A Human Proximal Tubule Kidney-Chip for Accelerated Therapeutic Development

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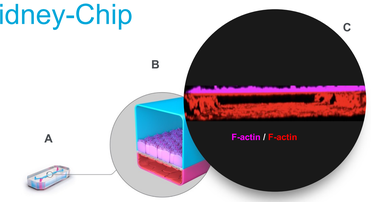
Abstract

Drug-induced nephrotoxicity accounts for a majority of acute kidney injury (AKI) cases and is a primary cause of clinical attrition for lead therapeutic candidates. The lack of predictive preclinical tools is a key factor in the failure to translate preclinical results into clinical outcome that is sensitive enough to detect early biological markers of kidney injury. There is significant evidence that the renal proximal tubule is the primary target for most nephrotoxic compounds. Therefore, there is a desperate need for more human-relevant proximal tubule models to evaluate early indicators of nephrotoxicological events. Here, we present an engineered Proximal Tubule Kidney-Chip that more accurately captures *in vivo* phenomena by recreating the natural tubular-peritubular interface. The Human Proximal Tubule Kidney-Chip features two fluidic channels separated by a porous membrane that is coated with extracellular matrix proteins, thereby creating a tubular epithelium and a vascular endothelium channel. The tubular epithelium is established with human primary renal proximal tubule cells (RPTECs) while the vascular endothelium consists of human primary renal microvascular endothelial cells (RMVECs) cultured under continuous physiological flow to form the Proximal Tubule Kidney-Chip. We have shown that the proximal tubule epithelium expresses transporters that are key to proper kidney function in vivo, which are typically absent in conventional culture systems. Using well-known nephrotoxicants, including cisplatin and gentamicin, we have also demonstrated toxic responses at physiologically relevant concentrations. This Proximal Tubule Kidney-Chip recreates key physiological features of the human kidney and is a promising predictive tool to assess drug safety.

The Proximal Tubule 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. Schematic representation of the Human Proximal Tubule Kidney-Chip: (**A**) Kidney-Chip cellular composition and channel orientation. (**B**) Cross-sectional representation of the chip with indicated cell types; and (**C**) F-actin immunostaining 3D representation of the Proximal Tubule Kidney-Chip, showing contiguous proximal tubule epithelium and vascular endothelium.

Proximal Tubule Kidney-Chip Characterization

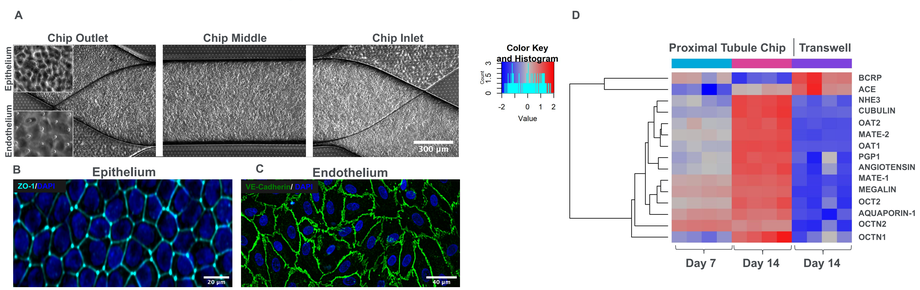


Figure 2. The cells in the Proximal Tubule Kidney-Chip form nice monolayers as shown by the bright field montage of the cells in the chip (**A**). Immunofluorescent staining for the junctional protein, ZO-1, reveal a defined and orderly network of epithelial tight junctions, indicative of polarized epithelial cells (**B**). VE-cadherin staining shows a healthy endothelium with uncompromised barrier (**C**). Transcriptomic analysis reveal that key transporters, such as Megalin (LRP2), PGP1 (ABCB1), OCT2 (SLC22A2), Aquaporin-1 (AQP1), and others necessary for renal tubular function have significantly higher expression levels in the Kidney-Chips compared to Transwells (**D**). Interestingly, transporter expression seems to improve over time in the chip. These results suggest that the microenvironment created by the chips allows the cells to reestablish most of their *in vivo* characteristics, which are lost in the process of cell isolation and in conventional culture systems.

Functional Assessment of Transporters

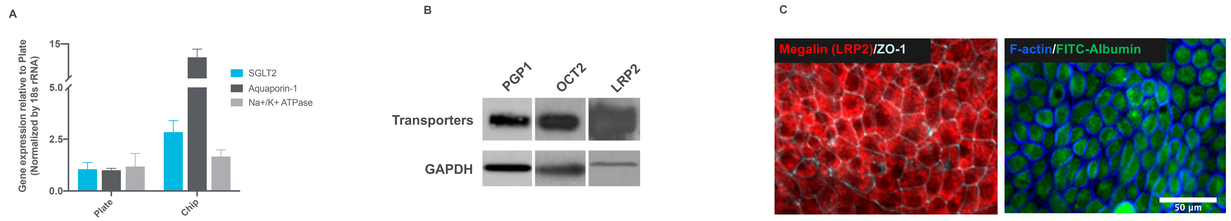


Figure 3. (**A**) Relative gene expression of key functional transporters, including SGLT2, AQP1, and Na^+/K^+-ATPase was measured in the Proximal Tubule Kidney-Chip compared to conventional plate culture. (**B**) Western blot analysis confirmed protein expression of uptake and efflux transporters such as PGP1 (ABCB1), OCT2 (SLC22A2), and Megalin (LRP2). (**C**) The Proximal Tubule Kidney-Chip stained positive for Megalin protein, further confirming both the expression and translation of this important transporter. Interestingly, Megalin expression seems to corelate with uptake of FITC-labeled human albumin, demonstrating the functional capability of the Proximal tubule Kidney-Chip.

Cisplatin-Induced Toxicity in Proximal Tubule Kidney-Chip

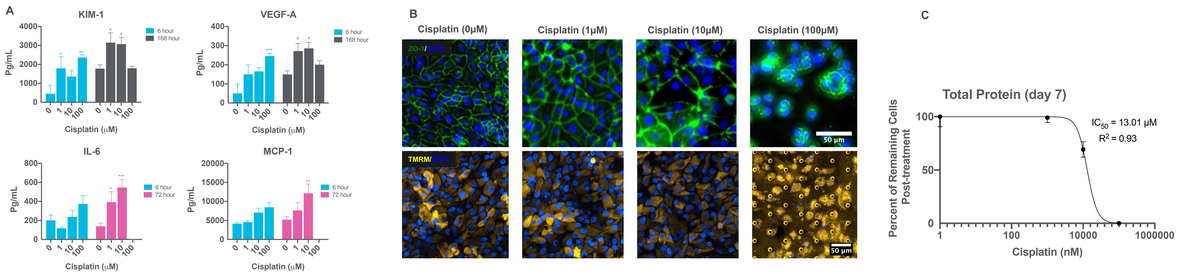


Figure 5. Proximal tubule Kidney-Chips were exposed for 24 hours to various cisplatin concentrations, over a 100-fold range. (**A**) Marked increase in kidney injury markers (KIM-1 and VEGF-A), and proinflammatory cytokines (IL-6 and MCP-1) was observed within 6 hours of cisplatin exposure. The observed increase in biomarkers continued 72 hours and 168 hours post cisplatin exposure for the inflammatory cytokines and the kidney injury markers, respectively. (**B**) We observed a concentration dependent loss of cellular cytoarchitecture as indicated by increased cell surface area and cell detachment as well as decreased mitochondrial function based on ZO-1 and TMRM immunostaining respectively, 7 days after exposure to cisplatin. (**C**) Analysis of cell viability by total protein quantification confirmed a concentration-dependent response with a calculated IC_{50} equal to 13.01µM. In line with this finding, the *in vivo* reported C_{max} of 20µM for cisplatin is known to result in acute kidney injury (AKI) in 20 to 30% of patients. (Statistical analysis: 2-way ANOVA followed by Dunnett's multiple comparisons test, significant at $p \leq 0.05$).

Gentamicin-Induced Toxicity in Proximal Tubule Kidney-Chip

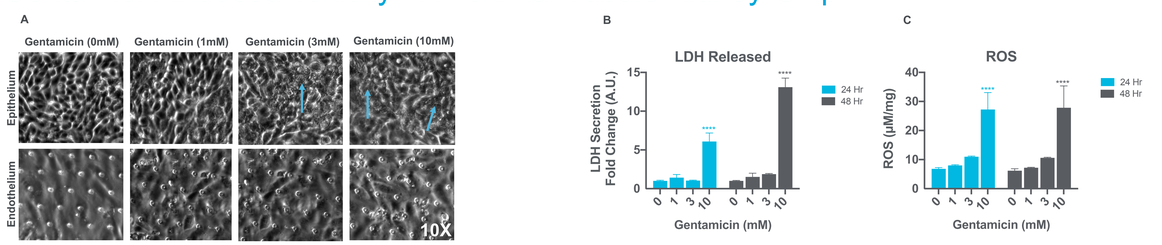


Figure 6. (**A**) Morphological analysis by bright field microscopy of the proximal tubular epithelium revealed significant cell damage with increased gentamicin concentration after 48 hours of treatment. (**B**) LDH release significantly increased at the highest gentamicin concentration (10mM) while (**C**) Radical Oxygen Species (ROS) activity was increased with increasing gentamicin concentration. (Statistical analysis: 2-way ANOVA followed by Dunnett's multiple comparisons test, significant at p ≤ 0.05).

Conclusion

This Proximal Tubule Kidney-Chip recreates an *in vivo* relevant microenvironment of the kidney proximal tubule. Fluorescent microscopy analysis of the cells in the chip demonstrated the formation of a polarized epithelium and an intact endothelium that reproduced *in vitro*-like baseline functions of the proximal tubule. Gene expression and western blot analyses coupled with fluorescent microscopy established expression and function of transporters that are very important for normal kidney functions. The chip replicates drug-induced cellular injuries at clinically relevant concentration of both cisplatin and gentamicin, promising a more physiologically relevant system for drug safety assessment.