Innovation in Kidney Disease Research: the Human Kidney on a Chip

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Why a Kidney on a Chip?

- Rationale
- Goals
- Approach
- Technology
- Progress to date
- Challenges
- Long term Plans
Unsolved Public Health Problems in Kidney Disease: 1960-2013

- Increasing incidence and prevalence of kidney disease
- Ongoing high morbidity, mortality and costs
- Lack of innovation in therapies
- Lack of high level evidence from clinical trials

USRDS Annual Data Report 2012
Population incidence of dialysis-requiring Acute Kidney Injury in the United States

Hsu R K et al. JASN 2013;24:37-42
Epidemic of Diabetic Kidney Disease

- Incidence doubled in past decade
- Twelve fold increased risk of End-Stage Kidney Disease
- Leading cause of End-Stage Kidney Disease (54% of incident cases)
- Current treatment can slow disease progression
- No additional new therapies proven beneficial in past 10 years
<table>
<thead>
<tr>
<th>Rank</th>
<th>Drug</th>
<th>Year</th>
<th>Indication</th>
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<tr>
<td>1</td>
<td>Icodextrin</td>
<td>2002</td>
<td>Peritoneal Dialysate</td>
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<tr>
<td>2</td>
<td>Cinacalcet</td>
<td>2004</td>
<td>Calcimimetic, Hyperparathyroidism</td>
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<td>3</td>
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<td>4</td>
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<td>2005</td>
<td>Vasopressin antagonist, Hyponatremia</td>
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<td>Mircera</td>
<td>2007</td>
<td>Erythropoietin stimulating agent, Anemia</td>
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<td>6</td>
<td>Tolvaptan</td>
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<td>Belatacept</td>
<td>2011</td>
<td>Immunosuppressive Drug, Transplantation</td>
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<td>8</td>
<td>Pegenesitide</td>
<td>2012</td>
<td>Erythropoietin stimulating agent, Anemia</td>
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Drug Therapy and Kidney Disease

- Renal blood flow rate is 20-25% of cardiac output.
- 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.
- Over 20 million adult Americans have kidney disease, which alters drug metabolism and elimination.
- Up to 20% of all hospital admissions for community acquired acute kidney injury are attributable to drug induced kidney injury.
- People with kidney disease are at greatly increased risk of adverse drug reactions.
The Nephron: the functional unit of the kidney

- **Renal corpuscle**
- **Efferent Arteriole**
- **Afferent Arteriole**
- **Arcuate artery**
- **Vasa recta**
- **Proximal convoluted tubule**
- **Glomerular capsule**
- **Interlobular artery**
- **Interlobular vein**
- **Afferent Arteriole**
- **Distal convoluted tubule**
- **Arcuate artery**
- **Peritubular capillary**
- **Collecting duct**
**Proximal Tubule**

- **Reabsorption**
  - Sodium (65%) and water (65%).
  - HCO₃ (80-90%)
  - Others: K (65%); Ca (80%); PO₄ (90%); Mg (20%); Glucose (100%); uric acid (90%)

- **Secretion**
  - Cations (Creatinine, drugs [e.g. trimethoprim, cimetidine])
  - Anions (hippurate; drugs [e.g. diuretics, penicillin, cephalosporins, salicylates])

- **Ammoniagenesis**
  - generation of NH₄⁺ from glutamine which is secreted into lumen by sodium hydrogen antiporter (NHE3)

- **1α-hydroxylation** of 25(OH)Vit D to form 1,25 (OH)₂-Vit D (calcitriol).
Kidney Drug and Toxin Clearance

\[
CL_R = \frac{\text{Excretion rate}}{\text{Plasma conc}} = (1 - \text{Frac Reabs}) \left[ \frac{\text{Filtration rate}}{\text{Plasma Conc}} + \frac{\text{Secretion rate}}{\text{Plasma conc}} \right]
\]
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 endogenous and exogenous toxicants.
Nortis Chip Technology

Disposable, chip-like devices for the creation of vascularized 3D microenvironments of human tissues/organs
Nortis 3-D Cell Culture Chip Technology

- Disposable microfluidic chips containing 3D micro-environments that are traversed by one or more tubular cell structures
- The setup allows for creation of compartmentalized tissue models: lumenal versus extracellular matrix (ECM) compartment
- Both, lumenal and ECM compartments can be independently perfused
- There are no artificial material surfaces to which cells must attach
- Lumenal 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
Platform for Perfusion and Environmental Control

• Designed to be used in a standard cell-incubator

• Fluid control accomplished using pressurized air (same composition as desired in cell incubator) to perfuse fluids through cell culture devices

• Many platforms (dozens of microfluidic chips) can be pneumatically powered via one common pressurized gas connection

• Flow rates can be varied by changing flow resistor, or by connecting a separate pressure regulator

• High flow resistor value means flow rate more consistent with changing biological system fluidic resistance or other variations

• Quick disconnects from pressurized gas allow the platform to be easily and safely moved from incubator to microscope

• Self-contained platform means system is insensitive to fluidic perturbation (unlike when liquid supply lines are run from an external pump to a microfluidic device)
Platform for Perfusion and Environmental Control

- Low-cost polycarbonate assembly provides tubing stabilization, mechanical support for pressure bottles, and cell culture device(s)
- Microscope-imaging friendly: Can image entire vessel in brightfield, phase contrast, fluorescence. Usable with objectives down to 0.65 mm working distance
- Autoclavable, easily cleaned
- Can be operated for long term experiments without much attendance (in contrast to other systems like gravity-feed perfusion, which require daily refilling of perfusate)
- Closed system: no problems with sterility or evaporation
- Can get 20x range of flow rates, probably higher. Typical range is 0.5 microliters/min to 10 microliters/min
- Needle guides on platform to regulate position and depth of needle injection
- Design-in-progress integrates pressure container into stage, eliminating large pressure bottles and excess swept volume. Makes use of 5mL Eppendorf tubes
Cell sources: Isolation and purification of primary cells from normal human kidney
Kidney microvessels

1. Cell sources:
   - Peritubular endothelial cells
     - Isolation – CD34⁺
     - Characterization: morphology, proliferation, marker expression
   - Perivascular cells
     - Isolation – PDGFRb, NG2, CD73
     - Characterization: morphology, marker expression

2. Microvessel formation
   - Survivability
   - Permeability
   - Endothelial cell junctions
   - EC-PC interactions

3. Microvessel – peritubules
Short Term Challenges to System Optimization

- Cell source
- Cell seeding and adhesion
- Extracellular matrix
- Kidney derived microvessels
- Flow dynamics
- Analytical chemistry
An integrated multi-organ systems instrument

Huh et al, Lab Chip, 2012, 12:2156-2164
Longer term plans and goals: three to five year vision

- Merging of ‘top down’ and ‘bottom up’ approaches
- Consistent cell sources (cell lines?, iPSCs?)
- Consistent high quality real time biomarker and imaging readouts
- Enhanced ADMET prediction
- Genetic analysis of transporters and drug metabolizing enzymes (personalized medicine)
- “Offer the ability to produce high quality/high content data at key stages of the drug discovery process” (Ingber group)
Potential Additional Uses of the Kidney on a Chip

- Improved drug dosing in kidney disease
- Tool for understanding uremia
- Tool for improving organ preservation for kidney transplantation
- Tool for drug development for kidney disease
- Step towards an implantable artificial kidney?