3D Primary Human Kidney Tissue Model For Nephrotoxicity

MATTEK
A BICO COMPANY

Abstract ID: 886
Poster#: P02-35

J. Finelli[1], Y. Kaluzhny[1], Z. Stevens[1], M. Klausner[1], A. Armento[1], S. Ayehunie[1], V. Karetsky[1]

[1]MatTek Corporation, Ashland, MA, USA

Abstract

Approximately 20% of drug failures in human clinical trials are associated with kidney damage. The proximal tubular (PT) region is the most common site for compound reabsorption and is highly susceptible to drug and toxin damage. The goal of this study is to develop a novel, physiologically relevant and functional three-dimensional (3D) organotypic tissue model that can accurately predict drug-induced nephrotoxicity. Human primary proximal tubular epithelial cells (PTEC) were isolated and expanded in a monolayer culture prior to seeding onto microporous membrane inserts. The reconstructed PTEC 3D tissues were analyzed by histology, TEER measurements, immunostaining, and qPCR. Also, receptor mediated FITC-albumin uptake and transpeptidase hydrolytic activity of glutamyl transpeptidase (GGT1) and leucine aminopeptidase (LAP) were assayed. The organotypic PTEC tissues organize into characteristic tubular structures, develop a barrier with mean TEER values of 169±33.4 Ω×cm^2 and stain positive for the tight junction proteins, ZO-1 and Claudin-1. Gene expression analysis shows brush border proteins together with water channel AQP1 and GGT1 on the apical side and sodium-potassium ATPase pump on the basolateral side. Real-time qPCR confirmed expression of PTEC-specific markers necessary for renal clearance, secretion, and reabsorption including aminopeptidase CD13, p-glycoprotein (PgP; MDR1), multidrug resistance proteins MRP1, 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. Concentration and time dependent receptor mediated uptake of FITC-albumin was observed by fluorescent microscopy. 3 Albumin uptake was inhibited by the addition of BSA (competitive binding utilizing a common receptor for albumin) or the drug chlorpromazine, an inhibitor of calthrindependent 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). Specific transpeptidase hydrolytic activity was inhibited by 88.8% (GPNA) and 35.0% (LLNA) in the presence of an irreversible inhibitor acivicin (1.2mM). Glucose uptake was enhanced by the addition of sodium chloride. Treatment of the 3D kidney model with Cisplatin, a known nephrotoxin, causes reduced TEER and reduced viability in a time and concentration dependent manner. Thus, the reconstructed 3D PTEC organotypic tissue is physiological in terms of structure, barrier properties, gene expression, and functionality mimicking the in vivo human PT region. This model will help establish confidence in modeling drug-induced kidney damage/injury and reduce animal use for experimentation.

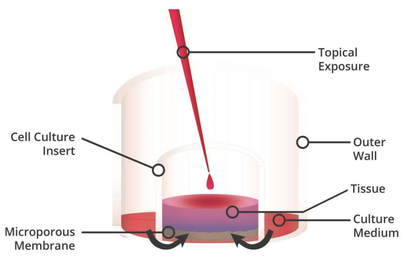
Methods

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 for 2h. FITC uptake was analyzed by confocal microscopy of the 3D tissues or the fluorescence spectroscopy (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.

Histology & Immuno-staining: Tissues were characterized by polarity of the renal proximal tubule epithelial cells (histology and immunohistochemistry). Reconstructed kidney tissue cultures were fixed in 10% formalin (overnight, room temperature), paraffin embedded, sectioned using a microtome, and stained with hematoxylin and eosin (H&E) according to standard procedures (Figure 2). Unstained slides or Formalin Fixed tissues (10 min, room temperature) were also used for immunohistochemistry (Fig. 3)



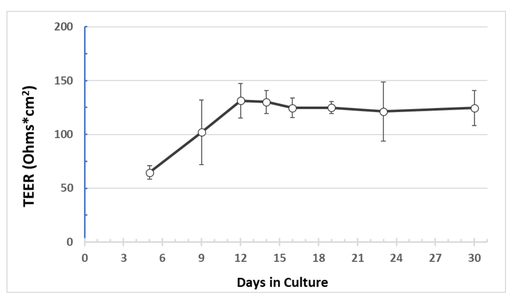


Figure 1: A. Schematic of the EpiKidney tissue model grown in cell culture inserts at air liquid interface (topical tissue surface is exposed to air) allowing for topical or systemic exposure to test materials; **B. Barrier Properties - Transepithelial Electrical Resistance (TEER)** of the EpiKidney tissues at different time points in culture (AVG±SD of 12 lots of tissues).

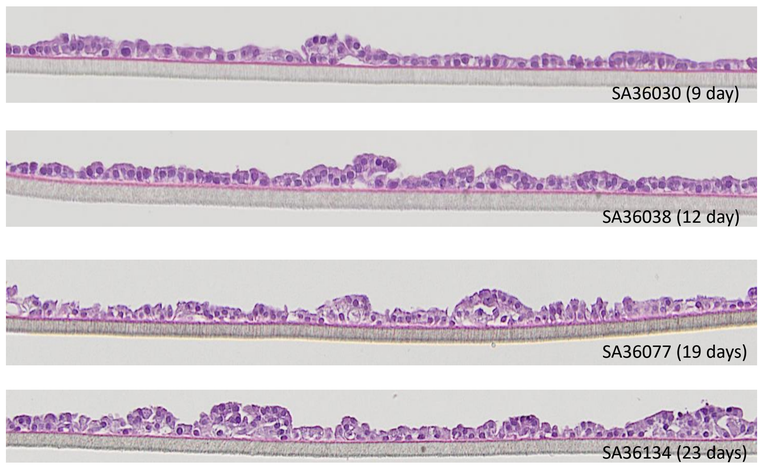


Figure 2: A. H&E-stained histological cross-sections of EpiKidney tissue model at different time in culture.

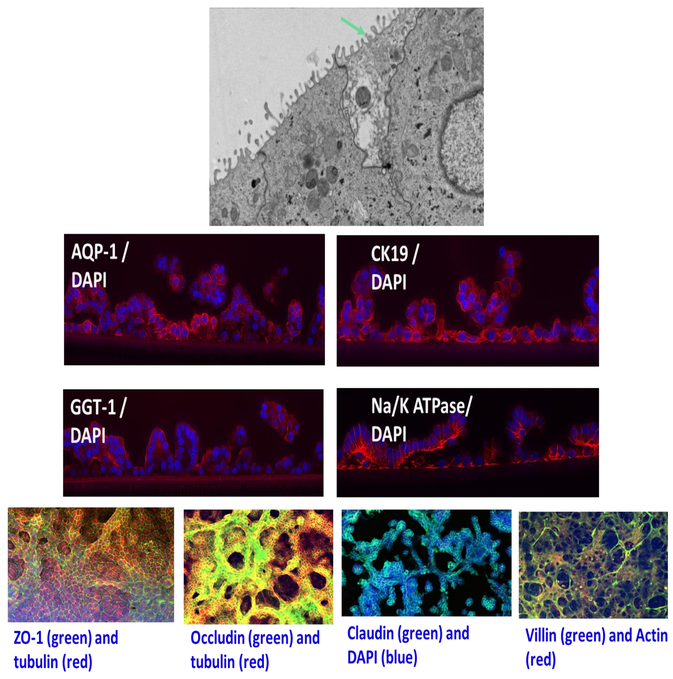


Figure 3: Expression of tight junction and brush border proteins (topical view) by EpiKidney tissues (day 14). Immunohistochemical staining of whole tissues; imaged with ECHO microscope Transmission Electron Microscopy (TEM) also showed brush border formation (Arrow)

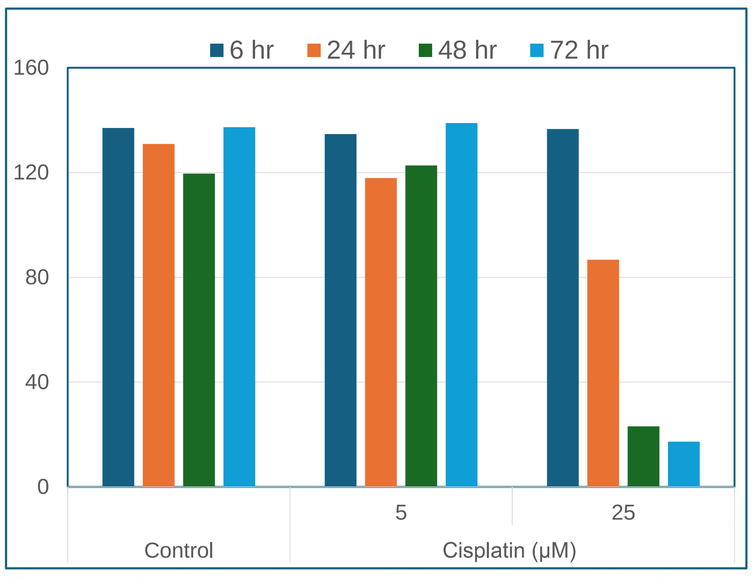


Figure 4: Barrier integrity as an indicator of cytotoxicity. Cisplatin known to cause kidney tubular cell death and tissue damage causes a decrease in tissue barrier integrity in a concentration and time dependent manner.

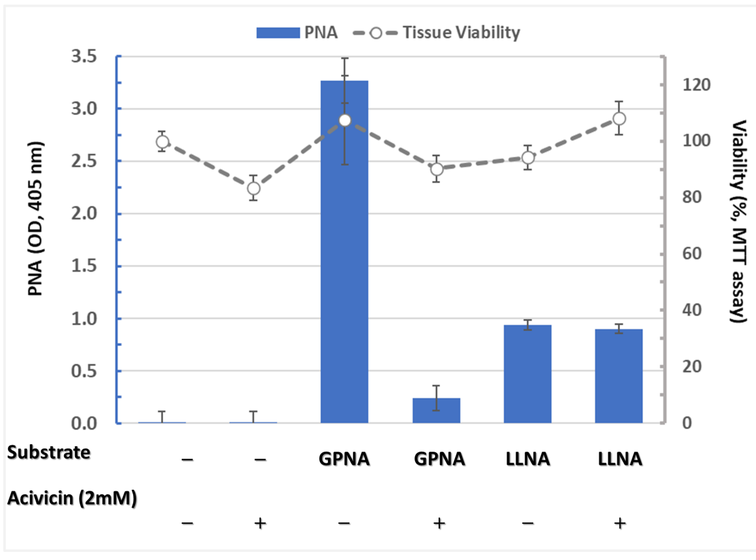


Figure 5: Transpeptidase hydrolytic activity of gamma – glutamyl transpeptidase (GGT-1) and leucine aminopeptidase (LAP) of the EpiKidney tissues was assayed on days 14 to 21. L-γ-Glutamyl-p-nitroanilide (GPNA) and L-leucine-p-nitroanilide (LLNA) were converted to PNA by GGT-1 and LAP enzymes. GGT-1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

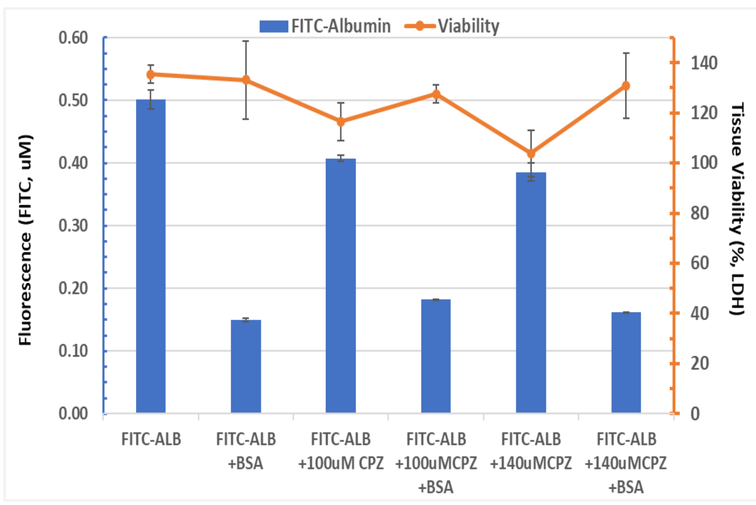


Figure 6: Receptor-mediated FITC-Albumin uptake. A and B. Topical view of the EpiKidney tissues after exposure to FITC-Albumin (2h, topical exposure); imaged with ECHO, 10x (A) and confocal microscope, 60x (B). C. FITC-albumin absorption was determined at different time in culture. Albumin absorption and the receptor-mediated saturation in the presence of 10 mM of BSA was detected for up to 28 days in culture.

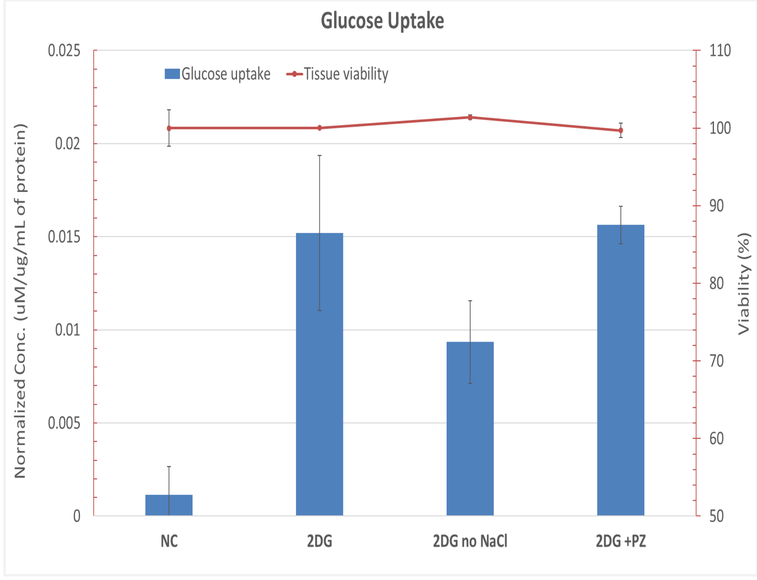


Figure 7: Glucose uptake by EpiKidney tissues. 2-Deoxyglucose (2-DG) uptake was determined on day 19. Data presented as averages of two independent experiments with n=3 tissues per condition. The sodium-glucose symport transport was inhibited in the absence of Na+ ions.

Conclusions

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, megalin, GGT-1) on the apical surface (**Fig. 3**).
   - ❑ Express water channel AQP-1 on the apical side and Na-K channel on the basolateral side (**Figures 3**). Brush border formation (**Fig.3**).
   - ❑ Transmission electron microscopy imaging showed
2. EpiKidney tissues exposed to the known nephrotoxin chemotherapeutic drug Cisplatin showed drug-induced cytotoxicity in a dose and time dependent manner (**Figure 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.