A 3D Bioprinted Model of the Renal Proximal Tubule for Evaluation of Drug-Induced Nephrotoxicity

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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.

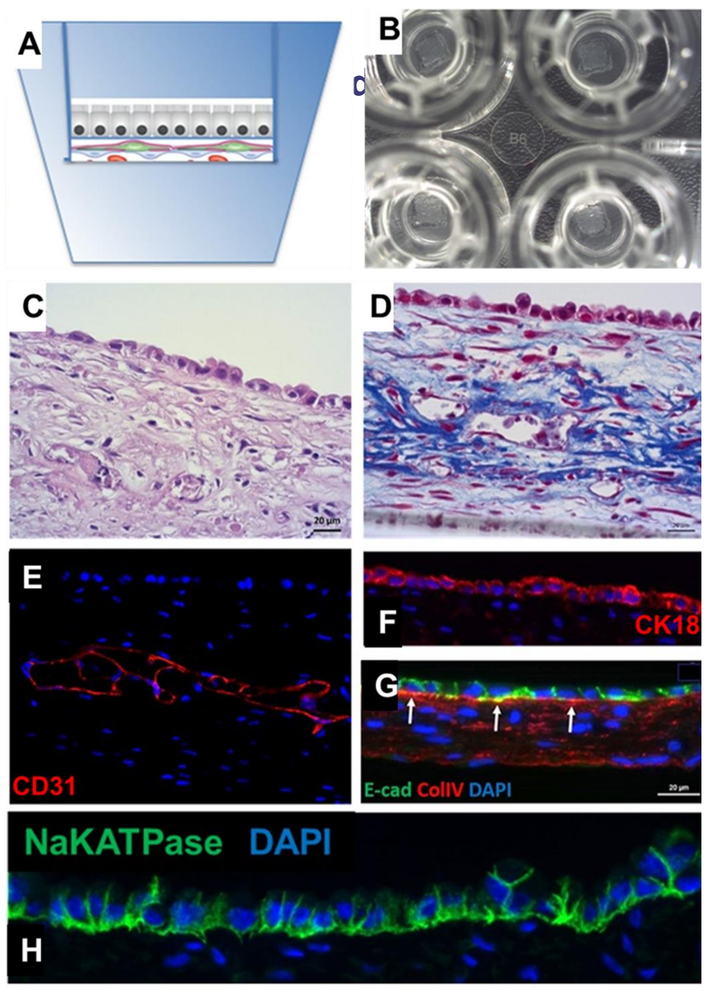


Figure 1: Characterization of ExVive Human Kidney Tissue. **A**, Schematic of 3D PT tissues comprising an interstitial layer of renal fibroblasts and endothelial cells supporting a polarized epithelial monolayer in standard Transwell® format. **B**, Bioprinted tissues are highly reproducible from well to well. **C**, H&E. **D**, Masson's trichrome stain for collagen (blue). **E**, CD31 staining for endothelial cell networks. **F**, Cytokeratin 18 staining for epithelial cells. **G**, E-cadherin staining (green) for junctions between epithelial cells and collagen IV staining (red) for basement membrane (arrows). **H**, Basolateral localization of Na^+K^+ATPase.

A

Culture Type	R (Ω*cm^2)
MDCK or LLC-PK1 monolayer	100-200 [1]
Rat or dog PT in vivo	6.6-11.6 [2,3]
3D bioprinted tissue	12-18

[1]Liang et al. Kidney Int 1999.
[2]Boulpaep EL et al. Am J Physiol 1971.
[3]Lutz MD et al. Am J Physiol 1973.

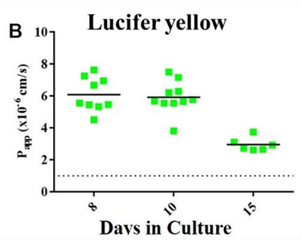


Figure 2: ExVive Human Kidney Tissue exhibits barrier function consistent with observed values for the *in vivo* PT. **A,** TEER values for 3D bioprinted kidney tissues measured in an Ussing chamber and compared to historical values. **B,** Passive permeability measurements following directional transport of Lucifer yellow. Dotted line indicates a P_{app} measurement of $1x10^{-6}$ cm/s, as observed for monolayer cultures.

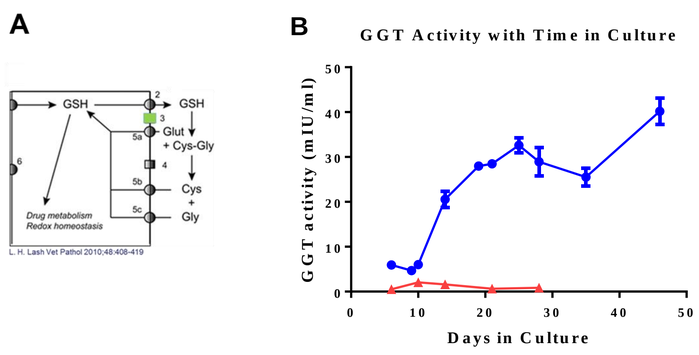


Figure 3: ExVive Human Kidney Tissue demonstrates gamma glutamyl transferase (GGT) activity for at least 30 days in culture. **A,** Schematic of the role of GGT activity at the apical membrane of PT epithelial cells for glutathione homeostasis and xenobiotic detoxification. **B**, GGT activity measured over time for 3D bioprinted tissues (blue) or 3D bioprinted interstitial tissues lacking epithelium (red).

Renal Transporter Expression and Function

Target Name	Day 12	Day 18	Day 24	Day 30
ABCB1 (MDR1, P-gp)	6.8	23.0	15.2	28.0
ABCG2 (BCRP)	0.2	1.6	0.3	0.1
AQP1	0.9	1.0	0.9	1.5
CUBN	17.0	23.8	17.9	33.5
LRP2 (megalin)	0.8	2.5	1.1	0.7
SLC22A2 (OCT2)	1.8	2.8	0.9	1.8
SLC22A6 (OAT1)	BLQ	BLQ	BLQ	BLQ
SLC22A8 (OAT3)	BLQ	BLQ	BLQ	BLQ
SLC47A1 (MATE1)	0.9	1.3	0.5	0.4
SLC47A2 (MATE2K)	6.0	2.7	2.9	6.2
SLC5A2 (SGLT2)	BLQ	0.7	2.3	3.4

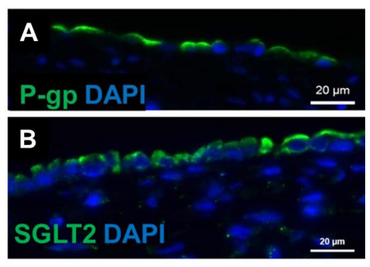


Table 1 and Figure 4: ExVive Human Kidney Tissue retains renal transporter expression over time in culture. **Table 1**, Tissues were assessed for expression of renal transporters by quantitative RT-PCR over time in culture. Data shown is the fold change of relative quantity (RQ; normalized to cytokeratin 18 (CK18) and GAPDH) versus Day 3. BLQ, below limit of quantitation. **A and B**, Tissues cultured for 14 days were stained for P-gp or SGLT2 localization to the apical membrane of the PT epithelium in 3D bioprinted tissues.

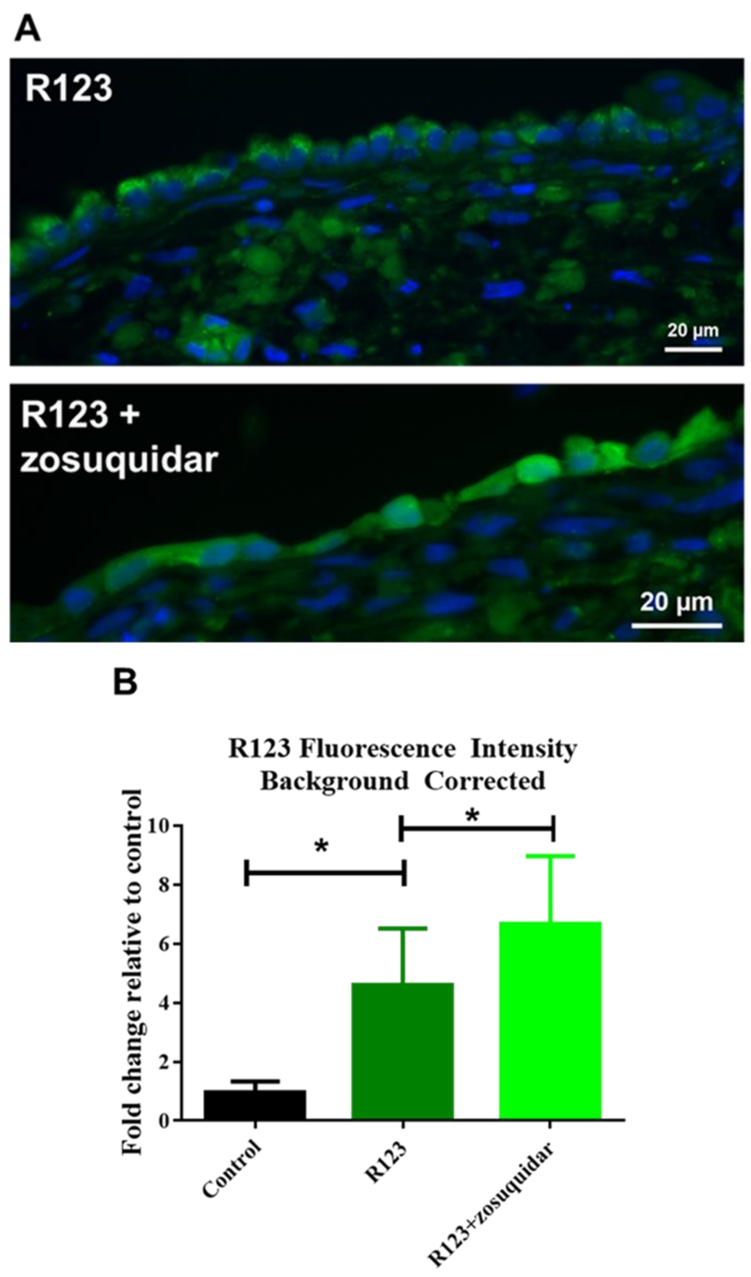
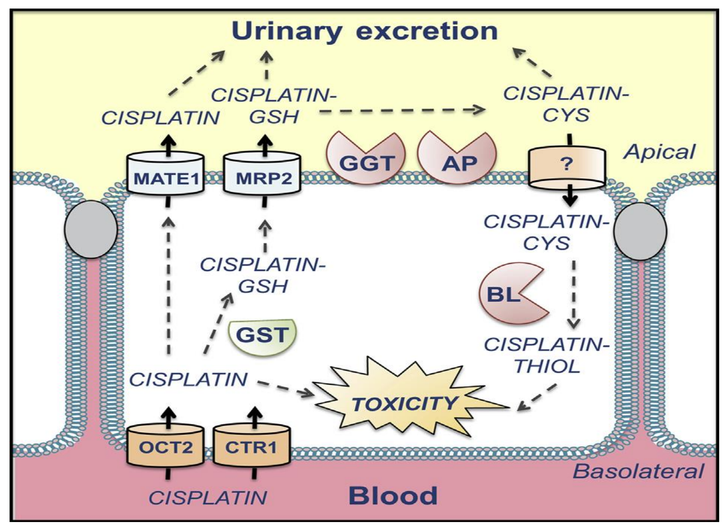


Figure 5: ExVive Human Kidney Tissue demonstrates retention of rhodamine 123 following inhibition of P-gp-mediated efflux. **A,** Tissues were loaded with the fluorescent substrate rhodamine 123 (R123; 1 µM) in the presence or absence of the P-gp inhibitor zosuquidar (5 µM). Images were taken at the same exposure time for comparison. **B,** Fluorescence intensity of individual epithelial cells was quantified in Image J following area and background correction. * indicates $P<0.05$ between groups.

Figure 6: ExVive Human Kidney Tissue demonstrates SGLT2-mediated uptake of the glucose analog 2-deoxyglucose (2-DG). **A,** Schematic showing localization and function of the Na^+/Glucose co-transporter at the apical surface of PT epithelial cells. **B,** 3D bioprinted tissues were starved overnight prior to stimulation with 1 µM insulin or 1 µM insulin plus the SGLT2 inhibitor canagliflozin (500 µM). Tissues were then loaded with 2-DG, homogenized, and assessed for 2-DG retention by colorimetric assay. * indicated $P<0.05$ between groups.

Cisplatin-induced nephrotoxicity



Wen et al., Am J Path 2014: 184 (5).

Figure 7: Schematic of mechanisms of cisplatin nephrotoxicity. Cisplatin can be actively transported into the proximal tubule epithelium by the uptake transporters CTR1 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 effluxed from the epithelium by the actions of MATE1 and MRP2.

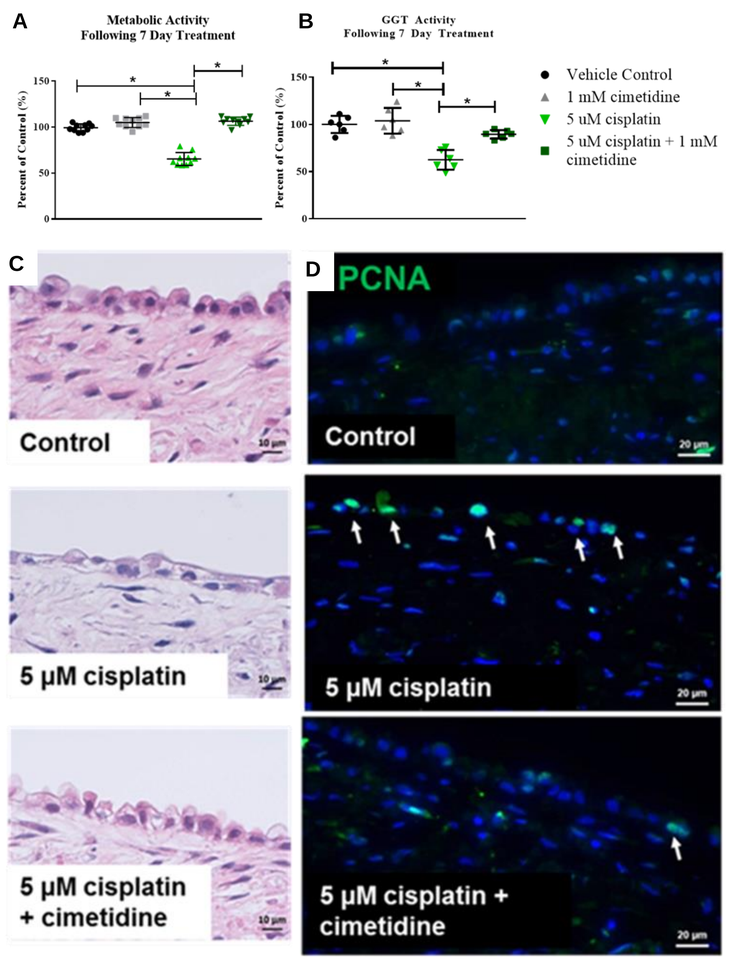


Figure 8: Toxic response of ExVive Human Kidney Tissue to cisplatin is rescued by the OCT2 inhibitor cimetidine. **A,** Tissues were dosed daily for 7 days and assessed for overall tissue metabolic activity by alamarBlue® assay. **B,** Epithelial function was measured by GGT activity. **C and D**, H&E 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).

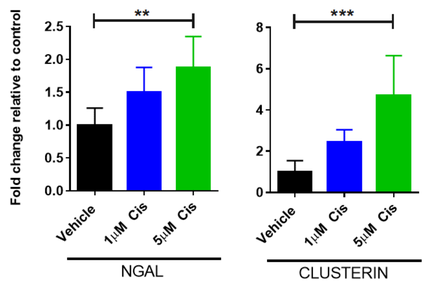


Figure 9: Release of neutrophil gelatinase-associated lipocalin (NGAL) or clusterin into media as biomarkers of kidney injury following 7 days of Cisplatin treatment (Cis).

Summary

- ExVive Human Kidney Tissue recapitulated 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.