Building a Bioartificial Kidney: From Silicon Chips to Renal Clearance

Paul Brakeman, MD, PhD
Medical Director, Pediatric Dialysis Unit
Assistant Professor
Department of Pediatrics
UCSF
Currently There Are > 540,000 Patients with End Stage Renal Disease in the United States

USRDS Annual Report, 2009
Currently There Are > 540,000 Patients with End Stage Renal Disease in the United States

> 350,000 patients receive dialysis

USRDS Annual Report, 2009
Currently There Are > 540,000 Patients with End Stage Renal Disease in the United States

USRDS Annual Report, 2009

- > 350,000 patients receive dialysis
- > 70,000 patients waiting for a renal transplant
Currently There Are > 540,000 Patients with End Stage Renal Disease in the United States

> 350,000 patients receive dialysis
> 70,000 patients waiting for a renal transplant
Only 18,000 transplants per year

USRDS Annual Report, 2009
Strategies to Increase Numbers of Available Organs

• Publicity campaigns to increase organ donation
• Extended criteria for identifying usable organs (Audard Trans Int 2007)
• Desensitized patients so they are more likely to be able to find a compatible kidney (Montgomery NEJM 2011)
Strategies to Increase Numbers of Available Organs

- Publicity campaigns to increase organ donation
- Extended criteria for identifying usable organs (Audard Trans Int 2007)
- Desensitized patients so they are more likely to be able to find a compatible kidney (Montgomery NEJM 2011)
- Engineer replacement organs
Strategies for Engineering Replacement Organs

• Generate humanized animals and use them as a source for organs
• Grow an organ using stem cells
• Use human kidney cells to reconstitute renal tissue
Strategies for Engineering Replacement Organs

- Generate humanized animals and use them as a source for organs
- Grow an organ using stem cells
- Use human kidney cells to reconstitute renal tissue
- Engineer a bioartificial organ using novel technology for hemofiltration and renal cells to provide cellular function
The Bioartificial Kidney Project
The Bioartificial Kidney Project
The Bioartificial Kidney Project
The Renal Filter Unit: the Nephron

- Glomerulus
- Proximal Tubule
- Loop of Henle
- Distal Tubule
- Collecting Duct
- Peritubular Capillary
The Renal Filter Unit: the Nephron

Glomerulus
~500,000-1,000,000 per kidney
Generate ~150L of filtrate per day

Peritubular Capillary

Distal Tubule

Collecting Duct

Loop of Henle
The Renal Filter Unit: the Nephron

Proximal Tubule
- Selectively reabsorbs ~80% of most solutes
- Reabsorbs ~80% of filtered water
- 1,25-(OH)2-Vit D3 activation

Peritubular Capillary

Glomerulus

Distal Tubule

Collecting Duct

Loop of Henle
The Renal Filter Unit: the Nephron

- Glomerulus
- Proximal Tubule
- Loop of Henle
- Peritubular Capillary
- Distal Tubule: Fine tunes solute excretion, Further water excretion
- Collecting Duct
Schematic of the Bioartificial Kidney

Blood → $\text{H}_2\text{O}$ → $\text{Na}^+$ → Urea → $\text{H}_2\text{O}$ → $\text{Na}^+$ → Urea

Filtrate → $\text{H}_2\text{O}$ → $\text{Na}^+$ → Urea → $\text{H}_2\text{O}$ → $\text{Na}^+$ → Urea

Waste

Blood ← $\text{H}_2\text{O}$ ← $\text{Na}^+$ ← Urea ← $\text{H}_2\text{O}$ ← $\text{Na}^+$ ← Urea

Blood → $\text{H}_2\text{O}$ → $\text{Na}^+$ → Urea → $\text{H}_2\text{O}$ → $\text{Na}^+$ → Urea

Blood ← $\text{H}_2\text{O}$ ← $\text{Na}^+$ ← Urea ← $\text{H}_2\text{O}$ ← $\text{Na}^+$ ← Urea
Schematic of the Bioartificial Kidney

Blood $\rightarrow$ H$_2$O $\rightarrow$ Na$^+$ $\rightarrow$ Urea $\rightarrow$ H$_2$O $\rightarrow$ Na$^+$ $\rightarrow$ Urea

Filtrate $\rightarrow$ H$_2$O $\rightarrow$ Na$^+$ $\rightarrow$ Urea $\rightarrow$ H$_2$O $\rightarrow$ Na$^+$ $\rightarrow$ Urea

Hemofilter

Waste

Blood $\leftarrow$ H$_2$O $\leftarrow$ Na$^+$ $\leftarrow$ H$_2$O $\leftarrow$ Na$^+$ $\leftarrow$ H$_2$O $\leftarrow$ Na$^+$ $\leftarrow$ H$_2$O $\leftarrow$ Na$^+$
Based on hemofiltration to eliminate need for dialysate
- Implantable to allow for continuous blood cleansing
- Incorporate of renal cells to reabsorb solutes and exclude toxins
- Renal cells provide some metabolic and hormonal functions
Innovations Required for Implantation of the RAD

Humes et al., Univ. of Michigan
Innovations Required for Implantation of the RAD

- Elimination of high pressure pumps
- Miniaturization of the hemofilter
- Better biocompatibility of the filter and blood path
- Intrinsic feedback to monitor patient’s homeostasis
- Stable function for months to years
- Adequate reabsorption of salt and water

Humes et al., Univ. of Michigan
Design Targets for the Bioartificial Kidney

- Targets
  - Hemofiltration-
    - 30 liters per day of filtrate produced
Design Targets for the Bioartificial Kidney

- **Targets**
  - Hemofiltration-
    - 30 liters per day of filtrate produced – why?
Improvement in Survival with Intensive Daily Dialysis

Design Targets for the Bioartificial Kidney

• **Targets**
  – Hemofiltration-
    • 30 liters per day of filtrate produced – how?
Improved Membrane Technology is Critical for Miniaturizing the Bioartificial Kidney

Standard dialysis membrane

Silicon nanopore membrane

Improved Membrane Technology is Critical for Miniaturizing the Bioartificial Kidney

- Very consistent pore sizes create highly selective pores and allow for exclusion of antibodies and medium to large size proteins for better biocompatibility
- High hydraulic permeability
- Smooth surface allows for coating the membranes to allow for low bioreactivity

Silicon nanopore membrane after 48 hours of *in vitro* exposure to whole blood

Improved Membrane Technology is Critical for Miniaturizing the Bioartificial Kidney

- Very consistent pore sizes create highly selective pores and allow for exclusion of antibodies and medium to large size proteins for better biocompatibility
- High hydraulic permeability
- Smooth surface allows for coating the membranes to allow for low bioreactivity

Silicon nanopore membrane after 48 hours of *in vitro* exposure to whole blood

3-Dimensional CAD Rendering of the Bioartificial Kidney

- Current hydraulic permeability of the silicon membrane is 10 μl/cm²/min/PSI.
- At this porosity the hemofilter requires 380 cm² of filter area to generate 30L per day of filtrate – About the size of two decks of cards.
Ex-vivo Hemofilter Design Testing

Computer Assisted Design

Manufacturing Prototype

Testing Prototype
Design Targets for the Bioartificial Kidney

• **Targets**
  – **Hemofiltration**-
    • 30 liters per day of filtrate produced
    • Biocompatibility of the filter and blood path
  – **Bioreactor**-
    • Barrier function to prevent reabsorption of toxins
    • 25 liters of water reabsorption
    • 3500 mM of sodium reabsorption
    • Metabolic function
Properties of an Ideal Renal Cell for the Bioreactor

- Barrier to reabsorption of toxins
- Adequate reabsorption of sodium, potassium, phosphorus and water
- Provides metabolic function including 1,25 OH Vitamin D production
- Stable for 3-4 months
- Non-immunogenic
Analysis of Cells in a Laminar Flow Bioreactor

Standard Tissue Culture

Tissue Culture Under Flow Conditions
Barrier Function of Commercially Available Proximal Tubule Cell Lines

- Creatinine Retention
- Urea Retention

OK
- Creatinine Retention: n = 6
- Urea Retention: n = 6

LLC-PK1
- Creatinine Retention: n = 6
- Urea Retention: n = 6
Water Transport of Proximal Tubule Cell Lines

![Graph showing transport rates of different cell lines.]

Ferrell et al. 2011, submitted
Primary human proximal tubule cells were transfected with human telomerase and clones were selected and characterized.

Green = Acetylated Tubulin

Green = β-catenin

Wieser, Grillari, AJPR, 2008
Barrier Function of RPTEC/TERT1 Cells

Percent retained creatinine

<table>
<thead>
<tr>
<th>Time</th>
<th>Percent Retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Month</td>
<td>97.0%</td>
</tr>
<tr>
<td>Two Months</td>
<td>97.5%</td>
</tr>
<tr>
<td>Three Months</td>
<td>98.0%</td>
</tr>
</tbody>
</table>
Salt Reabsorption of RPTEC/TERT1 Cells

Transport Rate mEq/cm²/day

<table>
<thead>
<tr>
<th>Condition</th>
<th>Transport Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Month Control</td>
<td>8</td>
</tr>
<tr>
<td>One Month Oubain</td>
<td>4</td>
</tr>
<tr>
<td>Two Months Control</td>
<td>12</td>
</tr>
<tr>
<td>Two Months Oubain</td>
<td>1</td>
</tr>
<tr>
<td>Three Months Control</td>
<td>16</td>
</tr>
<tr>
<td>Three Months Oubain</td>
<td>18</td>
</tr>
</tbody>
</table>
Salt Reabsorption of RPTEC/TERT1 Cells

ZO-1

Untransfected  Human AQP-1 Transfected

25 kD

20 kD

β-tubulin

10μM
Water Transport of Other Immortalized Human Renal Proximal Tubule Cells

Sanechika, NDT, 2011
Water Transport of Other Immortalized Human Renal Proximal Tubule Cells

** Actual reabsorption 17 uL/cm²/day

Sanechika, NDT, 2011
Salt and Water Transport in Renal Proximal Tubule Cells
Bioartificial Kidney Project

Silicon Nanofabrication

Cell Engineering
UCSF Bioartificial Kidney Team

- UCSF Team members
  - Brakeman Lab
    - Chao-Zong Lee
    - Natalie Spivak
    - Daniel Kaplan
  - Shuvo Roy Lab
    - Rishi Kant
    - Alex Heller
    - Peter Soler
    - Torin Yeager
  - Mark Wilson Lab
    - Steven Hetts
    - Loi Do
    - Mathem Saeed
    - Jeremy Durack
Bioartificial Kidney

• Non-UCSF Team members
  o William Fissell Lab (Vanderbilt School of Medicine)
    o Nicholas Ferrell, PhD
  o David Humes, MD (University of Michigan)
Project Team

Co-PI
William Fissell, MD
Cleveland Clinic

Kit Carquitan
Experien Group

Dave Brown, MD
UMichigan

Matt Simmons, MD, PhD
Cleveland Clinic

David Goldfarb, MD
Cleveland Clinic

Aaron Fleischman, PhD
Cleveland Clinic

Paul Brakeman, MD, PhD
UCSF

Roger Marchant, PhD
Case Western Reserve

Terry Conlisk, PhD
Ohio State

Advisors
Scientific and Medical
Commercial and Regulatory

Mark Goodin, MS
SimuTech Group

Robert Glines Hiemstra

Tejal Desai, PhD
UCSF

Ken Goldman, MS
H-Cubed

Project Director and Co-PI
Shuvo Roy, PhD
UCSF

Keith McCrea, PhD
ExThera Medical

Stephen Duffy, PhD
CRT

Andrew Zydney, PhD
Penn State

Project Manager

Administrative Assistant

Budget Analyst
Bioartificial Kidney Project

Silicon Nanofabrication

Cell Engineering