3D Primary Human Kidney Tissue Model for Nephrotoxicity

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Abstract ID # 4653 / Poster# 539

Abstract

Background: Currently renal toxicity accounts for only 2% of drug failure during the preclinical phase but nearly 20% of drug withdrawal is associated with kidney damage during human clinical trials. This discrepancy emphasizes the need for a more physiological and functional alternative assay system that can predict and recapitulate drug induced kidney injury/toxicity in humans. The proximal tubular (PT) region is the most common site for compound and nutrient reabsorption and is highly susceptible to drug and toxin damage. PT region is responsible for essential kidney functions, including reabsorption of low molecular weight proteins, solutes, and glucose; secretion of acids; and clearance of administered medications. The goal of this study is to develop a novel physiologically relevant and functional primary human kidney-cell-based three-dimensional (3D) organotypic tissue model that can accurately predict drug induced human nephrotoxicity.

Methods: Human primary proximal tubular epithelial cells (PTEC) were isolated and expanded in a monolayer culture prior to seeding onto microporous membrane inserts to reconstruct a 3D organotypic tissue model. 3D tissues were analyzed by histology, barrier integrity (transepithelial electrical resistance, TEER), immunostaining, and qPCR on days 9-23. Receptor mediated FITC-albumin uptake and transpeptidase hydrolytic activity of glutamyl transpeptidase (GGT1) and leucine aminopeptidase (LAP) were assayed on days 9-16.

Results: The PTEC organotypic tissues organize into characteristic tubular structures, develop a barrier with mean TEER values of 169±33.4 Ω*cm^2 between days 9 to 23 of the culture period. Histological features show epithelial layer with tubular structure. Immunohistochemical analysis of the 3D kidney model stained positive for tight junction proteins ZO-1, and clauidn-1. At a gene level, the polarized organotypic tissues express brush border proteins megalin, cubilin, and villin, together with water channel AQP1 and GGT1 on the apical side and sodium-potassium ATPase pump on the basolateral side.Transmission electron microscopy confirms brush border formation. Real-time qPCR also confirmed that tissues express a panel of PTEC-specific markers that are necessary for renal clearance, secretion, and reabsorption: aminopeptidase CD13, p-glycoprotein (PgP; MDR1), multidrug resistance proteins MRP 1, 3, 4, and 5), CYP450 enzymes, glucose transporters SGLT1/2, multidrug and toxin extrusion transporter MATE1, organic cation and anion transporters OCT1/2, OCTN1/2, and OATP4C1, urate transporter URAT1, and sodium phosphate co-transporter NP2. Specific concentration and time dependent receptor mediated uptake of FITC-albumin by the PTEC tissues was observed by fluorescent microscopy. Kidney uptake of albumin was inhibited by addition of BSA (competitive binding utilizing a common receptor for albumin) or the drug chlorpromazine, an inhibitor of the calthrin-dependent endocytosis. Hydrolytic activity was monitored by the conversion of γ-Glutamyl-p-nitroanilide (GPNA) and L-leucine-p-nitroanilide (LLNA), substrates for GGT1 and LAP using spectrophotometric assays of p-nitroaniline (PNA) following 30 min incubation. Specific transpeptidase hydrolytic activity was inhibited in the presence of an irreversible inhibitor acivicin (2mM) by 88.8% (GPNA) and 35.0% (LLNA). Glucose uptake was also enhanced by the addition of sodium chloride. Furthermore, treatment of the 3D kidney model with Cisplatin, a known nephrotoxin that causes acute and chronic kidney injury, shows compromise in PTEC barrier integrity and reduced viability of tissues in a time and concentration dependent manner.

Conclusions: The reconstructed *in vitro* 3D PTEC organotypic tissue is physiological in terms of structure, barrier properties, gene expression, and tissue functionality mimicking the in vivo human PT region. This model is anticipated to be a useful tool to evaluate human nephrotoxicity and to perform mechanistic studies that can improve the predictivity of human responses to pharmacological compounds. This model will help establish confidence in modeling drug induced kidney damage/injury and reduce animal use for experimentation.

Methods

Histology: Tissues were fixed in 10% formalin (overnight, RT), paraffin embedded, sectioned using a microtome, and stained with H&E.

Ultrastructural features: Transmission electron microscopy was used to examine microvilli/brush border in the 3D renal proximal tubule epithelial tissue model.

Immunohistochemistry: Immuno-staining was performed following de-paraffinization of tissue sections and antigen retrieval or on tissued fixed with 10% formalin. For 10 min.

Tissue barrier integrity: Barrier function of the EpiKidney tissue model was monitored using transepithelial electrical resistance (TEER) measurements. TEER measurements were made using an EVOM voltohmmeter equipped with an Endohm electrode chamber (World Precision Instruments, Sarasota, FL). TEER values (reported in Ohm * cm^2) were calculated by multiplying raw resistance measurements by the surface area of the tissue (0.6 cm^2). Tissues with TEER values of >100 Ohm * cm^2 were used in the various experiments.

FITC-albumin uptake: EpiKidney tissues were pre-incubated in DMEM/F12 medium for 2h followed by application of 300 µg/mL of FITC-Albumin conjugate (#A9771, Sigma) in the presence or absence of 10 mg/mL of BSA and chlorpromazine (CPZ, inhibitor of clathrin-dependent endocytosis) for 2h. FITC uptake was analyzed by fluorescence spectroscopy at excitation/emission 488/516 nm) of Tx-100 lysed tissues.

Glucose uptake: EpiKidney tissues were pre-incubated in a glucose-free buffer for 3h followed by 1h incubation in the presence of 1mM of 2-deoxyglucose (2-DG) in a buffer with or without of NaCl (137mM). 2-DG uptake was analyzed by a chromogenic assay following manufacturer's instructions by absorbance at 420nm (#CSR-OKP-PMG-K01TE, Cosmo Bio Co Ltd, Tokyo, Japan).

Hydrolase activity: L-γ-Glutamyl-p-nitroanilide (GPNA) and L-leucine-p-nitroanilide (LLNA) were used to determine γ-glutamyl transpeptidase (GGT1) and leucine aminopeptidase (LAP) hydrolytic activity via spectrophotometric monitoring of p-nitroaniline (PNA). EpiKidney tissues were incubated with 2.5 mM of GPNA or 3 mM of LLNA in the presence or absence of 2mM Acivicin for up to 30 min. The reaction was stopped, and hydrolytic activity was determined by absorbance at 405nm.

A: Topical Exposure; Cell Culture Insert; Outer Wall; Tissue; Culture Medium; Microporous Membrane

B: TEER (Ohms*cm²) vs. Days in Culture

Figure 1: A. Schematic of the EpiKidney tissue model culture (A) and Transepithelial Electrical Resistance (TEER) measurement of the EpiKidney tissues at different time points in culture (AVG±SD of 12 lots of tissues).

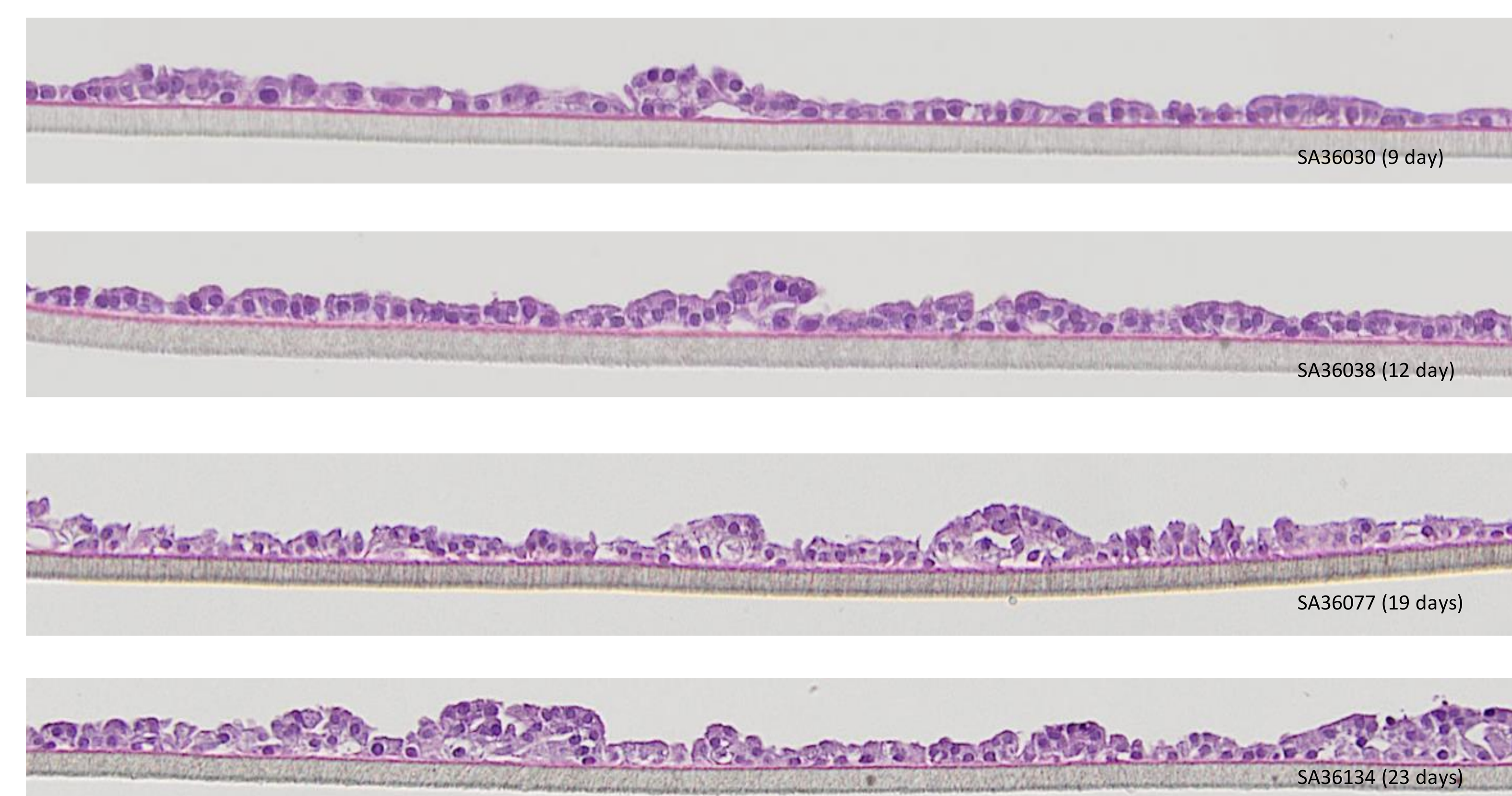
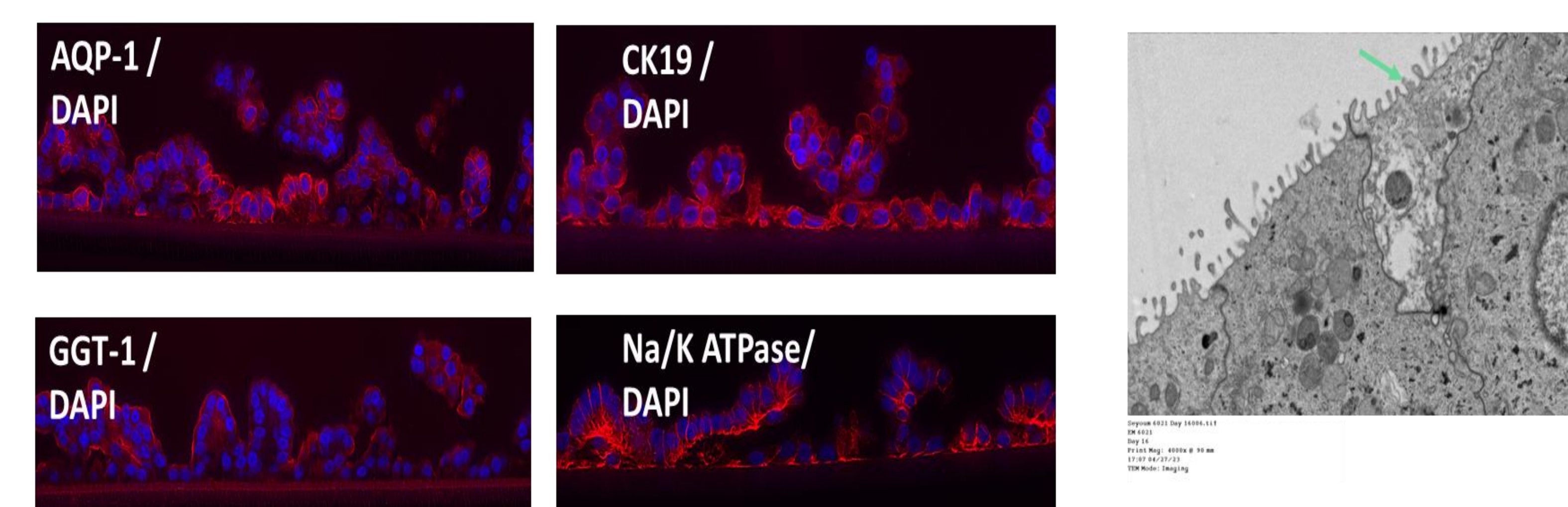


Figure 2: A. H&E-stained histological cross-sections of EpiKidney tissue model at different time in culture.



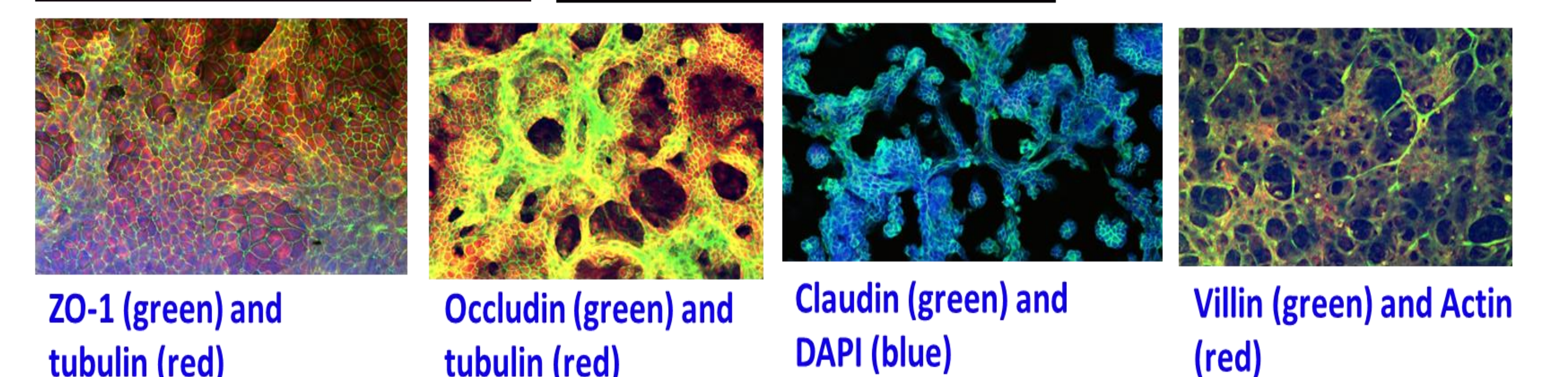


Figure 3: Expression of tight junction and brush border proteins (topical view) by EpiKidney tissues (day 14). Immunohistochemical staining of whole tissues; imaged with ECHO microscope Transmission Electron Microscopy (TEM) also showed brush border formation (Arrow)

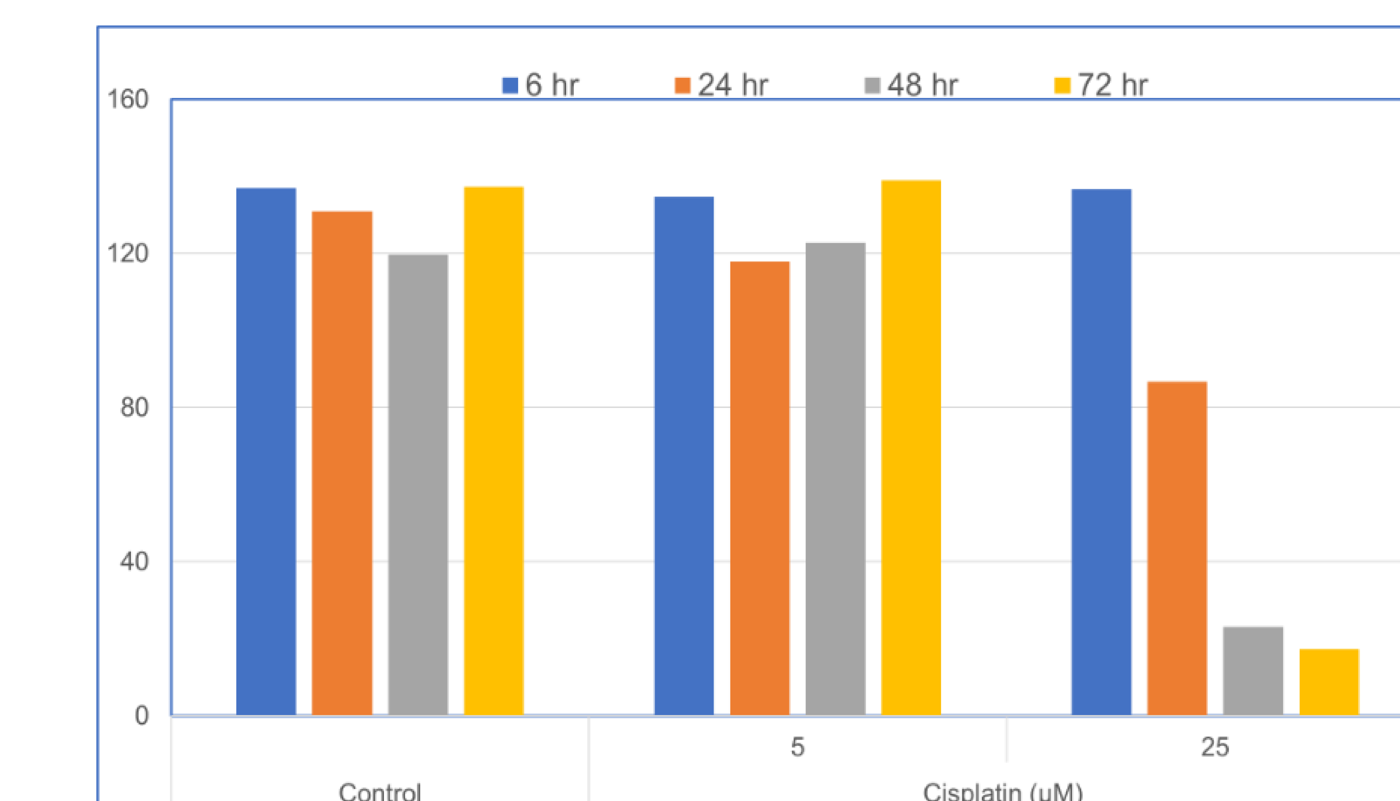


Figure 4: Barrier integrity as an indicator of cytotoxicity. Cisplatin, known to cause kidney tubular cell death and tissue damage, causes a decrease in tissue barrier integrity in a concentration and time dependent manner.

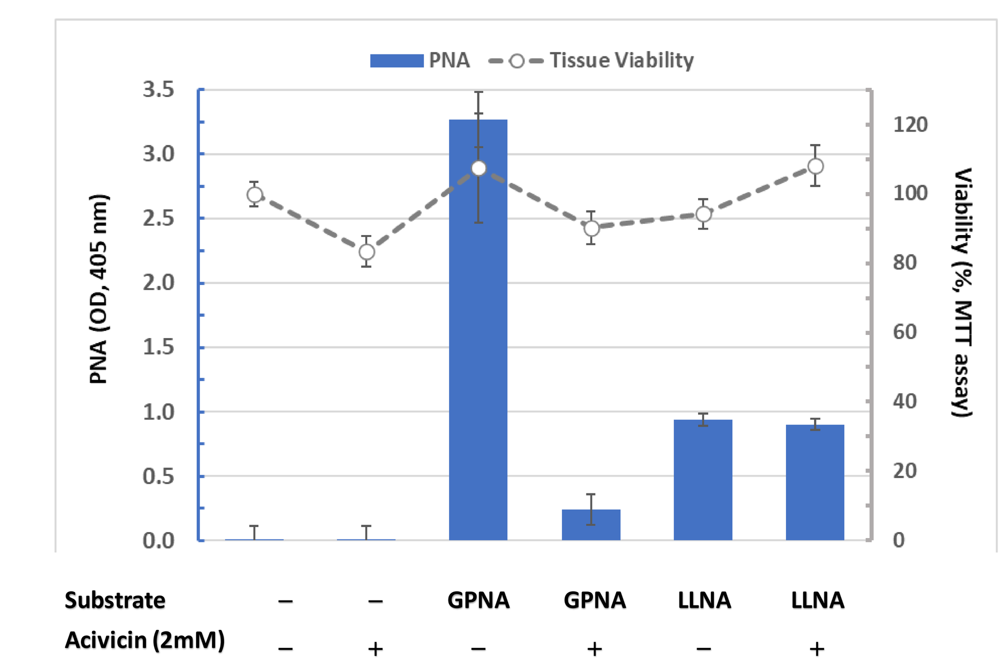


Figure 5 : Transpeptidase hydrolytic activity of gamma-glutamyl transpeptidase (GGT-1) and leucine aminopeptidase (LAP) of the EpiKidney tissues was assayed on days 14 to 21. L-γ-Glutamyl-p-nitroanilide (GPNA) and L-leucine-p-nitroanilide (LLNA) were converted to PNA by GGT-1 and LAP enzymes. **A.** GGT-1 activity was inhibited by acivicin, a specific inhibitor of gamma-glutamyl transferase; LAP activity was not inhibited. **B.**

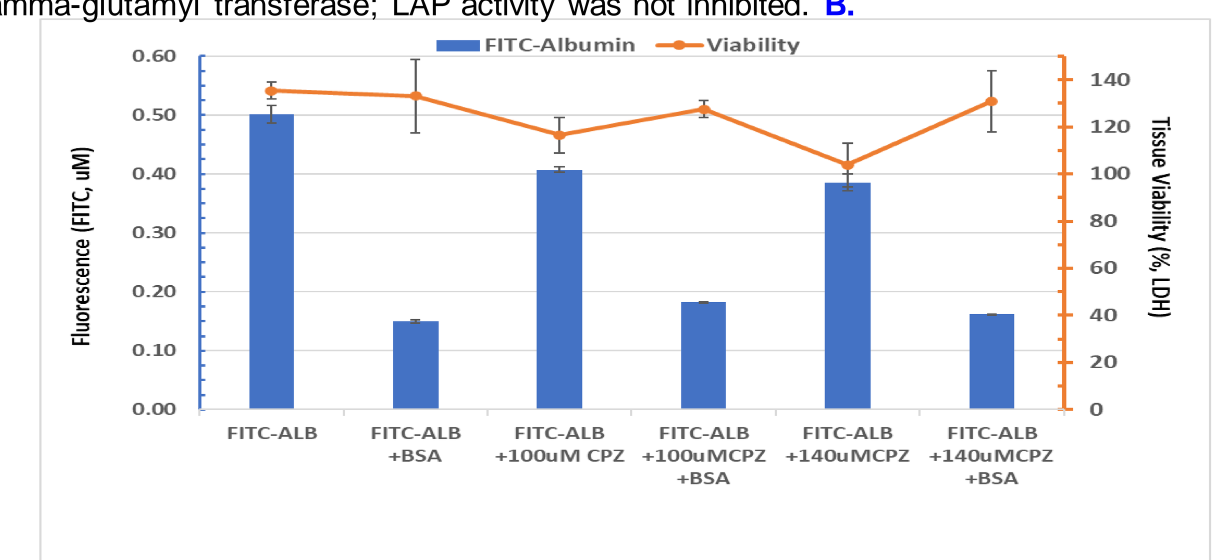


Figure 6: Receptor-mediated FITC-Albumin uptake. Showing albumin absorption and the receptor-mediated saturation in the presence/absence of 10 mM of BSA or chlorpromazine (CPZ, inhibitor of clathrin-dependent endocytosis). FITC uptake was analyzed by fluorescence spectroscopy at excitation/emission 488/516 nm) of Tx-100 lysed tissues.

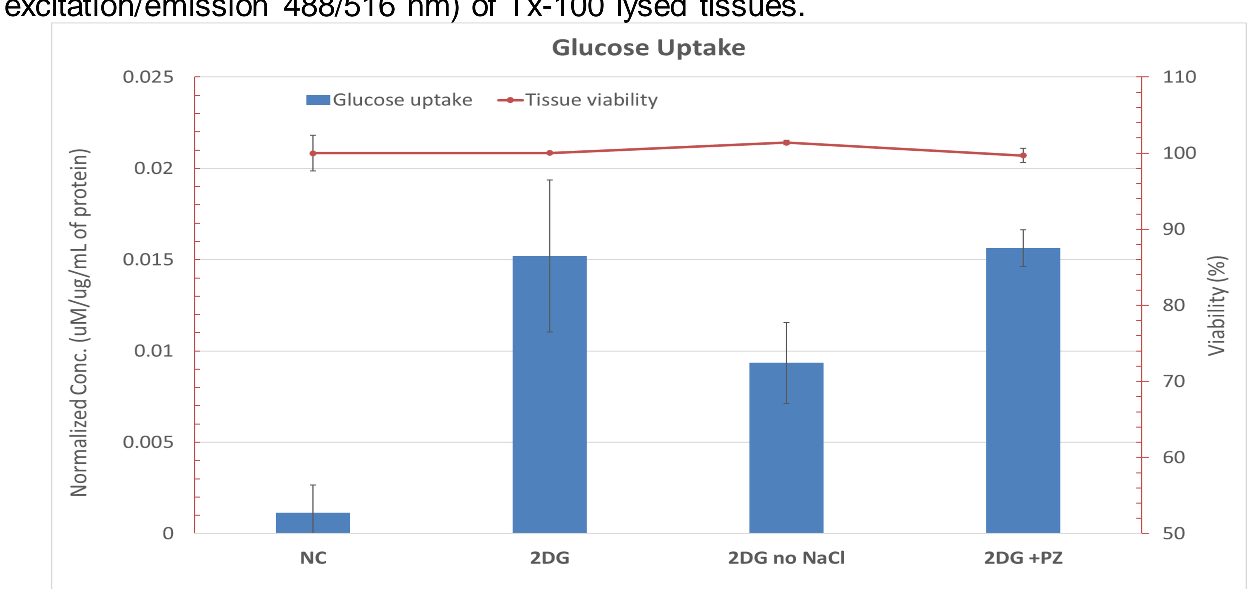


Figure 7: Glucose uptake by EpiKidney tissues. 2-Deoxyglucose (2-DG) uptake was determined on day 19. Data presented as averages of two independent experiments with n=3 tissues per condition. The sodium-glucose symport transport was inhibited in the absence of Na+ ions

Summary

1. EpiKidney tissues:
   - Resemble in vivo proximal tubular epithelium, which is polarized, tubular, and convoluted (**Figs. 2 & 3**).
   - Express tight junction proteins (ZO-1, claudin, occludin) and brush border proteins (villin), GGT-1) on the apical surface (**Fig. 3**).
   - Express water channel AQP-1 and villin on the apical side and Na-K channel on the basolateral side (**Fig 3**).
   - Transmission electron microscopy imaging shows the formation of brush border (Fig 3)
2. EpiKidney tissues exposed to the known nephrotoxin chemotherapeutic drug Cisplatin showed drug-induced cytotoxicity in a dose and time dependent manner (**Fig 4**).
3. EpiKidney tissues performed PT-specific functions:
   - Transpeptidase hydrolysis by γ-glutamyl transpeptidase (GGT1) and leucine aminopeptidase (LAP): enzyme-specific inhibition (**Figure 5**).
   - Albumin absorption: receptor-mediated endocytosis was saturable (**Figure 6**).
   - Glucose uptake: inhibition in the absence of sodium ions (**Figure 7**).
4. EpiKidney model is anticipated to be a valuable tool to improve the predictivity of human responses to pharmacological drug candidates, to study human nephrotoxicity and its mechanisms, and reduce animal usage in pre-clinical drug screening.