Regenerative medicine and Immunomodulatory Therapy: Insights From the Kidney, Heart, Brain, and Lung

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Regenerative medicine was initially focused on tissue engineering to replace damaged tissues and organs with constructs derived from cells and biomaterials. More recently, this field of inquiry has expanded into exciting areas of translational medicine modulating the body's own endogenous processes, to prevent tissue damage in organs and to repair and regenerate these damaged tissues. This review will focus on recent insights derived from studies in which the manipulation of the innate immunologic system may diminish acute kidney injury and enhance renal repair and recovery without the progression to chronic kidney disease and renal failure. The manner in which these interventions may improve acute and chronic organ dysfunction, including the heart, brain, and lung, will also be reviewed.

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As a primer to this review, which will focus on a novel immunomodulatory therapy using a selective cytopheretic device (SCD) in a variety of studies to treat various etiologies of acute and chronic organ dysfunction, the reader is referred to recent comprehensive reviews on the classical tissue engineering approach to renal replacement function.¹⁻³ In brief, the tissue engineering approach uses biological components (proteins, cells, tissues) as well as degradable or nondegradable biomaterials such as polymers as a support matrix or delivery vehicles to reconstruct damaged tissue in the body. In this review, a cell-processing approach will be predominantly featured that leverages the integral role of the immune system in the body's innate repair and regenerative mechanisms, which is modulated by an extracorporeal device to avoid abnormal pathology. Immunomodulation via continuous leukocyte processing from an extracorporeal circuit with a biomimetic membrane is shown to affect neutrophils and monocytes, altering the progression of inflammatory diseases, alleviating tissue damage caused in disease states without treatment, and enhancing repair dependent upon regenerative and remodeling processes. In this regard, the immunomodulation approach in regenerative medicine is vastly different from the tissue engineering approach, where damage to tissue can be limited to avoid abnormal pathology, and normal repair processes can be augmented rather than having to engineer and recapitulate tissue structure and function.

Role of Neutrophils in Acute Tissue/Organ Injury—Kidney, Brain, Heart, and Lung

Inflammation plays a central role in the development of acute and solid organ dysfunction. It is the excessive dysregulated leukocyte inflammatory processes in many acute and chronic disease states that lead to tissue damage, which results in organ/tissue dysfunction. In an example of acute systemic inflammation, systemic inflammatory response syndrome (SIRS) evolves into multiple organ dysfunction syndrome (MODS) due to excessive inflammation promoted by both activated circulating leukocytes and activated microvascular endothelial cells of solid organs. The interaction of activated leukocytes and activated endothelium results in poor tissue perfusion, with ischemic consequences to vital organs. The interaction of activated neutrophils and endothelium also leads to increased vascular permeability with fluid leakage from the intravascular space to tissue interstitium with resulting hypovolemia, hypotension, and cardiovascular instability. In the
Role of Monocytes/Macrophages on Chronic Organ Dysfunction—Kidney, Heart, Brain, and Liver

The monocyte/macrophage component of the innate immunological system is critical in the host response to invading pathogens and tissue repair and remodeling after tissue injury. The optimization of these processes is dependent upon a finely regulated and coordinated response of the monocyte/macrophage system. A balanced monocyte/macrophage response, both in phenotype and in timing, is necessary for optimal host defense and tissue repair. Monocytes are produced in the bone marrow and are continuously released into the circulation, constituting approximately 5% to 10% of the circulating leukocyte pool in humans. Human circulating monocytes are not a homogeneous population. Three subsets of monocytes have been identified and are based upon the expression of cell surface markers, CD14 (lipopolysaccharide [LPS] co-receptor) and CD16 (FcγRII). Within the population of monocytes, the majority are the classical subset with high CD14 but no CD16 expression (CD14hiCD16−), with the minority population further subdivided into the intermediate subset (CD14hiCD16+) and the nonclassical subset (CD14lowCD16++). The classical and intermediate monocytes have the ability for phagocytosis and production of inflammatory effectors, similar to Ly6c hi mouse monocytes. The nonclassical monocytes have a patrolling anti-inflammatory and reparative role similar to the Ly6c low mouse monocytes. The monocyte/macrophage system exists in at least 2 distinct phenotypes of differentiation: pro-inflammatory and anti-inflammatory. Upon inflammatory signals, promoted by infection or tissue injury, circulating monocytes infiltrate tissue and differentiate into the M1 (inflammatory) or M2 (anti-inflammatory, reparative) macrophage phenotype. The M1 macrophage is usually the initial responder to coordinate and accentuate the pro-inflammatory response to destroy invading pathogens and to digest cellular and tissue debris. The M2 macrophage becomes more prominent later in this process, to repair and remodel damaged tissue promoted by this vigorous inflammatory process.

Various chronic organ dysfunction disorders have been associated with chronic inflammation. Chronic heart failure (CHF), chronic kidney disease (CKD), and type 2 diabetes mellitus (T2D) have been shown to have an increase in pro-inflammatory CD14 hi monocytes compared to those in normal controls. In fact, an increase in CD14 hi monocytes in these chronic disease states correlate with worse clinical outcomes. The development of atherosclerosis in the general and CKD population, as well as in end-stage renal disease (ESRD) patients on chronic hemodialysis (HD), are associated with M1 macrophage phenotype. The circulating CD14 hi monocyte subset increases as renal function declines, with higher numbers of CD14 hi monocytes predicting adverse cardiovascular outcomes in ESRD patients undergoing chronic HD. Pro-inflammatory (CD14 hi) monocytes are also increased in T2D and correlate with progression to diabetic nephropathy and uremia. Furthermore, a higher level than normal of circulating inflammatory monocytes has been shown to result in worse clinical outcomes in both acute myocardial and brain injury. A persistent pro-inflammatory response also characterizes progressive organ dysfunction in CHF, CKD, and obesity-related T2D. The recognition of this relationship between the chronic pro-inflammatory monocyte/macrophage subtypes and disease progression have identified the monocyte/macrophage system as a therapeutic target for altering the clinical progression of chronic inflammatory disorders. Accordingly, a treatment that shifts the circulating monocyte pool from CD14 hi to CD14 low phenotype may have a clinical benefit to ameliorate the progression of various chronic inflammatory disorders.

Selective Cytopheretic Device Therapy and Acute Kidney Injury

Initial Clinical Insight and Proposed SCD Mechanism of Action

Selective cytopheretic device therapy (SCDtry) to treat inflammatory disorders originated from the clinical...
evaluation of a tissue-engineered renal assist device (RAD) containing adult human renal epithelial cells as a component of a bioartificial kidney. In the RAD phase Ila clinical study, subsets of patients were treated with a cell containing RAD or a sham (non—renal cell containing) RAD cartridge. The phase IIb study was a randomized, controlled, blinded, multicenter study in intensive care unit (ICU) patients with ARF secondary to AKI undergoing continuous renal replacement therapy (CRRT). The clinical study was suspended after an interim analysis due to an unanticipated high survival rate of the sham device arm. In retrospective analysis of the sham control groups, the improved survival rate was demonstrated in the presence of regional citrate anticoagulation (RCA) when compared to systemic heparin anticoagulation. Subjects were divided into the following 4 groups: RAD with citrate anticoagulation; sham device with citrate anticoagulation; RAD with heparin anticoagulation; and sham device with heparin anticoagulation. The 28-day survival rate in the heparin sham patient group was 50% versus 75% in the citrate sham group (n = 12 for each treatment arm), and the 90-day survival rate was 25% (heparin) versus 67% (citrate). The baseline demographics for the 2 subsets were comparable, with similar sequential organ failure assessment (SOFA) scores (13.4 ± 1.1 vs. 12.2 ± 0.9), organ failure number (4.17 ± 0.46 vs. 3.93 ± 0.36), and incidence of sepsis (58% vs. 58%) for the citrate versus heparin sham groups, respectively. This clinical result, although unexpected, was consistent with a potential clinical benefit of the fiber-based device without cultured renal cells (RAD sham), when used with RCA, which later became known as SDD therapy (SCD_sham) (Figure 1).

The therapeutic benefit afforded by this combination of a device and a pharmacological agent (citrate) on a systemic clinical disorder can be better understood from the following: (i) Microscopy of the sham cartridges (future SCD) after patient treatment demonstrated adherent leukocytes on the outer surface of the membranes of the cartridge along the blood flow path. The attached leukocytes were dominated by neutrophils and monocytes (Figure 2), which preferentially adhere, compared to other leukocytes such as lymphocytes, eosinophils, and basophils. The ability of leukocytes to adhere to the outer walls of the hollow fiber membranes rather than the inner walls, which is the conventional blood flow path for renal dialysis/hemofiltration applications, was due to the shear forces of blood flow. The shear stress of blood along the outer wall of the membrane was near capillary force of <1 dyne/cm² compared to the shear stress of 100 dyne/cm² for blood flowing along the conventional luminal surface of the hollow fiber membranes. (ii) RCA lowers the ionized calcium (iCa) in blood within the circuit to <0.4 mM, a level that inhibits the coagulation system, has an inhibitory effect on leukocyte and platelet activation, and also affects the calcium-dependent selectin- and integrin-mediated interactions between leukocytes and the membrane. Extravasation of neutrophils and monocytes from the systemic circulation into tissues is a highly regulated process. In a low—shear force environment like that found in capillaries or created within the SCD, neutrophils and monocytes roll along surfaces and are slowed via selectin binding followed by integrin-mediated, firm adhesion before diapedesis. An in vitro blood study using flow chambers to visualize leukocyte interactions with fiber materials suggests that leukocytes roll, then transiently adhere to fibers, are retained for a significant time period (referral to as sequestration), and are then released. Binding selectivity in the SCD is increased in the low-iCa environment, where calcium-dependent selectin rolling, integrin binding, and downstream conformational changes of attached cells are inhibited. Neutrophils and monocytes mobilize intracellular stores of CD11b, to the cell surface as they become (primed) activated. Measurement of CD11b, allows for real-time measurement of systemic acute neutrophil (priming) and monocyte activation. In addition, monocyte populations are heterogeneous in their expression of CD11b, with CD14hiCD16— being the highest and CD14lowCD16+ being the lowest (Figure 3). The selectivity of binding of the highest-activated leukocytes has been repeatedly observed in preclinical animal models in which systemic CD11b levels decrease through the treatment course. This effect was measured directly in a clinical trial by comparing the CD11b mean fluorescence intensity of the circulating cells in the peripheral blood to those directly associated with the SCD. These results, when taken together, suggest an SCD mechanism of action with a simultaneous, combination effect to transiently sequester activated circulating neutrophils and monocytes, with enhanced selectivity for inflammatory leukocytes, which alters the overall activation of bound and processed leukocytes. Clinical efficacy in AKI/multiple organ dysfunction (MOD) may be due to sequestration and immunomodulation of leukocytes in the SCD, which appears to block the inflammatory sequence associated with accumulation and aggregation of leukocytes in the peritubular capillaries and to reduce infiltration into interstitial spaces, which, when unchecked, promotes kidney injury following SIRS.
SCD Clinical Treatment in AKI

SCD Phase I/II: ARF Safety, Mortality, and Device Integrity Study Performed Outside the United States. The initial clinical study of the SCD was completed at the Huashan Hospital in Shanghai, China. This prospective, single-arm, single-center study was designed to evaluate the safety and efficacy of SCDRx on clinical outcomes in AKI requiring CRRT in the ICU. The patients enrolled in the trial were compared with historical case-matched controls with respect to age and SOFA score. The mortality for the case-matched controls was 7 of 9 or 78%, whereas the mortality in the SCDRx group was 2 of 9 or 22% \( P < 0.027 \). Multiple regression analysis identified treatment with SCD as the only significant variable affecting mortality among age, SOFA score, and average change in urine output over the first 7 days during or after treatment. Mean total urine output in the 9 SCDRx subjects increased from a baseline of \( \sim 500 \) to >2000 ml/d by day 7 of treatment. Total white blood cell (WBC) counts also declined with SCDRx, but not to leukopenic levels. Reported serious adverse events (SAEs) were consistent with the targeted patient group and did not result in any clinical sequela.

Figure 1. Schematic representation of (a) selective cytopheretic device therapy (SCD\(_{\text{Rx}}\)) and (bi–iii) current understanding of the mechanism of action (MoA) of the SCD, which involves leukocyte (LE)/fiber interactions. (i) Binding of activated LE (purple) with mobilized surface integrins (green); (ii) “reset” LE; (iii) release of immunomodulated LE. Erythrocytes are depicted in all panels as red.
SCD Phase II: Pilot Prospective Multi Center US Study (Investigational Device Exemption G090189; Protocol ARF-002). A prospective, single-arm, multicenter, US study was designed to evaluate the safety and efficacy of SCDRx on AKI requiring CRRT in the ICU. The study enrolled 35 subjects. The mean age was 56 ± 15 years. The average SOFA score was 11.3 ± 3.6. Death from any cause at day 60 was 31%. Renal recovery, defined as dialysis independence, was observed in all surviving subjects at day 60. The results of this pilot study indicate the potential for a substantial improvement in patient outcomes over standard-of-care therapy, which is associated with a greater than 50% 60-day mortality in the literature. There were no SCD-associated SAEs.

SCD Phase III: A Multicenter, Randomized, Controlled, Pivotal Study to Assess the Safety and Efficacy of an SCD in Patients With AKI (Investigational Device Exemption G090189, Protocol SCD-003). The primary objective of this study was to determine whether CRRT+SCDRx, compared to CRRT alone, would result in a clinically relevant and statistically significant improvement in all-cause mortality through day 60. Secondary objectives included assessment of renal replacement therapy (RRT) dependency at day 60, mortality at day 28, number of ventilator free days (VFD) at day 28, and mortality at day 60 in the subset of patients with severe sepsis. This was a 2-arm, randomized, open-label, controlled, multicenter pivotal study that enrolled 134 patients at 21 US medical centers. The ICU AKI patients of each participating hospital were randomized to treatment undergoing CRRT or CRRT+SCDRx. Each participating clinical site used its established RCA protocol for the CRRT+SCD circuits (study arm) and for the CRRT-only (control arm). The recommended iCa (riCa) level (measured after SCD) in the CRRT and SCD circuit was specified to be between 0.25 and 0.4 mmol/l.

During the second quarter of the enrollment period, a national calcium shortage occurred in the United States from FDA-related quality manufacturing issues of the major US supplier. Due to reliance of the SCD on a narrow intracircuit iCa range for functional efficacy and the concern that patients randomized to SCDRx were not getting effective therapy, the interim analysis was performed early after enrollment of 134 patients. Enrollment was paused on 24 May 2013, to assess the clinical impact of the calcium shortage on study endpoints. The shortage of calcium infusion solutions resulted in a tendency to minimize citrate infusion rates. Accordingly, iCa levels within the blood circuit tended to be above the riCa of 0.25 to 0.40 mmol/l. Subsequently, the injectable calcium shortage resulted in 9 of the 21 open clinical sites being unable to enroll patients because of low hospital inventories of injectable calcium, contributing to the early termination of the study. Of the 134 patients in the analysis, 69 received CRRT alone and 65 received SCDRx.

Figure 2. Micrographs of the sham, acellular cartridges as part of the regional citrate anticoagulation arm of the Renal Assist Device (RAD) clinical trial. Patient treatment demonstrated adherent leukocytes (LE) on the outer surface of the membranes of the cartridge along the blood flow path within the extracorporeal circuit, which translated into patient benefit. This was the basis for the treatment now referred to as SCDRx. (a–d) Light micrographs stained with hematoxylin and eosin. Low-power micrograph showing adherent cells around each fiber (a, original magnification ×160). (b,c) Higher-power micrographs showing clustering of bound LE (b and c, original magnification ×400). (d) High-power micrograph displaying predominance of NE and MO in the adherent cell clusters (original magnification ×1600). MO, monocyte; NE, neutrophil.
Human monocytes can be classified by CD14 and CD16 expression into classical (Q1: CD14+CD16+), intermediate (Q2: CD14+CD16-), and nonclassical (Q3: CD14lowCD16-) subsets using flow-cytometric techniques. Representative cytometric analysis of systemic human blood is shown, with CD14 and CD16 expression displayed as a dot plot of relative fluorescence intensity (RFI). The intensity of CD11b expression of each event is heat mapped according to the arrow in Q4 (blue indicates lowest and red indicates highest CD11b RFI). The subsets have differential CD11b expression according to phenotype, contributing to integrin-dependent selectcytopheretic device selectivity for pro-inflammatory, classical, and intermediate MO. MO, monocytes.

No significant differences were noted between the control and treatment groups in baseline characteristics. No statistically significant difference was found between the treated and control patients, with a 60-day mortality of 39% (27/69) and 36% (21/59), respectively, with 6 patients lost to follow-up. The amount of time that the patients in both the control and treatment groups were maintained in the riCa range (0.25–0.40 mmol/l), as specified in the study protocol, was substantially lower than expected due to the injectable calcium shortage. Of the 134 patients enrolled at the time of the interim analysis, 19 SCD patients and 31 control patients were maintained at riCa for greater than or equal to 90% of the therapy time. Furthermore, none of the SAE were considered device related per the principal investigator. Comparison of these subgroups of patients revealed that 60-day mortality was 16% (3/19) in the SCD group compared to 41% (11/27) in the control group (P = 0.11). Dialysis dependency showed a borderline statistically significant difference between the SCD versus control patients maintained for greater than 90% of the treatment in the protocol’s riCa target range with values of 0% (0/16) and 25% (4/16), respectively (P = 0.10). When the riCa SCD and control subgroups were compared for a composite index of 60-day mortality and dialysis dependency, the percentage in SCD subjects was 16% versus 58% in the control subjects (P < 0.01). When the riCa subpopulation was considered, a statistically significant difference was detected in several parameters: log urine output substantially increased, and WBC and neutrophil counts diminished in the SCD group versus the control group over time.

The observation that, in those patients who had the riCa level greater than 90% of the time of SCDRx, mortality improved from 41% to 16%, is clinically compelling. In addition, the observation, both in the pilot SCD-002 trial and in this SCD-003 study reported here, that no patient receiving appropriate SCDRx was dialysis dependent at day 60 is also compelling. Previous large, prospective clinical studies in AKI with MOD had a greater than 20% incidence of dialysis dependency of patients followed up for 60 or more days. The effect of SCDRx to modulate excessive leukocyte activation most likely plays a critical role in the recovery of renal function after a substantive AKI event. The relationship of ongoing inflammation in the kidney after AKI and chronic progressive kidney disease and dialysis dependency has been demonstrated. In this patient population, immunomodulation by SCDRx appears to positively promote kidney healing as evidenced by the lack of dialysis dependency at day 60. In addition, improvement in overall mortality may suggest improved immune balance that persists through the late SIRS process to ameliorate the compensatory anti-inflammatory response that follows the excessive systemic pro-inflammatory state in AKI and MOD. Furthermore, the significant decrease in WBC and neutrophil counts, as well as the improvement in urine output over time, corroborates findings in the mechanistic and pilot studies previously published.

SCD Phase II: Pilot Pediatric Patients With AKI. Similar to the adult AKI clinical trial, a 5-center US study of the SCD in a critically ill pediatric population (>20 kg, age up to 22 years) with AKI and MOD receiving CRRT as part of standard of care has been initiated and is ongoing under the FDA-approved investigational device exemption (IDE) G150179. Mortality rates in pediatric patients with AKI and MOD requiring CRRT have historically approached 50%. In this pilot-study clinical trial, pediatric patients have received SCDRx for up to 7 days or when CRRT is discontinued, whichever comes first. Results to date are as follows: 6 pediatric patients (3 female and 3 male) have been enrolled and have completed SCDRx. Patient age ranged from 6.5 to 17.5 years, with a Pediatric Risk of Mortality score
(PRISM II) range of 2 to 14. Admission diagnoses were as follows: 1 severe rhabdomyolysis (case study presented below), 3 septic shock, 1 shigatoxin-associated hemolytic-uremic syndrome, and 1 community-acquired pneumonia. Patients received 3 \( n = 2 \), 4 \( n = 1 \), 6 \( n = 1 \), or 7 \( n = 2 \) days of SCDRx. All 6 patients survived and were off CRRT at the time of hospital discharge. No SCD-related SAEs were recorded. 46

A case study of the first pediatric patient treated with SCD has been published, which describes the treatment course of an 11-year-old female patient with an uncommon reaction to anesthesia (propofol) during an elective surgery that resulted in MOD: severe AKF requiring CRRT, acute liver failure, acute respiratory failure requiring mechanical ventilation, and hemotologic failure from disseminated intravascular coagulation and rhabdomyolysis with oliguria. 42 After 24 hours of SCDRx, her liver injury and hematologic failure improved. After 4 days of therapy, her lung function improved, resulting in extubation. After 7 days of SCDRx, her kidney function improved, resulting ultimately in complete recovery of kidney function not requiring dialysis treatment. The patient was discharged from hospital with normal renal function. 42

This technology has been tested in several different clinical trials, as summarized in Table 1, which includes trials during the original development of the RAD, in which SCDRx emerged, and in 4 trials using the SCD in adult ICU patients with AKI requiring CRRT 28,44,45 and a pediatric trial. 42 These trials have demonstrated an excellent safety profile and suggestive efficacy impact. At the 28-day survival point, MOF ICU patients treated with the SCD, on average, had a full-day decrease in ventilator dependency. A supplemental IDE is currently FDA approved for a prospective, randomized, multicenter 175 patient clinical trial with a new primary composite endpoint of 60-day mortality or dialysis dependency. The analysis clearly demonstrated clinical efficacy without safety issues, providing substantive support to move this technology to other patient populations.

### SCDRx and ESRD in HD patients

Patients with ESRD on chronic HD suffer accelerated morbidity and mortality rates due to cardiovascular disease and infections. Chronic inflammation plays a critical role in these poor outcomes. The activated monocyte has become a prime therapeutic target to modulate this inflammatory process. SCDRx was evaluated to assess its effects on the circulating monocyte pool. A pilot trial was undertaken in 15 ESRD patients on HD with C-reactive protein (CRP) levels greater than 5 mg/dl (SCD Phase I/II ESRD Safety and Bio-inflamatory Assay Study, additional data added to the original submission of IDE G090189). In this study, ESRD patients were treated with one 4-hour session of SCD therapy, and an excellent safety profile was observed with no decline in leukocyte or platelet counts. The effect of SCDRx on monocyte phenotypes in these patients was determined on peripheral blood monocytes by using flow cytometry. SCDRx promoted a significant shift \( (P < 0.013) \) in monocyte phenotype, from predominantly CD14\(^{hi}\)expressing monocytes at baseline/pre-SCDRx to CD14\(^{low}\)-expressing monocytes post-SCDRx. 43 In a subset of patients \( n = 7 \) presenting with T2D, this persistent decline in monocyte CD14 expression was sustained for 2 weeks after therapy. 43 These results demonstrate that SCDRx has the potential to modulate the chronic pro-inflammatory state in ESRD patients.

### SCDRx in Preclinical Large-Animal Models of Acute Myocardial Infarction and Chronic Heart Failure

Ischemia/reperfusion injury (IRI), characterized by a vigorous inflammatory response immediately post-reperfusion via molecular signals generated by injured endothelium and cardiomyocytes, results in increased injury from leukocyte infiltration into the peri-infarct zone. This response becomes important in healing necessary to re-establish cardiac performance, but is excessive and maladaptive.

SCDRx was evaluated in a canine model of IRI, based on left circumflex coronary artery occlusion. Acute myocardial infarction was induced for 3 hours, and SCDRx was established using an extracorporeal blood circuit with RCA, 30 minutes before reperfusion and continued for up to an additional 3 hours afterward.

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**Table 1. SCDRx clinical application history**

| RAD phase | ARF and MOF receiving CVVH, 10 patients |
|-----------|----------------------------------------|
| I/II      |                                        |
| II        | ARF and MOF receiving CVVH, 58 patients |
| IIb       | ARF and MOF receiving CVVH, Citrate Arm comparable to SCDRx, 24 patients |
| IIb       | receiving Acellular Sham, 12 with heparin, 12 with RCA |
| SCD phase | ARF and MOF receiving CVVH, 9 patients (China) |
| I/II      | Protocol ARF-002, 35 patients |
| III       | Protocol SCD-003, 134 patients |
| SCD phase | FDA/IDE approved, adult AKI, 175 patients |
| I         | FDA/IDE approved, pediatric AKI, 16 patients |
| SCD phase | Safety and bioinflammatory assay ESRD study, 15 patients |

AKI, acute kidney injury; ARF, acute renal failure; CVVH, continuous veno-venous hemofiltration; ESRD, end-stage renal disease; FDA, US Food and Drug Administration; IDE, investigational device exemption; MOF, multiple organ failure; SCD, selective cytopheretic device; SCDRx, selective cytopheretic device therapy.
Systemic inflammation was monitored by CD11b expression of leukocyte populations by using flow cytometry, and leukocyte infiltration was evaluated by histology. Systemic cytokine and cardiac injury marker troponin–I (cTn-I) levels were assayed. Left ventricular (LV) function, infarct size and edema were evaluated in treated animals (n = 3) and compared to control animals (n = 4). SCDRx resulted in a 50% reduction of infarct size (19.2 ± 2.7 vs. 10.2 ± 4.5% of LV volume respectively, \( P < 0.05 \) (Figure 4), less edema (LV wall thickening) and 10 times lower cTn-I levels. Leukocyte infiltration was prominent in the peri-infarct zone of controls but was negligible in the SCD group.

The SCD immunomodulatory platform therapy was also evaluated in a canine chronic heart failure (CHF) model induced by chronic trauma via multiple sequential intracoronary embolizations with microspheres. Dogs were administered either three 6-hour therapy sessions over 1 week (n = 7) or no treatment (n = 5). Data from an interim analysis demonstrated that LV ejection fraction (EF) increased substantially in the treatment group, from 33.6 ± 1.3 (n = 5) to 43.3 ± 2.5 (n = 5; 6–48 hours posttherapy) and 37.0 ± 0% (n = 2; 4 weeks posttherapy), reflective of 29% and 10% increases, respectively. In the no treatment group (n = 5), EF% did not change. This effect was not due to a decline in systemic vascular resistance, which was similar in both groups. Ventriculograms demonstrated that SCDRx converted viable but noncontracting myocardium to contracting myocardium. This benefit was maintained throughout the 4-week posttherapy follow-up period (Figure 4). Of relevance to the immunomodulatory impact of SCDRx, elevated peritoneal macrophage absolute number, which is associated with CHF, was lower in SCDRx dogs compared to the no-treatment dogs. In addition, the macrophage phenotype shifted from pro-inflammatory M1 seen in the no-treatment dogs to the reparative/anti-inflammatory M2 phenotype seen in SCDRx dogs (unpublished results). Modulation of peritoneal macrophages demonstrates that the SCDRx impact is not only organ specific but also affords the benefit of systemic immunomodulation. In summary, in this CHF model, SCDRx improved myocardial contractility and modulated chronic inflammation, demonstrating strong support of SCDRx as an innovative treatment approach to treat CHF.

![Figure 4](image-url)
SCD<sub>RX</sub> in Preclinical Large-Animal Models of Traumatic Brain Injury and Intracranial Hemorrhage

Inflammation has recently been recognized as a central contributor to the pathobiology of stroke and traumatic brain injury (TBI), indicating that therapies that target inflammation may provide a new approach to the treatment of the acute phase of this disease process, particularly for intracranial hemorrhage (ICH), as there are currently no interventive therapies for this subset of stroke patients. Pilot preclinical studies were initiated to determine the SCD effect on the acute inflammatory cascade in ICH.

The acute effects of SCD<sub>RX</sub> were evaluated in a porcine model of ICH, for which thrombin was used as the inflammatory nidus. ICH was induced simultaneously with SCD<sub>RX</sub> by using an extracorporeal blood circuit with RCA. Therapy continued for 24 additional hours, during which systemic inflammation was monitored by assay of systemic cytokines. Platelet activation and CD11R3 expression of leukocyte populations were determined using flow cytometry. Postmortem coronal sections of frozen brain tissue were evaluated for leukocyte infiltration, and neuronal injury was evaluated by histology. SCD effects on lesion size and brain edema were evaluated in treated animals (n = 3) and compared to a contemporaneous control. SCD<sub>RX</sub> resulted in a reduction in edema (Figure 5), reduced expression of neuronal injury markers, and reduced leukocyte infiltration as evaluated in animals sacrificed at 24 hours after ictus. 61 Pilot studies demonstrate that immunomodulation with the SCD represents a novel therapy that has the potential to improve outcomes associated with ICH.

SCD<sub>RX</sub> in a Preclinical Large-Animal Model of Acute Lung Injury

Acute lung injury (ALI) is included within the clinical definition of acute respiratory distress syndrome (ARDS). 62 ARDS affects more than 190,000 Americans annually. 63 Mortality rates range from 26% to 58% and despite advances in supportive care; no pathophysiologically driven therapeutic intervention for ARDS is currently available. 64 ALI results from direct (pulmonary) and indirect (extrapulmonary) injury to the lungs. Pneumonia, aspiration, pulmonary contusion, inhalation injury, and fat emboli from fractured long bones constitute direct causes of ALI, whereas indirect lung injury resulting from SIRS is observed with polytrauma requiring multiple transfusions, severe sepsis, and burns. Sepsis remains the most common cause of ARDS, with 46% of cases triggered by primary pulmonary disorders.

Aside from the use of lung-protective ventilator strategies or extracorporeal membrane oxygenation (ECMO), which are purely supportive of gas exchange while attempting to limit further lung injury, there are no pathogenesis-directed therapies for ARDS, leaving a vast unmet medical need. Patients with lung injury more commonly than not encounter more than “one hit” modulating the immunological response to injury by increasing duration and amplitude of the inflammatory response. In fact, many second “hits” occur after proinflammatory responses (e.g., SIRS) have waned, and patients manifest compensatory anti-inflammatory responses (CARS) with suppressed immunity and diminished resistance to infection. This scenario seemingly places patients at risk for manifesting clinically significant ARDS and MODS. 65 Leukocytes are major contributors to the pathogenesis and progression of the inflammatory response and have been implicated in the pathogenesis of ARDS. Sequestration and infiltration in lung tissue progresses to diminish pulmonary gas exchange and to exacerbate disruption of the alveolar capillary membrane. 66 Therapeutic strategies to block inflammation are expected to decrease morbidity/mortality by limiting activity and tissue accumulation of leukocytes at sites of inflammation. 67, 68

Immunomodulatory effects of SCD<sub>RX</sub> during ARDS have been investigated in a pilot study using a porcine model of acid-induced ALI (Humes HD, Buffington DA, Transportable renal replacement therapy for battlefield applications DoD/TARTRC, 2011–2013 [proposal application]). In brief, pigs were anesthetized and ventilated when 0.4N HCl was delivered via a tracheal catheter. Pa:FiO<sub>2</sub> decreased to <300 in all pigs, which were then allocated to untreated or SCD<sub>RX</sub> cohorts and followed up for 6 hours. Pulmonary vascular resistance was lower in SCD<sub>RX</sub> pigs without a concomitant decrease in systemic vascular resistance. The extent of leukocyte infiltration into the lungs at 6 hours post-injury was reduced as determined by immunohistochemical staining. Using the number of CD11R3<sup>+</sup> events normalized for total cells identified through 4',6-diamidino-2-phenylindole (DAPI) staining, the extent of this reduction in leukocyte infiltration was quantitatively evaluated with ImageJ software (National Institutes of Health, Bethesda, MD). Leukocyte infiltration was reduced in the SCD pigs across all major lung lobes. This reduction in leukocyte infiltration into lung tissue has also been observed when assessing SCD<sub>RX</sub> in a porcine model of septic shock—associated MODS. 29 Fewer neutrophils were recovered in the bronchoalveolar lavage fluid of SCD<sub>RX</sub> versus untreated ALI pigs. In the clinical setting, the concentration of neutrophils collected in bronchoalveolar lavage fluid has been shown to correlate with the
severity and outcome of ARDS. Accordingly, the significant decrease in neutrophil extravasation could possibly lead to improved long-term lung function. The impact of SCD Rx on lung function has been demonstrated by an increase in ventilator-free days at 28 days during a SCD phase III clinical trial. Preclinical evaluation of SCDRx as a treatment for ALI in a porcine model of trauma with sepsis is currently being conducted (Humes HD, Assessment of a therapeutic device for treatment of acute lung injury using a combat-relevant porcine model. DoD/PRMRP, 2016–2019 [proposal application]).

**SCD Rx in a Preclinical Large-Animal Model of Type 2 Diabetes Mellitus**

T2D is a complex disease whereby insulin resistance is a critical pathophysiological disorder. Obesity is associated with tissue inflammation, which is now recognized as a critical etiology of insulin resistance. Circulating WBC counts, including absolute neutrophil and monocyte counts, are elevated in diabetic patients compared to nondiabetic patients. Not only do these cells of the innate immunologic system increase in absolute number, but they also exist in a persistently activated state. It is clear that recruitment of circulating monocytes to form tissue macrophages within adipose tissue is the initiating event in obesity-induced inflammation and insulin resistance. The internal environment of adipose tissue favors the M1 pro-inflammatory phenotype of adipocyte tissue macrophage, resulting in tissue inflammation and insulin resistance. Pro-inflammatory cytokines, produced by adipocyte tissue macrophages and other cells, have been shown to promote insulin resistance in a paracrine and endocrine fashion. Interventions with anti-inflammatory action therefore have beneficial effects to improve insulin sensitivity.

To evaluate the effect of SCD Rx on insulin resistance in a preclinical model of T2D, an Ossabaw miniature swine model of metabolic syndrome was used. When fed an excess-calorie atherogenic diet over several months, Ossabaw swine develop at least 5 of the 6 criteria for metabolic syndrome, including primary insulin resistance, obesity with significant visceral adipose expansion, hypertriglyceridemia and increased low-density lipoprotein:high-density lipoprotein cholesterol, mild hypertension, and coronary artery disease. Treatment with the SCD in this porcine model demonstrated a decline in circulating neutrophil activation parameters and monocyte counts. These changes were associated with improvements in insulin resistance as determined by i.v. glucose tolerance.
testing. Improvements were also reflected in lowering of homeostatic model assessment of insulin resistance (HOMA-IR) scores for up to 2 weeks after SCDRx.

Summary

Increasingly, clinicians have recognized the critical role that the immune system plays in the response after organ injury, and the dire ramifications of excessive, dysregulated inflammation in both acute and chronic disease states, as well as the resulting impact on solid organ function. This review article has examined a number of the peer-reviewed publications, as well as work in progress, to elucidate the mechanisms of action for a novel immunomodulatory therapy, SCDRx, to treat various inflammatory disease indications. This demonstrates a growing body of clinical and preclinical support for immunomodulatory interventions.

DISCLOSURE

HHD is a shareholder of Innovative BioTherapies Inc., and CytoPherx Inc., biotechnology spin-out companies of the University of Michigan. CJP, AJW, KAJ, and DAB are employees of Innovative BioTherapies Inc.

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SUPPLEMENTARY MATERIAL

Definitions of Abbreviations.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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