was measured by an albumin quantification assay using effluent samples. Quantification revealed that the level of albumin uptake of the proximal tubule epithelium was close to *in vivo*-relevant levels over the long term (**Figure 5B**).

Evaluation of active transport of the key transporters in the Proximal Tubule Kidney-Chip was performed by quantifying the transporter-mediated secretion of creatinine via organic cation transporter 2 (OCT2) (**Figure 6A**) and para-aminohippuric acid (PAH) via organic anion transporter 1 (OAT1) (**Figure 6B**). Metformin, a drug to treat diabetes that is renally excreted via OCT2, OCT1, multi-antimicrobial extrusion protein (MATE)1, and MATE2-K[10], was applied to the Proximal Tubule Kidney-Chip and efflux activities (the permeability from the vascular to the luminal channel/the permeability from the luminal to the vascular channel) was measured at days six and eight (**Figure 6C**). Analysis revealed that metformin, creatinine, and PAH are actively transported by their respective proximal tubule transporters along the Proximal Tubule Kidney-Chip (Efflux ratio ≥ 2). The efflux ratios of the Proximal Tubule Kidney-Chip-derived substrates—well in excess of 2—indicate the utility of the model for assessing the role of active transport in drug disposition as well as transporter-mediated drug-drug interactions, as per the FDA Guidance for Industry.[11]

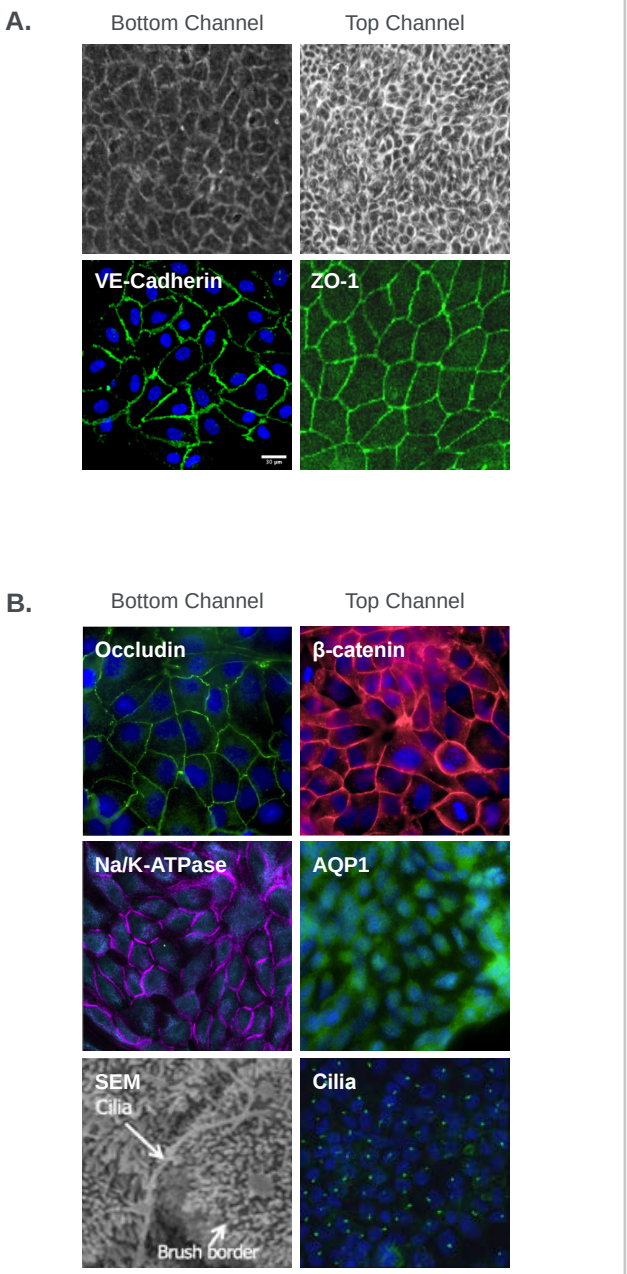


Figure 3. Morphology of cells on the Proximal Tubule Kidney-Chip. A*) Cells formed a polarized monolayer showing defined and orderly expression of the epithelial tight junction protein ZO-1 and the endothelial adherent protein VE-Cadherin.* ***B****) Polarized proximal tubular epithelial cells expressed specific markers, including beta-catenin, occludin, aquaporin 1 (AQP1), and Na/K-ATPase, as well as presented cilia and a brush border.*

Conclusion

The Proximal Tubule Kidney-Chip emulates functional characteristics of the intact human proximal tubule through polarized epithelium and endothelium, critical functional renal markers, and active transporter function. Recreation of the tubular-capillary interface was successful and allowed for paracrine communication and cross-regulation of these two cell types as observed *in vivo*. Long-term viability and function in culture for up to 14 days is possible, allowing for the measurement of mechanistic endpoints and biomarkers, which can enable the delineation of pathways and mechanisms leading to nephrotoxicity. Experimental advantages of the Proximal Tubule Kidney-Chip include the ability of continuous collection or sampling of effluents, easy access to the sample, and results that are accurate and reproducible.

Taken together, the Proximal Tubule Kidney-Chip allows for testing across a broad range of applications including the study of toxicity, drug-drug interactions, and treatment efficacy. Expression of a more *in vivo*-like phenotype and more human-like responses using the Proximal Tubule Kidney-Chip can provide an improved preclinical model for nephrotoxicity as well as insights into disease pathologies, detection of biomarkers, basic biological and physiological function, nutrient metabolism, and mechanisms of toxicities.

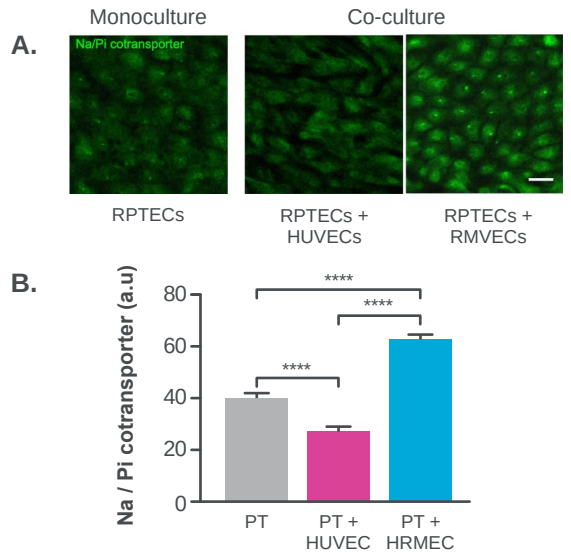


Figure 4. Na/Pi co-transporters on the Proximal Tubule Kidney-Chip. A) *Monoculture renal proximal tubule epithelial cells (RPTECS) and co-cultures of RPTECs (with human umbilical vein endothelial cells: HUVECs or renal microvascular endothelial cells: RMVECs) with and without renal-specific endothelial cells stained for transporter function.* ***B)*** *Quantitative analysis of sodium/phosphate (Na/Pi) co-transporters in monoculture and co-culture with RMVECs. ****p<0.0001.*

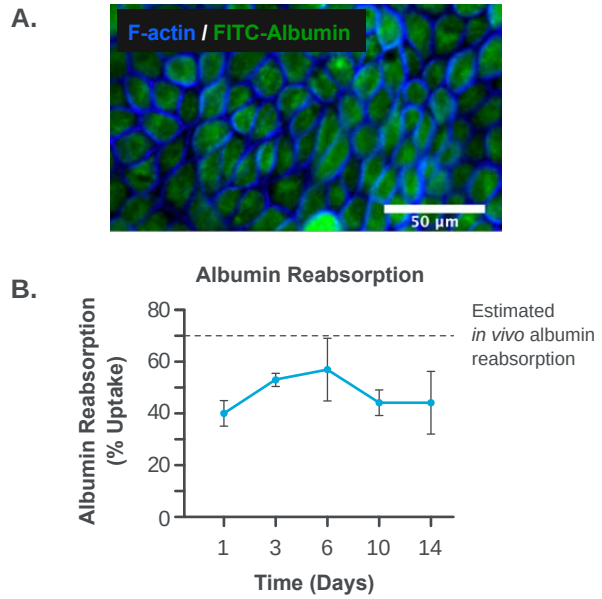


Figure 5. Albumin reabsorption in the Proximal Tubule Kidney-Chip. A) *Confocal image of FITC-labeled human albumin uptake.* ***B)*** *Quantitative analysis of albumin reabsorption from Chip effluent that was maintained over 14 days and validated using two endpoints, F-actin and FITC-albumin.*

References

1. Faria, J., Ahmed, S., Gerritsen, K. G. F., Mihaila, S. M. & Masereeuw, R. Kidney-based in vitro models for drug-induced toxicity testing. Archives of Toxicology vol. 93 3397–3418 (2019).

2. Bajaj, P., Chowdhury, S. K., Yucha, R., Kelly, E. J. & Xiao, G. Emerging kidney models to investigate metabolism, transport, and toxicity of drugs and xenobiotics. Drug Metabolism and Disposition vol. 46 1692–1702 (2018).

3. Yeung, C. K. & Himmelfarb, J. Kidneys on chips: Emerging technology for preclinical drug development. Clin. J. Am. Soc. Nephrol. 14, 144–146 (2019).

4. Soo, J. Y. C., Jansen, J., Masereeuw, R. & Little, M. H. Advances in predictive in vitro models of drug-induced nephrotoxicity. Nature Reviews Nephrology vol. 14 378–393 (2018).

5. Ewart, L. et al. Application of Microphysiological Systems to Enhance Safety Assessment in Drug Discovery. Annual Review of Pharmacology and Toxicology vol. 58 65–82 (2018).

6. Jang, K. J. et al. Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assesment. Integr. Biol. (United Kingdom) 5, 1119–1129 (2013).

7. Geuens, T., van Blitterswijk, C. A. & LaPointe, V. L. S. Overcoming kidney organoid challenges for regenerative medicine. npj Regenerative Medicine vol. 5 1–6 (2020).

8. Tian, P. & Lennon, R. The myriad possibility of kidney organoids. Curr. Opin. Nephrol. Hypertens. 28, 211–218 (2019).

9. Phillips, J. A. et al. A pharmaceutical industry perspective on microphysiological kidney systems for evaluation of safety for new therapies. Lab Chip 20, 468–476 (2020).

10. Heaf, J. Metformin in chronic kidney disease: Time for a rethink. Peritoneal Dialysis International vol. 34 353–357 (2014).

11. FDA Draft Guidance. In Vitro Metabolism and Transporter Mediated Drug-Drug Interaction Studies Guiance for Industry. (2017)

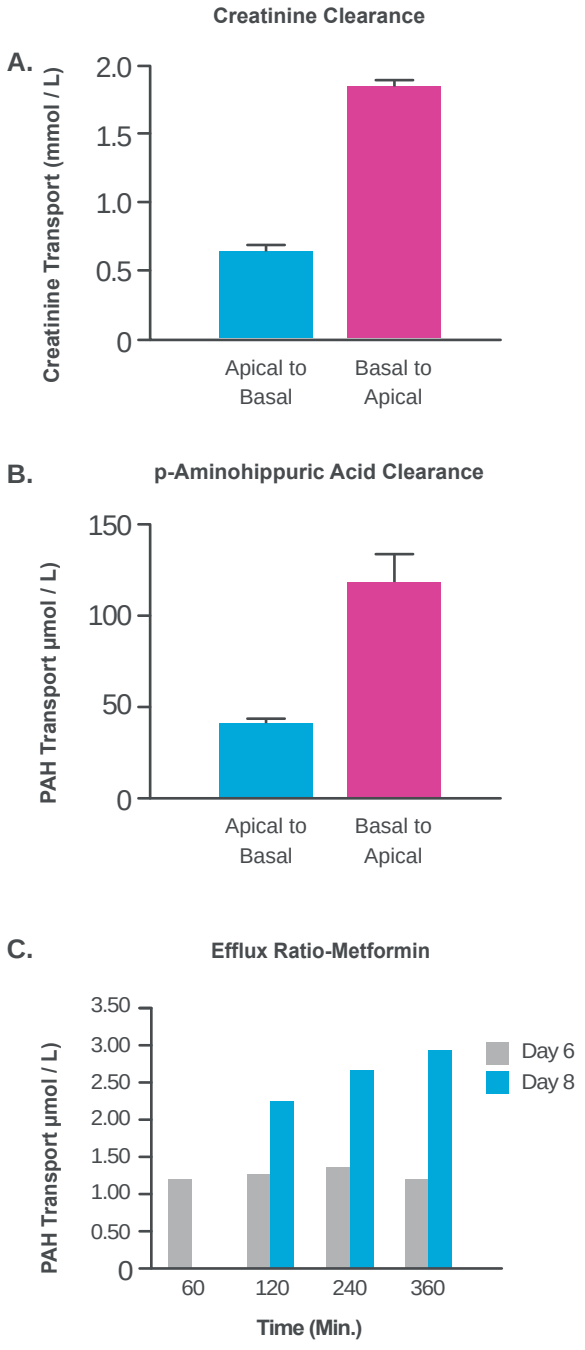


Figure 6. Transporter-mediated secretion from the Proximal Tubule Kidney-Chip. A*) Creatinine and* ***B****) p-aminohippuric acid (PAH) are secreted from the vascular channel to the luminal channel (basal to apical).* ***C****) Significant efflux of metformin from the vascular channel to the luminal channel was observed over time.*