Developing a Kidney on a Chip for Clinical and Translational Research

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Why a Kidney on a Chip?
Drug Therapy and Kidney Disease

- Kidney function plays a primary role in the elimination of 20-25% of drugs and their metabolites.
- The kidney is highly susceptible to injury from drugs.
- People with kidney disease are at greatly increased risk of adverse drug reactions.
- Up to 20% of all hospital admissions for community acquired acute kidney injury are attributable to drug induced kidney injury.
- Few new drugs have been successfully developed for the treatment of kidney disease
Kidney Drug and Toxin Clearance

\[ CL_R = \frac{\text{Excretion rate}}{\text{Plasma conc}} = (1 - \text{Frac Reabs}) \left[ \frac{\text{Filtration rate}}{\text{Plasma Conc}} + \frac{\text{Secretion rate}}{\text{Plasma conc}} \right] \]
Cell Sources: Isolation & purification of primary cells from normal human kidney
Nortis 3-D Cell Culture Chip Technology

• Disposable microfluidic chips containing 3D micro-environments that are traversed by one or more tubular cell structures

• Allows for creation of compartmentalized tissue models: luminal versus extracellular matrix (ECM) compartment

• Luminal and ECM compartments can be independently perfused. There are no artificial material surfaces to which cells must attach

• Luminal fluid flow leads to controlled shear force and other mechanical stimuli

• Septa allow for injection/extraction of fluids directly on the chip, and insertion of sensors

• Integrated bubble-traps
Kidney tubule functional characterization

- Cell viability
- Glucose reabsorption
- Kidney Injury Molecule 1 expression
- Glutathione synthesis
- Ammoniagenesis
- Vitamin D biotransformation
- Transporter expression
- Organic anion secretion
- Organic cation secretion
Human kidney tubules in 3D MPS

ZO-1
E-cadherin
Nuclei

LIVE DEAD
Phenotypic changes for kidney tubules in 3D MPS

SGLT2
KIM-1
Nuclei

ZO-1
KIM-1
Nuclei

KIM-1
SGLT2
Nuclei

SGLT2

KIM-1
Vitamin D Metabolic Pathway

- 7-dehydrocholesterol
  - Skin (UV) converts to Vitamin D
  - Liver CYP27A1, CYP3A4, CYP2R1 converts Vitamin D to 25OH-Vitamin D

- 25OH-Vitamin D is converted to:
  - 1,25 (OH)₂-Vit D by CYP27B1
  - 24, 25 (OH)₂-Vit D by CYP24
  - 4β, 25 (OH)₂-Vit D by CYP3A
25 Hydroxy Vitamin D₃ Biotransformation

Control

DAPI

CYP27B

CYP24A1

![Graphs showing the biotransformation of 25hydroxy vitamin D₃ with Day 1, 2, and 3](image_url)
Ammoniagenesis

- Ammonia (NH₃) and ammonium (NH₄⁺) transport between the lumen and blood.
- Gln (Glutamine) and Glu (Glucone) metabolism.
- αKG (α-Ketoglutarate) involvement.

Graph:
- Linear regression: \( y = 0.0031x + 0.0001 \) with \( R^2 = 0.9216 \)
- OD 670nm vs. nmol Ammonia
- Two pH conditions: pH 7.4 (green) and pH 6.9 (blue)

Bar chart:
- OD 670nm for Device Numbers 1732, 1733, 1734, 1739
- pH 7.4 and pH 6.9 comparison
**p-Amino Hippurate-Indoxyl Sulfate Interaction**

- Inhibitor
+Inhibitor

N=6 devices
Duration of experiment: 4 hr
C(PAH)=2uM
C(14-C PAH)= 1uCi/mL
C(indoxyl sulfate)= 1mM
Kidney microvessel functional characterization

- Cell viability
- Vessel integration
- Microvascular integrity
- Barrier function
- Non-thrombogenicity
- Matrix production
- Response to injury
Human kidney microvascular cells

Endothelial cells:
1\textsuperscript{st} Perfusable human kidney microvascular network system: alignment along flow direction.
Permeability of fenestrated kidney microvasculature

Permeability coefficient $K \sim 1 \times 10^{-5}$ um/s for 40kDa FITC-Dextran
Bioengineered kidney vasculature is non-thrombogenic

Before blood perfusion

\[ T = 10 \text{ mins after blood} \]

\[ T = 30 \text{ mins after PBS washing} \]

10 mins to 2 hour blood perfusion through the kidney microvessels
Red: CD41a. Minimal platelet adhesion, no red blood cell adhesion
Microvascular response to Cyclosporine A: Platelet & erythrocyte adherence

1 hour treatment of cyclosporine at 1ug/mL
Cross-linked kidney gels

Human Kidney Matrix supports EC growth
Human fetal kidney microvascular cells
Kidney on a Chip: Summary of Progress to date

• Robust, reproducible validation of physiological function
• 1st isolation and purification of human kidney microvascular cells
• 1st bioengineered human kidney microvessels
• 1st human kidney matrix hydrogel
• 1st quantification of human kidney transporters
• Developing signatures for accurate prediction of kidney toxicity
• Progress towards unique human PK Model of tubular secretion
• Developing systems pharmacology for ADME (PGRNseq, RNAseq)
• Demonstrated utility of Nortis MPS platform
• Active planning for integration of kidney, liver and intestine modules
• Beginning to develop novel alternative kidney cell sources
Challenges to Kidney Microphysiological System Optimization

- Cell source
- Cell seeding and adhesion
- Organ specific extracellular matrix
- Kidney derived microvessels
- Universal media
- Flow dynamics
- Analytical chemistry
- Non-destructive biosensors
- Vascular integration
- Scaling (allometric, other)
Plans and goals for next phase

- Optimize kidney on a chip performance
- Optimize MPS platform
- Measure functional and injury responses to established nephrotoxic drugs
- Molecular signatures for accurate prediction of kidney toxicity
- Complete a PK model of tubular secretion
- Integration of kidney, liver and intestine modules into a full ADME and toxicology model
- Develop alternate cell sources
Overview of Integration