Development of a Bioartificial Kidney
Bioartificial Kidney

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We need to do find better ways to treat kidney failure

Dear Dr. Humes

My 12 year old daughter, Kileen, was recently diagnosed with progressive chronic kidney disease. If you could kindly send me some literature and drawings of your developing bioartificial kidney work, I would like to share it with her to develop a project on this topic for the local science fair. I think that if she can see the potential advances in kidney replacement technology besides dialysis, she will see hope and promise to get her through these difficult times.

I wish you success in this important endeavor to help the many patients suffering from kidney failure and provide hope to those who live daily with this chronic affliction.
Unmet Medical Needs

- Acute Renal Failure
  Mortality Rate Exceeds 50%

- End Stage Renal Disease
  20% Annual Mortality Rate
  Self Withdrawal from dialysis

- Renal Transplantation Improves Survival
Artificial Renal Replacement is Suboptimal

- Non-convective solute removal
- Does not replace the metabolic, endocrine, and immunologic functions of the cellular components
FIGURE 1. Schematic of an extracorporeal circuit of a bioartificial kidney used to treat patients with acute renal failure. The first cartridge is a hemofiltration cartridge in series with a renal tubule cell assist device (RAD). The ultrafiltrate is delivered to the luminal compartment of the RAD which contains the cells and the post-filtered blood is pumped into the extracapillary space of the RAD. The processed luminal ultrafiltrate from the RAD is discarded to waste and the processed blood is returned to the patient.
Developmental Step #2 For A Bioartificial Kidney (BAK)

Wearable BAK

A circuit design for a WEBAK utilizing peritoneal fluid to maintain cell viability and functionality in the BRECS. Sorbent based technology is used to regenerate peritoneal dialysis fluid for uremic toxin removal and fluid / electrolyte balance.
Developmental Step #3 For A Bioartificial Kidney (BAK)

Implantable BAK

Implantable Bioartificial Kidney
Therapy is Provided By Cells In Conventional Delivery System

Renal Epithelial Cells in Culture

Fluorescence microscopy of epithelial cells on culture plate nuclei (blue), actin cytoskeleton (green)

Renal Epithelial Cells in Hollow Fiber

Fluorescence microscopy – cross section of cells on hollow fiber nuclei (blue), actin cytoskeleton (green)

Therapy Delivered in Hollow Fiber Cartridges

Conventional CVVH cartridge system with >4000 cell-containing hollow fibers
Renal Bio-Replacement Therapy

Advantages

<table>
<thead>
<tr>
<th>Function</th>
<th>Current Treatment</th>
<th>RBT</th>
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<tbody>
<tr>
<td>Waste Control</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Fluid Balance</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Immune Modulation</td>
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<tr>
<td>Host defense system</td>
<td>✓</td>
<td></td>
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<tr>
<td>Antigen presentation</td>
<td>✓</td>
<td></td>
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<tr>
<td>Cytokine production</td>
<td>✓</td>
<td></td>
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<tr>
<td>Metabolic/endocrine functions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hormone production</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Vitamin production</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Ca, Phos homeostasis</td>
<td>✓</td>
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RBI-01 replicates the structure and function of the nephron.
Does Renal Stem/Progenitor Tubule Cell Therapy Provide Better Renal Replacement Therapy

- Acute Renal Failure; short term, extracorporeal therapy
- Wearable Extracorporeal Therapy in Conjunction with Blood or Peritoneal Perfusion Systems
- Fully Implantable Bioartificial Kidney
Kidney as an Immunomodulatory Organ

- Acute Renal Failure results in an excessive systemic inflammatory response syndrome (SIRS)
- Chronic Renal Failure results in a chronic proinflammatory disorder
Ischemia/
Nephrotoxic
insult
Partially damaged, non-necrotic cells

Denuded tubular basement membrane

Necrotic cell debris
Inflammation
MICROVASCULAR INJURY

Mechanism of Action Working Hypothesis

↑ barrier dysfunction (GI, lung)

bacteria entry into blood

sepsis

vascular resistance (sludging)

lungs

kidney

liver

vascular leak

platelet consumption

inotropes & vasopressors

bleeding

arrhythmias

right heart strain

acute lung injury

acute renal injury

acute hepatic injury

right ventricular failure

respiratory failure

renal failure

hepatic dysfunction
Patient 001

28 year old male with a 2 year old son and 6 mos. old daughter developed leg cellulitis 3 days PTA. Presented to ER with toxic shock syndrome from S. Aureus infection. 5 organ failure: cardiac, respiratory, renal, liver and hematologic failure. 80% predicted mortality. Ventilated, 3 pressors, acute dialysis
Phase I-II Clinical Study*

Phase II Study Design

ICU patients with ARF and MOF
Randomized 2 : 1
CVVH + RAD vs. CVVH alone
Open label
Up to 72 h of RAD therapy

The Cox Proportional Hazard ratio was 0.49 indicating that the risk of death for patients in the CVVH + RBT group was ~ 50% of that observed in the CVVH alone group.
Cell Therapy Manufacturing: Effective but Difficult to Commercialize

- Cell Isolation
- Cell Expansion
- Device Fabrication
- Device Storage and Distribution
Growth Curves

Human REC

Yield/gram Cortex vs. Days in Culture
BRECS

- Contains 32 disks seeded with RECs
- Less than 10 centimeter diameter
WEARABLE BIOARTIFICIAL KIDNEY (WEBAK) FOR THE TREATMENT OF ESRD
Circuit diagram of version 1.0 of the wearable artificial kidney (WAK) showing micro-shuttle pump for countercurrent blood and dialysate flows, a series of sorbent cartridges, and minipumps for refreshing electrolyte solution, bicarbonate, heparin, and ultrafiltration control.
Victor Gura’s Wearable Artificial Kidney
Automated Wearable Artificial Kidney (AWAK)  Martin Roberts; David Lee

AWAK, which stands for Automated Wearable Artificial Kidney, is an ambulatory (portable) form of peritoneal dialysis. In peritoneal dialysis, dialysis fluid (or dialysate) is pumped into the peritoneal cavity, a space in the abdomen that surrounds the abdominal organs. Toxins and wastes are then cleared from the blood through the peritoneal membrane, the lining of the abdomen (or the peritoneum). The peritoneal membrane acts as a filter to remove impurities from the blood while at the same time preventing important components of the blood, such as the red and white blood cells and proteins, from leaking out into the dialysate. In essence, the peritoneal membrane serves the same function as the normal kidneys, which act to selectively filter out impurities from the blood and remove them in the urine (1). Spent dialysate is automatically drained from the peritoneal cavity and filtered through the sorbent cartridge where toxins are removed to produce regenerated dialysate (2). The regenerated dialysate is enriched with electrolytes & glucose from the Enrichment Module, before it is returned to the peritoneal cavity (3). Steps 1—3 are repeated until the Sorbent Cartridge is exhausted. The exhausted Disposable Module is replaced with a new Disposable Module and the cycle is repeated. In ultra filtration mode, the dialysate from the peritoneal cavity is emptied into the UF bag and a pre-determined amount of dialysate is returned to the peritoneal cavity (4). AWAK returns to the dialysis mode.
Wearable Artificial Kidney Belt
Requirement of Cell Therapy Devices

- Continuous fluid flow (blood or peritoneal fluid) to deliver oxygen and nutrients.
- Immunoprotection.
- Mitotic inhibitory.
BRECS Circuit Design for CAPD
LREC-seeded BRECS on Normal Sheep During Continuous Peritoneal Dialysis

Oxygen Consumption/ BRECS (nMoles/min)

Study Day

Note: Pink line shows average _in vitro_ oxygen consumption for reference.
Goal: Implantable Bioartificial Kidney
Fundamental Barrier to Miniaturization

- Current hollow-fiber membranes have limitations
  - thick porous polymer films have non-uniform pore sizes and degrade over time upon exposure to body fluids
Silicon Nanotechnology

- Robust membranes with very uniform pore size
  - membranes coated with biocompatible films can perform highly selective filtrations of blood at very low driving pressures

SEM – Silicon Membranes

9 nm Pore (x 200K)
2nd Generation Membranes

- High hydraulic permeability
  - up to 600 ml/hr/mmHg/m²
    - no pump needed
- Manufacturing compatibility
  - scalable for larger quantities
Phase II – Integration & Preclinical Tests

Hemofilter

Bioreactor

Blood in

Blood out

Effluent
Decellularized Kidney with reseeding with cells
Cell Therapy Approaches

- Cell replacement of lost cellular components due to acute or chronic disease process

- Cell removal or processing to diminish the detrimental effects of specific cell populations
Selective Cytopheretic Inhibitory Device

- Membrane device that replicates renal epithelial cells’ inhibitory immunologic effects
Multi-Organ Failure ARDS/ ARF

Poor Tissue Perfusion

Capillary Leak & Occlusion

Endothelial Dysfunction

Excessive Neutrophil Activation

Toxic Tissue Injury

Neutrophil Tissue Infiltration

Systemic Inflammatory Response Syndrome (SIRS)
Current hollow-fiber membranes to be used as a binding surface for activated leukocytes

Blood Purification 29:183-190, 2010
SCD Fibers Selectively Sequester Activated Neutrophils

400x

1600x

5µm
Leukocytes Adherent To Surface of Fibers In Dialysis Cartridge
Conventional single-pump extracorporeal circuit using a polysulfone hemofiltration cartridge along with the SCD

- Cardiac output
- Pulmonary vascular resistance
- Hematocrit
- Myeloperoxidase
- CD11b expression
- Immunohisto analysis of lung tissue
- Survival Time
Porcine Model of Septic Shock

Control

Treated
51
SCD treated
Control / Published

NAMSA subset
N = 1260

Discovery @ day 28
control
N = 12
treated
N = 12

Dose & Safety @ discharge
treated
N = 9

Pilot @ day 28
treated
N = 35
control
N = 172

Pivotal @ 60 day
treated
N = 172
Target

0.0% 10.0% 20.0% 30.0% 40.0% 50.0% 60.0%
Excessive inflammation plays an important role in the underlying pathophysiology of multiple diseases, contributing to high morbidity, mortality and cost to treat.
Other Clinical Indications

• Preventive: CPB, ECMO, Organ Transplant
• Acute Organ Injury: MI, Stroke, ALI/ARDS, Asthma
• Chronic Organ Dysfunction: ESRD, CHF, Type 2 Diabetes, Alzheimer’s
• Autoimmune: ANCA, SLE flare, RPGN
Porcine Model of Intracerebral Hemorrhage

• Injection of thrombin into cerebral cortex stereotactily via burr hole in calvarium

• 24 hour treatment

• Brain excised, fixed, and processed for inflammatory activity, edema formation and degree of injury
Coronal brain sections are shown at the site of thrombin injection (arrow). Area of damage (demarcated by an asterisk (*)) can be identified by the lack of defined white matter due to swelling (edema), and is clearly evident in the brain of the untreated (no SCD) control pig, but not in the brain of a representative SCD treated animal. Scale bar is 1cm.
Neutrophils (NE), normally not present in brain tissue, migrate into sites of injury causing further damage. NE, identified by immunohistochemistry using a CD11b specific antibody (Red), were more prevalent in the untreated animal, indicating that SCD therapy can limit damage from ICH. Nuclei of all cells are counterstained with DAPI (Blue). Scale bar is 100 microns.
Methods

• close-chest dogs with AMI produced by balloon occlusion of the proximal Circumflex coronary artery

• Coronary occlusion was maintained for 3 hours followed by reperfusion

• SCD therapy initiated 30 minutes prior to reperfusion and maintained for 2.5 hrs
SCD – REPERFUSION INJURY

CANINE MODEL CIRCUMFLEX CORONARY ARTERY OCCLUSION & REPERFUSION

Historical Control Dog

- RV Free Wall
- Septum
- LV Posterior Wall
- LV Anterior Wall

MI
CANINE MODEL CIRCUMFLEX CORONARY ARTERY OCCLUSION & REPERFUSION

Historical Control

20.5% of total LV mass

Infarct

SCD Treated

6.1% of total LV mass

Infarct
INFLAMMATION & CHRONIC ORGAN FAILURE

• End Stage Renal Disease
  – correlation with mortality and CRP, IL6
  – IL6 reduction from 25 to 12 pg/ml, lasting up to 2 weeks

• Chronic Heart Failure
  – cardiodepressant effects of TNF-a, IL-6
  – monocyte activation
  – correlation of poor outcomes and absolute monocyte counts
CHRONIC HEART FAILURE CANINE MODEL (AJP: H1379-84, 1991)

- Recurrent microembolization of small vessels in left ventricle over 6 weeks
- Reduction of ejection fraction from 55% to 25%
- Model has been consistent with efficacy of pharmacologic approaches in clinical CHF
EJECTION FRACTION IN CHF DOG MODEL

SCD - Heart Failure
Ventriculograms of a CHF dog heart are shown at baseline (before therapy) and at the end of the 4 hour therapy session. The red line depicts the border of the left ventricular diastolic silhouette (most relaxed state during filling) overlayed on the left ventricular systolic image (most contracted state), demonstrating improved contractility (black arrows) of the left ventricle after therapy.
Fractional Excretion (FE) Urea and Sodium (Na)

- Total Urine Sodium excretion doubled from control to 4hr SCD treatment.
Cardiorenal Syndrome Type 1

A 45 yr. old male with longstanding heart failure with an EF of 20% presented with a 20 lb. weight gain in 2 weeks with increasing shortness of breath and inability to walk more than 10 yards and increasing lower extremity edema. Over the course of 5 days in the ICU he had no net fluid removal despite intravenous dobutamine, milrinone, and lasix with controlled oliguria. His serum creatine (Scr) increased from a baseline Scr of 1.5 to 3.38. A transesophageal echocardiogram demonstrated a pulmonary capillary wedge pressure of 30mm Hg, cardiac output of 3L/min, and cardiac index of 1.44L/min. CRRT with SCD therapy was initiated and over the next 24hrs his urine output doubled and he had a net 3L removal due to increased urine sodium excretion and net ultrafiltration with CVVH. Over the next 5 days, a total of 14L of fluid was removed with improvement of his symptoms and peripheral edema.
PLATFORm TECHNOLOGY TO MODULATE INFLAMMATION

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<tr>
<th></th>
<th>Human MOF</th>
<th>Porcine ALI</th>
<th>Canine CHF</th>
<th>Porcine ICH</th>
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<td><strong>T + 24 hrs</strong></td>
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SUMMARY

Cell Therapy is the next therapeutic modality for hormonal and organ replacement technology.

Extracorporeal devices will achieve the earliest success.

Cell implants have numerous safety and durability hurdles but will have the greatest applicability.

Cell processing may be a new therapeutic opportunity.
Requirements to Develop New Therapies

3 C’s: Conviction, Creativity, Courage

3 P’s: Passion, Patience, Persistence,