Design and characterization of a multicellular, three-dimensional (3D) tissue model of the human kidney proximal tubule

Abstract #9212

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ABSTRACT

Renal toxicity is a major cause of drug attrition at the clinical trial stage, and the primary site of this toxicity is within the proximal tubule. Conventional renal cell culture models lack the complexity of native tissue and thus have a limited capacity for predicting tissue-level responses. In addition, the predictive potential of pre-clinical animal trials is limited due to species-specific differences between human and animal renal functions, including differential sensitivity to insults. We designed and fabricated a human three-dimensional (3D) tissue model of the tubulointerstitial interface in which human renal interstitial tissue is supporting proximal tubule epithelial cells to facilitate their optimal morphology and function. Histological characterization demonstrated that the interstitial layer is viable and well organized, containing well-developed CD31+ endothelial cell networks. Method optimization resulted in the formation of a polarized layer of renal epithelium on top of the interstitial layer, and formation of a basement membrane between the layers. Gene expression analysis showed that the renal tissues expressed key enzymes involved in metabolism and protein processing (CYPs, renin-angiotensin system), suggesting that physiologic function is retained. These bioprinted human tissues may provide an opportunity to accurately study how compounds affect the renal proximal tubule as well as modeling pathogenic processes that involve tubular transport, cell-cell interactions, and the development of tubulointerstitial fibrosis.

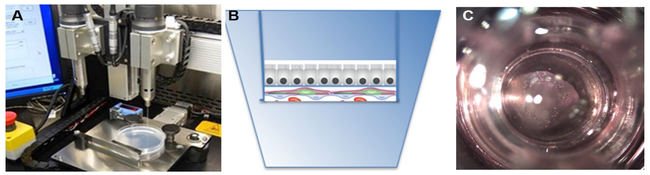

Figure 1. The Novogen MMX™ Bioprinter (A) deposits multiple types of cell aggregates in spatially-defined patterns. Following bioprinting, the cells secrete extracellular matrix that maintains relevant tissue microarchitecture in the absence of exogenous scaffold material. B, Schematic of 3D renal tissues comprising an interstitial layer of renal fibroblasts and endothelial cells supporting a polarized epithelial monolayer in standard transwell format. C, 3D Renal tissues 6 days after printing.

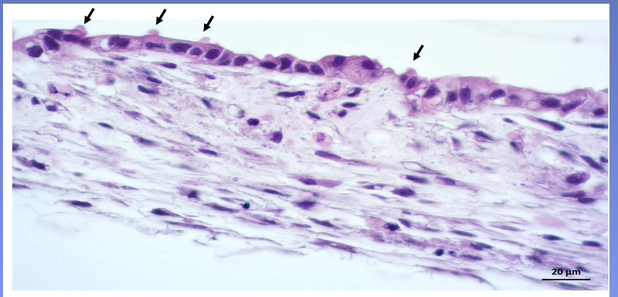

Figure 2. Hematoxylin and eosin staining of 3D renal proximal tubule tissues. The interstitial layer is approximately 100 um thick and is composed of renal fibroblasts and endothelial cells. Adjacent to the interstitium is a monolayer of renal proximal tubule epithelial cells (RPTEC). Putative brush borders are observed on the apical surface of some epithelial cells (arrows).

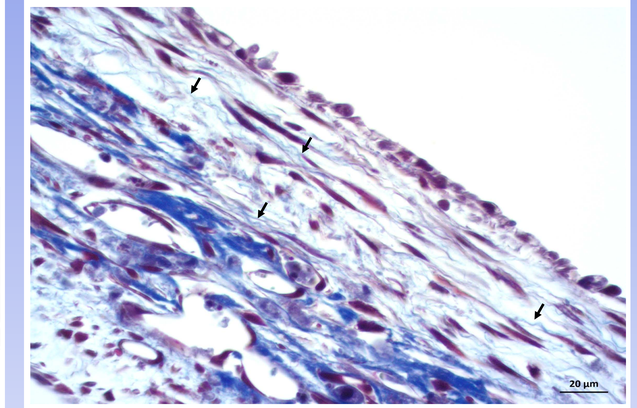

Figure 3. Masson's trichrome stain of 3D renal proximal tubule tissues. Abundant collagen deposition is evidenced by blue staining in the interstitial layer. Aligned fibrils (arrows) can be observed supporting extensive endothelial cell networks.

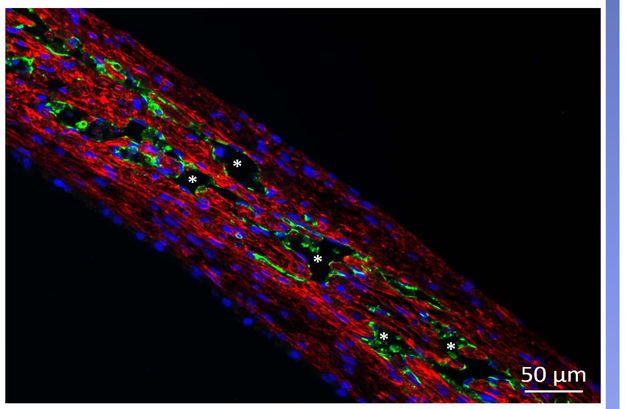

Figure 4. Extensive endothelial cell networks are observed in 3D renal proximal tubule tissues. Tissues were stained with antibodies against CD31 (green, endothelial cells) and TE7 (red, fibroblasts). Networks with putative lumens lined with endothelial cells are marked (*****).

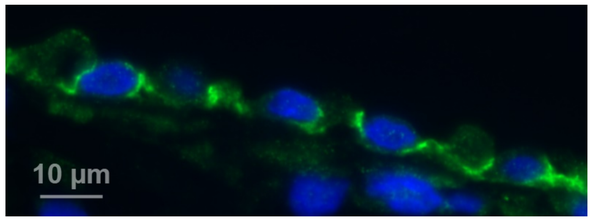

Figure 5. Renal proximal tubule epithelial cells in 3D renal tissues exhibit features of polarization. Tissues were stained with antibodies against E-cadherin (green). E-cadherin is observed at the lateral membranes between adjacent RPTEC cells, corresponding to localization at tight junctions.

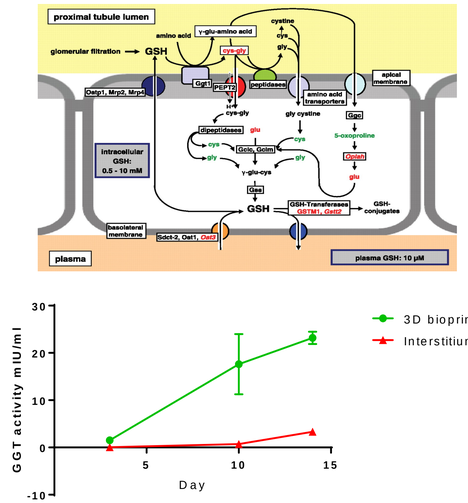

Figure 6. γ-glutamyl-transferase (GGT) activity over time in 3D renal tissues or tissues composed of interstitial cells alone. GGT is expressed on the apical surface of RPTEC cells and is involved in glutathione homeostasis and xenobiotic metabolism. GGT activity increases over time in culture for 3D renal tissues as assessed by a colorimetric assay. Schematic of pathway from IM Frey et al., Physiological Genomics 2007 28(3): 301-310.

CONCLUSIONS

- Morphologic and functional aspects of the human proximal tubule are recapitulated in the 3D renal tissues
  - Direct interface between tubular epithelium and renal interstitial cells
  - Polarized renal epithelium
  - Microvascular structures within the interstitial tissue
  - Stable / increasing tubular function (GGT activity) over a 2 week period
- This model may be useful in the preclinical assessment of human nephrotoxicity and drug-induced fibrosis