



- (51) International Patent Classification:
A61M 1/34 (2006.01)
- (21) International Application Number:
PCT/US2012/059615
- (22) International Filing Date:
10 October 2012 (10.10.2012)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/584,337 9 January 2012 (09.01.2012) US
- (71) Applicant: HUMES, H., David [US/US]; 2918 Parkridge Drive, Ann Arbor, MI 48103 (US).
- (72) Inventor; and
- (71) Applicant : BUFFINGTON, Deborah, A. [US/US]; 3453 Miller Road, Ann Arbor, MI 48103 (US).
- (74) Agents: KOMM, Crystal, A. et al.; Goodwin Procter LLP, Exchange Place, Boston, MA 02109 (US).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,

DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

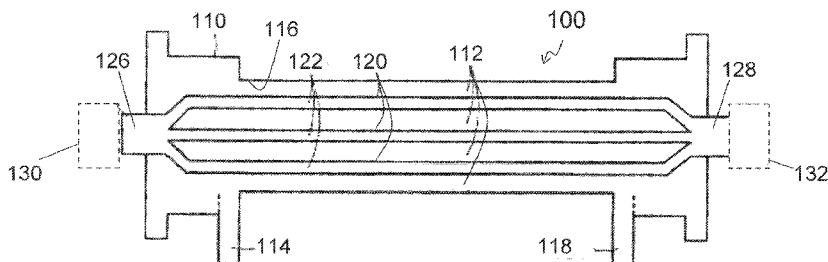
Published:
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
— without international search report and to be republished upon receipt of that report (Rule 48.2(g))



WO 2013/106109 A1

(54) Title: CARTRIDGE AND METHOD FOR INCREASING MYOCARDIAL FUNCTION

Figure 1A



(57) Abstract: The present invention relates to a cytopheretic cartridge for use in treating and/or preventing inflammatory conditions that affect myocardial function and to related methods. The cartridge can be used in treating a subject with myocardial dysfunction, such as a subject with chronic heart failure and/or acute decompensated heart failure.

- 1 -

CARTRIDGE AND METHOD FOR INCREASING MYOCARDIAL FUNCTION**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 61/584,337, filed January 9, 2012, the contents of which are incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under grant number W81XWH-10-2-0137, awarded by the US Army Medical Research and Material Command. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present invention relates to cartridges, systems, and methods for treating and/or preventing inflammatory myocardial conditions in a subject. More particularly, the present invention relates to cartridges and systems for sequestering and reducing the inflammatory activity of cells associated with myocardial inflammation, such as leukocytes and platelets, and to related methods for sequestering and reducing the inflammatory activity of such cells.

BACKGROUND

[0004] Various medical conditions are caused, exacerbated, and/or characterized by unwanted inflammation. For example, chronic inflammation is central to the development of a variety of acute organ failures, including those involving the heart, kidney, lung, and brain. Chronic inflammation is also a major contributing factor to chronic organ dysfunction, including those involving the heart and kidney as well as diabetes type 2. Several of these conditions, such as, for example, chronic heart failure (CHF) and acute decompensated heart failure (ADHF), through abnormal or excessive chronic activation of the immune system, may result in life threatening myocardial dysfunction.

- 2 -

[0005] Certain cell types are critical to the dysfunction of the cardiovascular and immune systems. For example, leukocytes such as neutrophils contribute to the pathogenesis and progression of various inflammatory conditions, including systemic inflammatory response syndrome (SIRS), sepsis, ischemia/reperfusion injury, acute respiratory distress syndrome (ARDS), CHF, and ADHF (see, *e.g.*, Kancider *et al.* (2006) FEBS J 273:4416-4424; Maroszynska *et al.* (2000) ANN. TRANSPLANT. 5(4):5-11). Other types of leukocytes, such as monocytes and tissue macrophages, have been identified as critical sources of systemic inflammation in CHF and cause a decrease in cardiac myocyte contractility (see, *e.g.*, Conraads *et al.* (2005) J. HEART LUNG TRANSPLANT. 24(7): 854-59; Simms *et al.* (1999) AM. J. PHYSIOL. 277: H253-60; Conraads *et al.* (2005) J. HEART LUNG TRANSPLANT, 24(7): 854-9; Simms *et al.* (1999) AM. J. PHYSIOL. 277: H253-60). In addition, activated platelets enhance leukocyte adhesion and promote leukocyte activation. While inflammation and a systemic immune response can be beneficial in certain circumstances, they can also be fatal.

[0006] Inflammatory injury in organs can result in microvascular damage induced by leukocyte activation and aggregation, as well as platelet activation and aggregation. These activated cells can contribute to microvascular stasis and reperfusion injury by releasing toxic compounds into a patient's tissue. Activated leukocytes additionally cause damage by extravasating across the endothelium into the tissue, where they release toxic agents normally intended to destroy invading microbes or clear out necrotic debris. Further, the interaction of activate leukocytes and the endothelium can lead to increased vascular permeability with fluid leakage from the intravascular space to the tissue interstitium with resulting hypovolemia, hypotension, and cardiovascular instability. Activated platelets additionally cause damage by enhancing the activation and endothelial transmigration of leukocytes. When these processes are not controlled, they can lead to tissue injury and death.

[0007] Cardiovascular disease is the leading cause of mortality in the United States, accounting for 45% of all deaths. Furthermore, in the United States, CHF affects 5 million people, with over 0.5 million new cases identified annually with direct hospital costs exceeding \$30 billion (see, *e.g.*, Association (2006) HEART DISEASE AND STROKE FACTS; Association (2002) 2003 HEART AND STROKE STATISTICAL UPDATE; Fonarow *et al.* (2003) 4: p. S21-30). In severe CHF, annual mortality rates can be as high as 50%. Currently, treatment of CHF generally involves a ventricular assist device or orthotropic heart transplant. Over the past decade, a number of therapeutic agents for treating CHF have been clinically tested in large

- 3 -

prospective trials. Endothelin receptor antagonists, adenosine A1-receptor antagonist, and vasopressin V2 receptor blocker have all failed to prove clinical efficacy (see, e.g., McMurray *et al.* (2007) JAMA 298(17): 2009-19; Massie *et al.* (2010) N. ENGL. J. MED. 363(15): 1419-28; Konstam *et al.* (2007) JAMA 297(12): 1319-31). The myocardial calcium sensitizing agent
5 (levosimendan) and the vasodilatory recombinant B-type natriuretic peptide (nesiritide) have also failed to meet clinical efficacy end points with an increase in risks of arrhythmias or hypotension ((see, e.g., Cohn *et al.* (1998) N. ENGL. J. MED. 339(25): 1810-6; Mebazza *et al.* (2007) JAMA 297(17): 1883-91; O'Connor *et al.* (2011) N. ENGL. J. MED. 365(1): 32-43).

[0008] Acute decompensated heart failure (ADHF) accounts for almost one million
10 hospitalizations per year, and rehospitalization within six months is as high as 50%. The annual mortality rate in patients frequently hospitalized with ADHF approaches 50%. Current therapeutic approaches for treating patients with ADHF focus on relieving these patients of the congestive symptoms of heart failure, usually with diuretics. However, such an approach results in, and is limited by further declines in renal functions.

15 [0009] Accordingly, there remains a need for improved treatments of inflammatory conditions that affect myocardial functions, such as chronic heart failure and acute decompensated heart failure.

SUMMARY OF THE INVENTION

[0010] Inflammatory conditions often arise from the activation of cells associated with inflammation, such as leukocytes and platelets. The present invention relates to methods and
20 cytopheretic cartridges for use in treating and/or preventing inflammatory conditions that affect various myocardial functions. The methods and/or cartridges of the invention extracorporeally sequester leukocytes and/or platelets and inhibit or deactivate their inflammatory action. For example, these cells can be deactivated and/or their release of pro-inflammatory substances can be inhibited.

25 [0011] In a first aspect, the invention provides a method of increasing myocardial function in a subject with chronic heart failure. The method comprises the step of (a) extracorporeally sequestering activated leukocytes and/or activated platelets present in a body fluid (for example, blood) of the subject in a cartridge comprising (i) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid outlet port, wherein the inner volume is in fluid flow

- 4 -

communication with the fluid inlet port and the fluid outlet port, and (ii) a solid support disposed within the housing and defining a fluid contacting surface with a surface area (SA) capable of sequestering activated leukocytes and/or activated platelets, if present in a body fluid entering the housing via the fluid inlet port. The body fluid (for example, blood) is introduced
5 into the housing via the fluid inlet port under conditions that permit sequestration of the activated leukocytes and/or activated platelets on the fluid contacting surface of the solid support. The method also comprises the step of (b) treating the sequestered leukocytes and/or platelets to inhibit release of a pro-inflammatory substance or to deactivate the leukocytes and/or platelets thereby to increase myocardial function of the subject when compared to the
10 myocardial function of the subject prior to treatment. The increase in myocardial function can be one or more functions selected from the group consisting of left ventricular ejection fraction, cardiac output, systemic vascular resistance, left ventricular stroke volume, aortic pressure, left ventricular pressure, peak rate of change of left ventricular pressure during isovolumic contraction and relaxation, left ventricular end-diastolic pressure, myocardial oxygen
15 consumption, and coronary flow reserve.

[0012] The first aspect of the invention can have any one or more of the following features or embodiments described herein.

[0013] In certain embodiments, the SA/IV ratio of the cartridge provided in step (a) is greater than 25 cm^{-1} , or is in the range of 25 cm^{-1} to $2,000 \text{ cm}^{-1}$, or is no greater than 80 cm^{-1} .
20 The solid support can be disposed within the housing at a packing density in the range from 20% to 65%.

[0014] In certain embodiments the solid support can be defined by one or more fibers (for example, fluid permeable fibers (for example, permeable hollow fibers) or fluid impermeable fibers (for example, solid fibers)), one or more planar support members, or any combination
25 thereof. The solid support can comprise one or more membranes. The solid support can be substantially parallel to the direction of fluid flow within the cartridge.

[0015] In certain embodiments, the SA of the cartridge provided in step (a) is in the range of from 0.1 m^2 to 10.0 m^2 , or in the range of from 0.1 m^2 to 5.0 m^2 . In certain embodiments, the IV is less than 300 cm^3 , less than 150 cm^3 , is in the range of from 10 cm^3 to 150 cm^3 , is in
30 the range of from 75 cm^3 to 150 cm^3 or is in the range of from 15 cm^3 to 120 cm^3 .

- 5 -

[0016] In certain embodiments, the method optionally further comprises permitting the body fluid to exit the cartridge via the fluid outlet port at a flow rate in the range of 10 cm³/minute to 8,000 cm³/minute.

[0017] In step (b), the leukocytes and/or platelets can be treated with an
5 immunosuppressant agent, a serine leukocyte inhibitor, nitric oxide, a polymorphonuclear leukocyte inhibitor factor, a secretory leukocyte inhibitor, or a calcium chelating agent, wherein the calcium chelating agent is one or more of the group consisting of citrate, sodium
10 hexametaphosphate, ethylene diamine tetra-acetic acid (EDTA), triethylene tetramine, diethylene triamine, o-phenanthroline, and oxalic acid. In a preferred embodiment, in step (b), the leukocytes and/or platelets are treated with a calcium chelating agent, for example, citrate. Each of the foregoing agents, including the calcium chelating agent, can be introduced into the body fluid of the subject prior to, during, or after step (a).

[0018] In certain embodiments, the leukocytes and/or platelets are treated over a period of at least 2 hours, at least 4 hours, at least 6 hours, at least 8 hours, or at least 12 hours, or over a
15 period of 2 to 48 hours, 2 to 24 hours, 2 to 12 hours, 4 to 48 hours, 4 to 24 hours, or 4 to 12 hours.

[0019] The subject may have myocardial dysfunction secondary to inflammatory cell penetration of heart tissue, and/or may have received a heart transplant. In certain
20 embodiments, the increased myocardial function is maintained for at least 6 hours or at least 24 hours after termination of the treatment in step (b).

[0020] In a second aspect, the invention provides a method of increasing myocardial function in a subject with chronic heart failure. The method comprises: (a) extracorporeally sequestering activated leukocytes and/or activated platelets present in a body fluid (for
25 example, blood) of the subject; and (b) treating the sequestered leukocytes and/or platelets to inhibit release of a pro-inflammatory substance or to deactivate the leukocytes and/or platelets thereby to increase myocardial function of the subject when compared to the myocardial function of the subject prior to treatment. The myocardial function can be selected from the group consisting of left ventricular ejection fraction, cardiac output, systemic vascular
30 resistance, left ventricular stroke volume, aortic pressure, left ventricular pressure, peak rate of change of left ventricular pressure during isovolumic contraction and relaxation, left ventricular end-diastolic pressure, myocardial oxygen consumption, and coronary flow reserve.

- 6 -

[0021] In a third aspect, the invention provides a method of increasing myocardial function in a subject with acute decompensated heart failure (ADHF). The method comprises: (a) extracorporeally sequestering activated leukocytes and/or activated platelets present in a body fluid (for example, blood) of the subject; and (b) treating the sequestered leukocytes and/or platelets to inhibit release of a pro-inflammatory substance or to deactivate the leukocytes and/or platelets thereby to increase myocardial function of the subject when compared to the myocardial function of the subject prior to treatment.

[0022] The second and third aspects of the invention can have any one or more of the following features or embodiments described herein.

[0023] For example, in each aspect the subject can have myocardial dysfunction secondary to inflammatory cell penetration of heart tissue, and/or may have received a heart transplant. In step (b), the leukocytes and/or platelets are treated with an immunosuppressant agent, a serine leukocyte inhibitor, nitric oxide, a polymorphonuclear leukocyte inhibitor factor, a secretory leukocyte inhibitor, or a calcium chelating agent, wherein the calcium chelating agent is one or more of the group consisting of citrate, sodium hexametaphosphate, ethylene diamine tetra-acetic acid (EDTA), triethylene tetramine, diethylene triamine, o-phenanthroline, and oxalic acid. In a preferred embodiment, in step (b), the leukocytes and/or platelets are treated with a calcium chelating agent, for example, citrate. Each of the foregoing agents, including the calcium chelating agent, can be introduced into the body fluid of the subject prior to or after step (a).

[0024] In each aspect, the leukocytes and/or platelets from the subject are treated over a period of at least 2 hours, at least 4 hours, at least 6 hours, at least 8 hours, or at least 12 hours, or are treated over a period of 2 to 48 hours, 2 to 24 hours, 2 to 12 hours, 4 to 48 hours, 4 to 24 hours, or 4 to 12 hours.

[0025] The myocardial function improved by the treatment can be one or more of the myocardial functions selected from the group consisting of left ventricular ejection fraction, cardiac output, systemic vascular resistance, left ventricular stroke volume, aortic pressure, left ventricular pressure, peak rate of change of left ventricular pressure during isovolumic contraction and relaxation, left ventricular end-diastolic pressure, myocardial oxygen consumption, and coronary flow reserve. The increase in myocardial function is maintained for at least 6 hours, or at least 24 hours after termination of the treatment in step (b).

- 7 -

[0026] In step (b), the activated leukocytes are sequestered (for example, bound) by introducing the body fluid into a cartridge comprising (i) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid outlet port, wherein the inner volume is in fluid flow communication with the fluid inlet port and the fluid outlet port, and (ii) a solid support
5 disposed within the housing and defining a fluid contacting surface with a surface area (SA) capable of sequestering activated leukocytes and/or platelets, if present in a body fluid entering the housing via the fluid inlet port. The body fluid is introduced into the housing via the fluid inlet port under conditions that permit sequestration (for example, binding) of the activated leukocytes and/or platelets on the fluid contacting surface of the solid support. It is understood
10 that, under certain circumstances, the activated leukocytes and platelets bind preferentially to the fluid contacting surface of the solid support relative to unactivated or deactivated leukocytes or platelets.

[0027] In certain embodiments, the SA/IV ratio of the cartridge provided in step (a) is greater than 25 cm^{-1} , greater than 80 cm^{-1} , or greater than 150 cm^{-1} , or is in the range of from
15 25 cm^{-1} to $1,500 \text{ cm}^{-1}$, or is in the range of from 80 cm^{-1} to $1,500 \text{ cm}^{-1}$. In other embodiments, the SA/IV ratio is no greater than 80 cm^{-1} . The solid support can be disposed within the housing at a packing density in the range from 20% to 65%.

[0028] In certain embodiments the solid support can be defined by one or more fibers (for example, fluid permeable fibers (for example, permeable hollow fibers) or fluid impermeable
20 fibers (for example, solid fibers)), one or more planar support members, or any combination thereof. The solid support can comprise one or more membranes. The solid support can be substantially parallel to the direction of fluid flow within the cartridge.

[0029] The SA of the cartridge provided in step (a) can be in the range of from 0.1 m^2 to 10.0 m^2 , in the range of from 0.1 m^2 to 5.0 m^2 . Furthermore, the inner volume of the cartridge
25 provided in step (a) is less than 300 cm^3 , less than 150 cm^3 , or is in the range of from 10 cm^3 to 150 cm^3 , in the range of from 75 cm^3 to 150 cm^3 , or in the range of from 15 cm^3 to 120 cm^3 . In certain embodiments, the method further comprises permitting the body fluid to exit the cartridge via the fluid outlet port at a flow rate in the range of $10 \text{ cm}^3/\text{minute}$ to $8,000 \text{ cm}^3/\text{minute}$.

30 [0030] In a fourth aspect, the invention provides a method of treating a subject having or at risk of developing an inflammatory condition associated with acute decompensated heart

- 8 -

failure (ADHF). The method comprises: (a) providing a cartridge comprising (i) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid outlet port, wherein the inner volume is in fluid flow communication with the fluid inlet port and the fluid outlet port; and (ii) a solid support disposed within the housing and defining a fluid contacting surface with a surface area (SA) capable of sequestering an activated leukocyte, if present in a body fluid entering the housing via the fluid inlet port; and (b) introducing a body fluid from a subject into the housing via the fluid inlet port under conditions that permit sequestration of an activated leukocyte and/or an activated platelet on the fluid contacting surface of the solid support. The method optionally further comprises the step of (c) treating the leukocyte and/or platelet sequestered in step (b) to reduce the risk of developing inflammation associated with the ADHF or to alleviate inflammation associated with the ADHF.

[0031] In a fifth aspect, the invention provides a method of treating a subject having or at risk of developing an inflammatory condition, wherein the inflammatory condition is chronic heart failure (CHF). The method comprises: (a) providing a cartridge comprising (i) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid outlet port, wherein the inner volume is in fluid flow communication with the fluid inlet port and the fluid outlet port; and (ii) a fluid permeable solid support disposed within the housing and defining a fluid contacting surface with a surface area (SA) capable of sequestering an activated leukocyte, if present in a body fluid entering the housing via the fluid inlet port, wherein the SA/IV ratio is no greater than 80 cm^{-1} ; and (b) introducing a body fluid from a subject into the housing via the fluid inlet port under conditions that permit sequestration of an activated leukocyte and/or an activated platelet on the fluid contacting surface of the solid support. The method optionally further includes the step of (c) treating the leukocyte and/or platelet sequestered in step (b) (for example, with a calcium chelator) to reduce the risk of developing inflammation associated with the inflammatory condition or to alleviate inflammation associated with the inflammatory condition.

[0032] The fourth and fifth aspects of the invention can have one or more of the following features or embodiments described herein.

[0033] For example, in certain embodiments, the leukocyte and/or platelet is sequestered for a time sufficient to deactivate the leukocyte and/or the platelet, for example, at least one

- 9 -

minute. Furthermore, the method optionally further includes the step of returning the leukocyte and/or the platelet produced in step (c) back to the subject.

[0034] In certain embodiments, the SA/IV ratio of the cartridge provided in step (a) is greater than 80 cm^{-1} , or is greater than 150 cm^{-1} or can be in the range of from 80 cm^{-1} to $1,500 \text{ cm}^{-1}$, or in the range of from 150 cm^{-1} to $1,500 \text{ cm}^{-1}$. In certain embodiments the solid support can be defined by one or more fibers (for example, fluid permeable fibers (for example, permeable hollow fibers) or fluid impermeable fibers (for example, solid fibers)), one or more planar support members, or any combination thereof. The solid support can comprise one or more membranes. The solid support can be substantially parallel to the direction of fluid flow within the cartridge.

[0035] In certain embodiments, the SA of the cartridge provided in step (a) is in the range of from 0.1 m^2 to 10.0 m^2 , or in the range of from 0.1 m^2 to 5.0 m^2 , or is in the range of from 0.1 m^2 to 0.4 m^2 , from 0.4 m^2 to 0.8 m^2 , from 0.8 m^2 to 1.2 m^2 , from 1.2 m^2 to 1.6 m^2 , from 1.6 m^2 to 2.0 m^2 , from 2.0 m^2 to 2.4 m^2 , from 2.4 m^2 to 2.8 m^2 , from 2.8 m^2 to 3.2 m^2 , from 3.2 m^2 to 3.6 m^2 , from 3.6 m^2 to 4.0 m^2 , from 4.0 m^2 to 4.4 m^2 , from 4.4 m^2 to 4.8 m^2 , from 4.8 m^2 to 5.2 m^2 , from 5.2 m^2 to 5.6 m^2 , from 5.6 m^2 to 6.0 m^2 , from 6.0 m^2 to 6.4 m^2 , from 6.4 m^2 to 6.8 m^2 , from 6.8 m^2 to 7.2 m^2 , from 7.2 m^2 to 7.6 m^2 , from 7.6 m^2 to 8.0 m^2 , from 8.0 m^2 to 8.4 m^2 , from 8.4 m^2 to 8.8 m^2 , from 8.8 m^2 to 9.2 m^2 , from 9.2 m^2 to 9.6 m^2 , or from 9.6 m^2 to 10.0 m^2 .

[0036] In certain embodiments, the inner volume of the cartridge provided in step (a) is less than 150 cm^3 , or is in the range of from 10 cm^3 to 150 cm^3 , or is in the range of from 75 cm^3 to 150 cm^3 , or is in the range of from 15 cm^3 to 120 cm^3 , or is in the range of from 20 cm^3 to 80 cm^3 .

[0037] In certain embodiments, the method further comprises the step of permitting the body fluid to exit the cartridge via the fluid outlet port at a flow rate in the range of $10 \text{ cm}^3/\text{minute}$ to $8,000 \text{ cm}^3/\text{minute}$, or in the range of $50 \text{ cm}^3/\text{minute}$ to $8,000 \text{ cm}^3/\text{minute}$. Furthermore, the method optionally further comprises the step of measuring the myocardial function of the subject prior to step (a) and/or after step (b).

[0038] In each of the foregoing aspects (including all five aspects of the invention) and embodiments, the leukocyte and/or platelet can be sequestered (for example, bound) for a time (e.g., at least one second, at least one minute, at least five minutes, at least fifteen minutes, or at least an hour) sufficient to inhibit the release of the pro-inflammatory substance or to deactivate

- 10 -

the leukocyte and/or the platelet. The activated leukocytes and/or activated platelets bind to a fluid contacting surface of the solid support. Under certain circumstances, the activated leukocytes and/or activated platelets bind preferentially to the fluid contacting surface of the solid support relative to unactivated or deactivated leukocytes and/or platelets.

5 [0039] In another aspect, the invention provides a cartridge for use in a method of improving myocardial function in a subject with chronic heart failure (CHF) or acute decompensated heart failure (ADHF). The cartridge comprises (i) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid outlet port, wherein the inner volume is in fluid flow communication with the fluid inlet port and the fluid outlet port, and (ii) a solid support
10 disposed within the housing in fluid flow communication with the inner volume and defining a fluid contacting surface with a surface area (SA) configured for sequestering activated leukocytes and/or platelets, if present in a body fluid entering the housing via the fluid inlet port.

[0040] In certain embodiments, the cartridge has a SA to IV ratio in the range of 25 cm^{-1} to
15 $2,000 \text{ cm}^{-1}$, or a SA/IV ratio of greater than 80 cm^{-1} , or a SA/IV ratio of no greater than 80 cm^{-1} . The cartridge can be disposed within sterile packaging, for example, where the sterile packaging comprises plastic packaging. The cartridge optionally further comprises a label disposed on an outer surface of the rigid housing. The cartridge optionally further comprises a cap sealing the fluid inlet port and/or the fluid outlet port. The surface area configured for
20 sequestering activated leukocytes and/or platelets binds the activated leukocytes and/or platelets, optionally preferentially binds the activated leukocytes and/or platelets relative to unactivated or deactivated leukocytes or platelets. It is understood that the cartridge can be used in any of the methods described herein.

[0041] In another aspect, the invention provides a calcium chelating agent for use in a
25 method of treating chronic heart failure or acute decompensated heart failure in a subject in need thereof, wherein the method of treating comprises administering the calcium chelating agent to extracorporeally sequestered activated leukocytes and/or activated platelets wherein the activated leukocytes and/or platelets are sequestered in any of the cartridges and methods using cartridges described herein.

30

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] The foregoing aspects and embodiments of the invention may be more fully understood by reference to the following detailed description and claims.

[0043] **Figure 1A** is a schematic, cross-sectional representation of an exemplary SCD cartridge containing a plurality of hollow fibers. **Figure 1B-1D** are schematic, cross-sectional representations of a SCD cartridge containing a plurality of solid fibers and/or planar support members.

[0044] **Figure 2A** is a schematic representation of a fluid circuit containing a SCD cartridge where the intracapillary space (ICS) has both ends capped. **Figure 2B** is a schematic representation of an embodiment similar to **Figure 2A** except that ultrafiltrate (UF) is collected from a SCD cartridge having only one end of the ICS capped. **Figure 2C** is a schematic representation of an embodiment of a fluid circuit containing a first device, for example, a hemofiltration device, and a SCD cartridge that includes an ICS with both ends capped. **Figure 2D** is a schematic representation of an embodiment similar to **Figure 2C** except that ultrafiltrate (UF) is collected from the SCD cartridge where only one end of the ICS is capped.

[0045] **Figures 3A and 3B** are schematic representations of embodiments of system configurations that can be used as a CPB circuit. In **Figure 3A** the circuit comprises a recirculation loop and in **Figure 3B**, the fluid circuit lacks a recirculation loop.

[0046] **Figure 4** is a schematic representation of an embodiment of a system configuration used in treating a subject with sepsis. The container to the left of the animal, below the hemofilter contains citrate. The container to the right of the animal, below the SCD cartridge contains calcium ions.

[0047] **Figure 5** is a graphical depiction of changes in cardiovascular parameters of subjects with sepsis treated with an F-40 SCD device in the presence of heparin (SCD-H); an F-40 SCD device in the presence of citrate (SCD-C, F-40); or an F-80A SCD device in the presence of citrate (SCD-C, F-80A). Results are shown for mean arterial blood pressure (**Figure 5A**); systemic vascular resistance (**Figure 5B**); renal vascular resistance (**Figure 5C**); cardiac output (**Figure 5D**); pulmonary vascular resistance (**Figure 5E**); and hematocrit (**Figure 5F**).

- 12 -

[0048] Figure 6 is a graphical depiction of changes in renal parameters of subjects with sepsis treated with an F-40 SCD device in the presence of heparin (SCD-H); an F-40 SCD device in the presence of citrate (SCD-C; F-40); or an F-80A SCD device in the presence of citrate (SCD-C; F-80A). Results are shown for blood urea nitrogen (BUN) (Figure 6A); renal blood flow (Figure 6B); creatinine (Figure 6C); and cumulated urine output (Figure 6D).

[0049] Figure 7 is a graphical depiction of survival times for subjects with sepsis treated with an F-40 SCD device in the presence of heparin (SCD-H) or with an F-40 or F-80A SCD device in the presence of citrate (SCD-C).

[0050] Figure 8 is a bar graph depicting survival times for subjects with sepsis treated an F-40 SCD device in the presence of heparin (SCD-H); an F-40 SCD device in the presence of citrate (F-40, SCD-C); or an F-80A SCD device in the presence of citrate (F-80A, SCD-C).

[0051] Figure 9 is a series of light microscopy photographs showing leukocyte attachment and aggregation along the outer surface of SCD membranes.

[0052] Figures 10A and 10B are bar graphs depicting the number (Figure 10A) and distribution (Figure 10B) of cells eluted from SCD membranes following their use in SCD devices to treat septic subjects. The subjects were treated with an F-40 SCD device in the presence of heparin (SCD-H); an F-40 SCD device in the presence of citrate (F-40 SCD-C); or an F-80A SCD device in the presence of citrate (F-80A SCD-C).

[0053] Figure 11 is a graphical depiction of levels of serum myeloperoxidase (Figure 11A) or systemic neutrophil activation, as measured by CD11b mean fluorescent intensity (Figure 11B) shows hematocrit levels in subjects with sepsis treated with an F-40 SCD device in the presence of heparin (SCD-H) or with an F-40 or F-80A SCD device in the presence of citrate (SCD-C).

[0054] Figure 12 is a graphical depiction of release of IL-8 (Figure 12A) and TNF- α (Figure 12B) from peripheral blood mononuclear cells isolated from subjects after 6 hours of treatment for sepsis with an F-40 SCD device in the presence of heparin (SCD-H); an F-40 SCD device in the presence of citrate (F-40 SCD-C); or an F-80A SCD device in the presence of citrate (F-80A SCD-C).

[0055] Figure 13 is a photograph of lung sections incubated with primary anti-CD11b antibody, followed by incubation with an anti-mouse IgG Alexafluor594 conjugate. Nuclei

- 13 -

were counterstained with DAPI. The left panel is from a subject treated for sepsis with an F-40 SCD device in the presence of heparin; the right panel is from a subject treated for sepsis with a SCD device in the presence of citrate. A significant decrease in CD11b-labeled cells was observed in the lungs of the patients whose regimen included citrate rather than heparin.

- 5 [0056] **Figure 14** is a bar graph depicting the number of CD11b-positive cells detected in non-septic subjects; septic subjects treated with an F-40 SCD device in the presence of citrate (F-40 SCD-C); septic subjects treated with an F-80A SCD device in the presence of citrate (F-80A SCD-C); or septic subjects treated with an F-40 SCD device in the presence of heparin (F-40 SCD-H).
- 10 [0057] **Figure 15** is a graphical depiction of systemic white blood cell counts (**Figure 15A**), systemic absolute neutrophil counts (**Figure 15B**), and systemic immature neutrophil counts (**Figure 15C**) over time in septic subjects treated with an F-40 SCD device in the presence of citrate (SCD-C, F-40), with an F-80A SCD device in the presence of citrate (SCD-C, F-80A), or with an F-40 SCD device in the presence of heparin (SCD-H).
- 15 [0058] **Figure 16** is a bar graph depicting the percentage of neutrophils that were detected as positive for annexin V, as an assessment of the apoptotic potential of the cells. Both systemic neutrophils and SCD-adherent neutrophils were measured following treatment of septic patients with an F-40 SCD (F-40 SCD-C) or an F-80A SCD (F-80A SCD-C) in the presence of citrate.
- 20 [0059] **Figure 17** is a bar graph depicting the relative numbers of leukocytes attaching to polysulfone in the presence of shear flow and in the presence or absence of lipopolysaccharides (LPS) and/or citrate.
- [0060] **Figure 18** is a schematic representation of an embodiment of a system configuration for use in treating a subject with chronic heart failure.
- 25 [0061] **Figure 19** is a graphical depiction of changes in cardiovascular parameters of subjects with chronic heart failure when treated with a SCD device in the presence of heparin (SCD-H) or a SCD device in the presence of citrate (SCD-C). Results are shown for ejection fraction (**Figure 19A**); cardiac output (**Figure 19B**); and systemic vascular resistance (**Figure 19C**).

- 14 -

[0062] Figure 20 is a graphical depiction of changes to certain renal functions upon treatment, including, the urine volume of subjects with chronic heart failure treated with a SCD device in the presence of heparin (SCD-H) or a SCD device in the presence of citrate (SCD-C) (Figure 20A); percent fractional excretion (FE) of sodium (Na) in subjects with chronic heart failure treated with a SCD device in the presence of heparin (SCD-H), or a SCD device in the presence of citrate (SCD-C), or a CHF sham control (Figure 20B), percent fractional excretion of urea in subjects with chronic heart failure treated with a SCD device in the presence of heparin (SCD-H), or a SCD device in the presence of citrate (SCD-C), or a CHF sham control (Figure 20C), and mean renal sodium excretion (mmol/hour) of subjects with chronic heart failure treated with a SCD device in the presence of heparin (Hep) or a SCD device in the presence of citrate (Cit) (Figure 20D).

[0063] Figure 21 shows ventriculograms of a heart of a dog with CHF shown at baseline (before therapy) (Figure 21A) and at the end of four hours of SCD therapy (Figure 21B). The solid black line (bordered by arrows) depicts the border of the left ventricular diastolic silhouettes (most relaxed state during filling) overlaid on the left ventricular systolic image (most contracted state) demonstrating improved contractility of the left ventricle (black arrows), especially at the apex of the left ventricle, after therapy (see Figure 21B versus Figure 21A).

DETAILED DESCRIPTION

[0064] Cells associated with inflammation, such as leukocytes (or white blood cells) and platelets, normally defend the body against infection and injury. However, during many disease states and medical procedures, these cells can become activated, which in turn can produce undesirable immune and inflammatory responses that can be fatal. It is understood that devices, referred to as selective cytopheretic devices, that extracorporeally sequester leukocytes and/or platelets and then inhibit their inflammatory actions can be useful in the prevention or treatment of a variety of inflammatory conditions, in particular inflammatory conditions mediated or facilitated by activated leukocytes and/or platelets. U.S. Patent No. 8,251,941 describes exemplary selective cytopheretic devices and their use in the prevention and/or treatment of certain inflammatory conditions.

- 15 -

[0065] It has now been discovered that cytopheretic devices can also be useful in increasing cardiac function, for example, left ventricular ejection fraction, cardiac output, systemic vascular resistance, *etc.*, in subjects having or at risk of having chronic heart failure (CHF) and acute decompensated heart failure (ADHF). The use of cytopheretic devices, such as those,
5 described herein may be useful in the treatment of such disorders, especially in situations where drug based therapies (for example, nesiritide and levosimendan, which have been developed for the treatment of chronic heart failure) have heretofore been unsuccessful.

[0066] As used herein, the term “cytopheresis” or “selective cytopheresis” refers to the sequestration of certain cells, for example, leukocytes (*e.g.*, activated leukocytes) or platelets
10 (*e.g.*, activated platelets) from a body fluid, for example, blood. The sequestered cells can be deactivated and/or the release of the pro-inflammatory substance from such cells can be inhibited. It should be understood that such deactivation and/or inhibition can occur before, during, and/or after sequestration (*e.g.*, the binding to a fluid contacting surface of a solid support). In a specific embodiment, selective cytopheresis refers to the sequestration of
15 leukocytes (*e.g.*, activated leukocytes) and/or platelets (*e.g.*, activated platelets) from blood. The term “blood” refers to any aspect of blood, for example, whole blood, treated blood, filtered blood, or any liquid derived from blood, for example, serum or plasma.

[0067] The terms, “selective cytopheresis device,” “selective cytopheretic device,” “selective cytopheresis inhibitory device,” and “SCD” each refer to a device that facilitates or
20 is capable of facilitating cytopheresis. Such a device can also facilitate deactivation and/or inhibit the release of pro-inflammatory substances from such cells before, during, and/or after sequestration. The SCD includes one or more SCD cartridges that facilitate selective cytopheresis. While the discussion in the sections that follow generally describe sequestration and inhibition and/or deactivation of a particular cell type (*e.g.*, leukocytes), it is understood
25 that the same principles apply to the sequestration and inhibition and/or deactivation of other cell types associated with inflammation (*e.g.*, platelets, such as activated platelets).

[0068] An “activated leukocyte” is understood to mean a leukocyte that, in response to a challenge, for example, when exposed to an endotoxin (*e.g.*, lipopolysaccharide), has an enhanced ability to elicit an immune response relative to a leukocyte that has not been
30 challenged. For example, an activated neutrophil (PMN), is a neutrophil that, in response to a challenge, for example, when exposed to an endotoxin (*e.g.*, lipopolysaccharide), has an

- 16 -

enhanced ability to migrate, phagocytose, and produce an oxidative burst response relative to a neutrophil that has not been challenged. Activation can also be determined via an up-regulation of cell surface CD11b. An activated monocyte is a monocyte that, in response to a challenge, for example, when exposed to an endotoxin (*e.g.*, lipopolysaccharide), has an enhanced ability to release cytokines relative to a monocyte that has not been challenged. An “activated platelet” is understood to mean a platelet that, in response to a challenge, for example, when exposed to an endotoxin (*e.g.*, lipopolysaccharide), becomes adherent to other platelets, to leukocytes, and to certain proteins, for example, coagulation factors. Platelet activation can be quantified by determining the percentage of circulating monocytes that have platelets adhered to their cell surface. Activated leukocytes also include primed leukocytes. For example, a primed neutrophil (PMN), is a neutrophil that, in response to a challenge, for example, when exposed to an endotoxin (*e.g.*, lipopolysaccharide), has an enhanced ability to undergo an oxidative burst response relative to a neutrophil that has not been challenged.

1. Indications

[0069] The SCD cartridges, circuits incorporating the SCD cartridges, and methods of the present invention can be used for treating and/or preventing a number of heart or cardiovascular conditions that are associated with inflammation or an inflammatory condition. In particular the SCD cartridges, circuits incorporating the SCD cartridges, and methods of the present invention can be used for treating and/or preventing a number of heart or cardiovascular conditions where a subject is experiencing myocardial dysfunction secondary to inflammatory cell penetration of heart tissue, for example, myocardial tissue. As used herein, the term “subject” refers to any animal (*e.g.*, a mammal), including, but not limited to, a human (*e.g.*, a patient) or a non-human mammal, for example, a non-human primate or other experimental animal, farm animal, companion animal, or the like, which is to be the recipient of a particular diagnostic test or treatment.

[0070] In particular, it has now been discovered that cytopheretic devices can be useful in increasing cardiac function, for example, left ventricular ejection fraction, cardiac output, systemic vascular resistance etc., in subjects with myocardial dysfunction secondary to inflammatory cell penetration of heart tissue (for example, the myocardial tissue) such as subjects having or at risk of having chronic heart failure or acute decompensated heart failure.

- 17 -

The inflammatory cells that can affect myocardial function include, for example, leukocytes (for example, monocytes or macrophages) or platelets.

[0071] As used herein, the term “inflammatory condition,” includes any inflammatory disease, any inflammatory disorder, and/or any leukocyte activated disorder wherein the organism’s immune cells are activated. Such a condition can be characterized by (i) a
5 persistent inflammatory response with pathologic sequelae and/or (ii) infiltration of leukocytes, for example, mononuclear cells and neutrophils, leading to tissue destruction.

[0072] Leukocytes, for example, neutrophils, are major contributors to the pathogenesis and progression of many clinical inflammatory conditions. Several different and diverse types
10 of leukocytes exist; however, they are all produced and derived from a pluripotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes, also referred to as white blood cells, are found throughout the body, including in the blood and lymphatic system. There are several different types of leukocytes including granulocytes and agranulocytes. Granulocytes are leukocytes characterized by the presence of differently staining granules in their cytoplasm
15 when viewed under light microscopy. These granules contain membrane-bound enzymes, which primarily act in the digestion of endocytosed particles. There are three types of granulocytes: neutrophils, basophils, and eosinophils, which are named according to their staining properties. Agranulocytes are leukocytes characterized by the absence of granules in their cytoplasm and include lymphocytes, monocytes, and macrophages.

[0073] Platelets, or thrombocytes, also contribute to inflammatory conditions, as well as to homeostasis. Upon activation, platelets aggregate to form platelet plugs, and they secrete cytokines and chemokines to attract and activate leukocytes. Platelets are found throughout the
20 body’s circulation and are derived from megakaryocytes.

[0074] The molecules that are primarily responsible for initiation of leukocyte and platelet
25 adhesion to endothelium are P-selectin and von Willebrand factor, respectively. These molecules are found in the same granules, known as Weibel-Palade bodies, in endothelial cells. Upon activation of endothelial cells, the Weibel-Palade bodies migrate to the cell membrane to expose P-selectin and soluble von Willebrand factor at the endothelial cell surface. This, in turn, induces a cascade of leukocyte and platelet activity and aggregation.

[0075] The procedures described herein employ a SCD device that is in fluid flow
30 communication with the subject, such that a body fluid (for example, blood) flows from the

- 18 -

subject to the SCD device, and after passing through the SCD device flows back to the subject. Activated leukocytes, for example, activated monocytes, and/or activated platelets are sequestered within the SCD device on the fluid contacting surface of a solid support (for example, the outer surface of hollow or solid fibers that contact fluid as it passes through the SCD device or the fluid contacting surfaces of a planar support). The activated leukocytes and/or platelets are deactivated by exposure to one or more leukocyte inhibiting agents that are discussed below.

[0076] The devices can be used to increase myocardial function in subjects experiencing myocardial dysfunction that is secondary to inflammatory cell penetration of heart tissue (for example, myocardial tissue). The methods and devices described herein can be used therapeutically or prophylactically to increase myocardial function in a subject with chronic heart failure and/or acute decompensated heart failure. Each of these disorders is considered to be an inflammatory condition that also affects myocardial function in the subject. In addition, the methods and devices described herein can be used to therapeutically or prophylactically treat subjects experiencing or at risk of experiencing organ/tissue rejection following transplantation of an or organ (for example, a heart, liver or kidney) or tissue.

[0077] The subjects that are candidates for this treatment can be identified using standard techniques. For example, myocardial dysfunction can be measured by measuring one or more cardiac parameters, which can include, for example, left ventricular ejection fraction, cardiac output, systemic vascular resistance, left ventricular stroke volume, aortic pressure, left ventricular pressure, peak rate of change of left ventricular pressure during isovolumic contraction and relaxation, left ventricular end-diastolic pressure, myocardial oxygen consumption, and coronary flow reserve. These parameters can be easily measured before, during and after treatment with a SCD device.

[0078] The improvement of cardiac function is demonstrated below in Example 5, where an improvement in left ventricular ejection fraction, cardiac output and systemic vascular resistance was observed in subjects with chronic heart failure following treatment with a SCD device and a leukocyte inhibiting agent (citrate).

[0079] In certain embodiments, treatment of a subject may improve the left ventricular ejection fraction by at least 1% (compared to the left ventricular ejection fraction prior to treatment). For example, treatment of a subject may improve the left ventricular ejection

- 19 -

fraction by at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 12%, at least 14%, at least 16%, at least 18%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%. The treatment may continue until the subject has attained a left ventricular ejection fraction of at least 30%, at least 31%, at least 32%, at least 33%, at least 34%, at least 35%, at least 36%, at least 37%, at least 38%, at least 39%, at least 40%, at least 41%, at least 42%, at least 43%, at least 44%, at least 45%, at least 46%, at least 47%, at least 48%, at least 49%, or at least 50%. The treatment may provide a residual improvement in the left ventricular ejection fraction for at least 5 minutes, at least 10 minutes, at least 20 minutes, at least 30 minutes, at least 45 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 12 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 10 days, at least 14 days, at least 21 days, or at least 28 days.

[0080] In certain embodiments, treatment of a subject may improve the cardiac output by at least 1% (compared to the cardiac output prior to treatment). For example, treatment of a subject may improve the cardiac output by at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 12%, at least 14%, at least 16%, at least 18%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%. The treatment may continue until the subject has attained a cardiac output of at least 2.5L/min, at least 3.0 L/min, at least 3.5 L/min, at least 4.0 L/min, at least 4.5 L/min, at least 5.0 L/min, or at least 5.25 L/min. The treatment may provide a residual improvement in the cardiac output for at least 5 minutes, at least 10 minutes, at least 20 minutes, at least 30 minutes, at least 45 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 12 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 10 days, at least 14 days, at least 21 days, or at least 28 days.

[0081] In certain embodiments, treatment of a subject may improve the left ventricular stroke volume by at least 1% (compared to the stroke volume prior to treatment). For example, treatment of a subject may improve the left ventricular stroke volume by at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 12%, at least 14%, at least 16%, at least 18%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%. The treatment may continue until the

- 20 -

subject has attained a left ventricular stroke volume of at least 27 ml, at least 30 ml, at least 35 ml, at least 40 ml, at least 45 ml, at least 50 ml, at least 55 ml, at least 60 ml, at least 65 ml, or at least 70 ml. The treatment may provide a residual improvement in left ventricular stroke volume for at least 5 minutes, at least 10 minutes, at least 20 minutes, at least 30 minutes, at least 45 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 12 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 10 days, at least 14 days, at least 21 days, or at least 28 days.

[0082] In certain embodiments, treatment of a subject may reduce the systemic vascular resistance by at least 1% (compared to the systemic vascular resistance prior to treatment). For example, treatment of a subject may reduce the systemic vascular resistance by at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 12%, at least 14%, at least 16%, at least 18%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%. The treatment may continue until the subject has attained a systemic vascular resistance of no more than 3500 dyn·s/cm⁵, no more than 3000 dyn·s/cm⁵, no more than 2500 dyn·s/cm⁵, no more than 2000 dyn·s/cm⁵, or no more than 1600 dyn·s/cm⁵. The treatment may provide a residual improvement in the systemic vascular resistance for at least 5 minutes, at least 10 minutes, at least 20 minutes, at least 30 minutes, at least 45 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 12 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 10 days, at least 14 days, at least 21 days, or at least 28 days.

[0083] In addition to assessing myocardial function directly through hemodynamic parameters, subjects can also be assessed by monitoring of biomarkers such as norepinephrine, n-terminal brain natriuretic peptide (BNP), atrial natriuretic peptide (ANP), galectin-3, C-reactive protein, tumor necrosis factor- α (TNF- α), interleukin-1, interleukin-6, and troponin-1.

[0084] Although the invention is generally described herein with regard to blood and blood-based body fluids, the invention is applicable to any sample of a body fluid that can flow through an extracorporeal circuit, such as any body fluid from a subject that contains leukocytes and/or platelets. Exemplary extracorporeal circuits are described, for example, in U.S. Patent Nos. 6,561,997 and 8,251,941; U.S. Patent Application No. 61/584,337, filed

- 21 -

January 9, 2012; International Patent Application No. PCT/US11/56469, filed October 14, 2011, and published as International Patent Application Publication No. WO 2012/051595; and International Application No. _____ entitled "Cartridge and Method for Increasing Myocardial Function," filed October 10, 2012 and identified by Attorney Docket No.

5 NPR-013PC2; the entire disclosures of each of which are incorporated herein by reference.

The terms "sample" and "specimen" are used in their broadest sense. On the one hand, they are meant to include a specimen or culture. On the other hand, they are meant to include both biological and environmental samples. Body fluids include, but not limited to, blood, serum, plasma, cerebrospinal fluid (CSF), lymphatic fluid, peritoneal fluid or ascites, pleural fluid, and
10 saliva.

[0085] The following sections discuss exemplary SCD cartridges, systems incorporating such SCD cartridges, and their use in increasing cardiac function in a subject in need thereof.

2. *Cartridge Considerations*

[0086] Although the underlying principles for an appropriate SCD are discussed in detail, it
15 is understood that SCD cartridges useful in the practice of the invention are not limited to the particular design configurations discussed herein.

[0087] One exemplary SCD cartridge useful in the practice of the invention comprises a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid outlet port. The inner volume is in fluid flow communication with both the fluid inlet port and the fluid outlet port.
20 The inner volume is also referred to herein as the fill volume, and also the extracapillary space or (ECS) in embodiments that contain hollow fibers. The inner volume can be determined by sealing either the fluid inlet port or the fluid outlet port of the rigid housing, filling the SCD cartridge with a liquid, for example, water, via the unsealed port and then measuring the volume of liquid that fills the housing to the top of the unsealed port. In addition, the cartridge
25 comprises a solid support disposed within the housing so at least a portion of the solid support is isolated between the fluid inlet port and the fluid outlet port and defining a fluid contacting surface with a surface area (SA) capable of sequestering an activated leukocyte and/or an activated platelet, if present in a biological fluid entering the housing via the fluid inlet port.

[0088] It is understood that the choice of surface area of the solid support in a SCD
30 cartridge capable of sequestering the leukocytes and/or the platelets, and the inner volume (also referred to as the fill volume) of the housing of the SCD cartridge that contains the solid

- 22 -

support can have a profound effect on the efficacy of the SCD in treating certain inflammatory conditions. (See PCT/US11/056469.) The surface area of the solid support should be sufficient to sequester a portion of the leukocytes and/or platelets to be effective but without sequestering too many leukocytes and/or platelets. The sequestration of too many leukocytes can result in leukocyte deficiency that in turn can result in life-threatening leucopenia. The sequestration of too many neutrophils can result in neutropenia, and the sequestration of too many platelets can result in thrombocytopenia or bleeding diathesis. Furthermore, it can be important to choose a housing with an appropriate inner volume (also referred to as the fill volume or the extracapillary space when the solid support is defined by hollow fibers) depending upon the subject to be treated. For example, in the case of infants, children and severely ill, hemodynamically unstable patients, it is important to choose housings with lower fill volumes so that less body fluid needs to be extracted from the subject to contact or bathe the solid support. Accordingly, the choice of a SCD cartridge having the appropriate ratio of active surface area of the solid support to the inner volume of the SCD cartridge housing containing the solid support can have a profound effect on the efficacy of treatment in a given patient. The age, weight, and infirmity of the subject can be important considerations when choosing a particular SCD cartridge.

[0089] Depending upon the device, the SA/IV ratio of the cartridge can be in the range from 25 cm⁻¹ to 2,000 cm⁻¹, 25 cm⁻¹ to 1,750 cm⁻¹, 25 cm⁻¹ to 1,500 cm⁻¹, 25 cm⁻¹ to 1,250 cm⁻¹, 25 cm⁻¹ to 1,000 cm⁻¹, 25 cm⁻¹ to 800 cm⁻¹, 80 cm⁻¹ to 2,000 cm⁻¹, 80 cm⁻¹ to 1,750 cm⁻¹, 80 cm⁻¹ to 1,500 cm⁻¹, 80 cm⁻¹ to 1,250 cm⁻¹, 80 cm⁻¹ to 1,000 cm⁻¹, 80 cm⁻¹ to 800 cm⁻¹, 100 cm⁻¹ to 2,000 cm⁻¹, 100 cm⁻¹ to 2,000 cm⁻¹, 100 cm⁻¹ to 1,750 cm⁻¹, 100 cm⁻¹ to 1,500 cm⁻¹, 100 cm⁻¹ to 1,250 cm⁻¹, 100 cm⁻¹ to 1,000 cm⁻¹, 100 cm⁻¹ to 800 cm⁻¹, from 125 cm⁻¹ to 2,000 cm⁻¹, 125 cm⁻¹ to 1,750 cm⁻¹, 125 cm⁻¹ to 1,500 cm⁻¹, 125 cm⁻¹ to 1,250 cm⁻¹, 125 cm⁻¹ to 1,000 cm⁻¹, or 125 cm⁻¹ to 800 cm⁻¹, 150 cm⁻¹ to 2,000 cm⁻¹, 150 cm⁻¹ to 1,750 cm⁻¹, 150 cm⁻¹ to 1,500 cm⁻¹, 150 cm⁻¹ to 1,250 cm⁻¹, 150 cm⁻¹ to 1,000 cm⁻¹, 150 cm⁻¹ to 800 cm⁻¹, 200 cm⁻¹ to 2,000 cm⁻¹, 200 cm⁻¹ to 1,750 cm⁻¹, 200 cm⁻¹ to 1,500 cm⁻¹, 200 cm⁻¹ to 1,250 cm⁻¹, 200 cm⁻¹ to 1,000 cm⁻¹, 200 cm⁻¹ to 800 cm⁻¹, 200 cm⁻¹ to 600 cm⁻¹, from 300 cm⁻¹ to 2,000 cm⁻¹, from 300 cm⁻¹ to 2,000 cm⁻¹, from 300 cm⁻¹ to 1,750 cm⁻¹, from 300 cm⁻¹ to 1,500 cm⁻¹, from 300 cm⁻¹ to 1,250 cm⁻¹, from 300 cm⁻¹ to 1,000 cm⁻¹, 300 cm⁻¹ to 800 cm⁻¹, from 400 cm⁻¹ to 1,200 cm⁻¹, from 400 cm⁻¹ to 1,000 cm⁻¹, from 400 cm⁻¹ to 800 cm⁻¹, from 500 cm⁻¹ to 1,200 cm⁻¹, from 500 cm⁻¹ to 1000 cm⁻¹, or from 500 cm⁻¹ to 800 cm⁻¹.

- 23 -

[0090] In certain embodiments, the SA/IV ratio of the cartridge is greater than 25 cm^{-1} , or 80 cm^{-1} , or 150 cm^{-1} . In certain embodiments, the SA/IV ratio of the cartridge is no greater than 80 cm^{-1} (i.e., is 80 cm^{-1} or less).

[0091] Furthermore, in certain embodiments, the solid support (which can comprise a plurality of fibers or planar sheets) is disposed within the housing at a packing density in the range from 20 % to 65 % (for example, from 20 % to 60 %, or from 30 % to 60 % or from 40 % to 55 %). As used herein, the term "packing density" is understood to mean the percentage of the total volume of the interior of a cartridge that is occupied by the solid support. The volume V_{supp} occupied by the solid support is understood to include, for example, the aggregate volume of all the fibers, sheets, or other elements defining the solid support. If the solid support includes hollow elements, such as hollow fibers, the volume occupied by the solid support is understood to include any hollow spaces (e.g., intracapillary spaces), as well as the volume occupied by the material of the solid support. The total volume of the interior of a cartridge is therefore the sum of the fill volume (IV) of the cartridge and the volume occupied by the solid support. The packing density is the volume occupied by the solid support "inner volume" divided by the total volume of the interior of the cartridge, and can be expressed as $V_{\text{supp}} / (IV + V_{\text{supp}})$, which can also be presented as a percentage. For example, if the volume of V_{supp} is 10 cm^3 , and the IV is 20 cm^3 , the packing density is 0.33 or 33%.

[0092] In other embodiments, the cartridge comprises (a) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid outlet port, wherein the inner volume is in fluid flow communication with the fluid inlet port and the fluid outlet port; and (b) a solid support disposed within the housing and defining a fluid contacting surface with a surface area (SA) capable of sequestering an activated leukocyte and/or an activated platelet if present in a body fluid entering the housing via the fluid inlet port, wherein the SA is greater than 2.6 m^2 (for example, from 3.0 m^2 to 10.0 m^2 or from 3.0 m^2 to 5.0 m^2).

[0093] In another embodiment, the cartridge comprises (a) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid outlet port, wherein the inner volume is in fluid flow communication with the fluid inlet port and the fluid outlet port; and (b) a solid support comprising a plurality of solid fibers disposed within the housing, the solid support defining a fluid contacting surface with a surface area (SA) capable of sequestering an activated leukocyte and/or an activated platelet if present in a body fluid entering the housing via the fluid inlet

- 24 -

port, wherein the SA/IV ratio is greater than 25 cm^{-1} (for example, greater than 80 cm^{-1} , greater than 150 cm^{-1} , or in the range from 150 cm^{-1} to $1,500 \text{ cm}^{-1}$, in the range from 80 cm^{-1} to 800 cm^{-1} , in the range from 25 cm^{-1} to 800 cm^{-1}).

[0094] Figure 1A shows a schematic, cross-sectional representation of an exemplary SCD cartridge 100. SCD cartridge 100 comprises a housing 110 that defines an inner volume or fill volume 112, a fluid inlet port 114, a fluid contacting inner surface 116, and a fluid outlet port 118. The fluid inlet port 114, inner volume (or fill volume) 112, and fluid outlet port 118 are in fluid flow communication with one another. As shown, the fluid inlet port 114 and the fluid outlet port 118 are disposed on the same side of the housing (*i.e.*, are ipsilateral). In this embodiment, the housing further comprises a solid support 120 defined by the exterior surface(s) of one or more hollow fibers. Figure 1A shows three hollow fibers. In this embodiment, the interior of the hollow fibers 120 together define an intracapillary space (“ICS”) 122, and the volume disposed between the fluid contacting inner surface 116 of the housing and the exterior surface of the hollow fibers 120 together define the inner volume 112, which is also referred to as the extracapillary space (“ECS”). Depending upon the particular embodiment, a fluid, for example, an ultrafiltrate, can be introduced into ICS 122 of the SCD 100 via an ICS inlet 126 which can then pass into or through ICS 122 and, if desired, exit housing 110 via ICS outlet 128. In certain embodiments, however, the ICS inlet 126 can be blocked or otherwise capped with end cap 130 and/or ICS outlet 128 can be blocked or otherwise capped with end cap 132. In this embodiment, at least a portion of solid support 120 is disposed within housing 110 between fluid inlet port 114 and fluid exit port 118.

[0095] During operation of this SCD cartridge, the fluid sample of interest is introduced into housing 110 via fluid inlet 114 into inner volume (or ECS) 112. The fluid then passes along the surface of solid support 120 (along the exterior surface of the hollow fibers) in a plane substantially parallel to the plane of the solid support 120, and then exits inner volume (or ECS) 112 via fluid exit port 118. During passage along solid support 120, activated leukocytes and/or platelets are sequestered and optionally deactivated. As a result, during operation, cells (for example, leukocytes) from the body fluid (for example, blood) associate with a particular region within the passageway defined by the cartridge housing, specifically, with the exterior surface of the hollow fibers. Accordingly, in certain embodiments, a passageway region configured to sequester leukocytes may include a porous membrane that permits smaller molecules to pass therethrough but forces larger molecules and/or cells to flow

- 25 -

along the membrane. Moreover, in certain embodiments, the passageway region configured to sequester leukocytes is bounded by a surface of a housing and is bounded by, and may include, the exterior surface or surfaces of hollow fibers configured such that the biological sample (e.g., a subject's blood or filtered blood) flows over these surfaces (i.e., over the hollow fibers).

5 See, for example, **Figure 1**. The hollow fibers may be porous, semi-porous, or non-porous and a different fluid (e.g., ultrafiltrate) may optionally flow or be present within the hollow fibers. The fibers can be formed from any suitable material described herein.

[0096] Accordingly, the invention also provides a method of using a cartridge (i) for processing an activated leukocyte, activated platelet or a combination thereof, or (ii) for
10 treating a subject at risk of developing or having an inflammatory condition. The method comprises providing a cartridge comprising (i) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid outlet port; and (ii) a solid support disposed within the housing so at least a portion of the solid support is isolated between the fluid inlet port and the fluid outlet port and defining a fluid contacting surface with a surface area (SA) capable of sequestering an
15 activated leukocyte, if present in a biological fluid entering the housing via the fluid inlet port. In certain embodiments, the method, the SA/IV ratio of the cartridge is greater than 80 cm^{-1} , whereas in certain other embodiments, the SA/IV ratio of the cartridge is no greater than 80 cm^{-1} . The method further comprises introducing a body fluid from a subject into the housing via the fluid inlet port under conditions that permit sequestration of an activated leukocyte and/or
20 an activated platelet on the fluid contacting surface of the solid support.

[0097] **Figure 1B** shows a schematic, cross-sectional representation of another exemplary SCD cartridge **100**. SCD cartridge **100** comprises a housing **110** that defines an inner volume **112**, a fluid inlet port **114**, a fluid contacting inner surface **116**, and a fluid outlet port **118**. The fluid inlet port **114** and the fluid outlet port **118** are disposed on the same side of the housing (i.e., are ipsilateral). In this embodiment, the housing further comprises a solid support **120**
25 defined by the exterior surfaces of a solid substrate, which can be, for example, one or more (a plurality of) solid fibers or one or more (a plurality of) planar supports (for example, a flat membrane). In this **Figure 1B**, which shows a cross-sectional representation of a SCD cartridge, the solid support is defined by three solid fibers or three sheets of a planar support member (for example, a planar membrane). However, it is understood that a plurality of solid
30 fibers or planar support members may together define the solid support. The volume disposed between the fluid contacting inner surface **118** of the housing and the exterior surface of the

- 26 -

solid fiber(s) or the planar support member(s) together define the inner volume (or fill volume) 112. In contrast to the embodiment shown in Figure 1A, the solid fibers or planar support members, because they are not hollow, do not define an ICS. In this embodiment, at least a portion of solid support 120 is disposed within housing 110 between fluid inlet port 114 and fluid exit port 118.

[0098] During operation of this SCD cartridge, the fluid sample of interest is introduced into housing 110 via fluid inlet part 114 into the inner volume (ECS) 112. The fluid then passes along the surface of solid support 120 (along the exterior surface of the solid fibers or planar support, or a combination of one or more solid fibers with one or more planar supports) in a plane substantially parallel to the plane of the solid support 120 and then exits inner volume 112 via fluid exit port 118. During movement of the body fluid along solid support 120, activated leukocytes and/or platelets are sequestered.

[0099] The SCD cartridges shown in Figures 1C and 1D are similar to the SCD cartridge shown in Figure 1B. In Figure 1C, the fluid inlet port 114 and fluid outlet port 118 are located at opposite sides of the housing (i.e., are contralateral). In Figure 1C, housing 110 has a first end and a second end opposite the first end, where fluid inlet port 114 is configured to permit fluid flow through first end and fluid outlet port 118 is configured to permit fluid flow through the second end.

[00100] The SCD cartridge can be configured in any of a variety of ways to sequester cells, for example, leukocytes. As will be discussed in more detail below, the SCD cartridge preferably is designed with a particular subject and indication in mind. For example, the surface area of the solid support should be sufficient to sequester a portion of the activated leukocytes and/or activated platelets to be effective without sequestering too many leukocytes, which potentially can cause life-threatening leukopenia, neutropenia, or too many platelets resulting in thrombocytopenia, or bleeding diathesis. Furthermore, it can be important to choose a housing with an appropriate inner volume depending upon the subject to be treated. For example, in the case of infants, children and severely ill, hemodynamically unstable patients, it is important to choose housings with lower fill volumes so that less body fluid needs to be extracted from the subject in order to contact or bathe the solid support. It is understood that the SCD cartridge can be configured in any of a variety of ways to sequester cells, for example, leukocytes, and to have the appropriate inner volume.

[00101] The solid support can be defined by any number of surfaces, for example, 1, 2, 3, 4, 5, 10, 20, 50, 100, or more different surfaces. Depending upon the subject and the indication to be treated, the surface area of the solid support is greater than about 0.09 m^2 , is greater than about 0.1 m^2 , is greater than about 0.2 m^2 , greater than 0.4 m^2 , greater than 0.6 m^2 , greater than 0.8 m^2 , greater than 1.0 m^2 , greater than 1.5 m^2 , or greater than 2.0 m^2 .

[00102] The surface area of the solid support can be in the range of 0.1 m^2 to 10.0 m^2 , or 0.1 m^2 to 5.0 m^2 . More specifically, the surface area of the solid support can be in the range from 0.1 m^2 to 0.4 m^2 , from 0.4 m^2 to 0.8 m^2 , from 0.8 m^2 to 1.2 m^2 , from 1.2 m^2 to 1.6 m^2 , from 1.6 m^2 to 2.0 m^2 , from 2.0 m^2 to 2.4 m^2 , from 2.4 m^2 to 2.8 m^2 , from 2.8 m^2 to 3.2 m^2 , from 3.2 m^2 to 3.6 m^2 , from 3.6 m^2 to 4.0 m^2 , from 4.0 m^2 to 4.4 m^2 , from 4.4 m^2 to 4.8 m^2 , from 4.8 m^2 to 5.2 m^2 , from 5.2 m^2 to 5.6 m^2 , from 5.6 m^2 to 6.0 m^2 , from 6.0 m^2 to 6.4 m^2 , from 6.4 m^2 to 6.8 m^2 , from 6.8 m^2 to 7.2 m^2 , from 7.2 m^2 to 7.6 m^2 , from 7.6 m^2 to 8.0 m^2 , from 8.0 m^2 to 8.4 m^2 , from 8.4 m^2 to 8.8 m^2 , from 8.8 m^2 to 9.2 m^2 , from 9.2 m^2 to 9.6 m^2 , or from 9.6 m^2 to 10.0 m^2 .

[00103] As a general guiding principle, it is contemplated that when treating subjects having a body weight less than 50 kg the surface area of the solid support preferably should be in the range of the from 0.4 m^2 to 0.8 m^2 , when treating subjects having a body weight greater than 50 kg but less than 100 kg, the surface area of the solid support preferably should be in the range of the from 0.8 m^2 to 1.6 m^2 , and when treating subjects having a body weight greater than 100 kg the surface area of the solid support preferably should be in the range of the from 1.6 m^2 to 5.0 m^2 . It is understood, however, that when therapy is initiated, if the patient shows symptoms of developing leukopenia and/or neutropenia, the SCD cartridge can be replaced with a cartridge with a lower surface area to avoid sequestering too many leukocytes and/or platelets.

[00104] The housing of the cartridge is not limited to a particular set of dimensions (e.g., length, width, weight, or other dimension) in order to achieve a particular fill volume. Depending upon the subject and the indication to be treated, the IV can be less than 300 cm^3 , or less than 150 cm^3 , or less than 100 cm^3 , or less than 80 cm^3 , or less than 60 cm^3 , or less than 40 cm^3 , or less than 20 cm^3 . In certain embodiments, the IV is in the range of from 10 cm^3 to 150 cm^3 , 75 cm^3 to 150 cm^3 , 20 cm^3 to 80 cm^3 , or 15 cm^3 to 120 cm^3 . In the case of infants, children, and severely ill, hemodynamically unstable patients, the inner volume can be less than 40 cm^3 , for example, in the range from 5 cm^3 to 50 cm^3 , from 1 cm^3 to 20 cm^3 or from 5 cm^3 to 30 cm^3 .

- 28 -

[00105] In certain embodiments, the SA/IV ratio is in the range from 25 cm⁻¹ to 2,000 cm⁻¹, 25 cm⁻¹ to 1,750 cm⁻¹, 25 cm⁻¹ to 1,500 cm⁻¹, 25 cm⁻¹ to 1,250 cm⁻¹, 25 cm⁻¹ to 1,000 cm⁻¹, 25 cm⁻¹ to 800 cm⁻¹, 80 cm⁻¹ to 2,000 cm⁻¹, 80 cm⁻¹ to 1,750 cm⁻¹, 80 cm⁻¹ to 1,500 cm⁻¹, 80 cm⁻¹ to 1,250 cm⁻¹, 80 cm⁻¹ to 1,000 cm⁻¹, 80 cm⁻¹ to 800 cm⁻¹, 100 cm⁻¹ to 2,000 cm⁻¹, 100 cm⁻¹ to 2,000 cm⁻¹, 100 cm⁻¹ to 1,750 cm⁻¹, 100 cm⁻¹ to 1,500 cm⁻¹, 100 cm⁻¹ to 1,250 cm⁻¹, 100 cm⁻¹ to 1,000 cm⁻¹, 100 cm⁻¹ to 800 cm⁻¹, from 125 cm⁻¹ to 2,000 cm⁻¹, 125 cm⁻¹ to 1,750 cm⁻¹, 125 cm⁻¹ to 1,500 cm⁻¹, 125 cm⁻¹ to 1,250 cm⁻¹, 125 cm⁻¹ to 1,000 cm⁻¹, or 125 cm⁻¹ to 800 cm⁻¹, 150 cm⁻¹ to 2,000 cm⁻¹, 150 cm⁻¹ to 1,750 cm⁻¹, 150 cm⁻¹ to 1,500 cm⁻¹, 150 cm⁻¹ to 1,250 cm⁻¹, 150 cm⁻¹ to 1,000 cm⁻¹, 150 cm⁻¹ to 800 cm⁻¹, 200 cm⁻¹ to 2,000 cm⁻¹, 200 cm⁻¹ to 1,750 cm⁻¹, 200 cm⁻¹ to 1,500 cm⁻¹, 200 cm⁻¹ to 1,250 cm⁻¹, 200 cm⁻¹ to 1,000 cm⁻¹, 200 cm⁻¹ to 800 cm⁻¹, 200 cm⁻¹ to 600 cm⁻¹, from 300 cm⁻¹ to 2,000 cm⁻¹, from 300 cm⁻¹ to 2,000 cm⁻¹, from 300 cm⁻¹ to 1,750 cm⁻¹, from 300 cm⁻¹ to 1,500 cm⁻¹, from 300 cm⁻¹ to 1,250 cm⁻¹, from 300 cm⁻¹ to 1,000 cm⁻¹, 300 cm⁻¹ to 800 cm⁻¹, from 400 cm⁻¹ to 1,200 cm⁻¹, from 400 cm⁻¹ to 1,000 cm⁻¹, from 400 cm⁻¹ to 800 cm⁻¹, from 500 cm⁻¹ to 1,200 cm⁻¹, from 500 cm⁻¹ to 1000 cm⁻¹, or from 500 cm⁻¹ to 800 cm⁻¹.

[00106] The housing of the cartridge can be fabricated from a variety of materials, but the material that defines that fluid contacting surface in the inner volume should be biocompatible. The SCD cartridge can be constructed from a variety of materials including, metals such as titanium, or stainless steel with or without surface coatings of refractory metals including titanium, tantalum, or niobium; ceramics such as alumina, silica, or zirconia; or polymers, such as polyvinylchloride, polyethylene, or polycarbonate.

[00107] The solid support can be defined by flat surfaces (*e.g.*, sheets), curved surfaces (*e.g.*, hollow tubes, hollow fibers, solid tubes, and solid fibers), patterned surfaces (*e.g.*, z-folded sheets or dimpled surfaces), irregularly-shaped surfaces, or other configurations to sequester cells. It is understood that the solid support can be defined by a variety of materials, which can include, for example, hollow fibers, solid fibers, planar support members (for example, planar membranes) or a combination of two or more of the foregoing (for example, a combination of hollow and solid fibers, a combination of hollow fibers and planar support members, or a combination of solid fibers and planar support members). In certain embodiments, the solid support is substantially parallel to the plane of fluid flow within the SCD cartridge from fluid inlet port 114 to the fluid exit port.

[00108] Depending upon the embodiment, the solid support can comprise a membrane. The term “membrane” refers to a surface capable of receiving a fluid on both sides of the surface, or a fluid on one side and gas on the other side of the surface. A membrane can be porous (*e.g.*, selectively porous or semi-porous) such that it is capable of fluid or gas flow therethrough. It is understood that the term “porous” as used herein to describe a surface or membrane includes generally porous, selectively porous and/or semi-porous surfaces or membranes. Moreover, additional surfaces that can facilitate leukocyte sequestration, such as particle (*e.g.*, bead) surfaces, surfaces of one or more projections into the passageway, or surfaces of one or more membranes exposed to the flowing biological sample.

10 [00109] It is understood that the solid support is not limited to a particular type, kind or size, and may be made of any appropriate material; however, the material should be biocompatible. For example, a surface of the solid support may be any biocompatible polymer comprising one or more of nylon, polyethylene, polyurethane, polyethylene terephthalate (PET),
15 polytetrafluoroethylene (PTFE), CUPROPHAN (a cellulose regenerated by means of the cuprammonium process, available from Enka), HEMOPHAN (a modified CUPROPHAN with improved biocompatibility, available from Enka), CUPRAMMONIUM RAYON (a variety of CUPROPHAN, available from Asahi), BIOMEMBRANE (cuprammonium rayon available from Asahi), saponified cellulose acetate (such as fibers available from Teijin or CD Medical), cellulose acetate (such as fibers available from Toyobo Nipro), cellulose (such as that are
20 regenerated by the modified cuprammonium process or by means of the viscose process, available from Terumo or Textilkombinat (Pirna, GDR) respectively), polyacrylonitrile (PAN), polysulfone, polyethersulfone, polyarylethersulfone, acrylic copolymers (such as acrylonitrile-NA-methallyl-sulfonate copolymer, available from Hospal), polycarbonate copolymer (such as GAMBRONE, a fiber available from Gambro), polymethylmethacrylate copolymers (such as
25 fibers available from Toray), and ethylene vinyl copolymer (such as EVAL, a ethylene-vinyl alcohol copolymer available from Kuraray). Alternatively, a surface may be nylon mesh, cotton mesh, or woven fiber. The surface can have a constant thickness or an irregular thickness. In some embodiments, surfaces may include silicon, for example, silicon nanofabricated membranes (see, *e.g.*, U.S. Patent Publication No. 2004/0124147). In some
30 embodiments, surfaces may include polysulfone fibers. Other suitable biocompatible fibers are known in the art, for example, in Salem and Mujais (1993) DIALYSIS THERAPY 2D ED., Ch. 5: Dialyzers, Eds. Nissensen and Fine, Hanley & Belfus, Inc., Philadelphia, PA.

- 30 -

[00110] Any technique or combination of techniques that facilitate sequestration (for example, binding) of the leukocytes and platelets can be used, including, for example, biological, chemical, mechanical and/or physical techniques. In some embodiments, biological or chemical techniques for sequestration can be used. Such techniques include using tissues, 5 cells, biomolecules (for example, proteins or nucleic acids), or small molecules to sequester leukocytes. In one embodiment, for example, the fluid contacting support of the solid support in the ECS can further comprise a cell adhesion molecule attached thereto to facilitate sequestration.

[00111] For example, when a leukocyte is activated, selectins are produced by the leukocyte. 10 This altered selectin production can facilitate binding between the leukocyte and other leukocytes. In turn, the binding between leukocytes can increase selectin production in the additionally bound leukocytes, yielding exponential binding of leukocytes. Thus, selectins may be useful to enhance sequestration. Proteins, protein complexes, and/or protein components known to bind leukocytes include CD11a, CD11b, CD11c, CD18, CD29, CD34, CD44, 15 CD49d, CD54, podocalyxin, endomucin, glycosaminoglycan cell adhesion molecule-1 (GlyCAM-1), mucosal addressin cell adhesion molecule-1 (MAdCAM-1), E-selectin, L-selectin, P-selectin, cutaneous lymphocyte antigen (CLA), P-selectin glycoprotein ligand 1 (PSGL-1), leukocyte functional antigen-1 (LFA-1), Mac-1, leukocyte surface antigen p150,95, leukocyte integrin CR4, very late antigen-4 (VLA-4), lymphocyte Peyer's patch adhesion 20 molecule-1 (LPAM-1), intracellular adhesion molecule-1 (ICAM-1), intracellular adhesion molecule-2 (ICAM-2), intracellular adhesion molecule-3 (ICAM-3), inactivated C3b (C3bi), fibrinogen, fibronectin, peripheral lymph node addressin (PNAd), endothelial vascular adhesion protein 1 (VAP-1), fractalkine, CCL19, CCL21, CCL25, and CCL27. Other large molecules known to bind leukocytes include hyaluronic acid, glycosaminoglycans (GAGs), and 25 fucosylated oligosaccharides and their precursors. In certain embodiments, small molecules or adherents used to sequester a leukocyte can include, but are not limited to, peptides, such as peptides comprising the amino acid sequence arginine-glycine-aspartic acid (RGD), and molecules comprising sialic acid. Accordingly, any of these materials can be used to enhance sequestration.

[00112] During use, any of these biological or chemical materials may be bound to the fluid 30 contacting surface of the solid support and/or the fluid contacting surface of the cartridge housing to facilitate or enhance sequestration. Alternatively, or in combination, any of these

- 31 -

materials may be used with other additional techniques to facilitate sequestration. For example, materials may be used to bind leukocytes in solution, causing them to agglomerate and to increase their overall size relative to the size of a single leukocyte. The agglomerated leukocytes then can be captured with a membrane having a particular pore size.

5 [00113] It should be understood that the sequestration techniques described herein also can apply to platelets. In the case of platelets, similar biological, chemical, mechanical and/or physical techniques as described above may be used to sequester platelets. In certain embodiments, agents used to sequester platelets include one or more of glycoprotein Iba (GPIba), glycoprotein IIb (GPIIb), glycoprotein IIIa (GPIIIa), CD41, CD61, von Willebrand
10 Factor, β_2 -integrin macrophage antigen-1, selectins such as P-selectin, and a cell-adhesion molecule.

[00114] In addition, sequestration can also be facilitated and/or enhanced by the control of certain mechanical forces that occur within the SCD cartridge. For example, leukocytes may be sequestered on one or more surfaces of (or in) a passageway or passageway region (*e.g.*, the
15 outside of a porous hollow fiber) by utilizing a flow rate and device configuration that minimizes shear force between the leukocytes and the surface(s), allowing the leukocytes to associate with the surface(s). For example, the housing is configured to create a low shear force environment to permit the cells of interest, for example, leukocytes, platelets, etc., to be sequestered on the solid support as body fluid traverses the inner volume.

20 [00115] More specifically, the cartridge is configured to facilitate shear forces between the flowing cells (for example, leukocytes or platelets) and the sequestration surface(s) less than 1000 dynes/cm², less than 500 dynes/cm², less than 100 dynes/cm², less than 80 dynes/cm², less than 60 dynes/cm², less than 40 dynes/cm², less than 20 dynes/cm², less than 10 dynes/cm², or less than 5 dynes/cm² when a biological fluid enters the cartridge housing through fluid inlet
25 port 114 and exits the cartridge housing through the fluid outlet port 118, for example, at a flow rate in the range of 10 mL (cm³)/minute to about 8,000 mL (cm³)/minute or from 50 mL/minute to about 8,000 mL/minute (for example, 1,000 cm³/minute). As a result, the fluid inlet port 114 and the fluid outlet port 118 are dimensioned to permit a flow rate through the housing in a range from 10 mL/minute to 8,000 mL/minute or from 50 mL/minute to 8,000 mL/minute. For
30 example, when treating certain inflammatory disorders, for example, inflammatory responses during cardiopulmonary bypass, it is understood that treating large flow rates can be tolerated,

- 32 -

for example, up to 7000 mL/minute. That said, when treating inflammatory responses associated with other indications, for example, chronic heart failure or acute decompensated heart failure, slower flow rates should be used, for example, less than about 500 mL/minute, from about 100 mL/minute to about 500 mL/minute, and from about 200 mL/minute to about 500 mL/minute. As a result, the inlet port 114 and the outlet port 118 are dimensioned to permit a desired volume of body fluid to pass through the SCD cartridge housing in a given amount of time. It is understood that the fluid inlet port 114 and the fluid outlet port 118 each have an internal diameter of no less than 0.1 cm to 2 cm, or 0.2 cm to 1 cm, or have a cross-sectional surface area of no less than 0.01 cm², no less than 0.1 cm², no less than 0.2 cm², no less than 0.4 cm², no less than 0.6 cm², no less than 0.8 cm², no less than 1.0 cm², no less than 2.0 cm², or no less than 3.0 cm². In certain embodiments, the inlet port, the outlet port, or both the inlet and outlet ports have a cross-sectional surface area of 0.01 cm² to 1 cm². The distance between the fluid inlet or fluid outlet to the nearest end of the housing (distance A), can be such that A divided by the length of the housing is between 0.01 and 0.25. It is also understood that the plane of the inlet and/or outlet port can range from 5 degrees to 90 degrees (*i.e.*, is perpendicular) to the plane defined by the longest dimension (usually the length) of the housing.

[00116] In certain embodiments, the fluid inlet port 114 and the fluid outlet port 118 are both disposed on one side of the housing 116, for example, as shown in Figures 1A and 1B. Alternatively, as shown in Figure 1C, the fluid inlet port 114 and the fluid outlet port 116 can be disposed on opposite sides of the housing 116. Other orientations of the fluid inlet port 114 and the fluid outlet port 116 are also envisioned. For example, if the housing comprises a first end and a second end opposite the first end, the fluid inlet port can be configured to permit fluid flow through the first end and/or the fluid outlet port can be configured to permit fluid flow through the second end. One such orientation is depicted in Figure 1D, in which fluid inlet port 114 permits fluid flow through the left end of housing 116, and fluid outlet port 118 permits the fluid to exit through the right end of housing 116.

[00117] It is understood that the size and shape of the housing of the SCD cartridge may be designed to provide the appropriate fill volume and to minimize turbulence when a fluid is passed through the SCD cartridge. Furthermore, it is understood that the size, shape and composition of the solid support located within the SCD cartridge may be designed to provide

the appropriate surface area and to minimize turbulence when a fluid is passed through the SCD cartridge.

[00118] By way of example, when solid fibers are used to create the solid support in the cartridge, if a cartridge having a total surface area of 1.8 m² to 2.5 m² is desired, the cartridge
5 can be designed to contain about 43,000 fibers when the fiber length is 26 cm and the fiber diameter is 50 μm, or about 22,000 fibers when the fiber length is 26 cm and the fiber diameter is 100 μm, or about 11,000 fibers when the fiber length is 26 cm and the fiber diameter is 200 μm, or about 43,000 fibers when the fiber length is 13 cm and the fiber diameter is 100 μm, or about 22,000 fibers when the fiber length is 13 cm and the fiber diameter is 200 μm.

10 Alternatively, if the cartridge having a total surface area of 3.6 m² to 5.0 m² is desired, the cartridge can be designed to contain about 87,000 fibers when the fiber length is 26 cm and the fiber diameter is 50 μm, or about 43,000 fibers when the fiber length is 26 cm and the fiber diameter is 100 μm, or about 87,000 fibers when the fiber length is 13 cm and the fiber diameter is 100 μm.

15 [00119] In contrast, and by way of example, when planar support members are used to create the solid support, if a cartridge with a total surface area of 1.8 m² to 2.5 m² is desired, the cartridge can contain, for example, a plurality of sheets having an average thickness of 50 μm and an average width of 5 cm (for example, about 115 sheets of a membrane about 12 cm in length, or 63 sheets of membrane about 26 cm in length). In contrast, if a cartridge with a total
20 surface area of 3.6 m² to 5.0 m² is desired, the cartridge can contain about 125 sheets of membrane having an average thickness of 50 μm, an average width of 5 cm, and average length of 26 cm. The sheets may be placed within the cartridge such that, in certain embodiments, the spacing between the sheets is about 50 μm or 100 μm.

[00120] In certain embodiments, the cartridge can be designed such that the solid support
25 (for example, the fibers or planar supports that constitute the solid support) is disposed within the housing at a packing density from 20% to 65%, 20% to 60%, from 30% to 60%, or from 40% to 55%. The packing density should be chosen to minimize the risk of clotting when blood is passed across the solid support disposed within the IV of the housing.

[00121] In certain embodiments, for example, when hollow fibers are used in the SCD
30 cartridge, the SA/IV ratio preferably is at least 80 cm⁻¹ or more. Exemplary SCD cartridges with a SA/IV ratio greater than 80 cm⁻¹ include the F-50, F-60, F-70 and F-80A cartridge,

which are available commercially from Fresenius Medical Care North America, Waltham, MA, U.S.A.) or Renaflo cartridges (PSH series) from Baxter (Deerfield, IL, U.S.A.). These cartridges have been approved by the USFDA for use in acute and chronic hemodialysis. The F-80A cartridge, for example, has a solid support (defined by the exterior surfaces in a bundle of hollow fibers) with a surface area capable of sequestering leukocytes and/or platelets of about 2.5 m², has an inner volume of about 250 mL, and a SA/IV ratio of about 100.

[00122] In certain embodiments, exemplary cartridges can have the features set forth in Table 1.

TABLE 1

Device	ECS SA (m ²)	ECS SA (cm ²)	ECS Fill (cm ³)	SA/V (cm ⁻¹)
	0.98	9800	130	75
2	2.5	25000	250	100
3	1.25	12500	125	100
4	2.5	25000	125	200
5	2.5	25000	109	230
6	2.5	25000	94	267
7	5	50000	93	536
8	5	50000	125	400
9	6.7	67000	125	537
10	10	100000	125	800

10

[00123] In certain embodiments, in particular, for pediatric uses, exemplary cartridges can have the features set forth in Table 2.

- 35 -

TABLE 2

Device	ECS SA (m ²)	ECS SA (cm ²)	ECS Fill (cm ³)	SA/V (cm ⁻¹)
1 - 1.5 cm case; 200μm fibers	0.17	1700	9	185
2 - 1.5 cm case; 100μm fibers	0.35	3500	9	392
3 - 1.5 cm case; 75μm fibers	0.47	4700	9	530
4 - 1.5 cm case; 50μm fibers	0.70	7000	9	784
5 - 2.5 cm case; 200μm fibers	0.49	4900	25	199
6 - 2.5 cm case; 100μm fibers	0.98	9800	25	399
7 - 2.5 cm case; 75μm fibers	1.30	13000	25	526
8 - 2.5 cm case; 50μm fibers	1.96	19600	25	797

[00124] In certain embodiments, a system can achieve sequestration by subjecting the leukocytes, platelets or cells of interest to a series of cartridges, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more cartridges (*e.g.*, hollow fiber cartridges), each comprising one or more sequestration passageways, or passageway regions, so as to increase the length of the region configured to sequester the leukocytes and the residence time of the leukocytes therein. In any of the aforementioned embodiments, the devices are configured to accomplish sequestration of leukocytes in a manner permitting inhibition of release of a pro-inflammatory substance from a leukocyte and/or deactivation of a leukocyte before, during, or after sequestering. Inhibition of release of a pro-inflammatory substance from a leukocyte and/or deactivation of a leukocyte can be achieved both during sequestration and during transport through a passageway, passageway region, or entire system of the present invention.

[00125] In some embodiments, the SCD cartridges or fluid circuits incorporating the SCD cartridges are configured to sequester the leukocytes for any desired amount of time, for example, from 1 to 59 seconds, from 1 to 59 minutes, from 1 to 24 hours, from 1 to 7 days, one or more weeks, one or more months, or one year or more. In some embodiments, the devices are configured to sequester leukocytes for an amount of time sufficient to permit the subsequent inhibition of release of a pro-inflammatory substance from the leukocytes and/or deactivation the leukocytes. In certain embodiments, leukocytes and/or platelets are sequestered within the SCD cartridge for a time (*e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 minutes or at least an hour)

- 36 -

sufficient to deactivate the leukocyte and/or inhibit the release of a pro-inflammatory substance.

5 [00126] It is understood that the fluid contacting surface of the solid support can sequester (for example, bind) activated leukocytes and/or activated platelets during operation. In certain embodiments, the fluid contacting surface can preferentially sequester (for example,
preferentially bind) activates leukocytes and/or platelets relative to unactivated or deactivated leukocytes or platelets.

10 [00127] In certain embodiments, leukocytes from the subject are treated over a period of at least 2 hours, at least 4 hours, at least 6 hours, at least 8 hours, or at least 12 hours. In other embodiments, leukocytes from the subject are treated over a period of 2 to 24 hours, 2 to 12 hours, 4 to 24 hours, or 4 to 12 hours.

15 [00128] It is understood that the SCD cartridge, once fabricated should be sterilized prior to use. Sterility can be achieved through exposure to one or more sterilizing agents, separately or in combination, such as high temperature, high pressure, radiation, or chemical agents such as ethylene oxide, for example. The SCD cartridge preferably is sterilized once it has been
packaged, for example, after it has been hermetically sealed within an appropriate container or packaging (*i.e.*, the cartridge is terminally sterilized). The packaging may comprise plastic, and may be entirely plastic or may comprise a pouch defined by plastic adhered to a planar support, for example, a paper support. The sterilization process preferably achieves a sterility assurance
20 level (SAL) of 10^{-3} or less; *i.e.* the probability of any given unit being nonsterile after the process is no more than 1 in 10^3 . More preferably, the sterilization process achieves an SAL of no more than 10^{-4} , no more than 10^{-5} , or no more than 10^{-6} . Furthermore, it is understood that the cartridge may comprise a cap sealing fluid inlet port 114 and/or a cap sealing fluid exit port
25 118. Caps disposed on the fluid inlet and outlet ports may help preserve the sterility of the inner volume of the cartridge prior to use, and can be removed before the cartridge is connected into a system with a fluid line that facilitates the flow of a body fluid from the subject to the cartridge and a fluid line that facilitates the flow of the body fluid from the cartridge back to the subject.

30 [00129] In certain embodiments, the cartridge comprises a label disposed on (for example, adhered to) an outer surface of the rigid housing. The label may comprise a lot number or bar code for identifying and/or tracking the cartridge.

2. System Configurations

[00130] It is understood that the SCD cartridges can be used in a variety of different fluid circuits depending upon the indication to be treated. See, for example, U.S. Patent No. 8,251,941 and International application WO2012/051595.

5 [00131] In some embodiments, fluid circuits incorporating the SCD cartridge optionally can also perform other blood treatments. For example, fluid circuits optionally can further include additional devices that can filter, oxygenate, warm, or otherwise treat the blood before or after the blood enters the SCD cartridge. Moreover, the SCD cartridge and/or additional devices in a system can include more than one component for treating blood in other or complementary
10 ways, for example, porous filters, oxygen pumps, and/or xenographic or allographic cells (for example, xenographic or allographic renal cells such as renal tubule cells). In certain embodiments, the SCD cartridge is free of such additional components. For example, a SCD cartridge may be free of cells such as xenographic or allographic cells (*e.g.*, xenographic or allographic renal cells). These basic principles are described in more detail below.

15 [00132] The fluid circuits are configured to accomplish selective cytopheresis. In basic form, the system includes a SCD cartridge, a fluid connection for blood to flow from a blood source (for example, a subject, such as a patient) to the SCD cartridge, and a fluid connection for treated blood to flow from the SCD cartridge to a receptacle (for example, back to the subject). The SCD cartridge acts to sequester cells, for example, leukocytes, such as activated
20 leukocytes, and facilitate inhibition of release of a pro-inflammatory substance from the leukocytes and/or deactivate the leukocytes. Sequestration of leukocytes can be achieved using the SCD cartridges described hereinabove. Inhibition of the release of a pro-inflammatory substance from the leukocytes and/or deactivation of the leukocytes can be achieved by any technique described in Section 3 below.

25 [00133] The leukocytes may become activated within the subject as result of a primary patient condition or secondary to other types of medical intervention, for example, during passage through a hemofilter (for example, as described hereinbelow, with reference to **Figures 2C and 2D**). The activated leukocytes then enter a SCD cartridge wherein the activated leukocytes are sequestered. In the case of the circuit in **Figure 2D**, replacement fluid equal to
30 the volume of the ultrafiltrate produced optionally is provided to the subject.

- 38 -

[00134] In other words, in the SCD cartridge, the activated leukocytes from the blood are sequestered, for example, by temporarily adhering to one or more surfaces inside the cartridge. Sequestration of the leukocytes can be achieved by a variety of approaches, for example, by association with molecules in a passageway or passageway region in the cartridge that bind
5 leukocytes, for example, activated leukocytes, or by setting blood flow within the device to provide low shear stress on leukocytes, allowing them to associate with one or more surfaces inside the SCD cartridge. These sequestered leukocytes then are exposed to an agent, for example, citrate, to deactivate the leukocytes or inhibit their release of pro-inflammatory substances. The cartridges can also be used to sequester and deactivate other cell types, such as
10 platelets.

[00135] It is believed that calcium chelators, for example, citrate, lead to a low Ca_i environment in the cartridge thereby inhibiting release of a pro-inflammatory substance from the leukocytes and/or deactivating the leukocytes. Pro-inflammatory substances may include destructive enzymes and/or cytokines from the leukocytes. This inhibition and/or deactivation
15 leads to an amelioration of the inflammatory state of the leukocytes. In this way, the SCD cartridge sequesters leukocytes, for example, neutrophils and monocytes, and inhibits release of a pro-inflammatory substance from the leukocytes and/or deactivates the leukocytes, for example, with citrate and/or a low- Ca_i environment. The sequestration and inhibition and/or deactivation of platelets can be achieved in a similar fashion.

[00136] It has been demonstrated that the addition of a calcium chelator, *e.g.* citrate, to a
20 device of the present invention including a housing containing hollow fibers that sequester leukocytes can improve myocardial function in a subject with myocardial dysfunction subsequent to infiltration by inflammatory immune cells. Accordingly, it is contemplated that the SCD cartridges of the invention can treat a variety of conditions associated with myocardial
25 inflammation, such as chronic heart failure and acute decompensated heart failure by directly treating blood from the subject. After treatment, the blood is returned to the subject.

2.A. Single Device System

[00137] As mentioned, a system can contain a SCD cartridge to accomplish selective
30 cytopheresis and, optionally, other blood treatments without additional treatment devices in the system (see **Figures 2A-2B**). In one embodiment, such a SCD cartridge is shown schematically in **Figure 1A**. During operation, leukocytes and/or platelets are sequestered

- 39 -

within the SCD cartridge, for example, at the external surface of the hollow fibers, and exposed to an agent, for example citrate, capable of inhibiting release of a pro-inflammatory substance from a leukocyte and/or deactivating a leukocyte. The agent can be infused into a line upstream of the fluid inlet 114 or may be infused into the SCD itself via a port. Alternatively, or in addition, the SCD cartridge can be prepared with the agent prior to its use. Flow rates in the ECS are chosen in the ranges described herein such that there is a low shear force (in the ranges described herein) at the surface of the fiber to allow leukocytes to associate therewith. In this way, inhibition and/or deactivation of the leukocytes and/or platelets is achieved or initiated. Then, the blood in the ECS exits the SCD via fluid outlet 118, which enters into an outflow line.

[00138] Figure 2A shows an exemplary SCD cartridge 100 of Figure 1A in an exemplary fluid circuit. Body fluid, for example, blood, from a subject enters a blood line and is moved through that line via a pump 204. On the same blood line, a leukocyte inhibiting agent (*e.g.*, citrate) can be infused at a port 206, optionally with a pump. The blood in the blood line then enters the inlet 114 and exits the SCD cartridge 100 at outlet 118. Blood lines at the inlet 114 and outlet 118, respectively, are attached using blood line connectors with locking mechanisms 256. Leukocytes are shown sequestered in the ECS 112 at the external surface of the solid support 120, which is depicted as a single hollow fiber. A blood outflow line from the outlet 118 returns blood to the subject. Another agent, such as calcium (*e.g.*, calcium chloride or calcium gluconate), can be infused at a port 258 on this blood outflow line to prepare the blood for re-entry into the subject. In certain embodiments, the ICS can contain xenographic or allographic cells, for example, renal tubule cells, cultured in a monolayer on the lining of the ICS 122 of each fiber to further aid in treatment of the blood. However, in other embodiments, the ICS is cell-free. In one embodiment of the circuit of Figure 2A, the lumen 122 of SCD cartridge 100 can be filled with saline.

[00139] The circuit of Figure 2B includes the same components as Figure 2A and operates in the same manner, except that Figure 2B utilizes a SCD cartridge 100 in which ultrafiltrate is produced. The SCD cartridge 100 contains a plurality of porous membranes, which are hollow fibers. The luminal space within the fibers is the ICS 122 and the surrounding space outside the solid support 120 (depicted as hollow fibers) and within the SCD cartridge housing 110 is the ECS 112. Body fluid, for example, blood containing leukocytes enters the inlet 114 and moves into the ECS 112 surrounding the hollow fibers and exits at the outlet 118. Leukocyte

- 40 -

sequestration and inhibition and/or deactivation can be achieved as described above. However, in this SCD, only the ICS inlet is capped with end cap 130. The ICS outlet 128 is not capped. Accordingly, depending on the characteristics of the porous hollow fibers (e.g., permeability and pore size), a portion of the blood in the ECS 112 can pass across the hollow fibers, and into the ICS 112 as ultrafiltrate (UF). A tube can be connected to the ICS outlet 128 for collecting ultrafiltrate (UF), which may be discarded as waste.

[00140] Flow rates and membrane characteristics for the embodiments shown in the circuits of Figures 2A-2B with the SCD of Figure 1A can be as described below. For example, the ECS flow rate may be from about 100 mL/minute to about 500 mL/minute. The flow rate of the ultrafiltrate waste (e.g., for the SCD cartridge shown in Figure 2B) may include, for example, flow rates from about 5 mL/minute to about 50 mL/minute. In the case of the circuit in Figure 2B, replacement fluid equal in volume to the ultrafiltrate waster produced can optionally be added to the subject.

2.B. *Selective Cytopheresis Inhibitory Device as part of a Hemodialysis or Hemofiltration System*

[00141] As mentioned, in some embodiments the SCD cartridge can be part of a system with other devices for treating blood. For example, the SCD cartridge can be a part of a hemofiltration system, a hemodialysis system and/or a hemodiafiltration system that includes one or more filtration cartridges separate from the SCD cartridge within the system. When describing the part of the system that is not the SCD, the term “hemofiltration” can refer to hemodialysis, hemodiafiltration, hemofiltration, and/or hemoconcentration, and “hemofilter” can include a device (e.g., a cartridge) for performing one or more of hemodialysis, hemodiafiltration, hemofiltration, and/or hemoconcentration. The hemofiltration cartridge(s) can be configured to be in parallel or series with a SCD within an extracorporeal blood circuit, and associated blood pumps and tubing can be used to move the blood through the extracorporeal circuit.

[00142] For example, as shown in Figures 2C and 2D, blood flows from a subject through a blood line. The blood is moved through the blood line via a pump 204. A leukocyte inhibiting agent (e.g., citrate) can be infused into the same blood line at a port 206, optionally with a pump before entering a conventional hemofilter 260. The blood then flows through hollow fibers 262 in hemofilter 260. Dialysate is infused into the ECS surrounding the hollow fibers

- 41 -

262 and within the housing of hemofilter 260, and dialysis occurs with solutes being removed as “waste” from the blood across the hemofilter filtration membrane 262 (the hollow fibers) and into the dialysate. The dialysate flows in a counter current fashion relative to the blood, and the dialysate is moved with a dialysate pump 264. Additionally, molecules and fluid from
5 the blood can pass across the hemofilter filtration membrane 262 (the hollow fibers) as ultrafiltrate, depending on the pore size through the membrane.

[00143] The exemplary system of Figure 2C shows a circuit with the SCD cartridge 100 of Figure 1A, in which the ICS inlet and outlet ports have been capped with end caps. Blood exits the hemofilter 260 and enters the SCD cartridge 100 at the inlet 114. The blood then is
10 processed through the SCD cartridge, which sequesters leukocytes on the solid support 120 (depicted as hollow fibers) and inhibits release of a pro-inflammatory substance from a leukocyte and/or deactivates a leukocyte in the manner described for Figures 2A-2B, above. The blood lines into and out of the SCD cartridge 100 are attached using a connection with a locking mechanism 256. The blood then is returned to the subject via a blood outflow line
15 from the outlet 118. Another agent, such as calcium, can be infused at a port 258 on this blood outflow line in order to prepare the blood for re-entry into the subject. In certain embodiments, the intracapillary space (ICS) of the SCD can contain xenographic or allographic cells, for example, renal tubule cells, cultured in a monolayer on the lining of the lumen of each fiber to further aid in treatment of the blood. However, in other embodiments the ICS is cell free. In
20 certain embodiments of the fluid circuit shown Figure 2C, the ICS 122 of the SCD 100 is filled with saline and the end ports of the ICS are capped with end caps 130 and 132.

[00144] The circuit of Figure 2D includes the same components as Figure 2C and operates in the same manner, except that Figure 2D utilizes a SCD cartridge 100 that produces ultrafiltrate (*i.e.*, the ICS outlet port is not capped with end caps). The flow of body fluid (*e.g.*,
25 blood) through the SCD cartridge 100 is described above in the context of Figure 2B. Additionally, SCD cartridge 100 functions as described above, in the context of Figure 2B. As noted above, SCD cartridge 100 has only the ICS inlet 126 capped with end cap 130. The ICS outlet 128 is not capped with an end cap. Accordingly, depending on the characteristics of the porous hollow fibers, a portion of the blood in the ECS 112 can pass across the hollow fibers,
30 and into the ICS as ultrafiltrate (UF). A tube can be connected to the ICS outlet 128 for collecting ultrafiltrate (UF), which may be discarded as waste.

- 42 -

[00145] Without wishing to be bound by theory, it is contemplated that the flow geometry in these embodiments of the SCD system (and those shown in **Figures 2A-2D** and **3A** and **3B**) allows leukocytes to exist in a low shear force environment in the ECS of the SCD cartridge and, therefore, associate with one or more internal surfaces in the SCD cartridge, for example, the hollow fibers. Conversely, in a typical use of a hemofiltration cartridge (for example, the first device **260** in the circuits of **Figures 2C** and **2D**), blood flow through the small diameter lumens of the hollow fibers yields a higher shear force (than that in the SCD) that prevents association of leukocytes with the hollow fibers and sequestration of leukocytes within the device. Accordingly, a hemofiltration device having the conventional flow circuit supporting its operation reversed (*i.e.*, blood flowing outside the hollow fibers rather than inside the hollow fibers) can act as a SCD to sequester potentially damaging and circulating activated leukocytes. These sequestered leukocytes can be treated with a leukocyte inhibiting agent (*e.g.* citrate).

[00146] Further, it is contemplated that the inflammatory response of sequestered leukocytes is inhibited and/or deactivated in the presence of low Ca_i (caused, for example, by citrate) before, during, and/or after sequestration. The low- Ca_i environment may inhibit the inflammatory activity of, or deactivate, the leukocytes.

[00147] In certain embodiments, the circuit of **Figure 2D** can be modified such that the dialysate produced by hemofilter **260** can be introduced into the ICS of SCD cartridge **100** via ICS inlet **126**. Although the ICS can be cell free, it is understood that this system optionally also can include cells within the ICS **122**, for example, renal tubule cells. The rate of the blood flow is chosen to have a sufficiently low shear force (in the ranges described herein) at the surface of the porous, hollow fibers to allow sequestration of leukocytes by association with the fibers, for example at a blood flow rate from about 100 mL/minute to about 500 mL/minute. Alternatively, the blood flow rate through the extracorporeal circuit, through the lumens of the hollow fibers in the hemofilter **260**, and through the ECS **112** of the SCD cartridge **100** can be about 120 mL/minute. The ultrafiltrate can be moved at rates in the ranges described herein, for example, at flow rates less than about 50 mL/minute, from about 5 mL/minute to about 50 mL/minute, and from about 10 mL/minute to about 20 mL/minute. Alternatively, the ultrafiltrate flow rate can be maintained at 15 mL/minute. Optionally, a balanced electrolyte replacement solution (*e.g.*, a solution containing bicarbonate base) can be infused into the bloodline on a 1:1 volume replacement for ultrafiltrate produced. The fluid (*e.g.*, ultrafiltrate)

- 43 -

and blood (or leukocyte-containing fluid) can flow in the same direction or in opposite directions.

[00148] In this and other embodiments, the blood flow configuration through the SCD cartridge is opposite the blood flow configuration through a typical hemofiltration cartridge.

5 That is, blood flows through the interior of the hollow fibers of the hemofiltration cartridge in its intended use versus around the outside of the hollow fibers of the SCD cartridge. This unconventional blood flow configuration through the SCD cartridge allows for a lower shear force within the ECS at the exterior surface of the hollow fiber relative to the higher shear force within the lumen of the hollow fibers of a hemofilter, thus facilitating sequestration of
10 leukocytes in the ECS of the SCD. Conversely, the blood flow through the interior of the hollow fibers of the hemofilter prohibits leukocyte sequestration due to high shear force created by blood flowing through the small diameter lumens of the hollow fibers. For example, the passage of blood within the interior of a hollow fiber of a hemofilter can create a shear force of 1.5×10^7 dynes/cm² whereas blood flow through the ECS of certain embodiments of a SCD
15 creates a shear force of 10 dynes/cm², or about 10^6 less shear force. For comparison, the shear force at a typical arterial wall is 6 to 40 dynes/cm² and the shear force at a typical vein wall is 1-5 dynes/cm². Thus, a capillary wall has a shear stress of less than 5 dynes/cm².

[00149] Accordingly, use of the SCD cartridge uses a sufficiently low shear force at a surface in a region of a passageway configured to sequester leukocytes to be able to associate
20 leukocytes with that surface and sequester leukocytes, such as activated leukocytes in the region. For example, in some embodiments a shear force of less than 1000 dynes/cm², or less than 500 dynes/cm², or less than 100 dynes/cm², or less than 80 dynes/cm², or less than 60 dynes/cm², or less than 40 dynes/cm², or less than 20 dynes/cm², or less than 10 dynes/cm², or less than 5 dynes/cm², is useful at a surface in the passageway region configured to sequester
25 leukocytes. It should be understood that these shear forces may be useful in any of the SCD embodiments described herein. In certain embodiments, having two devices, such as a hemofilter and a SCD, the difference in shear force between blood flowing in the hemofilter and blood flowing in the SCD can be at least 1000 dynes/cm².

[00150] In these and other embodiments, so long as the unconventional flow configuration is
30 followed (*i.e.*, blood flows outside of the hollow fibers, rather than inside the hollow fibers) to yield the requisite shear force, the SCD can be comprised of a conventional (*e.g.*, Model F-

- 44 -

80A, Fresenius Medical Care North America, Waltham, MA, U.S.A.), which is approved by the FDA for use in acute and chronic hemodialysis. Similarly, the extracorporeal perfusion circuit of this or any other embodiment can use standard dialysis arteriovenous blood tubing. The cartridges and blood tubing can be placed in any dialysate delivery pump system (*e.g.*,
5 Fresenius 2008H) that is currently in use for chronic dialysis.

[00151] In one exemplary system, the system includes tubing leading from a subject (a blood line) with a bag of a citrate solution infused into the tubing by an infuser. A first F-40 hemofilter cartridge (Fresenius Medical Care North America, Waltham, MA, U.S.A.) is connected with the blood line at a point after the citrate enters the blood line. Blood in the
10 blood line then flows through the interior of hollow fibers (the ICS) inside the cartridge, from an end port inlet to an end port outlet, and dialysate flows outside these hollow fibers and within the cartridge (the ECS) from one side port to a second side port in a countercurrent manner with respect to the blood flow. A dialysate/ultrafiltrate mixture exiting from the second side port is collected. Substantially no blood cells, platelets, or plasma cross from the ICS to
15 the ECS, and substantially no leukocytes adhere to the interior of the hollow fibers. The hollow fibers are disposed parallel to one another in a bundle, and each fiber has a diameter of approximately 240 micrometers. Furthermore, the pores of the hollow fibers are small enough to prevent passage of albumin, a molecule of about 30 angstroms, through the fibers, and the pores are generally this size across the entire fiber. The filtered blood then continues from the
20 end port outlet, through tubing, to a side port inlet of an F-80A-based cartridge (Fresenius Medical Care North America, Waltham, MA, U.S.A.), which operates as a SCD cartridge. The blood flows through the ECS of the F-80A-based cartridge and exits the cartridge at a side port outlet. Any ultrafiltrate that is produced in the F-80A-based cartridge enters the ICS and exits through an end port. The other end port of the cartridge is capped. Substantially no blood
25 cells, platelets, or plasma cross from the ECS to the ICS, and leukocytes adhere to the exterior of the hollow fibers for some period of time. Blood exiting the F-80A cartridge enters tubing where a calcium solution is infused into the blood using an infuser. Finally, the tubing returns the processed blood to the subject. In certain embodiments, the blood flow rate in the system does not exceed 500 mL/minute, and blood does not displace air in the system at any point.
30 Additionally, the pumping and infusion rates can be manually changed in view of bedside readings of electrolytes and white blood cell counts. An i-STAT[®] handheld monitoring device produces these readings from a small amount of blood withdrawn from the subject.

- 45 -

[00152] It is contemplated that the risk of using such a system is similar to the risk associated with hemodialysis treatment and includes, for example, clotting of the perfusion circuit, air entry into the circuit, catheter or blood tubing kinking or disconnection, and temperature dysregulation. However, dialysis machines and associated dialysis blood perfusion sets have been designed to identify these problems during treatment with alarm systems and to mitigate any clot or air embolism to the subject with clot filters and air bubble traps. These pump systems and blood tubing sets are FDA approved for this treatment indication.

[00153] As mentioned above, infusion of a leukocyte inhibition agent, for example, citrate, can be local to the SCD, regional, or throughout the system. In this or any embodiment, citrate can also be used as an anti-clotting agent, in which case perfusion throughout the system would be useful. Clinical experiences suggest that if clotting occurs within a hemofiltration system, it is initiated in the first dialysis cartridge. Anticoagulation protocols, such as systemic heparin or regional citrate, are currently established and routinely used in clinical hemodialysis.

2.C. *Selective Cytopheresis Inhibitory Device as part of a Cardiopulmonary Bypass System*

[00154] As shown in Figures 3A-3B, a SCD cartridge can be used within a cardiopulmonary bypass (CPB) circuit to treat and/or prevent inflammatory conditions secondary to surgeries (e.g., bypass surgery). Figures 3A and 3B show the SCD cartridge of Figure 1A in exemplary CPB systems. CPB is used to divert blood from both the left and right sides of the heart and lungs. This is achieved by draining blood from the right side of the heart and perfusing the arterial circulation. However, since systemic-to-pulmonary collaterals, systemic-to-systemic collaterals, and surgical site bleeding return blood to the left side of the heart, special drainage mechanisms of the left side of the heart are required during CPB. Optionally, cardioplegia can be delivered through a special pump and tubing mechanism. A standard CPB system has several features that can be broadly classified into three subsystems. The first subsystem is an oxygenating-ventilating subsystem that supplies oxygen and removes carbon dioxide from the blood. The second subsystem is a temperature control system. The third subsystem includes in-line monitors and safety devices.

[00155] As shown in the embodiment of Figure 3A, blood is moved via a venous cannula 300 from a subject into a blood line 310. Blood flows through the blood line 310, passing a recirculation junction 320, which is connected to a SCD outflow line 330. The SCD outflow

- 46 -

line 330 contains blood treated by the SCD device 100. The blood in the blood line 310 mixes with the SCD-treated blood and continues to a venous reservoir 350 and onto an oxygenator 360 where the blood is oxygenated. The oxygenated blood then flows from the oxygenator 360 to a junction 370 with a SCD inflow line 380. Here, where a portion of the blood in the blood line 310 is diverted to the SCD 100 via the SCD inflow line 380 for treatment by the SCD cartridge 100. The flow of blood through the SCD inflow line 380 is controlled by a pump 382. The SCD cartridge 100 is designed to sequester select cells associated with inflammation, for example, leukocytes or platelets. Blood containing leukocytes enters the inlet 114 and moves into the ECS 112 (see in Figure 1A) surrounding the hollow fibers. Leukocytes are sequestered in the device, for example, on the fluid contacting surface of solid support 120 (see in Figure 1A) (*i.e.*, the exterior surface of the hollow fibers). Flow rates at pump 382 can be chosen at ranges described herein such that there is a low shear force (in the ranges described herein) at the surface of the hollow fibers to allow leukocytes to associate therewith. Blood in the ECS 112 (see in Figure 1A) exits the SCD via outlet 118 and enters the SCD outflow line 330. At junction 370, a portion of the blood in the blood line 310 also continues to an arterial filter/bubble trap 390, before being returned to the subject at an arterial cannula 395.

[00156] Although no agents need be added to the blood, in one embodiment, a citrate feed 335 and citrate pump 336 add citrate to the blood in the SCD inflow line 380 and a calcium feed 345 and calcium pump 346 add calcium to the blood in the SCD outflow line 330. Citrate (or another leukocyte inhibiting agent described herein) is added to the blood flowing into the SCD cartridge 100 from the citrate feed 335 to inhibit and/or deactivate cells associated with inflammation, such as leukocytes. Calcium can be added back into the blood to prepare the blood for reentry into the subject.

[00157] The circuit shown in Figure 3B is different from the circuit of Figure 3A in that it does not recirculate blood within the circuit, for example, at a recirculation junction 320 (*see*, Figure 3A). Rather, as shown in Figure 3B, blood is moved via the venous cannula 300 from a subject into the blood line 310, where the blood flows directly to the venous reservoir 350 and onto an oxygenator 360 where the blood is oxygenated. The oxygenated blood then flows from the oxygenator 360 to the junction 370 with the SCD inflow line 380. Here, a portion of the blood in the blood line 310 is diverted to the SCD cartridge 100 via the SCD inflow line 380 for sequestration of leukocytes by the SCD cartridge 100, as described above for Figure 3A. Blood exiting the SCD cartridge 100 enters the SCD outflow line 330 and mixes with

- 47 -

oxygenated blood at junction 386. After blood from the SCD cartridge mixes with blood in the blood line 310 it continues in the blood line 310 to the arterial filter/bubble trap 390, before being returned to the subject at the arterial cannula 395.

[00158] A citrate feed 335 and citrate pump 336 to add citrate to the blood in the SCD inflow line 380 and a calcium feed 345 and calcium pump 346 to add calcium to the blood in the SCD outflow line 330. As described for Figure 3A, citrate or any other leukocyte inhibiting agent is added to the blood from the citrate feed 335 to inhibit and/or deactivate cells associated with inflammation, such as leukocytes. Calcium can be added back into the blood to prepare the blood for reentry into the subject.

2.D. Additional Features of Selective Cytopheresis Inhibitory Devices

[00159] In some embodiments, the SCD cartridges are configured for treating and/or preventing a certain disorder. It is understood, however, that a number of different configurations can be used to treat and/or prevent a particular disorder.

[00160] Moreover, the SCD cartridge can be oriented horizontally or vertically and placed in a temperature controlled environment. The temperature of a SCD cartridge containing cells preferably is maintained at about 37°C to about 38°C throughout the SCD's operation to ensure optimal function of the cells in the SCD cartridge. For example, but without limitation, a warming blanket may be used to keep the SCD cartridge at the appropriate temperature. If other devices are utilized in the system, different temperatures may be needed for optimal performance.

[00161] In some embodiments, the SCD cartridges and/or the fluid circuits incorporating the SCD cartridges are controlled by a processor (e.g., computer software). In such embodiments, a device can be configured to detect changes in activated leukocyte levels within a subject and provide such information to the processor (e.g., information relating to leukocyte levels and/or increased risk for developing an inflammation disorder). In some embodiments, when a certain activated leukocyte level is reached or a subject is deemed at a certain risk for developing an inflammation disorder (e.g., SIRS), the subject's blood is processed through a SCD for purposes of reducing the possibility of developing an inflammation disorder. In some embodiments, the fluid circuit can automatically process the subject's blood through the SCD in response to these measurements. In other embodiments, a health professional is alerted to

- 48 -

the elevated leukocyte level or increased risk within the subject, and the professional initiates the treatment.

[00162] It is contemplated that the cartridges of the present invention can be included with various kits or systems. For example, the kits or systems may include the SCD cartridges of the present invention, leukocyte inhibiting agents (*e.g.*, calcium chelating agents, such as citrate), allographic cells (*e.g.*, renal tubule cells), or other parts. Additionally, the SCD cartridges may be combined with various surgical instruments necessary for implanting the filtration device into a subject.

4. *Inhibition and/or Deactivation of Cells Associated with Inflammation*

[00163] The SCD cartridges are configured, and the methods of the present invention when performed inhibit release of a pro-inflammatory substance from leukocytes and/or deactivate leukocytes, such as activated leukocytes, in a subject's blood such that an inflammatory response within the subject is prevented and/or diminished. Various techniques can be used. For example, in some embodiments, the SCD cartridges and the fluid circuits incorporating one or more of the SCD cartridges can inhibit release of a pro-inflammatory substance from a leukocyte and/or deactivate a leukocyte by exposing the leukocytes (*e.g.*, sequestered activated and/or primed leukocytes) to leukocyte inhibiting agents. A leukocyte inhibiting agent can be bound, covalently or non-covalently, to a fluid contacting surface of the SCD cartridge, for example, a hollow fiber. Additionally or alternatively, a leukocyte inhibiting agent can be infused into the SCD cartridge or a circuit incorporating a SCD cartridge before, during, or after sequestration of the leukocytes, for example, at or near a membrane surface.

[00164] The present invention is not limited to a particular type or kind of leukocyte inhibiting agent. Leukocyte inhibiting agents include, for example, anti-inflammatory biological agents, anti-inflammatory small molecules, anti-inflammatory drugs, anti-inflammatory cells, and anti-inflammatory membranes. In some embodiments, the leukocyte inhibiting agent is any material or compound capable of inhibiting activated leukocyte activity including, but not limited to, non-steroidal anti-inflammatory drugs (NSAIDs), anti-cytokines, imatinib mesylate, sorafenib, sunitinib malate, anti-chemokines, immunosuppressant agents, serine leukocyte inhibitors, nitric oxide, polymorphonuclear leukocyte inhibitor factor, secretory leukocyte inhibitor, and calcium chelating agents. Examples of calcium chelating agents include, but are not limited to, citrate, sodium hexametaphosphate, ethylene diamine

- 49 -

tetra-acetic acid (EDTA), triethylene tetramine, diethylene triamine, o-phenanthroline, oxalic acid and the like. The leukocyte inhibiting agent can be any protein or peptide known to inhibit leukocytes or immune cells including, but not limited to, angiogenin, MARCKS, MANS, Complement Factor D, the disulfide C39-C92 containing tryptic angiogenin fragment

5 LHGGSPWPPC⁹²QYRGLTSPC³⁹K (SEQ ID NO: 1) and synthetic homologs of the same; the agent also can be those proteins, peptides, and homologs reported by Tschesche *et al.* (1994) J. BIOL. CHEM. 269(48): 30274-80, Horl *et al.* (1990) PNAS USA 87: 6353-57, Takashi *et al.* (2006) AM. J. RESPIRAT. CELL AND MOLEC. BIOL. 34: 647-652, and Balke *et al.* (1995) FEBS LETTERS 371: 300-302, that may facilitate inhibition of release of a pro-inflammatory substance

10 from a leukocyte and/or deactivate a leukocyte. Moreover, the leukocyte inhibiting agent can be any nucleic acid known to inhibit release of a pro-inflammatory substance from the leukocyte and/or deactivate the leukocyte. The leukocyte inhibiting agent can be in solution or lyophilized.

[00165] Any amount or concentration of leukocyte inhibiting agent can be used to inhibit the

15 release of pro-inflammatory substances from a leukocyte and/or deactivate the leukocyte. The leukocyte inhibiting agent can be introduced into a passageway, passageway region, device, device region, or system region of a system by any methods known in the art. For example, the leukocyte inhibiting agent can be infused at a port. The amount of leukocyte inhibiting agent infused in a passageway can be sufficient to inhibit release of a pro-inflammatory substance

20 from a leukocyte and/or deactivate a leukocyte sequestered within the same passageway or within an adjacent passageway. In some embodiments, a leukocyte inhibiting agent, for example, citrate, can be infused into the system, a region of the system, or one or more devices within the system, including devices that perform other functions and do not sequester leukocytes. More particularly, the leukocyte inhibiting agent (*e.g.* citrate) can be infused

25 upstream from, into, or downstream from a passageway that sequesters leukocytes. Alternatively, the leukocyte inhibiting agent can be contained in one or more passageways, passageway regions, devices, or system regions within a system. For example, a leukocyte inhibiting agent can be bound to a surface in the passageway configured to sequester leukocytes, or in another passageway, in an amount sufficient to inhibit release of a pro-

30 inflammatory substance from the leukocytes and/or deactivate the leukocytes.

[00166] The inhibition of release of a pro-inflammatory substance from a leukocyte and/or deactivation of a leukocyte can occur temporally before, during, and/or after sequestration of

- 50 -

the leukocyte. Moreover, the leukocyte can remain inhibited or deactivated for a period of time following sequestration. In certain embodiments, a leukocyte can be inhibited or deactivated during the period of time that the leukocyte is exposed to a target concentration of a leukocyte inhibiting agent or is exposed to a target concentration of Ca_i (typically from about 0.20
5 mmol/L to about 0.40 mmol/L) that results from exposure to a leukocyte inhibiting agent such as citrate. The period of time that the leukocyte is exposed to the target concentration of leukocyte inhibiting agent or target concentration of Ca_i can precede, include, and/or follow the period of time that the leukocyte is sequestered. In certain embodiments, the leukocyte can continue to become or remain inhibited or deactivated for a period of time following exposure
10 to the leukocyte inhibiting agent.

[00167] The time of exposure to the leukocyte inhibiting agent can vary depending upon the agent used, the extent of leukocyte activation, the extent of production of pro-inflammatory substances, and/or the degree to which the inflammatory condition has compromised patient health. Exposure can be, for example, from 1 to 59 seconds, from 1 to 59 minutes, from 1 to 24
15 hours, from 1 to 7 days, one or more weeks, one or more months, or one year or more. In certain embodiments, the leukocytes treated (for example, are permitted to be sequestered by the cartridge and/or exposed to the leukocyte inhibiting agent (for example, a calcium chelating agent) over a period of at least 2 hours, at least 4 hours, at least 6 hours, at least 8 hours, or at least 12 hours. In certain embodiments, the leukocytes from the subject are treated over a
20 period of 2 to 24 hours, 2 to 12 hours, 4 to 24 hours, or 4 to 12 hours.

[00168] The leukocyte inhibiting agent can be applied to the system before or during operation the system. In certain embodiments, the leukocyte inhibiting agent is applied during operation of the system and the amount of leukocyte inhibiting agent applied to the system is monitored.

25 [00169] In some embodiments, a leukocyte inhibiting agent can be titrated into the system (e.g., at a port 206 as shown in Figures 2A-2D or from a feed 335 and pump 336 as shown in Figures 3A and 3B). The titration can be adjusted relative to a monitored blood characteristic. For example, citrate can be titrated into the system to keep the Ca_i in the blood at a certain level, for example, at a Ca_i concentration of about 0.2 to about 0.4 mmol/L. Any type of citrate
30 that is biologically compatible can be used, for example, 0.67% trisodium citrate or 0.5% trisodium citrate. See, e.g., Tolwani *et al.* (2006) CLIN. J. AM. SOC. NEPHROL. 1: 79-87. In

- 51 -

some embodiments, a second solution can be added into the system following inhibition of the release of pro-inflammatory substances from a leukocyte and/or deactivation of the leukocyte (e.g., at port 258 as shown in Figures 2A-2D, or from a feed 335 and pump 336 as shown in Figures 3A and 3B), to readjust the blood for reentry into the subject. For example, in
5 embodiments in which a calcium chelating agent is used as the leukocyte inhibiting agent, calcium can be added back into the blood before reentry into the subject.

[00170] In one embodiment, a 1000 mL bag containing a citrate solution, for example ACD-A (Baxter Fenwal, Chicago IL; contents per 100 mL: dextrose 2.45 g, sodium citrate 2.2 g, citric acid 730 mg, pH 4.5 - 5.5 at 25° C) can be attached to an infusion pump and then attached
10 to an arterial line (outflow from subject to devices) of the system (e.g. at port 206; the outflow from a subject in a CPB situation is called a venous line, and infusion occurs from, for example, the feed 335 and pump 336). A negative pressure valve can be employed to facilitate citrate pump function (infusing into a negative pressure area proximal to the blood pump). The initial rate of citrate infusion can be constant, for example, about 1.5 times, in mL/hour, the
15 blood flow rate, in mL/minute (e.g., if the blood flow rate is about 200 mL/minute, then the initial constant rate of citrate infusion may be about 300 mL/hour). In addition, a calcium chloride infusion at a concentration of about 20 mg/mL may be added near the venous port of the system (e.g., port 258 of Figures 2A-2D); the analogous location in the CPB situation is shown as a feed 335 and pump 336 in Figures 3A and 3B). The initial calcium infusion can be
20 set at 10% of the citrate infusion rate (e.g., 30 mL/hour). The Ca_i can be monitored continuously or at various times, for example, every two hours for the first eight hours, then every four hours for the next sixteen hours, then every six to eight hours thereafter. The monitoring can be increased as needed and can be monitored at more than one location in the system, for example, after citrate infusion and after calcium infusion.

25 [00171] Exemplary citrate and calcium chloride titration protocols are shown in Table 3 and in Table 4, respectively. In this embodiment, the target Ca_i range in the SCD is from about 0.20 mmol/L to about 0.40 mmol/L, with the Ca_i target concentration achieved by infusion of citrate (e.g., ACD-A citrate solution). As this is a dynamic process, the rate of citrate infusion may need to be changed to achieve the target Ca_i range in the SCD. The protocol for doing so
30 is shown below, with infusion occurring at the infusion points described above.

TABLE 3

Citrate Infusion Titration Guidelines

Circuit Ionized Ca²⁺ (between the SCD and patient)	Infusion Adjustment with ACD-A citrate solution (as described above)
If circuit ionized Ca ²⁺ is less than 0.20 mmol/L	then decrease the rate of citrate infusion by 5 mL/hour
If circuit ionized Ca ²⁺ is 0.20 - 0.40 mmol/L (Optimal Range)	then make no change to the rate of citrate infusion
If circuit ionized Ca ²⁺ is 0.41 - 0.50 mmol/L	then increase the rate of citrate infusion by 5 mL/hour
If circuit ionized Ca ²⁺ is greater than 0.50 mmol/L	then increase the rate of citrate infusion by 10 mL/hour

TABLE 4

Calcium Infusion Titration Guidelines

5

Patient Ionized Ca²⁺ (drawn systemically from patient)	Ca²⁺ Infusion (20 mg/mL CaCl₂) Adjustment
If patient ionized Ca ²⁺ is greater than 1.45 mmol/L	then decrease the rate of CaCl ₂ infusion by 10 mL/hour
If patient ionized Ca ²⁺ is 1.45 mmol/L (maximum allowable amount)	then decrease the rate of CaCl ₂ infusion by 5 mL/hour
If patient ionized Ca ²⁺ is 0.9 mmol/L (minimum allowable amount)	then increase the rate of CaCl ₂ infusion by 5 mL/hour
If patient ionized Ca ²⁺ is less than 0.9 mmol/L	then administer a 10 mg/kg CaCl ₂ bolus and increase the rate of CaCl ₂ infusion by 10 mL/hour
Default Range (preferred target level)	1.0 – 1.2 mmol/L

[00172] It should be understood that the deactivation techniques described herein also can apply to platelets. In certain embodiments, agents used to deactivate a platelet and/or inhibit release of a pro-inflammatory substance from a platelet include, but are not limited to, agents that inhibit thrombin, antithrombin III, meglatran, herudin, Protein C and Tissue Factor Pathway Inhibitor. In addition, some leukocyte inhibiting agents can act as platelet inhibiting agents. For example, calcium chelating agents, such as citrate, sodium hexametaphosphate, ethylene diamine tetra-acetic acid (EDTA), triethylene tetramine, diethylene triamine, o-

10

- 53 -

phenanthroline, and oxalic acid can deactivate a platelet and/or inhibit release of a pro-inflammatory substance from a platelet.

[00173] In light of the foregoing description, the specific non-limiting examples presented below are for illustrative purposes and not intended to limit the scope of the invention in any way.

EXAMPLES

Example 1. Treatment of Inflammation Associated with Acute Sepsis in an Animal Model

[00174] Activated leukocytes, especially neutrophils, are major contributors to the pathogenesis and progression of sepsis as well as other clinical inflammatory disorders. This example describes *in vivo* experiments that evaluate the effect of different SCD cartridges on leukocyte sequestration and deactivation. The results demonstrate that the choice of a particular SCD cartridge can have a profound effect on the pathogenesis and progression of sepsis in a large animal model. In particular, the results demonstrate that a SCD cartridge having a larger sequestration area is more effective than a SCD cartridge having a smaller sequestration area in alleviating complications associated with sepsis and in prolonging survival.

(I) Methods and Materials

A. - Animal Model

[00175] The efficacy of the SCD cartridge in treating inflammation was evaluated in a well-established porcine model of acute septic shock. (See, *e.g.*, Humes *et al.* (2003) CRIT. CARE MED. 31:2421–2428.)

[00176] Pigs weighing 30–35 kg were utilized. After administration of anesthesia and intubation, the pigs underwent placement of an arterial catheter and a Swan-Ganz thermodilution catheter (which were connected to transducers) to monitor arterial blood pressure, cardiac output, and central venous pressures. An ultrasonic flow probe was placed on a renal artery for continuous assessment of renal blood flow (RBF).

[00177] To induce septic shock, the pigs received 30×10^{10} bacteria/kg body weight of *E. coli* into their peritoneal cavities. To better replicate the human clinical situation, the antibiotic Ceftriaxone (100 mg/kg) was administered 15 minutes after bacteria infusion. During the first

- 54 -

hour following bacteria infusion, all animals were resuscitated with 80 mL/kg of crystalloid and 80 mL/kg of colloid. All treatment groups received identical volume resuscitation protocols. No animal received vasopressor or inotropic agents.

B - Extracorporeal Circuit Containing the SCD Cartridge

5 [00178] Immediately after bacterial administration, the animals were connected to an extracorporeal circuit containing a standard continuous renal replacement therapy (CRRT) hemofilter and a SCD device, as depicted in Figure 4. The hemofilter was a Fresenius F-40 hemofiltration cartridge (Fresenius AG). The SCD cartridge (CytoPhex, Inc.) was connected to the blood port of the hemofilter through its side port using a special blood line connector.

10 Two types of SCD cartridges were tested. The first type of SCD cartridge (based on a Fresenius F-40 hemofiltration cartridge) had a membrane surface area of 1.0 m² facing the extracapillary space, which had an ECS fill volume of 130 mL. The second type of SCD cartridge (based on a Fresenius F-80A hemofiltration cartridge) had a membrane surface area of 2.5 m² facing the extracapillary space, which had an ECS fill volume of 250 mL. The F-40

15 and F-80A SCD cartridges each contained polysulfone hollow fibers with an inner diameter of 200 µm and a wall thickness of 40 µm. The pressure drop across the SCD was 70-75 mmHg. Either the Gambro AK-10 or the Fresenius 2008H dialysis pump system was utilized for these experiments. Extracorporeal blood flow was regulated at 100-150 mL/min.

[00179] A balanced electrolyte replacement solution (Na 150 mEq/L, Cl 115 mEq/L, HCO₃ 38 mEq/L, Ca 2.5 mEq/L, and Mg 1.6 mEq/L in Dextrose 5%) was infused into the blood line on a 1:1 volume replacement basis for the net ultrafiltrate which would exit the circuit. In addition, continuous volume resuscitation with normal saline at 150 mL/h was employed to maintain mean arterial pressure and cardiac output in the treated animals.

[00180] As a control, one group animals (n=3) underwent extracorporeal blood perfusion in a circuit containing the hemofilter alone but without the SCD device. These animals also

25 received regional citrate infusion and were referred to as the conventional citrate (Con-citrate) group. A second group of animals was treated similarly to the SCD group with citrate but without bacterial infusion. These animals were referred to as the non-septic control (NS-control) group.

30

C - Anticoagulation Process

[00181] The anticoagulation process was a critical variable in this series of experiments. One group of animals referred to as the SCD-heparin group (SCD-H, n = 12), received systemic heparinization to maintain patency of the extracorporeal circuit with targeted activated clotting times (ACTs) of 200–300 sec and treated with a SCD cartridge based on the Fresenius F-40 cartridge with a membrane surface area of 1.0 m² facing the extracapillary space. A second group of animals referred to as the SCD-citrate, F-40 group (SCD-C, F-40; n = 13) were treated with SCD cartridges based on the Fresenius F-40, cartridge with a membrane surface area of 1.0 m² facing the extracapillary space received regional citrate anticoagulation (Pinnick, R.V. *et al.*, (1983) N. ENGL. J. MED., 308(5): 258-261; Lohr, J.W. *et al.*, (1989) AM. J. KIDNEY DIS., 13(2):104-107; Tobe, S.W. *et al.* (2003) J. CRIT. CARE, 18(2): 121-129). In addition, a third group of animals also received regional citrate anticoagulation and were treated with SCD cartridges based on the Fresenius F-80A, with a membrane surface area of 2.5 m² facing the extracapillary space (SCD-C, 2.5; n=3). Regional citrate coagulation was achieved by infusing citrate dextrose-A (ACD-A, Baxter) pre-hemofilter at a rate of 2.5–5.0 mM citrate per 1000 mL whole blood. This essentially lowered iCa concentration in the circuit to 0.2–0.5 mmol/L. Calcium chloride was infused into the venous return of the circuit to maintain systemic iCa values of 1.1-1.3 mmol/L. iCa levels were monitored using an iSTAT reader (Abbott Labs).

D - Complete Blood Counts, Serum Chemistries, and Systemic Inflammation Parameters

[00182] Complete blood counts and serum chemistries were measured with a Hemavet automated analyzer (Drew Scientific) and a VET Test automated analyzer (IDEXX), respectively. Serum myeloperoxidase (MPO) activity was measured using a modified o-dianisidine assay containing 4-aminobenzoic acid hydrazide as a potent and specific inhibitor of MPO (Fietz S, *et al.*, (2008) RES. VET. SCI., 84(3):347-353). Cytokine concentrations, including IL-1 β , IL-6, IL-8, IL-10, TNF- α and IFN- γ , were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems.

E - Assessment of Leukocyte Activation

[00183] FITC-conjugated anti-porcine CD11b antibody (SeroTec) was added to pre-chilled peripheral blood. Red blood cells were lysed and the remaining leukocytes were fixed by addition of a FACS lysing solution (Becton-Dickinson). Cells were collected by centrifugation

- 56 -

and resuspended for flow-cytometric analysis. CD11b expression was quantitatively assessed as mean fluorescent intensity (MFI) with an Accuri flow cytometer.

[00184] Peripheral blood mononuclear cells (PBMCs) were isolated from the venous blood. Mononuclear cells were isolated using standard Ficoll-Hypaque gradient technique (Humes *et al.* (2003) CRIT. CARE MED. 31:2421–2428). These cells were then incubated for 24 hours in
5 culture plates containing RPMI-1640 medium supplemented with antibiotics in the absence or the presence of 1 µg/mL of lipopolysaccharide (LPS). The supernatants were collected and cytokine concentrations measured. The ratio of stimulated to unstimulated cytokine concentrations in the supernatants was then calculated.

10 F - Lung Histology and Immunohistochemistry

[00185] Lung samples were harvested post-mortem from septic pigs treated under SCD-citrate or SCD-heparin conditions. Two random sections from each of the 5 lobes of the lungs were processed for cryosections. Frozen lung samples were cut at 5-µm thickness and fixed with 4% paraformaldehyde on ice for 10 minutes. Tissues were stained with hematoxylin and
15 eosin for light microscopic examination, or for CD11b evaluation; nonspecific adsorption was minimized by incubating the section in goat serum in PBS for 1 hour.

[00186] For evaluation of CD11b expression, lung sections were incubated with primary anti-CD11b antibody at recommended dilutions for 1 hour at room temperature. This was followed by incubation with an anti-mouse IgG Alexafluor594 conjugate (1:200 dilution) at
20 room temperature for 30 minutes, and counterstaining the nuclei with DAPI. ImageJ software (Abramoff, M.D. (2004) Biophotonics International, 11(7): 36-42) was used to quantify the percentage of CD11b-positive areas in random 10x images taken with fixed capture settings. Cell number normalization was achieved by determining the percentage of DAPI-positive areas in the same picture. The results were expressed as the ratio of percent CD11b-positive area by
25 percent DAPI-positive area.

G - Cell Elution from SCD Cartridges

[00187] Prior to disconnecting the circuit, blood was returned to the pig by perfusion with replacement fluid. The SCD extracapillary space (ECS) was then continuously flushed with replacement fluid until the perfusate fluid was free of visible blood. After draining off the
30 replacement fluid, the cartridge was either fixed for histologic processing (Humes, H.D. *et al.*,

- 57 -

(2010) BLOOD PURIFICATION, 29:183-190) or exchanged with a stabilization buffer containing a calcium chelating agent. Adherent cells were mechanically removed from the SCD eluent for analysis. To ensure that all cells adherent to the device were eluted, several cartridges were digested after elution with a DNA isolation buffer (SDS and proteinase K). The DNA extracted
5 in this manner, on average, was less than 5 percent of the eluted DNA from the cartridge.

H - Statistical Analysis

[00188] Group comparisons at multiple time points utilized ANOVA with repeated measures. Otherwise, comparisons between groups used Students' T test, paired or unpaired, as appropriate. Statistical significance was defined as $p < 0.05$.

10 *(II) Results and Discussion*

A - Observations of Cardiovascular Parameters

[00189] The porcine model of septic shock was utilized to evaluate the effectiveness of SCD cartridges having different membrane surface areas combined with either systemic heparin or regional citrate anticoagulation. Specifically, one group of animals (SCD-H) was treated with
15 systemic heparin anticoagulation and either an F-40-based SCD or an F-80A-based SCD cartridge. A second group of animals was treated with regional citrate anticoagulation and an F-40-based SCD cartridge (SCD-C, F-40). A third group of animals was treated with regional citrate anticoagulation and an F-80A-based SCD cartridge (SCD-C, F-80A). A fourth group of animals received citrate without a SCD device (con-citrate).

20 [00190] As indicated in **Table 5** and **Figure 5A**, the intraperitoneal administration of bacteria induced a rapid and profound decline in mean arterial pressure (MAP) in all four groups of animals. This decline was progressive and ultimately fatal.

TABLE 5 – Cardiovascular Parameters

Parameter	0	1	2	3	4	5	6	7	8	9	10	11
Cardiac output, L/min												
SCD-Citrate F-40	4.3±0.3	4.9±0.2	4.7±0.2	4.4±0.3	3.7±0.2	2.7±0.3	2.3±0.2	2.1±0.3	1.7±0.1	1.0±0.3	1.1±0.1	1.1±0.1
SCD-Citrate F-80A	3.9±0.3	5.2±0.6	4.8±0.3	4.5±0.4	4.1±0.5	3.7±0.5	3.1±0.2	2.8±0.2	2.4±0.3	2.1±0.4	1.4±0.2	
SCD-Heparin	4.1±0.3	5.2±0.2	4.2±0.3	3.8±0.2	2.6±0.2	1.7±0.2	1.5±0.2	1.3±0.2	1.1			
Con-Citrate	4.5±0.3	4.7±0.5	5.2±1.2	3.6±0.5	3.8±0.5	2.6±0.4	1.5±0.3	1				
Systolic blood pressure, mmHg												
SCD-Citrate F-40	96.9±5.7	99.9±2.2	94.5±3.2	88.9±4.4	80.3±4.1	69.7±6.5	69.5±7.0	68.0±6.5	55.0±8.7	45.8±5.1	53.5±0.5	36.5±8.5
SCD-Citrate F-80A	118.7±29.2	98.7±9.7	65.7±4.4	70.3±4.1	69.0±5.1	67.0±4.6	59.3±4.5	60.7±8.7	61.7±8.1	51.0±4.5	33.3±7.9	
SCD-Heparin	96.6±4.7	104.9±4.8	94.4±6.5	88.0±4.4	76.4±6.3	58.4±4.4	52.4±8.4	41.0±12.1	55			
Con-Citrate	87.3±1.8	103.0±11.4	77.3±4.2	69.0±3.2	74.7±13.7	51.7±4.9	30.0±20.0					
Diastolic blood pressure, mmHg												
SCD-Citrate F-40	60.5±4.6	64.5±2.9	54.0±4.7	45.5±4.4	42.1±4.7	39.7±4.8	39.9±4.8	36.1±3.4	26.3±3.2	26.5±4.7	32.5±4.5	19.5±2.5
SCD-Citrate F-80A	89.3±25.9	70.0±6.1	40.3±6.6	40.0±1.0	39.3±1.2	36.7±1.2	29.0±0.6	30.3±1.8	27.3±1.9	25.0±2.9	17.0±3.5	
SCD-Heparin	61.4±3.3	75.6±4.5	61.7±6.6	48.3±3.4	38.6±3.6	27.6±3.4	26.1±5.1	24.0±7.3	36.5			
Con-Citrate	53.3±2.0	71.7±6.3	50.3±4.5	42.7±1.5	48.3±12.9	31.0±2.1	20.0±10.0					
Mean arterial pressure, mmHg												
SCD-Citrate F-40	72.2±4.8	75.8±2.6	67.2±4.1	59.9±4.2	54.8±3.9	47.1±6.4	49.5±4.5	46.5±3.7	35.7±4.9	34.3±5.3	28.4±10.1	23.3±2.7
SCD-Citrate F-80A	99.1±27.7	79.6±7.3	48.8±5.8	50.1±1.5	49.2±2.5	46.8±2.1	39.1±1.6	40.4±3.9	38.8±3.9	33.7±3.3	22.4±4.9	
SCD-Heparin	72.0±3.3	86.1±4.4	72.6±6.5	60.6±3.1	50.3±4.4	36.5±3.6	34.3±6.3	26.8±8.6	42.7±0.3			
Con-Citrate	64.7±1.7	82.1±8.0	59.3±4.1	51.4±1.1	44.5±0.5	37.9±2.9	23.3±13.3					

TABLE 5 (cont.)

Parameter	0	1	2	3	4	5	6	7	8	9	10	11
Systemic vascular resistance, dyn·s/cm⁵												
SCD-Citrate F-40	1288±119	1119±61	1027±73	994±72	110±64	1414±111	1601±143	1767±204	1701±179	2170±183	2856±722	1776±336
SCD-Citrate F-80A	1881±152	1073±23	710±143	784±59	874±114	926±131	884±59	1028±159	1134±186	1088±87	971	
SCD-Heparin	1371±137	1250±120	1268±110	1290±58	1412±75	1567±140	1552±242	1918±533				
Con-Citrate	1034±111	1149±94	1067±72	976±96	1174±103	1375±343	1274					
Pulmonary vascular resistance, dyn·s/cm⁵												
SCD-Citrate F-40	141±17	180±25	255±33	321±47	393±78	573±118	632±97	859±145	935±131	948±143	1602±242	1067±133
SCD-Citrate F-80A	164±13	228±83	207±86	281±63	317±55	377±55	475±61	543±54	634±49	694±58	552	
SCD-Heparin	268±102	287±51	384±46	525±58	763±76	1293±243	1024±198	1121±291	1504			
Con-Citrate	147±18	122±17	404±177	602±83	525±151	982±248	1199±14					
Pulmonary capillary wedge pressure, mmHg												
SCD-Citrate F-40	7.8±0.7	8.5±0.9	8.3±1.0	7.0±1.1	7.2±1.1	7.2±1.1	5.9±0.9	5.9±0.8	4.9±1.0	6.8±2.1	5.0±2.6	3.5
SCD-Citrate F-80A	8.3±0.9	11.3±2.4	10.7±3.7	7.3±1.2	6.3±0.9	5.7±0.9	6.0±0.6	6.3±0.7	6.3±0.7	6.0±0.6	12.0±5.5	
SCD-Heparin	7.0±0.8	8.5±1.2	7.2±0.8	6.6±0.7	7.3±1.4	6.3±1.0	5.7±1.0	6.8±1.0	5.5			
Con-Citrate	7.7±1.2	10.7±0.9	9.0±1.5	7.3±1.3	6.3±0.3	6.3±0.3	8.5±1.5					
Renal arterial blood flow, mL/min												
SCD-Citrate F-40	197.4±16.9	183.7±12.8	193.4±25.5	173.2±23.4	125.1±18.2	79.9±18.0	69.3±17.9	48.5±14.7	37.1±11.8	37.0±13.9	47.5±12.5	13.5±8.5
SCD-Citrate F-80A	152.0±15.5	141.0±2.3	170.7±31.5	173.3±33.5	153.0±23.9	131.3±26.9	103.0±23.5	83.0±13.1	67.5±8.2	49.7±9.2	30.5±24.5	
SCD-Heparin	207.0±22.8	155.2±15.7	152.0±21.7	148.5±18.8	111.8±21.4	53.4±13.6	37.6±13.8	45.8±20.1	24			
Con-Citrate	200.3±19.5	157.3±38.1	184.3±63.0	183.0±48.3	138.0±17.7	69.0±24.0	19.0±19.0					

TABLE 5 (cont.)

Parameter	0	1	2	3	4	5	6	7	8	9	10	11
Renal vascular resistance, mmHg/min/mL												
SCD-Citrate F-40	0.39±0.03	0.37±0.06	0.37±0.05	0.48±0.07	1.05±0.29	1.37±0.44	2.18±0.63	1.93±0.72	1.05±0.31	0.82±0.37	2.38±1.56	
SCD-Citrate F-80A	0.67±0.27	0.49±0.06	0.25±0.05	0.28±0.07	0.30±0.05	0.35±0.08	0.44±0.09	0.50±0.08	0.59±0.07	1.69±1.14		
SCD-Heparin	0.39±0.08	0.58±0.08	0.55±0.11	0.41±0.04	0.63±0.20	0.77±0.16	1.30±0.37	0.78±0.23	1.64±0.30			
Con-Citrate	0.30±0.02	0.52±0.12	0.33±0.08	0.26±0.05	0.28±0.04	0.67±0.31	0.75					

- 61 -

5 [00191] Cardiac outputs (CO) were also assessed. As depicted in **Figure 5B**, CO was significantly higher ($p < 0.02$) in the SCD-C groups. This increase in CO was not due to differences in left ventricular filling pressures, since pulmonary capillary wedge pressures were similar in all three groups. Rather, the increase in CO in the SCD-C groups was associated with lower levels of systemic vascular resistance (SVR; $p < 0.03$; **Figure 5C**) and pulmonary vascular resistance (PVR; $p < 0.001$; **Figure 5D**). Notably, the SCD-C, F-80A group consistently showed the most improvement in cardiac out and also had lower SVR, PVR, and renal vascular resistance (**Figure 5E**) when compared to the other groups.

10 [00192] As a quantitative measure of the systemic capillary leak induced by bacterial sepsis, changes in hematocrit (HCT) were assessed. As depicted in **Figure 5F**, the SCD-H group had a higher rate of HCT increase, reflective of larger rates of volume loss from the intravascular compartment. In comparison, HCT levels plateaued after 6 hours in the SCD-C groups. Notably, the SCD-C, F-80A group showed the most protection to the bacterially activated systemic capillary leak.

15 [00193] Renal parameters were also assessed. As shown in **Figure 6**, the SCD-C groups exhibited much better renal function than the SCD-H group as reflected in the lower BUN ($p < 0.02$) and serum creatinine levels ($p = 0.007$). Renal blood flow (RBF) was also much better preserved in the SCD-C, F-80A group as compared to the SCD-H group ($p < 0.05$). Furthermore, the SCD-C, F-80A group also exhibited must higher urine output ($p < 0.05$).

20 [00194] The improved cardiovascular and renal parameters observed with the SCD-C groups translated to longer survival time. As shown in **Figure 7**, the citrate-treated animals survived 8.8 ± 0.4 hours compared to 6.4 ± 0.3 hours for the SCD-H animals ($p = 0.0002$). Notably, the SCD-C, F-80A group had the longest survival times (11.5, 10, and 9.5 hours), as shown in **Figure 8**.

25 [00195] Only those animals treated with a combination of the SCD device and citrate exhibited improved cardiovascular parameters and organ function. The Con-citrate group of animals treated with a single hemofilter cartridge with citrate anticoagulation but without the SCD device demonstrated similar cardiovascular parameters as the SCD-H group, with a average survival time of 6.5 ± 0.5 hours. Thus, both the SCD cartridge and the citrate
30 anticoagulation protocol were required to provide a survival advantage. Furthermore, it was

- 62 -

found that the surface area for sequestration can have a profound effect on alleviating complications relating to sepsis and in prolonging survival time post infection.

B - Observations of Leukocyte Sequestration and Activation

[00196] To assess the sequestration of activated leukocytes along the SCD membranes, the SCD cartridges were processed for histologic evaluation at the conclusion of the porcine sepsis study. The light microscopy findings depicted in **Figure 9** clearly showed leukocyte attachment and aggregation along the outer surface of the SCD membranes. To determine the amount and type of adherent leukocytes, the devices were processed and cells eluted off the membrane at the end of the treatment period. The number of white blood cells (WBCs) eluted off the SCD-H and SCD-C, F-40 cartridges were $6.44 \pm 3.4 \times 10^8$ and $1.72 \pm 1.20 \times 10^8$ cells (**Figure 10A**) ($p < 0.05$), respectively, indicating that citrate anticoagulation reduced the number of adherent leukocytes. Furthermore, the distributions of eluted cells were $79 \pm 5\%$ neutrophils and $21 \pm 4\%$ monocytes in the SCD-H group as compared to $55 \pm 4\%$ neutrophils and $30 \pm 5\%$ monocytes in the SCD-C, F-40 group (**Figure 10B**). Surprisingly, an average of $1.88 \pm 1.21 \times 10^7$ cells were eluted off from the cartridges of the SCD-C, F-80A group (**Figure 10A**), which was about ten fold lower than the average number of eluted cells from the SCD-C, F-40 group. Thus, even though the substantially larger membrane surface area of the F-80A might have led to increased retention of leukocytes, the SCD cartridge's efficiency in deactivating leukocytes apparently led to a dramatic reduction in leukocyte retention by the end of the procedure. An average of 8×10^6 cells were eluted from the cartridges of non-septic control animals ($n = 2$), suggesting that most of the cells that were sequestered in the cartridges of the SCD-H and SCD-C groups were activated leukocytes. The SCD-C group had fewer than 2×10^4 cells eluted from lumens of the cartridges with luminal blood perfusion.

[00197] In order to determine whether the SCD cartridge with citrate anticoagulation can influence the activity of neutrophils in the systemic circulation, biomarkers of neutrophil activation were assessed. Activated neutrophils release various enzymes in response to invading microbes or tissue injury. Since the dominant enzyme released from neutrophil granules is myeloperoxidase (MPO) (Klebanoff, S.J., *et al.*, (2005) *LEUKOC. BIOL.* 77(5): 598-625), blood MPO levels reflect the level of neutrophil activation. As depicted in **Figure 11A**, plasma MPO levels in the SCD-C groups were significantly lower compared with the SCD-H group, reflective of a lower level of activated neutrophils. Furthermore, the SCD-C, F-80A

- 63 -

group showed the lowest level of MPO. Systemic circulating neutrophil activation was also assessed by measuring the amount of CD11b expression on circulating neutrophils. CD11b is a membrane protein involved in the adherence of leukocytes to activated endothelium at the site of inflammation (Fan, S.T., *et al.*, (1993) J. IMMUNOL., 150(7): 2972-2980). As depicted in 5 **Figure 11B**, the amount of CD11b expression on circulating neutrophils was dramatically decreased in the SCD-C groups compared to the SCD-H groups ($p = 0.03$), indicating a lower level of neutrophil activation.

[00198] To further assess the immunomodulatory effect of the SCD cartridge and regional citrate coagulation, systemic cytokine levels were evaluated. Serum levels of various cytokines 10 including IL-1 β , IL-6, IL-8, IL-10, TNF- α and IFN- γ were not significantly different between the SCD-H and the SCD-C groups, although the pro-inflammatory cytokines IL-1 β and IL-8 appeared to be slightly higher in the SCD-H group. Since the SCD device also sequesters monocytes, PBMCs were isolated and assessed for cytokine release. Prior to sepsis induction, PBMC release of TNF- α and IL-8 in response to LPS were 2.1 ± 1.8 and 6.5 ± 2.8 pg/10⁶ cells, 15 respectively, in the SCD-H group; in the SCD-C group, the release was 5.1 ± 0.9 and 18.7 ± 8.1 pg/10⁶ cells, respectively. At 6 hours post sepsis, PBMC release of TNF- α and IL-8 in response to LPS was significantly lower in the SCD-C groups as compared to the SCD-H group ($p < 0.05$) (**Figures 12A and 12B**). These results indicated that the overall pro-inflammatory cytokine profile in the septic state was dampened in the SCD-C groups. Again, it appeared that 20 the SCD device having a membrane surface area of 2.5 m² had the greatest immunomodulatory effect.

[00199] Previous studies have reported that the lung was the first organ targeted for activated leukocyte sequestration and infiltration after endotoxemia or sepsis (Welbourn, C.R. *et al.*, (1992), BR. J. SURG., 79(10): 998-1003; Andonegui, G., *et al.*, (2009), J. CLIN. INVEST., 25 119(7): 1921-1930). Thus, we evaluated the effect of the SCD device and citrate anticoagulation on the sequestration of activated leukocytes in lung tissues. As demonstrated in **Figure 13**, a significant decrease in CD11b-labeled cells in the lung was observed in the SCD-C group compared to the SCD-H group. Further, a histomorphometric analysis showed that the ratios of percent CD11b-positive area by percent DAPI-positive area in the SCD-C group and 30 SCD-H group were 0.114 ± 0.21 versus 0.334 ± 0.052 ($p = 0.007$), respectively (**Figure 14**). Together, these results indicated a reduced lung sequestration of activated leukocytes in animals treated with the SCD device and citrate.

- 64 -

[00200] White blood cell (WBC) kinetics may also provide insights into the manner in which the SCD device may influence leukocyte response to infection. To determine the kinetics of the circulating pool of leukocytes in the SCD-H and SCD-C groups, absolute WBC and neutrophil counts were measured (Figure 15). Both the SCD-H and SCD-C, F-40 groups reached a nadir of 1125 ± 240 and 1094 ± 166 neutrophils/mm³ at 3 hours post sepsis induction, respectively. These groups did not reach absolute neutropenia (defined as counts below 500) due to an increase in immature neutrophils from the bone marrow, as determined by manual examination of blood smears, beginning at 3 hours post sepsis induction. Notably, the SCD-C, F-80A, group consistently exhibited a low neutrophil count reaching a nadir of 457 ± 77 at 6 hours. This was due to a markedly diminished release of immature neutrophils from the bone marrow, suggesting that the SCD device with a larger surface area may function to alter the kinetics of bone marrow release of immature neutrophils. The Con-citrate F-40 group had a similar decline and rebound of leukocyte counts as the SCD-H F-40 group, whereas the NS-control animals tended to have neutrophilia, with neutrophil counts rising from approximately 4,000 to 14,000 over the 8-hour evaluation period.

[00201] Under septic conditions, activated neutrophils have an increased lifespan with a delay in apoptosis. The apoptotic potential of the circulating and adherent leukocytes isolated from the SCD-C groups was assessed. As shown in Figure 16, the SCD-C, F-80A group had a higher number of apoptotic circulating neutrophils as compared to the SCD-C, F-40 group, suggesting that this SCD device with the larger membrane surface area decreased the activation state of circulating neutrophils. On the other hand, the SCD-C, F-80A group had fewer apoptotic SCD-cartridge-adherent neutrophils, suggesting that this SCD device selectively sequestered activated neutrophils thus removing them from the circulating pool.

[00202] Together, the above results demonstrated the efficacy of the SCD device combined with citrate in ameliorating cardiovascular instability, reducing renal dysfunction, and improving survival time in a porcine model of septic shock. More importantly, these results demonstrated that a SCD cartridge having larger sequestration area is more effective in alleviating the complications associated with sepsis.

Example 2. In Vitro Studies of Leukocyte Sequestration and Deactivation

[00203] This example describes *in vitro* experiments to evaluate the effect of the SCD device on leukocyte sequestration and activation.

- 65 -

(I) Methods and Materials

A - In Vitro Assessment of Leukocyte Interaction with the Membrane of a SCD Cartridge

[00204] A custom microscopic flow chamber system was set up to enable microscopic analysis of leukocyte interaction with the SCD membrane. The flow chamber consisted of a polycarbonate housing with an inlet and outlet for perfusion. A polysulfone membrane was affixed to the polycarbonate block with a gasket which directed shear flow. The thickness of the gasket (100 μm) along with the length (2 cm) and the width of the channel (1.5 mm) determined the volume of the flow chamber. Microscopic imaging was accomplished through an optical window made up of a cover glass affixed to the bottom of the polycarbonate block. Either isolated blood or purified leukocytes were used for this study.

[00205] Isolated blood was prone to activation from excessive handling. Thus, 5 mL of fresh heparinized porcine blood was minimally manipulated prior to the flow chamber study. Briefly, leukocytes were fluorescently labeled using 50 $\mu\text{g}/\text{mL}$ of Hoechst 33342 dye. Further, the leukocytes were activated by adding 1 $\mu\text{g}/\text{mL}$ lipopolysaccharide (LPS) directly to the blood samples. Similarly, 125 μL of Anticoagulant Citrate Dextrose Solution USP (ACD) Formula A (Baxter) was added to 5 mL of isolated blood and ionized calcium levels were measured prior to microscopic flow analysis with i-stat EG-7+ cartridges. Blood passed through the flow chamber at a rate of 20 $\mu\text{L}/\text{min}$ with calculated shear forces between 1-10 dynes/cm^2 . For each isolated blood sample, sequences were acquired in triplicate.

[00206] Microscopic analysis of cell capture events was accomplished using either a Zeiss Axiovert 200M or Axio-Observer epifluorescence microscope equipped with a microscope stage-top incubator to control environmental temperature and CO_2 content. Fluorescence images were acquired with either a Zeiss MRm3 or an Icc1 camera at a frequency of 1 frame/second for 5 minutes, for analysis of leukocyte/membrane interaction, and at 1 frame/minute for 1 hour sequences, for analysis of long term leukocyte attachment. Frame by frame evaluation of leukocyte rolling, attachment and detachment of leukocytes was carried out to determine the total number and duration of these phenomena. An attachment event was defined as when a leukocyte appeared in the same location for multiple frames within a sequence. Detachment was defined as release events associated with previously defined attached leukocytes. Rolling events were defined by identifying the same leukocyte in multiple

- 66 -

sequence frames within a sequence where the leukocyte was not in same exact location, but in close proximity to the prior location.

B - Assessment of *In Vitro* Leukocyte Activation

[00207] Heparinized human whole blood was added to tubes with or without
5 lipopolysaccharide (LPS) (10 µg/mL) or formyl-Methionyl-Leucyl-Phenylalanine (fMLF, 50 nM). Citrate anticoagulation was achieved by adding citrate dextrose solution (ACD) to the tubes (Damsgaard, C.T., (2009) J. IMMUNOL. METHODS, 340(2): 95-101; Wutzler, S., (2009) J. TRAUMA, 66(5): 1273-1280). The release of IL-6, IL-8, or IL-10 was measured using commercially available ELISA kits from R&D Systems. The release of elastase was measured
10 using a commercially available ELISA kit from Bender MedSystems. The release of lactoferrin was measured using a commercially available ELISA kit from EMD Chemicals. The iCa levels were measured using an I-STAT reader and were confirmed to be ≤ 0.25 mM and 1.25 mM in the citrate treated or nontreated samples, respectively. Samples were incubated for various times at 37 °C and 5% CO₂. CD11b activation was measured using an
15 FITC-conjugated mouse anti-human antibody (AbD Serotech) and evaluated on an Accuri C6 flow cytometer.

(II) Results and Discussion

A - Observation of Leukocyte Parameters

[00208] To assess the interactions of leukocytes and the SCD polysulfone membranes, a
20 customized flow chamber with video microscopy was set up. The addition of citrate lowered blood iCa level from 1.32 ± 0.05 mmol/L to 0.32 ± 0.05 mmol/L. Analysis of leukocyte attachment events confirmed that LPS activation of the leukocytes in the absence of citrate significantly increased leukocyte attachment to polysulfone membranes during shear flow ($p < 0.05$, **Figure 17**). In citrate-treated, low ionized calcium flow chambers, a statistically
25 significant decrease in leukocyte attachment was observed ($p < 0.05$), suggesting that leukocyte adhesion to polysulfone membranes may be ionized calcium dependent. These results were consistent with the *ex vivo* data in the above-described sepsis porcine model, in which citrate-treated membrane cartridges had fewer adherent leukocytes at the end of the studies. In addition, preliminary analysis of 1 hour sequences demonstrated far fewer persistent leukocyte
30 adhesion events for LPS and citrate treated blood compared to blood treated with LPS only. However, there was an observed increase in rolling events for the LPS and citrate treated blood.

This suggested a catch and release phenomena when leukocytes interact with the polysulfone membrane in the presence of citrate.

[00209] Experiments were carried out to assess the effects of citrate-promoted reductions in blood iCa on leukocyte activity. Specifically, an *in vitro* whole blood assay system was utilized (Damsgaard, C.T., (2009) J. IMMUNOL. METHODS, 340(2): 95-101; Wutzler, S., (2009) J. TRAUMA, 66(5): 1273-1280) to assess the effects of lowered blood iCa levels on leukocyte cytokine production (IL-6, IL-8, IL-10) and the release of preformed inflammatory proteins from neutrophil exocytotic vesicles (lactoferrin, elastase). The results are summarized in Table 6.

10 **TABLE 6 – Effect of citrate on leukocyte activation parameters**

Baseline	IL-6 (ng/mL) n = 7	IL-8 (ng/mL) n = 5	IL-10 (ng/mL) n = 4	Lactoferrin (mg/mL) n = 4	Elastase (mg/mL) n = 5	CD11b (MFI x 10 ³) n = 3
Heparin	0.18 ± 0.04	0.0 ± 0	0.11 ± 0.07	8.47 ± 1.54	2.73 ± 0.29	22.55 ± 1.06
Citrate	0.38 ± 0.15	0.59 ± 1.51	0.01 ± 0.01	1.67 ± 0.29*	0.94 ± 0.14§	7.32 ± 0.47§
Stimulated (LPS, MLE)						
Heparin	65.42 ± 19.77	34.18 ± 6.66	3.74 ± 0.94	12.42 ± 1.08	4.52 ± 0.54	53.43 ± 3.12
Citrate	28.99 ± 7.60*	3.45 ± 2.30†	2.06 ± 0.84†	3.43 ± 0.18§	0.91 ± 0.28**	28.72 ± 2.95§

*p < 0.05; †p < 0.02; **p < 0.005; §p < 0.002, as determined with paired t-test between heparin and citrate groups.

[00210] As shown in Table 4, lowering iCa with citrate inhibited the release of cytokines (IL-6, IL-8, IL-10) and neutrophil exocytotic proteins, suggesting that a low iCa environment promoted the deactivation of leukocytes.

Example 3. Use of SCD Device During Cardiopulmonary Bypass Surgery

[00211] Systemic Inflammatory Response Syndrome (SIRS) can occur in association with cardiopulmonary bypass (CPB) surgery, resulting in multiple organ dysfunction (MOD). Activated neutrophils have been implicated as major inciting factors in this process. This example describes *in vitro* and *in vivo* experiments that evaluate the effect of SCD cartridges for use during CPB surgery. The results demonstrate that the usage of SCD cartridges may disrupt the systemic leukocyte response during CPB surgery, leading to improved outcomes for CPB-mediated MOD.

(I) Background

[00212] Leukocytes, especially neutrophils, are major contributors to the pathogenesis and progression of many clinical inflammatory disorders, including systemic inflammatory response syndrome (SIRS), sepsis, ischemia/reperfusion injury, acute respiratory distress syndrome (ARDS) and acute kidney injury (AKI). Cardiac surgical advances have been dependent upon the techniques for cardiopulmonary bypass (CPB). It has been recognized that a systemic inflammatory response occurs in association with CPB, resulting in multiple organ dysfunctions (MOD) following surgery. Multiple insults during CPB have been shown to initiate and extend this inflammatory response, including artificial membrane activation of blood components (membrane oxygenator), surgical trauma, ischemia-reperfusion injury to organs, changes in body temperature, blood activation with cardiotomy suction, and release of endotoxin. These insults promote a complex inflammatory response, which includes leukocyte activation, release of cytokines, complement activation, and free-radical generation. This complex inflammatory process often contributes to the development of acute lung injury, acute kidney injury, bleeding disorders, altered liver function, neurologic dysfunction, and ultimately MOD.

[00213] The mechanisms responsible for MOD following CPB are numerous, interrelated and complex, but growing evidence suggests a critical role in the activation of circulating blood leukocytes, especially neutrophils in the development of ARDS in CPB-induced post-pump syndrome. Sequestered and activated neutrophils migrate into lung tissue, resulting in tissue injury and organ dysfunction. The importance of activated leukocytes and microvascular dysfunction has also been demonstrated to be important in acute kidney injury.

[00214] In this regard, the use of leukocyte depleting filters within an extracorporeal blood circuit during CPB has been developed and evaluated in preclinical animal models and clinical studies. While filters remove leukocytes *in vitro*, they do not appear to consistently deplete leukocyte concentrations *in vivo*. The majority of papers reported no significant reduction in circulating leukocytes, a conclusion similarly drawn by meta-analysis. Acknowledgement of "filter exhaustion," a progressive decrease in leukocyte reduction efficiency during CPB has been repeatedly observed during experimental evaluation.

[00215] The instant invention utilizes a biomimetic membrane called the selective cytopheretic device (SCD) and regional citrate anticoagulation to promote a decrease in

- 69 -

activated leukocytes in animals and patients suffering from acute inflammation. Early pre-clinical and clinical results, suggest that the device ameliorates the MOD effects of SIRS and impacts the mortality rate of multiorgan failure in intensive care unit (ICU) patients. Results described herein demonstrate that the SCD reduces the circulating level of neutrophils and
5 reduces markers of neutrophil activation, both *in vitro* and *in vivo*.

(II) Methods and Materials

A - Selective Cytopheretic Device (SCD)

[00216] The SCD tested was a polycarbonate housing containing porous polysulfone hollow
10 fibers with an inner diameter of 200 μm , a wall thickness of 40 μm , and a molecular weight cutoff of 40 to 50 kDa. Blood flow was directed to the extracapillary space (ECS). The SCDs used had outer membrane surface area (SA) of 2.2 m^2 and 2.6 m^2 , and surface area/inner volume (SA/IV) ratios of 486 cm^{-1} and 508 cm^{-1} , respectively. The SCDs were supplied by CytoPherx, Inc. (Ann Arbor, MI).

B - *In Vitro* Blood Circuit Studies

[00217] *In vitro* blood circuit studies were initiated to compare two leukocyte reducing
membrane systems, the Pall Leukogard LGB (Ann Arbor, MI) and the SCD device in a series of 10 paired studies. Fresh, heparinized bovine blood (5-6L) was collected in a 7L silicone
20 drain bag (B Braun Medical Inc. Bethlehem, PA) with 90,000 IU sodium heparin (Clipper Distributing LLC, Saint Joseph, MO) and divided evenly into two identical drain bags, which served as reservoirs for two separate blood circuits, each to test the respective device. The *in vitro* blood circuits utilized FDA approved Tygon lines (Cole-Parmer, Vernon Hills, IL). The circuits were set up to monitor temperature with type T thermocouples, and pressure
25 measurements with a 4 channel 90XL (Mesa Labs, Lakewood, CO), pre- and post- device during perfusion. Both blood reservoirs were warmed in the same water bath (34.5°C) to insure identical heating behavior, and a handheld IR-pyrometer was employed to measure internal temperatures (approximately 31°C) within each device tested. Peristaltic blood pumps (Fresenius 2008H, Walnut Creek, CA) maintained a constant flow rate of 300 mL/min in both
circuits.

30 [00218] Blood samples were obtained every 15 minutes to measure total white cell, neutrophil, and platelets as previously described, as well as for other assays. For plasma

- 70 -

myeloperoxidase (MPO) and free hemoglobin (Hgb) analysis, blood samples were immediately cooled and centrifuged free of cells. Plasma hemoglobin concentration was chemically determined using a colorimetric assay with 3,3', 5,5', tetramethylbenzidine (TMB), and MPO was measured by ELISA. At the end of the experiment, the circuit was disconnected and
5 normal saline flushed continuously through the extracapillary space (ECS) of the SCD until fluid was free of visible blood, and then the SCD was eluted to quantify adherent cells as previously described. A similar process was also conducted to elute LGB filters.

C - In Vivo Cardiopulmonary Bypass Model

[00219] Wisconsin calves (100-110kg) were premedicated with atropine (0.04 mg/kg), and
10 ketamine (25 mg/kg) administered by intramuscular (IM) injection, and then anesthetized with 5 µg/kg of thiopental. After intubation with an endotracheal tube (Mallinckrodt Company, Mexico City, Mexico), ventilation was established with a volume cycle ventilator. Anesthesia was maintained by continuous infusion of 5 mg/kg/h of thiopental and 20 µg/kg/h of fentanyl. Muscle relaxation was induced with 0.2 mg/kg of pancuronium followed by intermittent
15 reinjections at 0.1 mg/kg. Polyethylene monitoring lines were placed in the external jugular vein and the femoral artery and vein. Median sternotomy was performed. A 16 to 20 mm Transonic perivascular flow probe was placed on the main pulmonary artery, and Millar microtip pressure transducers were placed in the pulmonary artery and left atrium. Prior to initiating cardiopulmonary bypass, baseline pulmonary artery pressure and flow rate and left
20 atrial pressure readings were taken for determination of cardiac output. After systemic heparinization (300 U/kg), an 18F Medtronic DLP arterial cannula was placed in the left carotid artery and a 24F Medtronic DLP single-stage venous cannula was placed in the right atrium.

[00220] The CPB circuit was primed with 1,000 mL of lactated Ringer's solution and 25
25 mEq of NaHCO₃. The circuit consisted of a Sarns roller blood pump, a Medtronic Affinity hollow fiber oxygenator with integral heat exchanger, and a cardiotomy reservoir. A Medtronic Affinity 38-µm filter was placed in the arterial limb to capture particulate debris. The left ventricle was vented using a 12-Ga Medtronic standard aortic root cannula with vent line connected to a Sarns roller pump and the cardiotomy reservoir. Cardiopulmonary bypass
30 was initiated, ventilation was discontinued, and systemic perfusion maintained at 2.4 L/min/m² body surface area. Moderate perfusion hypothermia (32°C rectal temperature) was used, and mean aortic pressure kept at 60-80 mmHg by modification of flow and intravenous

- 71 -

phenylephrine infusion (0-2 $\mu\text{g}/\text{kg}/\text{min}$). The ascending aorta was cross clamped. CPB was maintained for 255 minutes.

[00221] Three groups of animals were evaluated: CPB circuit without SCD, CPB circuit with SCD, and CPB circuit with SCD with citrate/calcium regional perfusion to provide a low ionized calcium (iCa) blood environment only along the SCD circuit. The SCD circuit blood flow was controlled at 200 mL/min with an AK12 blood pump system (Gambro). Citrate/calcium infusion was based upon well developed clinical protocols for citrate regional anticoagulation, as previously described.

[00222] Similar to the in vitro blood circuit studies, for all sample times systemic blood was used to assess CBCs. The SCD or LGB was routinely removed at T = 225 minutes, with a final blood sample taken 15 minutes after removal to evaluate post therapy dynamics. Total manual white cell counts were determined using the Unopette system (BD Biosciences) and manual differentials were determined from blood smears after ethanol fixation and Wright stain (Richard-Allen Scientific). After each study, if a SCD or LGB was used, adherent cells were eluted and quantified as previously described.

D - Statistical Analysis

[00223] Analysis of variance (ANOVA) was conducted for all studies with statistical significance of $p < 0.05$.

(III) Results and Discussion

A - In vitro blood circuit studies

[00224] The temperature of the blood was similar between the SCD and LGB circuits throughout the study, averaging 31.1 ± 0.4 °C and 31.1 ± 0.3 °C, respectively. The pressure profile across the devices were 92.0 ± 49.1 and 29.2 ± 16.2 mmHg for pre- and post- SCD with a pressure drop of 62.9 ± 39.8 , and 98.8 ± 71.5 and 40.1 ± 17.1 pre- and post- LGB, with a pressure drop of 31.3 ± 3.9 mmHg. The variability in pressures was related to differences in the hematocrit of blood in the circuit, which averaged $31.1 \pm 3.9\%$.

[00225] The total white cell counts for the LGB circuits dropped by greater than 50% within the first 15 minutes and remained steady to the end of the experiment. This decline is largely the result of a more than an 80% drop in circulating neutrophils. The SCD circuits showed a substantial, but smaller drop in total white cells and neutrophils during the experiment, with the neutrophil counts declining between 40% and 60%. Differential white blood cell counts from

- 72 -

each device were evaluated. Monocyte and eosinophil concentrations also declined, but due to their low percentages in circulating blood, accurate quantification was challenging. A substantial decline in the number of platelets was observed, with the SCD and LGB in particular, displaying a relative platelet reduction of greater than 80% at 15 minutes. However, in both cases the platelet count rebounds to a level equivalent to approximately 50% of the platelet counts enumerated prior to beginning the experiment.

B - *In vitro* blood circuit device elution

[00226] The total number of cells eluted from LGB and SCD were counted. Twice as many cells were recovered from LGB than the SCD. The percentage of neutrophils, monocytes, and eosinophils in the closed circulation loop that were recovered from each device were calculated. The total number of each leukocyte population recovered from each device was divided by the total number of each leukocyte population present in blood prior to the initiation of each experiment. The Mean \pm SEM for neutrophils, monocytes, and eosinophils are shown for 10 SCD and 10 LGB. Neutrophils outnumbered monocytes roughly 2 to 1, while eosinophils were present at a variable and much smaller number and percentage from both leukocyte filters. More neutrophils and monocytes were eliminated from LGB versus SCD.

[00227] Total cell numbers remaining in the blood at the termination of each experiment were added to the cell numbers eluted from the device and compared with the number of cells present in the blood sample at the beginning of the experiment. The difference in these numbers is reported as the "change of total cell number" and is most likely to indicate the number of cells destroyed during the four hour circulation experiment. Significantly more cells were unaccounted for in the circuits employing the LGB than in the case of the SCD ($P < 0.05$). The data are presented as the mean \pm SEM of 10 paired experiments.

C - *In vitro* blood circuit blood biocompatibility

[00228] Neutrophil released myeloperoxidase (MPO) activity was assayed as the mean \pm SEM for SCD (N=8), and for LGB (N=10) in $\mu\text{g/ml}$. Plasma MPO activity was significantly higher for the LGB relative to the SCD, with a peak at the first sampling time after circuit initiation ($7.45 \pm 3.02 \mu\text{g/mL}$) and continued to be elevated for the remainder of the experiment ($p < 0.05$). SCD circuit MPO values remained below $0.4 \mu\text{g/mL}$ at all times. Free hemoglobin (Hgb) in plasma, a measure of hemolysis is also assessed, as the mean \pm SEM for LGB (N=10) and SCD (N=10) in mg/mL , with a peak at the first sampling time after circuit

- 73 -

initiation (0.06 ± 0.04 mg/mL) and elevated levels throughout. SCD circuit free hemoglobin values remained below 0.005 mg/mL at all times.

D - *In vivo* bovine calf model of CPB

[00229] Systemic white blood cell (WBC) counts are assessed for the CPB *in vivo* bovine studies. In the CPB No SCD control group, WBC increased above the baseline level counts after 90 minutes and peaked with nearly double the baseline WBC. For device treated groups, WBC counts decreased in the first hour of CPB. In the SCD heparin treatment group, following this initial reduction, the WBC gradually increased after 60 minutes, and throughout CPB, with a sharp raise after removing SCD (routinely at $t = 225$ min) for the final measurement 15 minutes thereafter. Similar results were observed when LGB was placed in the circuit rather than the conventional arterial line filter (data not shown). In SCD citrate group, WBCs were low throughout CPB, and even after the SCD was removed.

[00230] Quantification of the neutrophil population during cardiopulmonary bypass (CPB) surgery without a SCD showed an approximate 5-fold rise in the systemic levels. SCD treatment with only systemic heparin coagulation during CPB dramatically reduced the systemic neutrophil concentration during the first 120 min, but was followed by a steady rise until SCD removal (routinely at $t = 225$ min), with a larger increase 15 minutes after SCD removal. SCD with regional citrate during CPB resulted in a systemic neutrophil concentration approximately 75% lower than the pre-SCD level, which persisted throughout CPB, and remained low 15 minutes after SCD removal.

[00231] At the conclusion of SCD therapy, SCD were thoroughly washed and bound leukocytes were eluted and enumerated. On average 8×10^7 and 1.63×10^9 leukocytes were eluted from the SCD employing regional citrate or systemic heparin, respectively. Eluted cells were of the granulocytic lineage independent of the use of regional citrate, on average consisting of approximately 80% neutrophils, 20% monocytes, and variable amounts of eosinophils, typically $< 2\%$, similar to distributions reported in *in vitro* blood circuit studies. Preliminary results from the quantification of immature neutrophils by manual counts demonstrate a trend of low counts for the SCD-Citrate group at the end of 240 minutes of CPB (230, 0 per μL , $n = 2$) whereas SCD-Heparin (1630, 6300, 1390 per μL , $n = 3$), No SCD (160, 2660 per μL , $n = 2$) and LGB (1760, 3880 per μL , $n = 2$) groups all have cases of increased amounts of immature neutrophils.

- 74 -

E - Discussion

[00232] CPB promotes SIRS often resulting in MOD. This inflammatory disorder arises from multifactorial processes, but circulating leukocyte activation is postulated to play a central role. Therapeutic interventions directed toward leukocyte depletion during CPB have been
5 evaluated both in pre-clinical and clinical studies. The results have been inconsistent with regards to reducing circulating leukocyte counts and alleviating progression to MOD.

[00233] An *in vitro* test circuit was developed to assess leukocyte depletion in a circulating heparinized blood circuit between 31°C and 34.5°C and comparable blood flow rates of 300 ml/min. When integrated into the blood circuit, both the LGB and SCD prompted a significant
10 reduction in circulating white blood cell and neutrophil counts with the LGB group having a greater effect to lower WBC counts compared to the SCD. This reduction in leukocyte counts in the LGB group compared to the SCD group was due to both a higher degree of sequestration in the device (eluted cells), and a higher degree of destruction of leukocytes (by mass balance). Destruction of cellular elements within the blood was reflected in the higher free hemoglobin
15 and MPO levels in the LGB versus SCD. Platelet dynamics with over an 80% reduction within the first 15 minutes followed by a recovery to 50% of the pre-study platelet concentration, are suggestive of rapid initial phase of platelet binding to circuit components, followed by subsequent release.

[00234] To further assess the influence of the SCD to lower circulating leukocyte counts, a
20 bovine model utilizing CPB was examined. CPB performed without SCD demonstrated a small, but not statistically significant reduction of WBC counts in the first 60 minutes of CPB perfusion most likely due to non-specific attachment along the artificial membranes and blood tubing of the perfusion circuit. After 60 minutes, the WBC counts increased two-fold, and neutrophils increased up to five-fold relative to starting values. When the SCD was placed in
25 the circuit utilizing systemic heparinization, leukocyte reduction was achieved for 2 hours, but led to a large increase in neutrophils at later time points and following SCD removal. When the SCD perfusion circuit was regionally perfused with citrate to lower ionized calcium to 0.25 to 0.40 mM, leukocyte and neutrophil counts remained low throughout CPB, even after removal of the SCD (routinely at $t = 225$ min) for the final measurement 15 minutes after SCD removal.

[00235] The WBC and neutrophil kinetics in these bovine studies also provide insight into the
30 manner in which SCD treatment may influence the leukocyte response to CPB. The number of

- 75 -

neutrophils sequestered in the SCD was approximately 10^8 cells, a small percentage of the circulating and marginated pool. However, the magnitude of neutrophil release from bone marrow and marginated stores in response to the systemic insult of CPB was blunted with SCD, especially with regional citrate infusion, suggesting that SCD-C treatment may alter the kinetics of neutrophil apoptosis and/or signals required for recruitment of neutrophils from marginated or bone marrow pools.

[00236] Further, the finding that the number of leukocytes eluted from the SCD during citrate infusion was 10-fold less than in the heparin condition, while maintaining lower leukocyte concentration in blood suggests that the low-iCa environment may promote the adhesion of activated leukocytes, followed by release after a time period of sequestration and deactivation. The kinetics of this “catch and release” phenomenon is supported with published and ongoing studies utilizing *in vitro* shear chambers. These *in vitro* and *ex vivo* studies suggest that the SCD devices of the invention may ameliorate the natural progression of SIRS by blunting the systemic leukocyte response leading to improved cardiovascular stability, respiratory performance and renal function. This study demonstrates a preventative therapeutic approach to ameliorate CPB promoted leukocyte response and lessen progression to MOD. The *in vitro* and *ex vivo* data provided herein demonstrates the safety and efficacy of the SCD for CPB applications.

Example 4. Exemplary SCD Cartridge for Use in Treating an Inflammatory Condition in a Subject

[00237] To demonstrate the efficacy of the SCD cartridges of the invention, subjects (for example, porcine animal model or a human subject) with various inflammatory conditions may be treated with a SCD device listed below in Table 7 using the protocols described above to improve cardiovascular and/or renal parameters.

TABLE 7 – Exemplary SCD Cartridges

Device	ECS SA (m ²)	ECS SA (cm ²)	ECS Fill (cm ³)	SA/V (cm ⁻¹)
1	0.98	9800	130	75
2	2.5	25000	250	100
3	1.25	12500	125	100
4	2.5	25000	125	200
5	2.5	25000	109	230
6	2.5	25000	94	267
7	5	50000	93	536
8	5	50000	125	400
9	6.7	67000	125	537
10	10	100000	125	800

[00238] The SCD cartridges of the invention may also be adapted for treating small subjects (for example, pediatric patients) with inflammatory conditions. Table 8 depicts various SCD cartridges that may be useful in such applications.

TABLE 8 – Exemplary SCD Cartridges

Device	ECS SA (m ²)	ECS SA (cm ²)	ECS Fill (cm ³)	SA/V (cm ⁻¹)
1 – 1.5 cm case; 200µm fibers	0.17	1700	9	185
2 - 1.5 cm case; 100µm fibers	0.35	3500	9	392
3 - 1.5 cm case; 75µm fibers	0.47	4700	9	530
4 – 1.5 cm case; 50µm fibers	0.70	7000	9	784
5 - 2.5 cm case; 200µm fibers	0.49	4900	25	199
6 - 2.5 cm case; 100µm fibers	0.98	9800	25	399
7 - 2.5 cm case; 75µm fibers	1.30	13000	25	526
8 - 2.5 cm case; 50µm fibers	1.96	19600	25	797

Example 5. Treatment of Chronic Inflammation Associated with Chronic Heart Failure in an Animal Model

[00239] Chronic heart failure (CHF) is recognized as associated with chronic systemic inflammation, especially monocyte/macrophage activation (Conraads *et al.* (2005) J. HEART LUNG TRANSPLANT. 24(7): 854-59). This example describes *in vivo* experiments that evaluate the effect of SCD cartridges on the chronic inflammatory state associated with CHF. This example further describes experiments that assess the acute and chronic effects of SCD cartridges on the cardiovascular and renal functions in an animal model of CHF. The results demonstrate that the SCD improved cardiovascular parameters and altered the pro-inflammatory phenotype of monocytes.

(I) Methods and Materials

A - Animal Model

[00240] The efficacy of the SCD cartridge in treating chronic inflammation and in improving cardiorenal functions was evaluated in a canine model of CHF.

[00241] CHF in this model is induced by multiple sequential intracoronary embolizations with polystyrene Latex microspheres (approximately 90 μm in diameter) that lead to loss of viable myocardium. The model manifests many of the sequelae of CHF in humans including profound systolic and increased systemic vascular resistance (SVR) and decreased cardiac output (CO) (Sabbah *et al.* (1991) AM. J. PHYSIOL. 260: H1379-84). The model also possesses the nearly entire spectrum of cellular, biochemical and molecular abnormalities that have been shown to occur during the development of CHF (See *e.g.*, Kono *et al.* (1992) CIRCULATION 86(4): 1317-22; Imai *et al.* (2007) J. AM. COLL. CARDIOL. 49(21): 2120-28; Morita *et al.* (2006) AM. J. PHYSIOL. HEART CIRC. PHYSIOL. 290(6): H2522-7). Further, long-term therapy with ACE inhibition, beta-adrenergic blockade, aldosterone blockade and angiotensin-I receptor blockade in this model elicits benefits that are identical to those reported in human patients with CHF (Morita *et al.* (2002) CARDIOVASC. DRUGS THER. 16(5): 443-9; Sabbah *et al.* (1994) Circulation 89(6): 2852-9; Suzuki *et al.* (2003) BR. J. PHARMACOL. 138(2): 301-9; Suzuki *et al.* (2002) Circulation 106(23): 2967-72). Accordingly, this model provides the ability to predict efficacy of new therapies for treatment of CHF.

- 78 -

[00242] Three groups of animals with advanced CHF were utilized for this study: one group was treated with the SCD cartridge and systemic heparin anticoagulation to maintain patency of the extracorporeal circuit (SCD-H; n=2); a second group was treated with the SCD cartridge and regional citrate anticoagulation (SCD-C; n=3), which provided patency and the additional therapeutic benefit associated with low iCa environment within the extracorporeal circuit; and a third group was treated with a cartridge without any hollow fibers (sham control, n=3).

[00243] In all studies, extracorporeal circuits (*see* Figure 18) were maintained for 4 hours, and then discontinued with the removal of the circuit and its blood volume for 2 hours. Hemodynamic and ventricular function parameters were measured at baseline and at 2, 4, and 6 hours after initiation of SCD (heparin or citrate) therapy or with sham control. Blood samples were obtained at baseline and at 2, 4 and 6 hours for the assessment of various biologic parameters.

(II) Results and Discussion

A - Observations of Cardiovascular Parameters

[00244] The canine model of chronic heart failure was utilized to evaluate the effectiveness of SCD cartridges having with either systemic heparin or regional citrate anticoagulation. Specifically, one group of animals (SCD-H) was treated with systemic heparin anticoagulation, and a second group of animals (SCD-C) was treated with regional citrate anticoagulation.

[00245] As depicted in Figure 19A, Left ventricular (LV) ejection fraction (EF) increased in the SCD-C group within 5 minutes of starting treatment. Further, LV EF of increased substantially in the SCD-C group from $34 \% \pm 2.3 \%$ to near normal values of $48 \% \pm 3.7 \%$ while the SCD-H and sham control did not change. In particular, the SCD-C group increased to near normal EF values at 2 and 4 hours of treatment and was sustained during the 2 hour post therapy. This effect was not due to a decline in systemic vascular resistance which was similar in all groups. Stroke volume (SV) also increased within 5 minutes of starting treatment and increased from 26.7 ± 4.9 to 35.3 ± 7.3 mL in the SCD-C group (data not shown). The SCD-H group showed a decline in SV of from 26 ± 1.4 to 25 ± 2.1 mL after 4 hours of treatment to 20 mL following 2 hours post treatment.

[00246] Cardiac output (CO) was also assessed (Figure 19B). CO increased within 4 hours of SCD-C treatment from 2.40 ± 0.15 to 2.77 ± 0.95 L/min, and this elevation was maintained

- 79 -

for 2 hours post-treatment. In comparison, SCD-H treatment resulted in a decline in CO from 2.22 ± 0.5 to 1.97 ± 0.03 L/min within four hours of treatment, and further reduced to 1.56 L/min during the 2 hour post-treatment period.

5 [00247] Systemic vascular resistance (SVR) showed a modest decline in both the SCD-C and SCD-H groups with baseline values at 2985 ± 215 and 2898 ± 62 to 2415 ± 847 and 2599 ± 76 dynes/sec/cm⁻⁵ at 4 hours of therapy, respectively (Figure 19C). At 2 hours post-treatment, SVR in the SCD-C group returned to baseline levels, while the SVR in the SCD-H group was slightly elevated compared to baseline. No episodes of arrhythmias or hypotension were observed during the treatment period.

10 [00248] Ventriculograms demonstrated the SCD-C to convert viable but non-contracting myocardium into contracting myocardium. Exemplary ventriculograms are shown in Figure 21 in a dog model before (Figure 21A) and after (Figure 21B) treatment. The red line depicts the border of the left ventricular diastolic silhouettes (most relaxed state during filling) overlaid on the left ventricular systolic image (most contracted state) demonstrating
15 significantly improved contractility of the left ventricle (black arrows), especially at the apex of the left ventricle, after therapy (Figure 21B versus Figure 21A). The results are consistent with the results of increased cardiac output following SCD-citrate therapy.

[00249] The renal effects were also substantive. Urine volume increased immediately within the first hour of SCD-C treatment and continued to be higher than SCD-H treatment for the
20 entire 4 hours of treatment (see, Figure 20A). The fractional excretion (FE) of sodium nearly doubled in the SCD-C compared to SCD-H increasing from $2.2 \pm 0.8\%$ to $5.3\% \pm 0.8\%$ (see, Figure 20B) and FE of urea went from $59\% \pm 3.1\%$ to $81\% \pm 11.3\%$ (see, Figure 20C). No adverse events of arrhythmia or hypotension were observed during treatment. Total urine sodium excretion (see, Figure 20D) was also increased during the 4 hours of SCD-C treatment
25 compared to SCD-H.

[00250] Collectively, these data indicate that SCD-C treatment significantly improved cardiac contractility and function.

B - Observations of Leukocyte Sequestration and Activation

30 [00251] To evaluate the effect of SCD influence on the activation process of circulating monocytes, a variety of biomarkers were measured in isolated peripheral blood monocytes after

LPS stimulation at various times during the treatment periods with established methods (Simms *et al.* (1999) AM. J. PHYSIOL. 277: H253-60). As indicated in **Table 9**, SCD-C treatment resulted in a decline in LPS stimulated monocyte release of IL-6 and TNF- α , demonstrating an immunomodulatory effect of SCD-C treatment on the systemic pool of circulating monocytes.

5 **TABLE 9 -- LPS stimulated Cytokine Release by Isolated Monocytes (MNC)**

Timeline	Baseline	2 hour	4 hour	Washout (2 hour)
IL6 (ng/10 ⁶ MNC/24hr)	4.56± 0.91	3.37 ± 1.31	2.10 ± 0.30	1.92 ± 0.67
TNF α (ng/10 ⁶ MNC/24hr)	6.53± 0.53	2.88 ± 0.27	4.08 ± 1.82	1.61 ± 0.17

[00252] To assess the sequestration of activated leukocytes along the SCD membranes, the SCD cartridges were processed at the end of the treatment period and the types of adherent leukocytes were determined using established methods (Ding *et al.* (2011) PLOS ONE 6(4): e18584). The number of eluted cells in the SCD-C and SCD-H groups were 1.06 x 10⁹ and 7.2 x 10⁹ leukocytes, respectively. The types of leukocytes were 68% and 80% neutrophils, 28% and 16% monocytes, and 4% and 4% eosinophils in the SCD-C and SCD-H groups, respectively. Of note, the ratio of eluted monocytes to neutrophils was four times greater than the baseline ratio of circulating monocytes to neutrophils. Specifically, the number of monocytes eluted from the SCD membrane after 4 hours of SCD-C therapy was 90% of the baseline absolute number of circulating monocytes. These results indicate that replacement of the circulating systemic monocytes may have occurred from the mobilization of a non-circulating monocyte pool, most likely the spleen (Swirski *et al.* (2009) SCIENCE 325(5940): 612-6). The results also suggest that SCD-C treatment affects the circulating pool of leukocytes and alters the pro-inflammatory phenotype of monocytes.

[00253] The change in inflammatory parameters was associated with dramatic increases in EF and CO in SCD-C treated CHF animals compared to SCD-H controls. Collectively these data suggest that SCD-C treatment reduces the cardiodepressant state of chronic inflammation associated with CHF.

Example 6. Case Study of Subject with Acute Decompensated Heart Failure After Undergoing SCD-Citrate Therapy

[00254] A 45 year old male patient presented with acute decompensated heart failure. In particular, the patient presented with longstanding systolic heart failure (ejection fraction of about 20%) after gaining 18 pounds of weight in two weeks. The patient had a history of diabetes, sleep apnea, chronic kidney disease, atrial fibrillation and implantable cardiac defibrillator (ICD) placement. The subject has increasing shortness of breath being unable to walk 10 yards and increasing lower extremity edema.

[00255] The patient was treated with intravenous dobutamine infusion and a Lasix (furosemide, a high potency diuretic) drip with persistent oliguria. The patient’s blood urea nitrogen (BUN) value and serum creatine (Scr) value were 64 and 3.38 (baseline Scr 1.5), respectively. A transoesophageal echocardiogram (TEE) was performed, which showed that the subject had a pulmonary capillary wedge pressure (PCWP) of 30 (normally less than about 15), a cardiac index (CI, a measure of cardiac output per weight of an individual) of 1.4 (normally in the range of 2.5-3.0), a cardiac output (C.O.) of 3 L/minute on Milrinone (normally greater than 5 L/minute), and an ejection fraction (EF) of 10%. The patient was started on therapy with continuous venovenous hemofiltration (CVVH) with SCD-citrate for about 5 days with the SCD cartridge being changed once every 24 hours. The fluid output was measured on each of the five days of SCD therapy and then for three follow-up days post SCD therapy, and the results are summarized in Table 10.

TABLE 10

	SCD Treatment Day						Follow Up Day		
	0	1	2	3	4	5	1	2	3
Urine Output (mL)	575	1,000	925	1,020	1,230	540	135	390	500
Net Fluid Balance (mL)	Not available	-3,071	+269	-5,837	-4,449	-1,466	1,994	1,809	

[00256] Net fluid balance represents the sum of urine volume and the volume of ultrafiltrate removed by the CVVH minus the fluid (e.g., saline) added back to the patient. The results show that on day 1 of the treatment the net fluid balance was -3,071 mL, which peaked at about

- 82 -

-5,837 mL on day 3. On day 2, the subject was partially rehydrated before removing additional fluid. During the five days of therapy on the SCD with regional citrate, the net fluid balance decreased by 14.5 L. During the follow-up days the patient was rehydrated. These findings demonstrate that the SCD device with regional citrate was able to remove fluid from the subject
5 that was not possible without the SCD cartridge. The SCD therapy improved the cardiovascular performance of the patient resulting in fluid removal not readily attained with current therapy.

INCORPORATION BY REFERENCE

[00257] The entire disclosure of each of the publications and patent documents referred to herein is incorporated by reference in its entirety for all purposes to the same extent as if each
10 individual publication or patent document were so individually denoted.

EQUIVALENTS

[00258] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing
15 description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

[00259] What is claimed is:

- 1 1. A method of increasing myocardial function in a subject with chronic heart failure, the
2 method comprising:
- 3 (a) extracorporeally sequestering activated leukocytes and/or activated platelets present
4 in a body fluid of the subject in a cartridge comprising
- 5 (i) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid
6 outlet port, wherein the inner volume is in fluid flow communication with the fluid inlet
7 port and the fluid outlet port, and
- 8 (ii) a solid support disposed within the housing and defining a fluid contacting
9 surface with a surface area (SA) capable of sequestering activated leukocytes and/or
10 activated platelets, if present in a body fluid entering the housing via the fluid inlet port,
11 and the body fluid is introduced into the housing via the fluid inlet port under conditions
12 that permit sequestration of the activated leukocytes and/or activated platelets on the
13 fluid contacting surface of the solid support; and
- 14 (b) treating the sequestered leukocytes and/or platelets to inhibit release of a pro-
15 inflammatory substance or to deactivate the leukocytes and/or platelets thereby to increase
16 myocardial function of the subject when compared to the myocardial function of the subject
17 prior to treatment, wherein the myocardial function is selected from the group consisting of left
18 ventricular ejection fraction, cardiac output, systemic vascular resistance, left ventricular stroke
19 volume, aortic pressure, left ventricular pressure, peak rate of change of left ventricular
20 pressure during isovolumic contraction and relaxation, left ventricular end-diastolic pressure,
21 myocardial oxygen consumption, and coronary flow reserve.
- 1 2. The method of claim 1, wherein the SA/IV ratio of the cartridge provided in step (a) is
2 greater than 25 cm^{-1} .
- 1 3. The method of claim 1 or 2, wherein the SA/IV ratio of the cartridge provided in step
2 (a) is in the range of 25 cm^{-1} to $2,000 \text{ cm}^{-1}$.
- 1 4. The method of claim 1 or 2, wherein the SA/IV ratio of the cartridge provided in step
2 (a) is no greater than 80 cm^{-1} .
- 1 5. The method of any one of claims 1-4, wherein the solid support is disposed within the
2 housing at a packing density in the range from 20% to 65%.

- 84 -

- 1 6. The method of any one of claims 1-5, wherein the solid support comprises a membrane.
- 1 7. The method of any one of claims 1-6, wherein the solid support comprises a planar
2 support member.
- 1 8. The method of any one of claims 1-7, wherein the solid support comprises a fiber.
- 1 9. The method of any one of claims 1-8, wherein the SA of the cartridge provided in step
2 (a) is in the range of from 0.1 m^2 to 10.0 m^2 .
- 1 10. The method of claim 9, wherein the SA of the cartridge provided in step (a) is in the
2 range of from 0.1 m^2 to 5.0 m^2 .
- 1 11. The method of any one of claims 1-10, wherein the inner volume of the cartridge
2 provided in step (a) is less than 300 cm^3 .
- 1 12. The method of claim 11, wherein the inner volume of the cartridge provided in step (a)
2 is less than 150 cm^3 .
- 1 13. The method of any one of claims 1-12, wherein the inner volume is in the range of from
2 10 cm^3 to 150 cm^3 .
- 1 14. The method of claim 13, wherein the inner volume is in the range of from 75 cm^3 to 150
2 cm^3 .
- 1 15. The method of claim 14, wherein the inner volume is in the range of from 15 cm^3 to 120
2 cm^3 .
- 1 16. The method of any one of claims 1-15, further comprising permitting the body fluid to
2 exit the cartridge via the fluid outlet port at a flow rate in the range of $10 \text{ cm}^3/\text{minute}$ to $8,000$
3 $\text{cm}^3/\text{minute}$.
- 1 17. The method of any one of claims 1-16, wherein the solid support is substantially
2 parallel to the direction of fluid flow within the cartridge.
- 1 18. The method of any one of claims 1-17, wherein, in step (b), the leukocytes and/or
2 platelets are treated with an immunosuppressant agent, a serine leukocyte inhibitor, nitric
3 oxide, a polymorphonuclear leukocyte inhibitor factor, a secretory leukocyte inhibitor, or a
4 calcium chelating agent, wherein the calcium chelating agent is one or more of the group
5 consisting of citrate, sodium hexametaphosphate, ethylene diamine tetra-acetic acid (EDTA),
6 triethylene tetramine, diethylene triamine, o-phenanthroline, and oxalic acid.

- 85 -

- 1 19. The method of any one of claims 1-18, wherein, in step (b), the leukocytes and/or
2 platelets are treated with a calcium chelating agent.
- 1 20. The method of claim 19, wherein the calcium chelating agent is citrate.
- 1 21. The method of claim 19, wherein the calcium chelating agent is introduced into the
2 body fluid of the subject prior to step (a).
- 1 22. The method of any one of claims 1-21, wherein the leukocytes and/or platelets are
2 treated over a period of at least 2 hours, at least 4 hours, at least 6 hours, at least 8 hours, or at
3 least 12 hours.
- 1 23. The method of any one of claims 1-22, wherein the leukocytes and/or platelets from the
2 subject are treated over a period of 2 to 48 hours, 2 to 24 hours, 2 to 12 hours, 4 to 48 hours, 4
3 to 24 hours, or 4 to 12 hours.
- 1 24. The method of any one of claims 1-23, wherein the subject has myocardial dysfunction
2 secondary to inflammatory cell penetration of heart tissue.
- 1 25. The method of any one of claims 1-24, wherein the subject has received a heart
2 transplant.
- 1 26. The method of any one of claims 1-25, wherein the body fluid is blood.
- 1 27. The method of any one of claims 1-26, wherein the increased myocardial function is
2 maintained for at least 6 hours after termination of the treatment in step (b).
- 1 28. The method of claim 27, wherein the increased myocardial function is maintained for at
2 least 24 hours after termination of the treatment in step (b).
- 1 29. A method of increasing myocardial function in a subject with chronic heart failure, the
2 method comprising:
- 3 (a) extracorporeally sequestering activated leukocytes and/or activated platelets present
4 in a body fluid of the subject; and
- 5 (b) treating the sequestered leukocytes and/or platelets to inhibit release of a pro-
6 inflammatory substance or to deactivate the leukocytes and/or platelets thereby to increase
7 myocardial function of the subject when compared to the myocardial function of the subject
8 prior to treatment, wherein the myocardial function is selected from the group consisting of left
9 ventricular ejection fraction, cardiac output, systemic vascular resistance, left ventricular stroke

10 volume, aortic pressure, left ventricular pressure, peak rate of change of left ventricular
11 pressure during isovolumic contraction and relaxation, left ventricular end-diastolic pressure,
12 myocardial oxygen consumption, and coronary flow reserve.

1 30. A method of increasing myocardial function in a subject with acute decompensated
2 heart failure (ADHF), the method comprising:

3 (a) extracorporeally sequestering activated leukocytes and/or activated platelets present
4 in a body fluid of the subject; and

5 (b) treating the sequestered leukocytes and/or platelets to inhibit release of a pro-
6 inflammatory substance or to deactivate the leukocytes and/or platelets thereby to increase
7 myocardial function of the subject when compared to the myocardial function of the subject
8 prior to treatment.

1 31. The method of claim 29 or 30, wherein the subject has myocardial dysfunction
2 secondary to inflammatory cell penetration of heart tissue.

1 32. The method of any one of claims 29-31, wherein the subject has received a heart
2 transplant.

1 33. The method of any one of claims 29-32, wherein the body fluid is blood.

1 34. The method of any one of claims 29-33, wherein, in step (b), the leukocytes and/or
2 platelets are treated with an immunosuppressant agent, a serine leukocyte inhibitor, nitric
3 oxide, a polymorphonuclear leukocyte inhibitor factor, a secretory leukocyte inhibitor, or a
4 calcium chelating agent, wherein the calcium chelating agent is one or more of the group
5 consisting of citrate, sodium hexametaphosphate, ethylene diamine tetra-acetic acid (EDTA),
6 triethylene tetramine, diethylene triamine, o-phenanthroline, and oxalic acid.

1 35. The method of any one of claims 29-34, wherein, in step (b), the leukocytes and/or
2 platelets are treated with a calcium chelating agent.

1 36. The method of claim 35, wherein the calcium chelating agent is citrate.

1 37. The method of claim 35, wherein the calcium chelating agent is introduced into the
2 body fluid of the subject prior to step (a).

1 38. The method of any one of claims 29-37, wherein leukocytes from the subject are treated
2 over a period of at least 2 hours, at least 4 hours, at least 6 hours, at least 8 hours, or at least 12
3 hours.

1 39. The method of any one of claims 29-38, wherein leukocytes from the subject are treated
2 over a period of 2 to 48 hours, 2 to 24 hours, 2 to 12 hours, 4 to 48 hours, 4 to 24 hours, or 4 to
3 12 hours.

1 40. The method of any one of claims 30-39, wherein the myocardial function is selected
2 from the group consisting of left ventricular ejection fraction, cardiac output, systemic vascular
3 resistance, left ventricular stroke volume, aortic pressure, left ventricular pressure, peak rate of
4 change of left ventricular pressure during isovolumic contraction and relaxation, left ventricular
5 end-diastolic pressure, myocardial oxygen consumption, and coronary flow reserve.

1 41. The method of claim 29 or 40, wherein the increased myocardial function is maintained
2 for at least 6 hours after termination of the treatment in step (b).

1 42. The method of claim 41, wherein the increased myocardial function is maintained for at
2 least 24 hours after termination of the treatment in step (b).

1 43. The method of any one of claims 29-42, wherein, in step (b), the activated leukocytes
2 are sequestered by introducing the body fluid into a cartridge comprising

3 (i) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid outlet
4 port, wherein the inner volume is in fluid flow communication with the fluid inlet port and the
5 fluid outlet port, and

6 (ii) a solid support disposed within the housing and defining a fluid contacting surface
7 with a surface area (SA) capable of sequestering activated leukocytes and/or platelets, if present
8 in a body fluid entering the housing via the fluid inlet port, wherein the body fluid is introduced
9 into the housing via the fluid inlet port under conditions that permit sequestration of the
10 activated leukocytes and/or platelets on the fluid contacting surface of the solid support.

1 44. The method of claim 43, wherein the SA/IV ratio of the cartridge provided in step (a) is
2 greater than 25 cm^{-1} .

1 45. The method of claim 44, wherein the SA/IV ratio of the cartridge provided in step (a) is
2 greater than 80 cm^{-1} .

- 88 -

1 46. The method of claim 45, wherein the SA/IV ratio of the cartridge provided in step (a) is
2 greater than 150 cm^{-1} .

1 47. The method of any one of claims 29-46, wherein the SA/IV ratio of the cartridge
2 provided in step (a) is in the range of from 25 cm^{-1} to $1,500 \text{ cm}^{-1}$.

1 48. The method of claim 47, wherein the SA/IV ratio is in the range of from 80 cm^{-1} to
2 $1,500 \text{ cm}^{-1}$.

1 49. The method of claim 43 or 44, wherein the SA/IV ratio is no greater than 80 cm^{-1} .

1 50. The method of claim 29-49, wherein the solid support is disposed within the housing at
2 a packing density in the range from 20% to 65%.

1 51. The method of any one of claims 29-50, wherein the solid support comprises a
2 membrane.

1 52. The method of any one of claims 29-51, wherein the solid support comprises a planar
2 support member.

1 53. The method of any one of claims 29-52, wherein the solid support comprises a fiber.

1 54. The method of any one of claims 29-53, wherein the SA of the cartridge provided in
2 step (a) is in the range of from 0.1 m^2 to 10.0 m^2 .

1 55. The method of claim 54, wherein the SA of the cartridge provided in step (a) is in the
2 range of from 0.1 m^2 to 5.0 m^2 .

1 56. The method of any one of claims 29-55, wherein the inner volume of the cartridge
2 provided in step (a) is less than 300 cm^3 .

1 57. The method of claim 56, wherein the inner volume of the cartridge provided in step (a)
2 is less than 150 cm^3 .

1 58. The method of any one of claims 29-57, wherein the inner volume is in the range of
2 from 10 cm^3 to 150 cm^3 .

1 59. The method of claim 58, wherein the inner volume is in the range of from 75 cm^3 to 150
2 cm^3 .

1 60. The method of claim 59, wherein the inner volume is in the range of from 15 cm^3 to 120
2 cm^3 .

1 61. The method of any one of claims 29-60, further comprising permitting the body fluid to
2 exit the cartridge via the fluid outlet port at a flow rate in the range of 10 cm³/minute to 8,000
3 cm³/minute.

1 62. The method of any one of claims 29-61, wherein the solid support is substantially
2 parallel to the direction of fluid flow within the cartridge.

1 63. A method of treating a subject having or at risk of developing an inflammatory
2 condition associated with acute decompensated heart failure (ADHF), the method comprising:

3 (a) providing a cartridge comprising

4 (i) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid
5 outlet port, wherein the inner volume is in fluid flow communication with the fluid inlet
6 port and the fluid outlet port; and

7 (ii) a solid support disposed within the housing and defining a fluid contacting
8 surface with a surface area (SA) capable of sequestering an activated leukocyte, if
9 present in a body fluid entering the housing via the fluid inlet port; and

10 (b) introducing a body fluid from a subject into the housing via the fluid inlet port
11 under conditions that permit sequestration of an activated leukocyte and/or an activated
12 platelet on the fluid contacting surface of the solid support.

1 64. The method of claim 63, further comprising

2 (c) treating the leukocyte and/or platelet sequestered in step (b) to reduce the risk of
3 developing inflammation associated with the ADHF or to alleviate inflammation associated
4 with the ADHF.

1 65. A method of treating a subject having or at risk of developing an inflammatory
2 condition, wherein the inflammatory condition is chronic heart failure (CHF), the method
3 comprising:

4 (a) providing a cartridge comprising

5 (i) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid
6 outlet port, wherein the inner volume is in fluid flow communication with the fluid inlet
7 port and the fluid outlet port; and

- 90 -

8 (ii) a fluid permeable solid support disposed within the housing and defining a
9 fluid contacting surface with a surface area (SA) capable of sequestering an activated
10 leukocyte, if present in a body fluid entering the housing via the fluid inlet port, wherein
11 the SA/IV ratio is no greater than 80 cm^{-1} ; and

12 (b) introducing a body fluid from a subject into the housing via the fluid inlet port
13 under conditions that permit sequestration of an activated leukocyte and/or an activated
14 platelet on the fluid contacting surface of the solid support.

1 66. The method of claim 65, further comprising

2 (c) treating the leukocyte and/or platelet sequestered in step (b) to reduce the risk of
3 developing inflammation associated with the inflammatory condition or to alleviate
4 inflammation associated with the inflammatory condition.

1 67. The method of any one of claims 63-66, wherein the leukocyte and/or platelet is
2 sequestered for a time sufficient to deactivate the leukocyte and/or the platelet.

1 68. The method of any one of claims 63-67, wherein the leukocyte and/or the platelet is
2 sequestered for at least one minute.

1 69. The method of any one of claims 63-68, further comprising the step of returning the
2 leukocyte and/or the platelet produced in step (c) back to the subject.

1 70. The method of any one of claims 64 or 66-69, wherein, in step (c), a calcium chelator
2 deactivates the leukocyte and/or the platelet.

1 71. The method of any one of claims 63, 64 or 67-70, wherein the SA/IV ratio of the
2 cartridge provided in step (a) is greater than 80 cm^{-1} or is greater than 150 cm^{-1} .

1 72. The method of any one of claims 63, 64 or 67-70, wherein the SA/IV ratio of the
2 cartridge provided in step (a) is in the range of from 80 cm^{-1} to $1,500 \text{ cm}^{-1}$.

1 73. The method of claim 72, wherein the SA/IV ratio is in the range of from 150 cm^{-1} to
2 $1,500 \text{ cm}^{-1}$.

1 74. The method of any one of claims 63-73, wherein the solid support comprises a
2 membrane.

1 75. The method of any one of claims 63-74, wherein the solid support comprises a planar
2 support member.

- 1 76. The method of any one of claims 63-75, wherein the solid support comprises a fiber.
- 1 77. The method of any one of claims 63-76, wherein the SA of the cartridge provided in
2 step (a) is in the range of from 0.1 m^2 to 10.0 m^2 .
- 1 78. The method claim 77, wherein the SA of the cartridge provided in step (a) is in the
2 range of from 0.1 m^2 to 5.0 m^2 .
- 1 79. The method of claim 78, wherein the SA is in the range of from 0.1 m^2 to 0.4 m^2 , from
2 0.4 m^2 to 0.8 m^2 , from 0.8 m^2 to 1.2 m^2 , from 1.2 m^2 to 1.6 m^2 , from 1.6 m^2 to 2.0 m^2 , from 2.0
3 m^2 to 2.4 m^2 , from 2.4 m^2 to 2.8 m^2 , from 2.8 m^2 to 3.2 m^2 , from 3.2 m^2 to 3.6 m^2 , from 3.6 m^2
4 to 4.0 m^2 , from 4.0 m^2 to 4.4 m^2 , from 4.4 m^2 to 4.8 m^2 , from 4.8 m^2 to 5.2 m^2 , from 5.2 m^2 to
5 5.6 m^2 , from 5.6 m^2 to 6.0 m^2 , from 6.0 m^2 to 6.4 m^2 , from 6.4 m^2 to 6.8 m^2 , from 6.8 m^2 to 7.2
6 m^2 , from 7.2 m^2 to 7.6 m^2 , from 7.6 m^2 to 8.0 m^2 , from 8.0 m^2 to 8.4 m^2 , from 8.4 m^2 to 8.8 m^2 ,
7 from 8.8 m^2 to 9.2 m^2 , from 9.2 m^2 to 9.6 m^2 , or from 9.6 m^2 to 10.0 m^2 .
- 1 80. The method of any one of claims 63-79, wherein the inner volume of the cartridge
2 provided in step (a) is less than 150 cm^3 .
- 1 81. The method of any one of claims 63-79, wherein the inner volume is in the range of
2 from 10 cm^3 to 150 cm^3 .
- 1 82. The method of claim 81, wherein the inner volume is in the range of from 75 cm^3 to 150
2 cm^3 .
- 1 83. The method of claim 82, wherein the inner volume is in the range of from 15 cm^3 to 120
2 cm^3 .
- 1 84. The method of claim 83, wherein the inner volume is in the range of from 20 cm^3 to 80
2 cm^3 .
- 1 85. The method of any one of claims 63-84, further comprising permitting the body fluid to
2 exit the cartridge via the fluid outlet port at a flow rate in the range of $10 \text{ cm}^3/\text{minute}$ to $8,000$
3 $\text{cm}^3/\text{minute}$.
- 1 86. The method of claim 85, wherein the flow rate is in the range of $50 \text{ cm}^3/\text{minute}$ to $8,000$
2 $\text{cm}^3/\text{minute}$.
- 1 87. The method of any one of claims 1-86, further comprising measuring the myocardial
2 function of the subject prior to step (a).

1 88. The method of any one of claims 1-87, further comprising measuring the myocardial
2 function of the subject after step (b).

1 89. The method of any one of claims 1-88, wherein the activated leukocytes and/or
2 activated platelets bind to a fluid contacting surface of the solid support.

1 90. A cartridge for use in a method of improving myocardial function in a subject with
2 chronic heart failure (CHF) or acute decompensated heart failure (ADHF), the cartridge
3 comprising (i) a rigid housing defining an inner volume (IV), a fluid inlet port and a fluid outlet
4 port, wherein the inner volume is in fluid flow communication with the fluid inlet port and the
5 fluid outlet port, and (ii) a solid support disposed within the housing in fluid flow
6 communication with the inner volume and defining a fluid contacting surface with a surface
7 area (SA) configured for sequestering activated leukocytes and/or platelets, if present in a body
8 fluid entering the housing via the fluid inlet port.

1 91. The cartridge of claim 90, wherein the cartridge has a SA to IV ratio in the range of 25
2 cm^{-1} to 2,000 cm^{-1} .

1 92. The cartridge of claim 90 or 91, wherein the cartridge has a SA/IV ratio of greater than
2 80 cm^{-1} .

1 93. The cartridge of claim 90 or 91, wherein the cartridge has a SA/IV ratio of no greater
2 than 80 cm^{-1} .

1 94. The cartridge of any one of claims 90-93, wherein the cartridge is disposed within
2 sterile packaging.

1 95. The cartridge of claim 94, wherein the sterile packaging comprises plastic.

1 96. The cartridge of any one of claims 90-95, wherein the cartridge comprises a label
2 disposed on an outer surface of the rigid housing.

1 97. The cartridge of any one claims 90-96, wherein the cartridge further comprises a cap
2 sealing the fluid inlet port and/or the fluid outlet port.

1 98. The cartridge of any one of claims 90-97, wherein the surface area configured for
2 sequestering activated leukocytes and/or platelets binds the activated leukocytes and/or
3 platelets.

- 93 -

1 99. The cartridge of any one of claims 90-98 for use in the method of any one of claims 1-
2 89.

1 100. A calcium chelating agent for use in a method of treating chronic heart failure or acute
2 decompensated heart failure in a subject in need thereof, wherein the method of treating
3 comprises administering the calcium chelating agent to extracorporeally sequestered activated
4 leukocytes and/or activated platelets in a cartridge recited in any one of claims 1-98.

Figure 1A

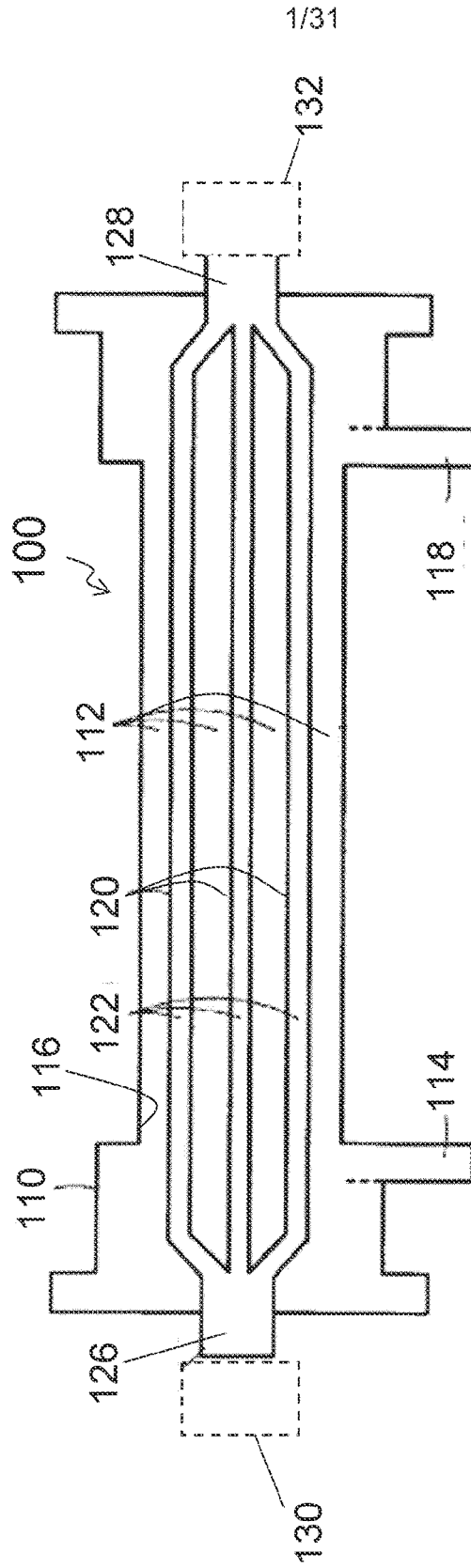


Figure 1B

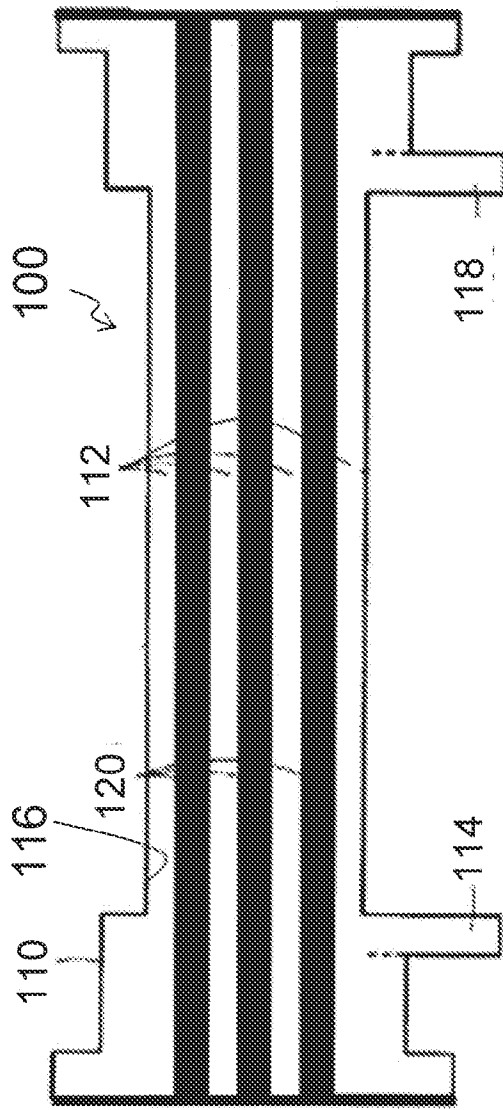


Figure 1C

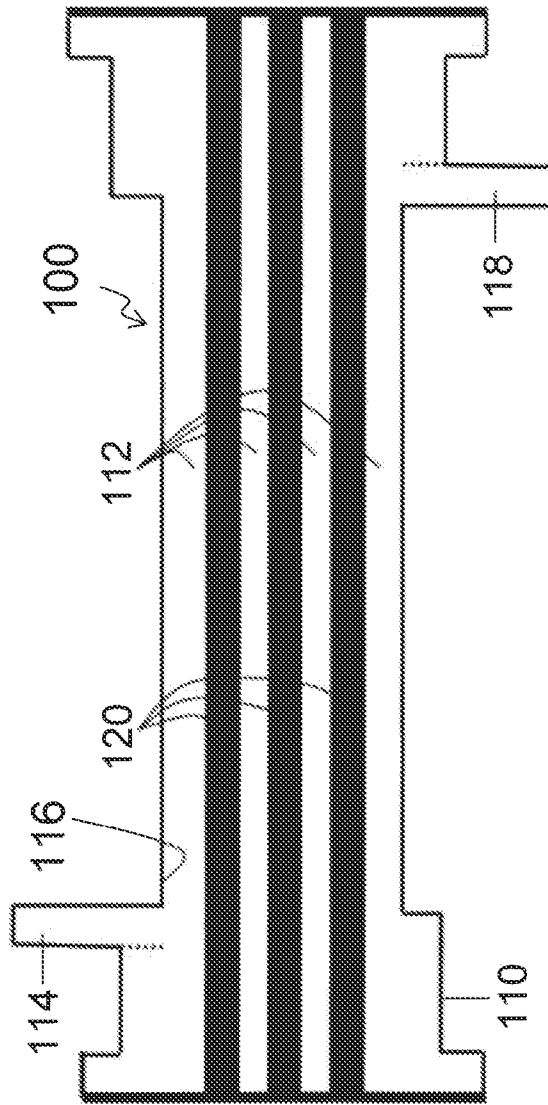


Figure 1D

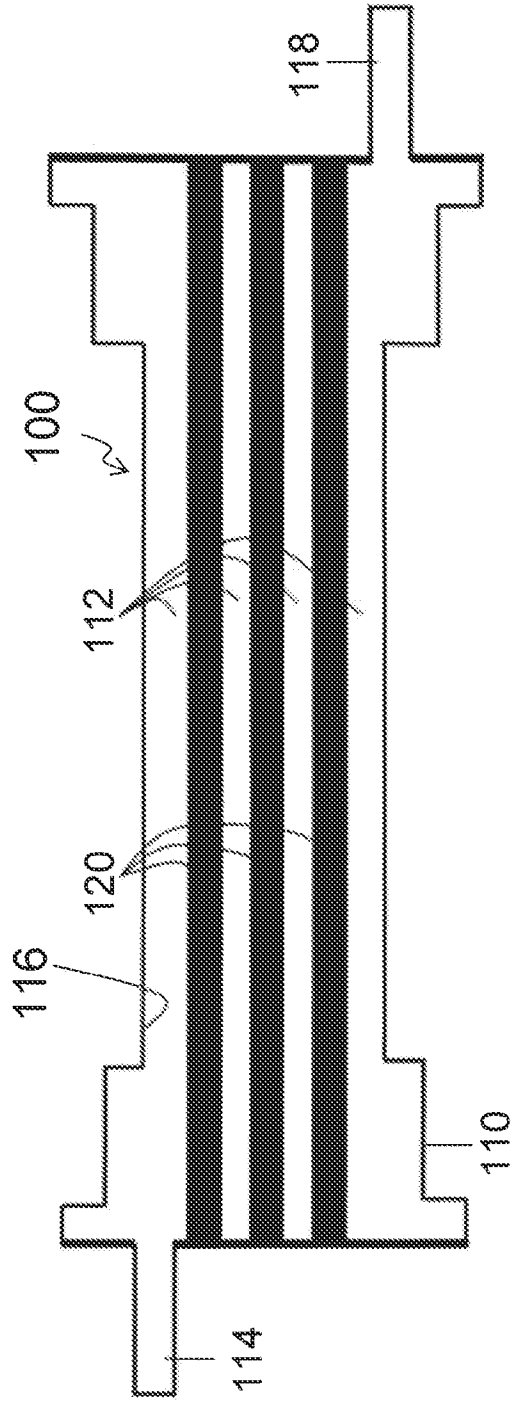


Figure 2A

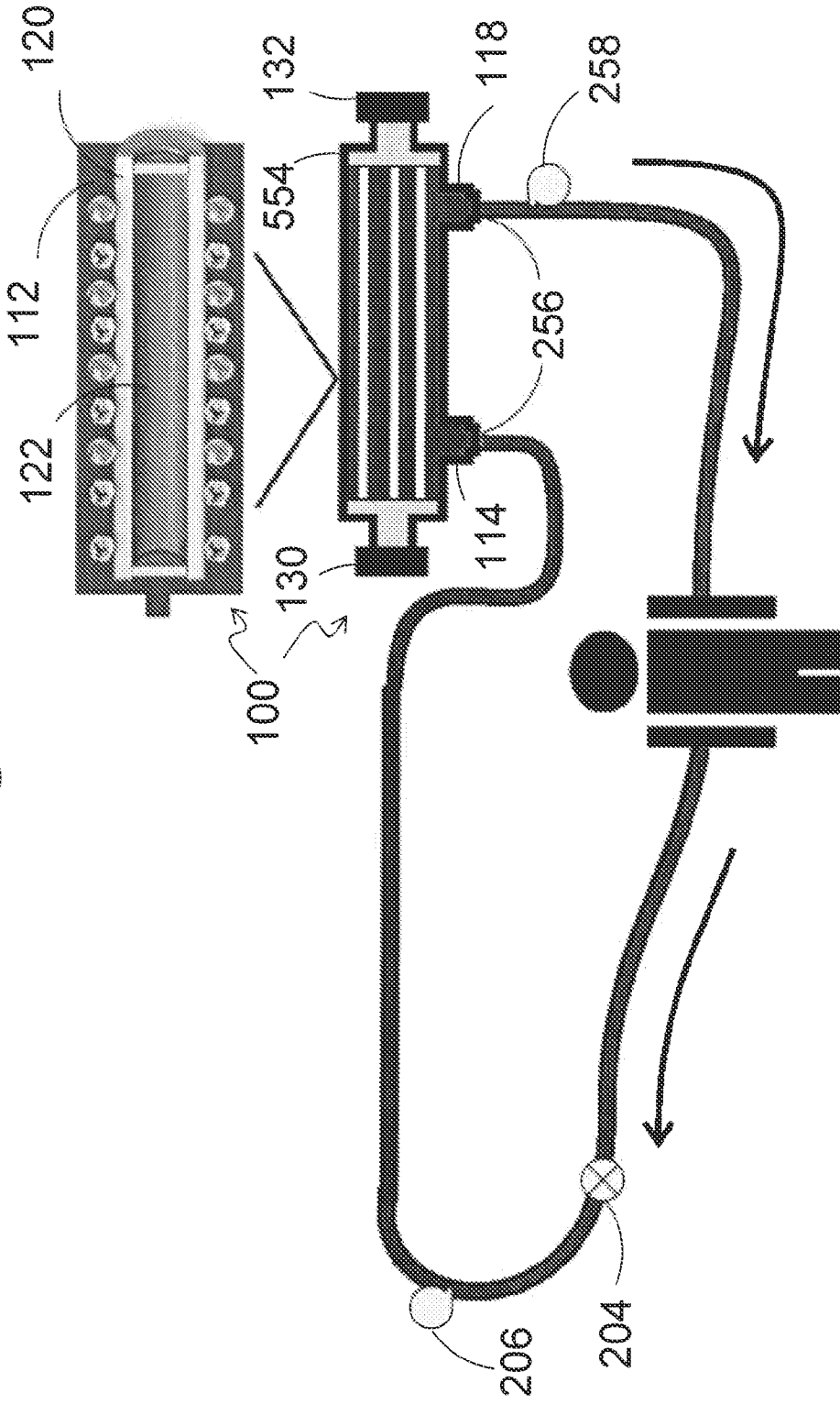


Figure 2B

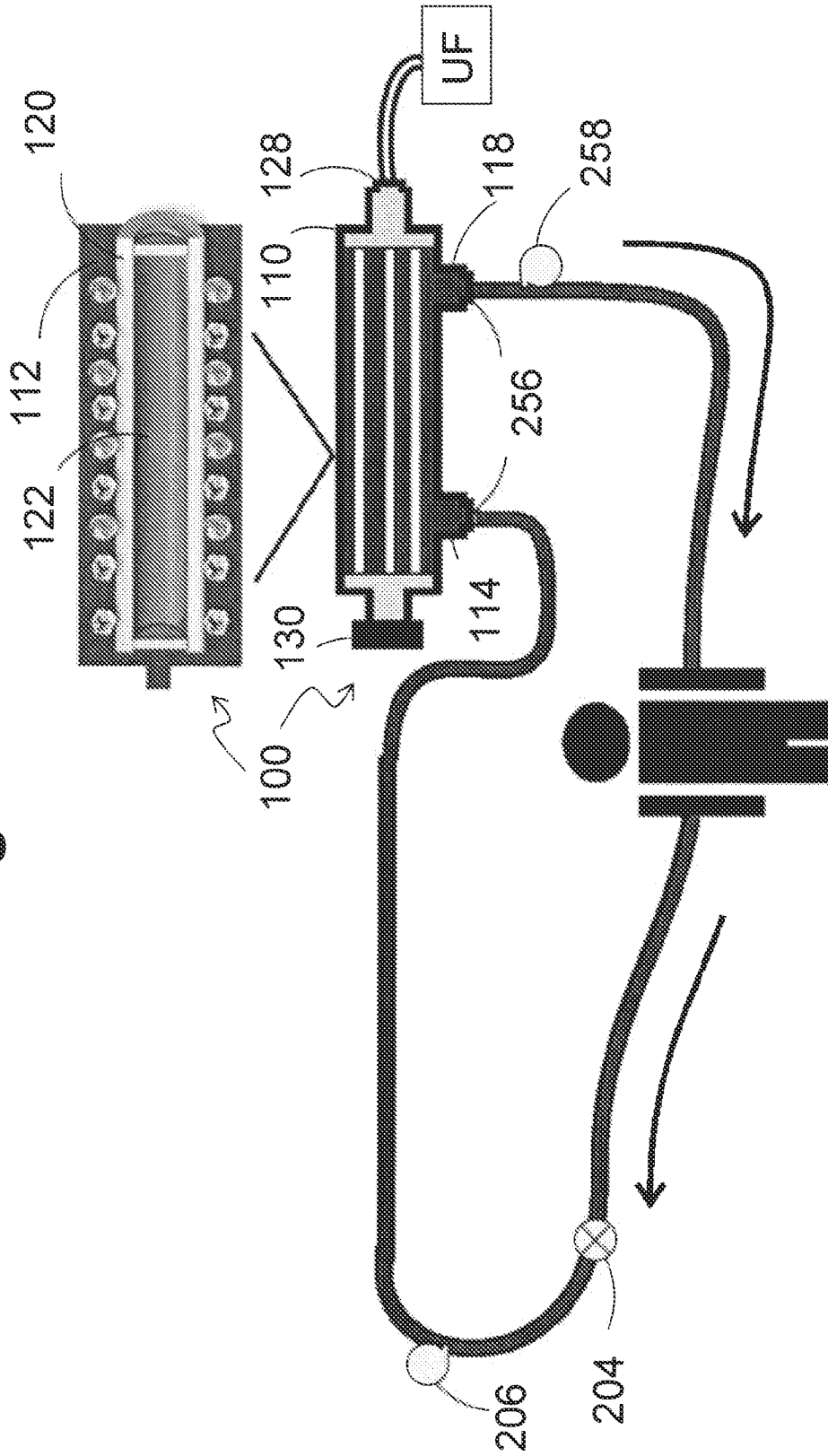


Figure 2C

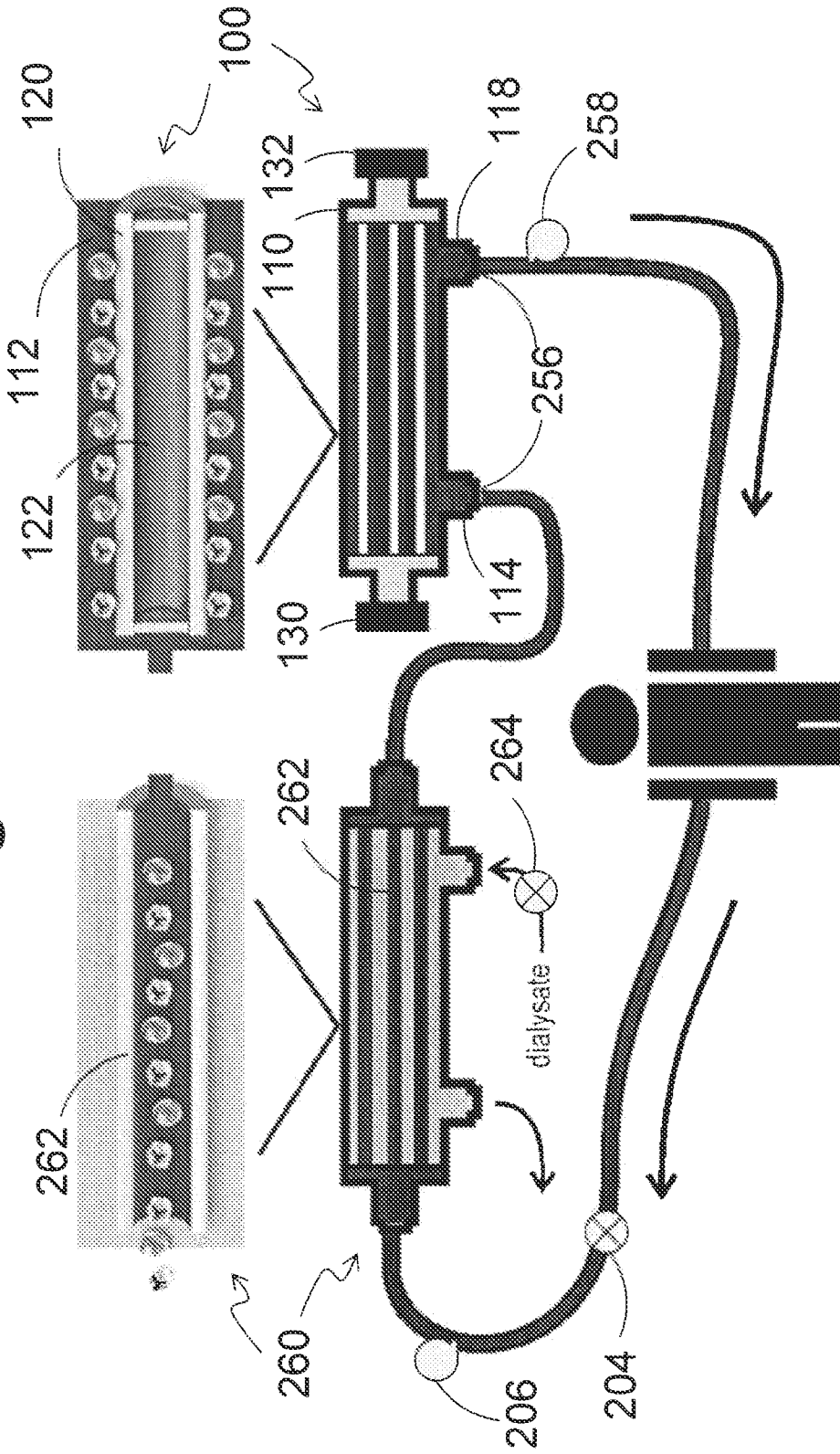
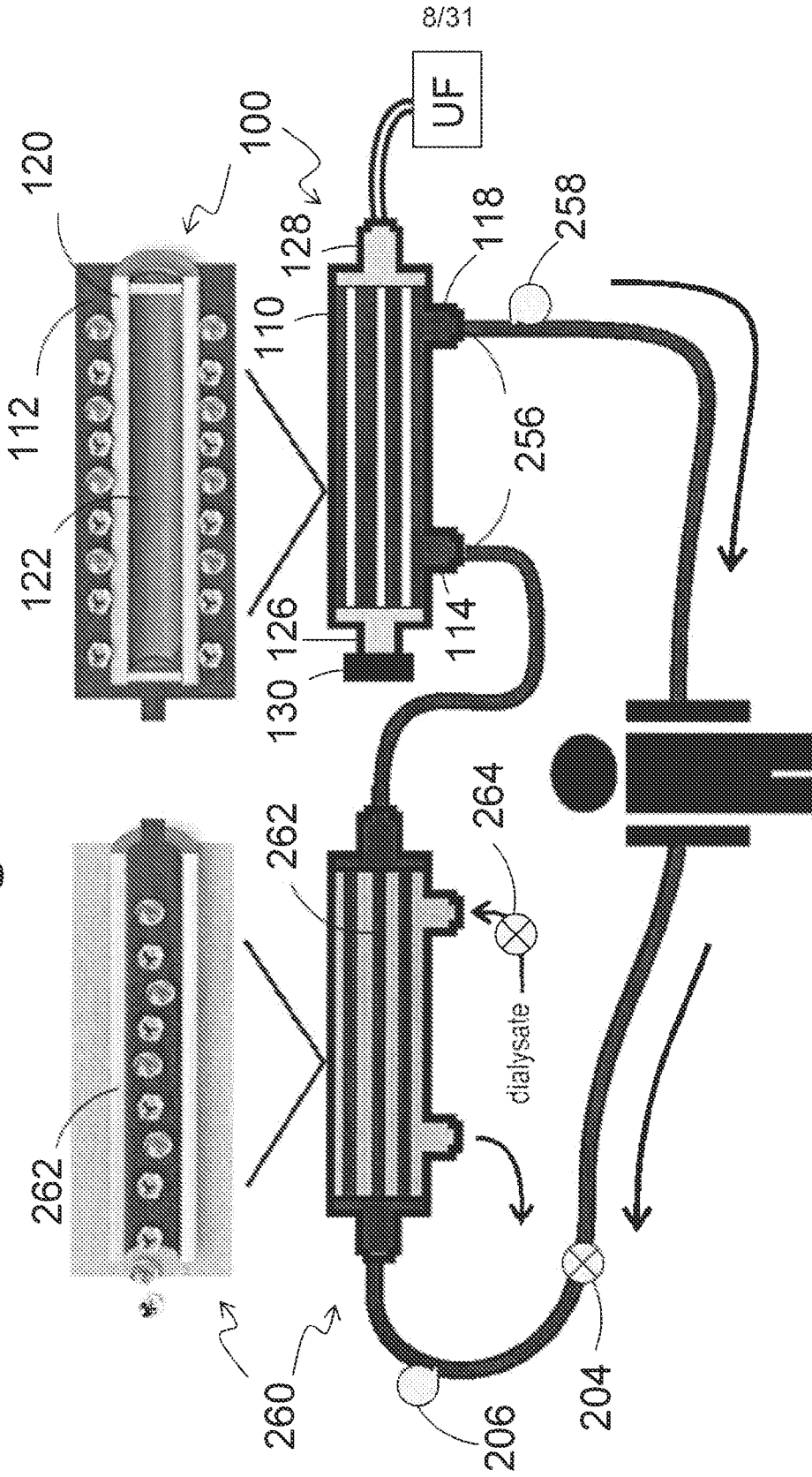
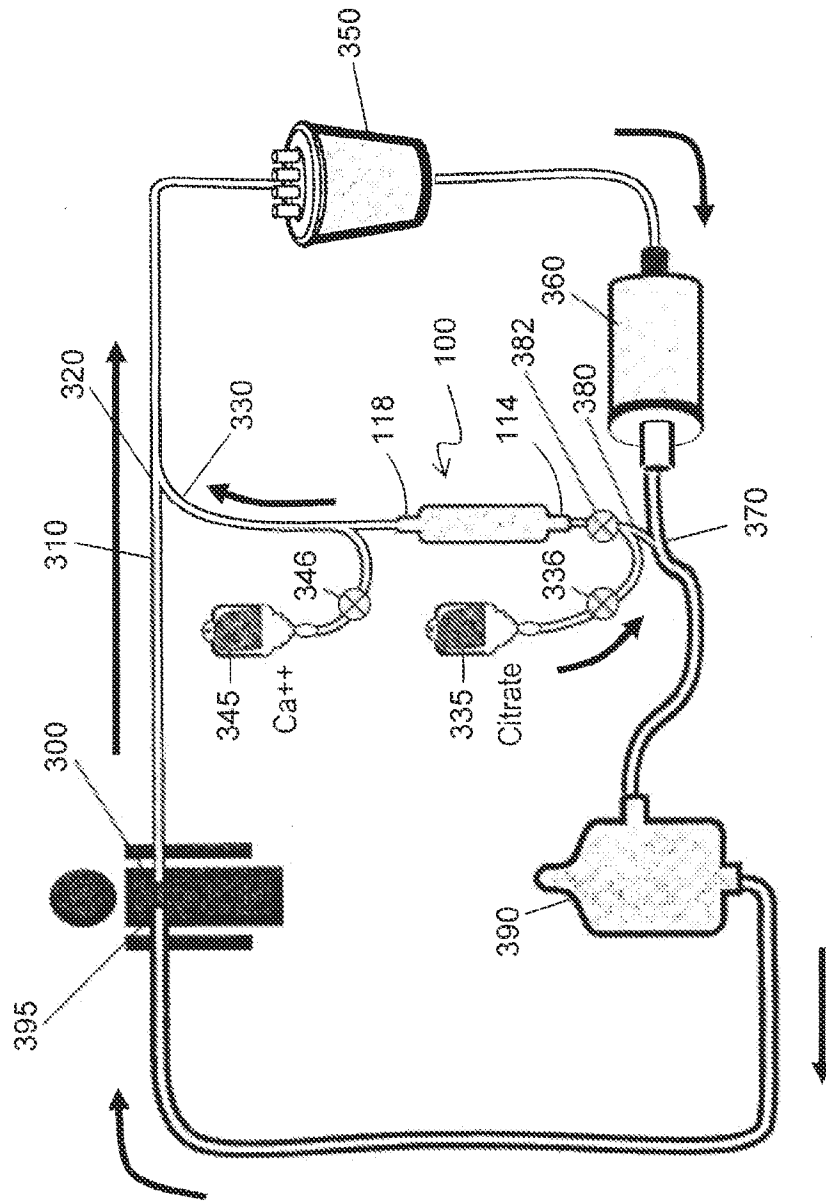


Figure 2D



9/31

Figure 3A



10/31

Figure 3B

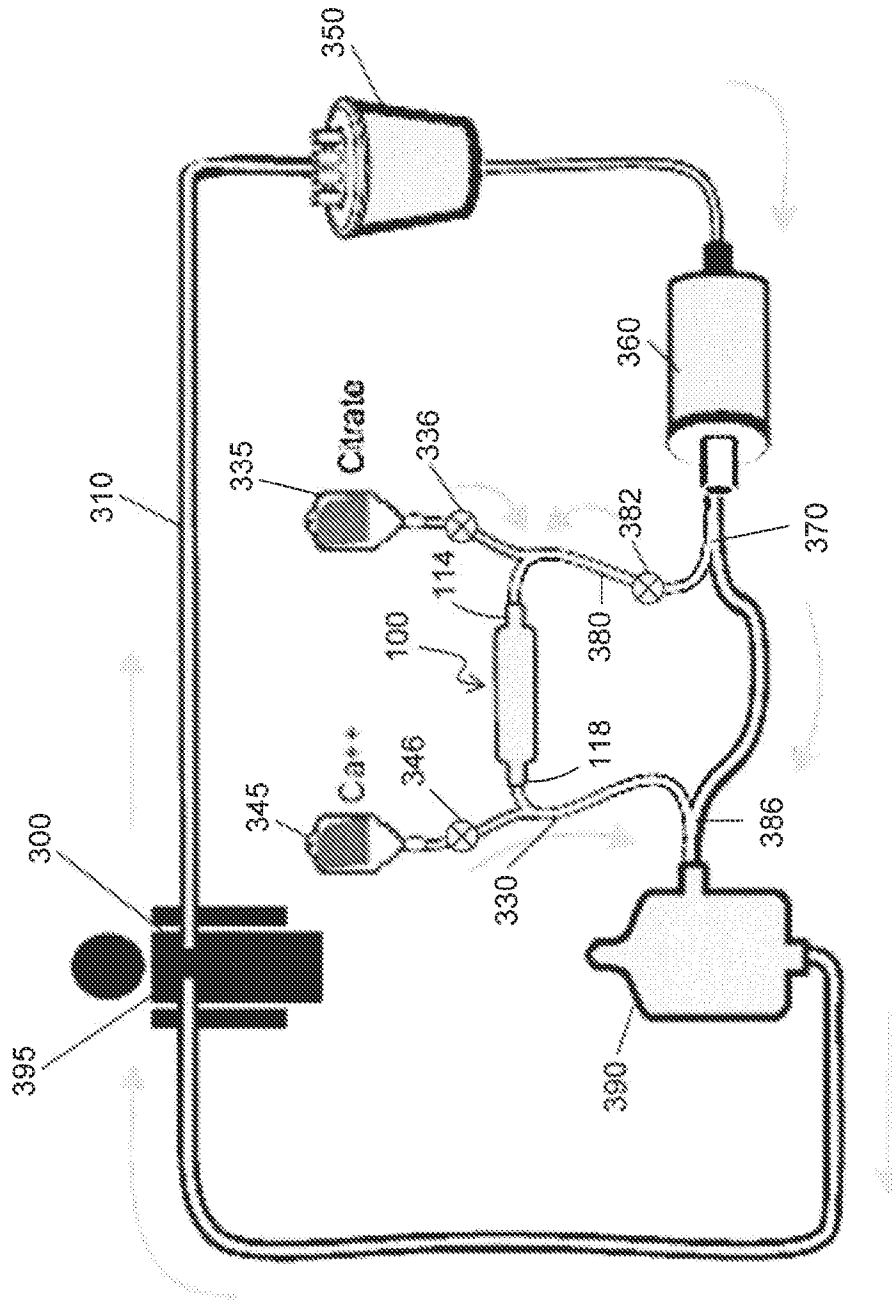
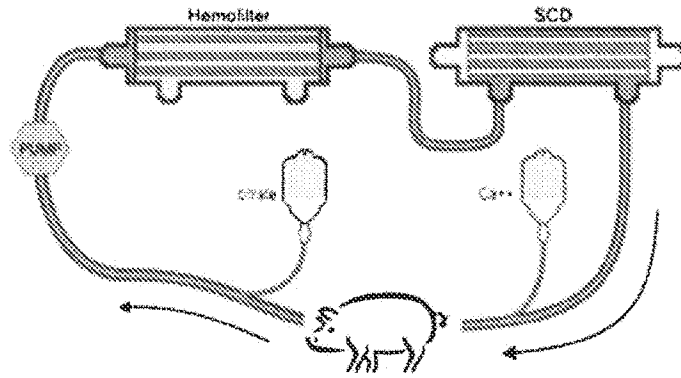


Figure 4



12/31

Figure 5

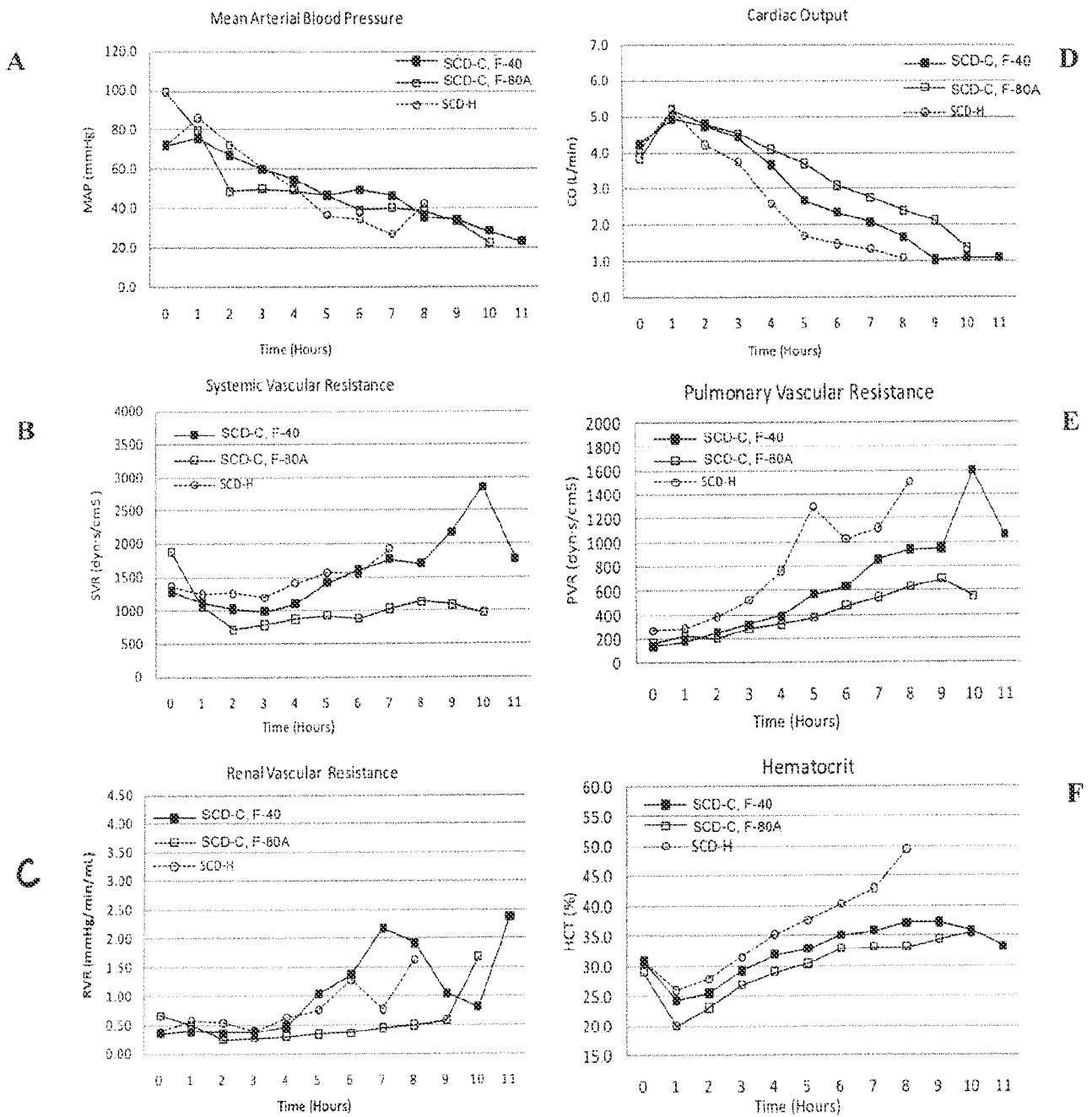
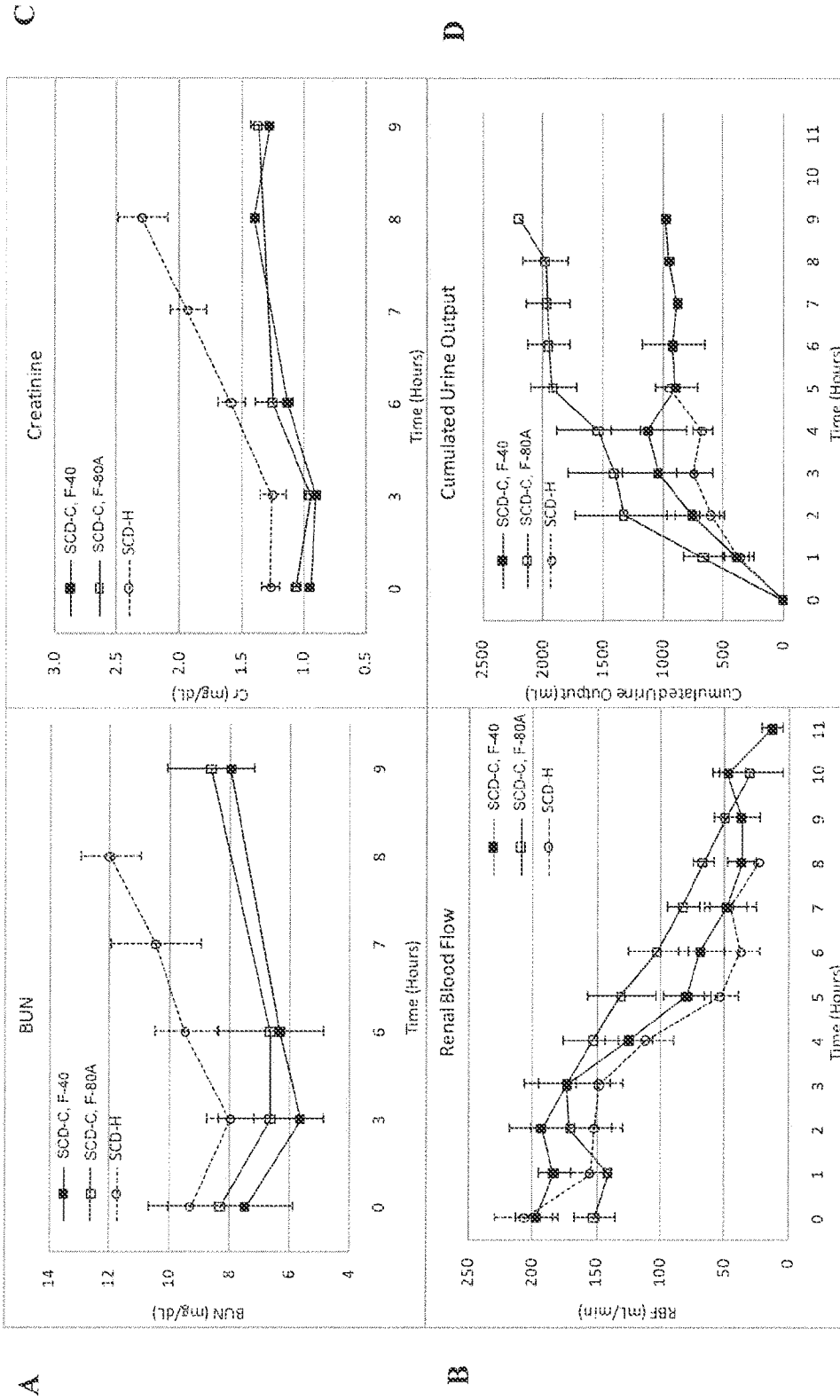


Figure 6



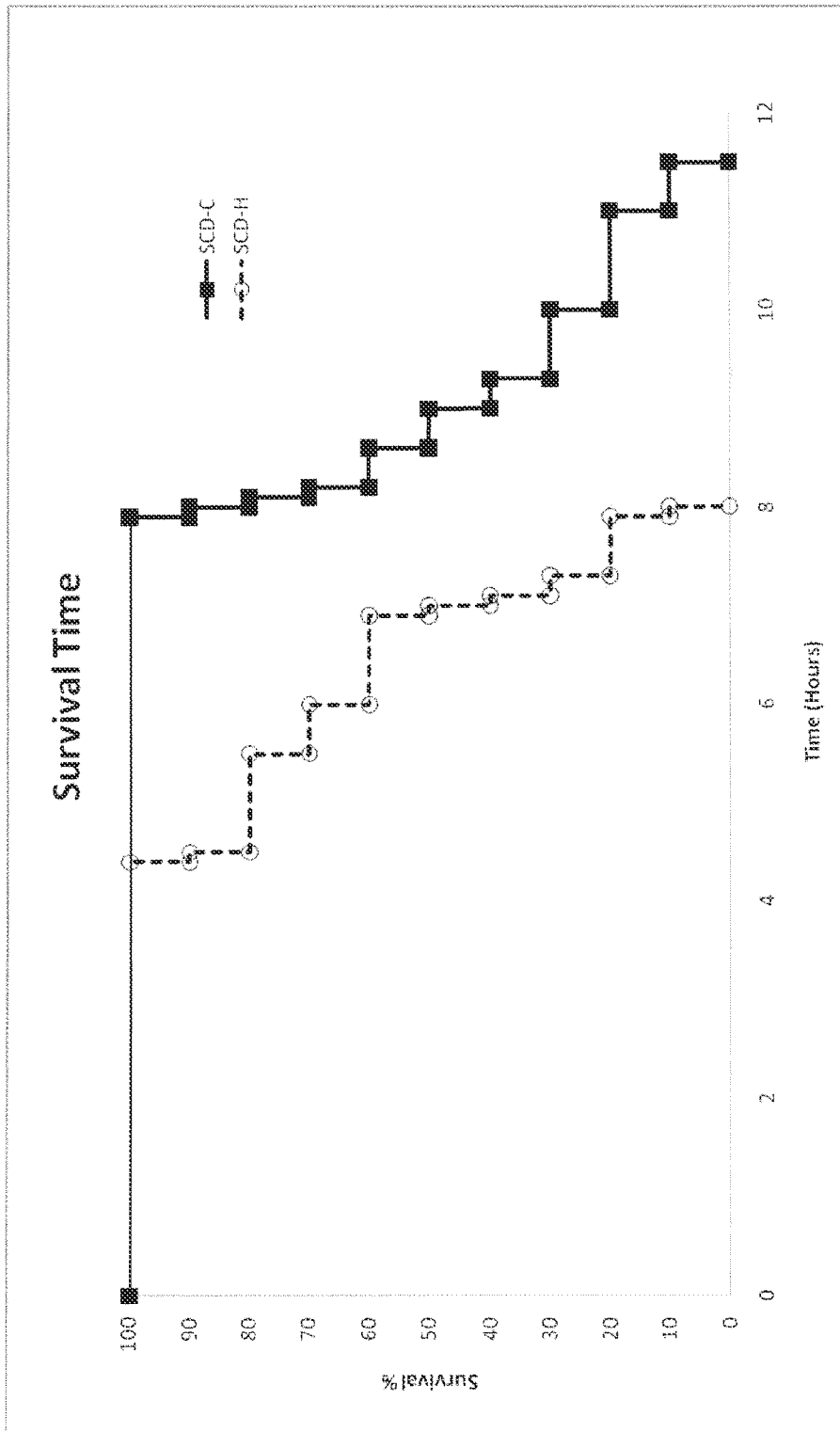


Figure 7

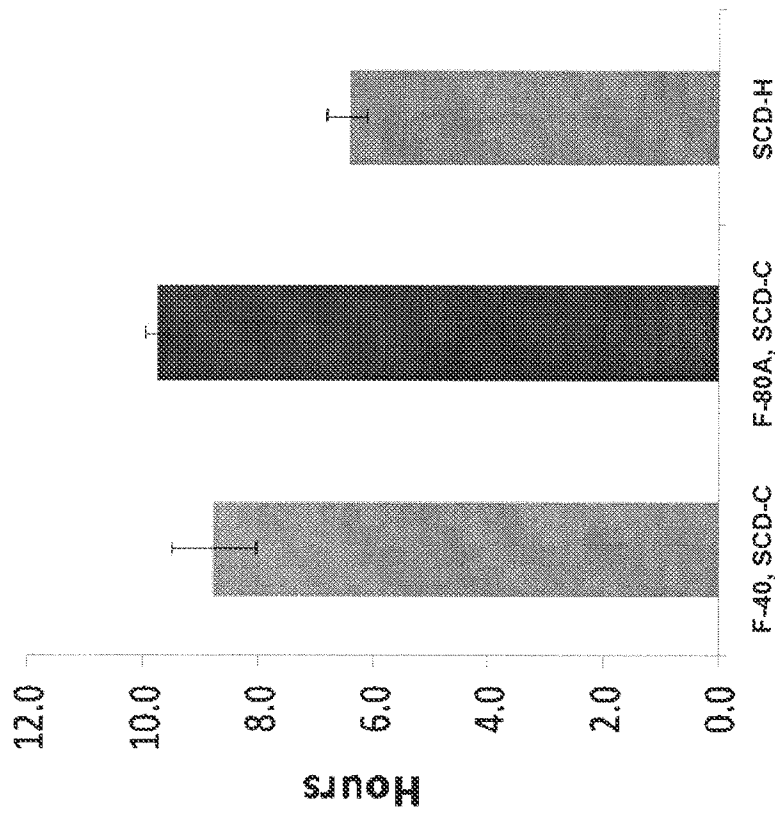
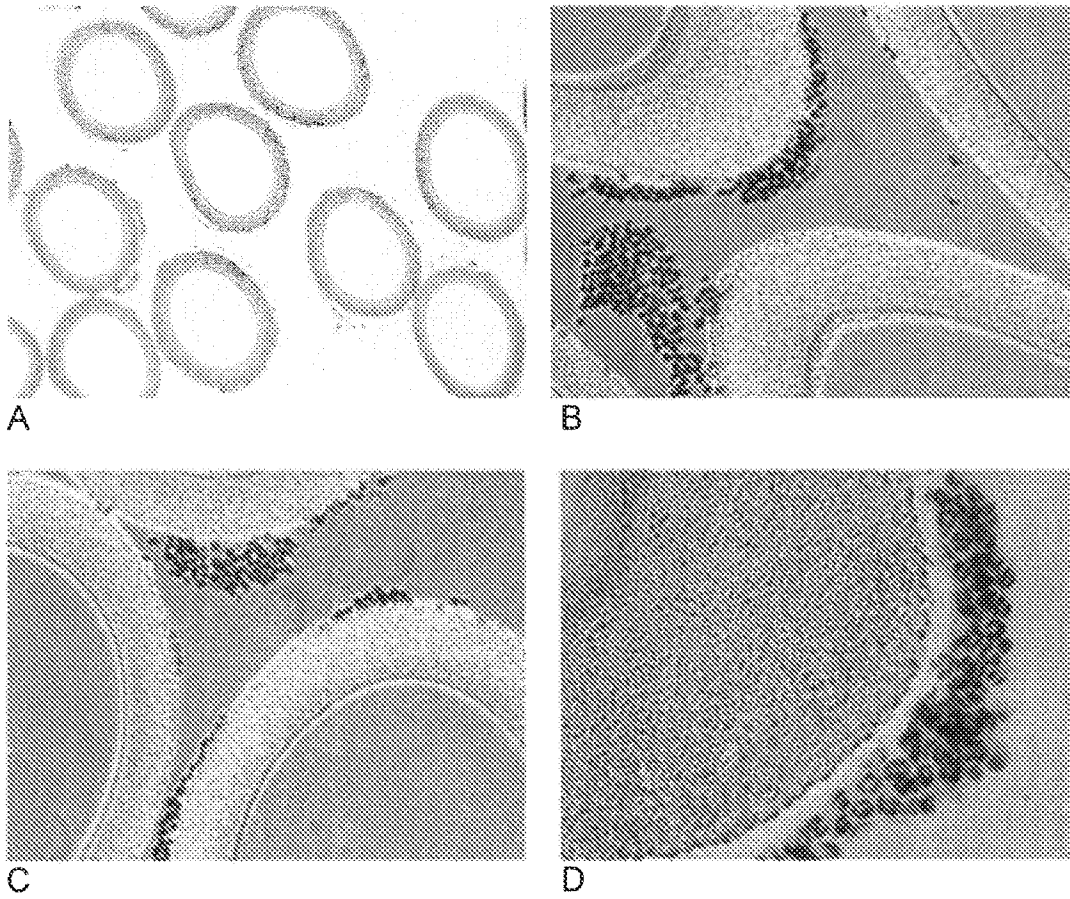


Figure 8. Impact of SCD-C vs. SCD-H on Survival Time in the septic pig. $p < 0.005$ (F-40) and < 0.0004 (F-80A)

Figure 9



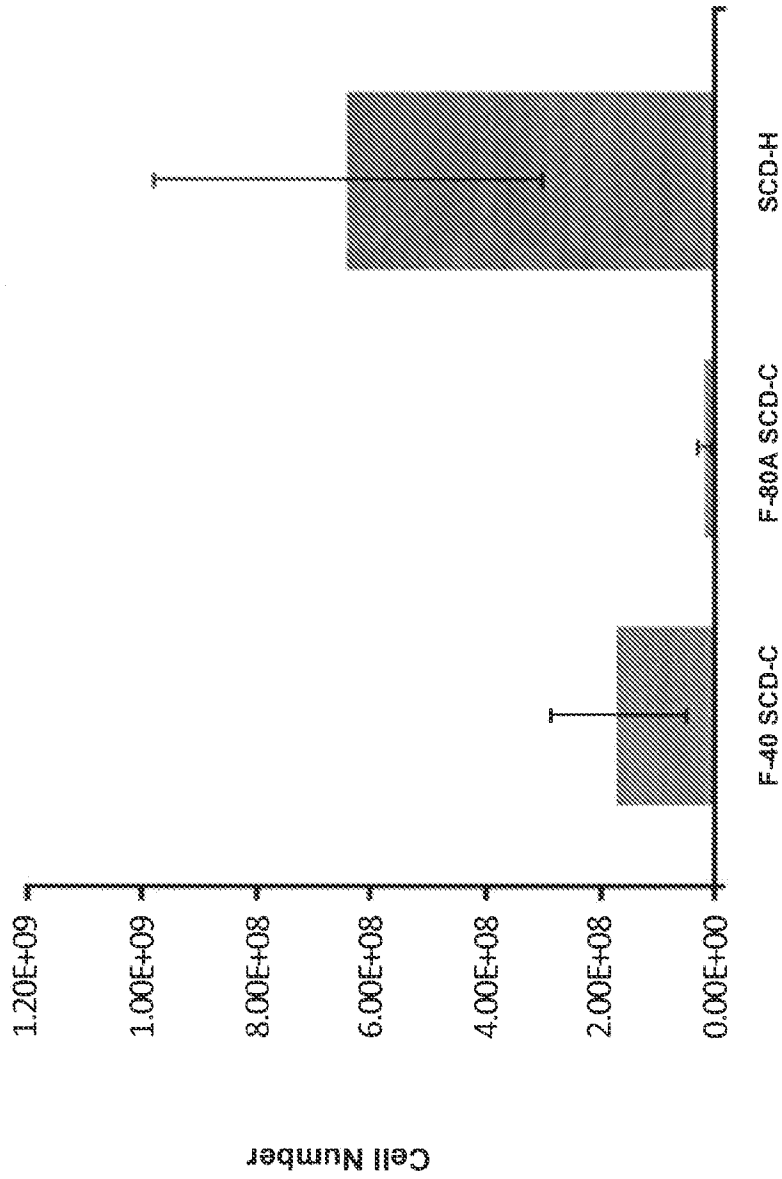


Figure 10A. Adherent cells eluted from SCD-C and SCD-H cartridges after septic pig study end point.

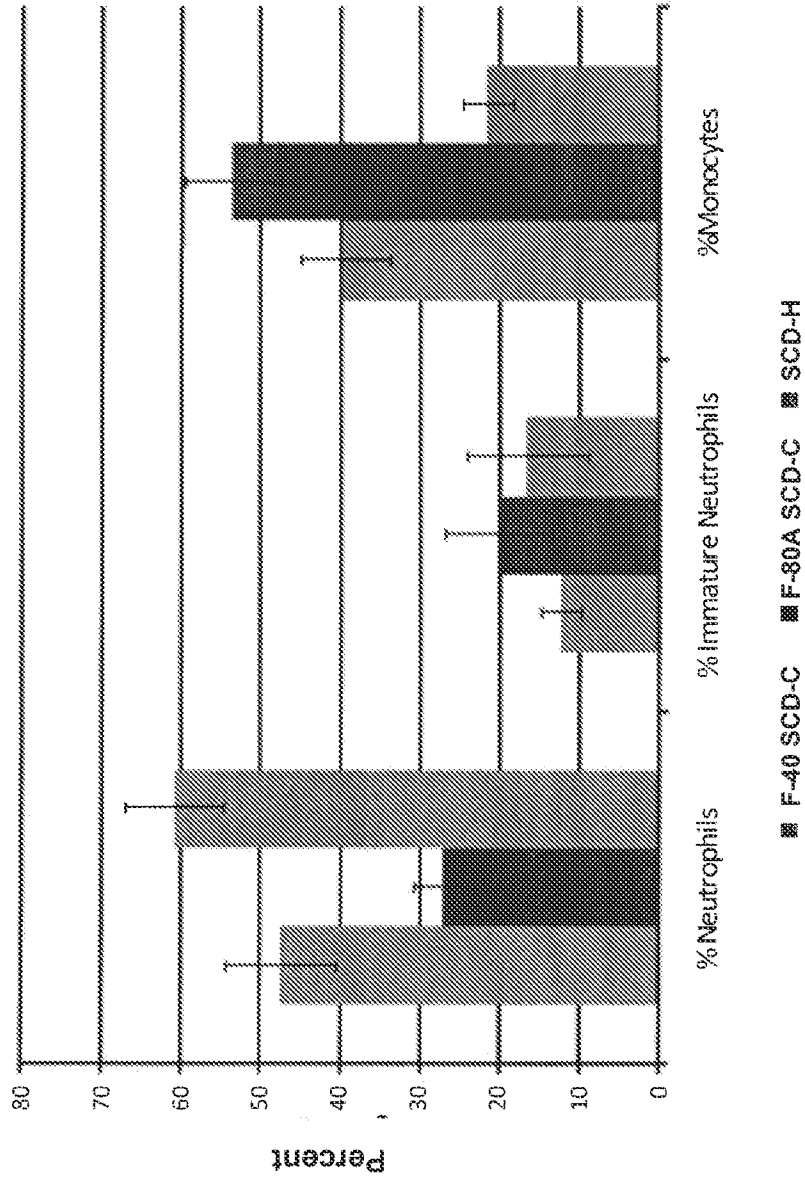


Figure 10B. SCD Eluted Cell Differentials

Figure 11A

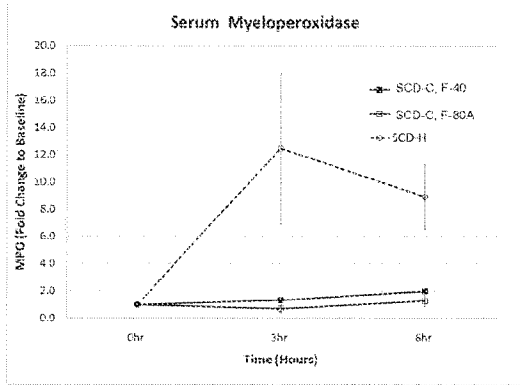
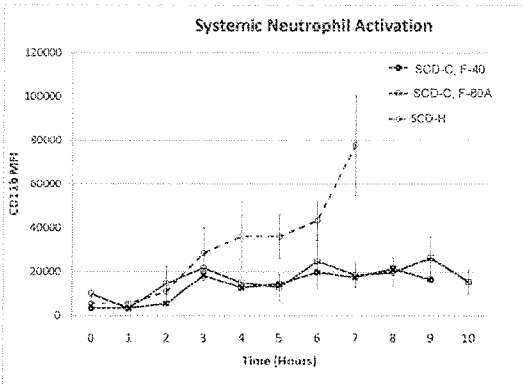


Figure 11B



20/31

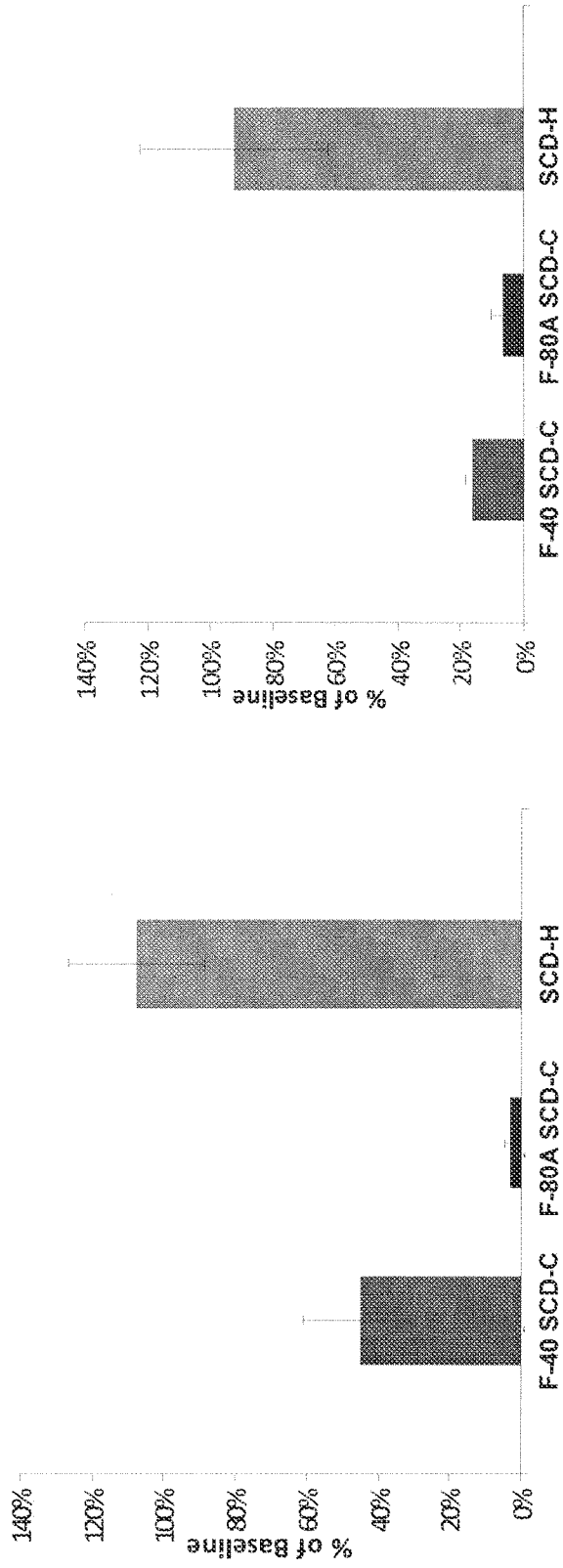


Figure 12A. Stimulated MNC IL-8 Secretion.

Figure 12B. Stimulated MNC TNF-a

Secretion. All values are expressed as the LPS-stimulated 6 hour Septic Time Point Percent of LPS-stimulated 0 hour Baseline, p < 0.05 for all SCD-C groups compared the SCD-H group for IL-8 and TNF-a.

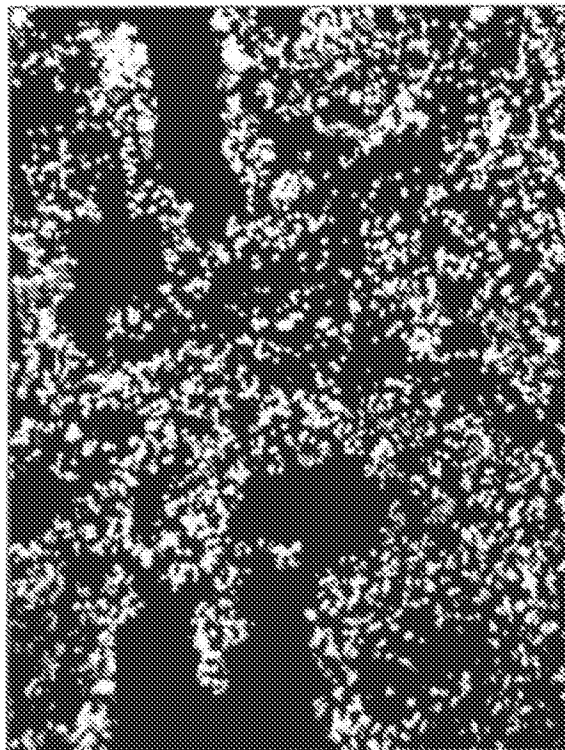
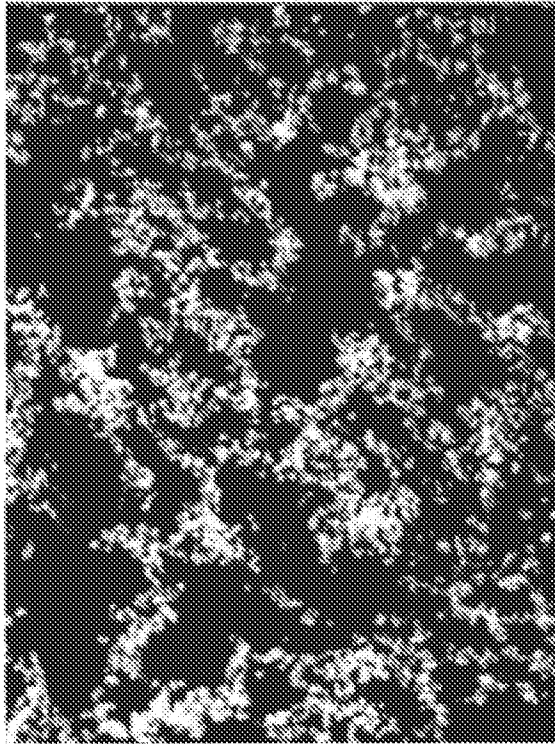


Figure 13

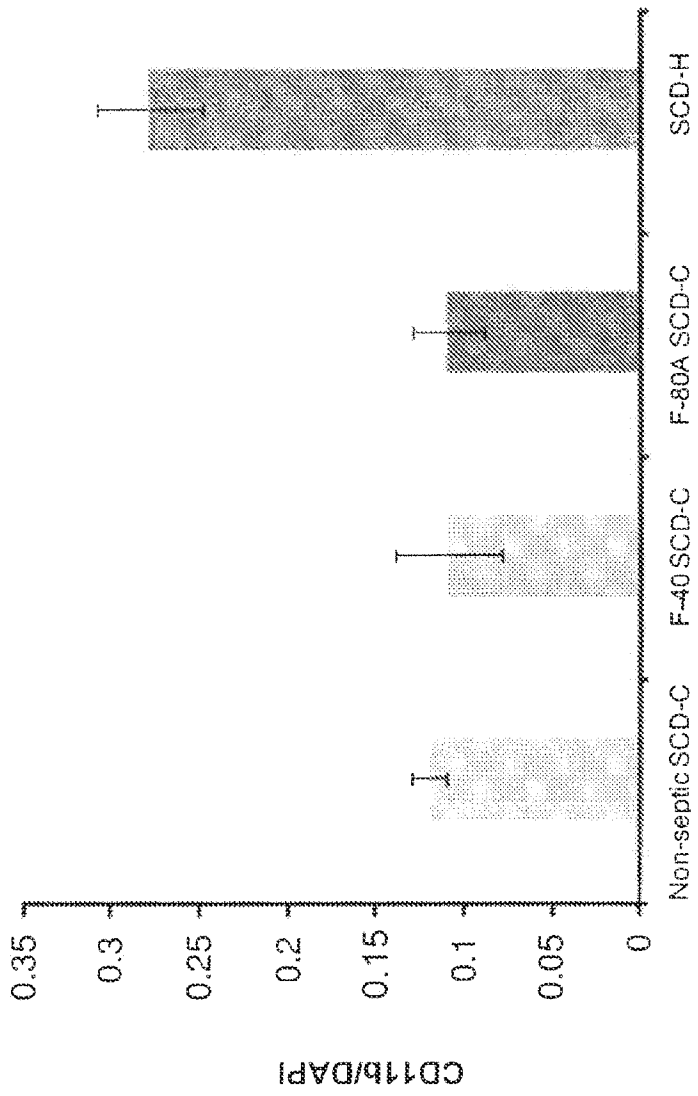
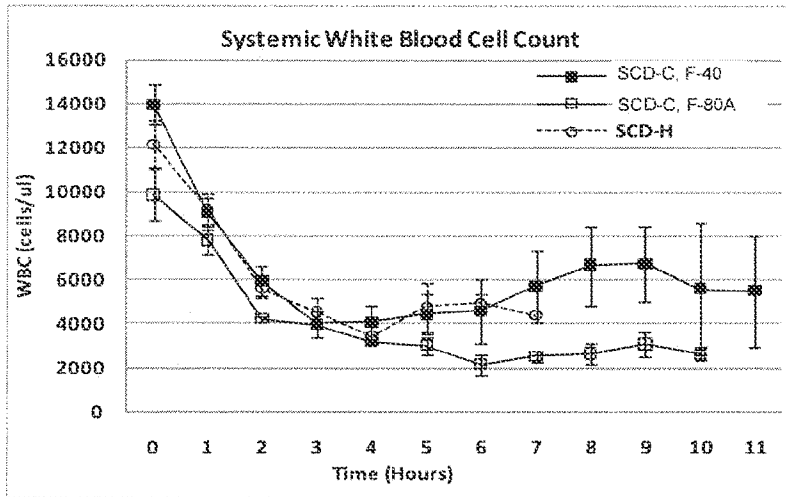


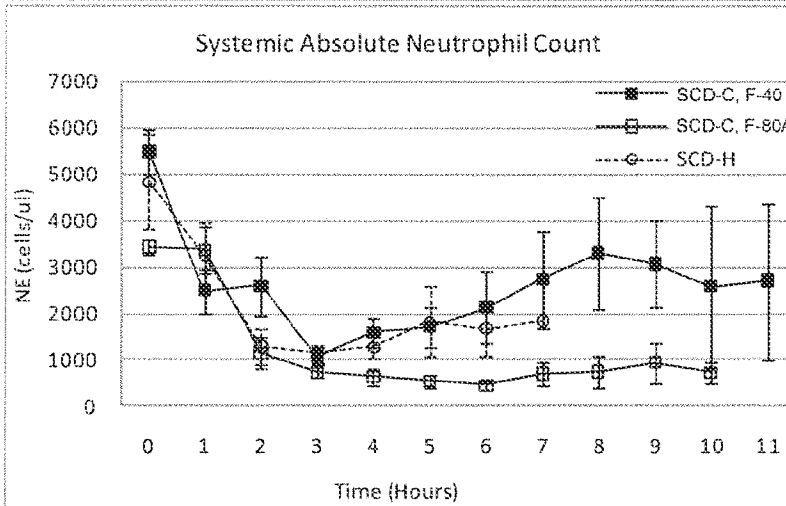
Figure 14. Lung Tissue Leukocyte Aggregation expressed as # of CD11b positive cells/# of DAPI stained nuclei after IHC staining, $p < 0.01$ for all SCD-C groups compared to the SCD-H group.

Figure 15

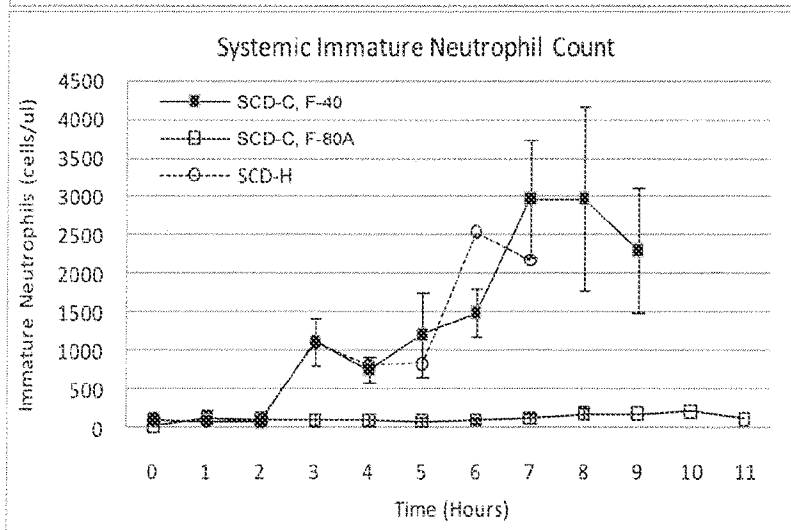
A



B



C



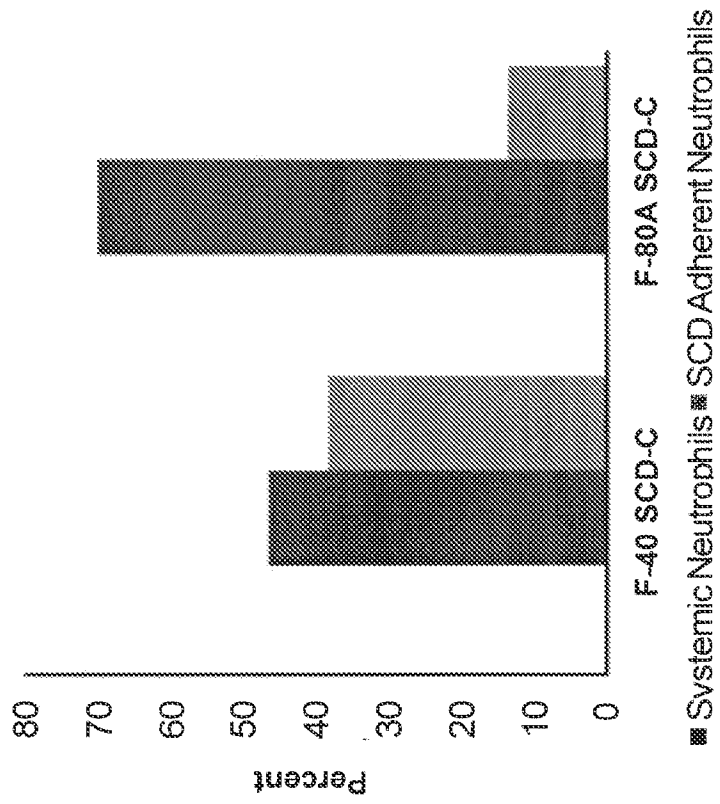


Figure 16. Apoptotic Potential assessed by percent Annexin V positive NEs.

Figure 17

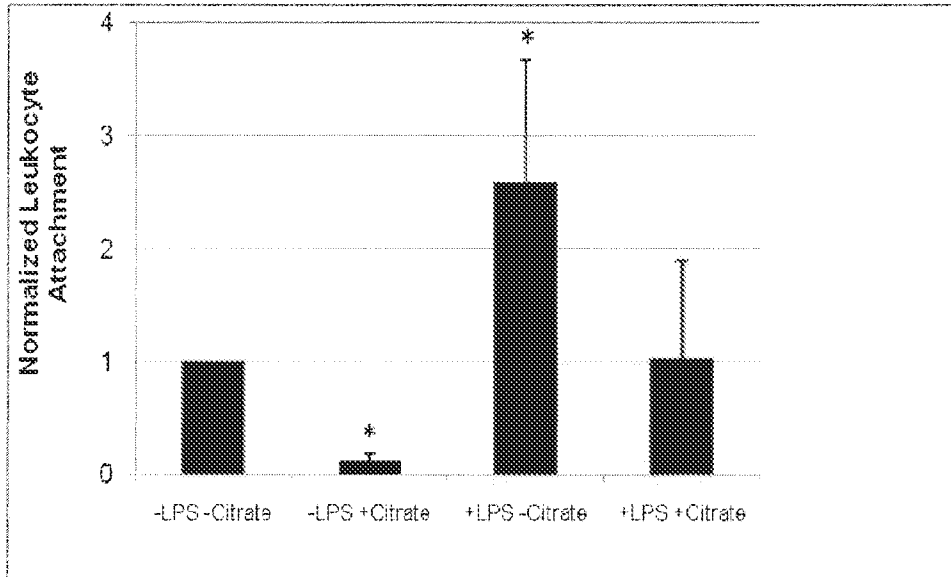
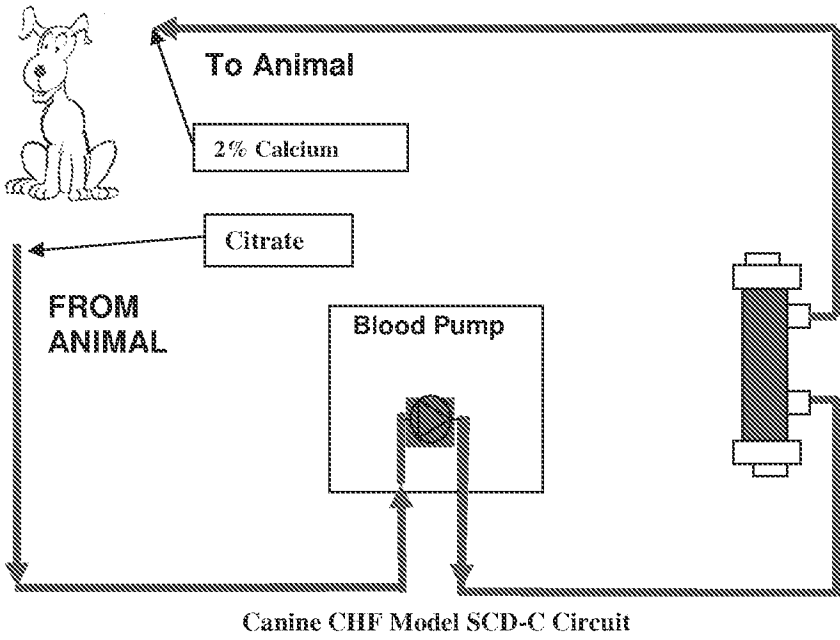


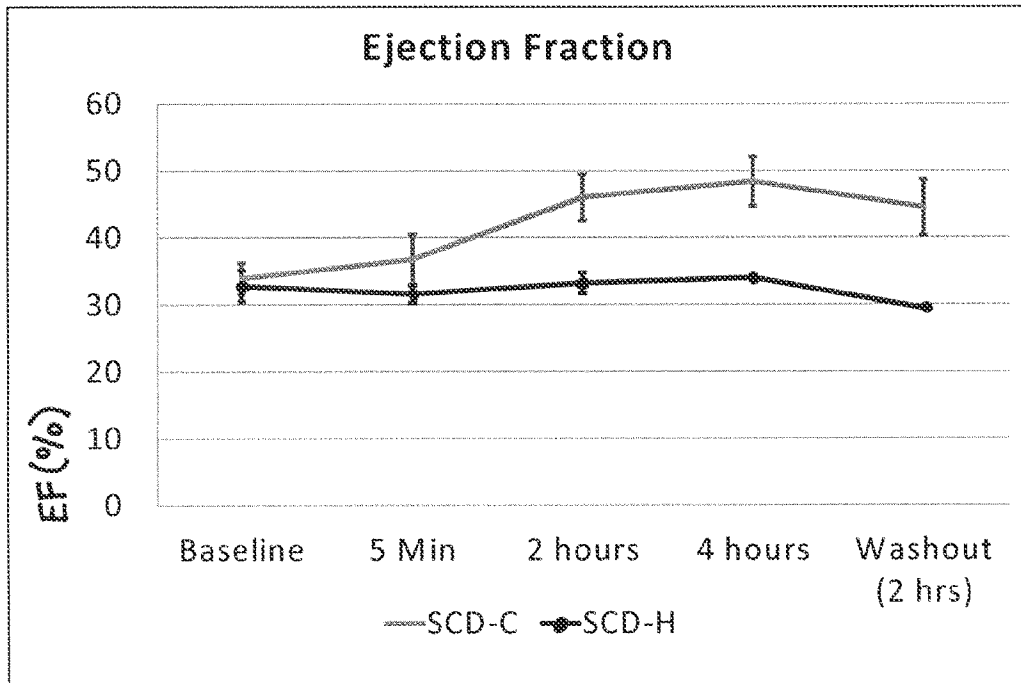
Figure 18



27/31

Figure 19

A.



B.

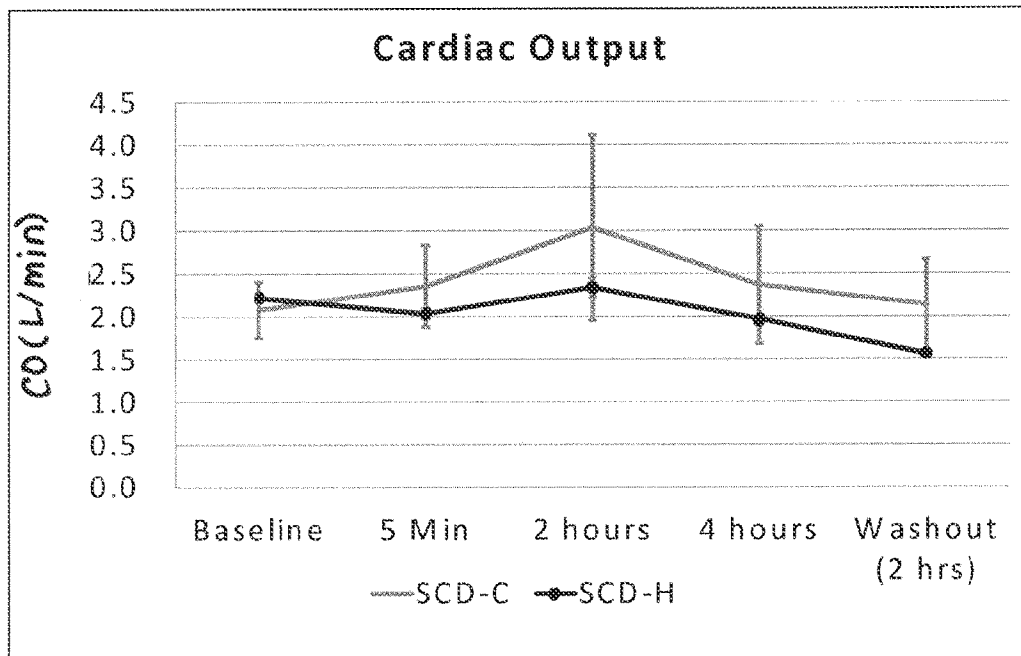
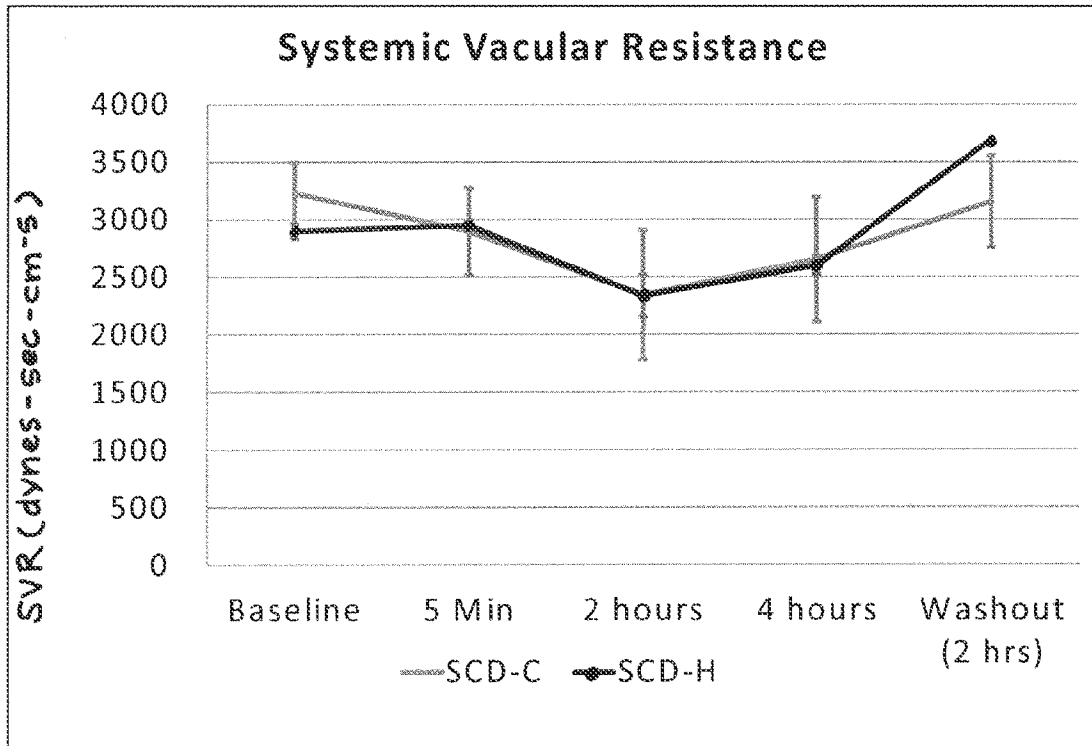


FIGURE 19

C.



29/31

Figure 20A

URINE VOLUME (ML) SCD CHF

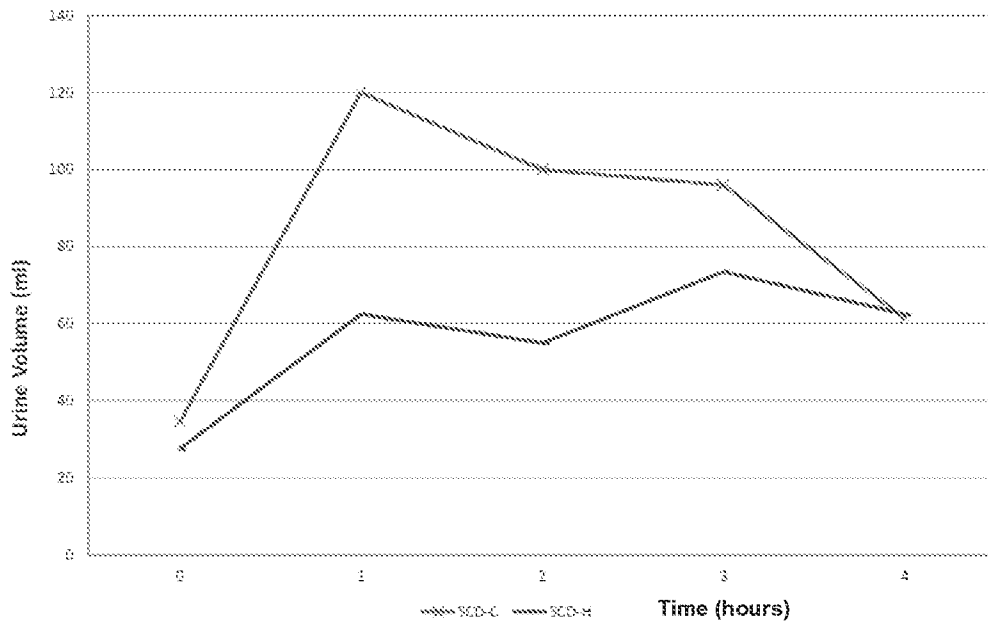
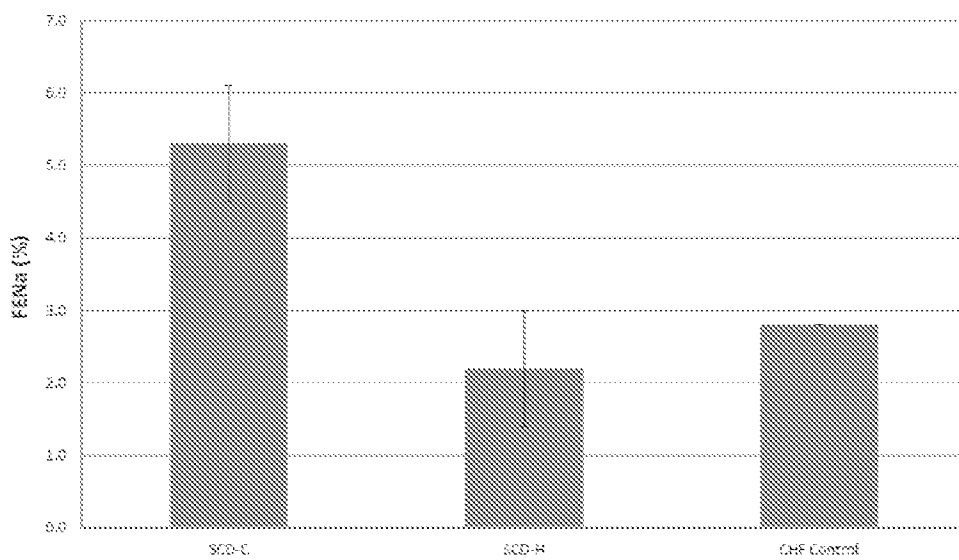


Figure 20B

FENa (%) in CHF



30/31

Figure 20C

FEUrea (%) in CHF

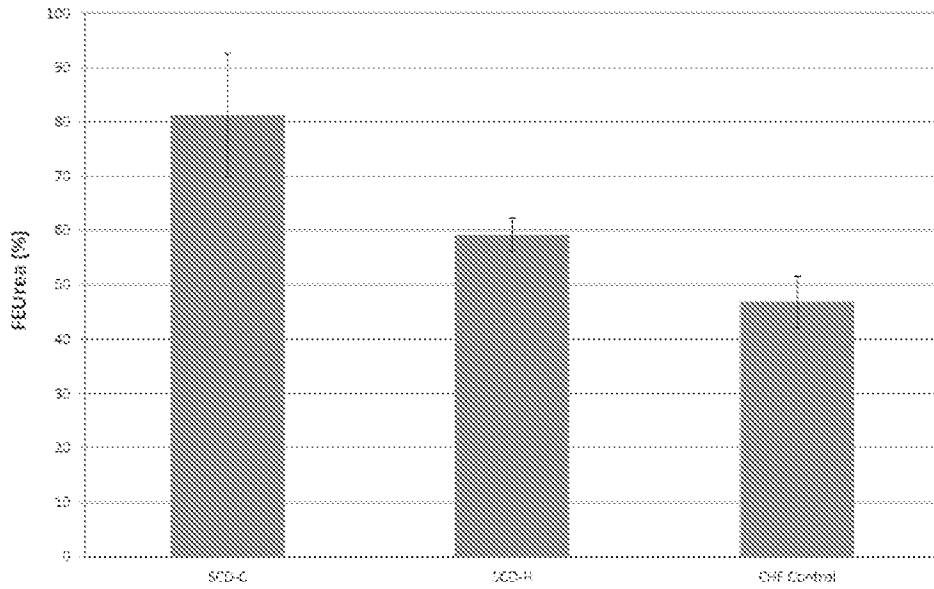


Figure 20D

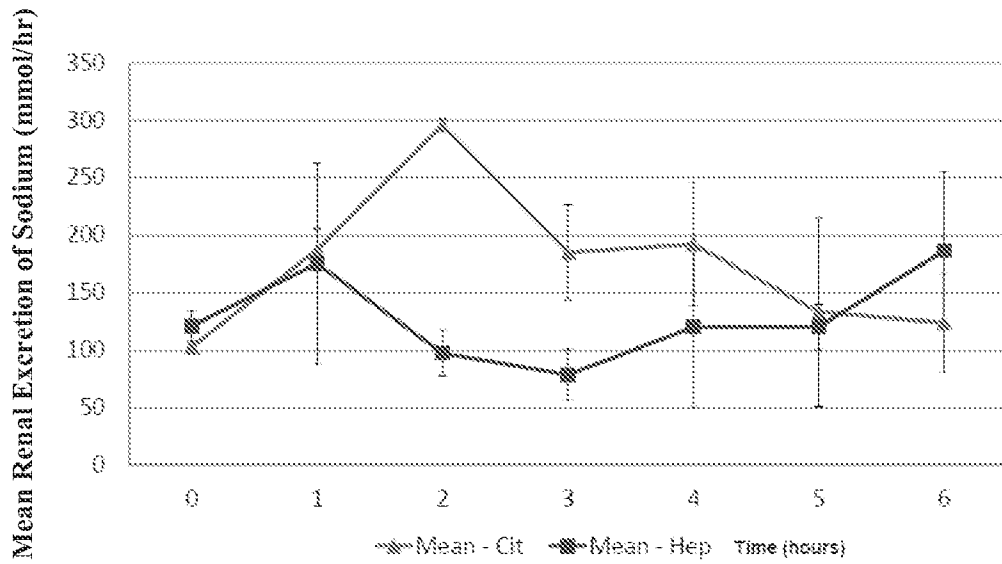


Figure 21A

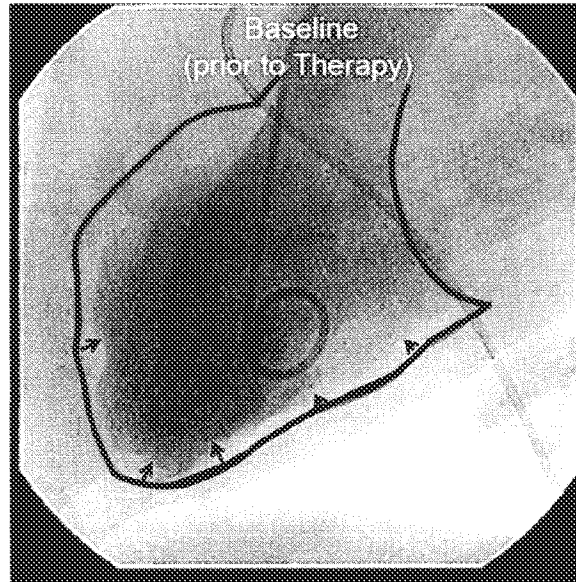
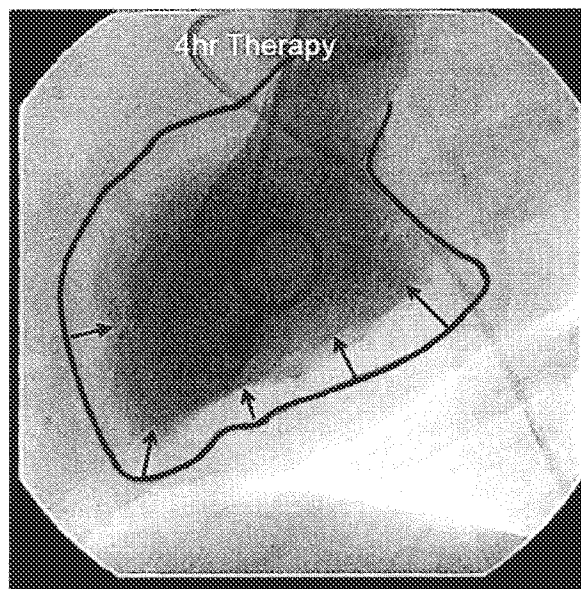


Figure 21B



INTERNATIONAL SEARCH REPORT

International application No. PCT/US2012/059615
--

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61M 1/34 (2013.01)
USPC - 604/4.01
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC(8) - A61M 1/02, 1/34, 1/36; B01D 27/00, 61/00 (2013.01)
 USPC - 210/645; 422/44; 604/4.01, 5.01, 6.01

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
 CPC - A61M 1/3633; 2202/0439 (2013.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 PatBase, Google Patents, Google

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2011/0196280 A1 (HUMES et al) 11 August 2011 (11.08.2011) entire document	1, 29-31, 63, 64, 67, 90 ----- 2-4, 65, 66, 91-93
Y	US 2004/0142463 A1 (WALKER et al) 22 July 2004 (22.07.2004) entire document	2-4, 65, 66, 91-93

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 05 June 2013	Date of mailing of the international search report 17 JUN 2013
---	--

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774
---	---

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/059615

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

- 2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

- 3. Claims Nos.: 5-28, 32-62, 68-89, 94-100
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- 1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
- 2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
- 3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

- 4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.