Genetically-engineered pig kidney xenotransplantation

David K. C. Cooper

The pig kidney is a 'ready-made' replacement for a human kidney, and will possibly never be matched by any 'artificial kidney', i.e., by a mechanical or biomechanical device. It is implantable, and could be replaced whenever necessary. The current data suggest that a pig kidney is likely to fulfill all or most of the essential functions of a human kidney. If any functional differences are identified, these can be corrected by judicious genetic engineering of the pig. The major barrier is the human immunobiological response to a pig kidney, and this has been largely overcome by (i) the deletion of expression of pig xenoantigens against which humans have performed natural antibodies, and (ii) the transgenic expression of human 'protective' proteins, e.g., complement-regulatory, coagulation-regulatory, and anti-inflammatory, in the pig organs. Survival of kidneys from pigs with multiple genetic manipulations in nonhuman primates is now being measured in months or even >1 year. Because conventional immunosuppressive regimens used in allotransplantation have been demonstrated to be ineffective in allowing prolonged survival of pig kidney grafts, novel immunosuppressive and anti-inflammatory regimens are required. This barrier has been overcome by regimens based on blockade of the CD40/CD154 costimulation pathway, with the evidence suggesting that agents that target CD154 are more effective than those that target CD40. In addition, there is increasing evidence that therapy aimed at reducing the effects of selected cytokines (IL-6, TNF) is associated with increased graft survival. My understanding is that the US Food and Drug Administration (FDA) will require evidence of good function and consistent survival of 6 months in at least 6 nonhuman primates with life-supporting pig kidney transplants. In view of my extensive experience in the pig-to-baboon kidney transplantation model, I suggest that this milestone can be readily achieved within 12-18 months, if sufficient funding is available. We currently have funding from the NIH NIAID (for the years 2020-2025) that will enable my group to carry out possibly 4 pig kidney transplants per year, but additional funding would enable us to make more rapid progress. It is proposed that 10 transplants of kidneys from pigs with 8 genetic manipulations in baboons receiving an anti-CD154-based immunosuppressive regimen should be carried out with follow-up for 6 months. Multiple tests of pig kidney function will be carried out at intervals, and renal biopsies will be taken at 3 months and at necropsy (at 6 months) for histopathological examination. It is anticipated that these studies will enable sufficient data to be obtained to satisfy the FDA that a limited clinical trial can be initiated. Three major requirements are needed to meet the FDA milestones \u2013 (i) the optimal genetically-engineered pigs (which are now currently available), (ii) a biosecure 'clean' pig facility (several of which are now available), and (iii) an anti-CD154 agent (which is available to me from Viela Bio, Gaithersburg, MD), which is currently in clinical trials for the treatment of autoimmune diseases. Pig kidney xenotransplantation will provide a treatment option for many patients with end-stage renal failure.

MI-TRAM: Smart Module for Implantable, Wearable, Portable or Bed-side Artificial Kidneys

imec USA Nanoelectronics Design Center, Inc.

Hemodialysis (HD) is a life-sustaining kidney replacement therapy that partially replaces glomerular filtration, but not tubular function of the kidney. As such, toxin removal efficiency of conventional HD decreases with molecule size & hydrophobicity and stops at the size of albumin (which must be retained in the blood). Extremely difficult to remove are the so-called protein-bound uremic toxins (PBUTs), due to their strong binding with plasma proteins like albumin. PBUTs affect a host of biological systems that contribute to the uremic syndrome, mainly inflammation and calcification, producing cardiovascular damage. PBUTs have also been recently linked to cognitive function decline in CKD patients. Thus, any artificial kidney would profit from

improved PBUT removal, which is one of the goals in the Kidney Health Initiative (KHI) innovation roadmap. Importantly, partners in the current consortium, (RWTH Aachen University, Germany) have shown that strong high frequency electromagnetic (EM) fields shift the dynamic equilibrium of protein-binding for toxins further to a non-bound state, thereby enabling removal of the freed toxins via the dialysis membrane. This core technology has been patented. Producing such strong EM fields currently requires large and expensive devices, which limits present use to non-portable/non-implantable devices. To disruptively solve this, another partner in the current consortium (IMEC, located in Kissimee, Florida) has recently patented a miniature system-on-chip, that enables producing the required EM fields across the pores of any hemodialyzer membrane, thereby loosening PBUTs exactly at the location where they can be filtered out. This system-on-chip is so small that it can be used in implantable artificial kidneys. Importantly, its use is not limited to implantable artificial kidneys: Touchless "through-the-wall" capacitive coupling makes the device "clip-on compatible" with any implantable technical dialysis membrane, but also with all existing dialysis filters used outside the body. This enables rapid introduction of this technology for all HD patients. The technology can quickly generate income from a license to conventional HD that can finance integration into implantable or wearable artificial kidneys. The system-on-chip also supports fluid load monitoring via optical hematocrit level sensing and bioimpedance spectroscopy. The chip offers internal data storage, a digital processor, a cyber-secure bi-directional wireless data connection and wireless charging capability. Additional consortium partners, University Medical Center Utrecht (UMCU) together with Utrecht University (UU), jointly have vast clinical experience on innovative kidney replacement therapies and in research on bioengineered kidneys & PBUTs removal. Further, UMCU has in-house ISO 13485 certified medical device manufacturing facility and a well-described uremic large animal (goat) model. This enables rapid in vivo testing. MI-TRAM realizes a miniature module to enhance the removal of PBUTs with embedded fluid load & temperature monitoring. The system-on-chip is cheaply mass-producible by semiconductor industry and offers highly flexible incorporation in any artificial kidney. Human trials are foreseen for phase II of the KidneyX artificial kidney prize competition. Further, we can provide functional platform evaluation kits to other KidneyX consortia to test integration with their implantable or wearable hemodialysis solutions. The device design will be transferable for licensed low-cost mass-production by industry (anti-shelving approach).

Xenotransplantation: A treatment for Kidney Failure

Makana Therapeutics

Our solution to the KidneyX Artificial Kidney Prize is the development of a pig kidney which can restore renal function when implanted into a human being. Kidney transplants offer patients suffering from renal failure a higher quality and longer life than dialysis typically permits. A key failing of transplantation is that the number of patients far exceeds the numbers of available kidneys. Therefore, we are attempting to bridge this gap by using pigs as donors instead of humans. Pigs are ideal for this purpose for several reasons: (i) Pigs reproduce quickly making it possible to quickly generate many organs. (ii) The anatomical and physiological similarities between the two species suggest that pig kidneys will adequately restore renal function to humans. (iii) Recent improvements in genetic engineering have made it possible to produce modified pigs to further increase their biological compatibility with human patients. In principle, the pig kidney is an excellent replacement organ as it has evolved to perform each physiologic activity. However, executing these functions requires integration of various renal and extrarenal mechanisms (e.g. hormonal pathways) to achieve the proper regulation of each process. Understanding whether the interaction of cells and molecules from both species can effectively regulate the essential kidney functions has been difficult to discern. Kidney xenotransplant studies have typically failed because the recipient, a non-human primate, fails to maintain healthy tissue in the transplanted organ, and the renal functions become dysregulated. This happens because primates, like humans, have anti-pig antibodies that develop prior to the transplant. Consequently, upon circulating recipient blood through the

organ, these anti-donor antibodies rapidly attack and destroy the implanted kidney. Though advances in xenotransplantation, have overcome some of these obstacles, the immune system ultimately develops an anti-pig response resulting in the same destruction of the kidney over a period of weeks to months. To overcome these issues, we have combined genetic engineering to minimize the expression of antigens which are initially targeted by the donor-specific antibodies. In addition, we have developed assays to carefully identify ideal recipients who lack, residual antibodies towards the modified organ. Finally, we have identified an effective immunosuppressive protocol to enable long-term function of the transplanted kidney in non-human primates. These advances have enabled kidney xenotransplants to provide life-sustaining function for more than a year. These studies have shown that pig kidneys can effectively replace many physiologic functions of the primate kidney. With these successes, a human clinical trial can now be considered. In phase I of the artificial kidney competition, we intend to pursue three activities to enhance the safety and efficacy of a xenokidney. First, we will develop the capabilities of identifying kidney failure patients who may benefit from a transplant, but with little to no opportunity to receive a human organ. Second, we will refine our histocompatibility assays using this specific population of patients in need. Third, we will begin the development of additional diagnostic assays which can help monitor the health of the transplanted pig kidney.

iBAK - Implantable Bio-Artificial Kidney for Continuous Renal Replacement Therapy

UC San Francisco (UCSF)

We are developing a self-monitoring and self-regulating implantable bioartificial kidney (iBAK) that will provide end-stage renal disease (ESRD) patients with continuous treatment and total mobility. There are almost 4 million people worldwide, including over 750,000 Americans affected by ESRD. While kidney transplantation offers excellent outcomes, the supply of donor organs is severely limited - less than 20% of patients waiting for a transplant ever receive one. For the vast majority of patients, in-center dialysis is the only treatment option. This modality is not only cumbersome and expensive to implement, but patient quality of life and outcomes are poor, such that less than 50% of patients survive after 5 years.The iBAK will serve as a universal donor organ for the vast majority of ESRD patients who will never receive a transplant. It consists of a compact hemofilter comprised of highly efficient silicon membranes that use the body's own blood pressure to perform filtration, without pumps or a power supply, and a renal tubule cell bioreactor to ensure volume homeostasis and other metabolic functions. The device will operate without the need for systemic anticoagulation or immunosuppression drugs. By incorporating physical and biochemical sensors, the iBAK will be able to detect device malfunctions and monitor blood flow, urea clearance, and electrolyte balance. Information on device performance and the patient's health status can be wirelessly sent to the patient and/or healthcare provider. Over the last decade, we developed small-scale versions of the hemofilter and bioreactor and demonstrated their individual operation in the preclinical setting. The implanted hemofilters functioned without the use of systemic anticoagulation to achieve toxin clearance and maintained patent blood flow paths. Encapsulated renal cells in the bioreactors were protected from the host immune system and remained healthy despite the lack of immunosuppression. Based on these successes, we have recently combined the hemofilters and bioreactors to create functional iBAK prototypes. These subtherapeutic devices were preclinically implanted to confirm feasibility of operation. Going forward, we will scale up the hemofilter and bioreactor designs into clinical size devices and demonstrate their sustained performance. The clinical-sized hemofilters and bioreactors will be integrated into therapeutic iBAK devices and evaluated for their ability to provide total renal replacement therapy in preclinical models of kidney failure. The preclinical performance data from the therapeutic iBAK devices will be combined with standardized safety test results and submitted for regulatory and ethics approval to initiate a pilot clinical trial in ESRD patients.

The Wearable AKTIV: Artificial Kidney to Improve Vitality

University of Washington Center for Dialysis Innovation

Our vision for the future of dialysis is the AKTIV: the Ambulatory Kidney to Improve Vitality, a wearable hemodialysis device that restores patient mobility and dramatically improves quality of life. Today's hemodialysis systems require connection to an external water source and use hundreds of liters of water per dialysis session, a key barrier to wearable dialysis. To translate our vision to reality, we developed a novel urea removal technology that successfully addresses the #1 obstacle to truly wearable dialysis: regeneration of the dialysate in a closed-loop circuit that does not require an external water source and operates with a liter of dialysate. The Center for Dialysis Innovation (CDI) is charting a new path, applying a method never before used in dialysis: the AKTIV is enabled by our novel Dialysate Regeneration Module (DRM), based on CDI's proprietary photo-oxidation technology. The DRM removes urea and other non-urea uremic toxins dialyzed from the blood and catalytically converts them to carbon dioxide and nitrogen that harmlessly disperse into the atmosphere, regenerating the dialysate and allowing it to be recirculated through the system. Thus, a small volume of recirculated dialysate (1 liter) will suffice. Maintenance dialysis today, where dialysis system components are used for a single treatment only, is environmentally unfriendly, using huge amounts of water and generating medical waste disposal issues. Sorbent columns used with most wearable concepts to remove urea also lead to large amounts of medical waste, are expensive, heavy, and can generate toxic products. The AKTIV does not use sorbent columns to remove urea, and we envision AKTIV dialyzers and tubing sets will last one week or longer before needing replacement due to the improved blood compatibility and non-fouling properties of our proprietary surface coating. Blood access has been called the "Achilles heel of dialysis" and current approaches are intensely disliked by patients. The AKTIV system will incorporate a new blood access strategy allowing the patient to safely plug in and disconnect without repeated skin punctures and greatly minimizing the potential for touch contamination and infection. On-line sensors will control the AKTIV and notify patients of issues. Such sensors open the possibility for personalized (precision) dialysis. Since people are different in body size, metabolic parameters and comorbidities, everyone should not have identical dialysis sessions. With tele-physician monitoring and on-line sensors, dialysis sessions can be tuned to the needs of the patient. The high costs associated with dialysis can be traced to "bricks and mortar" in-center dialysis, staffing costs, and hospitalization complications. The wearable AKTIV will minimize these expenses. All aspects of the AKTIV are designed and engineered with direct patient participation. If patients are not satisfied with the AKTIV it will never succeed. Human factors design plays a major part in our AKTIV development. Overall, the wearable AKTIV hemodialysis system is engineered to embrace 21st century technology and address concerns we have with today's dialysis systems that have been only incrementally improved since the first chronic dialysis in 1960.

Development of a Dialysate-Free Waterless Portable and Implantable Artificial Kidney

US Kidney Research Corporation

Current clinical approaches to treat patients with end stage renal disease (ESRD) include hemodialysis, peritoneal dialysis, and renal transplantation. Here we describe the design and operation of a novel dialysis-free and waterless artificial kidney technology that has the potential to mimic the filtration properties of the renal glomerulus and the ion/water transport processes in the nephron. Importantly, the device does not utilize external water/dialysate or living cells. This waterless technology creates more than a replacement for dialysis, it allows increased freedom for patients. Since the technology does not utilize a dialysate, the portable device we have created is self-enclosed and can ultimately be taken anywhere and operated without having to worry about where to source or dispose of dialysate and water. Additionally, for providers, our waterless technology will completely eliminate the massive water infrastructure and associated costs currently needed to perform standard dialysis treatments. The technologic advances and approaches employed in this proposal can be potentially utilized in various configurations that include standalone, portable and implantable artificial kidney

devices to treat patients with compromised kidney function. The device couples for the first time newly designed multiple mesh activated wafer electrodeionization (AWEDI) technology for ion transport that responds functionally using ion sensors to changes in blood chemistry, with pressure driven low-fouling ultrafiltration, nanofiltration and reverse osmosis modules specifically developed or selected for this project. The technology we describe represents a paradigm shift in the field of renal replacement therapy that compares with the introduction of dialysis as a therapeutic modality over 70 years ago. We have initiated animal testing of our current portable artificial kidney device and have produced the world's first artificial urine. In approximately two years we anticipate having completed the proof-of-concept phase of animal testing in preparation for the FDA and clinical trials. The novel technology we have developed in our waterless portable artificial kidney device will be used to create for the first time a completely implantable artificial kidney that simulates the filtration and ion transport properties of the native kidney.