BC KIDNEY DAYS

Hosted by BC Transplant and the BC Renal Agency

2013

Development of a Bioartificial Kidney

Bioartifical Kidney

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University of Michigan

Disclosures: CytoPherx, Inc.; Innovative BioTherapies, Inc., Renal Solutions, Inc.

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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

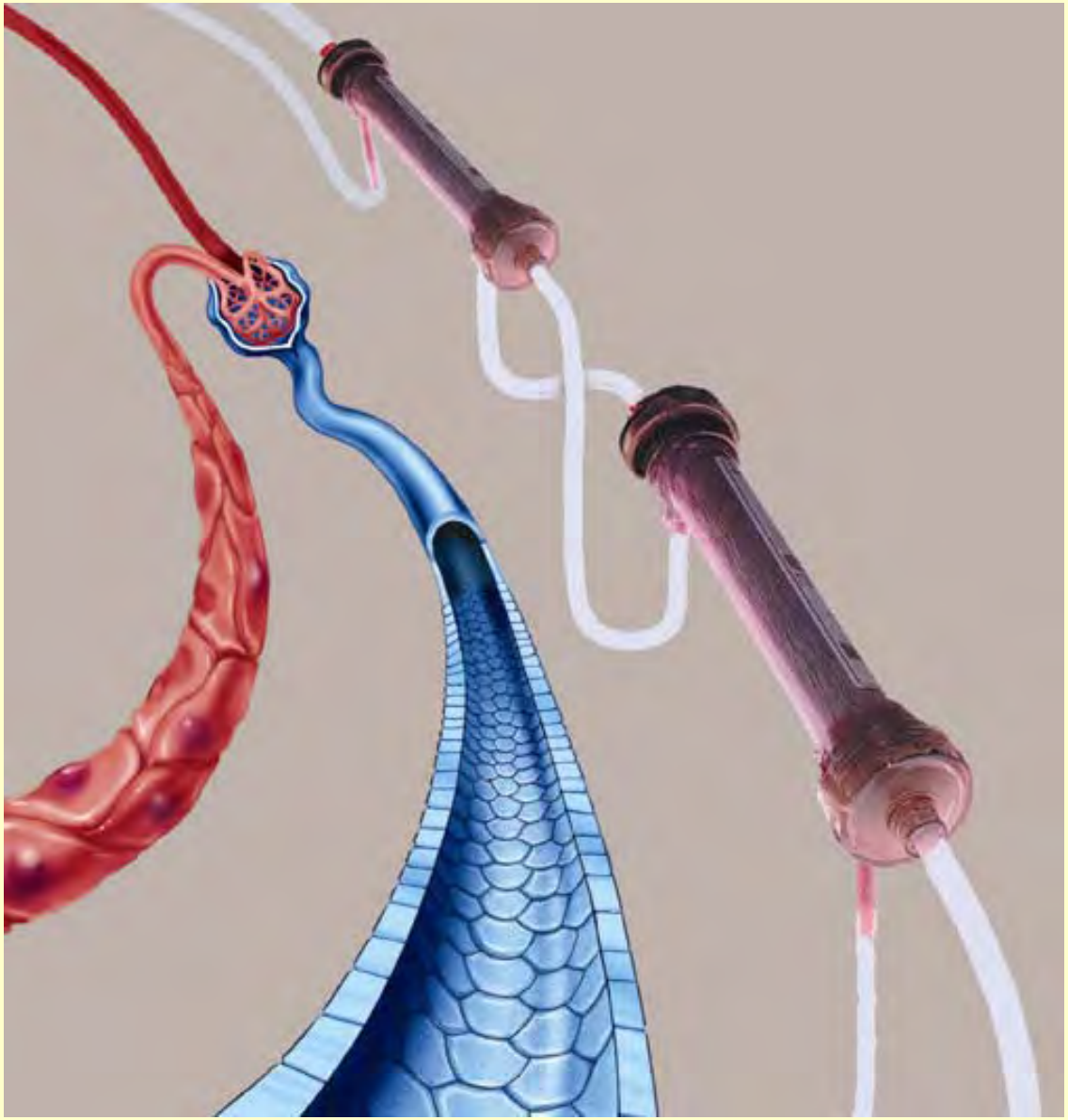
  20% Annual Mortality Rate

  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



Developmental Step #1 For A Bioartificial Kidney (BAK): Acute Extracorporeal RAD

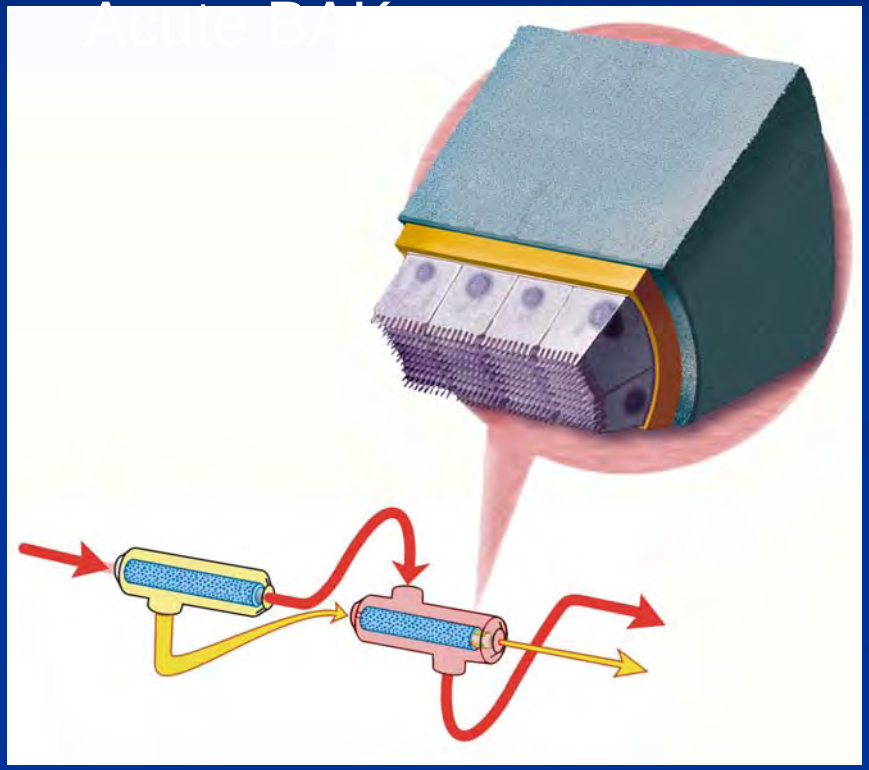
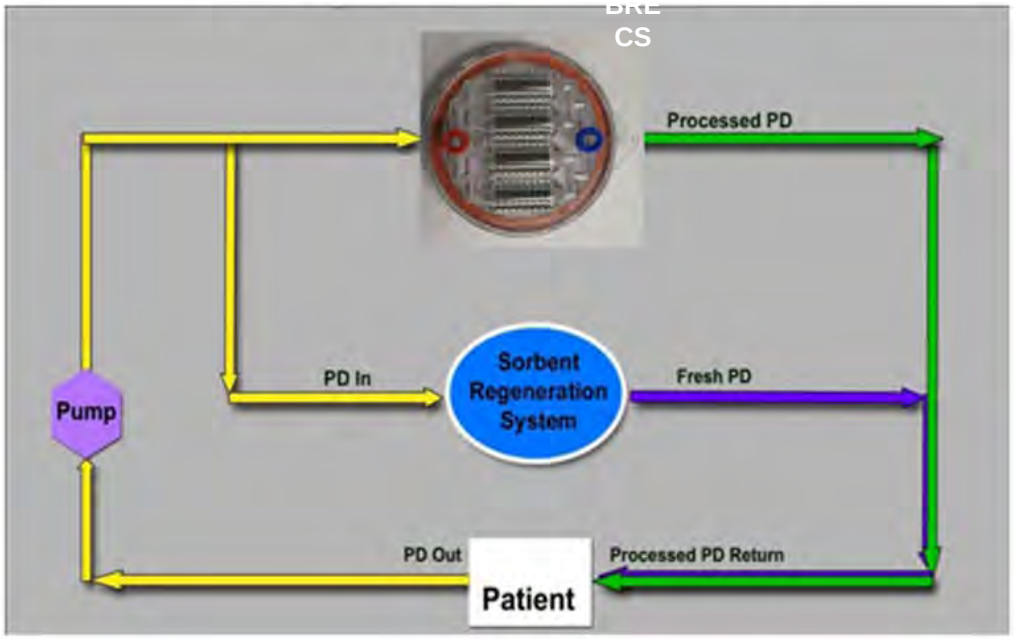


FIGURE 1. Schematic of an extracorporeal circuit of a bioartificial kidney used to treat patients with acute renal failure. The first cartridge is a hemofiltration cartridge in series with a renal tubule cell assist device (RAD). The ultrafiltrate is delivered to the luminal compartment of the RAD which 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)

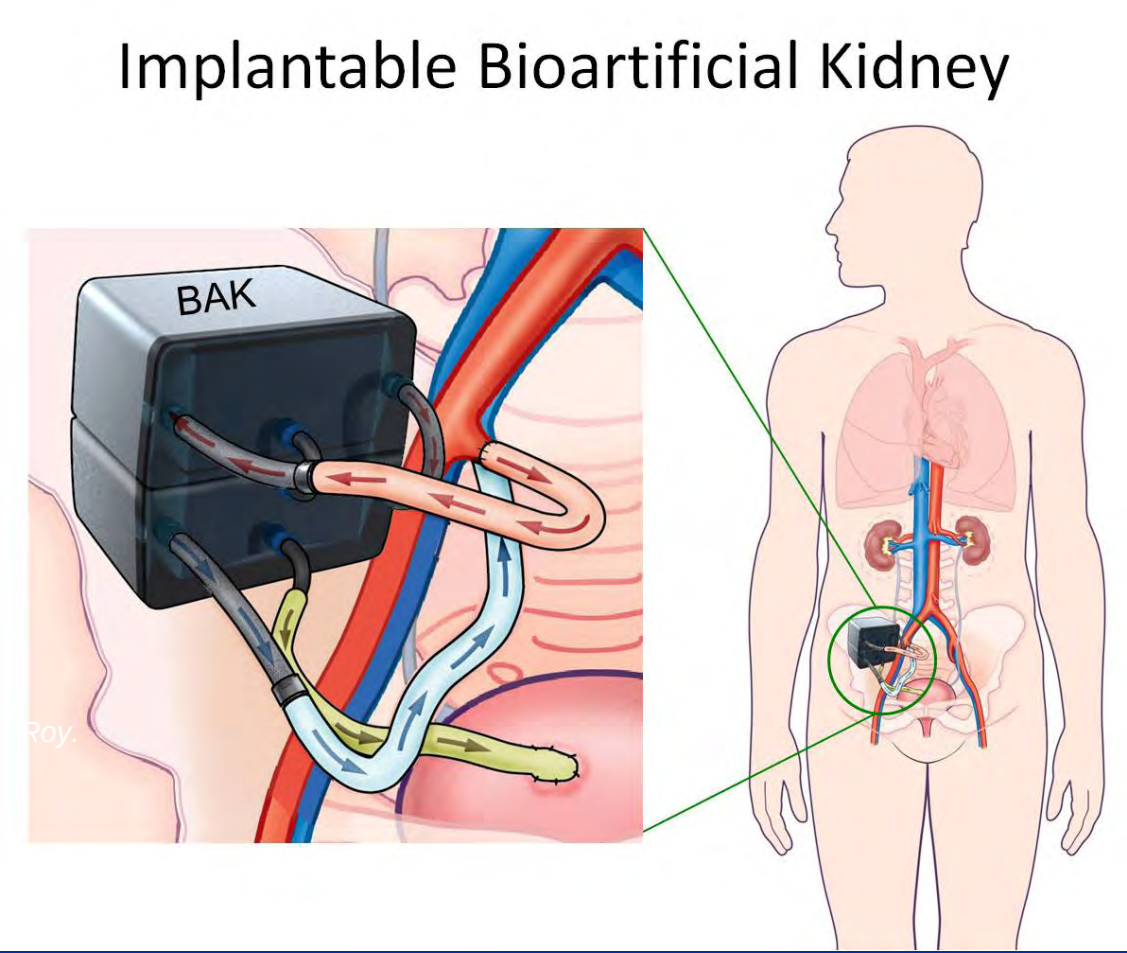
Wearable BAK



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.

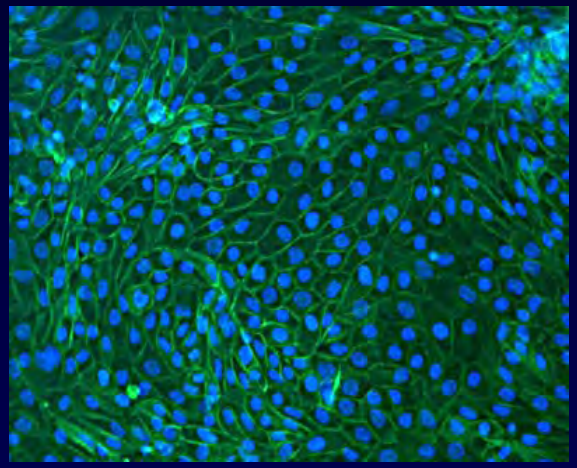
Developmental Step #3 For A Bioartificial Kidney (BAK)

Implantable BAK



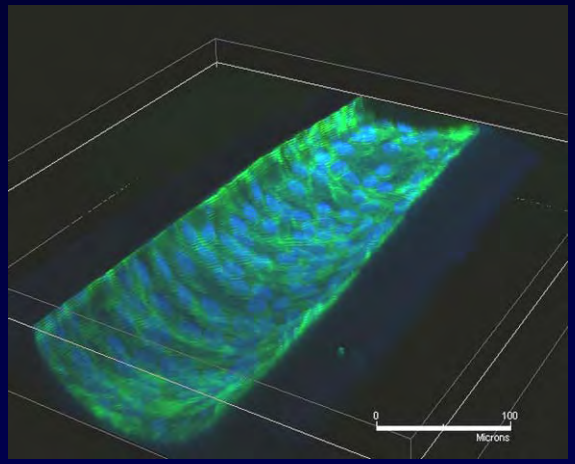
Therapy is Provided By Cells In Conventional Delivery System

Renal Epithelial Cells in Culture



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

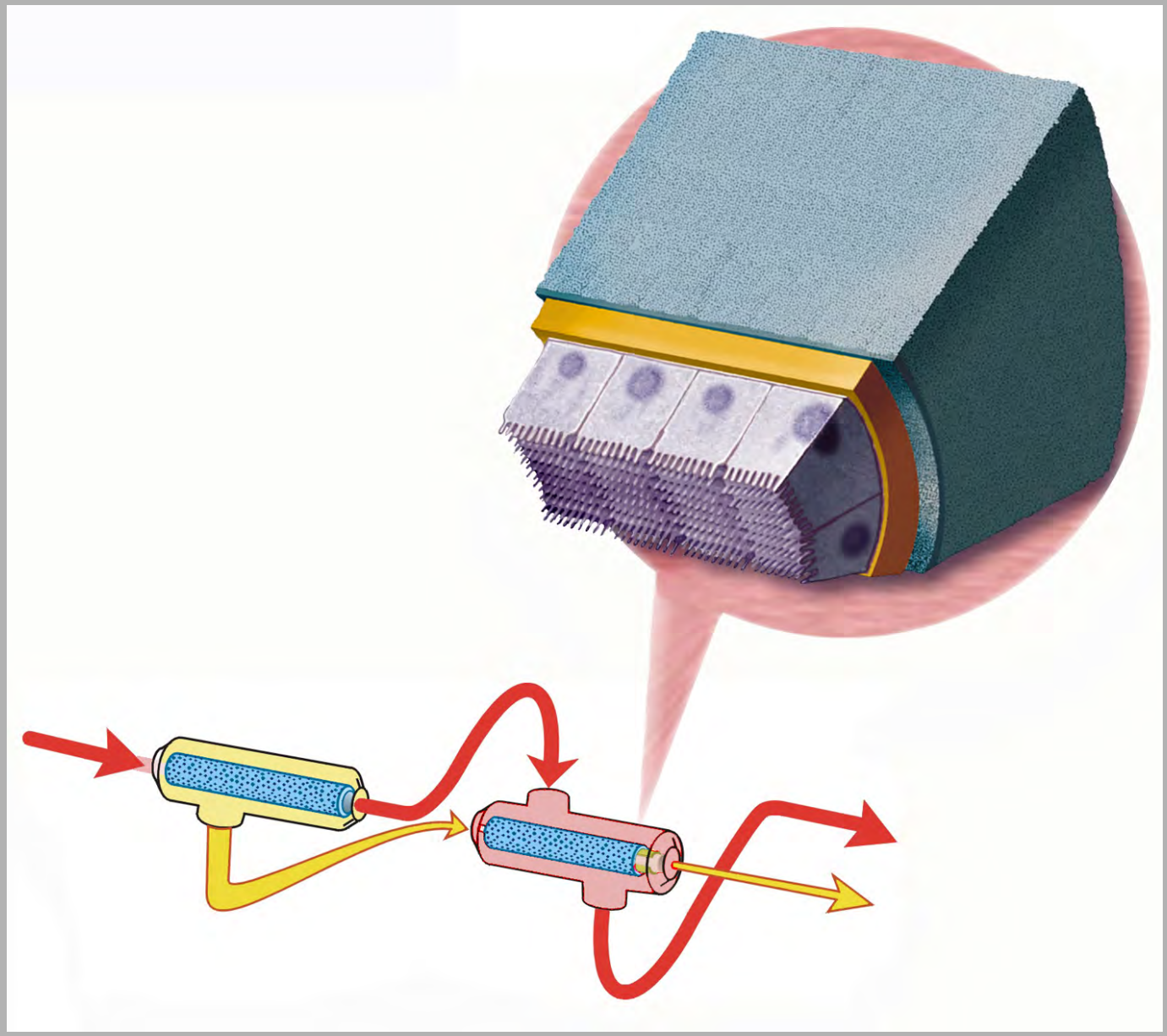
Renal Epithelial Cells in Hollow Fiber



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

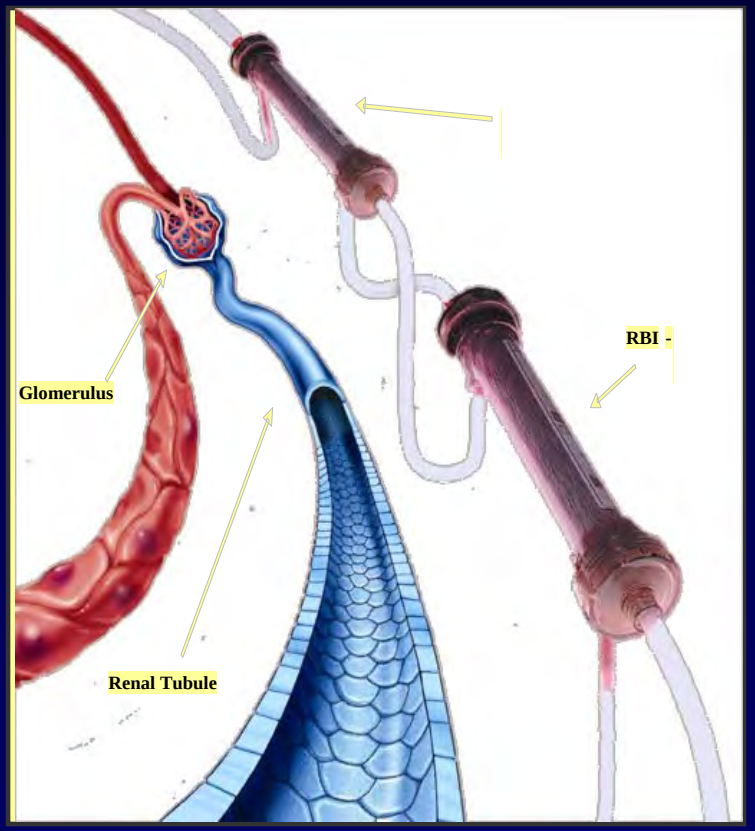


Nature Biotechnology 17:451-455, 1999

Renal Bio-Replacement Therapy Advantages

	Current Treatment	RBT
Waste Control	✓	✓
Fluid Balance	✓	✓

Immune Modulation	
Host defense system	✓
Antigen presentation	✓
Cytokine production	✓
Metabolic/endocrine functions	
Hormone production	✓
Vitamin production	✓
Ca, Phos homeostasis	✓



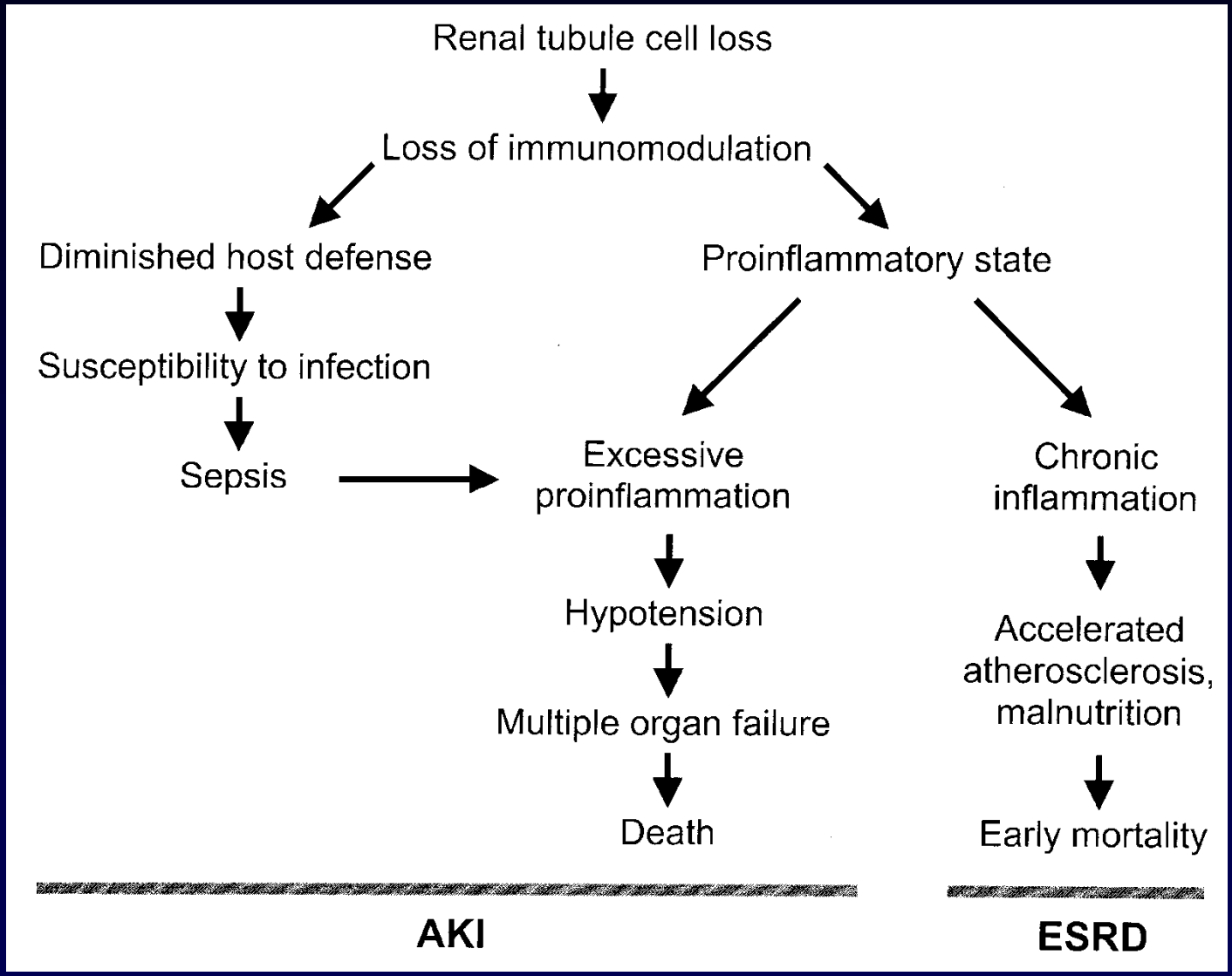
RBI-01 replicates the structure and function of the nephron

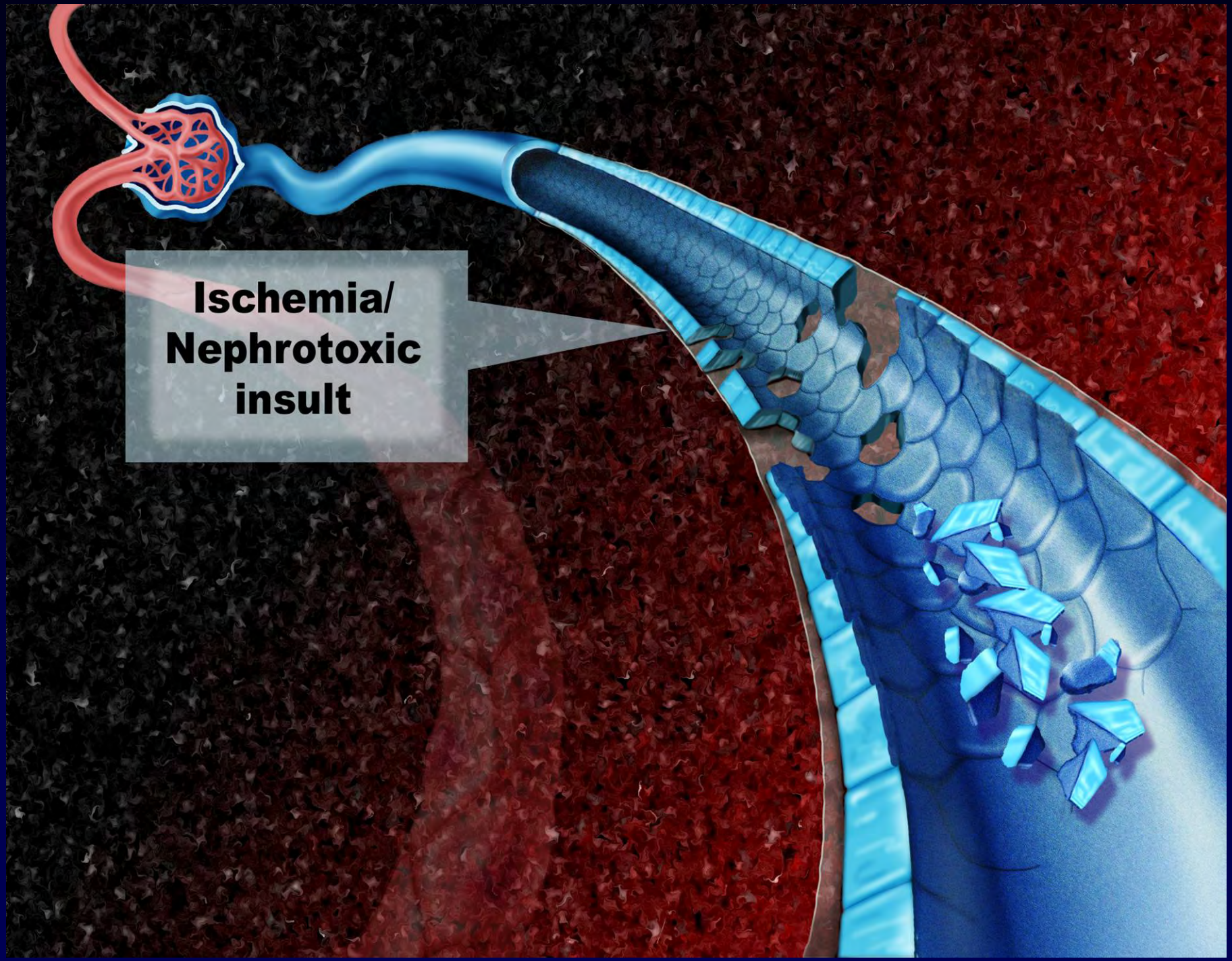
Does Renal Stem/Progenitor Tubule Cell Therapy Provide Better Renal Replacement Therapy

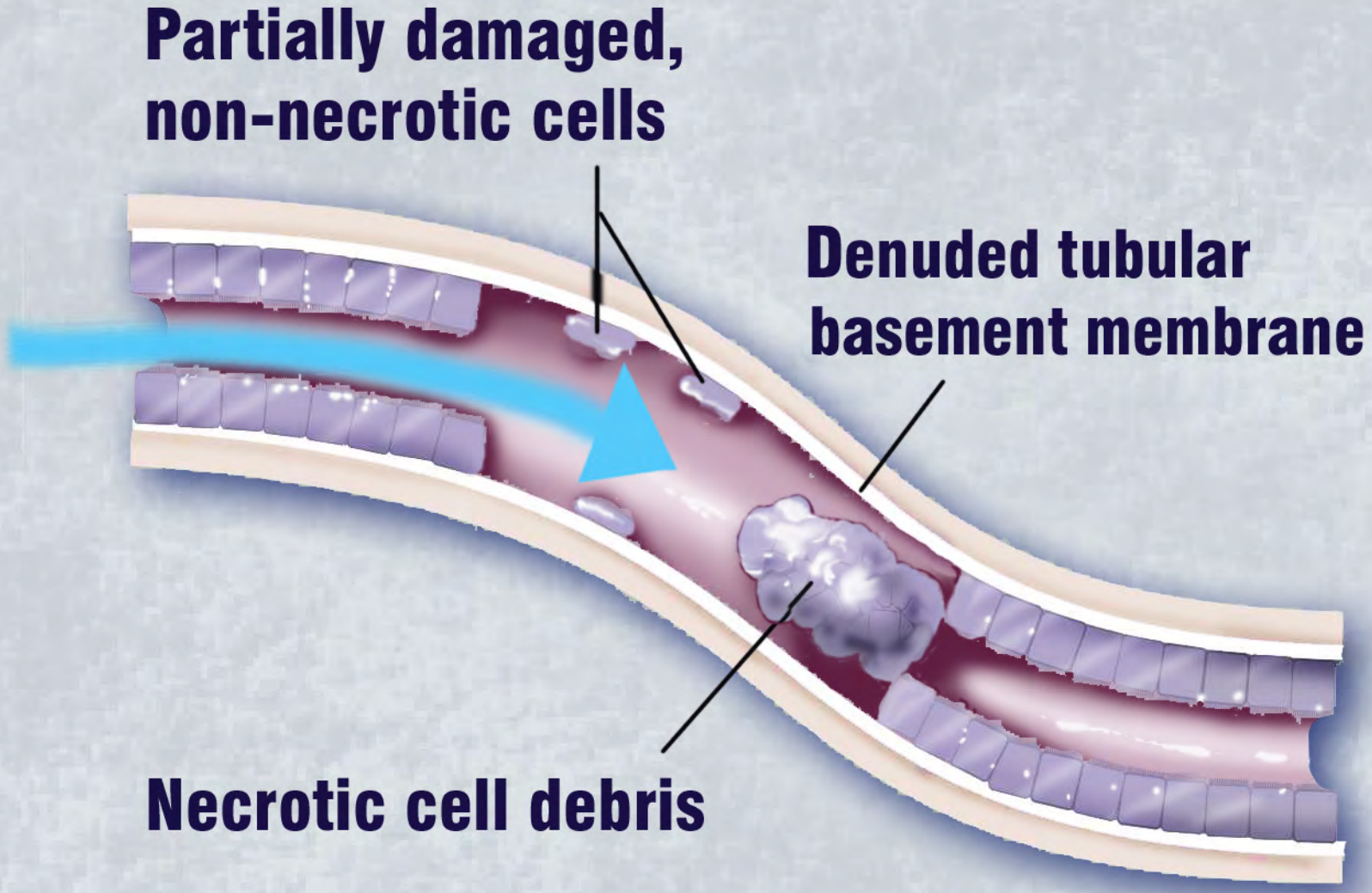
- Acute Renal Failure; short term, extracorporeal therapy
- Wearable Extracorporeal Therapy in Conjunction with Blood or Peritoneal Perfusion Systems
- Fully Implantable Bioartificial Kidney

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


Renal tubule cell loss
Loss of immunomodulation
Diminished host defense
Proinflammatory state
Susceptibility to infection
Sepsis
Excessive proinflammation
Chronic inflammation
Hypotension
Accelerated atherosclerosis, malnutrition
Multiple organ failure
Death
Early mortality
AKI
ESRD


Ischemia/
Nephrotoxic
insult

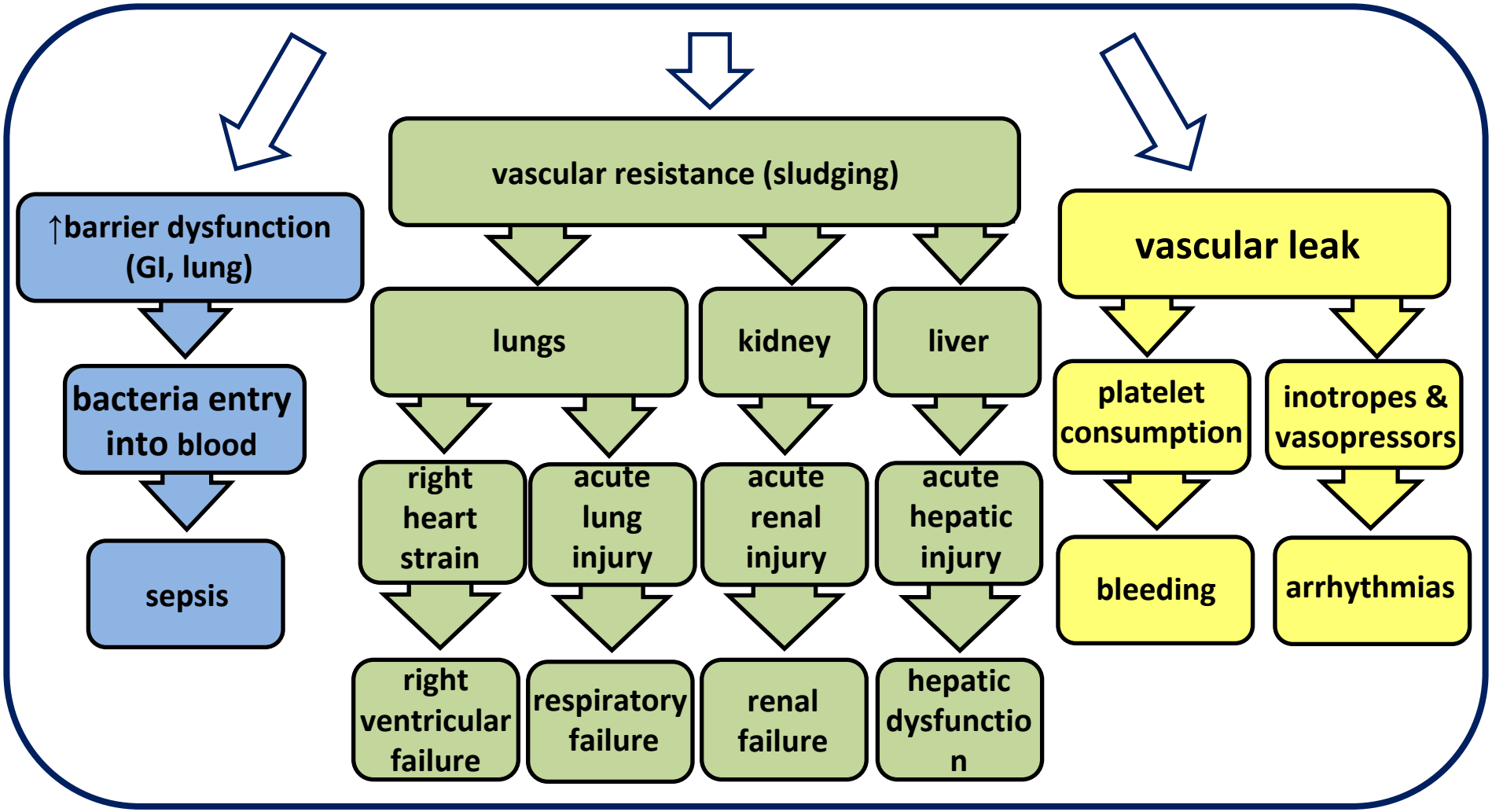

Partially damaged, non-necrotic cells
Denuded tubular basement membrane
Necrotic cell debris

Inflammation

Calor Rubor Tumor Dolor

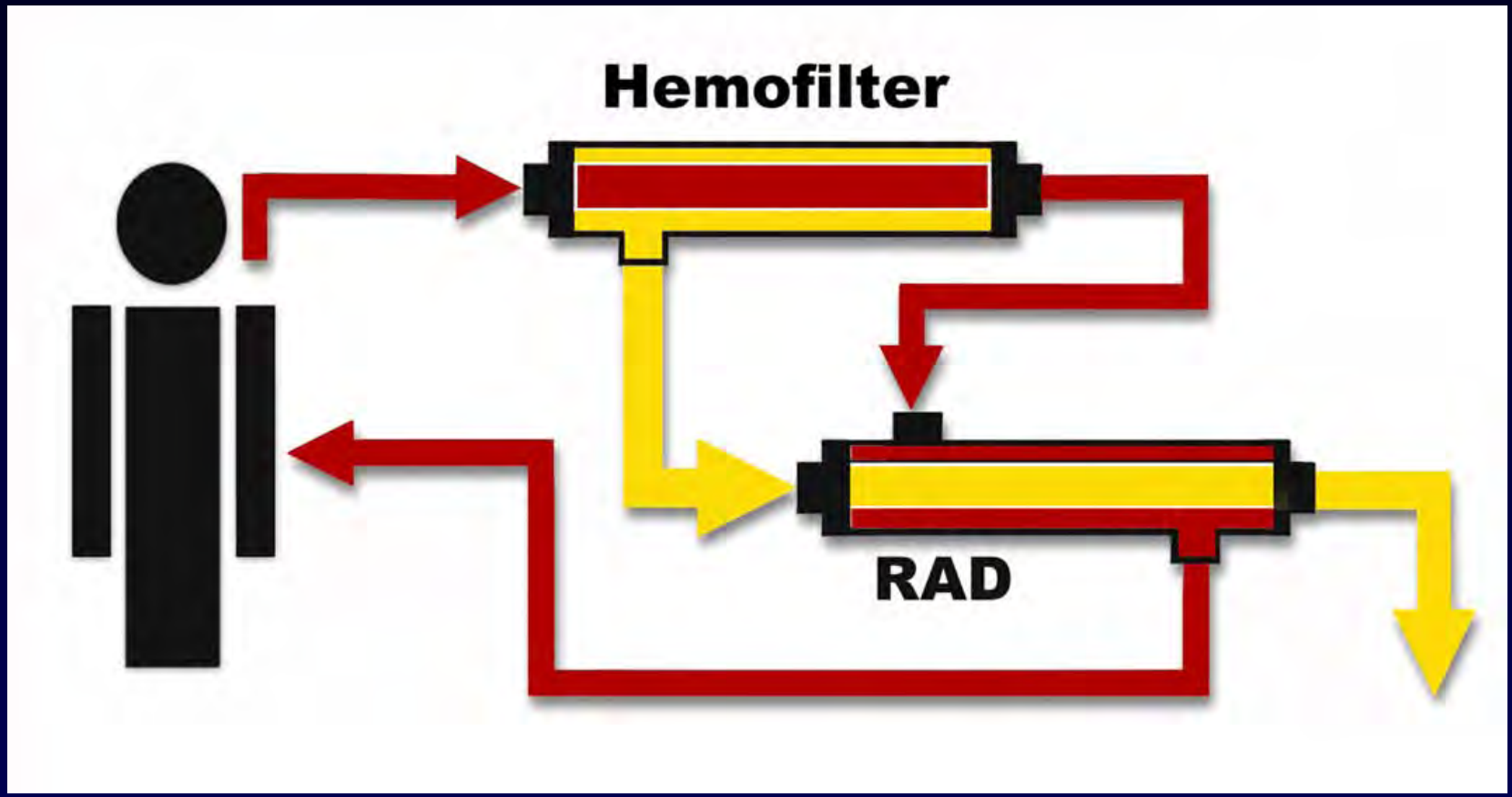
Mechanism of Action Working Hypothesis

MICROVASCULAR INJURY

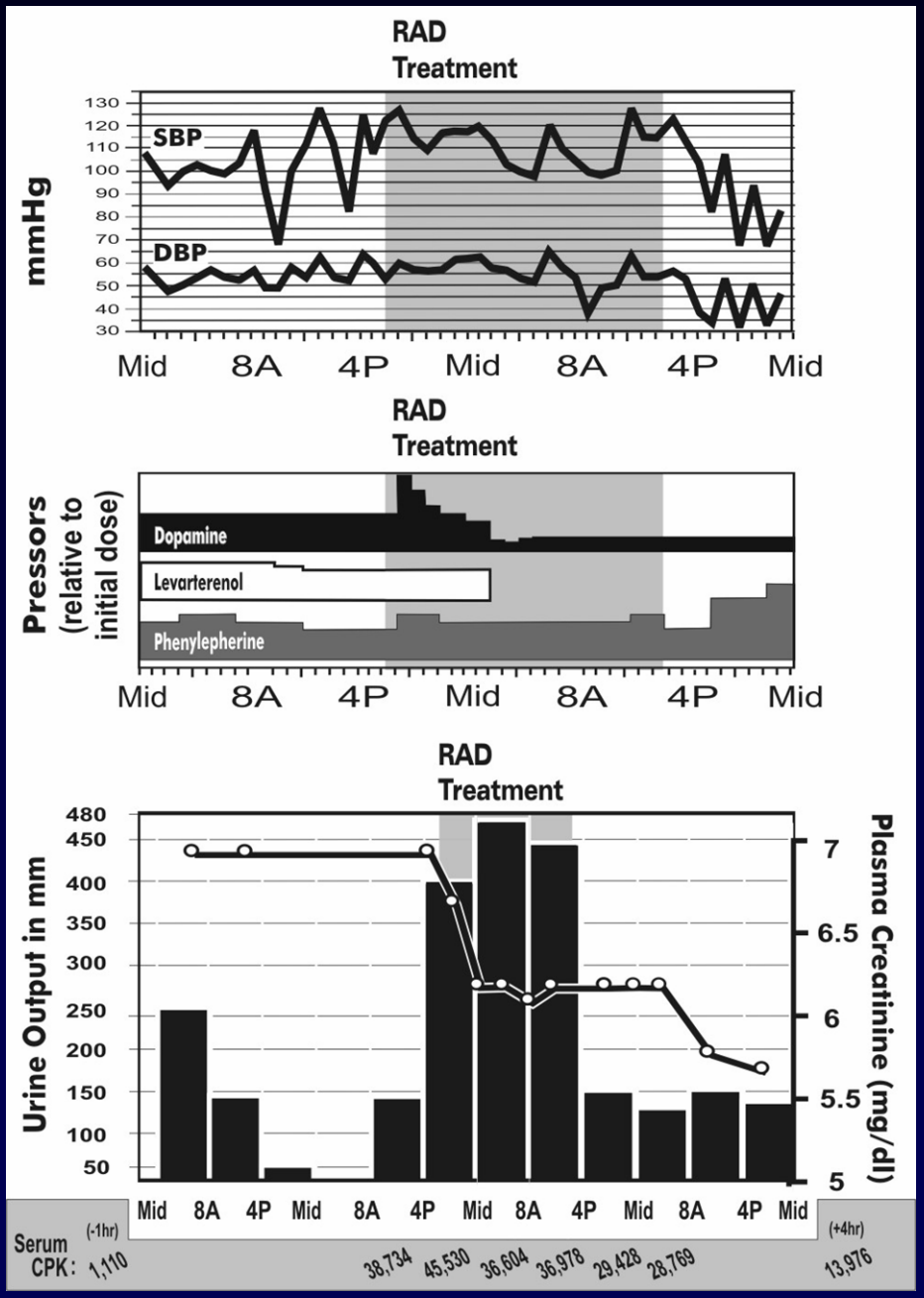


Patient 001

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


Hemofilter
RAD

Phase I-II Clinical Study*



* *Blood Purif* 2003;21:64-71.

Phase II Study Design

ICU patients with ARF and MOF

Randomized 2 : 1

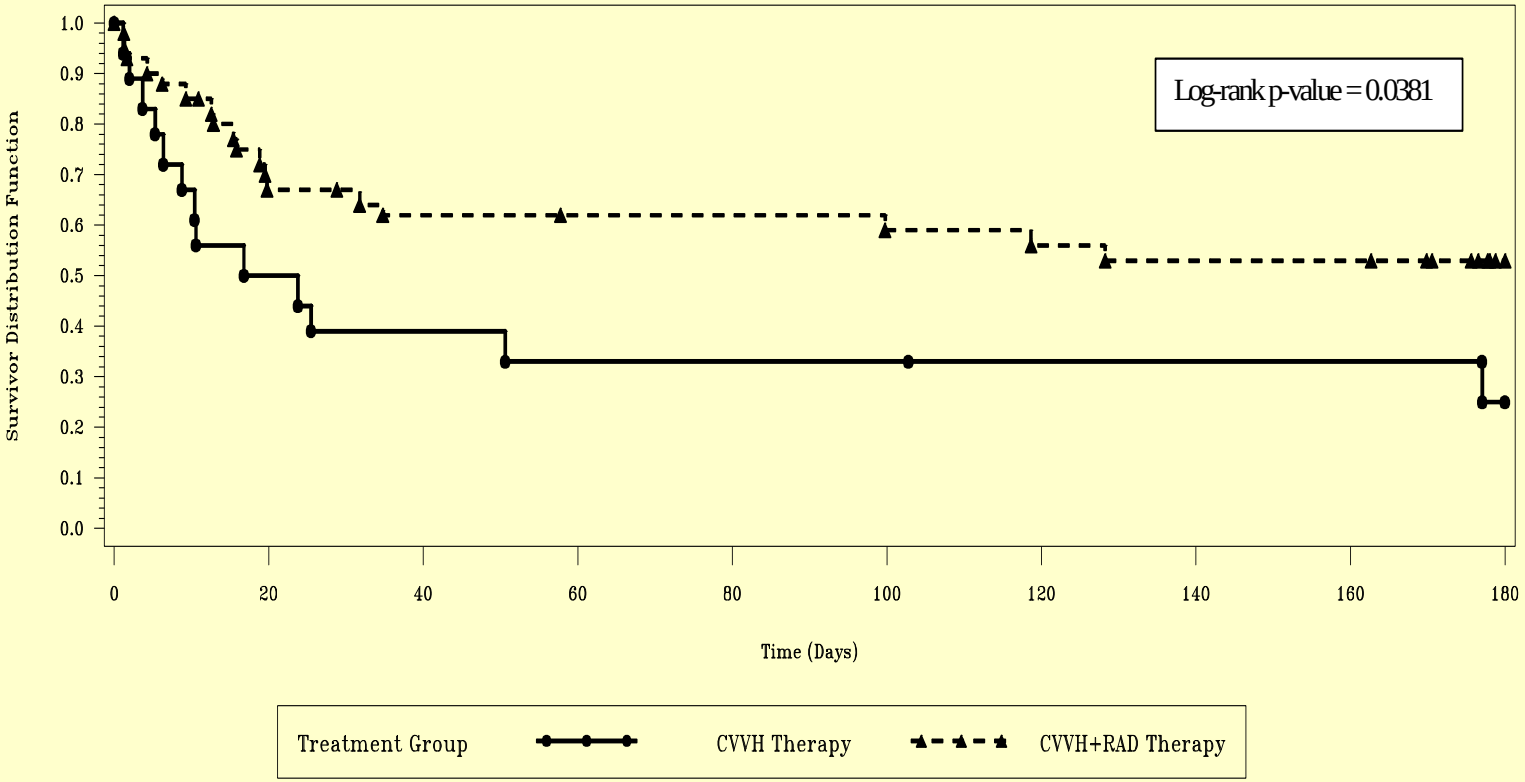
CVVH + RAD vs. CVVH alone

Open label

Up to 72 h of RAD therapy

J Am Soc Neph 19:1034-1040, 2008

Kaplan-Meier Survival Curve



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

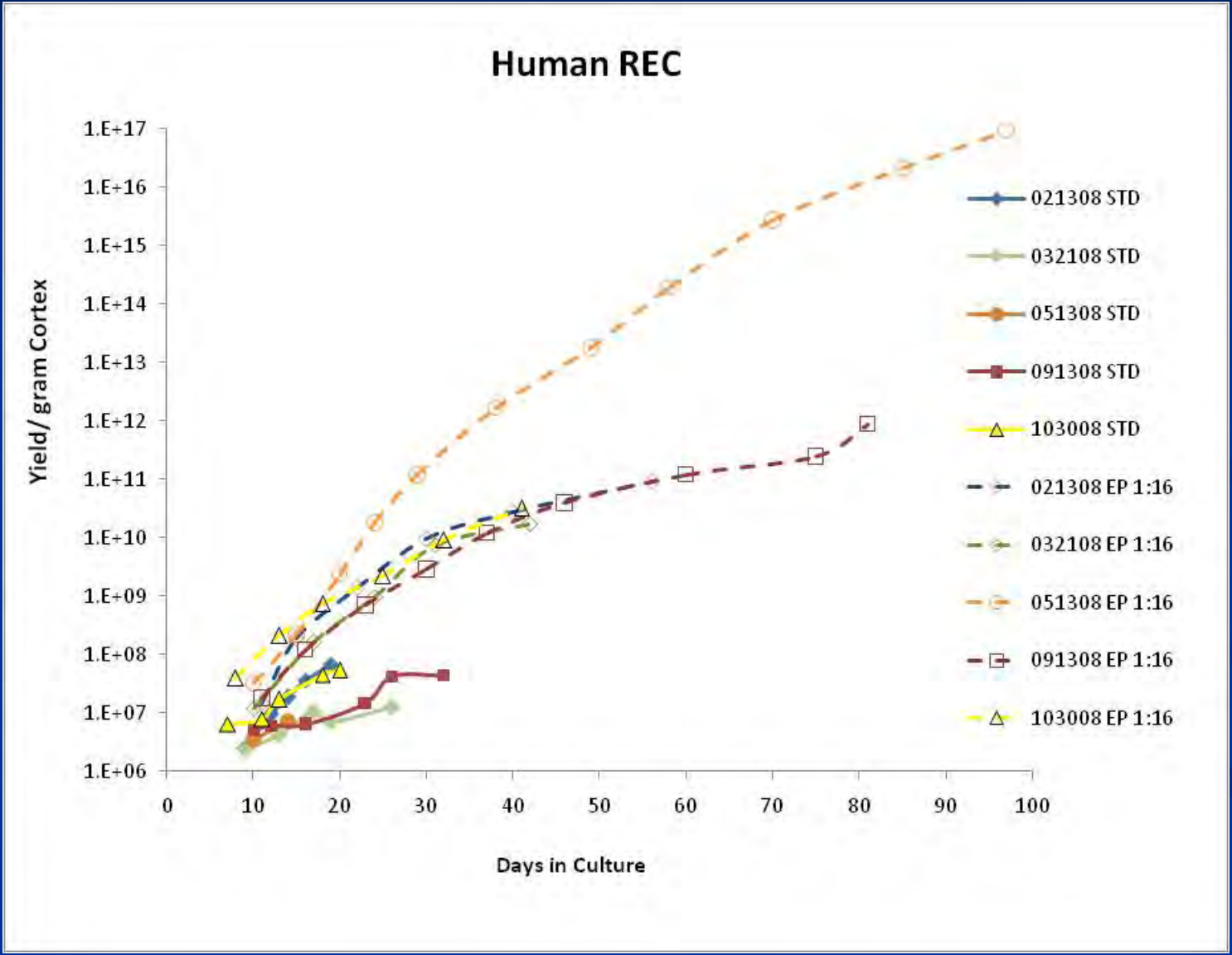
⬇

Device Fabrication

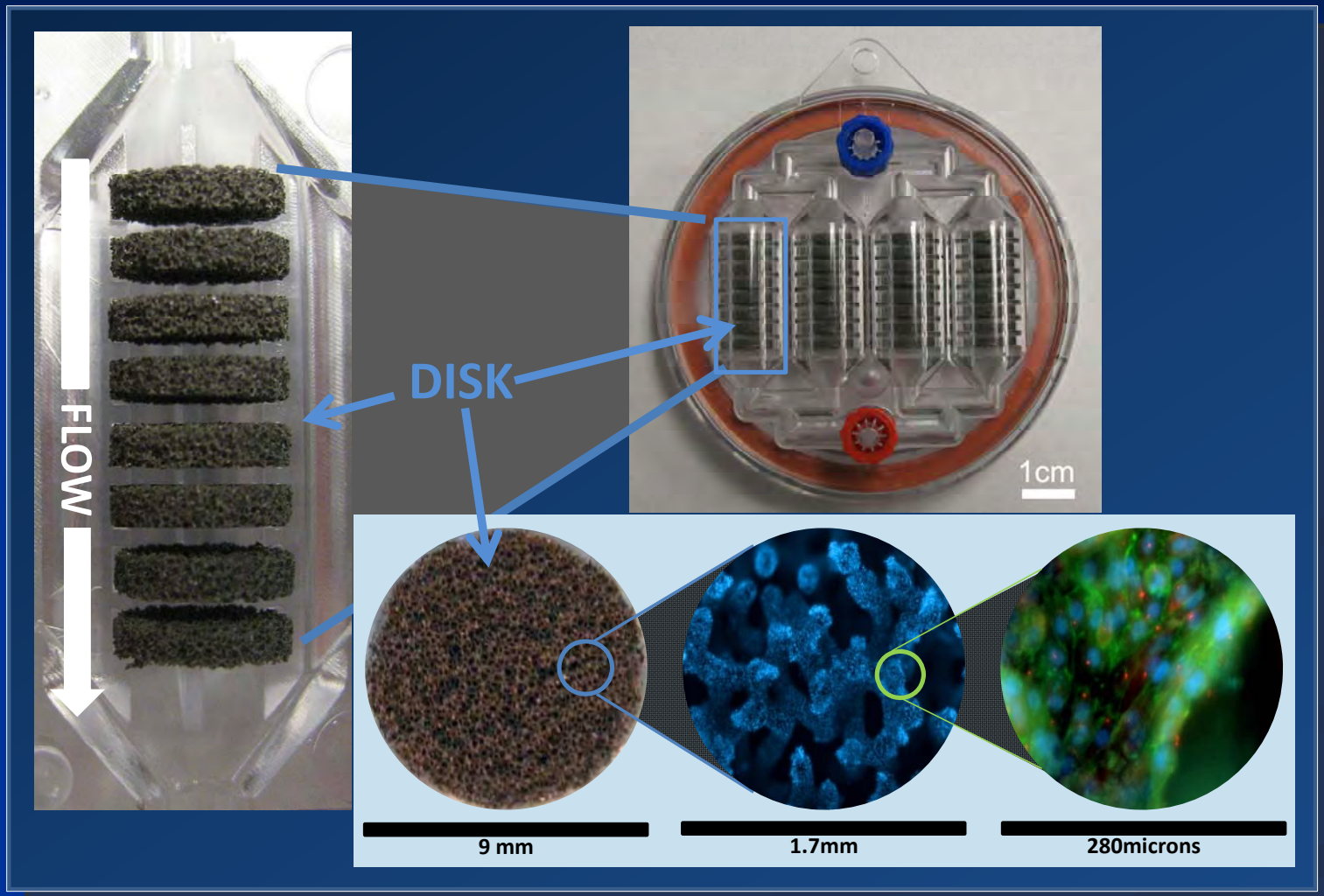
⬇

Device Storage and Distribution

Growth Curves



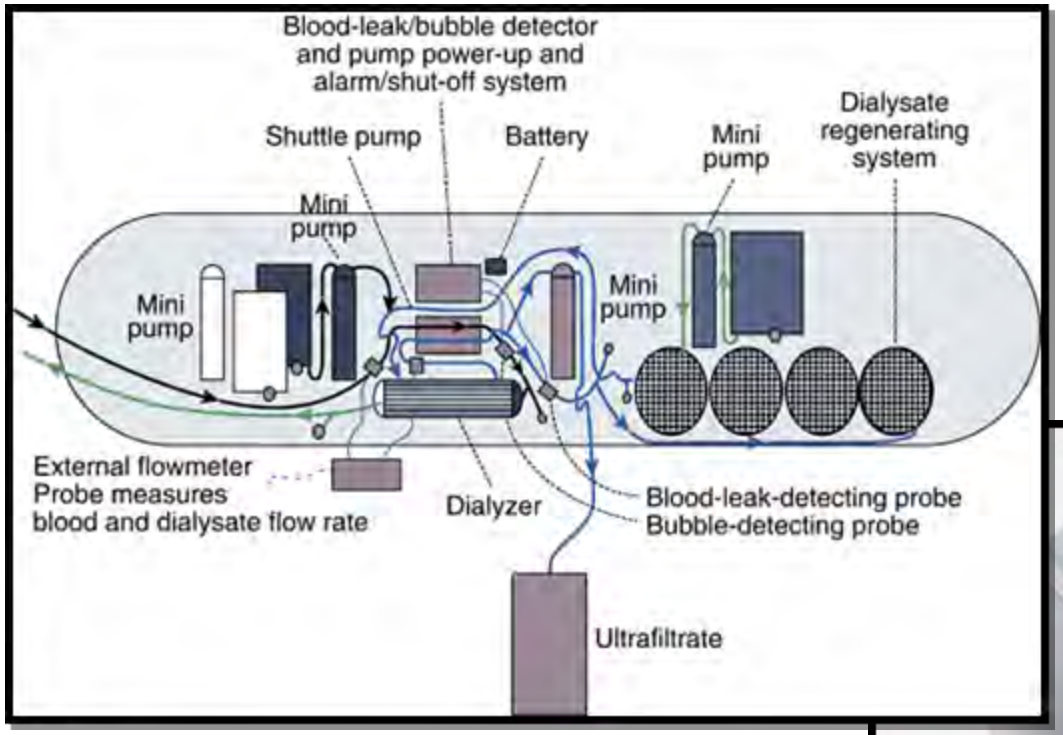
BRECS



- Contains 32 disks seeded with RECs
- Less than 10 centimeter diameter

WEARABLE BIOARTIFICIAL KIDNEY (WEBAK) FOR THE TREATMENT OF ESRD

Blood Circuit Requirement

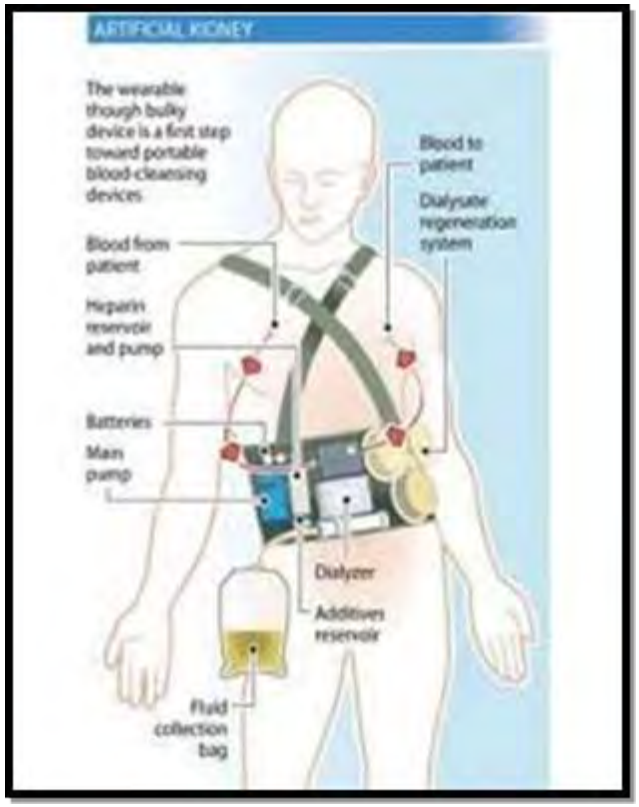


Circuit diagram of version 1.0 of the wearable artificial kidney (WAK) showing micro-shuttle pump for countercurrent blood and dialysate flows, a series of sorbent cartridges, and minipumps for refreshing electrolyte solution, bicarbonate, heparin, and ultrafiltration control.

Wearable Artificial Kidney

Victor Gura

Victor Gura's Wearable Artificial Kidney

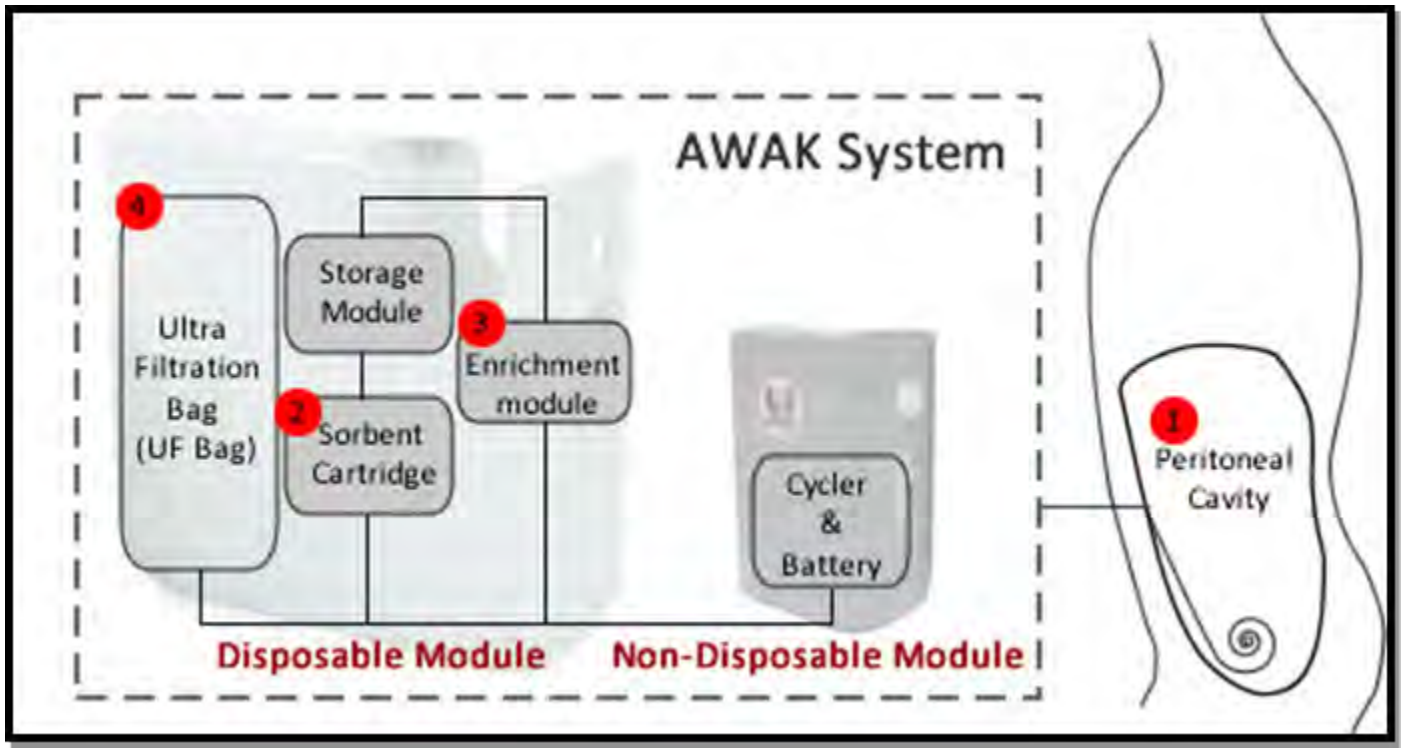


Automated Wearable Artificial Kidney (AWAK) Martin Roberts; David Lee

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.

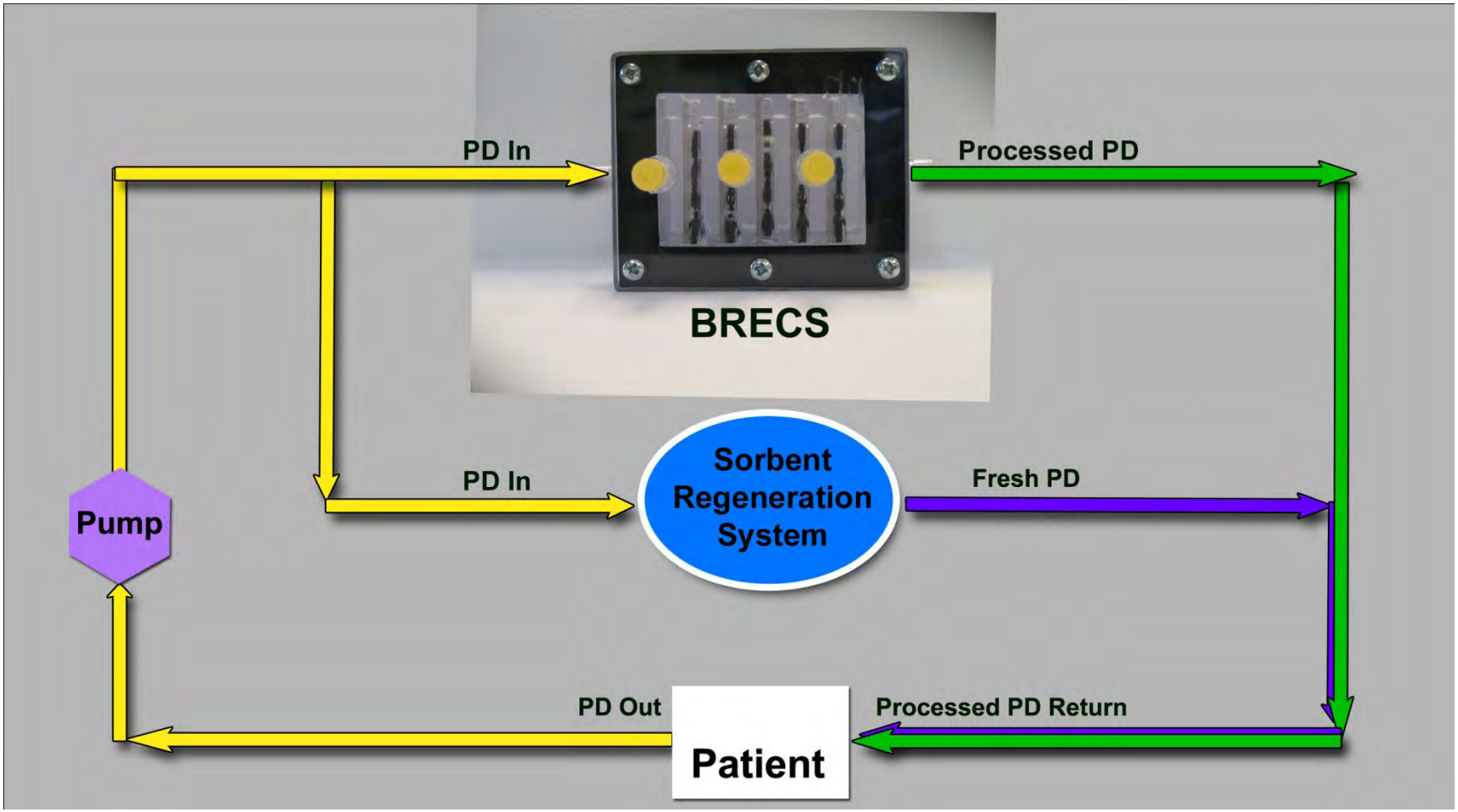


Wearable Artificial Kidney Belt

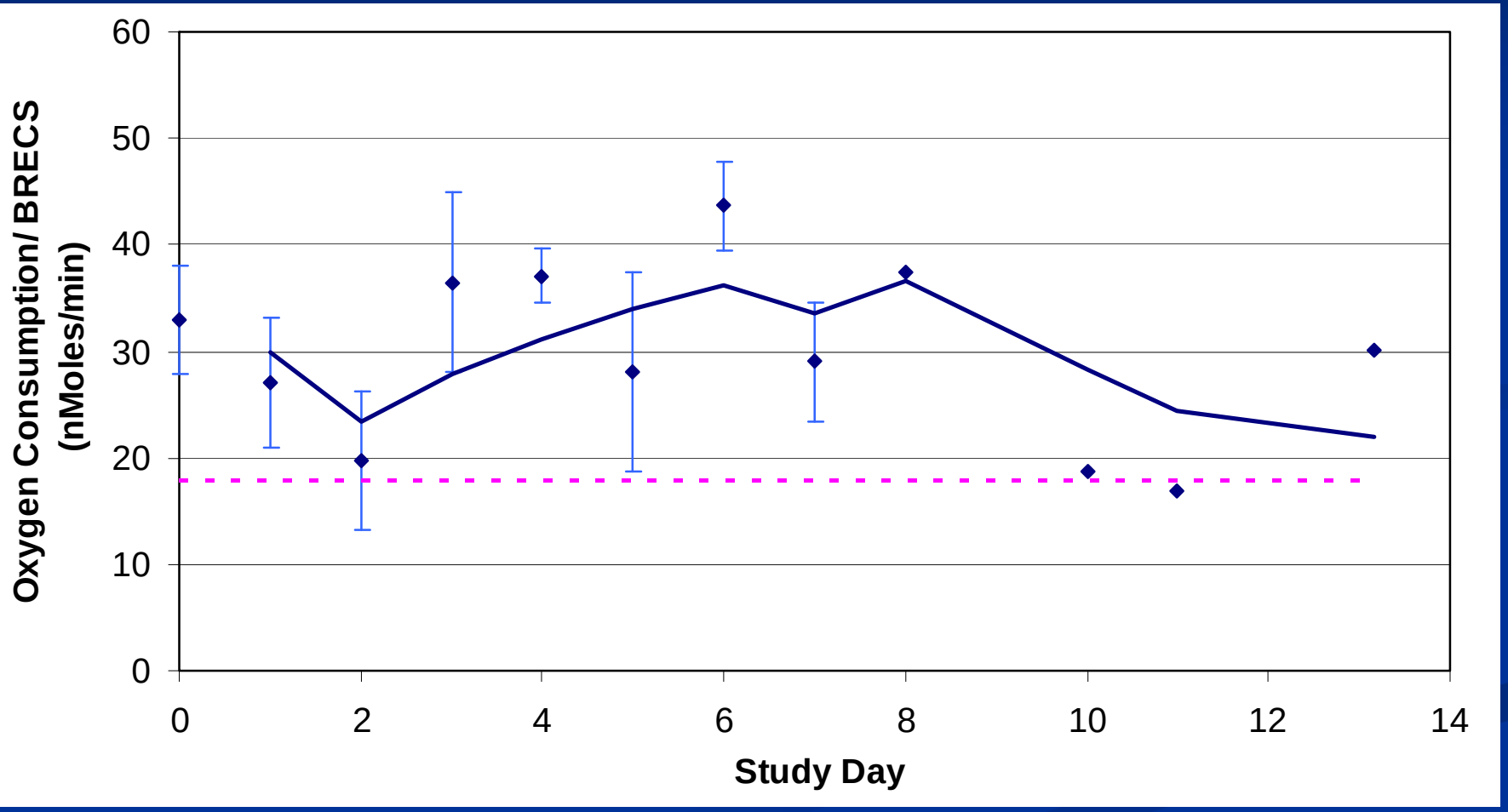
Requirement of Cell Therapy Devices

- 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

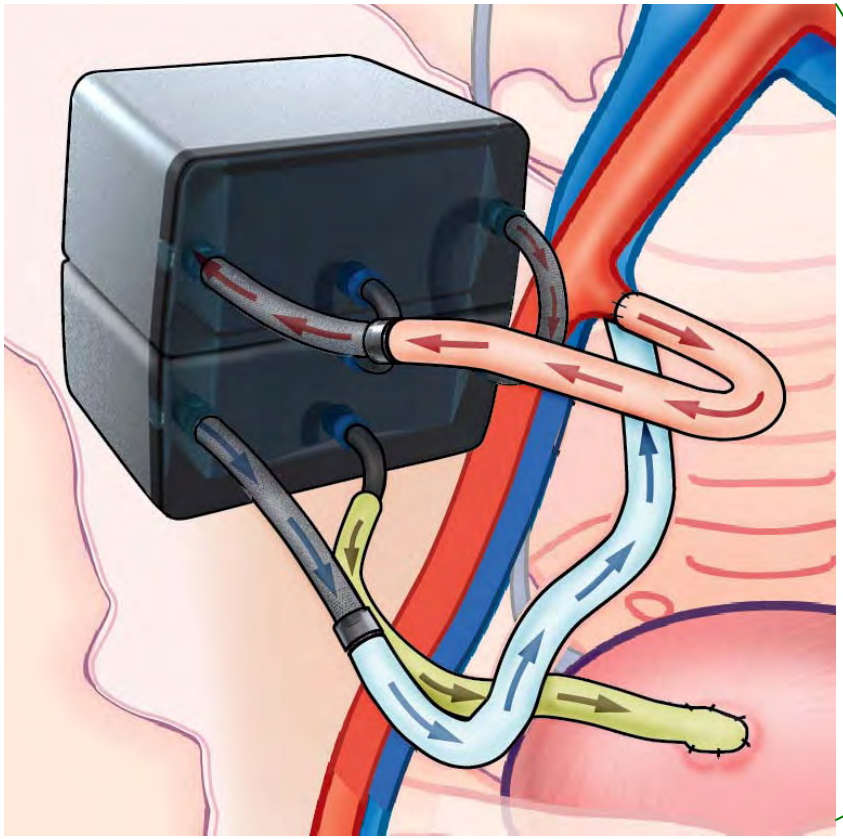


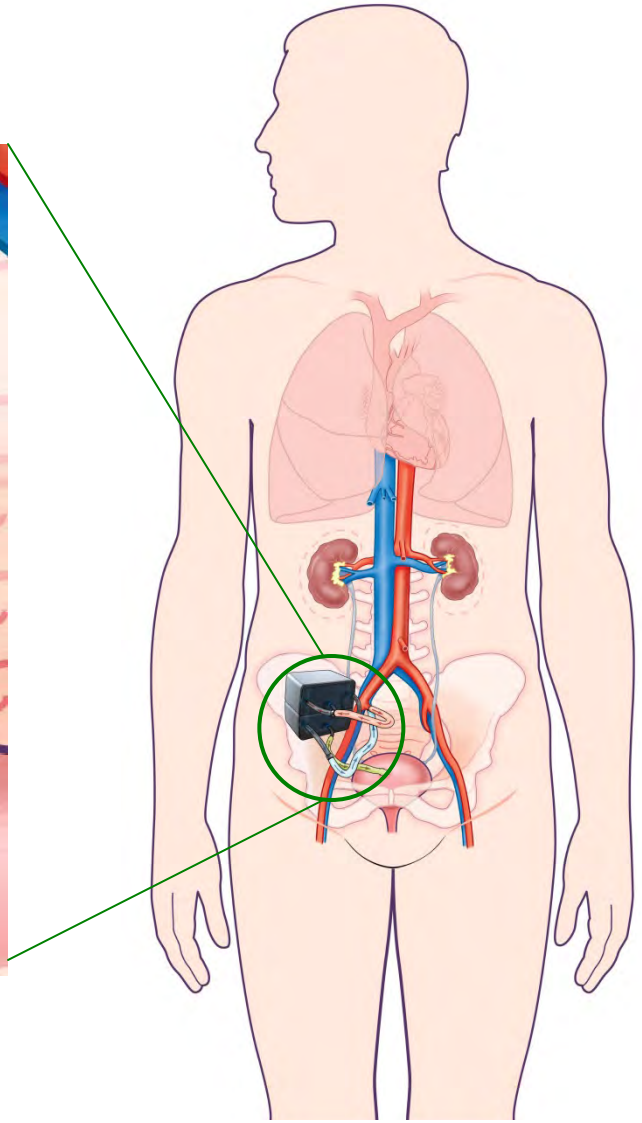
Note: Pink line shows average *in vitro* oxygen consumption for reference.

Goal: Implantable Bioartificial Kidney

UCSF University of California San Francisco

Shuvo Roy
Bill Fissell

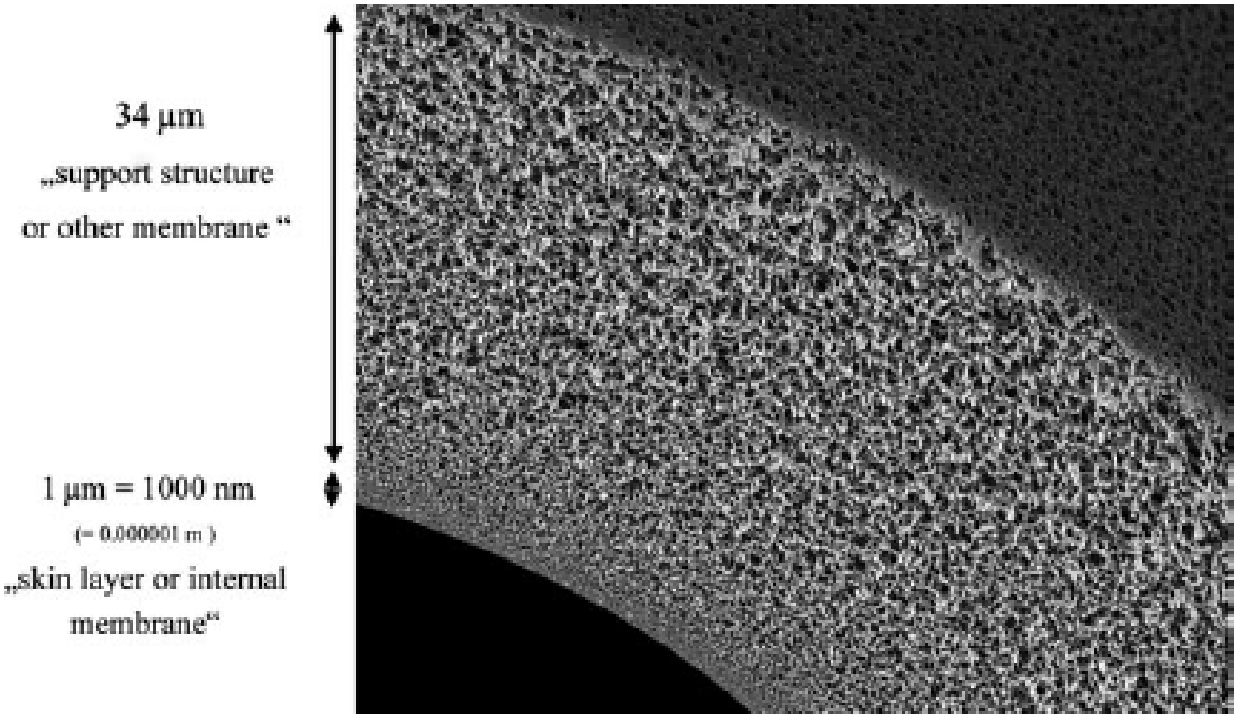




Fundamental Barrier to Miniaturization

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

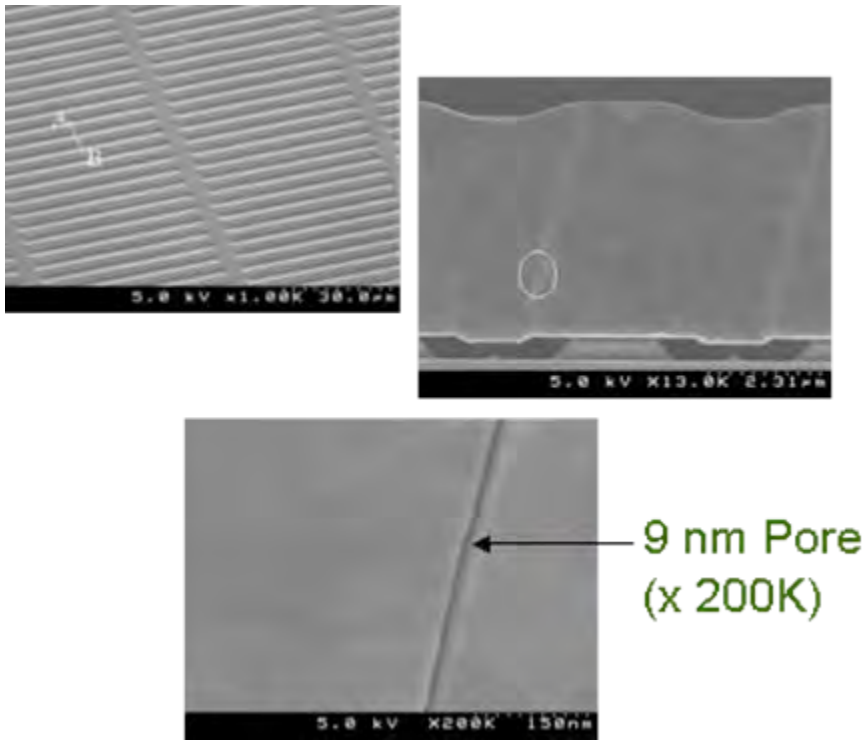
SEM – Polymer Membrane



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

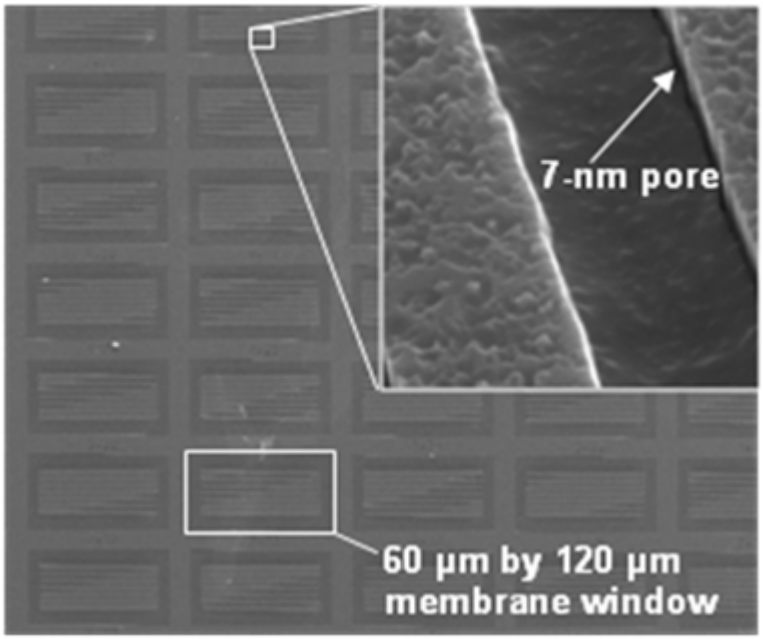
SEM – Silicon Membranes

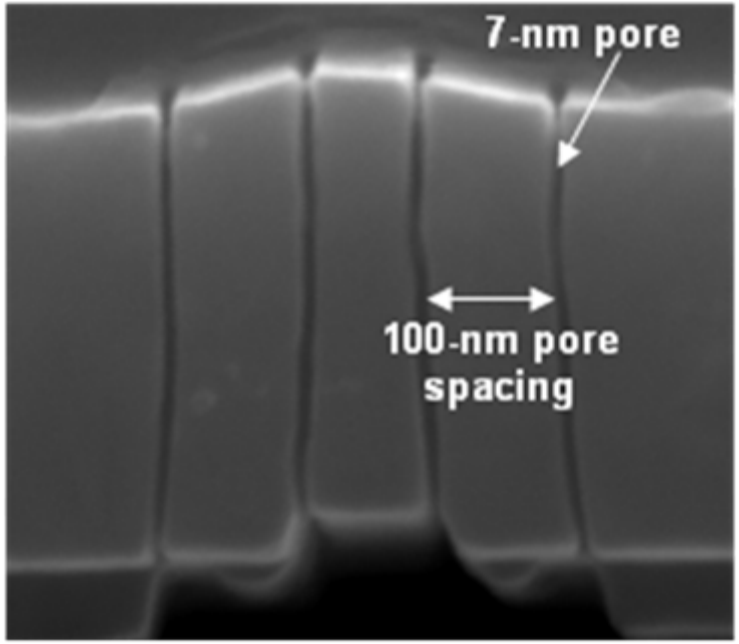


UCSF
University of California
San Francisco

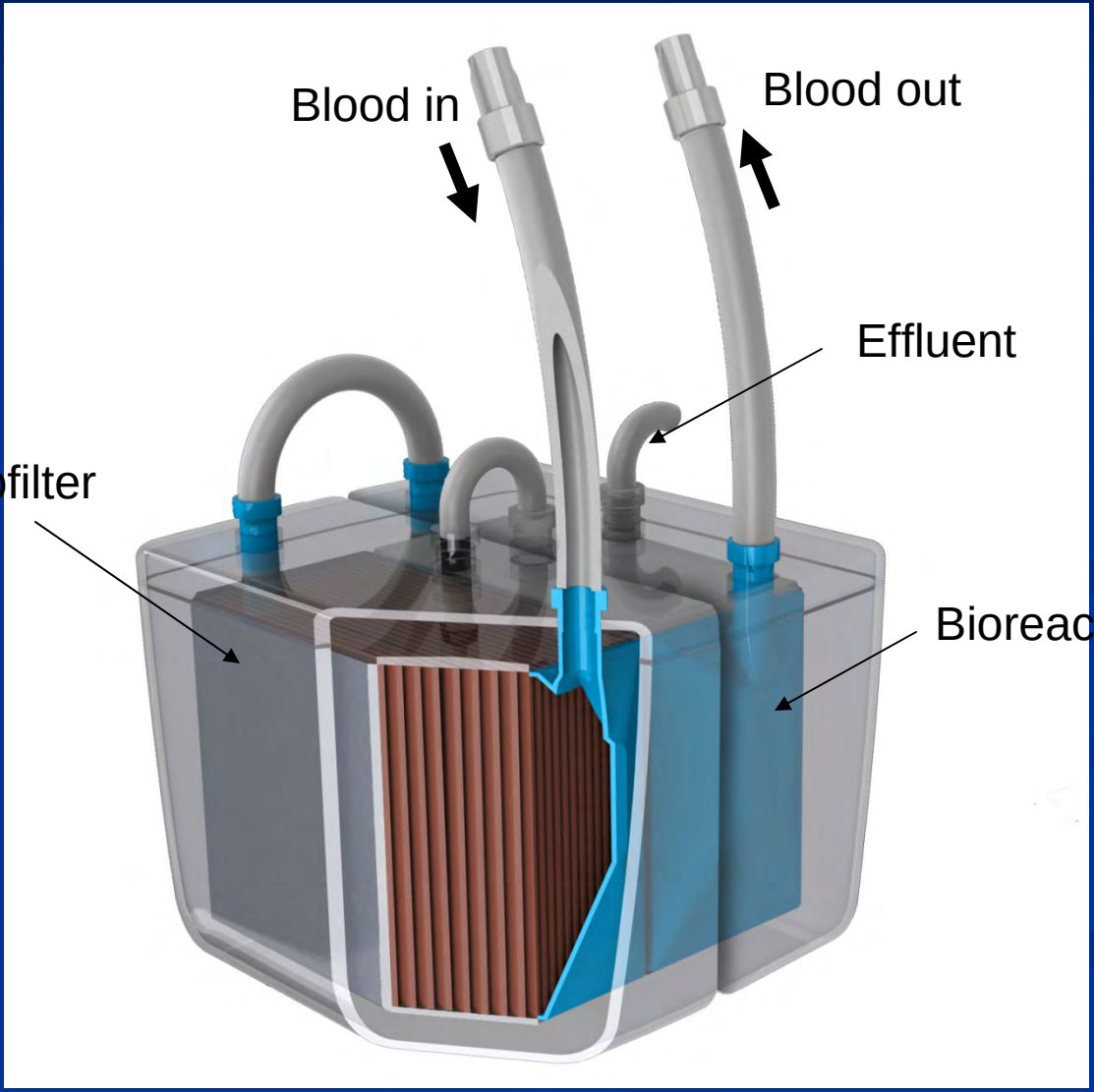
2nd Generation Membranes

- **High hydraulic permeability**
  - up to 600 ml/hr/mmHg/m^2
    - no pump needed
- **Manufacturing compatibility**
  - scalable for larger quantities

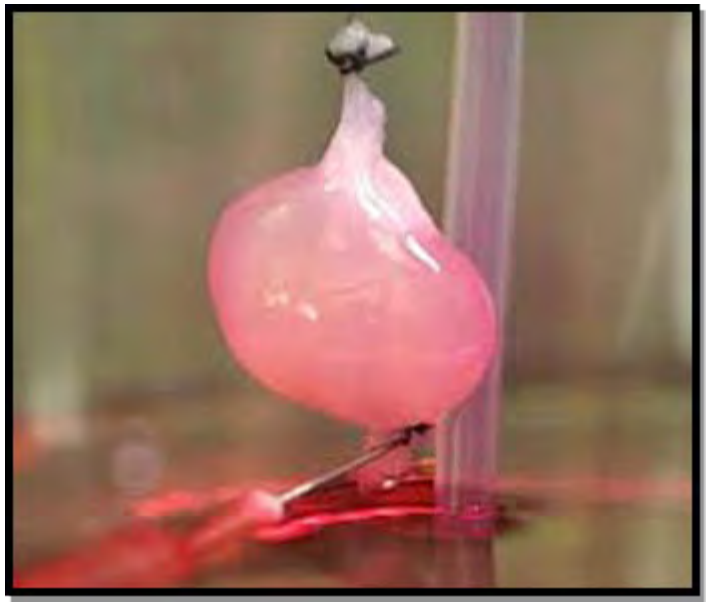




Phase II – Integration & Preclinical Tests



Decellularized Kidney with reseeding with cells



Cell Therapy Approaches

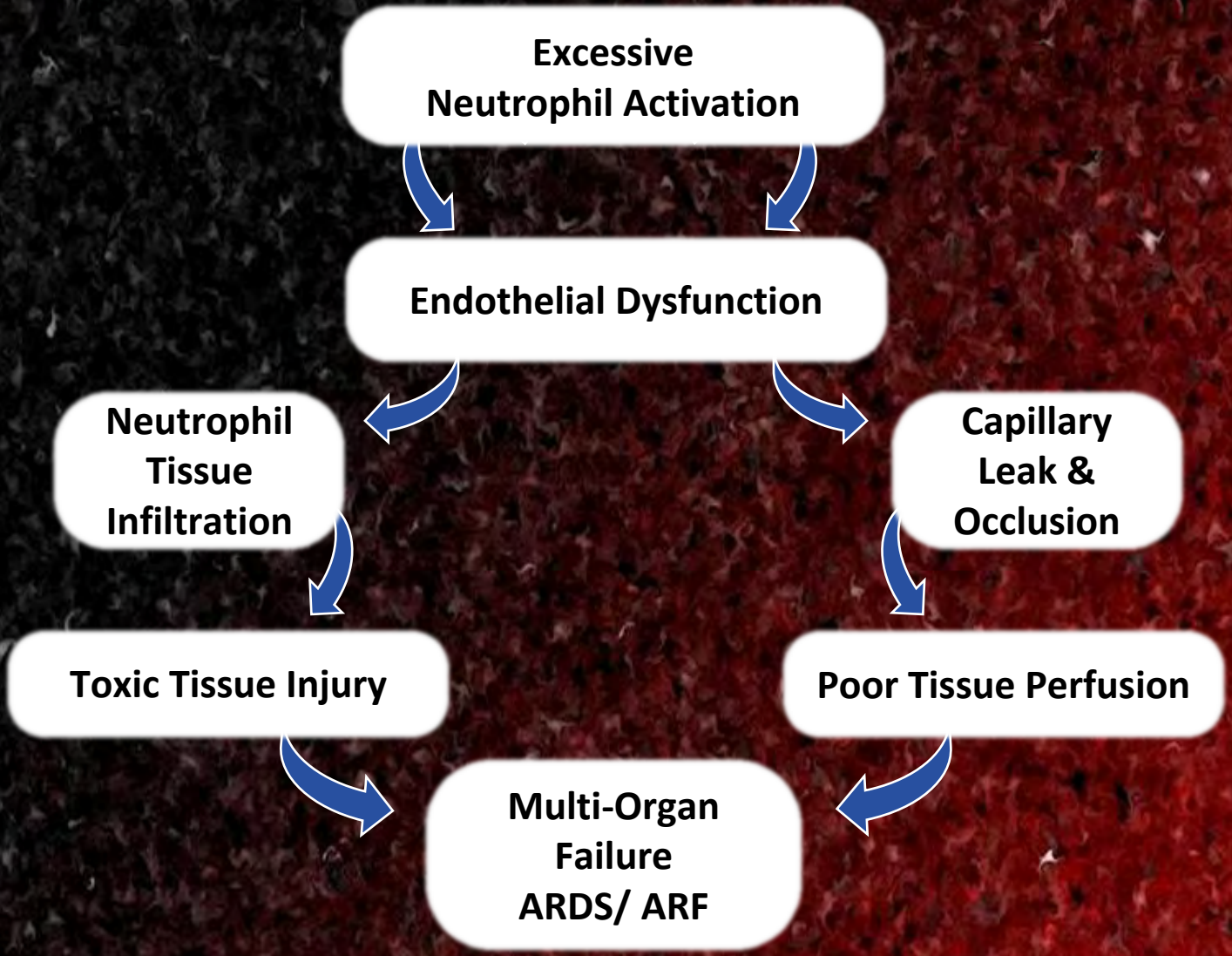
- Cell replacement of lost cellular components due to acute or chronic disease process

- 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

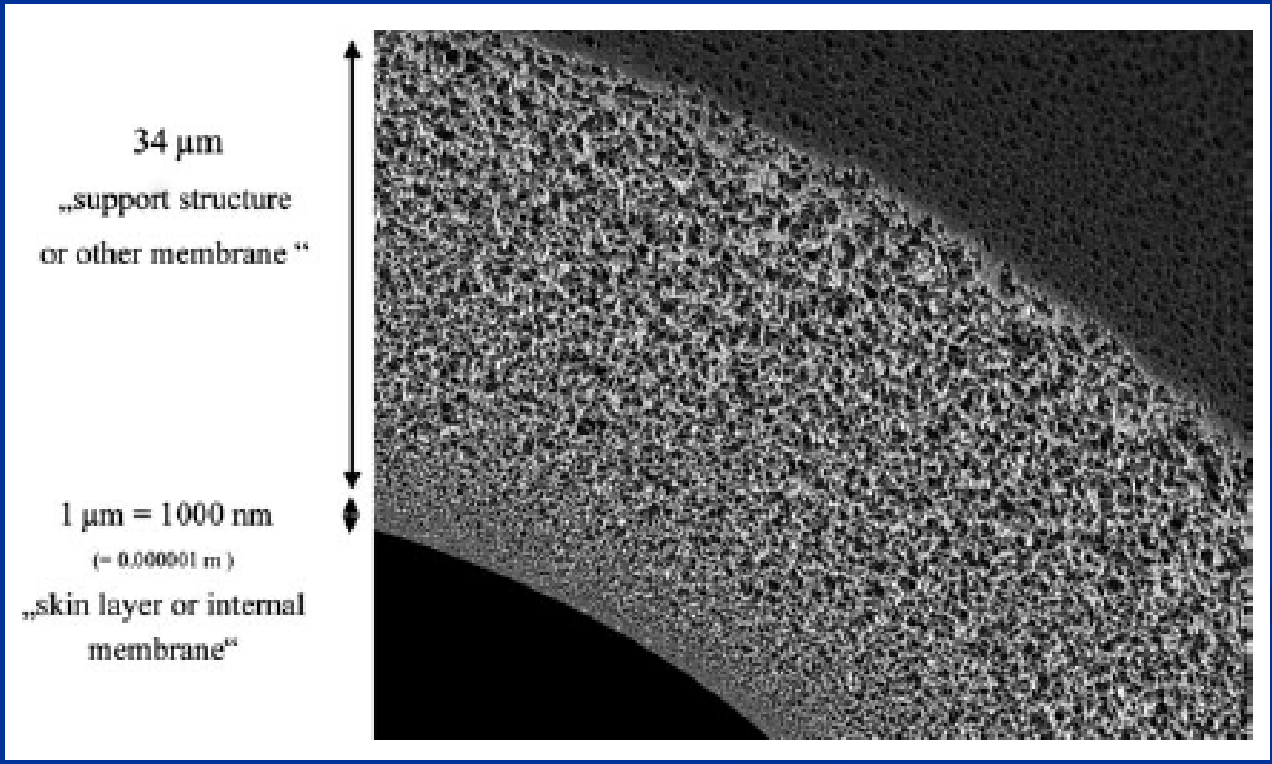
Systemic Inflammatory Response Syndrome SIRS

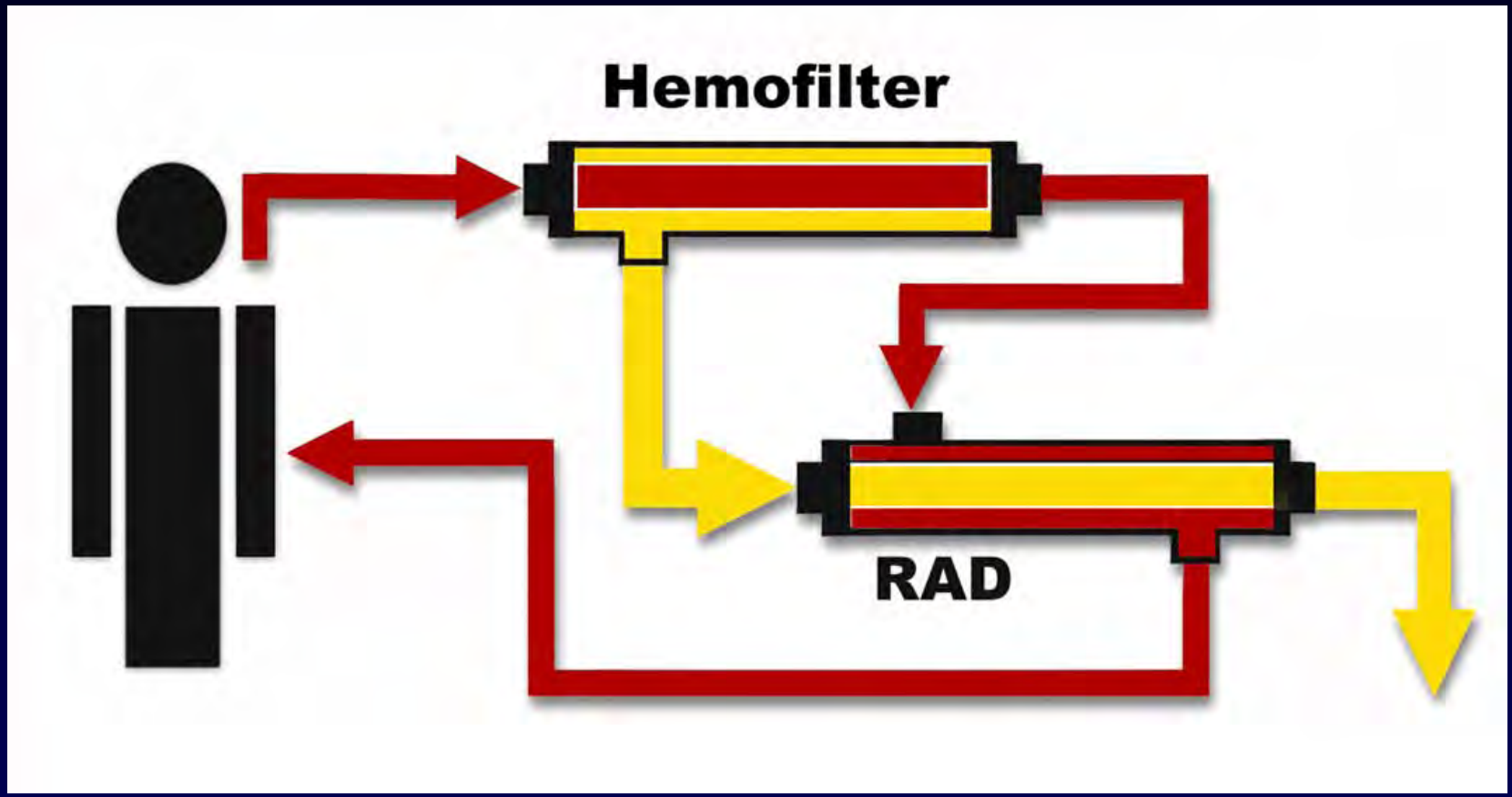


- Current hollow-fiber membranes to be used as a binding surface for activated leukocytes

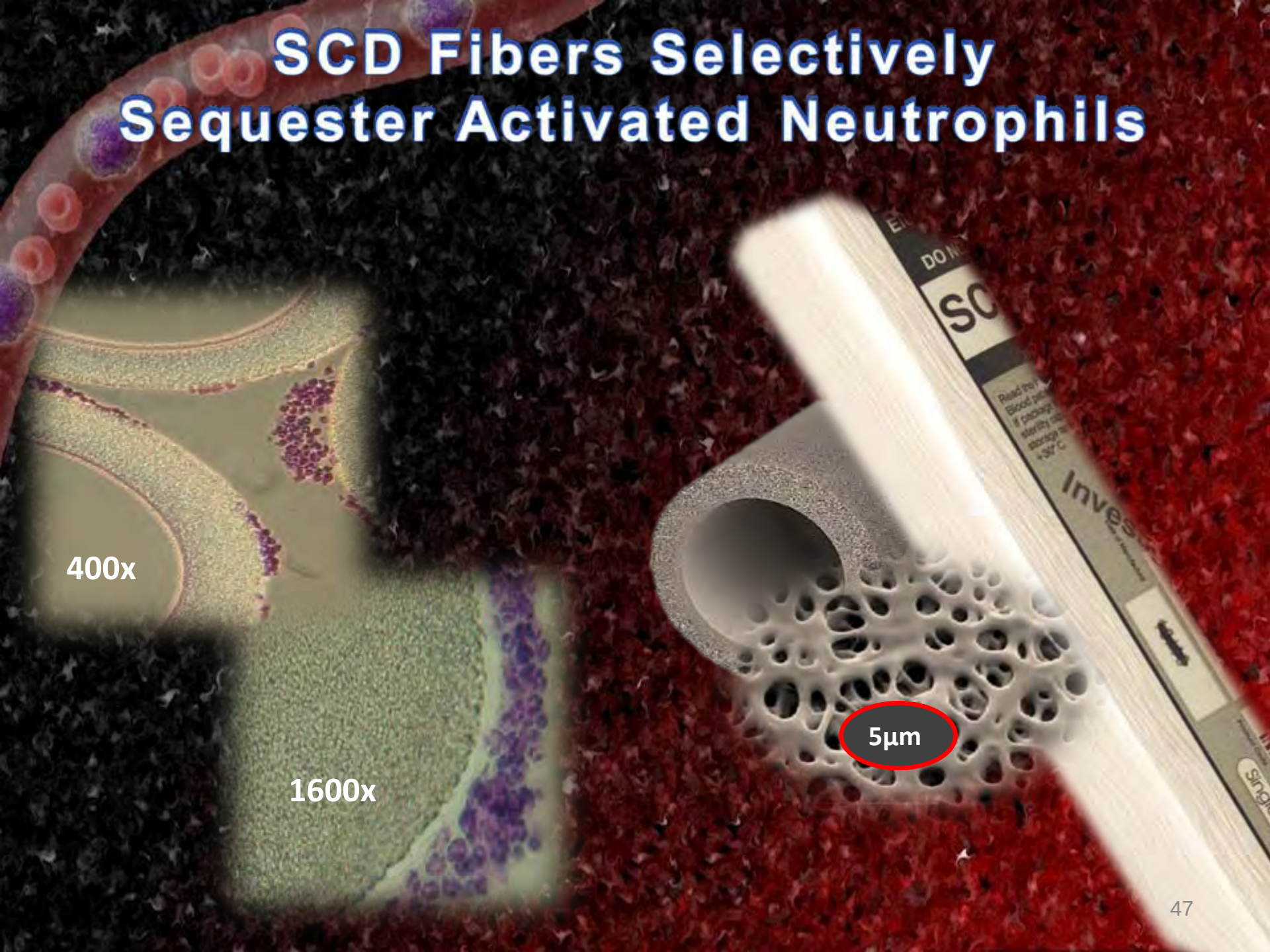
  Blood Purification 29:183-190, 2010

SEM – Polymer Membrane

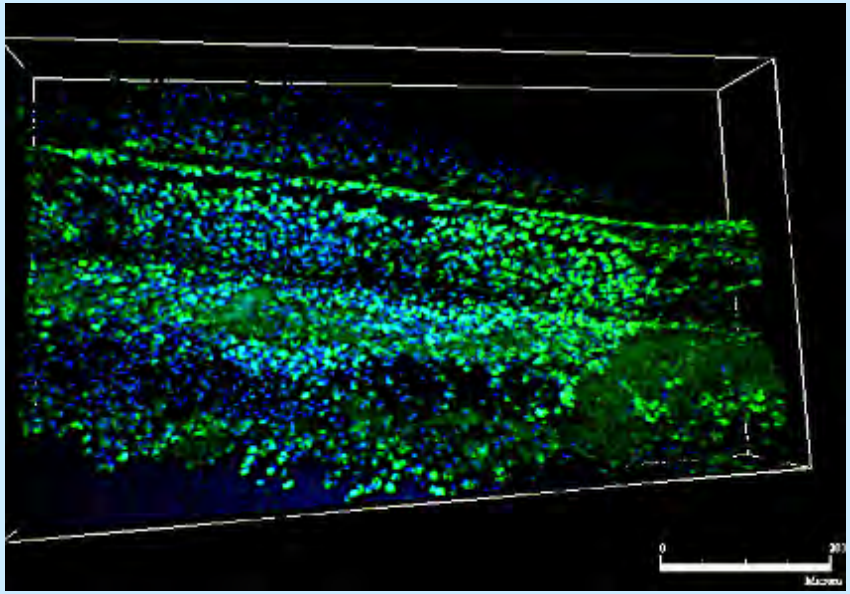


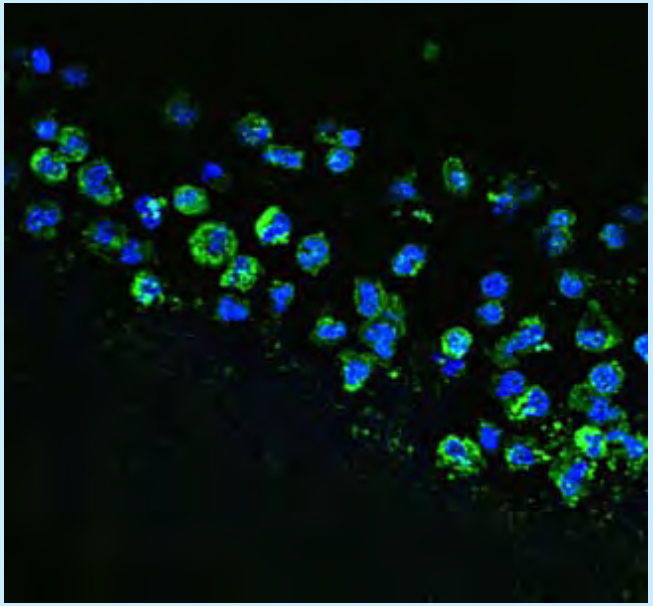

Hemofilter
RAD

SCD Fibers Selectively Sequester Activated Neutrophils

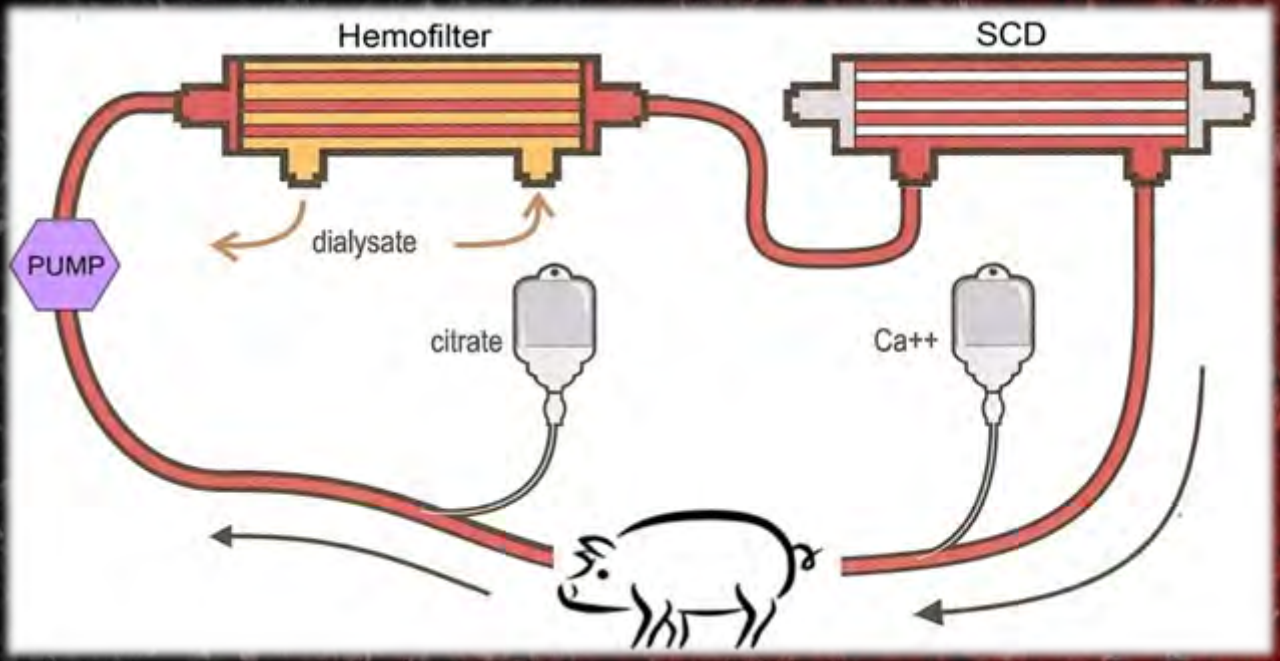


Leukocytes Adherent To Surface of Fibers In Dialysis Cartridge





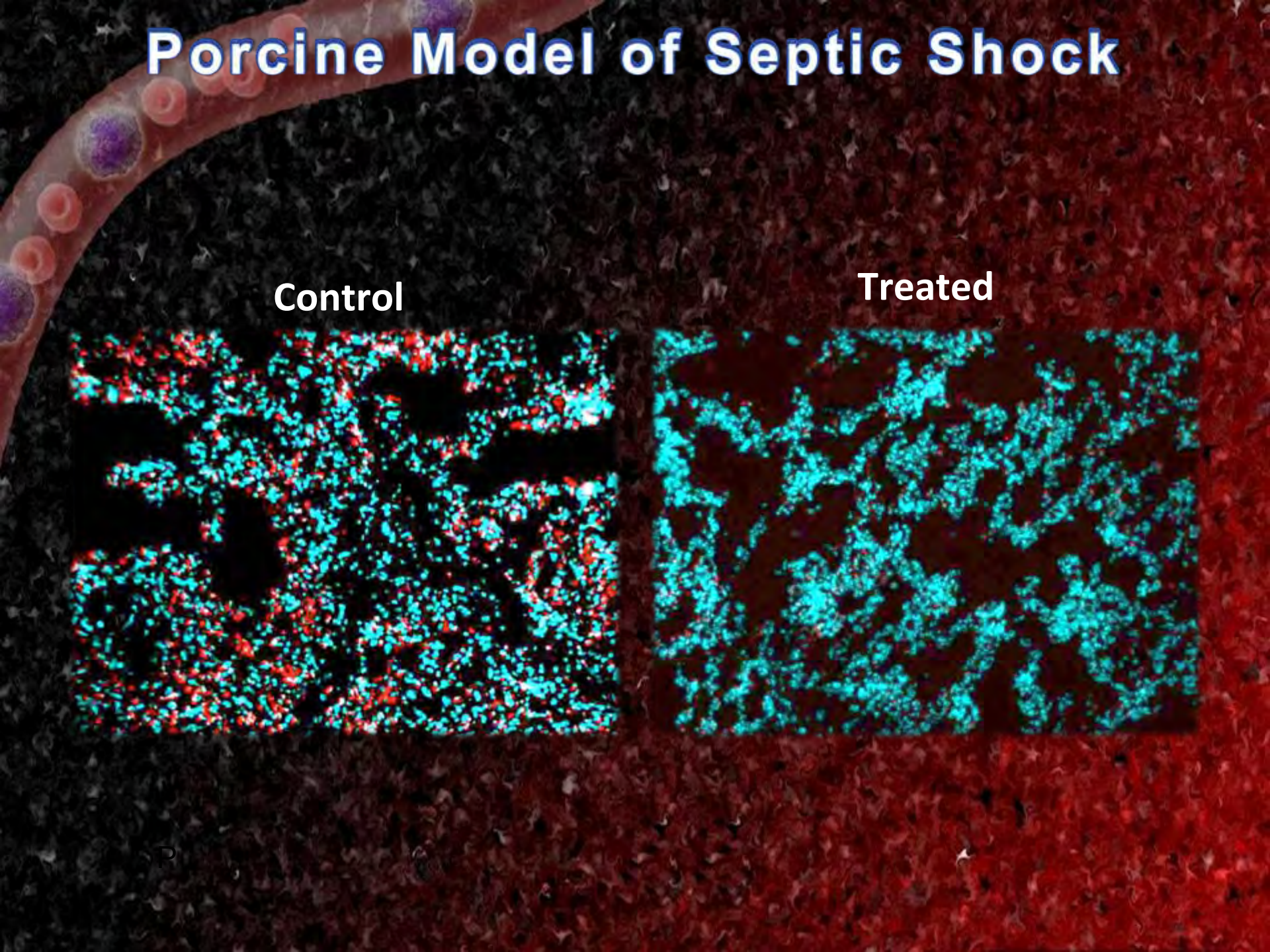
Porcine Model of Septic Shock
PLoS One 6:e18584, 2011



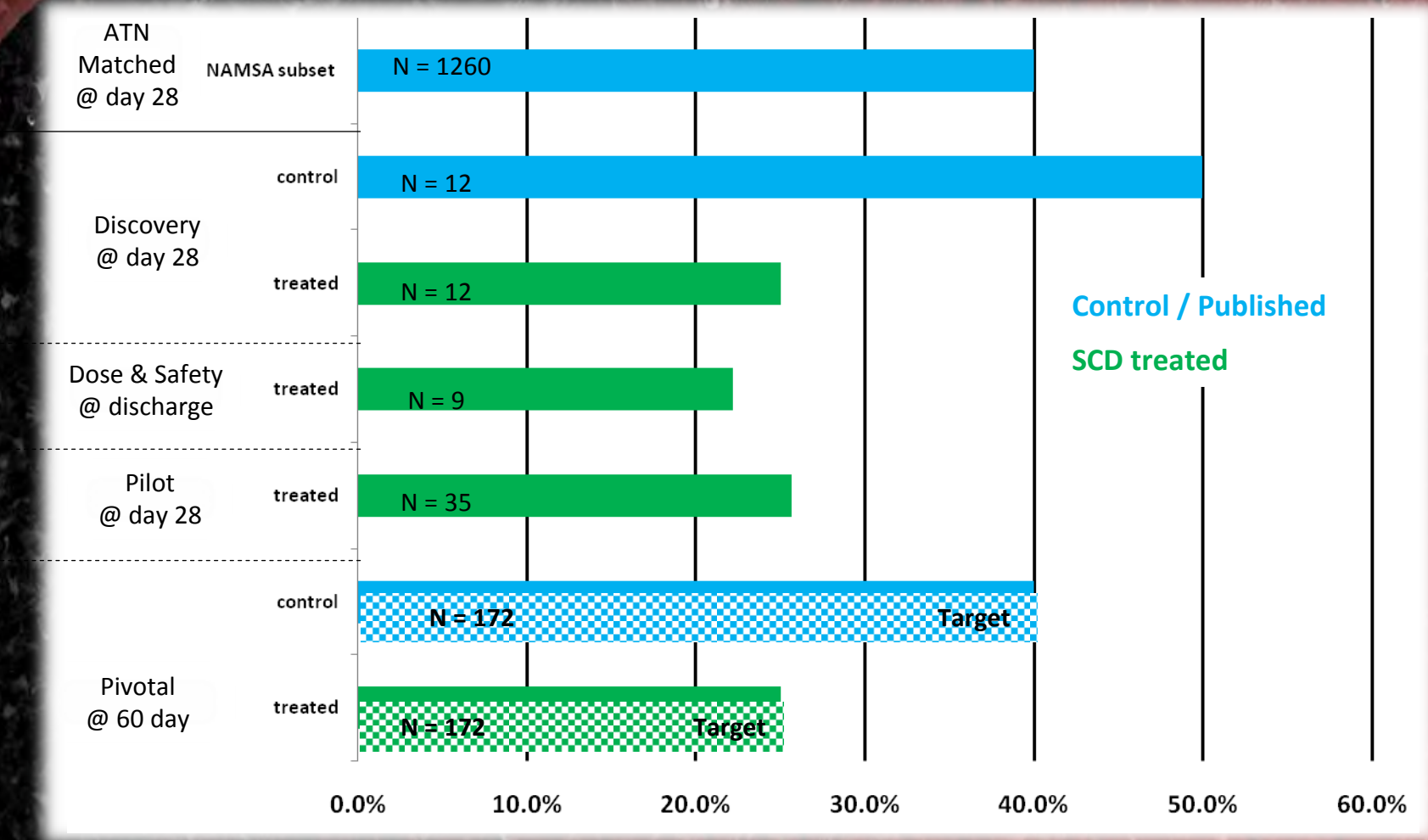
Conventional single-pump extracorporeal circuit using a polysulfone hemofiltration cartridge along with the SCD

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

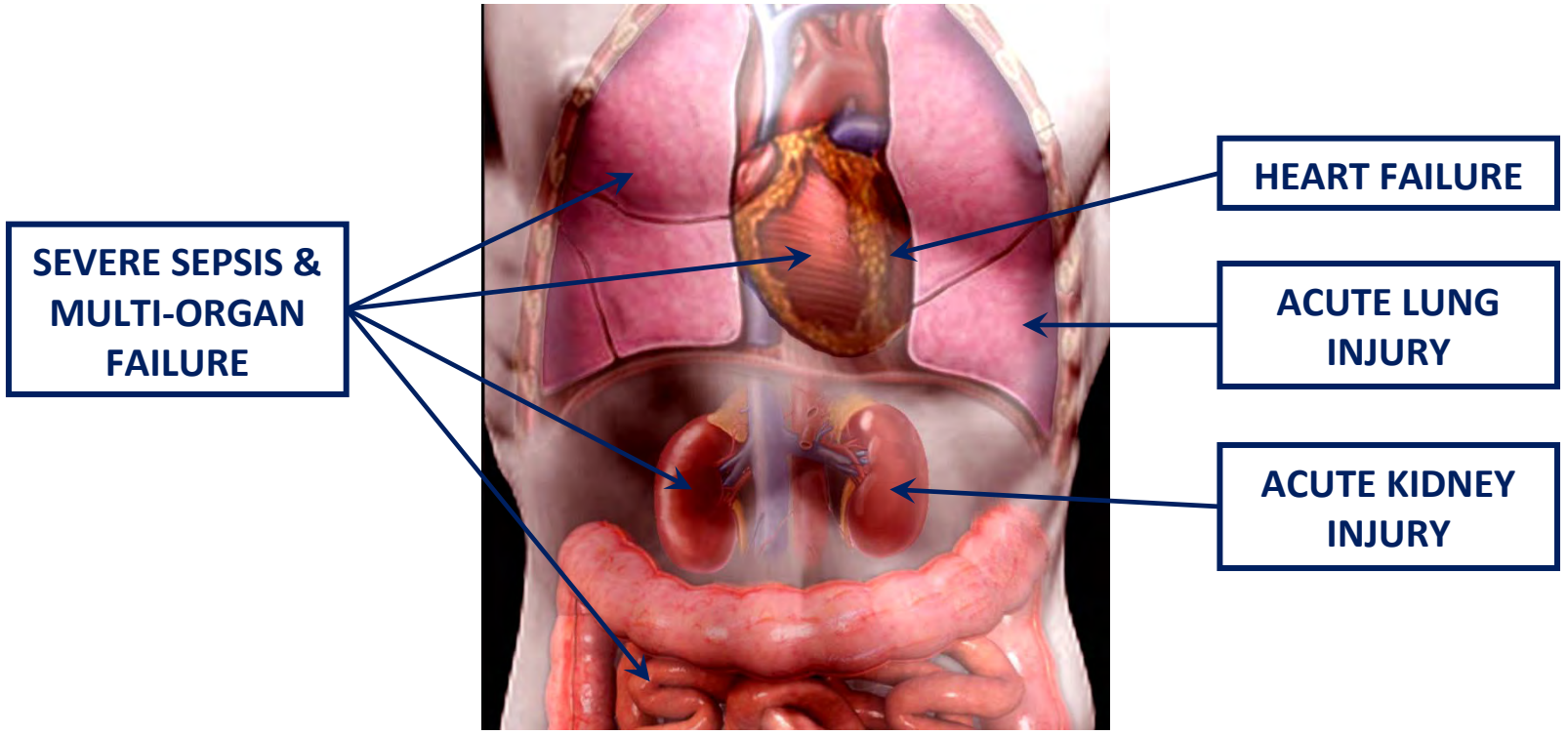
Porcine Model of Septic Shock



COMPELLING CLINICAL EVIDENCE



Excessive inflammation plays an important role in the underlying pathophysiology of multiple diseases, contributing to high morbidity, mortality and cost to treat.



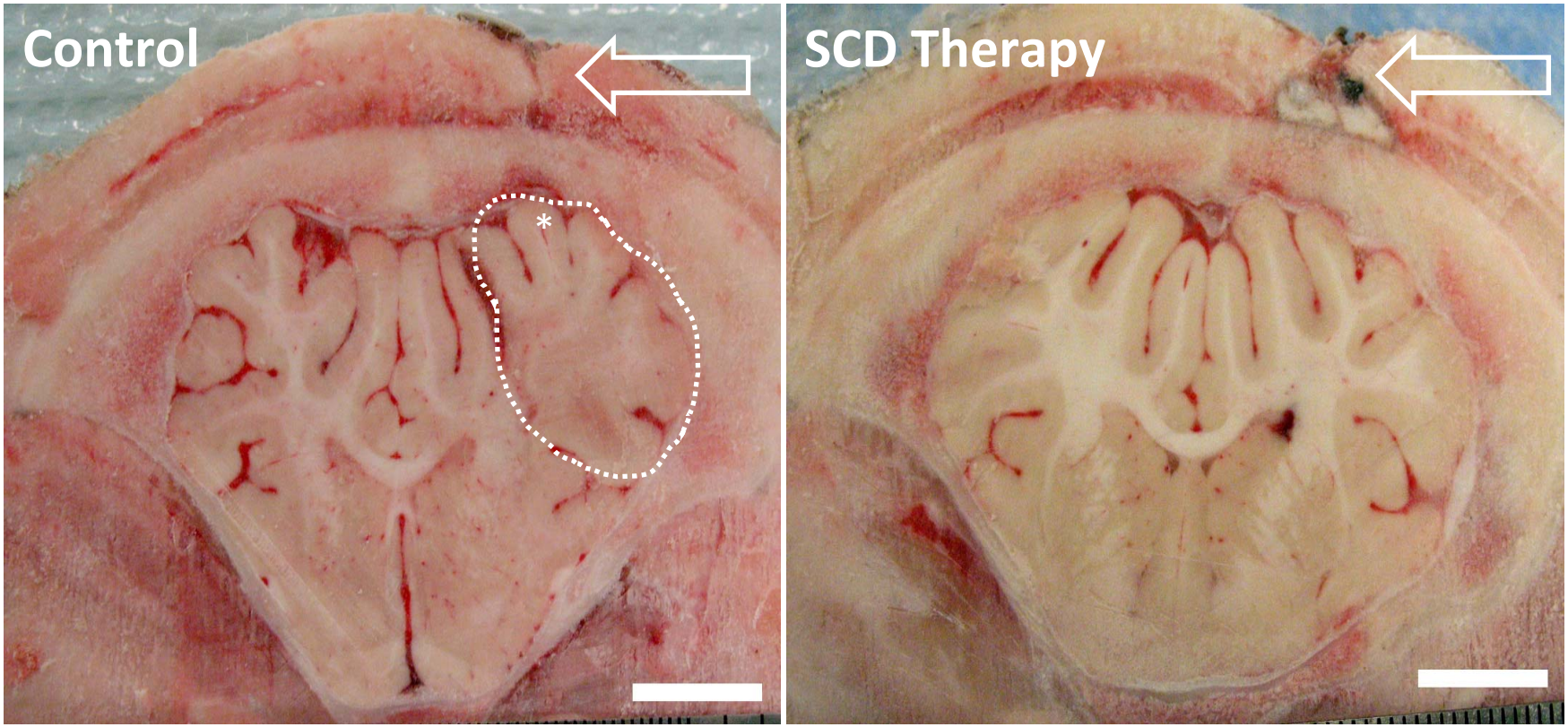
Other Clinical Indications

- 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

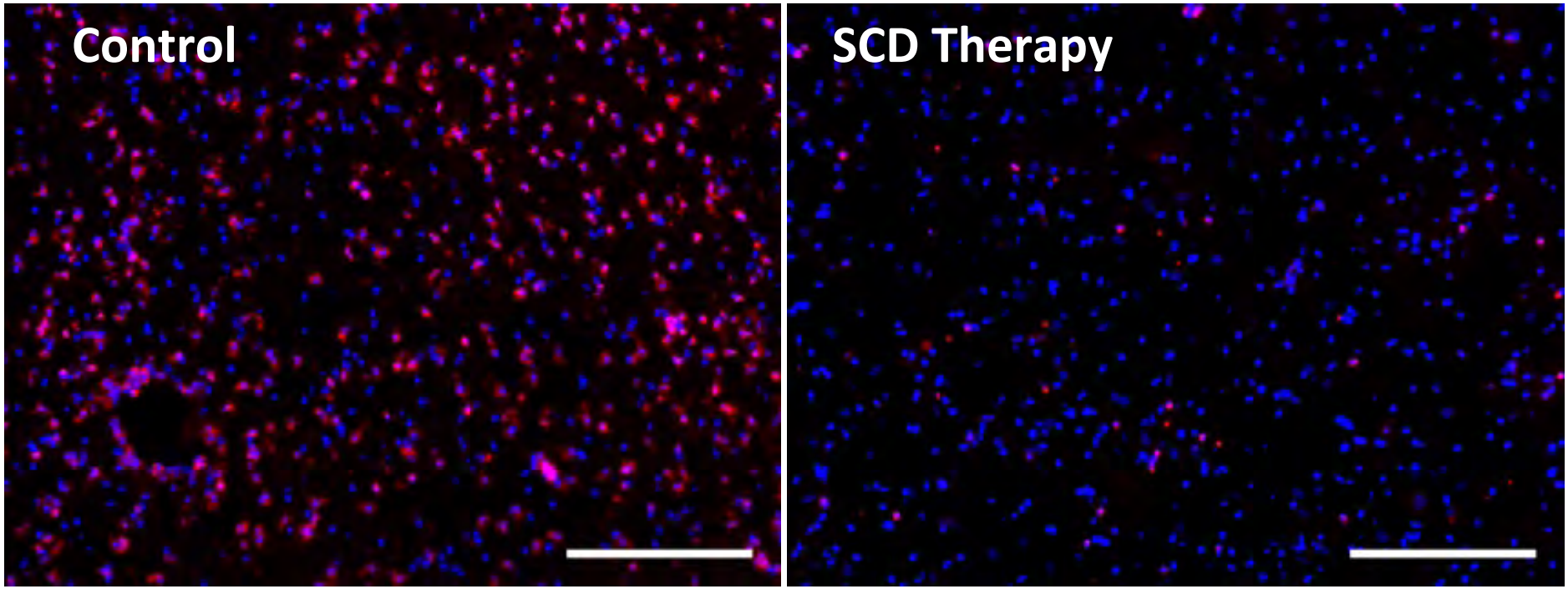
- Injection of thrombin into cerebral cortex stereotactily via burr hole in calvarium
- 24 hour treatment
- Brain excised, fixed, and processed for inflammatory activity, edema formation and degree of injury

BRAIN INJURY EVALUATED BY EDEMA 24HOURS POST INSULT



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.

BRAIN INJURY EVALUATED BY PRESENCE OF NEUTROPHILS IN EDEMATUOUS TISSUE 24HOURS POST INSULT



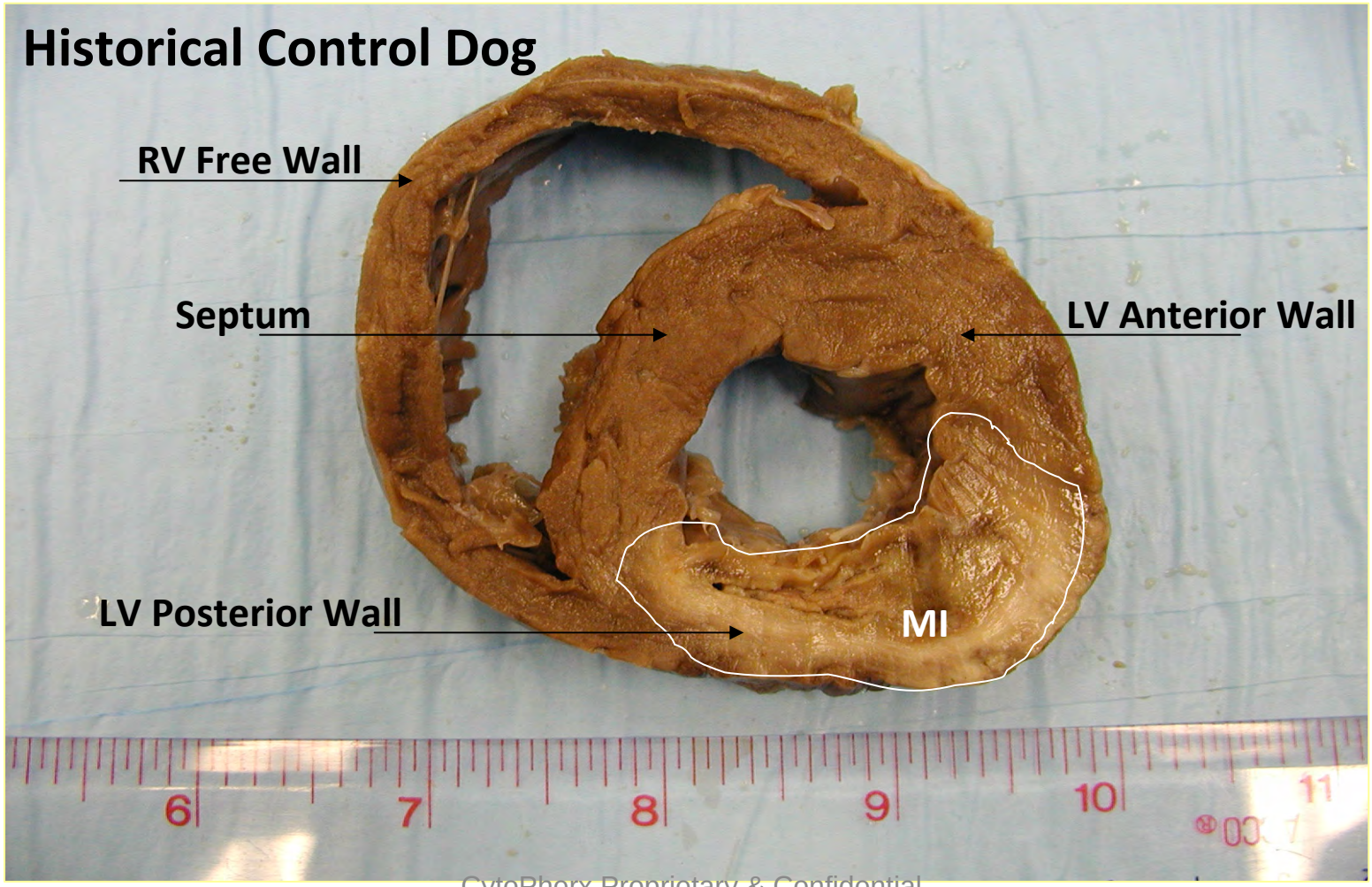
Neutrophils (NE), normally not present in brain tissue, migrate into sites of injury causing further damage. NE, identified by immunohistochemistry using a CD11b specific antibody (Red), were more prevalent in the untreated animal, indicating that SCD therapy can limit damage from ICH. Nuclei of all cells are counterstained with DAPI (Blue). Scale bar is 100 microns.

CANINE MODEL CIRCUMFLEX CORONARY ARTERY OCCLUSION & REPERFUSION

Methods

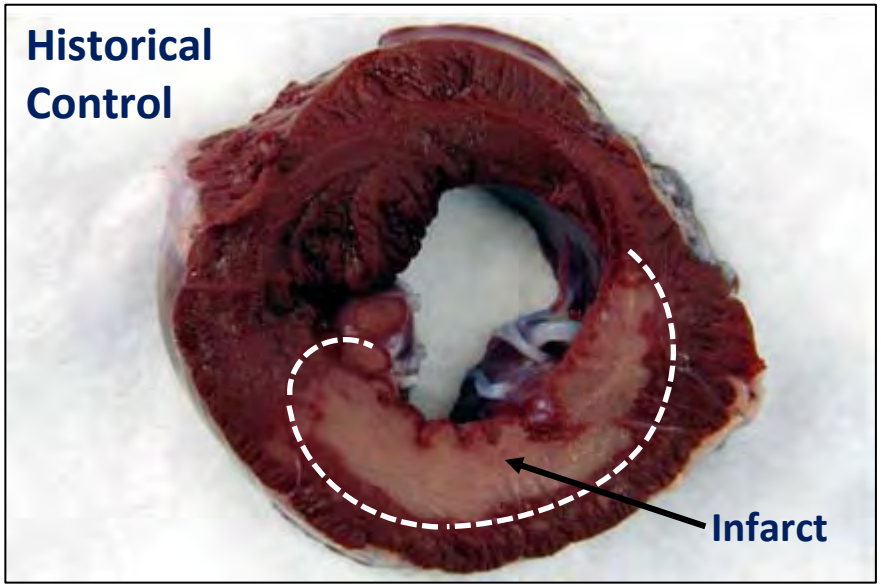
- close-chest dogs with AMI produced by balloon occlusion of the proximal Circumflex coronary artery
- Coronary occlusion was maintained for 3 hours followed by reperfusion
- SCD therapy initiated 30 minutes prior to reperfusion and maintained for 2.5 hrs

CANINE MODEL CIRCUMFLEX CORONARY ARTERY OCCLUSION & REPERFUSION

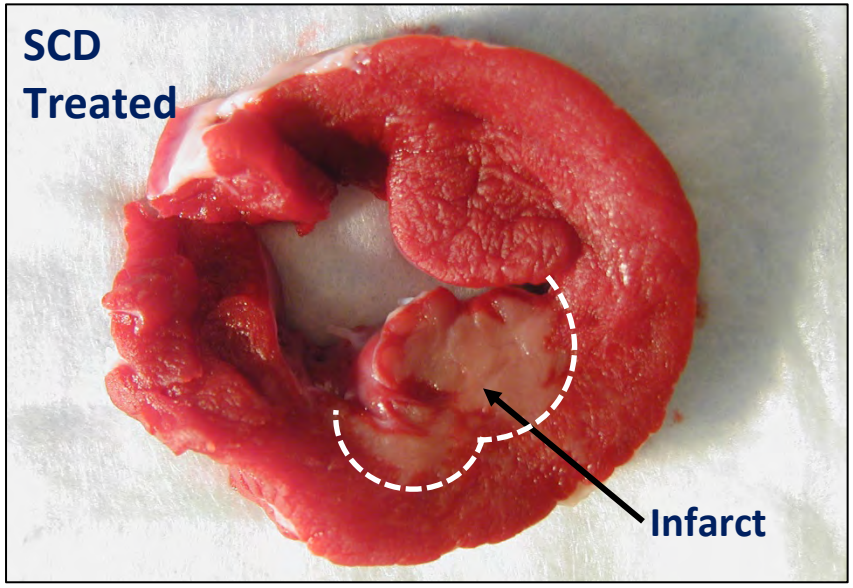


SCD – REPERFUSION INJURY

CANINE MODEL CIRCUMFLEX CORONARY ARTERY OCCLUSION & REPERFUSION



20.5% of total LV mass



6.1% of total LV mass

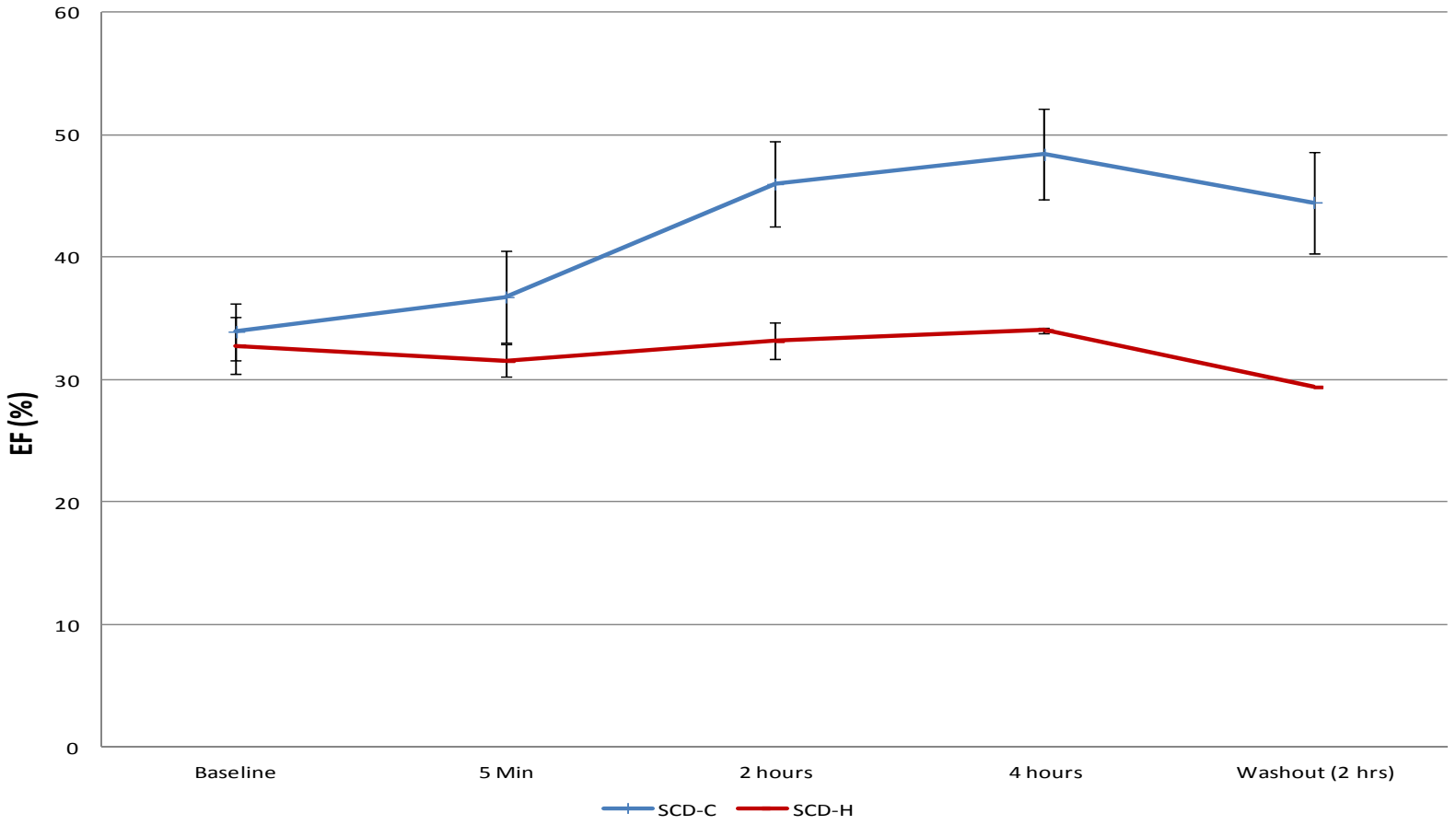
INFLAMMATION & CHRONIC ORGAN FAILURE

- 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

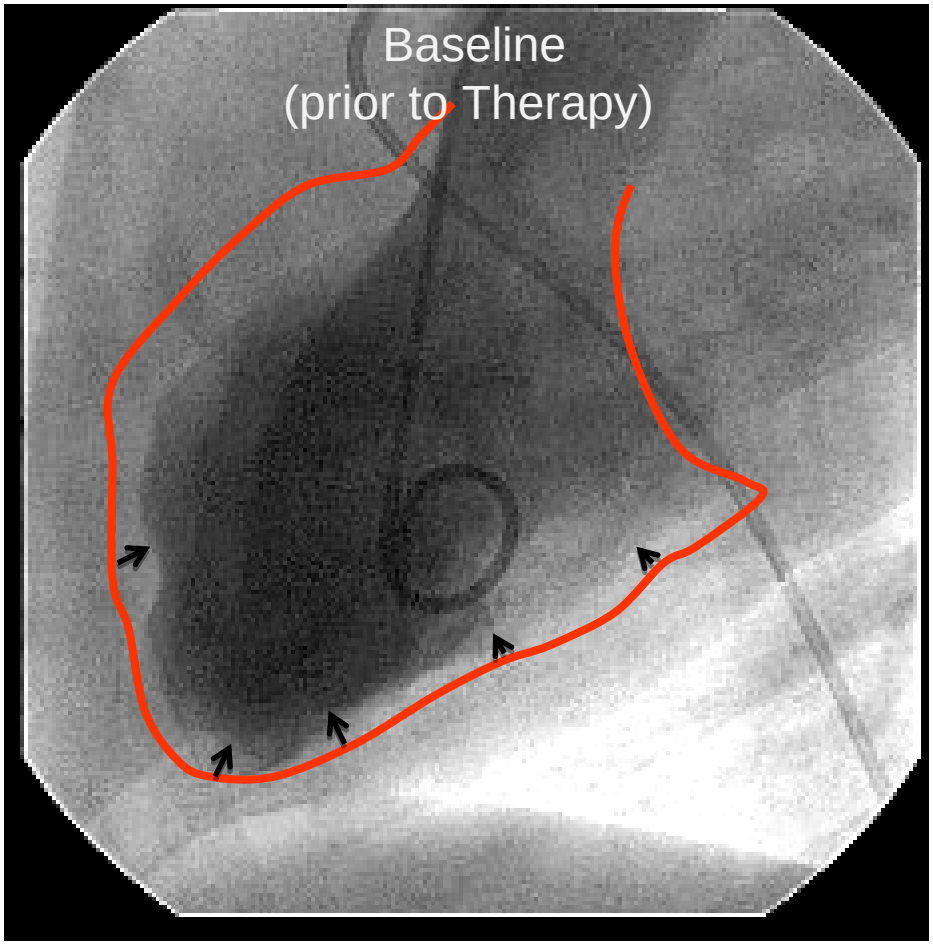
CHRONIC HEART FAILURE CANINE MODEL (AJP: H1379-84, 1991)

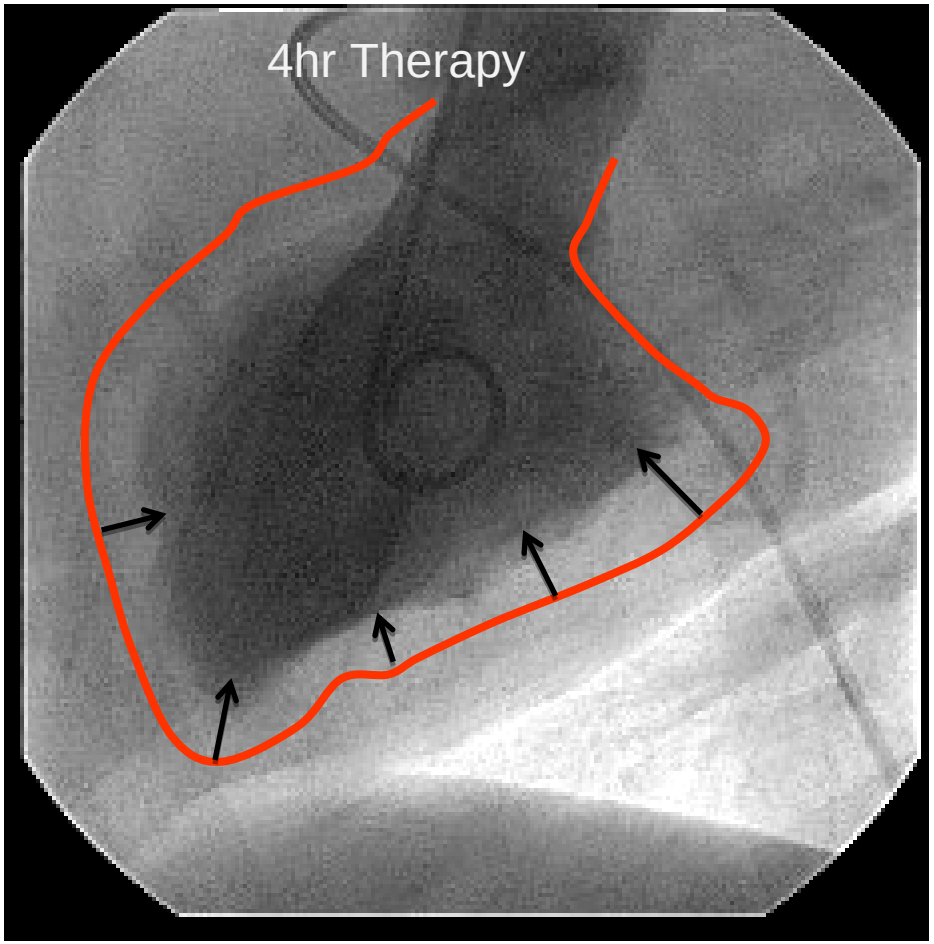
- Recurrent microembolization of small vessels in left ventricle over 6 weeks
- Reduction of ejection fraction from 55% to 25%
- Model has been consistent with efficacy of pharmacologic approaches in clinical CHF

EJECTION FRACTION IN CHF DOG MODEL



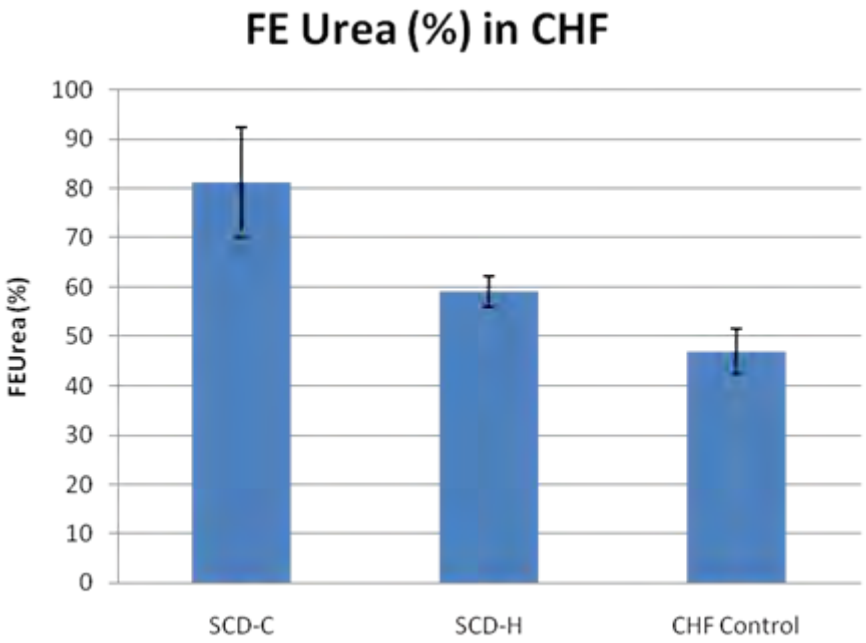
Therapy Impact on Cardiac Contractility

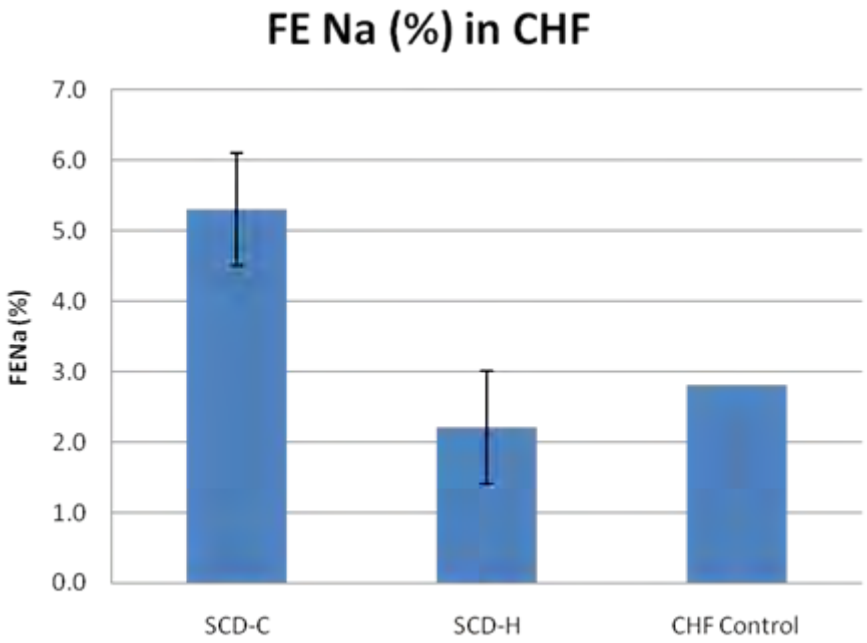




Ventriculograms of a CHF dog heart are shown at baseline (before therapy) and at the end of the 4 hour therapy session.
The red line depicts the border of the left ventricular diastolic silhouette (most relaxed state during filling) overlayed on the left ventricular systolic image (most contracted state),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

Fractional Excretion (FE) Urea and Sodium (Na)



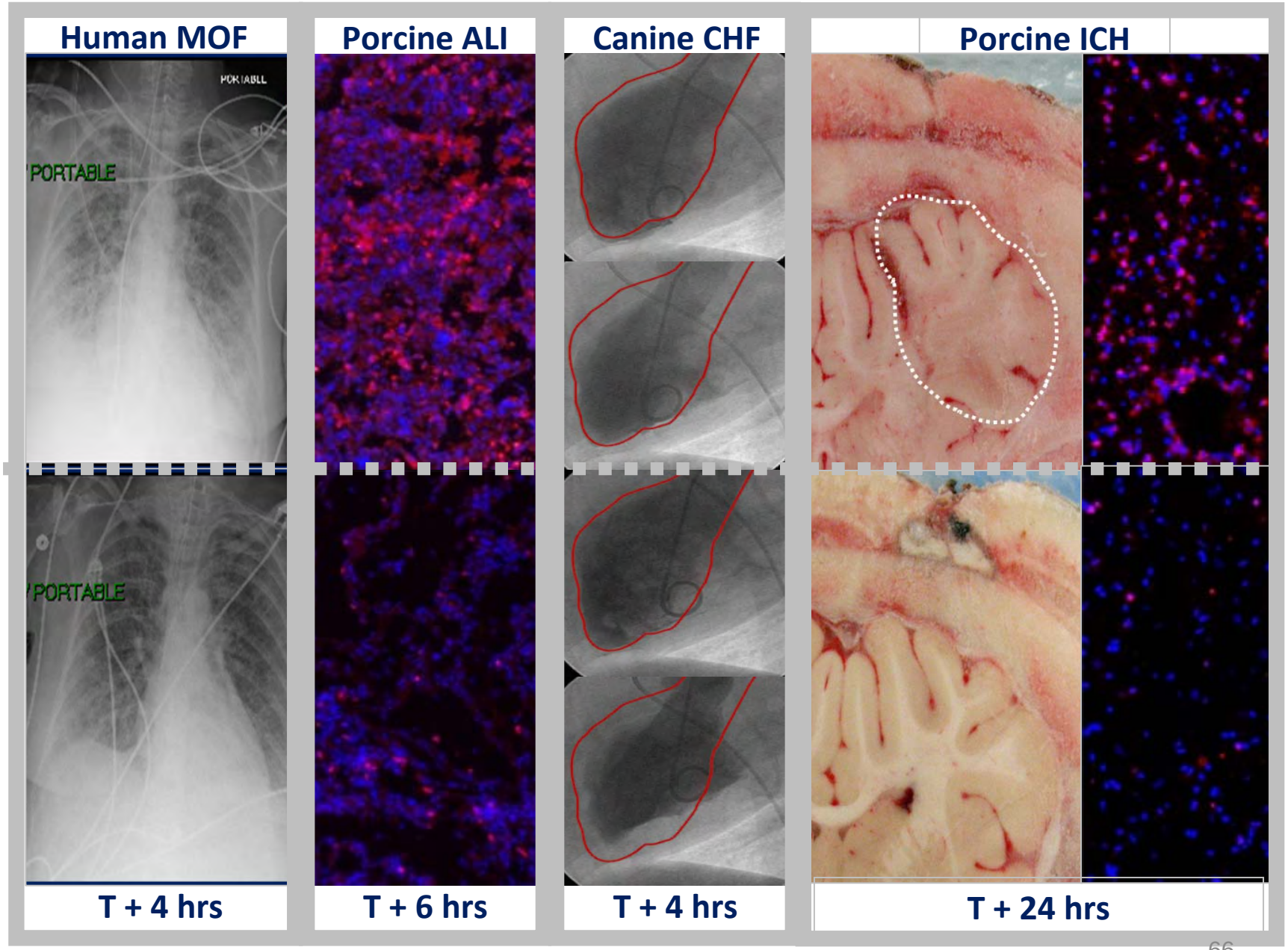


- Total Urine Sodium excretion doubled from control to 4hr SCD treatment.

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



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,