Development of Human Proximal Tubule Kidney-Chip to study Transporter-Based Drug-Drug Interactions

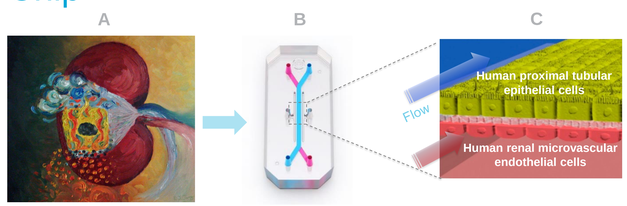
Sauveur Jeanty[1], Ananth Nookala[2], Hyoungshin Park[1], Sonalee Barthakur[1], Brian Zuckerman[1], Gang Luo[2], Ville Kujala[1], Josiah Sliz[1], Tanvi Shroff[1], Geraldine A. Hamilton[1], Kyung-Jin Jang[1], Donald Mckenzie[2]

[1] Emulate, Inc., Boston MA, USA, [2] Covance Laboratories Inc., Madison WI, USA

Introduction

The kidney plays a key role in elimination of xenobiotics and endogenous compounds through its complex and efficient uptake and efflux transporting systems. It is, therefore, very critical that drug interactions with renal tubular transporters be investigated systematically to increase our understanding of drug disposition and toxicity, and predict potential drug-drug interactions in human. However, current cell-based models often fail to predict renal transporter activity and are not scalable to a predictive clinical outcome due to *in vitro-in vivo* discrepancy. Here, we developed a human Proximal Tubule Kidney-Chip for assessment of renal transporter-based drug-drug interactions. The chip features two fluidic channels separated by a porous membrane that is coated with extracellular-matrix proteins, thereby creating an apical (luminal) channel and a basal (vascular) channel. Primary epithelial cells isolated from the human proximal tubule are cultured on the luminal channel, while primary human glomerular endothelial cells are cultured on the basal channel and serve as the vasculature. These cells are exposed to fluidic flow that recapitulates key functions of the human proximal tubule. This human Proximal Tubule Kidney-Chip that recreates the natural tissue-tissue interface of the kidney proximal tubule and the peritubular capillary may offer a new way to assess renal transporter-based drug-drug interactions and test for drug-associated kidney toxicities.

Kidney-Chip



To construct the human Proximal Tubule Kidney-Chip, we used a chip made of polydimethylsiloxane (PDMS). The chip contains two parallel channels: an upper channel (1 mm high × 1 mm wide) and a lower channel (0.2 mm high × 1 mm wide). The channels are separated by a porous PDMS membrane, which is coated with optimized extracellular matrix (ECM). The upper channel serves as a tubular lumen and is lined by primary human epithelial cells seeded on the ECM coated membrane. The lower channel, lined with endothelial cells, represents the peritubular vasculature.

Figure 1. Above is a schematic representation of the Proximal Tubule Kidney-Chip: **A** is an artistic rendering of the kidney. **B** is an image with emphasis on the channels of the chip; and **C** is a cross-section representation of the Proximal Tubule Kidney-Chip.

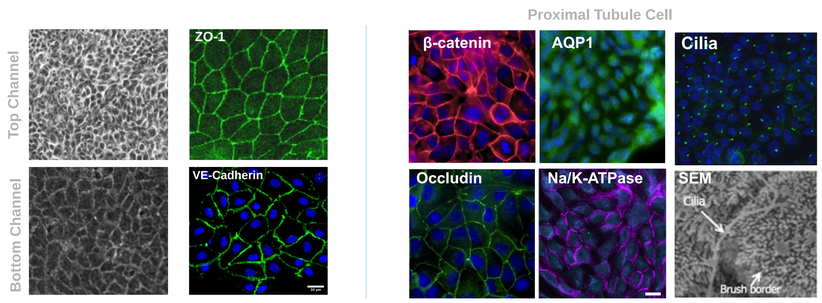


Figure 2. The Proximal Tubule Kidney-Chip formed a polarized monolayer showing defined and orderly expression of the epithelial tight junction protein ZO-1 and the endothelial adherent protein VE-Cadherin.
Polarized proximal tubular epithelial cells expressed specific markers known to be abundant along the proximal tubule, including beta-catenin, occludin, aquaporin 1 (AQP1), and Na/K-ATPase, and presented cilia and brush border.

Results

Functional Assessment of Various Transporters

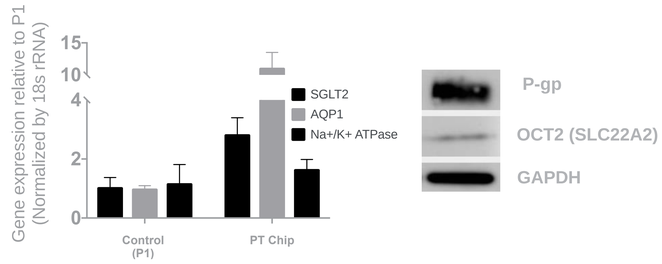


Figure 3. Relative gene expression of SGLT2, AQP1, and Na+/K+ ATPase was measured in control passage 1 (P1) proximal tubule cell vs Proximal Tubule Kidney-Chip. Western blot analysis confirmed expression of uptake and efflux transporters such as P-glycoprotein (P-gp) and OCT2 (SLC22A2).

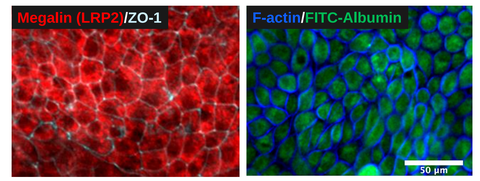


Figure 4. The Proximal Tubule Kidney-Chip exhibited abundant megalin protein expression and the resorptive capability of the proximal tubule epithelium was demonstrated by uptake of FITC-labeled human albumin

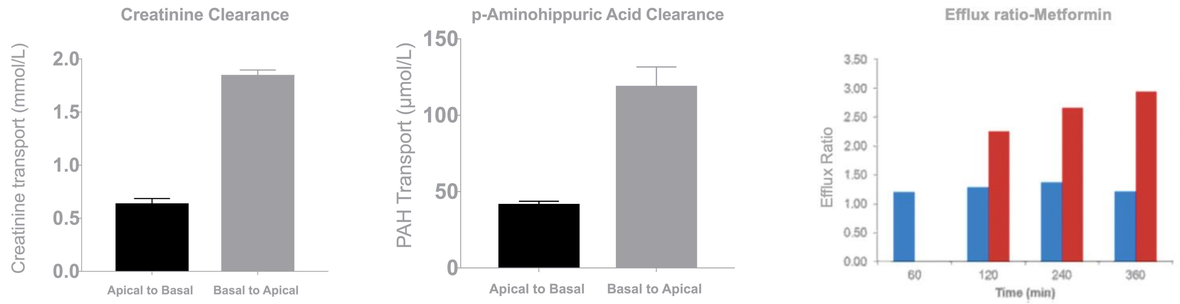
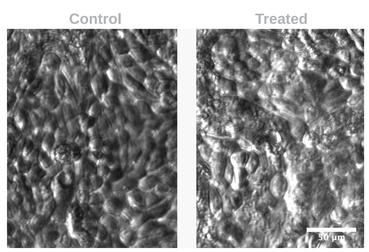


Figure 5. Transporter-mediated secretion of p-aminohippuric acid (PAH) and creatinine from the vascular channel to the luminal channel (basal to apical) was measured on chip. Additionally, significant efflux of Metformin from the vascular channel to the luminal channel was measured in a time-dependent manner. These results suggest that metformin, creatinine, and PAH are actively transported by their respective proximal tubule transporters along the Proximal Tubule Kidney-Chip.

Gentamicin Toxicity in Proximal Tubule-Chip



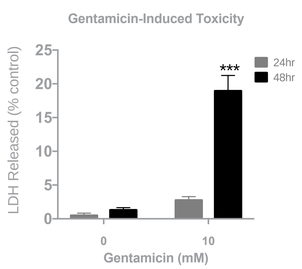


Figure 6. Microscopic analysis of the proximal tubular epithelium and LDH release revealed significant cell damage after 10 mM of gentamicin treatment for 48 hours.
(*** p<0.0.01)

Conclusion

We created a Proximal Tubule Kidney-Chip that recapitulates *in vivo* relevant tissue-tissue interface of the kidney proximal tubule. This chip exhibited polarized epithelium and endothelium that reproduced baseline functions of the proximal tubule *in vitro* and demonstrated active transporters functions that are critical for normal kidney functions. These results suggest that the Proximal Tubule Kidney-Chip could represent a more physiologically relevant system for drug discovery and development applications.

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