

Title: A Bioartificial Renal Epithelial Cell System Conveys Survival Advantage in a Porcine Model of Septic Shock

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1. Introduction

Acute kidney injury arises from toxic or ischemic (usually simultaneous) tubule damage from antibiotics, chemo-therapeutic agents, or results from a systemic inflammatory response syndrome (SIRS) subsequent to infection or major operative procedures [1, 2]. The development of AKI in hospitalized patients results in a 5 to 8 fold higher risk of death [3, 4] with overall mortality rates exceeding 50% and a high prevalence of sepsis. However, if the patient survives the episode of AKI, the regenerative repair processes inherent to the kidney can result in a return of kidney function in 90 to 95% of patients with this acute disorder [5, 6]. The prototypical clinical disorder of SIRS is sepsis with a high prevalence of AKI [7-9]. In the US, sepsis affects one million people annually, develops in one half to three-quarters of critically ill patients, and is the leading cause of death among this patient group [10]. The cause of death in septic patients with AKI requiring continuous renal replacement therapy (CRRT) is usually the development of SIRS, with resulting cardiovascular collapse, ischemic damage to vital organs, and multi-organ dysfunction (MOD) [8, 9]. The supportive role of kidney function

in immunoregulation is demonstrated in that patients with AKI have a propensity to develop SIRS and end stage renal disease patients have an increased risk of infection [11]. In the case of AKI associated SIRS, the prevalence of kidney tubule damage and accompanying organ dysfunction, suggests that early kidney dysfunction may lead to an exacerbation of the SIRS, which in turn may contribute to the cascade that leads to MOD and death. Attempts have been made using single target molecules to modulate the proinflammatory cascade evidenced early in SIRS. Treatment has not been successful, largely due to complications arising from the subsequent development of the compensatory anti-inflammatory response, exacerbated by anti-inflammatory therapy [12].

The potential success of renal cell therapy for AKI associated SIRS lies in the growing appreciation that most disease processes are not due to the lack of a single protein but develop due to alterations in complex interactions of a variety of cell products. In addition to glomerular filtration, the kidney's roles of endocrine, metabolic and excretory function are becoming more accepted as essential to homeostasis. A less recognized role of the kidney, particularly renal tubule cells, is immunoregulatory function [13]. In the acute situation, early intervention with renal cell therapy in sepsis may have therapeutic

benefit by replacing cell function lost by tubule injury occurring early in the septic cascade. The initial approach to test this hypothesis was to add renal cell therapy as a supplement to the current standard of care of renal substitution with hemodialysis and hemofiltration [8]. To this end, the therapeutic potential of a bioartificial renal tubule was clearly demonstrated with the hollow fiber based renal assist device (RAD). Constructed of biomatrix-coated hollow fiber membranes with a luminal renal epithelial cell monolayer, the RAD was placed in the extracorporeal circuit, providing nutrients and allowing cells to respond to the host system. RAD therapy has proved efficacious in preclinical animal models of septic shock (SS) where increased survival time, stabilization of cardiovascular parameters and amelioration of MOD was detected compared to a sham device [14-20]. In the clinical setting, the RAD demonstrated individualized responses in patients, depending on their unique pathophysiologic conditions [14]. Clinical trials culminated with a multicenter Phase IIa trial involving 58 patients, resulting in significant survival impact [21]. Of importance, relative to the choice of the porcine SS model used for the present study, the incidence of sepsis in this clinical trial was 73% in the RAD treated group and 67% in acellular controls. The efficacy of the RAD in patients with sepsis suggests

that renal cell therapy may moderate the SIRS accompanying sepsis in the clinical setting.

Further development of RAD therapy was suspended due to manufacturing and distribution issues. Major issues included: the ability to identify a robust cell source, and the ability to solve the storage, distribution, and reconstitution of cell devices for therapeutic use at point of care facilities [22].

To this end, the bioartificial renal epithelial cell system (BRECS) has been developed. The BRECS is made of durable carbon-based disks in an injection molded, perfusable, polycarbonate housing. The BRECS represents the first all-in-one culture vessel, cryostorage device and cell therapy delivery system, thus eliminating the shortcomings of the hollow fiber-based RAD design [23]. Additionally, the technology for the enhanced propagation and differentiation of human renal epithelial progenitor cells from available donor kidneys has been developed to potentially provide the necessary therapeutic cell population for both AKI and ESRD targeted therapies [24]. The primary study objective was to confirm that therapeutic efficacy was conserved after cell sourcing and design modifications were made to allow for clinical delivery, by evaluating the ability for BRECS therapy to prolong survival in a porcine model of SS, as demonstrated in the pre-clinical evaluation of the

RAD. Assessment of efficacy of BRECS efficacy in this model would provide a crucial step toward reinitiating the evaluation of renal cell therapy and transition in to the clinical setting.

2. Materials and Methods

2.1. Bioartificial Renal Cell System (BRECS)

The BRECS, consisting of niobium-plated trabeculated carbon disks (CY-908, Cytomatrix, Belmont Victoria, Australia) colonized with 2×10^8 human REC, has been bioengineered to allow for adherence to current Good Manufacturing Practice (cGMP) guidelines through seeding, perfusion, cryopreservation for storage and distribution, and thaw, for on demand use [23]. The BRECS design evolved from a larger, block-style housing, culminating in a mass-produced, injection-molded (IM) design that was fitted with monitoring systems and temperature probes allowing for the real time monitoring of oxygen consumption [25]. The BRECS is roughly 8.5 cm x 8.5 cm x 1.5cm, with a fill volume of 10 mL. The BRECS weight without disks is 49 g, with cell covered disks is 52 g, and total fluid filled weight is 62 g.

REC were derived from a recently developed enhanced propagation (EP) technique that exploits the natural regeneration potential of renal progenitor cells to differentiate into mature, metabolically active renal epithelium [24].

Human kidneys required for the manufacture of BRECS were obtained from the National Disease Research Interchange (NDRI). The BRECS provided oxygen and nutrients to the REC via delivery of 10 mL/min of UF derived from venous blood. For the final BRECS prototype, temperature was controlled at 37°C and oxygenation was maximized by insertion of a 12 foot loop of thin-walled gas permeable tubing, which allowed for the pO₂ of the UF to reach atmospheric values prior to being perfused through the BRECS. The final prototype was employed for all 4 animals with >16 hour survival.

2.2. Animal Welfare Statement

The study adhered to the principles stated in the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (1996), and was approved by the University of Michigan's Committee on Use and Care of Animals (UCUCA).

2.3. Animal Model and Extracorporeal Circuit

A porcine model of *Escherichia coli* induced SS, previously described in detail [15], results in clinically defined SS [26], which, without intervention, results in death at 6-8 hours. This model is a rapid mortality model in which only volume and not vasopressor interventions are employed. Due to the

need of a large animal model in which CRRT blood circuits are required, a short duration model must be used so that intervention and death occur in less than 24 hours to meet UCUCA standards. Briefly, pigs weighing 30-40kg were anesthetized and artificially ventilated. An ultrasonic flow probe was placed on one renal artery to monitor renal blood flow. Arterial and Swan-Ganz thermodilution catheters were placed and connected to transducers to monitor cardiovascular parameters. Continuous veno-venous hemofiltration (CVVH) was instituted with a Fresenius F40 filter (Waltham, MA) and anticoagulation maintained by systemic heparin. Extracorporeal blood flow was maintained at 150mL/min and ultrafiltrate (UF) production was controlled at 15mL/min. The circuit blood volume was 152-155 mL and the circuit ultrafiltrate volume was 192-202mL. Once the animals were hemodynamically stable, SS was induced by infusion of 4×10^{11} colony forming units of *Escherichia coli* (ATCC #19138. Serotype O6:K2:H1) per kg body weight into the peritoneal cavity. Therapy was initiated simultaneous to bacterial administration, with UF flow directed to the BRECS to create a homeostatic feedback loop. Of the 15mL/min UF created by the first F40, 5mL/min was diverted to waste (replenished with standard replacement fluid) and 10mL/min was directed through the BRECS. BRECS processed UF was

returned to the animal through a second F40 which served as an immunoisolating filter (Figure 1). Preliminary studies demonstrated that CRRT initiation at 2-3 hours after bacterial instillation resulted in a rapid cardiovascular collapse. Therefore, all experiments were performed with CRRT initiation at the time of bacterial infusion and continued until death or a predetermined maximum duration of 16 hours. All animals received standardized broad spectrum antibiotic (Ceftriaxone, 100 mg/kg), administered 15 minutes after bacterial instillation to replicate the clinical situation. Hemodynamic support of 80mL/kg crystalloid (saline) and 20mL/kg colloid (6% hetastarch) were infused over the first hour for all animals regardless of cohort and maintenance fluid of 5mL/min replacement fluid given after hour three. Animals did not receive vasopressors or inotropic agents.

Complete blood counts and serum chemistries were monitored with a Hemavet automated analyzer (Drew Scientific, Waterbury, CT) and Vet Test automated analyzer (IDEXX, Westbrook, ME), respectively. Systemic blood was drawn for analysis of cytokine concentrations (including IL-6, IL-8, IL-10 and TNF α) using assay kits reactive to porcine cytokines (R&D Systems, Minneapolis, MN) and for monitoring blood urea nitrogen and creatinine

using colorimetric assays (Stanbio, Boerne, TX). Additionally, at baseline and 6 hours, monocytes were isolated from systemic blood using discontinuous percoll gradient [27] , plated at 10^6 cells per mL and incubated for 24h in culture plates containing RPMI-1640 medium supplemented with antibiotics in the presence of $1\mu\text{g/mL}$ lipopolysaccharide (LPS). Monocyte supernatants were collected and cytokine concentrations measured as described for serum. Cohorts included: BRECS with human EP derived cells (n=10) and acellular controls, with no cell therapy. (n=15).

2.4. Statistics:

Comparisons between groups used student's t test and two way repeated measure analysis of variance (ANOVA) as appropriate. The results are presented as mean \pm SE. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Survival

The intraperitoneal instillation of high-dose gram negative bacteria (*E. coli*) produced a profound decline in arterial blood pressure and concomitant reduction in vital organ perfusion, resulting in SS associated MOD. Without intervention, death occurred in acellular controls at 7.6 ± 0.5 hours. The

Kaplan-Meier survival chart for each cohort is shown in Figure 2A. For survival calculations of cohorts receiving BRECS therapy, animals surviving through the predetermined study endpoint of 16 hours, (indicated by arrow), were assigned a value corresponding to 16 hours. The calculated survival time was extended to 13.0 ± 0.9 ($p < 0.001$) hours with BRECS therapy (Figure 2B).

3.2. Cardiovascular Parameters

A complete summary table of cardiovascular and renal functional parameters is provided in Table 1. Significant differences between cohorts were observed in the parameters of cardiac output (CO), systolic blood pressure, diastolic blood pressure, mean arterial blood pressure (MAP) and hematocrit. Baseline cardiovascular measurements taken at initiation of CVVH were variable due to individualized responses to anesthesia, bacterial instillation and the standardized fluid resuscitation that was completed prior to hour 1. Therefore, differences between groups were most apparent when data from each animal was normalized to the one hour value prior to group comparisons. Increase in survival time coincided with stabilization of capillary leak as assessed by hematocrit. Hematocrit increased rapidly in acellular controls, but remained comparatively steady in the BRECS treated group,

with normalized differences reaching statistical significance at 2 hours ($p < 0.01$) through study termination. MAP and CO declined over the septic time course for both cohorts, but both were better maintained in BRECS treated animals. Improved cardiovascular maintenance was demonstrated by the significant divergence of calculated values (normalized to hour 1) for MAP hours 4-6 ($p < 0.05$) and cardiac output hours 4-9 ($p < 0.05$) (Figure 3).

3.3. Renal Function

A rapid deterioration of renal function was evidenced by a reduction in renal arterial blood flow and urine output that occurred in both cohorts by four hours (Table 1). Urine output from hour 5 to hour 6 was present in 50% of BRECS treated animals, while in the acellular cohort only 13% of animals had detectable urine output during this time frame. For the BRECS cohort, renal arterial blood flow was better maintained, and was significantly higher than acellular controls at 4-6 hours ($p < 0.05$). For both groups, blood urea nitrogen (BUN) and creatinine initially decreased with fluid resuscitation then increased steadily starting at 3 hours. At 6 hours, average BUN levels were 13.5 ± 1.0 and 12.7 ± 0.9 mg/dL (not significant) and serum creatinine levels were 1.9 ± 0.1 and 1.4 ± 0.2 mg/dL ($p < 0.05$) for acellular and BRECS cohorts respectively.

3.4. Immune Response

The impact of BRECS therapy on the initial SIRS was evaluated by measuring alterations of serum cytokine levels, including IL-6, IL-8, IL-10 and TNF α in response to sepsis. Plots for acellular and BRECS treated pigs are truncated at 6 and 12 hours respectively because data may be misleading since it is weighted by the few animals within each group that survived past these time points (Figure 4). For TNF α , a spike in the systemic level was observed at 2-3 hours post introduction of *E. coli*, and significantly reduced concentrations ($p < 0.05$) were detected in the BRECS cohort during this acute response phase. IL-6 and IL-8 are cytokines with increased concentrations associated with the proinflammatory cascade. For these cytokines, levels increased through the septic time course and averages were lower with BRECS therapy. Differences in IL-6 which had much less variability within cohorts compared to IL-8, were significant at hours 3 and 6. For the BRECS treated cohort, IL-10 exhibited a biphasic response curve, peaking at 3 hours then increasing again in animals surviving longer than 6 hours. In this study, the anti-inflammatory cytokine IL-10 was the only cytokine with average plasma levels that were not reduced with BRECS therapy.

The response to severe bacterial infection is biphasic in that the initial SIRS is followed by a hypoinflammatory state during which immunoparalysis has been observed in systemic monocytes isolated from patients with severe sepsis, and demonstrated by the inability for these monocytes to secrete cytokines upon *ex vivo* stimulation with LPS [28]. To assess the effect of BRECS therapy on monocyte anergy, the secretory expression profile of isolated monocytes in response to LPS stimulus was performed in 5 BRECS treated and 5 acellular BRECS treated animals. The secretory profiles of cytokines (IL-6, IL-8, IL-10, and TNF α) were equivalent for both cohorts at baseline, but monocytes isolated from control animals at 6 hours post induction of sepsis had altered secretory abilities in response to LPS compared to monocytes from BRECS treated animals. Monocytes isolated from BRECS treated animals better retained the ability to respond to stimuli. The LPS stimulated cytokine secretion for IL6 was 3 ± 3 vs 153 ± 73 ; and for IL8 was $1\ 715 \pm 1\ 279$ vs $17\ 118 \pm 1\ 279$ ug/ 10^6 MNC/24hours for control and BRECS treated cohorts respectively (both $p<0.05$). TNF α secretion followed the same trend but did not reach significance. IL-10 secretion was not changed (Figure 5).

4. Discussion

Currently there is a large unmet need for renal replacement therapy for both acute and chronic applications. The supportive role of kidney function in immunoregulation is demonstrated in that patients with acute kidney injury (AKI) have a propensity to develop SIRS and end stage renal disease patients have an increased risk of infection. In the acute situation, early intervention with renal cell therapy in sepsis may have therapeutic benefit by replacing cell function lost by tubule injury occurring early in the sepsis disorder. This hypothesis was supported in animal models of acute SS and clinically in AKI patients coincident with sepsis. In spite of these positive results, major obstacles in the widespread clinical use of cell-based devices must be overcome by demonstrating: the ability to maintain a robust cell source, ability to cryopreserve the device for storage and distribution, and ability to reconstitute the device for therapeutic use at point of care facilities [22]. Though septic patients stand to receive therapeutic impact from early renal cell therapy, ideally initiated prior to evidence of renal insufficiency, prompt delivery is hampered because of the difficulty of maintaining a cell-based biologic device at point of care facilities. To overcome these hurdles, the renal cell therapy device was transitioned from the hollow fiber based RAD containing renal cells originating from differentiated cultures, to a

trabeculated carbon disk based BRECS, containing cells derived from kidney progenitor cells using enhanced propagation methods to provide the necessary biomass from available transplant discards [24]. The BRECS can be cryopreserved for distribution and storage at point of care facilities where, upon indication, it can be reconstituted by thawing and inserted into an extracorporeal circuit to provide therapy.

Due to the multi-faceted mechanisms by which survival benefit is conveyed by a living, biologically active device, the porcine SS model was used to confirm conservation of efficacy through this two phase transition from the hollow fiber based RAD, containing cells derived from differentiated renal tubule cell populations, to the carbon disk-based BRECS, containing cells derived from kidney progenitor cells expanded prior to differentiation. In this study, BRECS containing up to 2×10^8 human REC derived from EP, conveyed a significant survival advantage over contemporaneous acellular controls. Data indicate that this survival advantage is potentially due to a complex response by the REC to provide both cardiovascular support and systemic immunomodulation. Cardiovascular improvement was due to improvement of endothelial function resulting in less capillary leak as indicated by a more stable hematocrit. The maintenance of cardiovascular

function was also reflected in significantly improved MAP and cardiac output.

For these acute studies, the precise mechanisms by which endothelial integrity is preserved have yet to be elucidated. The association of renal disease and endothelial dysfunction in the resulting cardiovascular disease progression is well recognized [29].

Systemic immunomodulation was evidenced by alterations in systemic cytokines. Cytokine patterns are complex and often not predictive of outcomes [30], but systemic IL-6 concentrations and IL-6/IL-10 ratio have been found to have prognostic value in the overall outcome of sepsis and injury induced SIRS [31, 32]. In these studies, even though the systemic cytokine response was highly variable between individual animals, the average concentrations for the proinflammatory cytokines TNF α and IL-6 were significantly lower in BRECS treated animals while the anti-inflammatory cytokine IL-10 was not reduced, providing further evidence that replacement of some aspects of normal tubule function via the BRECS, promotes balance of the pro- versus anti-inflammatory cascade. Monocytes isolated from systemic blood six hours after induction of sepsis from BRECS treated animals better retained the ability to secrete cytokines in response to

stimuli, unlike the anergy observed in acellular controls, indicating that normal monocyte function was retained by BRECS therapy [28].

Pre-clinical testing of a human cell-based device in an animal model raised concerns that results would be altered due to xenographic differences between secreted proteins. However, as seen previously with RAD using porcine cells in this model, BRECS therapy using EP derived human REC provided a significant advantage over acellular controls. Additionally, as observed previously for the RAD [15], BRECS therapy resulted in significant lowering of systemic IL-6 during the initial proinflammatory cascade. SIRS is multifaceted in that dysregulated activation of both leukocytes and endothelium precipitate the accumulation of blood phagocytes in organs that contribute to MOD [33]. In this series of studies, suggestive evidence of endothelial functional improvement was observed with BRECS therapy, but did not demonstrate reduced neutrophil CD11b expression (data not shown). Of note, a second immunomodulating apparatus, termed the selective cytopheretic device (SCD), which is based on an acellular biomimetic membrane platform technology, has also been developed by our laboratory [34]. With SCD therapy, a significant decrease in neutrophil activation was observed as detected by expression of CD11b and return to normal

apoptosis patterns [34]. These important findings support the hypothesis that the immunomodulation afforded by the BRECS and SCD occurs by means of different pathways and introduce the possibility that the two therapies may be additive or synergistic. Current studies are evaluating an additive effect of these two approaches to SIRS.

The maintenance of capillary integrity, better sustained cardiac output, systemic and monocyte cytokine profiles, and increased survival time, are similar to the results observed in pre-clinical RAD testing. The results of this study confirm that with the incorporation of manufacturing changes, including both enhanced propagation of REC and device fabrication using niobium-coated carbon scaffolds, biologic efficacy associated with renal cell therapy is maintained by the BRECS. Accordingly, plans are being made to use this new formulation of renal cell therapy to transition to clinical evaluation.

5. Conclusions

Bacterially induced SS results in early renal tubule injury, which may contribute to progression of the sepsis syndrome. The results of this study provide further evidence that early intervention with renal cell therapy may provide a novel therapeutic approach to sepsis to improve outcomes. Clinical

administration of renal cell therapy was deterred previously by device manufacturing, storage and distribution hurdles, requiring the transition away from the hollow fiber based RAD, to the carbon disk based BRECS. Due to limited human kidney availability, the development of enhanced propagation techniques to create the necessary biomass was required to solve cell sourcing issues. The demonstration of conserved efficacy with BRECS therapy in a porcine model of SS represents a crucial first step toward returning renal cell therapy to the clinical setting, initially targeting septic patients with AKI.

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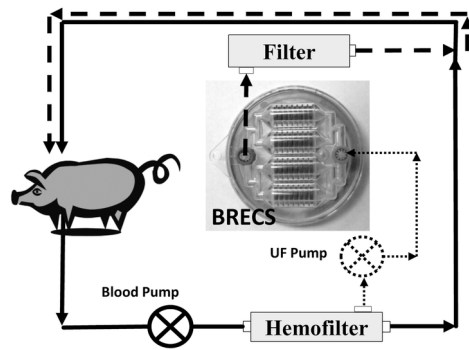
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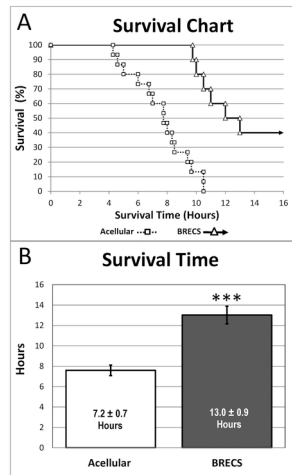
Author

Parameter	Baseline	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6	Hour 7	Hour 8	Hour 9	Hour 10	Hour 11	Hour 12
Cardiac Output, L/min													
Acellular	4.4 ± 0.3	5.1 ± 0.2	4.8 ± 0.3	4.0 ± 0.3	3.2 ± 0.3	2.1 ± 0.2	2.0 ± 0.2	2.0 ± 0.1	1.6 ± 0.2	1.5 ± 0.2	1.0		
BRECS	3.8 ± 0.3	4.1 ± 0.3**	4.6 ± 0.5	3.8 ± 0.4	3.4 ± 0.4	3.1 ± 0.4*	2.6 ± 0.3	2.4 ± 0.3	2.1 ± 0.3	1.8 ± 0.2	1.7 ± 0.2	1.5 ± 0.2	1.5 ± 0.2
Systolic Blood Pressure, mmHg													
Acellular	97 ± 2	105 ± 3	84 ± 4	74 ± 4	62 ± 3	52 ± 5	48 ± 5	44 ± 7	46 ± 2	39 ± 5	31		
BRECS	98 ± 3	101 ± 4	82 ± 4	71 ± 5	68 ± 4	64 ± 4	58 ± 4	57 ± 4	51 ± 3*	49 ± 3*	46 ± 5	48 ± 7	49 ± 3
Diastolic Blood Pressure, mmHg													
Acellular	65 ± 2	75 ± 2	54 ± 4	42 ± 3	33 ± 2	29 ± 2	26 ± 2	24 ± 3	22 ± 2	22 ± 3	20		
BRECS	70 ± 4	73 ± 3	56 ± 3	39 ± 5	43 ± 4*	39 ± 4**	35 ± 3*	32 ± 3*	27 ± 2	27 ± 2	25 ± 3	27 ± 3	27 ± 2
Mean Arterial Pressure, mmHg													
Acellular	74 ± 3	83 ± 3	63 ± 4	51 ± 4	42 ± 3	39 ± 3	34 ± 3	33 ± 4	32 ± 1	29 ± 2	36		
BRECS	81 ± 4	83 ± 3	65 ± 4	54 ± 3	52 ± 4*	48 ± 4	43 ± 3	40 ± 3	37 ± 2	34 ± 2	32 ± 3	34 ± 4	34 ± 2
Systemic Vascular Resistance, dyn-s/cm⁵													
Acellular	1239 ± 95	1167 ± 73	1004 ± 64	901 ± 64	1058 ± 98	1213 ± 128	1278 ± 105	1266 ± 154	1420 ± 148	1524 ± 331	1413		
BRECS	1537 ± 165	1456 ± 97	1018 ± 79	890 ± 74	1075 ± 70	1138 ± 71	1217 ± 73	1240 ± 87	1242 ± 102	1295 ± 111	1368 ± 133	1535 ± 142	1557 ± 156
Pulmonary Vascular Resistance, dyn-s/cm⁵													
Acellular	157 ± 17	222 ± 49	412 ± 58	418 ± 46	698 ± 100	743 ± 93	652 ± 82	638 ± 70	863 ± 173	948 ± 243	1360		
BRECS	213 ± 24	332 ± 95	362 ± 55	459 ± 69	545 ± 92	621 ± 129	601 ± 108	705 ± 133	754 ± 146	846 ± 152	934 ± 197	918 ± 212	1093 ± 234
Pulmonary Capillary Wedge Pressure, mmHg													
Acellular	9.0 ± 0.6	11.7 ± 0.7	9.3 ± 0.7	8.1 ± 0.5	7.3 ± 0.4	7.8 ± 1.1	7.4 ± 0.7	6.3 ± 0.3	6.4 ± 0.6	5.7 ± 1.3			
BRECS	9.5 ± 0.8	10.4 ± 1.0	9.7 ± 1.0	8.1 ± 1.2	7.1 ± 0.6	7.3 ± 0.7	6.3 ± 0.7	5.5 ± 0.6	6.0 ± 0.9	5.9 ± 0.8	6.0 ± 0.8	5.5 ± 0.8	6.2 ± 0.5
Renal Arterial Blood Flow, mL/min													
Acellular	179 ± 14	159 ± 13	187 ± 14	163 ± 17	115 ± 15	77 ± 15	46 ± 10	42 ± 10	40 ± 7	29 ± 8	15		
BRECS	213 ± 26	171 ± 22	211 ± 23	189 ± 14	162 ± 21*	124 ± 22*	103 ± 20**	81 ± 19	56 ± 13	49 ± 12	42 ± 13	43 ± 10	43 ± 8
Renal Vascular Resistance, mmHg/min/mL													
Acellular	0.45 ± 0.08	0.53 ± 0.06	0.32 ± 0.04	0.34 ± 0.06	0.52 ± 0.19	0.94 ± 0.41	0.98 ± 0.19	1.27 ± 0.43	0.73 ± 0.11	1.10 ± 0.30	1.99		
BRECS	0.39 ± 0.05	0.49 ± 0.05	0.30 ± 0.04	0.26 ± 0.02	0.32 ± 0.05	0.46 ± 0.11	0.56 ± 0.14	0.86 ± 0.28	0.76 ± 0.14	0.82 ± 0.16	1.44 ± 0.51	1.07 ± 0.50	0.74 ± 0.15
Hematocrit, %													
Acellular	30.3 ± 2.3	24.7 ± 1.8	27.7 ± 1.8	31.1 ± 2.0	32.2 ± 2.3	34.6 ± 2.8	34.7 ± 4.0	37.1 ± 4.4	39.9 ± 5.1	42.4 ± 8.1	56.8		
BRECS	30.5 ± 2.6	26.4 ± 2.2	27.2 ± 2.5	29.0 ± 3.1	29.2 ± 3.6	29.4 ± 3.7	30.8 ± 3.4	30.9 ± 3.5	30.1 ± 3.2	29.8 ± 3.0*	29.2 ± 3.2	27.4 ± 1.4	27.3 ± 1.2
Stroke Volume, mL													
Acellular	44.5 ± 2.9	49.6 ± 2.5	46.2 ± 3.0	42.0 ± 2.6	30.6 ± 2.9	23.1 ± 3.3	19.5 ± 2.9	17.6 ± 2.3	15.3 ± 1.9	13.9 ± 3.6	18.9		
BRECS	41.3 ± 2.8	44.0 ± 3.2	46.6 ± 3.5	38.9 ± 3.4	35.7 ± 4.4	30.7 ± 3.8	26.2 ± 3.3	24.1 ± 2.9	20.6 ± 2.4	18.3 ± 2.0	16.0 ± 2.3	16.1 ± 3.1	15.4 ± 2.6
Cumulative Urine Volume, mL													
Acellular	0 ± 0	678 ± 112	1062 ± 131	1089 ± 112	1192 ± 145	1087 ± 224	1035 ± 115	No Additional Urine Produced					
BRECS	0 ± 0	700 ± 107	1078 ± 179	1327 ± 193*	1608 ± 192*	1744 ± 203*	1990 ± 115*	2229	2231	2238	2238	2243	2248
Survival Number (n) at given time point from which data was generated													
Acellular	15	15	15	15	15	13	12	10	8	5	1	0	0
BRECS	10	10	10	10	10	10	10	10	10	10	9	7	6

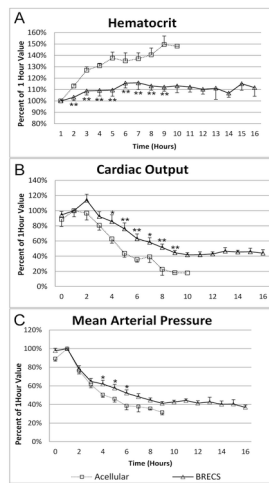
Table 1. Hemodynamic measurements in animals with peritoneal infusion of *E. coli* treated with the bioartificial renal epithelial cell system (BRECS). Significant parameters measured in human renal epithelial cell (BRECS n=10) containing BRECS groups compared to acellular controls (n=15) are shown at hourly intervals across rows. Significance is indicated by *p<0.05 and **p<0.01. The number of data points available at each hourly time point (shown in bottom rows) was dependent on survival time.



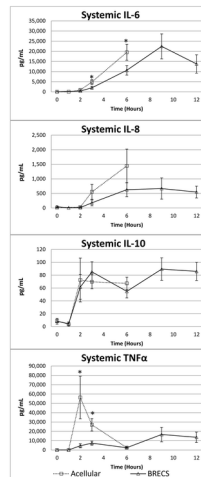
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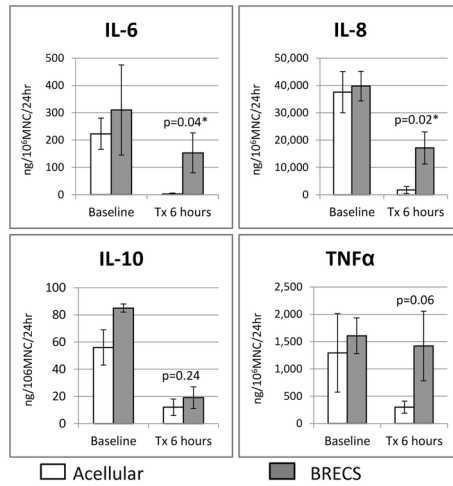
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TERM_1961_F4.tif



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