Opening Remarks

“Update of Renal MPS Screening Platform for DDIs”

Kenneth E. Thummel, PhD,
University of Washington, Seattle, WA

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Limits of Current Preclinical DDI Testing

- Quantitation of complex interactions
  - Intestinal efflux and first-pass metabolism
  - Hepatic uptake, metabolism, efflux
  - Sequential metabolism
- Impact of organ flow in clearance/first-pass process
  - Intestinal first-pass extraction
  - Hepatic uptake efficiency
  - Renal secretion efficiency (model – dependent)
- Lengthy, time-dependent interactions
  - Induction by complex mechanisms

Kidney Tubulo-Interstitium on a Chip

- The primary goal is to design, implement and test a tissue engineered human kidney microphysiological system.
- The system will be developed to fully evaluate uptake, metabolism and elimination of xenobiotics in a human tissue derived, in vitro 3-dimensional system that accurately reflects human physiology.
- The microphysiological system can be used to assess the response to organ injury inflicted by toxicants, drug clearance and DDIs.
Sources of Proximal Tubular Epithelial Cells

Cell Seeding Into MPS

- Human cortical epithelial cell isolation
- 2D culture growth
- Injection into MPS

PTEC-MPS Culture

- Continuous media flow

Human primary renal tubular epithelial cells form an *in vivo*-like tubule structure
PTEC-MPS: *In Vivo Like Environment*

- Cells form a tubular structure
- Cells tend to align by the flow
- Excellent cell viability
- Slow turnover (low KIM-1)
- Low oxidative stress

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**Critical Characteristics of PTEC-MPS**

- Cell viability and basic characteristics
- Glucose reabsorption
- Kidney Injury Molecule 1 (KIM-1) expression
- Glutathione reclamation
- Ammoniagenesis
- Vitamin D biotransformation
- Transporter expression
- Organic anion secretion
- Organic cation secretion
Epithelial Cell Marker E-Cadherin: Cell Self-Assembly

Blue: Nuclei
Red: E-Cadherin

Epithelial Cell Marker ZO-1: Tight Junction Formation

Blue: Nuclei
Green: ZO-1
3D System Induced Phenotypic Changes

Renal Tubular Secretion of Anionic Solutes: Co-Culture System
HUVEC and PTEC Co-culture

Phase contrast and immunocytochemistry (ICC) of proximal tubule epithelial cells (PTEC) and human umbilical vein endothelial cells (HUVEC) after 5 days in co-culture in MPS device. In secretion experiments, HUVEC channel serves as a pseudo-vascular channel into which a solute is introduced. The diameter of each channel is approximately 120 um. The distance between two channels is 1000 um. Cells were stained for nuclei (DAPI), CD13 (epithelial cell marker), CD31 (marker of epithelial and endothelial cells), 40x magnification.

Transporter Expression in PTEC

Immunocytochemistry (ICC) of proximal tubule epithelial cells (PTEC) after 7-14 days in MPS device. PTECs expressed OAT1 (left image) and OAT3 transporter. Images were taken on a fluorescent microscope (100x magnification).
**Transporter Expression in PTEC**

Immunocytochemistry (ICC) of proximal tubule epithelial cells (PTEC) after 7-14 days in MPS device. PTECs expressed OAT1 (left) and OAT3 (right) transporter. Images were taken on a confocal microscope (400x magnification).

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**Renal PAH Transport**

Time profile of the secretion of 2uM [14C]p-aminohippurate (PAH) in the presence or absence of 2mM probenecid by PTEC in MPS device. Data were generated from a total of 11 MPS devices (6 without inhibitor, 5 with inhibitor).
Future Directions

- Reduce interstitial space between vascular and epithelial channels
  - Induce formation of capillary bed between main channel and epithelial basement membrane.
- Replace HUVECs with human renal microvascular endothelial cells
- Induce formation of a more dense apical brush border
- Further characterize secretory and reuptake functions and inhibitory effects of endogenous and exogenous solutes
  - Using fluorescently tagged substrates, dissect differential effects of inhibitor on basolateral versus apical transport, overall trans-epithelial transport kinetics and intracellular drug accumulation