The bioartificial kidney

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Renal failure has an exceedingly high mortality rate despite advances in dialysis technology. Current renal replacement therapies (RRTs) restore only the filtration function of the kidney. Replacing the critical transport, metabolic, and endocrine functions of the kidney may provide more complete RRT, changing the natural history of these disease processes. Primary human renal epithelial cells (RECs) have been isolated and expanded under conditions that enhance propagation, resulting in maximum cell yield for use in bioengineered applications. These RECs demonstrate differentiated absorptive, metabolic, and endocrine functions of the kidney when tested under *in vitro* and preclinical *ex vivo* animal studies. When incorporated into bioengineered systems, RECs have proved to provide effective RRTs in both preclinical and clinical studies. These engineered "bioartificial kidneys" demonstrate metabolic activity with systemic effects and improvement of survival in patients with acute kidney injury and multiorgan failure. Results also indicate REC therapy influences systemic leukocyte activation and the balance of inflammatory cytokines, suggesting that this REC therapy may improve morbidity and mortality by altering the proinflammatory state of patients. This innovative approach for treating renal and inflammatory disease states may become a groundbreaking, transformative platform to current standard-of-care therapies, enabling the advancement of numerous life-saving technologies. (Translational Research 2014;163:342–351)

Abbreviations: AKI = Acute kidney injury; BAK = Bioartificial kidney; BRECS = Bioartificial renal epithelial cell system; EP = Enhanced propagation; ESRD = End-stage renal disease; FDA = Food and Drug Administration; IL = Interleukin; IND = Investigational new drug; MOF = Multiorgan failure; PD = Peritoneal dialysis; QA = Quality assurance; QC = Quality control; RAD = Renal assist device; REC = Renal epithelial cell; RRT = Renal replacement therapy; SSMOD = Septic shock-associated multiorgan dysfunction

An evolving field in the treatment of acute and chronic disease is the area of biomedical engineering, especially in the development of bioengineered cell-based therapeutics.[1] The potential clinical impact of this therapeutic approach is based on the emerging understanding that the majority of disease processes develop as a result of interactions between complex biosystems involving numerous types of cell products, rather than the deficiency of a single protein. Cell-based therapeutics are dependent on cell and tissue culture methodologies that expand specific cells that have the ability to replace important differentiated processes deranged or lost in various disease states. Recent approaches have made progress by seeding cells into hollow fiber bioreactors or encapsulating membranes as a means to deliver cell activities to a patient.[2,3] A natural extension of this approach, in the area of renal replacement therapy (RRT), would

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be the addition of renal cell-based therapeutics to the current renal substitution processes of hemodialysis, hemofiltration, and peritoneal dialysis (PD).[4-6] Acute hemodialysis or hemofiltration has yet to reduce the mortality rate of acute kidney injury (AKI) to less than 50%, despite advances in synthetic materials and extracorporeal circuits.[5,6] Consequently, AKI may be especially amenable to cell therapy in conjunction with continuous hemofiltration techniques, particularly because AKI develops predominantly as a result of injury and necrosis of renal proximal tubule cells. Early replacement of the functions of these cells during an episode of AKI, in conjunction with the current standard of care of hemodialysis, could potentially provide near-complete RRT.

In addition to renal cell-based therapy providing therapeutic efficacy for renal indications, both preclinical large-animal study results[7-11] and clinical trial outcomes[12-14] have indicated this cell-based therapy may play an important immunoregulatory role in the treatment of septic shock. With respect to this, death in patients with AKI is frequently preempted by the onset of systemic inflammatory response syndrome, most often secondary to sepsis, resulting in cardiovascular collapse, ischemic damage to vital organs, and multiorgan failure (MOF).[15-17] The predisposition of patients with AKI to develop systemic inflammatory response syndrome and sepsis suggests that renal function, specifically renal tubule cell function, plays a critical immunomodulatory role in individuals under clinically stressed disease states.

To appreciate more fully the vast potential of renal cell-based therapy, one needs first to understand the role of the renal tubule epithelial cell in, not only the well-accepted processes of renal metabolism, but also a less recognized function of the kidney and the renal tubule cell: immunoregulation of systemic inflammatory responses. During development, the kidney is derived embryologically from dorsal mesoderm, a collection of cells also essential in the development of bone marrow stem cells.[18,19] Of note, the maturation of cells responsible for erythropoietin synthesis and activation of 1,25-dihydroxyvitamin D_3 in the kidney is reflective of this embryonic origin. Phylogenetically, in bony fish and amphibians without lymph systems, the kidney is the major antibody-producing organ.[20,21] Predicatively, as part of the evolutionary process, mammalian renal proximal tubule cells are active immunologically. They are antigen-presenting cells[22-24] that have costimulatory molecules[25] and synthesize and process a variety of pro- and anti-inflammatory cytokines.[26-30] In support of this, patients with end-stage renal disease (ESRD) are typically in a chronic proinflammatory state, indicative of a reduction in immunologic function.[31-33] The degree of inflammation in patients with ESRD has been shown to be highly correlated with mortality rates.[32-36] Diminished renal tubular cell metabolic function, rather than reduced renal filtration and clearance, may very well be the cause of the inflammatory dysregulation observed in these patients. These factors, taken into consideration collectively, are the foundation for the development of the bioartificial kidney (BAK).

This review article details the development, to date, of the BAK, the regulatory and manufacturing challenges encountered during this development process, solutions to these challenges, and future expectations for this exciting, emerging field of bioengineered RRT. In addition to the BAK, there are various other regenerative medicine approaches for the treatment of renal disease indications. A stem cell-based therapeutic intervention has been recently assessed during a phase I clinical trial targeting patients undergoing cardiac surgery who were at high risk for AKI.[37] Preliminary findings from this phase I trial demonstrated allogeneic mesenchymal stem cell therapy to be delivered safely to patients, in addition to showing protection of renal function and reduced hospital bed days. For more detailed summaries of other stem cell approaches to treat kidney disease, and progress of nanotechnology approaches for implantable tissue-engineered RRT, there are several excellent and comprehensive reviews on these topics.[38-41]

DEVELOPMENT OF A BIOARTIFICIAL KIDNEY

The kidney is unique in that it was the first organ for which long-term *ex vivo* replacement therapy was established, with lifesaving outcomes. Renal failure before the inception of hemodialysis and transplantation resulted in certain death, and this dismal outcome of renal failure is still common outside the industrialized world. Even under optimal current RRT, mortality rates in patients presenting with AKI and MOF can be as high as 80%.[42,43] As a result of the prevalence of AKI in hospitalized patients, and this very poor clinical prognosis, recent focus has been directed toward alternate or adjunct RRTs. One of these focused efforts has been to develop a BAK to replace the functions of the renal tubule cell that are diminished during AKI.

Renal assist device. The first and, to date, only BAK that has been tested in a Food and Drug Administration (FDA)-approved clinical trial—the renal assist device (RAD)—consisted of an extracorporeal system that used a standard hemofiltration cartridge seeded with up to 10^8 renal tubule cells grown as monolayers along the inner surface of the hemofiltration cartridge hollow

fibers.[8-11] The fiber nonbiodegradability and pore size enabled the hollow fiber membranes to act as both a scaffold for the cells and an immunoprotective barrier. *In vitro* studies of the RAD demonstrated that the renal cells retained differentiated active transport properties, differentiated metabolic activities, and key endocrine processes.[44] In preclinical large-animal studies, the RAD effectively, when incorporated in series with a hemofiltration cartridge in an extracorporeal blood perfusion circuit, replaced filtration, transport, metabolic, and endocrine functions of the kidney in acutely uremic dogs.[8,9] The RAD was also shown to ameliorate endotoxin shock in acutely uremic animals.[10] Of clinical relevance, RAD therapy, using human renal epithelial cells (RECs), demonstrated efficacy in a phase I/II clinical trial in the treatment of acute renal failure in intensive care unit patients.[12-14]

The RAD system consisted of a filtration device (a conventional hemofilter) followed in series by the renal cell-based RAD.[8] Specifically, blood pumped from the body via a hemodialysis blood pump was directed into the fibers of a conventional hemofilter, where generated ultrafiltrate was then delivered downstream into the lumen of the fibers within the RAD. Processed ultrafiltrate exiting the lumen of the RAD was discarded as "urine." The filtered blood exiting the conventional hemofilter lumen was directed to the extracapillary space of the RAD, where it was dispersed throughout the RAD fibers. A portion of the processed ultrafiltrate, which contained the cell-based therapeutic products, passed through the membrane wall of the RAD hollow fibers where it merged with the filtered blood from the conventional hemofilter. The processed blood with therapeutic ultrafiltrate was then delivered back to the body. The temperature of the RAD was maintained at 37°C throughout the entire treatment period to ensure optimal functionality of the cells. The cells within the RAD maintained viability via metabolic substrate and low-molecular weight growth factor delivery from the ultrafiltrate in the lumen and the blood in the extracapillary space. Immunoprotection of the RAD cells was garnered by the molecular weight cutoff of the conventional hemofilter hollow fiber walls, which restricts passage of immunoglobulins and immunologically competent cells. Rejection of the cells, therefore, did not occur. In addition, the animal or patient was immunoprotected from the potential release of renal cells by the molecular weight cutoff of the RAD hollow fiber walls. This arrangement allowed the ultrafiltrate from the conventional hemofilter to enter the renal cell-lined hollow fibers of the RAD for metabolic processing, followed by regulated transport through the walls of the RAD hollow fibers into the systemic blood and back to the animal or patient.

RAD: preclinical large-animal studies. To assess efficacy of the RAD in the treatment of acute renal failure-associated indications, a series of preclinical studies was carried out in several large-animal models, including a canine model of uremia,[8,9] a canine model of lipopolysaccharide-induced endotoxic uremia,[10] a canine model of bacterial-induced septic uremia,[11] and a porcine model of septic shock-associated multiorgan dysfunction (SSMOD).[7] These studies demonstrated the RAD (1) to replace effectively the filtration, transport, metabolic, and endocrinologic functions of the kidney in acutely uremic dogs; (2) to have measurable effects on circulating mediators of inflammation and on hemodynamic stability in endotoxic-challenged uremic dogs, which was supported further by improved metabolic renal function—provided by RAD therapy—in septic uremic dogs; and (3) to improve cardiovascular performance associated with changes in cytokine profiles in septic shock-associated AKI pigs, resulting in a significant survival advantage.

RAD: clinical trials. With the success of the preclinical RAD large-animal studies, an investigational new drug (IND) application was obtained from the FDA authorizing the RAD to be tested under a phase I/II clinical trial in intensive care unit patients with AKI and MOF receiving continuous venovenous hemofiltration.[12,13] This initial study, consisting of 10 patients, demonstrated safe use of RAD therapy for up to 24 hours. Cardiovascular stability was maintained, and improvement in native renal function, as indicated by increased urine output, was correlated temporally with RAD treatment. All patients were critically ill with AKI and MOF, with predicted hospital mortality rates between 80% and 95%. Under RAD therapy, an actual mortality rate of 40% was demonstrated, with 6 of the 10 treated patients surviving past 30 days. The human RECs contained in the RAD demonstrated differentiated metabolic and endocrinologic activity in this *ex vivo* clinical situation, including glutathione degradation and endocrinologic conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3. Plasma cytokine levels suggested that RAD therapy produces dynamic and individualized responses in patients depending on their unique pathophysiological conditions. For the subset of patients who had excessive proinflammatory levels, RAD treatment resulted in significant declines in granulocyte-colony stimulating factor, interleukin (IL)-6, IL-10, and especially the IL-6/IL-10 ratio, suggesting a greater decline in IL-6 relative to IL-10 levels and a less proinflammatory state. These favorable phase I/II trial results led to subsequent FDA-approved, randomized, controlled phase II investigations at 12–15 clinical sites to determine whether this cell therapy approach

alters patient mortality. The early results from the phase II trials were as compelling as the phase I/II results. RAD therapy improved the 28-day mortality rate from 61% in the conventional hemofiltration-treated control group to 34% in the RAD-treated group.[14] This survival impact continued through the 90-day and 180-day follow-up periods ($P < 0.04$), with the Cox proportional hazard ratio indicating that the risk of death was 50% of that observed in the conventional RRT group.

Although RAD therapy demonstrated very encouraging clinical outcomes, the clinical trial was halted during the phase IIb interval analysis because of suboptimal clinical protocol design and several fabrication and manufacturing hurdles. These hurdles presented challenges that required resolution before more clinical trial testing of renal cell-based therapies could be conducted.

CHALLENGES IN THE DEVELOPMENT OF A BAK

There are several key stages in the development of a biologic to move it from inception to a robust product for commercialization. These include performing *in vitro* bench testing, conducting *ex vivo* preclinical animal testing for safety and efficacy, establishing robust Good Manufacturing Practices manufacturing and distribution procedures that incorporate appropriate quality assurance (QA) and quality control (QC), obtaining FDA approval to test under an IND, determining initial positive safety and efficacy clinical trial outcomes followed by manufacturing scale-up for final pivotal clinical trial testing, obtaining FDA biologic license application approval, and then, and only then, and only with adequate financial backing, beginning manufacture for market distribution. At each point during the development process, critical regulatory and manufacturing guidelines and controls must be considered carefully. When the biologic is a cell-based medical device, the regulatory and manufacturing milestones required to achieve an FDA-approved biologic license application for a marketable product are even more rigorous. Critical to achieving these milestones are (1) establishing protocols that yield a robust cell source with desired therapeutic characteristics that can pass specific lot release criteria, (2) developing fabrication procedures that allow the end product (the cell-based device) to be QA/QC tested after final manufacture manipulation, (3) having on-demand clinical use, (4) creating a manufacturing process that has multiple stop points that allow for efficient use of labor and resources, and (5) having a product that can be administered safely to the desired patient population.

Hurdles met during RAD development. During the initial RAD clinical trials demonstrating promising therapeutic efficacy, a careful evaluation of the RAD manufacturing process was initiated. It became clear that for REC-based therapy to become a viable, robust therapeutic, several changes were necessary with respect to cell source, the fabrication process, device design, and distribution to the clinical site.

Challenge: tissue source and supply. Critical to providing organ function replacement through cell therapy is the isolation and *in vitro* expansion of specific cells from adult tissue. As such, the first obstacle encountered during the development of the RAD was with respect to choice of species of the kidney source for the cells to be used in the RAD. Cells of choice must have stem cell-like characteristics, with a high capacity for self-renewal and the ability to differentiate under defined conditions into specialized cells, capable of developing the correct functional components of a physiological organ system. Methodology to isolate and expand renal proximal tubule progenitor cells from adult pig kidneys was well established,[45,46] and was therefore a natural first choice as the tissue source for the cells to be seeded into the RAD. Furthermore, the pig has been considered the best source of organs for both xenotransplantation and cell therapy devices because of its anatomic and physiological similarities to human tissue, and the relative ease of breeding large numbers of pigs in closed herds. However, at the RAD pre-IND meeting with the FDA, concern over reports of the ability of porcine endogenous retroviruses to infect human cells in coculture *in vitro* was raised. The potential risk of transmission of viral elements from porcine tissue to humans in xenogenic-based cell therapy devices[47,48] was the catalyst for moving to human RECs, isolated from kidney transplant discards, for seeding the RAD. Preclinical *in vitro* benchtop and *ex vivo* large-animal studies demonstrated these human cells to perform well with respect to viability, durability, and physiological performance.[8]

The number of RAD units that could be generated from a human kidney was limited to 4–5 units. In addition, only 5–10 kidneys from organ transplant discards were available for RAD generation per month, yielding an average of 20–50 RAD units per month—significantly less than monthly clinical need.

Challenge: fabrication and design. Fabrication and maintenance of the RAD was very labor intensive, specifically because, once fabricated, hands-on specialized technical support and constant maintenance at 37°C was required at a central manufacturing facility, with 37°C shipment to clinical sites also a necessity. These requirements delayed treatment and added to the overall cost of therapy. In addition, there was no

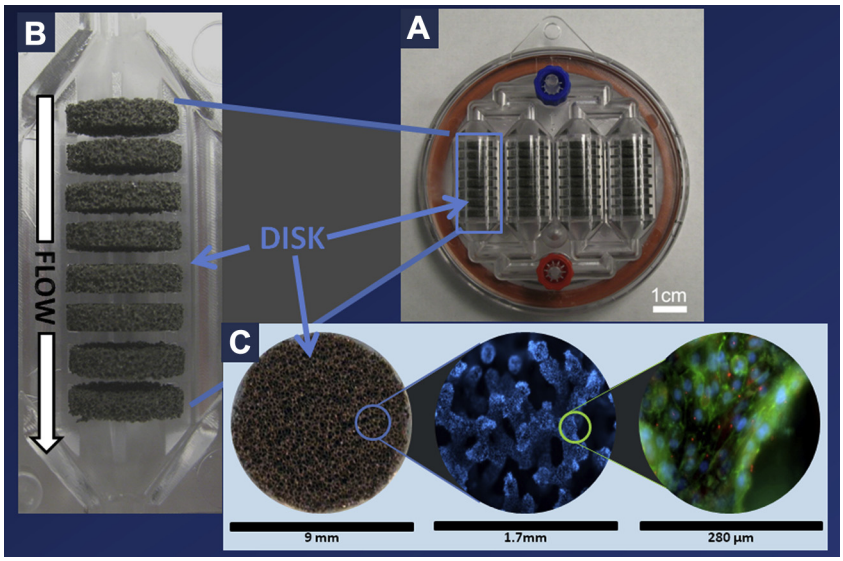

Fig 1. (**A–C**) The compact, freezable bioartificial renal epithelial cell system (BRECS) design (**A**), with an enhanced view of the carbon disk stack channel (**B**) and additional views of a human renal cell seeded carbon disk (**C**, 9-mm insert) and the cells on the carbon disk (**C**, 1.7-mm and 280-μm inserts). The cells depicted in (**C**) are human renal epithelial cells derived from the enhanced propagation (EP) method of cell expansion that allows more than 5000 BRECS to be fabricated from a single kidney, eliminating the issue of maintaining an adequate cell source for clinical applications. After culture, the previously cryopreserved cells show coverage (**C**, right; blue 4′,6-diamidino-2-phenylindole (DAPI)-labeled nuclei, 1.7-mm insert) and the correct renal phenotype with antibodies to tight junctions (**C**, right; Zona occludens protein 1 (ZO-1); green web pattern, 280-μm insert) and central cilia (**C**, right, insert; Acetylated alpha-tubulin 1 (AT-1); red dots, 280-μm insert). (For interpretation of references to color in this figure legend, the reader is referred to the web version of this article.)

manufacturing stop point, after final manufacture manipulation, for the rigorous QA/QC analyses required by federal regulations for biologics. Optimally, a cell-based device should have the ability to be held in a cryopreserved state to allow for postfinal manufacture QA/QC testing. Cryopreservation would also allow for distribution to and storage at the clinical site for on-demand deployment.[49] The RAD could not withstand the process of cryopreservation because the polysulfone hollow fibers would fracture during the freeze process.

The RAD, originally designed as adjunct therapy to existing dialysis treatment in patients with AKI, has a large footprint (26 × 3-cm cylinder) and significant blood/ultrafiltrate fill volume (175 mL), requiring an additional extracorporeal circuit and pump for delivery of blood and plasma ultrafiltrate. In addition to being a complex system, not easily set up or managed by existing, untrained standard-of-care hospital staff, the substantial blood volume requirement would not be tolerated safely by many critically ill patients. Because of these limitations, development of the RAD was discontinued, with a redirection of focus toward a more robust BAK format: the bioartificial renal epithelial cell system (BRECS; Fig 1).

THE BIOARTIFICIAL RENAL EPITHELIAL CELL SYSTEM

The improvements required to move REC-based therapy forward, toward a commercially viable, robust bioengineered device mandated a completely new approach in the design and fabrication process from that used for the RAD. This new approach leveraged the knowledge base gained during the development of RAD therapy to initiate an improved BAK: the BRECS.[50] Several iterations in the design platform occurred during the course of the BRECS development process. The final design stage of the current BRECS included (1) machining of an injection mold die for cost-effective, mass production of the BRECS housing units; (2) validation that the injection-molded housing units could support viable REC seeded on a porous scaffold; (3) demonstration that the REC-seeded injection-molded BRECS could tolerate the cryopreservation/reconstitution process; and (4) confirmation the REC-seeded injection-molded BRECS was both safe and therapeutically efficacious in preclinical animal studies.

Solutions to the RAD development hurdles. The first significant advancement in the BRECS development was with respect to cell supply. To achieve a robust cell supply, and because human donor tissue is limited, focus

was directed toward improving renal cell yield per human kidney. An enhanced propagation (EP) method to expand renal epithelial progenitor cells from available adult human kidney transplant discards was developed and implemented successfully to provide the biomass necessary for renal cell-based therapeutics.[51] The EP method was successful in generating renal progenitor cells using kidneys from a wide range of donors, including suboptimal health profiles consisting of long-term diabetes, hypertension, age older than 70 years, donation postcardiac death with prolonged warm ischemia, and 1 donor with ESRD on dialysis for more than 2 years. Under EP, cell yields are consistently higher than 10^{11} cells/g cortex, allowing for the manufacture of more than 100,000 BRECS per donor kidney for use in acute disease indications. In addition, more than 1.8×10^{10} progenitor cells/g cortex were isolated from the 1 ESRD kidney tested, which strongly supports that autologous therapy may be possible using cells derived from a renal sample taken early during the course of chronic renal failure. In this regard, a conservative 0.1-g renal cortex sample excised from a patient with ESRD would allow for the fabrication of at least 18 BRECS containing 10^8 cells/unit. The cells isolated using the EP method compared favorably, with respect to metabolic function and morphologic phenotype, to the cells used in the RAD therapy clinical trials. Cryopreservation of the EP isolated RECs, which is necessary to allow for processing of these large cell yields, has been advanced using FDA-compliant procedures. Cell death after thawing has been minimized, with the therapeutic potential of progenitor-derived RECs conserved, as evaluated using a surrogate efficacy panel consisting of lipopolysaccharide-stimulated IL-8 secretion, γ-glutamyl transpeptidase enzyme activity, and 25-hydroxyvitamin D_3 1 alpha-hydroxylase enzyme activity.

The second critical challenge that was addressed during the development of the BRECS was that of cryopreservation of the entire cell device, allowing for (1) the required QA/QC testing postmanufacture stop point, (2) long-term storage with limited ongoing resource expenditure, and (3) distribution to and storage at clinical sites for rapid, on-demand deployment for acute applications. As indicated, development of a cell device that can be cryopreserved and stored at clinical sites is a critical element for market penetration. With this in mind, cryopreservation was considered carefully at each step of the BRECS design process. Porous niobium-coated carbon disks were selected as the scaffold for the RECs to attach to and grow on. This choice was a result of the high porosity of the carbon disk, allowing for significant surface area for cell expansion[52-54] and ability to undergo the cryopreservation process without compromise. The BRECS housing components—including a thin polycarbonate top and bottom for optimized heat transfer; a gasket allowing for a sterile, airtight snap-fit closure; and inlet/outlet ports—were selected carefully and tested systematically to resist structural damage under cryogenic temperatures, so that the internal housing would retain an uncompromised, sterile state. To date, BRECS have been cryopreserved successfully for as long as 6 months, followed by reconstitution, with the cells in the thawed BRECS demonstrating similar metabolic and phenotypic profiles as that of cells in noncryopreserved, fresh BRECS. Recent studies have shown thawed BRECS to maintain viability both in an acute porcine model of SSMOD and a chronic ovine model of uremia.[52]

Additional improvements were made to allow for ease of implementation at clinical sites, including (1) a compact footprint with a low-profile (1.5 cm) housing unit (diameter, 8.5 cm), (2) reduced fill volume (10 mL), and (3) a simplified, bloodless circuit for administration of therapy. These improvements will also enable use of this technology for a broader scope of clinical indications, including severely ill patient populations that cannot tolerate large extracorporeal blood volumes, and use in nonblood-based circuits such as that used during PD.

BRECS: preclinical large-animal studies. The BRECS was designed for perfusion with either ultrafiltrate from hemofiltered blood or body fluids, such as peritoneal fluid, to minimize the numerous problems associated with maintaining a continuous acute or chronic extracorporeal blood-based circuit. During BRECS therapy, immunoisolation is afforded by 65-kDa molecular weight cutoff filters, placed before and after the BRECS, that allow systemic circulating factors (such as small proteins and hormones) to be presented to the cells of the therapy device while protecting both the device from immune system components from the patient and the patient from any large molecular compounds secreted by the BRECS or cells released from the BRECS that could illicit an adverse reaction. Therapeutic products, such as REC-secreted small proteins and hormones, are allowed to pass through the post-BRECS immunoisolation filter and return to the patient. The uremic state can be controlled by modulating the small solute clearance through hemofiltration dose by adjusting the prefilter surface area, ultrafiltration rate, and/or percent flow to waste.

In preclinical studies, BRECS extracorporeal therapy compared with sham therapy demonstrated efficacy in the treatment of SSMOD pigs, with improved cardiovascular performance; modulation of activated

neutrophils; 50% reduction in the extravasation of neutrophils, which then infiltrate the lungs; and prolonged survival time.[55] In the most recent preclinical study series, under optimized therapy design, BRECS treatment actually improved survival outcomes to achieve a predetermined 16-hour end point, as opposed to death of the animal. In an ovine model of uremia, BRECS therapy, delivered via a continuous-flow PD circuit with recycling PD, demonstrated an enhancement in oxidative burst and modulation in neutrophil activated state, which was sustained through the 48-hour postcessation of therapy predetermined study end point. More important, all BRECS remained viable, with detectable oxygen consumption and glutathione degradation rates, for the entire therapeutic period, for as long as 7 days.[56]

BRECS: potential clinical applications. Although BRECS technology has not yet advanced from the preclinical to the clinical trial stage, the conceptualization for BRECS therapy in both acute and chronic indications is in place. As was the case with the RAD, AKI and/or SSMOD will most likely be the first acute indication targeted for assessing the BRECS therapeutic impact under the IND regulatory pathway. The envisioned approach for delivery of BRECS therapy will be via a modified continuous venovenous hemofiltration circuit. A portion of the posthemofilter ultrafiltrate (currently BRECSs require 10 mL/min) will be channeled through the cell-seeded disks of the BRECS. This "conditioned" ultrafiltrate, containing BRECS therapeutic products, will be recombined with the hemofiltered blood and directed back to the patient (Fig 2).

Success under an acute clinical application will open the regulatory door for BRECS therapy in the treatment of chronic indications, of which the ESRD treatment course of PD could be engineered to incorporate BRECS therapy. Establishing BRECS/PD therapy for clinical use would motivate the development of a wearable bioartificial kidney. The small BRECS footprint allows for placement on a "belt-style" system, with a battery-operated, miniaturized pulsatile pump driving the BRECS/PD circuit to pass from the patient outlet PD catheter through the BRECS and back to the patient via the inlet PD catheter. To replace the excretory function of the kidney, the wearable BAK would incorporate microengineered sorbent-based technologies that have a significantly reduced water requirement and can regenerate spent dialysate.[57,58] A wearable continuous PD system, similar to that conceptualized for the BRECS, has been described by Ronco and Fecondini.[59]

As with all bioengineered organ replacement therapies, the ultimate goal is that of an implantable BAK that incorporates the filtrative, metabolic, absorptive, and endocrinologic functions of the kidney. An implantable "biohybrid" or bioartificial device has the potential to avoid both supply limitations to renal transplant and the burden of therapy of intensive maintenance dialysis. The innovative combination of an implanted hemofilter and an implantable BRECS would provide continuous small-solute clearance along with metabolic functions of the proximal tubule, without the clinical complications and burden of catheters and dialysis. The production of silicon nanopore membranes with defined, slit-shaped pores that are tailored for implementation in a BAK is feasible using microelectromechanical systems and nanotechnology. Specific means for patients to self-monitor and reprogram device function will be critical for truly independent self-care using an implanted device. Life cycle management of the device, including recognition of impending device failure and a minimally invasive approach to renew or replace failed components, modules, or cartridges are also essential elements required for the implantable BAK to become a viable clinical approach in the treatment of chronic renal disease.

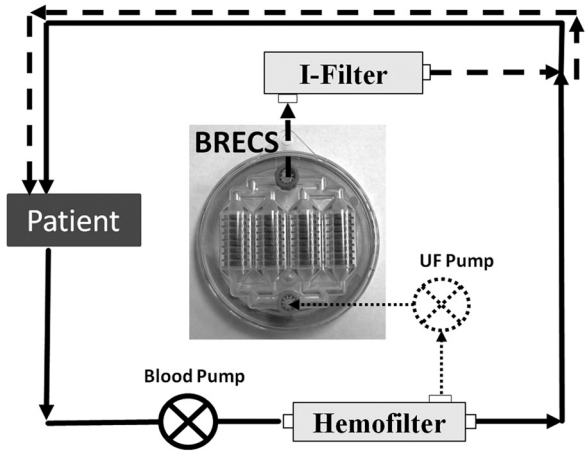

Fig 2. The bioartificial renal epithelial cell system (BRECS) extracorporeal circuit schematic for future clinical treatment of acute renal failure. Blood from the patient (solid line) flows through a standard dialysis hemofilter, with ultrafiltrate (UF) generated by the hemofilter (dotted line) being directed through the BRECS. The 65-kDa molecular weight cutoff of the hemofilter provides immunoprotection to the cells in the BRECS from any immunoreactive molecules and/or cells from the patient. "Conditioned" UF containing BRECS cellular therapeutic products (dashed line) passes from the BRECS and is channeled back to the blood returning to the patient (combined solid and dashed lines). Before being merged with the returning blood, the conditioned UF passes through an immunoisolation filter (I-Filter), which protects the patient from any large molecular by-products and/or cells released by the BRECS that could cause an adverse reaction.

CONCLUSIONS

Bioengineered RRT is a rapidly advancing field. The innovative technologies discussed in this review have advanced the bioengineered kidney from concept to

Table I. Bioartificial kidney: steps toward clinical application

Hurdle	Solution status
FDA approval for first in human clinical trial testing.	Renal cell therapy has demonstrated safety and efficacy in preclinical (RAD and BRECS) and clinical studies (RAD).
Robust cell supply required for commercially viable product.	Enhanced propagation methods have been developed that generate a robust renal cell supply for bioengineered RRT applications.
Bioengineered cell system for mass production.	A compact BRECS has been developed for use in renal and inflammatory chronic and acute indications.
Bioengineered cell system capable of passing rigorous regulatory QA/QC testing.	The ability of the BRECS to be cryopreserved provides a manufacturing stop point, allowing for QA/QC lot release before clinical use.
Bioengineered cell system capable for onsite, on-demand clinical use.	The BRECS can be cryopreserved for transport, storage, and reconstitution at clinical sites.

Abbreviations: BRECS, bioartificial renal epithelial cell system; FDA, Food and Drug Administration; QA, quality assurance; QC, quality control; RAD, renal assist devise; RRT, renal replacement therapy.

actual clinical application. Critical challenges along the course of this process have been identified, with many solutions implemented. Both the RAD and BRECS preclinical results and the RAD clinical trial outcomes demonstrate potential clinical application of REC therapy in the treatment of, not only AKI, but also inflammatory disorders, such as septic shock. The current BRECS design lends itself toward therapeutic application to treat acute disease indications. Furthermore, the portability of the BRECS, because of its small size, allows for future incorporation into a wearable dialysis system for chronic indications. Although no current wearable dialysis system exists, recent advancements in pump miniaturization and technology to reduce the volume of regenerated dialysate during continuous PD are important steps to an engineered solution.[60-62] In this regard, the current BRECS cell durability suggests that it could be used for at least 6 months in a chronic-use application without need for replacement, which is ideal for a BAK in a continuous PD circuit.[40]

Although the current BRECS design addresses the key shortcomings of the RAD, it does lack the potential of basic vectorial transport activity, which the RAD, because of its hollow fiber component, is capable of. In addition, although significant advances have been achieved with the BRECS toward a commercially robust BAK (Table I), before initiation of clinical trials, the cryopreservation/reconstitution process must be optimized further for ease of use at clinical sites, with proper safety, quality, and efficacy standards in place.

With these directives in mind, to achieve a BAK with metabolic, endocrinologic, and reabsorption functions of the kidney, the next-generation BAK will need to incorporate systems that allow for the basic transport properties of the RAD while maintaining the improved metabolic function and cryopreservability of the BRECS.

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