The Kidney Project

Annual Report 2014

Accomplishments, next steps, and requirements.

UCSF
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Letter from the Directors

Highlights. In reviewing the past year, The Kidney Project has made significant strides in progress. We completed Phase I and have moved into Phase II with success; specific highlights include the initiation of animal studies and demonstration of keeping kidney cells alive in the bioreactor for over 60 days, among other accomplishments.

Report. This report will present Phase I and II accomplishments thus far, as well as the next steps and requirements for successful completion of the device. We also present the philanthropic support we've received in the past year, as these gifts have been instrumental in our success.

Looking Ahead. The Kidney Project is an ambitious project and is not without its challenges. In the next year, our primary challenge is funding the research. A significant hurdle is procuring enough money in order to proceed further through the preclinical studies, which would allow us to build full-scale prototypes to generate data that will support the first round of human studies.

Shuvo Roy, PhD
Technical Director

William H. Fissell, MD
Medical Director

Kidney Failure: Current Statistics

The Problem. Chronic Kidney Disease (CKD), which is the slow, progressive loss of kidney function over time, affects more than 25 million people in the United States and countless worldwide. In the last stage of CKD, known as End Stage Renal Disease (ESRD), the kidneys fail completely. Kidneys not only filter blood and excrete wastes, but they also perform many vital biological functions including the regulation of blood pressure, fluid volume, serum osmolality, red blood cell mass, and electrolytes. In addition, the kidneys also play roles in antigen presentation, cytokine synthesis, and dissipating oxidative stress. The diversity of important kidney functions means that patients with renal failure, most frequently attributable to high blood pressure, diabetes, autoimmune disease, injury, and toxins, are extremely ill. ESRD affects almost 600,000 people per year in the United States, and the number of patients diagnosed with the condition is increasing at around 5% annually.

Transplant. The best current treatment for ESRD is kidney transplant; yet there is an acute shortage of donor organs. In addition, some patients have sensitive immune systems that reject any transplanted tissue. By the end of 2014, there were over 100,000 ESRD patients on the transplant wait list in the United States, yet there were less than 20,000 donor organs available for transplant. Further, the availability of donor organs has leveled off at a time when the donor wait list continues to grow at 10% per year. For those fortunate patients who receive kidney transplants, the outlook is positive; the five-year survival rate for transplant recipients is over 83%.

Dialysis. At present, the only alternative to transplant is dialysis, which is a procedure fraught with morbidity and eventual mortality. A typical dialysis schedule is three sessions per week, for three to five hours per session, during which blood is pumped through an external circuit for filtration of uremic toxins. Dialysis does not provide the other biological functions of kidneys. This procedure is exhausting for patients and they experience pervasive complications, such as infection and metabolic deficiencies. The mortality for dialysis patients is high; only 35% of patients are alive after five years.

Cost. In the United States, dialysis costs $82,000 per patient annually, which accounts for $29 billion of the overall Medicare budget. The 1% of the Medicare population with ESRD, approximately 600,000 individuals, inordinately account for 7% of the budget. The societal costs are even greater, since only 10% of dialysis patients have regular employment. By contrast, for those individuals fortunate to receive transplants, the annual health care cost is $29,000 per patient.

The Kidney Project: Overview

The Kidney Project is developing a device that offers the health benefits of a kidney transplant while addressing the limited number of donors. The device is a bioartificial kidney, the size of a coffee cup, which filters toxins from the blood, while also providing other biological functions of a healthy kidney. After a single surgery to establish a permanent blood connection, the bioartificial kidney processes blood continuously for 24 hours per day, which mitigates the inconveniences and morbidities associated with intermittent hemodialysis.

The implantable bioartificial kidney builds upon the earlier extracorporeal Renal Assist Device (RAD) developed by Dr. H. David Humes at the University of Michigan. The RAD is a bioartificial kidney that combines a membrane hemofilter and a bioreactor of human renal tubule cells to mimic many of the metabolic, endocrine, and immunological functions of a healthy kidney.

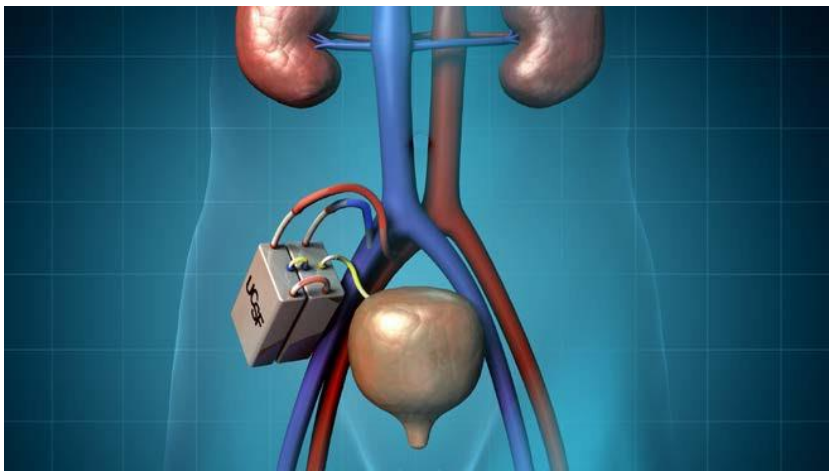
While clinical trials confirmed that the RAD could safely treat acute renal failure in a critical care setting, its adoption for routine treatment of ESRD patients is hindered by its labor-intensive and complex operation, large size, and high marginal cost.

The ultimate goal of The Kidney Project is to apply microelectromechanical systems (MEMS) and nanotechnology to miniaturize the extracorporeal RAD into a surgically implantable, self-monitoring and self-regulating bioartificial kidney.

The bioartificial kidney is a two-stage system that consists of:

- a pump-less silicon hemofilter to remove toxins and excess water, and
- a renal tubule cell bioreactor to provide other biological functions of a healthy kidney.

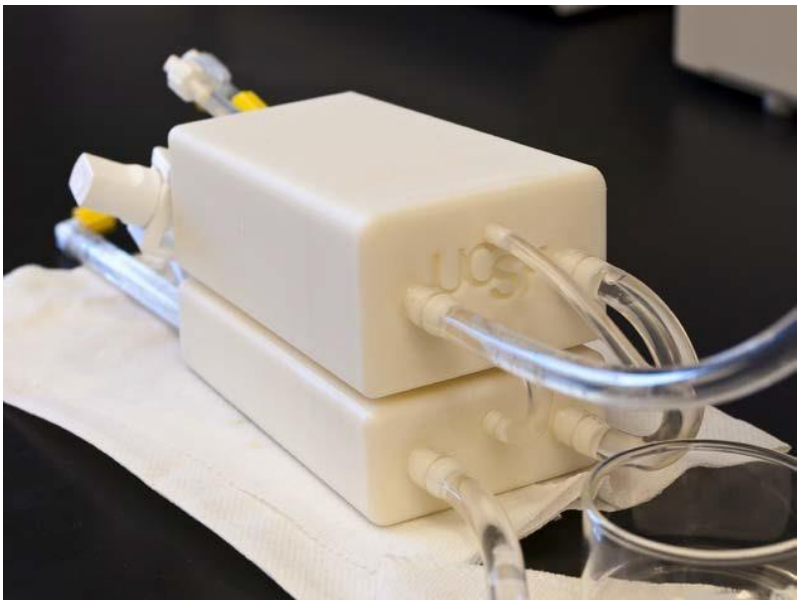
Because the kidney is not merely an excretory organ, this two-stage system will replicate much of the transport, homeostatic, metabolic, endocrinological, and immunoregulatory functions of a healthy kidney.



Phase I: Accomplishments

In Phase I, once we established concept feasibility by testing the external large-scale system of the filter and bioreactor on ICU patients, we developed the membrane technology. Our team fabricated robust silicon membranes using semiconductor manufacturing techniques and assessed them for safety and blood compatibility. We also developed thin film coatings to enhance blood biocompatibility and conducted successful short-term testing of blood compatibility of small implanted prototypes in rats and pigs.

For the cell bioreactor, recent advances in the field of tissue engineering are being leveraged to grow renal tubule cells, which will impart biological activity such as autoregulation of blood pressure and production of Vitamin D. By better mimicking healthy kidney function, the bioartificial kidney alleviates the necessity of constant physician oversight and a lifelong regimen of immunosuppressant medication. Research completed during the first phase isolated and expanded renal proximal tubule cells from human kidneys, demonstrated viable cryopreservation of cells for long-term storage, and confirmed metabolic function of small wearable prototypes in pigs and sheep.



Phase II: Progress and Requirements

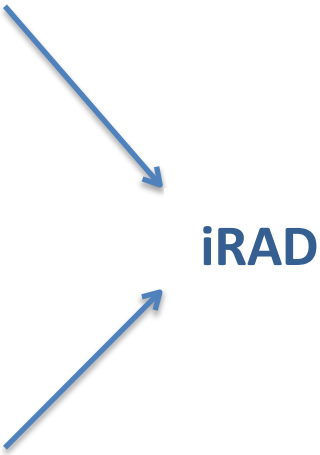
To continue advancing through Phase II, the creation of the bioartificial kidney, we must move forward with the development of both the hemofilter and the bioreactor. We require $8 million to complete prototype development and preclinical studies. The hemofilter will likely be a spin-off product by itself as a replacement filter for dialysis. The funding will ensure ongoing progress during the "Valley of Death" phase—the pivotal period between proof of concept and early investment by venture capitalists and corporate partners.

Reference Material. Published abstracts detailing the scientific progress made in the year 2014 can be reviewed in Appendix A, pages 12 to 25.

Next Steps. Phase II for the Hemofilter and Bioreactor, respectively, we need to accomplish the following primary tasks.

Hemofilter	I.	Membrane Design and Manufacturing Scale-up *$750,000*
	II.	Biocompatible Coating Selection *$300,000*
	III.	Hemofilter Design and Production *$550,000*
	IV.	Implanted Hemofilter Animal Studies *$1,400,000*

Bioreactor	I.	Cell culture optimization *$850,000*
	II.	Bioreactor design and production *$2,500,000*
	III.	Mock-Loop Assessment of Water and Electrolyte Transport *$850,000*
	IV.	Engineer HRTCs for Enhanced Water and Salt Reabsorption *$800,000*



*Amounts ($) indicated are approximate costs required to complete each component.

Phase II: Timeline

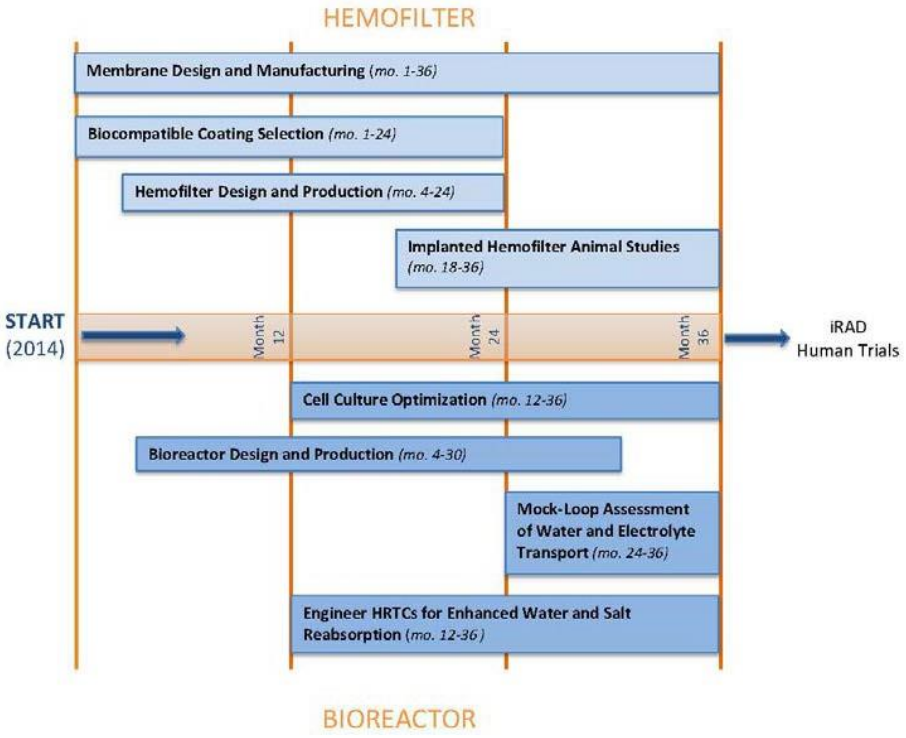
During Phase II of The Kidney Project, which is already underway, the research team is finishing the engineering refinements to the device components, integrating them into a full bioartificial kidney, testing it for safety, and implanting the device in humans.

Specifically, the team will finish fine-tuning both the hemofilter and the bioreactor and combine these two components into scaled-up prototypes and test increasingly complex prototypes of the device in stages. The work will involve a combination of engineering improvements to silicon membranes, bench-top experiments with the combined hemofilter and cell bioreactor, and packaging of the device for preclinical testing.

The Food and Drug Administration (FDA) selected The Kidney Project for participation in its new Innovation Pathway 2.0 program. This partnership with the FDA will address regulatory hurdles and create a more efficient roadmap, and in turn, accelerate the timeline to clinical trials and market approval.

If we raise all of the necessary funding in a timely fashion, and we do not encounter any unanticipated development challenges, we expect to have a device ready for clinical trials in 2017.

The nature of the results of the first round of clinical trials will largely influence the timing of release and industrial-scale manufacturing. Typically, there are at least two cycles of clinical testing required for all medical devices. We estimate that the clinical trials will be complete by the year 2020. During the clinical trials, we will be working with manufacturers to discuss and manage the details of production. Once the clinical trials are complete, the device will be immediately available for patients.



Donors: Honor Roll

From January 1, 2014 to December 31, 2014.

Benefactors

Donors of $50,000 or more

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- Wildwood Foundation (New Jersey)

Patrons

Donors of $20,000 - $49,999

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- Charities Angels

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- Lauren Stifelman
- Danny Torres
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- Jaclyn Weissman
- Pamela and Francis Wilbur

Team: Leadership

The Kidney Project is led by Drs. Shuvo Roy and William H. Fissell, from University of California, San Francisco (UCSF) and Vanderbilt University, respectively.

Shuvo Roy, Ph.D., principal investigator of The Kidney Project, is a bioengineer whose research is focused on smart medical devices, with an emphasis on implantable and wearable systems. He is leading a team of multidisciplinary specialists from UCSF, Vanderbilt University, Cleveland Clinic, Ohio State University, Penn State University, and University of Michigan, along with small business partners, to bring together the necessary scientific, engineering, and medical expertise needed to achieve the Project's ambitious goal. He is a professor in the UCSF Department of Bioengineering and Therapeutic Sciences (BTS), a joint department of the UCSF Schools of Pharmacy and Medicine, and director of the UCSF Biomedical Biodesign Laboratory. Dr. Roy is also a founding member of the UCSF Pediatric Device Consortium, whose mission is to accelerate the development of innovative devices for children's health, and a faculty affiliate of the California Institute for Quantitative Biosciences (QB3).

Dr. Roy earned a B.S. degree, magna cum laude, with general honors for triple majors in Physics, Mathematics (special honors), and Computer Science from Mount Union College in Alliance, Ohio. In 1995, he earned an M.S. in Electrical Engineering and Applied Physics and in 2011, he earned a Ph. D. in Electrical Engineering and Computer Sciences, both from Case Western Reserve University in Cleveland, Ohio.

William H. Fissell, M.D., is the co-principal investigator of The Kidney Project and has pioneered the application of silicon nanotechnology to hemodialysis and hemofiltration. He is an Associate Professor of Medicine in the Division of Nephrology and Hypertension at Vanderbilt University. Previously, he served as the Director of the Renal Nanotechnology Laboratory and the Innovations Center for Extracorporeal Therapy at the Cleveland Clinic in Cleveland, Ohio. His clinical practice focuses on the care of critically ill patients with renal disease.

Dr. Fissell received S.B. degrees in Physics and Electrical Engineering from the Massachusetts Institute of Technology, and he went on to apply his engineering training to treatment of patients with kidney disease first at Case Western Reserve University, University Hospitals of Cleveland, and the Louis B. Stokes Cleveland Veteran's Administration Hospital, where he completed medical school and residency.

Dr. Fissell moved to University of Michigan, Ann Arbor, for his fellowship in nephrology, where he trained with H. David Humes, M.D., pioneer of the first bioartificial kidney used in clinical trials. He remained at the University of Michigan for his first two faculty appointments before moving to the Cleveland Clinic in 2007. In 2012, Dr. Fissell was recruited to Vanderbilt University in Nashville, Tennessee, where he is Associate Professor of Medicine in the Division of Nephrology and Hypertension.

Contact Information

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Facebook: facebook.com/ArtificialKidney

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Appendix A: Abstracts

Comparative Evaluation of Nanoscale Surface Coatings on Silicon Substrates for Implantable Device Applications

Zohora Iqbal,[1] Brooke Benner,[1] Charles Blaha,[1] Jaehyun Park,[1] Eun Jung Kim,[1] Steven Kim,[2] William H. Fissell,[3] Shuvo Roy.[1]

[1]*Bioengineering, UCSF*

[2]*Nephrology, UCSF*

[3]*Nephrology, Vanderbilt University*

Background:

Highly uniform silicon nanopore membranes (SNM) are under investigation for use in implantable devices for organ replacement functions. A robust, readily scalable, non-fouling surface coating is required to enhance SNM biocompatibility while preventing pore occlusion. We evaluated a number of thin-film surface coatings compatible with silicon processing techniques. Five candidate biocompatible coatings were optimized for sub-5 nm deposition and evaluated for protein resistance.

Methods:

Silicon surfaces were modified using 1) surface grafted self-assembled monolayer polyethylene glycol (PEG); 2) RF plasma polymerized PEG; 3) poly(sulfobetaine methacrylate) (pSBMA) grown on surface via atom-transfer radical polymerization (ATRP); 4) poly(2-methacryloyloxyethyl phosphorylcholine) (pMPC) coated via ATRP; and 5) titanium oxide (TiO_2) deposited via atomic layer deposition. Coating thickness was determined by ellipsometry. Protein adsorption was determined by enzyme-linked immunosorbent assays after 2-hour incubation in single protein solutions of human fibrinogen and albumin. All adsorption data was normalized to uncoated silicon substrates.

Results:

Average coating thickness for the coatings ranges between 0.7 nm to 4.7 nm (Figure 1). All coatings exhibited reduced protein adsorption compared to non-coated substrates (Figure 2). TiO_2-coated silicon had the highest overall fouling, while pSBMA exhibited least fouling with an 84% reduction in human albumin adsorption and 98% reduction in human fibrinogen adsorption compared to bare silicon. The results demonstrate that pSBMA-coated surface exhibit superior non-fouling characteristics that make it suitable for application to SNM-based devices.

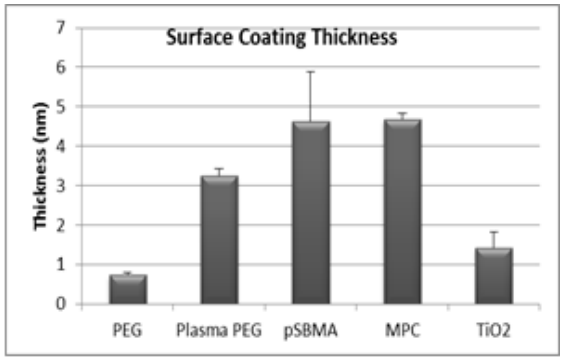

Figure 1: Surface modification coating thickness.

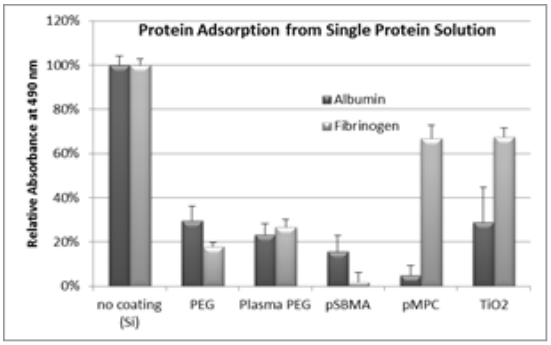

Figure 2: Normalized protein adsorption of human albumin and fibrinogen from single protein solutions.

Appendix A: Abstracts

Shear Stress Enhanced Proximal Tubule Cell Bioreactor Systems

Peter Soler[1,5], Nicholas Ferrell[2], Dorian Liepmann[3], Paul Brakeman[4], William Fissell[2], Shuvo Roy[5]
[1]*Department of Chemical & Biomolecular Engineering, UCB*
[2]*Departments of Medicine, Biomedical Engineering, Vanderbilt University*
[3]*Department of Bioengineering, UCB*
[4]*Department of Pediatrics, UCB*
[5]*Department of Bioengineering & Therapeutic Sciences, UCSF*

Background:
A compact parallel-plate system based on silicon nanopore membranes (SNM) is under development for an implantable bioartificial kidney. This study investigates two parallel-plate flow system bioreactors and resulting enhancement of cellular reabsorption by microenvironmental shear stress manipulation.

Methods:
The two systems were developed to characterize proximal tubule cell function in a planar flow geometry as shown in figures 1 and 2. Both systems utilized a 400 μm thick gasket defined flow path. System A is compatible with commercially available Corning Snapwell inserts and System B utilized a polycarbonate porous membrane incorporated into the device at the time of assembly. System B allows for SNM to be embedded in the flow cell. For both systems Lewis Lung Cancer Porcine Kidney Cells (LLC-PK1) were statically cultured on the porous membranes before assembly into the devices and exposing cells to physiological shear stress levels.

Results:
System A maintained LLC-PK1 barrier function with 3.5 fold increase in reabsorption performance with increasing shear stress rates, as shown in the figure 3. System B maintained LLC-PK1 viability on SNM for up to 1 week with sustained creatinine and urea barrier performance, as shown in figure 4. Barrier performance is calculated by normalizing concentration difference between apical and basal sides using (Capical – Cbasal)/ Capical.

Conclusion:
In summation, the two bioreactor systems have been developed and demonstrated potential for long-term cell viability, toxin barrier performance and enhanced reabsorption by LLC-PK1 cells under shear stress in a parallel-plate flow system geometry.

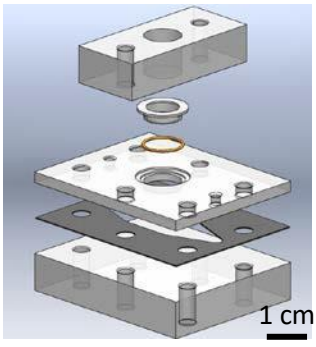

Figure 1 – System A – Snapwell insert compatible shear stress bioreactor assembly

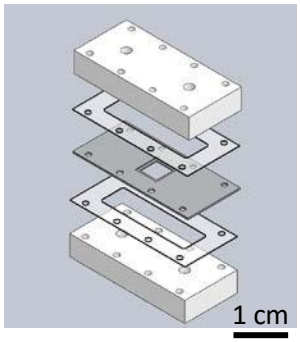

Figure 2 – System B – SNM-based shear stress bioreactor assembly

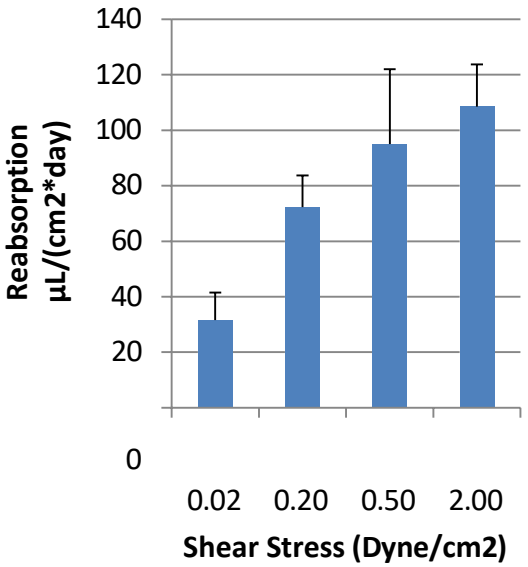

Figure 3 – Water reabsorption of System A

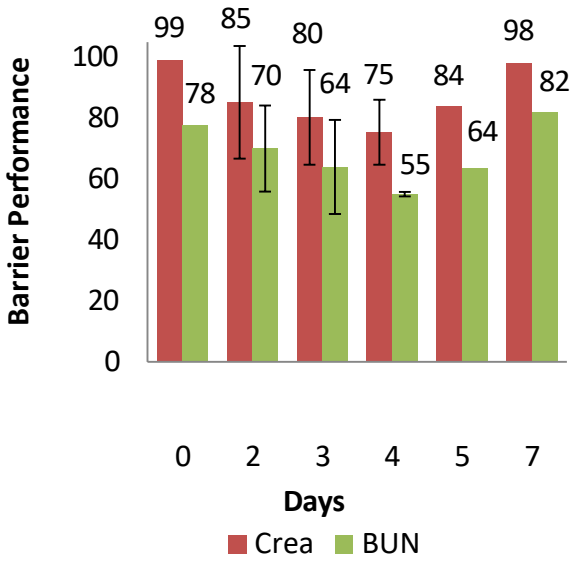

Figure 4 – System B maintanence of barrier function

Hemocompatibility Enhancement of Silicon Nanopore Membranes (SNM) using Optimized Deposition of Thin-Film Poly(Sulfobetaine Methacrylate) (pSBMA)

Zohora Iqbal[1], Steven Kim[1,2], Brooke Benner [1], Eun Jung Kim[1], Aaron Dolor[1], William H. Fissell[3], Shuvo Roy[1].

[1]Bioengineering, UCSF

[2]Nephrology, UCSF

[3]Nephrology, Vanderbilt University

Background:
The precision geometry and manufacturability of SNM is fundamentally enabling for the development of an implantable bioartificial kidney. To enhance long-term hemocompatibility, we optimized the deposition of sub-5nm thick pSBMA, a zwitterionic polymer to provide an anti-thrombogenic non-pore-occluding surface coating on SNM. This work details the optimization parameters for pSBMA deposition and presents hemocompatibility results under static and flow conditions.

Methods:
By varying the starting reagent concentrations, 2,2'-bipyridyl (BPY) and copper (II) bromide ($CuBr_2$) from 0 to 0.3 M, and 0 to 0.4 M, respectively, and polymerization time (PT) from 10 to 60 min, we optimized for sub-5 nm pSBMA coating and minimal protein adsorption (fibrinogen and albumin). Coating thickness was determined by ellipsometry. Hydraulic permeability was measured for SNMs before and after coating. Fibrinogen adsorption was determined by enzyme-linked immunosorbent assays after 2 hour incubation and FITC-labeled bovine serum albumin adsorption was examined by fluorescent microscopy after 1 hour incubation. pSBMA-coated silicon (Si) was compared to bare-Si after 2 hour fresh heparinized human blood flow (20 ml/min and 73.3/s wall shear rate). Platelet activation (CD62) (immunohistochemistry) and cell attachment (scanning electron microscopy) on surface were analyzed.

Results:
The optimized protocol (0.3 M BPY and 0.01 M $CuBr_2$ with 15 min PT) produces a ~4.6 nm thin-film pSBMA coating that significantly reduced protein adsorption compared to bare-Si. For static conditions and relative to bare-Si, albumin adsorption was reduced by 52%, while fibrinogen adsorption was reduced by 64%. Under flow conditions, there was a qualitative decrease in both platelet activation and cell adhesion. This study shows that pSBMA performance can enhance SNM hemocompatibility in both static protein solutions and under blood flow conditions.

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8-Day Implantation of Silicon Nanopore Hemofilter

Clark D. Kensinger[1], Seth Karp[2], Joseph J. Groszek[3], Phil Williams[1], Rashi Kant[5], Torin Yeager[5], Shuvo Roy[5], William Fissell[1,6]

[1]*Department of Surgery, Vanderbilt University*
[2]*Division of Transplantation, Vanderbilt University*
[3]*Division of Nephrology and Hypertension, Vanderbilt University*
[4]*Division of Surgical Research, Vanderbilt University*
[5]*Department of Bioengineering and Therapeutic Sciences, UCSF*
[6]*Department of Medicine, Vanderbilt University*

Background:
End-stage renal disease (ESRD) patients face a severely limited supply of donor organs and disappointing mortality and morbidity on maintenance dialysis. Existing hollow-fiber membranes are thrombogenic, foul quickly, and require high driving pressures. An implantable artificial kidney could improve outcomes and quality of life in ESRD. Our work is focused on development of new ultrathin, uniform pore size, silicon membrane technology. We report the first implant of the high efficiency filtration membrane.

Methods:
Parallel plate hemofiltration cartridges were designed using computational fluid dynamics to guide geometry. A silicon nanopore membrane (SNM) with porous surface area of 0.36 cm^2 and critical pore dimension of 5.6 nm was surface-modified with polyethylene glycol (PEG) and mounted in a cartridge. We implanted the cartridge in an adult purpose bred dog. All experiments were approved by the Institutional Animal Care and Use Committee. 6 mm PTFE grafts were anastomosed to the aorta and common Iliac as inflow and outflow conduits to the device. Low-molecular weight heparin (1.0 mg/kg) was administered perioperatively. Graft patency was serially assessed by Doppler ultrasound. On post-op day 8 the animal was euthanized by protocol and the cartridge explanted.

Results:
We report the first successful prolonged implantation of an SNM hemofilter. The animal displayed no signs of distress during the postoperative period. Evidence of graft patency was noted on each post-op observation. At explant, brisk flow was noted through the grafts and 27.5 mL of ultrafiltrate were collected. There were no signs of thrombus within the device. These findings demonstrate meticulous attention to surface chemistry and conduit geometry can permit implementation of this novel technology

Predicting Clot Formation in Implanted Hemofilters

Amanda K. W. Buck[1,2], Joseph J. Groszek[3], Shuvo Roy PhD[4], William Fissell MD[3]
[1]*Department of Radiology and Radiological Sciences, Vanderbilt University*
[2]*Vanderbilt University Institute of Imaging Science, Vanderbilt University*
[3]*Nephrology and Hypertension, Vanderbilt University*
[4]*Department of Bioengineering and Therapeutic Sciences, UCSF*

Background:
A common mode of failure of a vascular device is clotting related to the local hemodynamic environment.
Computational fluid dynamics (CFD) models can be used to predict flow fields, and we have used this approach to model flow in an implantable hemofilter using a realistic flow waveform. We hypothesized that CFD simulations of blood flow would demonstrate pathophysiologically-relevant flow patterns that coincide with locations of clot formation.

Methods:
We designed an implantable blood conduit to test hemofiltration membranes *in vivo*. Blood flow in the device was modeled with pulsatile flow boundary conditions based on *in vivo* ultrasound measurements. Regions of recirculation and slow near-wall flow were identified in each computational model. Hemofilter models were implanted in large animals anastamosed to iliac artery and vein for 30 days, or until thrombosis, then explanted.

Results:
CFD simulations predicted two persistent recirculation regions: one along the outer wall of the proximal curve, and one zone of near-wall recirculating/slow flow along the inner wall of the distal bend (Fig. 1). Of 42 implants, 2 showed localized clot formation and two more diffuse thrombosis. Clot formation was identified in the same two areas in which CFD models predicted recirculation (Fig 1).

Conclusion:
Pulsatile flow simulations predicted pathophysiologically relevant flow patterns in the same regions in which clot formation occurred. In the future, similar physiologically realistic simulations may prove useful for eliminating recirculation regions in subsequent device design.

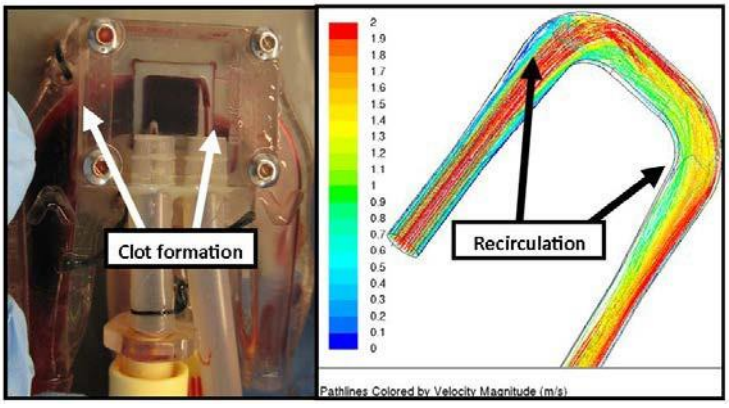

Figure 1

Appendix A: Abstracts

Long-Term Water Transport and Barrier Function of Proximal Tubule Cells Cultured Under Apical Shear Flow Condit

Peter Soler[1], Nick Ferrell[2], William Fissell[3], Shuvo Roy[4], Paul Brakeman[5]
[1]*Department of Chemical & Biomolecular Engineering, UCB*
[2]*Department of Medicine, Vanderbilt University*
[3]*Division of Nephrology, Vanderbilt University*
[4]*Department of Bioengineering & Therapeutic Sciences, UCSF*
[5]*Department of Pediatrics, UCSF*

Background:
Development of a bioartificial kidney (BAK) comprising a hemofilter and a proximal tubule cell bioreactor for treatment of chronic kidney disease requires long-term survival of proximal tubule cells under shear flow conditions. Previous work has only characterized transport properties of proximal tubules cells for 2-3 weeks in perfusion culture. We investigated fluid transport characteristics of proximal tubule cells in a shear flow bioreactor in long-term culture for >60 days.

Methods:
LLC-PK1 cells were cultured on polycarbonate filter supports for 14 days and then exposed to apical shear stress. Cells were perfused with media containing urea (40 mg/dL) and creatinine (10 mg/dL) on the apical side and media alone was used on the basal side. The change in volume of the compartments and concentrations of sodium, potassium, phosphorus, urea and creatinine were measured.

Results:
Water transport for proximal tubule cells under shear flow conditions increased significantly from 34+/-10 ul/cm2/day on day 7 of low shear flow (0.2 dyn/cm2) to 119+/-12 ul/cm2/day on day 63 (p=.002) with high shear flow (2 dyn/cm2) and was stable for 14 days at high shear flow from days 49 to 63. We characterized the barrier function of the cells by looking at the leakage of creatinine. On day 63, the leakage of creatinine was 0.012+/- 0.009 mg/cm2/day and did not differ significantly from the leakage of creatinine on day 7.

Conclusion:
Proximal tubule cells can maintain barrier function and water transport under shear flow conditions for >60 days. Water transport increases with increased shear flow and time in culture.

Appendix A: Abstracts

Implantable Hemofilter: 32 day Patency in a Canine Surgical Model

Clark Kensinger MD[1], Seth Karp MD[1], Joseph Groszek[2], David Laneve[3], Phil Williams[3], Baoxia Mi PhD[4], Mark Goodin[5], Rishi Kant[6], Torin Yeager[6], Shuvo Roy PhD[6,7], William Fissell MD[2]

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Background:

Transplantation offers the best treatment option in end stage renal disease in terms of cost, survival, and quality of life, but is limited by donor organ supply. An implantable artificial kidney using high-efficiency silicon nanopore membranes is in development to provide the benefits of transplantation to all dialysis patients. The major challenge in implementation of chronic blood-contacting devices is thrombosis. We report a successful 32-day device implant in a canine model without systemic anti-coagulation.

Methods:

A small-scale single-channel parallel-plate hemofilter (design membrane area ~2 cm^2) was manufactured from medical grade polycarbonate. In order to understand the influence of the hemofilter blood path on hemolysis, thrombosis and long-term patency, two silicon chips coated with biocompatible polymer thin films (sulfobetaine methacrylate) were mounted in the hemofilter in lieu of the planned silicon nanopore membranes.

In regards to the operation, the retroperitoneal vasculature was exposed via a midline laparotomy. 6 mm Polytetrafluoroethylene (PTFE) grafts were anastomosed in standard fashion with a running 6-0 prolene suture to the common iliac artery and vein to be used as inflow and outflow conduits to the device. Therapeutic heparin (100U/kg) was administered intra-operatively. The dog was housed without restrictions post-operatively. The dog received lovenox (0.5mg/kg) once a day at a venous thromboembolic prophylactic dose, rather than a therapeutic dose to demonstrate device patency in the absence of full anticoagulation. Graft patency and flow velocity were serially assessed post-operatively with a pulse wave, color flow doppler ultrasound.

The device was explanted at Day 32.

Results:

On serial ultrasound evaluations, the inflow and outflow PTFE grafts showed patent, pulsatile flow throughout the 32 day experiment. Operative findings on device explant noted that the device was patent without thrombosis formation. The dog had no complications during the course of the experiment.

Conclusion:

The 32 day patency of the device in the absence of therapeutic anti-coagulation highlights successful surgical technique, non-thrombogenic blood flow conduit geometry, and the biocompatibility of the manufacturing techniques and materials which provides the foundation for further preclinical canine experiments.

Appendix A: Abstracts

Fluid Shear Stress Alters Primary Renal Tubule Epithelial Cell Phenotype

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Background:
Primary cells cultured *in vitro* gradually lose features characteristic to the *in vivo* cell type, variously termed "senescence" or "culture stress". Culture conditions that help maintain cell-specific phenotype are advantageous. Here we evaluated the impact of applying apical fluid shear stress on the phenotype of primary kidney tubule epithelial cells.

Methods:
Renal tubule epithelial cells were isolated from donor kidneys not suitable for transplant. Cells were cultured on Transwell inserts under static conditions or on an orbital shaker at a rotation frequency producing fluid shear stresses of 2 dyn/cm^2. Transepithelial resistance was measured daily. After 2 weeks in culture, expression of tubule-specific markers was measured by PCR and western blot. Mitochondria were counted in TEM sections.

Results:
Shear stress significantly increased steady state transepithelial resistance from 284±23 to 477±20 Ω-cm^2 ($p<0.001$) and resulted in significantly increased gene expression of tubule epithelial cell-specific markers (GGT1, COL4A1, COL4A2, ITCH, and PEX14) with fold increases in expression of 4.9±0.2, 5.6±0.6, 3.4±0.2, 2.6±0.6, and 2.3±0.4, respectively. Cells grown under shear also showed increased protein expression of gamma glutamyl transpeptidase.

Conclusion:
Primary cells grown under physiologic levels of fluid shear stress appear to express proximal tubule markers more than cells grown in static conditions. This may be due to increased nutrient delivery and waste removal with improved mixing at the apical brush border, or due to specific gene regulation related to mechanotransduction. Further mechanistic insight may allow investigators to develop more accurate *in vitro* models of disease.

Appendix A: Abstracts

Apical Shear Stress Enhances Organic Cation Transport in hOCT2/hMATE1 Transfected MDCK Cells

Aishwarya Jayagopal[1], Peter Soler[1], Nicholas Ferrell[2], Paul Brakeman[3], Deanna Kroetz[1], William Fissell[2], Shuvo Roy[1]

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[3]*Pediatrics, UCSF*

Background:

Active transport by renal proximal tubules plays a significant role in human drug disposition and is therefore important to model when developing drugs. However, current *in vitro* drug testing methods fail to mimic important physiological cues, such as flow induced shear stress. In this study, the effect of shear stress on active transport was investigated using a parallel plate bioreactor cultured with MDCK cells expressing human organic cation transporters.

Methods:

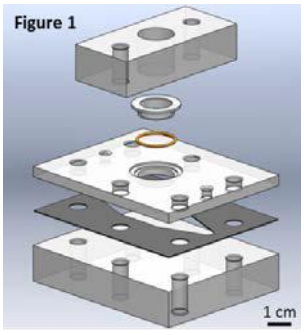
A polycarbonate parallel plate bioreactor compatible with Snapwell inserts was used in these experiments (Fig. 1). The device provides a flow path across the apical side of the cells and a static media reservoir on the basal side. MDCK cells transfected with a pair of uptake and efflux transporters (hOCT2/hMATE1) were grown on Snapwells under static conditions until confluence and then placed in the bioreactor. Media flow was increased over 7 days until shear stress of 0.2 dynes/cm^2 was achieved. Uptake of 25µM 4-(4-dimethylamino)styryl-N-methylpyridinium (ASP+), a fluorescent OCT2/MATE1 substrate, was measured for 1 hour with or without pretreatment (30 minutes) with 500µM imipramine, an OCT2/MATE1 inhibitor. Control cells were cultured under static conditions.

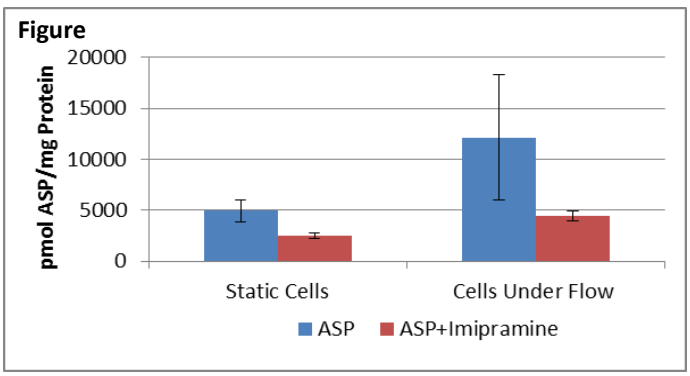
Results:

Cells maintained under flow showed a 2.2 fold increase in protein concentration over static controls, confirming previous observations of shear stress effects. Furthermore, cells cultured under shear stress showed a 2.4 fold increase in ASP+ uptake when compared to cells cultured under static conditions and a 63.4±3.7% inhibition with imipramine compared to 48.6±5.5% inhibition in static cells (Fig. 2).

Conclusions:

These results indicate that exposure to shear stress increases uptake of the active transport substrate ASP+ compared to static growth conditions.





Appendix A: Abstracts

Slit Nanotopography on Silicon Nanopore Membranes Resists Protein Deposition and Cell Attachment

Eun Jung Kim[1], William Fissell[2], Tejal Desai[1] and Shuvo Roy[1]
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Background:
Silicon Nanopore Membranes (SNM) with compact geometry and uniform pore size distribution are under development for the hemofiltration unit in an implantable bioartificial kidney.[a] Key concerns for long-term membrane function are centered on protein deposition and cell attachment that can result in surface fouling and thrombotic occulusion.[b]

Methods:
In this study, we investigated the influence of surface coatings and nanotopography on protein deposition and cell growth on SNM substrates.

SNM substrates consisting a 6 x 6 mm slit-array patterned hemofiltration region area in the center surrounded by a 2 mm unpatterned, smooth border, were modified by physically adsorbing either collagen type I (Col I-SNM) or covalently immobilizing RGD peptide (RGD-SNM). Atomic force microscopy (AFM)was used to characterize the roughness of modified SNM surfaces. The propensity of protein adsorption on SNM surfaces was evaluated using fluorescein isothiocyanate labeled bovine serum albumin (**FITC-BSA)**. Human umbilical vein endothelial cells growth on both modified and unmodified (Control) SNM were analyzed using immunohistochemistry.

The surface roughness (RMS) of RGD-SNM (12.5nm) was greater than that of Col I-SNM (7.8 nm)and Control(6 nm). In unpatterned regions, FITC-BSA adsorbed strongly to the Col I as well as RGD, and RGD-SNM was found to significantly enhance cell growth (1500 % on day 7) compared to Col I-SNM (120%) and Control (100 %). In patterned area of all modified SNMs, however, FITC-BSA protein adsorption and cell growth are strongly attenuated (below 10 % on day 7). In addition, significant actin impairment and cell detachment were observed on the patterned regions.

Results:
These results suggest that RGD is superior to Col I coatings for cell attachment. However, protein deposition and cell attachment on the slit-array region was significantly attenuated despite favorable coatings. This work will inform the development of SNM-based hemofiltration unit.

[a] *Fissell W et al., Semin Dial 22: 665-670, 2009*
[b] *Conlisk A et al., Ann Biomed Eng 37: 722-736, 2009*

Appendix A: Abstracts

9 Day Filtration by an Implantable Hemofilter

Clark Kensinger MD[1], Seth Karp MD[1], Joseph Groszek[2], David Laneve[3], Phil Williams[3], Mark Goodin[4], Rishi Kant[5], Torin Yeager[5], Shuvo Roy PhD[5], William Fissell MD[2]

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Background:
Patents with end-stage renal disease (ESRD) have high mortality and morbidity rates on dialysis. ESRD patients could experience better quality of life if implanted with an artificial kidney. A new ultrathin membrane with highly controlled pores made by sacrificial silicon oxide techniques allows size selective sieving in vivo. In a canine model, we have demonstrated albumin sieving through an implantable, high-efficiency hemofiltration membrane over 9 days.

Methods:
The device comprised of a single channel blood conduit with parallel-plate hemofiltration membranes. The membranes were bench tested for Ficoll sieving before implantation. *In vivo,* the device was attached to PTFE grafts and anastomosed to the common iliac artery and vein. Filtrate was collected in two implanted reservoirs. The animal was housed without restriction and received thromboembolic prophylactic doses of Lovenox (0.5mg/kg) once a day. After 9 days, filtrate was sampled from subcutaneous ports connected to the reservoirs. Albumin concentration in filtrate was measured by colorimetric assay. Filtration rates were estimated by indicator dilution.

Results:
Effluent sampled on day 9 had albumin sieving coefficients (bag 1: 0.13, and bag 2: 0.24). Ficoll sieving coefficients at equivalent Stokes radius were 0.14 and 0.19 respectively. *In vivo* filtration rates were consistent with *in vitro* measurements after correction for plasma oncotic pressure.

Conclusion:
A high efficiency hemofiltration device is capable of filtration over 9 days in a canine surgical model. Albumin sieving in vivo correlate well to Ficoll sieving collected at the bench indicating unchanged filtration characteristics, while filtration rates are commensurate with the expected pressure gradient across the hemofiltration membrane.

Appendix A: Abstracts

Evaluation of Next-Generation Silicon Nanopore Membranes Optimized for Diffusive Clearance

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Background:

Silicon nanopore membranes (HF-SNM) designed for hemofiltration have demonstrated remarkable permeability and selectivity. However, diffusive clearance was hindered by their thickness. Here we report hemodialysis-SNM (HD-SNM) with enhanced diffusive clearance.

Methods:

A new MEMS (microelectromechanical systems) fabrication protocol utilizing nested etch-back techniques was used to decrease the effective SNM thickness (HD-SNM 100um vs. HF-SNM 400 um). Diffusive clearances of polyethylene glycol coated HD-SNM and HF-SNM with sub-10 nm pore sizes were tested in a parallel plate flow cell. PBS with Cr 10 mg/dL, BUN 90 mg/dL, and albumin 3 g/dL was recirculated (45ml), while dialysate (160 mEq NaCl) was recirculated in a counter-current fashion. At Q_d=Q_b=10 ml/min and zero transmembrane pressure (TMP) clearance was independent of flow rate. Solute clearance (K) was calculated by fitting concentrations measured at 0, 2, 4 hrs (n=3) to an exponential decay function: $C(t)=C_i\, e^{-Kt/V}$. C(t): conc at time *t* ,C_i: initial conc, V: volume. Filtration was tested in water and fetal bovine serum at various TMP (1, 2, 4psi) using cross flow velocities at 0.1, 0.5 and 3ml/min. Platelet adhesion and activation were evaluated by immunohistochemistry (IHC) and scanning electron microscopy (SEM) after flowing human blood for 2 hrs at 2ml/min.

Results:

HD-SNM had a ~2.5 fold improvement in K, consistent with mathematical models. Creatinine, BUN and phosphorus clearances were 232.5±17.2, 314.6±15.6, 191.4±6.3ml/min/m^2(HD-SNM) and 85.5±10.6, 135.3±22.9, 75.5±12.8ml/min/m^2(HF-SNM), respectively. HD-SNM maintained mechanical integrity at over 200mmHg. The HD-SNM also showed comparable filtration rates (71.5 ± 21.3ml/hr/mmHg/m^2) and selectivity to HF-SNM. IHC for CD62 and SEM images showed similar levels of platelet activation and adhesion.

Conclusion:

These preliminary studies demonstrate significant improvement in diffusive clearance with the HD-SNM while still maintaining mechanical robustness, selectivity, permeability and hemocompatibility.

Appendix B: Budget

		Year 1	Year 2	Year 3	Total ($)
Hemofilter	Membrane Design and Manufacturing	500,000	100,000	150,000	750,000
	Biocompatible Coating Selection	150,000	150,000	-	300,000
	Hemofilter Design and Production	350,000	200,000	-	550,000
	Implanted Hemofilter Animal Studies	-	550,000	850,000	1,400,000
	TOTAL				**3,000,000**
Bioreactor	Cell Culture Optimization	400,000	300,000	150,000	850,000
	Bioreactor Design and Production	1,000,000	750,000	750,000	2,500,000
	Mock-Loop Assessment	-	375,000	475,000	850,000
	Engineering HRTCs	400,000	200,000	200,000	800,000
	TOTAL				**5,000,000**

*All amounts indicated are in US dollars ($).

Appendix C: News and Events

Highlights from 2014

May 1, 2014	Capitol Congressional Briefing: The Kidney Caucus
July 3, 2014	New FAQ released
June 17-21, 2014	Team presented at the annual American Society for Artificial Internal Organs Conference
November 11-15, 2014	Team presented at the annual American Society of Nephrology Conference