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RESEARCH ARTICLE

Development of a wearable bioartificial kidney using the Bioartificial Renal Epithelial Cell System (BRECS)

Kimberly A. Johnston[1], Angela J. Westover[1], Alvaro Rojas-Pena[2], Deborah A. Buffington[1], Christopher J. Pino[1], Peter L. Smith[1] and H. David Humes[1,3]*
[1]*Innovative BioTherapies Incorporated, Ann Arbor, MI, USA*
[2]*The University of Michigan, Ann Arbor Section of Transplantation Surgery, Ann Arbor, MI, USA*
[3]*University of Michigan, Ann Arbor Department of Internal Medicine-Nephrology, Ann Arbor, MI, USA*

Abstract

Cell therapy for the treatment of renal failure in the acute setting has proved successful, with therapeutic impact, yet development of a sustainable, portable bioartificial kidney for treatment of chronic renal failure has yet to be realized. Challenges in maintaining an anticoagulated blood circuit, the typical platform for solute clearance and support of the biological components, have posed a major hurdle in advancement of this technology. This group has developed a Bioartificial Renal Epithelial Cell System (BRECS) capable of differentiated renal cell function while sustained by body fluids other than blood. To evaluate this device for potential use in end-stage renal disease, a large animal model was established that exploits peritoneal dialysis fluid for support of the biological device and delivery of cell therapy while providing uraemic control. Anephric sheep received a continuous flow peritoneal dialysis (CFPD) circuit that included a BRECS. Sheep were treated with BRECS containing 1×10^8 renal epithelial cells or acellular sham devices for up to 7 days. The BRECS cell viability and activity were maintained with extracorporeal peritoneal fluid circulation. A systemic immunological effect of BRECS therapy was observed as cell-treated sheep retained neutrophil oxidative activity better than sham-treated animals. This model demonstrates that use of the BRECS within a CFPD circuit embodies a feasible approach to a sustainable and effective wearable bioartificial kidney.

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Keywords **artificial kidney; kidney failure; end-stage renal disease; peritoneal dialysis; cell therapy; sheep model**

1. Introduction

Renal replacement therapy (RRT) using haemodialysis or peritoneal dialysis (PD) has become a mainstay therapy for patients with end-stage renal disease (ESRD). Although this approach is life sustaining, it remains suboptimal with poor clinical outcomes. Current RRT utilizes semi-permeable membranes to substitute for the small solute clearance function of the renal glomerulus but they do not replace the transport, metabolic, and endocrinological functions of the tubular cells, rendering them only a partial substitutive therapy at best. The better outcomes in patients treated with kidney transplant compared with chronic dialysis emphasizes the benefits of full renal replacement for ESRD (US Renal Data System 2008; Tonelli *et al*., 2011).

Renal cell therapy incorporated into conventional RRT has shown metabolic, immunological and survival benefits in acute renal failure (ARF) in preclinical (Humes *et al*., 1999, 2002, 2003a; Fissell *et al*., 2003; Huijuan *et al*., 2007; Wang *et al*., 2010; Westover *et al*., 2014) and clinical studies (Humes *et al*., 2003b, 2004; Tumlin *et al*., 2008). The first renal cell therapy device used in patients with ARF, the renal assist device (RAD), demonstrated that allogeneic renal epithelial cells (REC) maintained within an extracorporeal environment could provide therapeutic benefit to patients requiring continuous RRT and improve survival (Humes *et al*., 2004; Tumlin *et al*., 2008). The limitations of cell expansion and device design made manufacture, storage and distribution of the RAD a challenge to meet clinical demand. Enhanced REC propagation methodologies (Westover *et al*., 2012), along with improved fabrication techniques have overcome these challenges, giving rise to a second-generation device, the Bioartificial Renal Epithelial Cell System (BRECS), which was designed to surmount a number of obstacles preventing widespread use of cell-based therapies. This approach resulted in the ability to maintain a dense population of RECs within a compact, portable and cryopreservable format for on-demand deployment in clinical situations (Buffington *et al*., 2012). The BRECS is a bioreactor containing adult RECs seeded onto porous carbon disks within a polycarbonate housing. Cell viability is maintained by perfusion culture through the disks, allowing cells to respond to alterations in the perfusate milieu, potentially releasing metabolic and endocrinological products with therapeutic value.

In addition to previously mentioned limitations for global use of cell enhanced therapies, the difficulty in maintaining continuous extracorporeal blood circulation without thrombosis, despite continuous anticoagulation

*Correspondence to: H. D. Humes, Innovative BioTherapies Inc., 650 Avis Road, Suite 300, Ann Arbor, MI 48108, USA. E-mail: dhumes@innbio.com

strategies, remains an impediment to development of a sustainable portable cell device for treatment of ESRD. Elimination of the blood circuit inherent to haemodialysis-based therapies could facilitate delivery. To this end, a wearable bioartificial kidney (WeBAK) that exploits peritoneal fluid to maintain oxygen and nutrient delivery to a BRECS was conceived and tested in an ovine model. The BRECS devices were monitored during and after extracorporeal culture and serial blood sampling was performed on sheep to assess the metabolic, endocrinological and immunological impact of BRECS therapy. The project aimed to provide insight into the feasibility and potential impact of delivering renal cell therapy in this manner for treatment in ESRD.

2. Materials and methods

2.1. Bioartificial renal epithelial cell system (BRECS)

The BRECS comprises porous carbon disks colonized with up to 2×10^8 human RECs within a perfusable polycarbonate housing. Perfusate flows over and through the disks to sustain cell viability and export cell products. An injection-moulded design allows BRECS to be fitted with monitoring systems, permitting the monitoring of temperature and oxygen consumption in real time. The total weight of a cell-seeded and fluid-filled device is approximately 62 g.

Primary RECs were isolated and expanded from human kidneys unsuitable for transplantation because of anatomical or fibrotic defects (procured from the National Disease Research Interchange) following an established method (Westover *et al*., 2012). Details of REC isolation, seeding onto carbon disks for use in BRECS units and maintenance of BRECS *in vitro* have been described previously (Buffington *et al*., 2012).

Metabolic activity of RECs within BRECS was evaluated using oxygen consumption rates (OCR) and non-destructive glutathione (GSH) metabolism. Oxygen measurements were performed either using an i-STAT analyser (Abbott Point of Care, Princeton, NJ, USA) on media collected from closed units or by oxygen sensors. RedEye patches (Ocean Optics, Dunedin, FL, USA) or PSt3 patches (PreSens, Regensburg, Germany) were set up in recirculating, closed, oxygen-impermeable circuits. To determine average OCR, a linear regression approximation of slope was used. The GSH metabolism was determined by the rate of degradation of exogenously added GSH. The BRECS were completely filled with 20 μM GSH (Sigma-Aldrich, St. Louis, MO, USA) in Ultra MDCK media and samples collected at baseline, 10 min and 30 min. Samples were analysed by the method of Tietze (1969). After extracorporeal culture, a qualitative assessment of cell viability was made by adding 1 μg/ml fluorescein diacetate and propidium iodide to individual disks from disassembled BRECS within well plates. Living and dead cells were visualized immediately with a Zeiss Axiovert 200 inverted fluorescence microscope (Carl Zeiss, Inc. Thornwood, NY, USA) equipped with corresponding filter sets, and micrographs obtained using Zeiss AxioCam MRm and ICc1 cameras.

2.2. Experimental animals and the extracorporeal WeBAK circuit

Animal use adhered to principles stated in the Guide for Care and Use of Laboratory Animals (Clark *et al*., 1996) and procedures were performed under protocols approved by the institutional committee for care and use of animals at the University of Michigan, USA. Female sheep (35–45 kg) were maintained under standard laboratory conditions until onset of continuous flow peritoneal dialysis (CFPD), during which they were confined in customized stanchions while connected to the extracorporeal circuit. Sheep were instrumented with separate ingress and egress PD catheters and nephrectomy was staged to permit healing around the catheters, which were placed concurrently with removal of the first kidney. Antimicrobial and analgesic medication was administered perioperatively. CFPD was instituted within 24 h of complete nephrectomy using commercially available 4.25% glucose dialysate solution (Dianeal®; Baxter Healthcare, Deerfield, IL, USA) with nafcillin (100 mg/l) and gentamicin (4 mg/l) added. An intraperitoneal instillation of 2–3 l dialysate was continuously recirculated at 80–100 ml/min through a primed extracorporeal circuit of ¾ inch (1.9 cm) ID Tygon® tubing using a rotary pump with a segment of collapsible tubing to prevent generation of negative abdominal pressure (M-pump; MC3 Inc., Ann Arbor, MI). A polysulfone haemofilter with a 65 kDa molecular size cut-off provided immuno-isolation for the cells by generating ultra-filtered PD that was directed by a peristaltic single channel pump (Masterflex®; Cole-Palmer, Vernon Hills, IL, USA) through a fluid warming system (Hotline®; Smith Medical, Dublin, OH, USA) at 38°C for parallel perfusion of the BRECS (Figure 1). Total circuit volume was approximately 1000 ml. Dialysate was refreshed by continuous flow into and out of the circuit plus daily exchanges of the abdominal content. BRECS containing approximately 1×10^8 RECs or acellular sham devices were cultured under identical conditions *in vitro* before placement in an extracorporeal WeBAK circuit. Devices were deployed subsequent to dialysate equilibration to physiological pH and were maintained by *ex vivo* perfusion with peritoneal fluid for up to 7 days.

Serum chemistry and electrolyte values were determined using automated veterinary analysers (IDEXX VetTest®, IDEXX VetLyte®; IDEXX Laboratories Inc., USA) according to the manufacturer's directions. Complete blood counts and differentials were determined by a Hemavet® analyzer (Drew Scientific, Waterbury CT). Neutrophil (NE) counts and quantification of intracellular reactive oxygen species (ROS) were assessed as markers of immunologic status. The NEs were isolated on a discontinuous Percoll gradient, incubated with the reactive dye, 5-(and 6)-chloromethyl-2′,7′-dichlorodihydroflourescein

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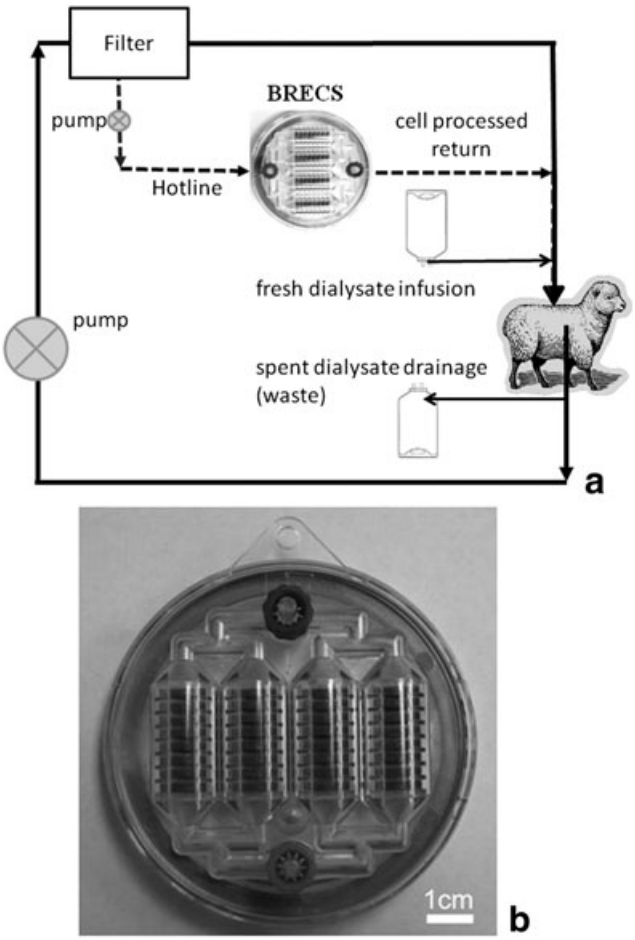

Figure 1. Schematic representation of the wearable bioartificial kidney. (a) An extracorporeal circuit was devised to permit continuous flow of peritoneal fluid to provide uraemic control for nephrectomized sheep and simultaneous perfusion of a Bioartificial Renal Epithelial Cell System (BRECS) for cellular enhanced therapy. Circuit flows of 50–80 ml/min were maintained using a rotary pump. Filtered and temperature regulated peritoneal fluid was pumped through the BRECS at 10–50 ml/min providing oxygen and nutrients to the bioreactor and the delivery of cell therapy to the sheep. Flow rates into and out of the circuit could be adjusted to attain the desired amount of small solute clearance and ultrafiltration. A bypass loop (not pictured) permitted recirculation of fluid *ex vivo* to retain continuous perfusion of BRECS during the daily dialysate exchange. (b) The BRECS is an attached cell bioreactor comprised of renal epithelial cells seeded onto porous carbon disks enclosed within a polycarbonate housing. Cell viability is maintained by perfusion culture with filtered body fluids (i.e. peritoneal fluid) enabling use in a blood-free extracorporeal circuit, as depicted.

diacetate, acetyl ester (CM-H2D CFDA, 10 μM; Molecular Probes, Eugene, OR, USA) in Roswell Park Memorial Institute (RPMI) 1640 media 15 min at 37°C, followed by incubation (±) phorbol-12-myristate-13-acetate (PMA), 1 μM, at 37°C for 30 min. The NEs were labelled with mouse anti-bovine unconjugated CD11b (ABD Serotech, Kidlington, UK)/Anti-IgG conjugate mouse 647 (Life Technologies, Carlsbad, CA, USA) to allow gating then fixed. Mean fluorescent intensity was determined using an Accuri Flow Cytometer.

Endocrine support by BRECS was assessed using vitamin D3. To ensure an adequate source of substrate, 3000 units/day of 25-hydroxyvitamin D3 (25VitD3; Sigma Aldrich) was infused into the CFPD circuit pre-BRECS. Sheep serum samples were frozen and sent to Heartland Assays, Inc. (Ames, IO, USA) for quantification of 1α,25-dihydroxyvitamin D3 (1,25VitD3) by radioimmunoassay.

2.3. Statistical analyses

Comparisons between groups were made using Student's *t*-test assuming equal variance. Significance was set at $p < 0.05$.

3. Results and discussion

3.1. Establishing a WeBAK using CFPD in an ovine model

Tissue engineered devices, such as the RAD and the BRECS, have revealed the therapeutic value in replacing the lost metabolic, endocrine and immunological functions of the kidney, however impediments to long term delivery of cell therapy remain. As technological advancements are made in miniaturization of medical devices, interest in portable dialysis systems is increasing as is recognition of PD as a viable platform for a sustainable wearable artificial kidney (Kim and Ronco 2011; Davenport, 2012; Armignacco *et al.*, 2015). A large animal model of uremia sustained using 24 h continuously recirculating CFPD was developed to demonstrate a WeBAK based on this modality. Pilot animals ($n = 13$) were needed to establish instrumentation and PD protocols. Catheter complications were a primary obstacle to a recirculating CFPD regimen, with catheter dysfunction occurring in 23 of the 34 total animals used. Leakage of fluid around catheters and catheter occlusion by omental wrapping were leading causes for disruption or failure of

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CFPD. Inclusion of a purse-string suture in the peritoneum at catheter implantation, a 14-day healing period before onset of PD and Oreopoulos–Zellerman catheters that limit omental wrapping were found collectively to minimize catheter malfunction in this model. Fibrin accumulation within the circuit was universal and the filter was replaced as needed to maintain circuit patency. Culture of bacteria from PD fluid of five pilot animals prompted the empirical addition of antimicrobials to the dialysate. Bacterial culture of PD fluid was positive for a single sheep in each of the study groups but no microbial contamination of BRECS units was identified, demonstrating efficacy of the WeBAK design to maintain an aseptic environment for the BRECS.

Upon overcoming the challenges of the model, a stable uraemic state could be established with CFPD, enabling at least 9 days of study with anephric sheep (Figure 2). Dialysate flow rates into the circuit and to waste were set at 420 ml/h and 470 ml/h respectively, providing a calculated weekly creatinine clearance of 55 l/week and ultrafiltrate generation up to 1 l/day. As the study aims were to determine feasibility and effects of maintaining the biological device within the WeBAK, no further characterization or optimization of dialysis efficiency using the CFPD circuit was attempted. Uraemic control was dependent on dialysis dose and therefore not different between animals treated with cellular or sham devices.

3.2. Extracorporeal maintenance of BRECS

Bioartificial Renal Epithelial Cell Systems containing approximately 1×10^8 RECs or acellular sham devices were cultured under identical conditions for an average of 21 ± 9 days *in vitro* according to BRECS protocol before placement in an extracorporeal WeBAK circuit. Devices were maintained in the WeBAK by perfusion with ultrafiltered peritoneal fluid for up to 7 days then returned to *in vitro* culture for 48 h before dismantling for histology. Cell BRECS demonstrated relatively consistent metabolic activity throughout the duration of extracorporeal therapy, confirming that RECs within the BRECS remained viable and metabolically active when maintained by the nutrients provided by CFPD. At the commencement of extracorporeal culture, the OCR in BRECS averaged 129 nmol/min and by day 7, average OCR was 85 nmol/min (Figure 3a). The OCR measured on days 5–7 trended toward being slightly lower than initial measurements; however, these values were not significantly lower ($p = 0.65$, $p = 0.53$ and $p = 0.59$ for days 5, 6 and 7, respectively). Non-destructive GSH degradation by γ-glutamyltranspeptidase (γGT) was chosen as a representative measurement of BRECS metabolic function: γGT, which is expressed on the apical brush border membrane of renal proximal tubules, catalyses the salvage of GSH. Depletion of GSH in patients receiving maintenance dialysis contributes to the oxidative stress of ESRD patients. Average GSH degradation in BRECS was 1194 nmol/h at day 0 and 898 nmol/h on day 7 (Figure 3b). Both OCR and GSH degradation increased toward pre-study values when BRECS were removed from sheep and returned to *in vitro* culture. No metabolic activity was detected in sham devices. Histological staining of individual disks removed from cell BRECS at the conclusion of the therapy period showed high densities of living cells up to 7 days of extracorporeal culture (Figure 4), with few dead cells.

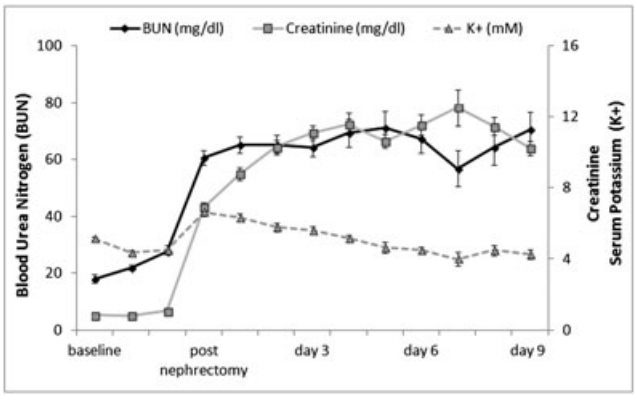

Figure 2. Control of uraemia using the wearable bioartificial kidney (WeBAK) in an ovine model of end stage renal disease. Following bilateral nephrectomy, sheep were maintained for up to 9 days with WeBAK comprised of a continuous flow peritoneal dialysis (CFPD) circuit and a Bioartificial Renal Epithelial Cell System (BRECS). Systemic blood sampling on uraemic animals was performed before the daily dialysate exchange. Transient hyperkalaemia developed in all sheep following complete nephrectomy but was well tolerated and resolved by increasing the frequency of dialysate exchanges during the first 48 h of therapy. Blood urea nitrogen (BUN) and creatinine were fairly stable by day 4. The graph depicts the mean ± SE for each analyte for the entire study group.

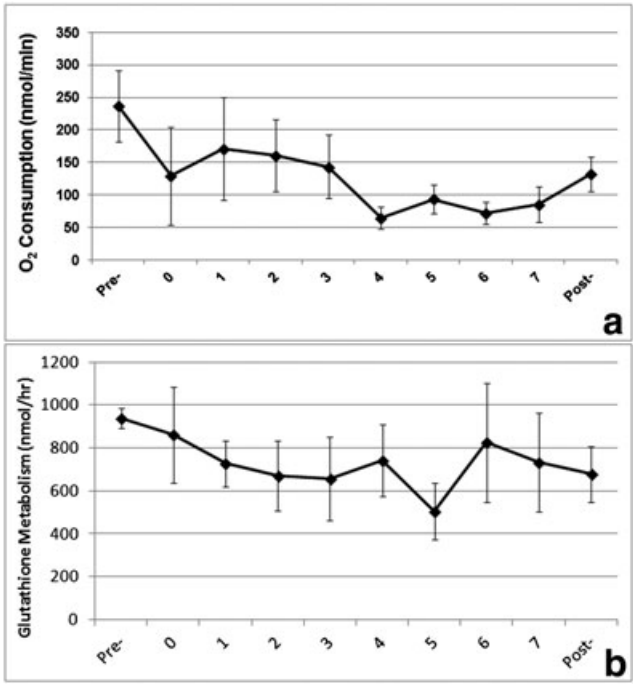

Figure 3. Metabolic activity measurements on the Bioartificial Renal Epithelial Cell System (BRECS) cell units during extracorporeal therapy in an ovine model. Oxygen consumption rate (OCR, a), and glutathione (GSH) metabolism (b), are depicted as the mean ± SE on each day during extracorporeal therapy. Before and after measurements were obtained from BRECS cell units during *in vitro* culture for comparison.

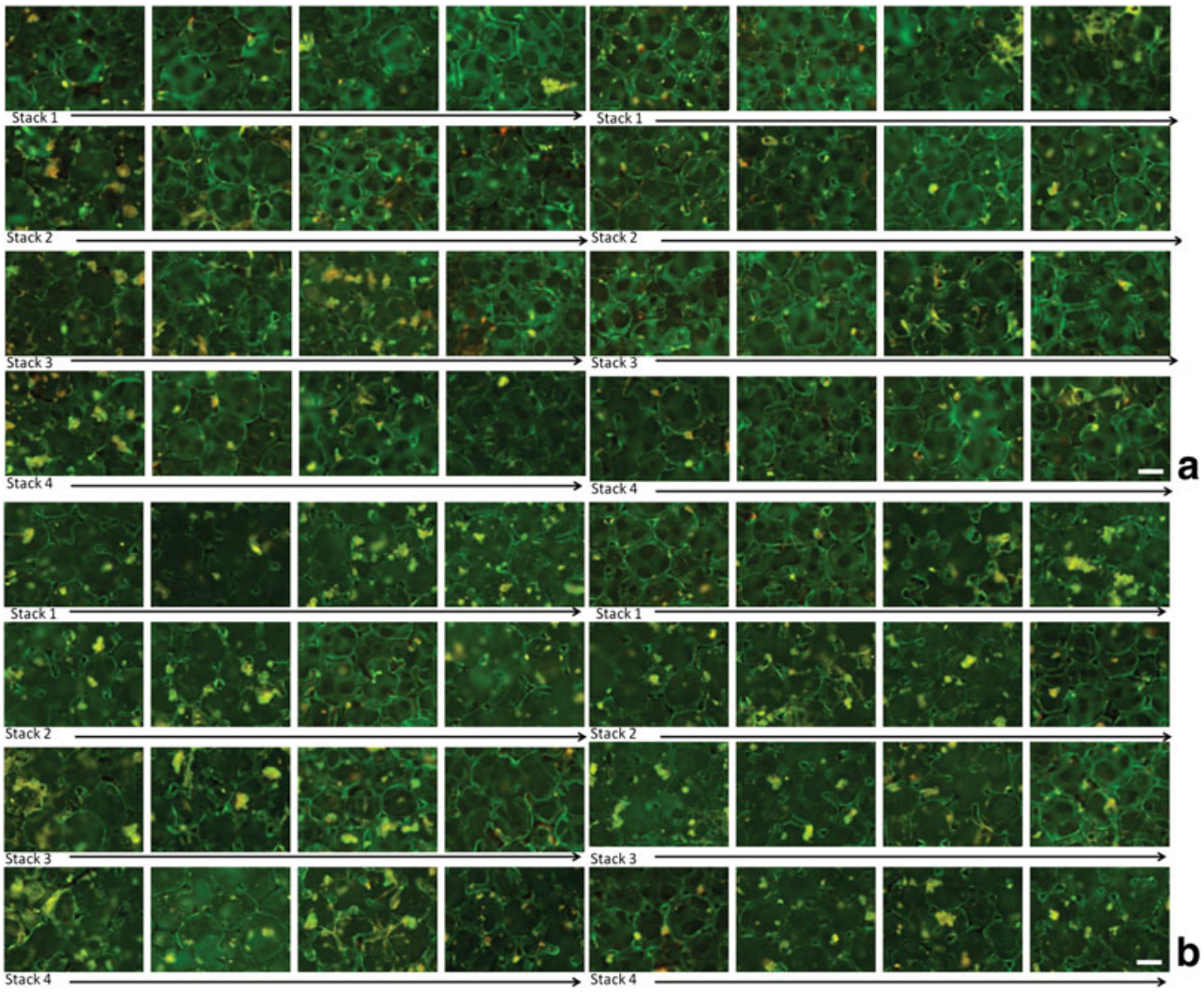

Figure 4. Cell viability of renal epithelial cells on porous disks within the Bioartificial Renal Epithelial Cell System (BRECS) was maintained after extracorporeal therapy in an ovine model. Living cells were stained with fluorescein diacetate (bright green) and dead cells were stained with propidium iodide (red). Overlapping and non-specific auto fluorescence appears yellow-orange. (a) A representative field from the front side of each of the 32, cell-covered disks organized in four stacks of eight disks; (b) shows a field from the rear side of each disk. Disks were imaged after extraction from a single BRECS device. The three-dimensional structure of the disks makes quantification of living vs. dead cells using image analysis problematic. Bar: 500 μm. [Colour figure can be viewed at wileyonlinelibrary.com]

3.3. Impact of BRECS therapy

The BRECS was designed to be the first all-in-one culture vessel, cryostorage device and cell therapy delivery system for clinical perfusion with either ultrafiltered blood or body fluids. A BRECS, maintained in a haematogenous circuit has demonstrated therapeutic impact in a preclinical model of acute kidney injury (Westover *et al.*, 2014), but this delivery method has limitations, particularly for chronic indications. With the ability of cells within BRECS to be sustained by *ex vivo* peritoneal fluid, the potential therapeutic impact of cell-enhanced therapy delivered in this manner for ESRD was investigated.

Anephric sheep either received cell BRECS (n = 13) or sham device (n = 8) therapy via the CFPD circuit starting day 1 post-nephrectomy. Therapy was continuous for up to 7 days, then devices were removed and sheep sustained with CFPD alone for an additional 48 h post-therapy. Blood was sampled before removal of each kidney then at a minimum of every 48 h to assess endocrinological, metabolic and immunological parameters. The inability to maintain adequate flow in the CFPD circuit to perfuse devices for a full 7 days caused some animals to be terminated before all sampling was completed and so data was averaged over each of three phases of the study: non-uraemic, therapy and post-therapy. Nine cell BRECS and five sham-treated sheep completed the entire study time-course.

Vitamin D3 levels were measured as an indicator of the endocrinological support that could potentially be provided by the BRECS. Western blotting of isolated RECs has confirmed the presence of 1-alpha hydroxylase, the enzyme that controls the final conversion of 25VIT D3 to biologically active 1,25VIT D3. Systemic 1,25VIT D3 levels decreased in all sheep post-nephrectomy and were highly variable in uraemic sheep over the BRECS therapy period with no difference detected between cohorts (data not shown). This may be attributable to seasonal (sunlight)

variation which impacts vitamin D kinetics and the individual animal response to exogenous precursor supplementation with 25VIT D, which can increase extra-renal 1-alpha hydroxylase activity in whole animal systems (Richart *et al*., 2007). Notably, activity of 1-alpha hydroxylase was not detectable in BRECS under normal tissue culture conditions, but when phosphate was omitted from the culture media 1-alpha hydroxylase activity was upregulated inside 24 h (unpublished data). Further investigation is needed to evaluate factors that may influence delivery of vitamin D by BRECS (i.e. phosphate levels in PD fluid).

Circulating NE counts were stable in cell BRECS-treated sheep (2.88 ± 0.35 non-uraemic, 4.20 ± 1.44 K/μl post-therapy) while NE counts in sham-treated animals were increased, significant at post-therapy compared with the non-uraemic state (6.26 ± 2.0 and 3.01 ± 0.69 K/μl respectively, $p = 0.017$). The differences between cohorts were not significant at any point. As uraemia has been associated with NE dysfunction (Haag-Weber and Horl, 1996; Anding *et al*., 2003), the oxidative activity of isolated systemic NE was assessed by quantification of intracellular ROS. Results are summarized in Figure 5 with values reported as mean fluorescent intensity ± SE both with and without PMA stimulation. Data on ROS were not available from four of the cell-treated sheep. The pre-therapy values (basal and stimulated) were not different between groups. Basal ROS level was maintained and the ROS production of stimulated NEs was significantly greater in sheep receiving BRECS cell treatment both during (cell 198 ± 69, sham 64 ± 20, $p = 0.033$) and after therapy (cell 232 ± 64, sham 37 ± 14, $p = 0.003$). Exclusion of immunological data from the two sheep with septic peritonitis did not significantly alter these findings.

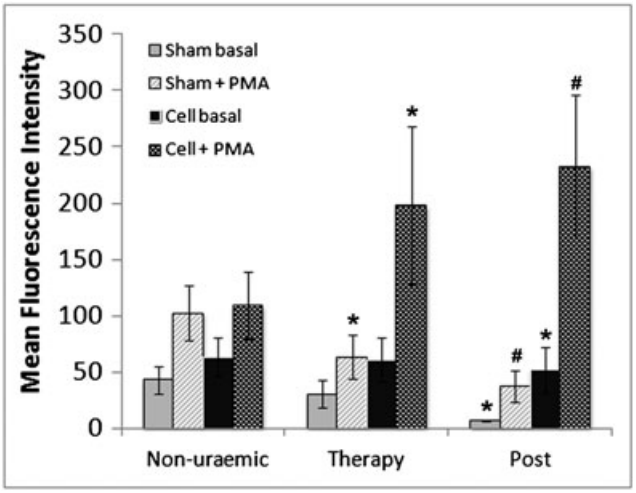

Figure 5. Oxidative activity of systemic neutrophils was retained by uraemic sheep that received renal cell therapy. Neutrophils isolated from whole blood were incubated with 5-(and 6)-chloromethyl-2 ,7 -dichlorodihydroflourescein diacetate (CM-H2D CFDA) to assess the production of intracellular reactive oxygen species in the presence or absence of phorbol myristate acetate (PMA) stimulation. Mean fluorescence intensity was determined by flow cytometry and values were averaged for each time-period. The detected oxidative activity of neutrophils (basal and stimulated) isolated from sheep before bilateral nephrectomy and treatment with the wearable bioartificial kidney was not different between groups. Basal oxidative activity was better retained in neutrophils from uraemic sheep that received Bioartificial Renal Epithelial Cell System (BRECS) cell therapy ($^*p < 0.05$) and the stimulated oxidative activity of isolated neutrophils was higher both during ($^*p < 0.05$) and after BRECS therapy ($^{\#}p < 0.01$) in cell-treated sheep compared with sham-treated animals. Some animals were not available for post-therapy sampling ($n = 9$ cell, $n = 8$ sham for non-uraemic and therapy; $n = 6$ cell, $n = 5$ sham for post-therapy).

4. Conclusions

The BRECS offers the possibility of full renal replacement therapy by providing the transport, endocrinological, metabolic and immunological activity that is lacking in conventional dialysis modalities. BRECS therapy has demonstrated therapeutic benefit in a preclinical model of acute kidney injury, and BRECS design overcomes a number of obstacles that have deterred clinical administration of renal cell therapy. A key feature of BRECS is the ability to maintain the cells using body fluids other than blood. Renewed interest in the utilization of PD as the basis for an artificial kidney with increased longevity and portability for chronic indications lends a platform for employment of the BRECS to provide cell therapy for patients with ESRD. In the present study, it is shown that a CFPD regimen was able to manage the uraemic state of anephric sheep and sustain cellular viability and functionality within BRECS, as shown by oxygen consumption and glutathione metabolism during therapy. Viability stains post-therapy confirmed REC survival for up to 7 days when maintained in this manner. These experiments demonstrate proof of concept of a WeBAK free from the constraints of an anticoagulated blood circuit to provide cellular enhanced therapy for chronic renal failure indications.

Fluid dynamics of the BRECS have been optimized so that perfusion rates as low as 10 ml/min are able to provide adequate nutrient and oxygen delivery to cells for survival without the use of an oxygenator. Lower metabolic output by REC within BRECS, although not statistically significant, was observed over the 1-week course of WeBAK therapy in this model. Correlation of GSH metabolism in BRECS and cell number via DNA isolation (reported previously) suggests that the reduction of metabolic output likely was caused by a combination of viable cell loss along with lower metabolism by the remaining cells when maintained with peritoneal dialysis perfusate. A decrease in oxygen consumption is also likely a combination of fewer cells consuming oxygen at a lower rate and there may be some shift from aerobic to anaerobic metabolism. Further investigation is needed to determine if metabolic output from BRECS during extracorporeal culture is predictably altered and to determine if the changes in metabolic output have therapeutic implications. Notably, it has been found that including a segment of oxygen-permeable silicone tubing pre-BRECS can increase the oxygen tension in the perfusate to approximate room air, although further studies are needed to determine if this modification has any effect on metabolic output from BRECS during *ex vivo* perfusion with PD fluid.

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While the importance of renal tubule cells in glutathione metabolism and activation of vitamin D3 and are well described, these cells also possess a less recognized, but important role in immunoregulatory function. Demonstrated immunological effects of renal cell therapy in the setting of acute kidney injury have included differences in the concentrations of plasma cytokines, alterations in the release of cytokines from isolated leukocytes and improved survival of sepsis (Humes *et al*., 1999, 2002, 2003a, 2003b, 2004; Fissell *et al*., 2003; Huijuan *et al*., 2007; Westover *et al*., 2014). Concomitant immune activation, systemic inflammation and immune deficiency have been linked to ESRD (Kato *et al*., 2008; Vaziri *et al*., 2012; Betjes 2013). Uraemia appears to impair the phagocytic and killing functions of circulating granulocytes (Porter *et al*., 1997; Anding *et al*., 2003; Sardenberg *et al*., 2006; Vaziri *et al*., 2012), although conflicting results for the generation of ROS by NE isolated from ESRD patients, particularly among those receiving different dialysis modalities, have been described (Porter *et al*., 1997; Anding *et al*., 2003; Morena *et al*., 2005; Sardenberg *et al*., 2006; Yoon *et al*., 2007). Infection is the second most common cause of death in ESRD, approaching 25% of annual mortality rate in haemodialysis' patients (US Renal Data System, 2003). This infection complication rate is not diminished with higher dialysis dose or high flux membrane utilization. In the present project, it was found that basal ROS production of isolated NEs declined in sheep over the uraemic time-course but was better maintained in the animals receiving cell therapy. Moreover, NE counts remained stable and the stimulated oxidative potential of NE was retained during and after BRECS treatment, signifying that the ability to have an appropriate immune response to stimuli may be preserved with REC therapy.

The results of this preliminary study demonstrate that a 24h CFPD regimen is able to provide a stable uraemic state in nephrectomized sheep. A BRECS is readily incorporated into an extracorporeal CFPD circuit using peritoneal fluid for perfusion of the device, providing cell survival and maintenance of function while eliminating the requirement for an anticoagulated blood circuit. Cell therapy using BRECS contributes to improved immunological homeostasis during the uraemic state, and endocrine support in the form of 1,25 vitamin D3 may become an added benefit. Advancement of CFPD (ideally with a dialysate regeneration system based upon sorbent technology) that couples clearance of uremic toxins with perfusion to a cell therapy device, such as the BRECS, embodies a feasible approach to a wearable bioartificial kidney for treatment of ESRD.

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Conflicts of Interest

H.D.H. is a shareholder of Innovative BioTherapies, Inc. A. W., D.B., K.J., P.S. and C.P. are employees or former employees of Innovative BioTherapies, Inc.

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