Introduction

- The kidney proximal tubule (PT) is a primary site of nephrotoxicity and resulting drug attrition in the development pipeline.
- Using Organovo’s proprietary bioprinting platform, we have developed a fully-human in vitro model of the PT to potentially enable more accurate prediction of tissue-level clinical outcomes.
- ExVive™ Human Kidney Tissue is created in a standard 24-well Transwell® plate by spatially-controlled deposition of cell aggregates in the absence of exogenous scaffold. Tissues are composed of an interstitial layer of renal fibroblasts and endothelial cells supporting a monolayer of human primary PT epithelial cells.

Renal Transporter Expression and Function

Table 1 and Figure 4: ExVive Human Kidney Tissue retains renal transporter expression over time in culture. Table 1. Tissues were measured for expression of renal transporters by quantitative RT-PCR over time in culture. Data shown is the fold change of relative quantity (RQ) normalized to cyclophilin A (CycA) and GAPDH versus Day 1. BQ, below limit of quantitation. A and B. Tissues cultured for 14 days were stained for P-gp or SGLT2 and localization to the apical membrane of the PT epithelium in 3D bioprinted tissues.

Cisplatin-induced nephrotoxicity

Figure 7: Schematic of mechanisms of cisplatin nephrotoxicity. Cisplatin can be actively transported into the proximal tubule epithelium by the uptake transporters CTnT and OCT2, where it can become concentrated in the epithelium and available for biotransformation. Reactive intermediates can induce intracellular damage through generation of reactive oxygen species. Cisplatin or its detoxified glutathione conjugate can be effused from the epithelium by the actions of MATE1 and MRP2.

Figure 8: Toxic response of ExVive Human Kidney Tissue to cisplatin is rescued by the OCT2 inhibitor cimetine. A. Tissues were dosed daily for 7 days and assessed for overall tissue metabolic activity by alamarBlue® assay. B. Epithelial function was measured by GGT activity. C and D, HE&E shows flattening of epithelial cells in response to Cisplatin, rescued in part by Cimetidine co-treatment. PCNA (green) shows proliferation of epithelial cells in response to damage (white arrows).

Figure 9: Release of neutrophil gelatinase-associated lipocalin (NGAL) or clustatin into media as biomarkers of kidney injury following 7 days of Cisplatin treatment (Cs).

Summary

- ExVive Human Kidney Tissue recapitulates key aspects of the physiology of the PT for at least 30 days in culture, including GGT activity, barrier function, and expression and function of renal transporters.
- The role of OCT2-mediated uptake and concentration of the nephrotoxin cisplatin in PT epithelial cells was confirmed in the 3D tissues histologically and biochemically.
- Together, these results support the use of this novel human 3D tissue model of the PT for assessment of human renal toxicity over extended time in culture.