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(56) Fremdragne publikationer:
US-A1- 2002 107 469
K WONG ET AL: "Nitric oxide infusion alleviates cellular activation during preparation, leukofiltration and storage of platelets", TRANSFUSION AND APHERESIS SCIENCE, vol. 30, no. 1, 1 February 2004 (2004-02-01), pages 29 - 39, XP055002745, DOI: 10.1016/j.transci.2003.07.001
M SCHOLZ ET AL: "Fas/FasL interaction: A novel immune therapy approach with immobilized biologicals", MEDICINAL RESEARCH REVIEWS, vol. 25, no. 3, 1 May 2005 (2005-05-01), pages 331 - 342, XP055002682, DOI: 10.1002/med.20025
A G KIDANE ET AL: "Anticoagulant and Antiplatelet Agents: Their Clinical and Device Application(s) Together with Usages to Engineer Surfaces", BIOMACROMOLECULES, vol. 5, no. 3, 1 May 2004 (2004-05-01), pages 798 - 813, XP055002740, DOI: 10.1021/bm0344553
H D HUMES ET AL: "A Selective Cytopheretic Inhibitory Device to Treat the Immunological Dysregulation of Acute and Chronic Renal Failure", BLOOD PURIFICATION, vol. 29, no. 2, 1 January 2010 (2010-01-01), pages 183 - 190, XP055002785, DOI: 10.1159/000245645
F DING ET AL: "A Biomimetic Membrane Device That Modulates the Excessive Inflammatory Response to Sepsis", PLOS ONE, vol. 6, no. 4, 1 January 2011 (2011-01-01), pages E18584 - E18584, XP055002585, DOI: 10.1371/journal.pone.0018584
S A KOZEK-LANGENECKER ET AL: "Effect of prostacyclin on platelets, polymorphonuclear cells, and heterotypic cell aggregation during hemofiltration", CRITICAL CARE MEDICINE, vol. 31, no. 3, 1 March 2003 (2003-03-01), pages 864 - 868, XP009132291

Fortsættes ...

E BUCHA ET AL: "Hirudin in haemodialysis", THROMBOSIS RESEARCH, vol. 60, no. 6, 15 December 1990 (1990-12-15), pages 445 - 455, XP022878783, DOI: 10.1016/0049-3848(90)90229-6
H D HUMES ET AL: "Replacement of renal function in uremic animals with a tissue-engineered kidney", NATURE BIOTECHNOLOGY, vol. 17, no. 5, 1 May 1999 (1999-05-01), pages 451 - 455, XP009162214, DOI: 10.1038/8626
ANONYMOUS: "Dialysis", WIKIPEDIA, 12 August 2007 (2007-08-12), XP055766677, Retrieved from the Internet <URL:https://en.wikipedia.org/w/index.php?title=Dialysis&oldid=150773177> [retrieved on 20210119]
H D HUMES ET AL: "Experience With Pediatric Medical Device Development", FRONTIERS IN PEDIATRICS, vol. 8:79, 7 April 2020 (2020-04-07), XP055766489, DOI: 10.3389/fped.2020.00079

DESCRIPTION

Description

RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application Serial No. 60/969,394, filed August 31, 2007.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Grant Nos. DK080529 and DK074289 awarded by the National Institutes of Health. The government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention relates to systems and devices, which can be used to treat and/or prevent inflammatory conditions within a subject. More particularly, the present invention relates to systems and devices that can be used to sequester cells associated with inflammation, such as leukocytes and platelets, and then reduce their inflammatory activity.

BACKGROUND

[0004] Various medical conditions are caused, exacerbated, and/or characterized by unwanted inflammation. Infections, such as bacterial, viral, and fungal infections; trauma, such as from falls, automobile accidents, gun and knife wounds; cardiovascular events, such as aneurysms and ischemic events often associated with surgery; and endogenous inflammatory reactions, such as pancreatitis and nephritis, often lead to profound dysfunction of the homeostatic mechanisms involved in regulating cardiovascular and immune system function. Several of these conditions, such as ischemia and infections, through abnormal or excessive activation of the immune system, may result in cardiovascular dysfunction that can develop over a period of hours to days, and which, under certain circumstances, can be life threatening or even fatal.

[0005] Certain cell types are critical to the dysfunction of the cardiovascular and immune systems. For example, leukocytes, especially neutrophils, contribute to the pathogenesis and progression of various inflammatory conditions, including systemic inflammatory response syndrome (SIRS), sepsis, ischemia/reperfusion injury and ARDS (see, e.g., Kaneider et al. (2006) FEBS J 273:4416-4424; Maroszynska et al. (2000) Ann. Transplant. 5(4):5-11). 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. In acute inflammation, activated leukocytes and platelets interact as a gel-like structure within the vessel. This leads to poor perfusion of the tissue, which normally is supplied with oxygen and nutrients by the capillaries. 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. 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] SIRS is the thirteenth leading cause of death in the United States of America. Severe sepsis with SIRS occurs in 200,000 patients annually in the U.S. with a mortality rate of 30-40%, even with use of intensive care units and broad spectrum antibiotics. SIRS is diagnosed largely on observed physiological changes such as increase (fever) or decrease (hypothermia) in body temperature, increased heart rate (tachycardia), increased respiration rate (tachypnea), elevated or diminished white blood cell counts, and inadequate perfusion of tissues and organs. A decrease in blood pressure is a complication associated with SIRS that occurs late in the course of the syndrome. Specifically, a decrease in blood pressure can reflect the development of shock and contribute to multiple organ failure, which is a leading cause of death in these patients. Septic shock is a condition that includes the clinical observations of the presence of an infection and a drop in blood pressure despite fluid resuscitation and proper cardiac blood output. A similar condition, sepsis syndrome, includes similar physiological signals with no evidence of any type of infection. Other insults, which induce a sepsis-like condition include pancreatitis, burns, ischemia, multiple trauma and tissue injury (often due to surgeries and transplants), haemorrhagic shock and immune-mediated organ dysfunction.

[0008] The standard therapies for SIRS and septic shock involve administration of antibiotics to bring the infection under control and fluid/colloid therapy to maintain circulating blood volume. Frequently, drugs that help maintain blood pressure, such as dopamine and vasopressin, are also administered.

[0009] Cardiopulmonary bypass (CPB) strongly induces SIRS, activating complement and coagulation systems and stimulating cytokine production. A large number of therapeutic approaches are under investigation to limit the activation and accumulation of leukocytes during CPB. In fact, animal and early clinical data suggest amelioration of lung and kidney damage during CPB surgery with the use of leukocyte depletion filters (see, e.g., Gu et al. (1996) J. Thorac. Cardiovasc. Surg. 112:494-500; Bolling et al. (1997) J. Thorac. Cardiovasc. Surg. 113:1081-1090; Tang et al. (2002) Ann. Thorac. Surg. 74:372-377; Alaoja et al. (2006) J. Thorac. Cardiovasc. Surg. 132:1339-1347). It appears, however, that dialysis can produce transient neutropenia (see Kaplow et al. (1968) JAMA 203:1135).

[0010] Recent strategies for developing more targeted therapies for the treatment of sepsis have been disappointing. In addition, many molecules in the new generation of anti-septic agents are very expensive and can produce adverse immunological and cardiovascular reactions, which make them contra-indicated in some cases, such as non-bacteremic shock.

[0011] Different devices for blood and their use in therapy are known in the art. For example, it has been demonstrated that the combination of a synthetic hemofiltration device and a renal tubule cell therapy device containing porcine renal tubule cells in an extracorporeal perfusion circuit successfully replaces filtration, transport, metabolic, and endocrinologic functions of the kidney in acutely uremic dogs (see Humes et al. (1999) Nat. Biotechnol. 17:451-455). Further, the usability of recombinant hirudin as an anticoagulant agent in haemodialysis has been investigated in nephrectomized dogs with different capillary dialyzers (see Bucha et al. (1990) Thromb. Res. 60:445-455). Independently, US 2002107469 A1 discloses apheresis devices for sequestering all kinds of blood components including platelets and leukocytes.

[0012] There remains a need for an effective treatment of inflammatory conditions, such as, cardiovascular shock, sepsis, systemic inflammatory response syndrome and anaphylaxis.

SUMMARY OF THE INVENTION

[0013] An inflammatory condition in a subject arises, in part, from the activation of cells associated with inflammation, such as leukocytes and platelets. The present invention relates to systems and devices,which can be used to treat and/or prevent this condition by sequestering leukocytes or platelets and inhibiting or deactivating their inflammatory action. The device of the invention can be used to extracorporeally sequester one or both of leukocytes and platelets and inhibit their inflammatory actions. For example, these cells can be deactivated and/or their release of pro-inflammatory substances can be inhibited. Although there are many ways to practice the invention, one approach is to sequester one or both of leukocytes and platelets in the interior of a device according to the claims, and providing an agent capable of deactivating the cells and/or inhibiting the release of a pro-inflammatory substance. Citrate may be provided to deactivate the cells and/or prevent the release of a pro-inflammatory substance. Experiments conducted using this and other approaches with the device of the present invention provide unprecedented and surprising success in maximizing subject survival. These results exemplify the compelling utility of the device of the invention and its related systems and uses across a range of inflammatory diseases and conditions.

[0014] Accordingly, the invention provides a device that can be used to sequester activated leukocytes and/or platelets from a biological sample, wherein the device comprises a housing containing hollow fibres having an intracapillary space (ICS) and also comprises a passageway in the form of an extracapillary space (ECS) defined by the interior of the housing and the exterior surface of the hollow fibres, an inlet into the ECS and an outlet from the ECS, the device being configured to provide a sufficiently low shear force in said passageway so that, when the device is in use with the biological sample being pumped through the passageway, activated leukocytes and/or platelets from the biological sample will adhere to the exterior surface of the hollow fibres; wherein the ICS of the hollow fibres is capped at one end. In some embodiments, the device is configured to provide an agent to the passageway, wherein the agent is capable of inhibiting the release of a pro-inflammatory substance from the leukocytes or platelets, or deactivating the leukocytes or platelets.

[0015] This aspect of the invention can have one or more of the following features. The leukocyte can be activated and/or primed. The device can be configured in a system which may further include a second device in series with the device defining the passageway. The agent can be associated with a surface of the passageway. In certain circumstances, the agent can be infused into the passageway. The agent can comprise an immunosuppressant, a serine leukocyte inhibitor, nitric oxide, a polymorphonuclear leukocyte inhibitor factor, a secretory leukocyte inhibitor, and a calcium chelating agent, wherein the calcium chelating agent can be citrate, sodium hexametaphosphate, ethylene diamine tetra-acetic acid (EDTA), triethylene tetramine, diethylene triamine, o-phenanthroline, or oxalic acid. However, the agent preferably is a calcium chelating agent, such as citrate.

[0016] The region configured to sequester the leukocyte and/or platelet includes hollow fibres. The hollow fibres can be porous, semi-porous, or non-porous the biological sample being pumped through the passageway. In some embodiments, the hollow fibres are porous, having pores that allow ultrafiltrate to pass into the intracapillary space, but that do not allow leukocytes or platelets from the biological sample to pass into the intracapillary space. In some embodiment, the external surface of the hollow fibres provide a surface area of at least 0.2 m^2 to which activated leukocytes or plaelets can adhere. The device is configured such that the shear force within the passageway is sufficiently low to allow the leukocyte and/or

platelet to adhere to the exterior surface of the hollow fibres, when the device is in use with In some embodiments, the device is configured so that a shear force of less than 1000 dynes/cm^2 is provided in the ECS when the bilogical samples is pumped through the passageway. Alternatively and/or in conjunction, the passageway can comprise a cell-adhesion molecule to allow the leukocyte and/or platelet to remain in the passageway longer than another component of the blood or fluid.

[0017] The device of the invention may be used for processing a leukocyte contained within a body fluid. The processing may include (a) sequestering extracorporeally a primed or activated leukocyte, and (b) treating the leukocyte to inhibit the release of a pro-inflammatory substance from the leukocyte and/or deactivate the leukocyte. The leukocyte can be sequestered for a time sufficient to inhibit release of the pro-inflammatory substance from the leukocyte and/or deactivate the leukocyte, and/or for a prolonged period of time, and/or for at least one hour. The leukocyte produced in step (b) may be returned back to a subject. In step (b), a calcium chelating agent can be used to inhibit release of the pro-inflammatory substance and/or deactivate the leukocyte. Step (a) can be performed using a device of the invention

[0018] The device of the invention may be used for treating a subject at risk of developing or having an inflammatory condition. The treatment may comprise (a) sequestering extracorporeally a primed or activated leukocyte from the subject and (b) treating the leukocyte to reduce the risk of developing inflammation associated with the inflammatory condition or to alleviate inflammation associated with the inflammatory condition. The inflammatory conditions that can be treated include, but are not limited to, systemic inflammatory response syndrome (SIRS), cardiopulmonary bypass syndrome, acute respiratory distress syndrome (ARDS), sepsis, rheumatoid arthritis, systemic lupus erythematosis, inflammatory bowel disease, multiple sclerosis, psoriasis, allograft rejection, asthma, chronic renal failure, cardiorenal syndrome, hepatorenal syndrome, acute organ failure from ischemic reperfusion injury to myocardium, central nervous system, liver, kidney, or pancreas, and acute organ failure due to toxic injury, for example, chemotherapy. Step (a) can be performed using a device of the invention.

[0019] The device of the present invention and its related systems and uses are not limited to a particular type or kind of leukocyte inhibiting agent. The leukocyte inhibiting agent may be any agent that is able to inhibit release of a pro-inflammatory substance from the leukocyte and/or deactivate the leukocyte. Examples of leukocyte inhibiting agents include, but are not limited to, immunosuppressants, serine leukocyte inhibitors, nitric oxide, polymorphonuclear leukocyte inhibitor factor, and secretory leukocyte inhibitor. In some embodiments, the leukocyte inhibiting agent is a calcium chelating agent (e.g., citrate). The present invention is not limited to a particular type or kind of calcium chelating agent, which include, but are not limited to, citrate, sodium hexametaphosphate, ethylene diamine tetra-acetic acid (EDTA), triethylene tetramine, diethylene triamine, o-phenanthroline, oxalic acid and the like.

[0020] It is understood that any of the above-identified aspects or embodiments of the present invention can be equally applied to the sequestration and deactivation or inhibition of leukocytes platelets (e.g., activated platelets), or the combination of leukocytes and platelets. The device of the invention can be used to treat a subject at risk of developing or having an inflammatory condition. The treatment may comprise (a) selectively sequestering extracorporeally a primed or activated cell associated with inflammation from the subject; and (b) treating the cell to reduce the risk of developing inflammation associated with the inflammatory condition or to alleviate inflammation associated with the inflammatory condition. The activated cell associated with inflammation can be selected from the group consisting of a platelet and a leukocyte. The primed cell associated with inflammation may be a leukocyte.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] The foregoing aspects and embodiments of the invention may be more fully understood by reference to the following detailed description and claims.

[0022] Figure 1 is a schematic diagram of a section of devices in a system described herein. In the Figure, (1) primed leukocytes from a subject's blood are (2) activated by an upstream device in the system, for example, a hemofiltration device. In the upstream device, blood flows through the internal space of a hollow chamber and ultrafiltrate (UF) is filtered through the wall of the chamber. Upon exiting the first device, blood then flows inside a second device, for example, a selective cytopheresis inhibitory device (SCID), along the outside of hollow fibers, while UF flows through the internal space of the hollow fibers. The blood flowing along the outside of the hollow fibers is exposed to conditions (3) that permit leukocytes in the blood to be sequestered, for example, by adhering to the external surface of the hollow fibers, thereby facilitating (4) inhibition of release of a pro-inflammatory substance from the leukocytes and/or deactivation of the leukocytes with a leukocyte inhibiting agent, for example, citrate, which decreases ionized calcium (Ca_i).

[0023] Figure 2A is a schematic representation of a system comprising a SCID **555** that is the only device in the system and that includes an intracapillary space (ICS) with both ends capped. **Figure 2B** is a schematic representation of a system similar to **Figure 2A** except that ultrafiltrate (UF) is collected from a SCID **655** having only one end of the ICS capped. **Figure 2C** is a schematic representation of a system comprising a first device, for example, a hemofiltration device **210,** and a SCID **555** that includes an ICS with both ends capped. **Figure 2D** is a schematic representation of a system similar to **Figure 2C** except that ultrafiltrate (UF) is collected from a SCID 655 having only one end of the ICS capped.

[0024] Figure 3 is a schematic representation of a system including a SCID **755** without a cap on its ICS.

[0025] Figures 4A-4F are schematic representations of system configurations as utilized in a CPB circuit. In **Figures 4A-4C,** blood treated by a SCID **555** with the ICS capped at both ends **(Figures 4A and 4B),** or by a SCID **655** with one end capped, is recirculated into the portion of the circuit prior to the venous reservoir **450** and oxygenator **460.** In **Figures 4D-4F,** blood treated by a SCID **555** with the ICS capped at both ends is recombined with blood in the portion of the circuit following the oxygenator **460.** HF/HC represents a hemofilter/hemoconcentrator, P represents a pump **504,** and UF represents a reservoir to collect ultrafiltrate.

[0026] Figure 5 shows a schematic representation of an embodiment of a SCID **555** of the present invention having an ICS with both ends capped.

[0027] Figure 6 shows a schematic representation of an embodiment of a SCID **655** of the present invention having an ICS with one end capped.

[0028] Figure 7 shows a schematic representation of a SCID **755**, which is not claimed herein, having an ICS inlet **745** and ICS outlet **746,** neither of which is capped.

[0029] Figure 8 shows a SCID **855**, which may form part of the present invention.

[0030] Figure 9 shows the mean arterial pressure for porcine model groups treated with a system, as described in Example 1.

[0031] Figure 10 shows the cardiac output in porcine model groups treated with a system, as described in Example 1.

[0032] Figure 11 shows hematocrit levels in porcine model groups treated with a system as described in

Example 1.

[0033] Figure 12 shows survival curves of porcine model groups treated with a system as described in Example 1.

[0034] Figure 13 shows the average total white blood cell counts with time of exposure to a SCID after bacterial challenge in each animal group (n = two to three for each group), as described in Example 1.

[0035] Figures 14A-14D show light micrographs of a SCID containing hollow fiber membranes stained with H&E from three different animals. **Figure 14A** is a low power micrograph showing adherent cells around each hollow fiber (160×). **Figures 14B and 14C** are higher power micrographs demonstrating leukocyte clustering along the outer surface of hollow fibers (400×). **Figure 14D** is a high-power micrograph displaying predominantly polymorphonuclear cells along with mononuclear cells in the adherent cell clusters (1600×).

[0036] Figure 15 is a graph showing the difference in survival rate in subjects treated with a SCID and either citrate or heparin treatment.

[0037] Figures 16A and 16B are graphs comparing the number of white blood cells (WBC) and neutrophils, respectively, in one pump and two pump system configurations

[0038] Figure 17 is a graph showing the amount of platelets in two exemplary system configurations

[0039] Figure 18 is a graph showing the average myeloperoxidase (MPO) levels in animals treated with either a SCID and citrate or a SCID and heparin.

[0040] Figure 19 is a graph showing the expression of CD11b, a neutrophil membrane protein responsible for neutrophil binding to endothelium, in animals treated with either a SCID and citrate or a SCID and heparin.

[0041] Figure 20 is a graph showing the number of neutrophils in the arterial and venous lines of systems described herein, in animals treated with either a SCID and citrate or a SCID and heparin.

[0042] Figure 21 is a graph showing the percentage of septic animals surviving as a function of time in animals treated with either a SCID and citrate or a SCID and heparin.

[0043] Figures 22A-22F are graphs showing the concentration of systemic total white blood cells (WBC), systemic neutrophils, systemic lymphocytes, systemic monocytes, systemic eosinophils, and systemic platelets, respectively, in animals subjected to cardiopulmonary bypass surgery and treated with a system that included a SCID and citrate.

[0044] Figures 23A and 23B are graphs showing systemic and circuit Ca_i, respectively, in animals subjected to cardiopulmonary bypass surgery and treated with a system that included a SCID and citrate.

DETAILED DESCRIPTION

[0045] Cells associated with inflammation, such as leukocytes (or white blood cells) and platelets, normally defend the body against infection and injury. However, many disease states and medical procedures can activate and/or prime these cells, which in turn can produce undesirable immune and inflammatory responses that can be fatal. The present invention relates to a device configured to treat and/or prevent

inflammatory conditions within a subject, and related system and uses. The device of the invention can be used to extracorporeally sequester one or both of leukocytes and platelets and inhibit their inflammatory actions. Specifically, the present invention includes a device for sequestering leukocytes and/or platelets, such as activated and/or primed leukocytes, and inhibiting release of a pro-inflammatory substance from the leukocytes and/or deactivating the leukocytes, which may then be returned to the subject. The device of the invention can also be used for sequestering platelets (e.g., activated platelets) and inhibiting release of a pro-inflammatory substance from these cells, which may then be returned to the subject.

[0046] Although there are many ways to practice the invention, one way is to sequester one or both of leukocytes and platelets in the interior of a device according to the claims that provides a surface with which these cells may associate and to provide an agent capable of deactivating the cells and/or inhibiting release of a pro-inflammatory substance. According to the invention, the device contains hollow fibers, and the cells associate with the exterior of these fibers. Citrate may be provided to deactivate the cells and/or prevent the release of a pro-inflammatory substance. Although the invention is described herein with regard to blood, the invention is applicable to any biological sample that can flow through an extracorporeal circuit, such as any fluid from a subject's body containing these cells. Exemplary extracorporeal circuits are described, for example, in U.S. Patent Serial No. 6,561,997.

1. ***Overview***

[0047] The device of the present invention arose from the unexpected observation that particular device configurations not only can sequester activated and/or primed leukocytes but also can inhibit their inflammatory activity, thereby reducing the multi-organ effects of inflammatory diseases and conditions, such as sepsis and SIRS. These acute effects may also have an influence on chronic pro-inflammatory states, such as the chronic pro-inflammatory state associated with end stage renal disease (ESRD). These devices also showed effective sequestration of platelets. Experiments conducted using embodiments of the present invention provide unprecedented and surprising success in maximizing a subject's survival (see, for example, Example 3) and exemplify the compelling utility of these devices, and their related systems and uses across a range of diseases and conditions for therapeutic, diagnostic, and research applications.

[0048] A schematic representation of one exemplary system is shown in **Figure 1.** As shown, blood is exposed to a first device. Thereafter, the leukocytes become activated (and/or primed). The activated (and/or primed) leukocytes then enter a device, generally referred to as a selective cytopheresis inhibitory device (SCID), wherein the activated leukocytes are sequestered. It is understood that rather than being activated by a first device, the leukocytes may be activated (and/or primed) as a result of a primary patient condition or secondary to other types of medical intervention.

[0049] In other words, in the SCID, the activated (and/or primed) leukocytes from the blood are sequestered, for example, by temporarily adhering to one or more surfaces inside the SCID. 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 SCID that bind leukocytes, for example, activated and/or primed 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 SCID. These sequestered leukocytes then are exposed to an agent, for example, citrate, to deactivate the leukocytes or inhibit their release of pro-inflammatory substances. These systems and devices also can apply to platelets.

[0050] Without being bound by theory, it is believed that calcium chelators, for example, citrate, lead to a low Ca_i environment in the device, 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 leads to an amelioration of the inflammatory state of the leukocytes. In this way, the SCID 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.

[0051] It has been demonstrated that the addition of a calcium chelator, e.g. citrate, to a device of the present invention including a housing containing hollow fibers that sequester leukocytes had the unexpected result of improving a subject's innate immunologic system. Accordingly, the device of the present invention can be used to treat or prevent a variety of inflammatory conditions (either as primary disease states or as a result of medical intervention) by directly treating a subject's blood that includes leukocytes (e.g., activated and/or primed leukocytes) or platelets (e.g., activated platelets). Following treatment, the blood may be returned to the subject.

[0052] The following sections describe (1) configurations of systems that may be used to treat an inflammatory condition, (2) examples of how cells associated with inflammation can be sequestered, (3) examples of how such cells can be deactivated and/or inhibited from releasing a pro-inflammatory substance, and (4) the inflammatory conditions that can be treated using the methods, devices, and systems described herein. 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 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).

2. System Configurations

[0053] As used herein, the term "cytopheresis" or "selective cytopheresis" refers to the sequestration of certain particles from blood. Selective cytopheresis is used to sequester certain cells, such as leukocytes (e.g., activated and/or primed leukocytes) or platelets (e.g., activated platelets) from blood for purposes of facilitating inhibition of release of a pro-inflammatory substance from such cells and/or deactivation of such cells. It should be understood that such inhibition and/or deactivation can occur before, during, and/or after sequestration.

[0054] "Selective cytopheresis device," "selective cytopheresis inhibitory device," "SCD," and "SCID" refer to devices of the present invention that sequester leukocytes (e.g., activated and/or primed leukocytes) and/or platelets (e.g., activated platelets). These devices can also be configured to deactivate and/or inhibit release of pro-inflammatory substances from such cells before, during, and/or after sequestration.

[0055] The SCID devices of the present invention may be configured within a system to accomplish selective cytopheresis. In basic form, the system includes a SCID, a fluid connection for blood to flow from a blood source (for example, a subject, such as a patient) to the SCID, and a fluid connection for treated blood to flow from the SCID to a receptacle (for example, back to the subject). The SCID acts to sequester leukocytes and/or platelets, such as activated and/or primed leukocytes, and facilitate inhibition of release of a pro-inflammatory substance from the leukocytes or platelets and/or deactivate the leukocytes or platelets. Sequestration of leukocytes and/or platelets can be achieved by any technique described in Section 3 below. Inhibition of the release of a pro-inflammatory substance from the leukocytes or platelets and/or deactivation of the leukocytes or platelets can be achieved by any technique described in Section 4 below.

[0056] A system can include a SCID, which optionally can also perform other blood treatments, without

additional treatment devices. See, for example, **Figures 2A-2B** and **Figure 8.** A system can include a SCID, which optionally can perform other blood treatments, as well as additional devices that treat blood. See, for example, **Figures 2C-2D** and **Figures 4A-4F.** For example, the additional devices can filter, oxygenate, or otherwise treat the blood before or after the blood enters the SCID. Moreover, the SCID and/or additional devices in a system can include more than one component for treating blood in other or complementary 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). The device or devices in the system that facilitate selective cytopheresis may be free of such additional components. For example, a SCID of the present invention 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.

2.A. Single Device System

[0057] As mentioned, a system can contain a SCID to accomplish selective cytopheresis and, optionally, other blood treatments without additional treatment devices in the system (see **Figures 2A-2B).** One embodiment of such a SCID is shown schematically in **Figure 5.** In **Figure 5,** a SCID **555** contains a plurality of porous membranes, which are hollow fibers **552** (only one is labeled for clarity). The luminal space within these fibers is called the intracapillary space ("ICS") **540.** In this embodiment, the ICS inlet and ICS outlet are capped **544.** The space **542** surrounding the hollow fibers **552** and within a housing **554** of the SCID **555** is called the extracapillary space ("ECS"). Blood containing leukocytes enters the ECS inlet **548** and moves into the ECS **542** surrounding the fibers **552** (i.e., moves into a passageway). Leukocytes are sequestered in the device, for example, at the external surface of the hollow fibers **552,** 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 ECS inlet **548** or may be infused into the SCID itself via a port. Alternatively, or in addition, the SCID can be prepared with the agent, prior to using the SCID. Flow rates in the ECS **542** 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 **552** to allow leukocytes to associate therewith. In this way, inhibition and/or deactivation of the leukocyte is achieved or initiated. Then, the blood in the ECS exits the SCID via the ECS outlet **550,** which enters into an outflow line.

[0058] Figure 2A shows the exemplary SCID **555** of **Figure 5** in a circuit. 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 ECS inlet **548** and exits the SCID **555** at the ECS outlet **550.** Blood lines at the ECS inlet **548** and outlet **550,** respectively, are attached using blood line connectors with locking mechanisms **256.** Leukocytes are shown sequestered in the ECS **542** at the external surface of the hollow fiber **552.** A blood outflow line from the ECS outlet **550** may return 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. The ICS can contain xenographic or allographic cells, for example, renal tubule cells, cultured in a monolayer on the lining of the ICS **540** of each fiber to further aid in treatment of the blood. Alternatively, the ICS may be cell-free. In the circuit of **Figure 2A,** the lumen **540** of the SCID **555** is filled with saline.

[0059] The circuit of **Figure 2B** includes the same components as **Figure 2A** and operates in the same manner, except that **Figure 2B** utilizes SCID **655** shown in **Figure 6** and ultrafiltrate is produced by this SCID **655.** The SCID **655** contains a plurality of porous membranes, which are hollow fibers **652.** The luminal space within the fibers is the ICS **640** and the surrounding space outside the fibers **652** and within the SCID housing **654** is the ECS **642.** Blood containing leukocytes enters the ECS inlet **648** and moves into the ECS **642** surrounding the fibers **652** and exits at the ECS outlet **650.** Leukocyte sequestration and

inhibition and/or deactivation can be achieved as described above. However, in SCID **655,** only the ICS inlet is capped **644.** The ICS outlet **646** is not capped. Accordingly, depending on the characteristics of the porous hollow fibers **652** (e.g., permeability and pore size), a portion of the blood in the ECS **642** can pass across the hollow fibers **652,** and into the ICS **640** as ultrafiltrate (UF). A tube can be connected to the ICS outlet **646** for collecting ultrafiltrate (UF), which may be discarded as waste.

[0060] A system with a single treatment device, the SCID can be a device as shown in **Figure 8.** Blood enters one end **810** of the SCID **855** and travels through hollow fibers **802** through which ultrafiltrate passes into a hollow space **804.** The filtered blood from the hollow fibers **802** passes into an ECS **806** and surrounds hollow fibers **808** containing ultrafiltrate, which was passed from the hollow space **804.** The blood in the ECS flows over the hollow fibers **808** filled with ultrafiltrate, and leukocytes are sequestered thereon. Flow rates are chosen in the ranges described herein to develop a shear force (in the ranges described herein) at the surface of the ultrafiltrate hollow fibers **808** that permit leukocytes to associate with the fibers. Blood ultimately exits the device at a side port **812,** and ultrafiltrate exits as waste via an end port **813.** The interior of the ultrafiltrate hollow fibers **808** optionally contain renal tubule cells. This SCID can be placed in a circuit as described for the SCID of **Figures 2A-2B.**

[0061] Flow rates and membrane characteristics for the circuits of **Figures 2A-2B** with the SCID of **Figures 5, 6,** or **8** 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 SCIDs shown in **Figures 6** and **8)** may include, for example, flow rates from about 5 mL/minute to about 50 mL/minute.

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

[0062] The SCID may be part of a system with other devices for treating blood. For example, the SCID 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 SCID within the system. When describing the part of the system that is not the SCID, 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 SCID within an extracorporeal blood circuit, and associated blood pumps and tubing can be used to move the blood through the extracorporeal circuit. For example, as shown in **Figures 2C and 2D,** blood may flow 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. The blood then flows through hollow fibers **214** in a conventional hemofilter **210.** Dialysate is infused into the ECS surrounding the hollow fibers **214** and within the hemofilter **210** housing, and dialysis occurs with solutes being removed as "waste" from the blood across the hemofilter filtration membrane **214** (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 **218.** Additionally, molecules and fluid from the blood can pass across the hemofilter filtration membrane **214** (the hollow fibers) as ultrafiltrate, depending on the pore size through the membrane.

[0063] The exemplary system of **Figure 2C** shows a circuit with the SCID **555** of **Figure 5.** Blood exits the hemofilter **210** and enters the SCID **555** at the ECS inlet **548.** The blood then is processed through the SCID, which sequesters leukocytes on the hollow fibers **552** 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 SCID **555** are attached using a connection with a locking mechanism **256.** The blood may then be returned to the subject via a blood outflow line from the ECS outlet

550. Another agent, such as calcium, can be infused at a port **258** on the this blood outflow line in order to prepare the blood for re-entry into the subject. The intracapillary space (ICS) of the SCID 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. Alternatively the ICS may be cell free. In the circuit of **Figure 2C,** the ICS **540** of the SCID 555 is filled with saline and the end ports of the ICS are capped **544.**

[0064] The circuit of **Figure 2D** includes the same components as **Figure 2C** and operates in the same manner, except that **Figure 2D** utilizes the SCID **655** of **Figure 6** and ultrafiltrate is produced by this SCID **655.** The flow of blood through the SCID **655** is described above in the context of **Figure 2B.** Additionally, SCID **655** functions as described above, in the context of **Figure 2B.** As noted above, SCID **655** has only the ICS inlet capped **644.** The ICS outlet **646** is not capped. Accordingly, depending on the characteristics of the porous hollow fibers **652,** a portion of the blood in the ECS **642** can pass across the hollow fibers **652,** and into the ICS as ultrafiltrate (UF). A tube can be connected to the ICS outlet **646** for collecting ultrafiltrate (UF), which may be discarded as waste.

[0065] Without wishing to be bound by theory, it is contemplated that the flow geometry in these SCID systems (and those shown in **Figures 1**, **2A-2B**, **3**, and **4A-4F** allows leukocytes to exist in a low shear force environment in the ECS of the SCID and, therefore, associate with one or more internal surfaces in the SCID, for example, the hollow fibers. Conversely, in a typical use of a hemofiltration cartridge (for example, the first device **210** 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 SCID) that prohibits 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 SCID to sequester potentially damaging and circulating activated leukocytes. These sequestered leukocytes can be treated with a leukocyte inhibiting agent (e.g. citrate).

[0066] 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.

[0067] Rather than both dialysate and ultrafiltrate being produced by the hemofilter (e.g., the hemofilter **210** of **Figures 2C** and **2D),** only ultrafiltrate may be produced. During ultrafiltration, blood is separated into ultrafiltrate, which has been filtered through a medium, such as a membrane, and a retentate, which does not pass through the medium. One example of this type of system is the SCID **755** of **Figure 7** in the system of **Figure 3.** Briefly, in this system the blood flows in through the ECS inlet **748** of the SCID **755,** into the ECS **742** defined by the SCID housing **754** and hollow fibers **752,** and out through the ECS outlet **750** in the SCID **755.** Additionally, an ultrafiltrate line **320** from the hemofilter **210** is in communication with the ICS **740** of the SCID **755** via an ICS inlet **745** and provides ultrafiltrate to the ICS **740.** The filtered blood (in the ECS **742)** and the ultrafiltrate (in the ICS **740)** are separate but can interact with one another across the membranes of the hollow fibers **752.** The ultrafiltrate in the ICS **740** and the filtered blood in the ECS **742** of the SCID **755** can flow in a cocurrent or countercurrent manner. Processed ultrafiltrate exits the ICS **740** at the ICS outlet **746** of the SCID **755** and can be discarded as a waste product. Accordingly, in this system the ICS inlet **745** and ICS outlet **746** are not capped, but the SCID 755 is otherwise substantially the same as the one shown in **Figure 5** and **Figure 6.**

[0068] More particularly, in the system of **Figure 3** using the SCID **755** according to **Figure 7,** blood may be moved from a subject (for example, a patient or any animal) in a blood line. Blood is pumped through the

blood line with a pump **204.** A leukocyte inhibiting agent, such as citrate, can be infused at port **206,** optionally with a pump. The blood then enters hollow fibers of a hemofilter **210** and deposited into the ECS of the hemofilter **210** in a manner described for **Figures 2C-2D** above. Ultrafiltrate is produced across the hollow fibers of the hemofilter **210** and is deposited into the ECS of the hemofilter **210.** The ultrafiltrate then passes through an ultrafiltrate line **320** from the hemofilter **210** and enters the SCID **755** at an ICS inlet **745.** The ultrafiltrate moves through the ICS **740** of the hollow fibers **752** and exits at the ICS outlet **746.** The hollow fibers can be porous, semi-porous, or non-porous membranes.

[0069] The filtered blood remaining in the ICS of the hollow fibers of the hemofilter **210** (i.e., the lumens of the hollow fibers in the hemofilter **210)** exits the hemofilter **210** and is pumped with pump **300** into the ECS inlet **748** of the SCID **755.** Optionally, this pump can be placed on the blood line between the SCID and the subject or a third pump (not shown) can be placed on the blood line between the SCID and the subject. The blood flows into the ECS **742** surrounding the hollow fibers **752** (i.e., moves in a passageway). Leukocytes, such as activated and/or primed leukocytes, are sequestered in the device, for example, at the external surface of the hollow fibers **752.** Blood then exits the SCID **755** at the ECS outlet **750** and may be returned to the subject. Blood line connectors **256** with a locking mechanism attach the blood lines to the ECS inlet **748** and the ECS outlet **750.** Another agent, such as calcium, can be infused at a port **258** on the blood outflow line returning to the subject to prepare the blood for re-entry into the subject. Also, an ultrafiltrate pump **304** moves ultrafiltrate from the ICS **740** to waste. However, depending on the pump flow rates in the system, none, some, or all of the ultrafiltrate can cross the hollow fibers **752** and return to the filtered blood in the ECS **742.**

[0070] The use of the SCID of **Figure 7** in the circuit shown in **Figure 3** has been evaluated in over 100 large animals in pre-clinical testing and in nearly 100 patients in Phase I, IIa, and IIb clinical studies with no unanticipated adverse events related to the SCID and the perfusion circuit. Although the ICS can be cell free, it is understood that this system optionally also can include cells within the ICS **740,** 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 **210,** and through the ECS **742** of the SCID **755** 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) and blood (or leukocyte-containing fluid) can flow in the same direction or in opposite directions.

[0071] The blood flow configuration through the SCID is opposite the blood flow configuration through a typical hemofiltration cartridge. 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 SCID. This unconventional blood flow configuration through the SCID 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 leukocytes in the ECS of the SCID. 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, tests have shown that blood within the interior of a hollow fiber of a hemofilter creates a shear force of 1.5×10^7 dynes/cm^2 while blood flow through the ECS of certain embodiments of a SCID creates a shear force of 5.77 dynes/cm^2, or 10^6 less shear force. For comparison, the shear force at a typical arterial wall is 6 to 40

dynes/cm^2 and the shear force at a typical vein wall is 1-5 dynes/cm^2. Thus, a capillary wall has a shear stress of less than 5 dynes/cm^2.

[0072] Accordingly, the present invention uses a sufficiently low shear force in the passageway so that, when the device in use with the biological sample being pumped through the passageway, activated leukocytes and/or platelets from the biological sample will adhere to he exterior surface of the hollow fibres. For example, a shear force of less than 1000 dynes/cm^2, or less than 500 dynes/cm^2, or less than 100 dynes/cm^2, or less than 10 dynes/cm^2, or less than 5 dynes/cm^2, is useful at an exterior surface of the hollow fibers in the passageway. It should be understood that these shear forces may be useful in any of the SCID embodiments described herein. When a system comprises two devices, such as a hemofilter and a SCID, the difference in shear force between blood flowing in the hemofilter and blood flowing in the SCID can be at least 1000 dynes/cm^2.

[0073] So long as the unconventional flow configuration is followed (i.e., blood flows outside of the hollow fibers, rather than inside the hollow fibers) to yield the requisite shear force, the SCID can be comprised of a conventional 0.7 m^2 polysulfone hemofilter (e.g., Model F40, 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 can use standard dialysis arteriovenous blood tubing. The cartridges and blood tubing can be placed in any dialysate delivery pump system (e.g., Fresenius 2008H) that is currently in use for chronic dialysis.

[0074] In one exemplary system, the system includes tubing which may lead from a subject (a blood line) with a bag of a citrate solution infused into the tubing by an infuser. A first F40 hemofilter cartridge is connected with the blood line at a point after the citrate enters the blood line. Blood in the 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 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 end port outlet, through tubing, to a side port inlet of a second F40 cartridge (i.e., the SCID). The blood flows through the ECS of the second F40 cartridge and exits the cartridge at a side port outlet. Any ultrafiltrate that is produced in the second F40 cartridge enters the ICS and exits through an end port. The other end port of the cartridge is capped. Substantially no blood 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 second F40 cartridge enters tubing where a calcium solution is infused into the blood using an infuser. Finally, the tubing may return the processed blood to the subject. Preferbly, 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. 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.

[0075] 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.

[0076] As mentioned above, infusion of a leukocyte inhibition agent, for example, citrate, can be local to the SCID, regional, or throughout the system. 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

[0077] As shown in **Figures 4A-4F** and as described in Examples 8 and 9 herein, a SCID can be used within a cardiopulmonary bypass (CPB) circuit to treat and/or prevent inflammatory conditions secondary to surgeries (e.g., bypass surgery). **Figures 4A, 4B, 4D, 4E, and 4F** show the SCID of **Figure 5** in exemplary CPB systems. **Figure 4C** shows the SCID of **Figure 6** in an exemplary CPB system. 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.

[0078] As shown in **Figure 4A**, blood may be moved via a venous cannula **400** from a subject into a blood line **410.** Blood flows through the blood line **410,** passing a recirculation junction **420,** which is connected to a SCID outflow line **430.** The SCID outflow line **430** contains blood treated by the SCID device **555.** The blood in the blood line **410** mixes with the SCID-treated blood and continues to a venous reservoir **450** and onto an oxygenator **460** where the blood is oxygenated. The oxygenated blood then flows from the oxygenator **460** to a junction **470** with a SCID inflow line **480.** Here, where a portion of the blood in the blood line **410** is diverted to the SCID **555** via the SCID inflow line **480** for treatment by the SCID **555.** The flow of blood through the SCID inflow line **480** is controlled by a pump **504.** The SCID **555** is designed to sequester select cells associated with inflammation, leukocytes or platelets. In this example, no leukocyte inhibiting agent is added to the blood entering the SCID **555.** Blood containing leukocytes enters the ECS inlet **548** and moves into the ECS **542** surrounding the hollow fibers **552.** Leukocytes are sequestered in the device, at the external surface of the hollow fibers **552.** Flow rates at pump **504** 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 **552** to allow leukocytes to associate therewith. Blood in the ECS **542** exits the SCID via the ECS outlet **550** and enters the SCID outflow line **430.** At junction **470,** a portion of the blood in the blood line **410** also continues to an arterial filter/bubble trap **490**, it may then be returned to the subject at an arterial cannula **495.**

[0079] The circuit in **Figure 4B** flows in the same fashion as the circuit in **Figure 4A,** with the additional features of a citrate feed **435** and citrate pump **436** to add citrate to the blood in the SCID inflow line **480** and a calcium feed **445** and calcium pump **446** to add calcium to the blood in the SCID outflow line **430.** Citrate (or another leukocyte inhibiting agent described herein) is added to the blood flowing into the SCID **555** from the citrate feed **435** 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.

[0080] The circuit in **Figure 4C** functions in a similar fashion as the circuit in **Figure 4B,** with additional features associated with a hemofilter/hemoconcentrator (HF/HC) **476.** Specifically, the portion of the oxygenated blood that is diverted at junction **470** toward the SCID **655** via the SCID inflow line **480** is further split at junction **472** into a portion that flows to the SCID **655** and a separate portion that flows to the HF/HC **476** via a HF/HC inflow line **474.** The HF/HC can filter or concentrate the blood, with ultrafiltrate passing from the device via a waste tube **477.** The filtered or concentrated blood exits the HF/HC **476** via a HF/HC outflow line **479** that returns the filtered or concentrated blood to the SCID outflow line **430** at a junction **444.** The SCID shown in **Figure 4C** is the SCID of **Figure 6,** as described above. Blood flows from the SCID inflow line **480,** into the ECS inlet **648,** through the ECS, out the ECS outlet **650,** and into the SCID outflow line **430.** Ultrafiltrate may be produced across the hollow fibers in the SCID (from the ECS to the ICS), with ultrafiltrate passing from the SCID at the ICS outlet **646** into a waste tube **478.**

[0081] Blood flow to the SCID **655** can be controlled by the pump **504.** Pump **504** is preferred to maintain constant flow in circuits that infuse agents, such as citrate, that inhibit or deactivate the leukocytes, and/or another agent, such as calcium, following SCID treatment. Alternatively, blood flow to the SCID can be controlled by selecting a smaller caliber of the SCID inflow line **480** between junction **472** and the SCID **655** relative to the caliber of the HF/HC inflow line **474,** so that only about 200 mL/5L (about 4% of the flow volume) is diverted to the SCID at the junction **472.** This results in low shear force in the SCID, which can facilitate sequestration.

[0082] The circuits shown in **Figures 4D-4F** are different from the circuits of **Figures 4A-4C** in that they do not recirculate blood within the circuit, for example, at a recirculation junction **420.** Rather, as shown in **Figure 4D,** blood may be moved via the venous cannula **400** from a subject into the blood line **410,** where the blood flows directly to the venous reservoir **450** and onto an oxygenator **460** where the blood is oxygenated. The oxygenated blood then flows from the oxygenator **460** to the junction **470** with the SCID inflow line **480.** Here, a portion of the blood in the blood line **410** is diverted to the SCID **555** via the SCID inflow line **480** for sequestration of leukocytes by the SCID **555,** as described above for **Figure 4A.** Blood exiting the SCID **555** enters the SCID outflow line **430** and mixes with oxygenated blood at junction **422.** After blood from the SCID mixes with blood in the blood line **410** it continues in the blood line **410** to the arterial filter/bubble trap **490**. It may then be returned to the subject at the arterial cannula **495.**

[0083] The circuit in **Figure 4E** flows in the same fashion as the circuit in **Figure 4D,** with the additional features of a citrate feed **435** and citrate pump **436** to add citrate to the blood in the SCID inflow line **480** and a calcium feed **445** and calcium pump **446** to add calcium to the blood in the SCID outflow line **430.** As described for **Figure 4B,** citrate or any other leukocyte inhibiting agent is added to the blood from the citrate feed **435** 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.

[0084] The circuit in **Figure 4F** flows in a similar fashion as the circuit in **Figure 4E,** except that the junction that diverts a portion of the blood from the blood line **410** to the SCID inflow line **480** and the junction which returns SCID-treated blood via the SCID outflow line **430** to the bloodflow line **410,** are positioned after the arterial filter/bubble trap **490** in the circuit. These junctions are labeled **492** and **494,** respectively. **Figure 4F** also depicts other subsystems and features, such as heat exchangers, additional pumps, gas meters and exchangers, and monitors, that can be used in any of the above-identified circuits. Moreover, the SCID in any of the circuits described in **Figures 4A-4F** can be configured with characteristics (e.g., configurations of devices such as the SCID, membrane characteristics, flow rates) in accordance with any embodiment described herein.

2.D. Additional Features of Selective Cytopheresis Inhibitory Devices

[0085] The devices of the present invention may be 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.

[0086] Moreover, the SCID of any embodiment can be oriented horizontally or vertically and placed in a temperature controlled environment. The temperature of a SCID containing cells preferably is maintained at about 37°C to about 38°C throughout the SCID's operation to ensure optimal function of the cells in the SCID. For example, but without limitation, a warming blanket may be used to keep the SCID at the appropriate temperature. If other devices are utilized in the system, different temperatures may be needed for optimal performance.

[0087] The devices of the present invention and their related systems may be controlled by a processor (e.g., computer software). 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 as to leukocyte level and/or increased risk for developing an inflammation disorder). 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 may be processed through a SCID for purposes of reducing the possibility of developing an inflammation disorder. The device or system may automatically processe the subject's blood through the SCID in response to these measurements. Alternatively, a health professional may be alerted to the elevated leukocyte level or increased risk within the subject, and the professional initiates the treatment.

[0088] It is contemplated that the devices of the present invention can be included with various kits or systems. For example, the kits or systems may include the devices of the present invention or various parts of the devices, for example, hollow fiber hemofilter cartridges, leukocyte inhibiting agents (e.g., calcium chelating agents, such as citrate), allographic cells (e.g., renal tubule cells), or other parts. Additionally, the kits or systems may be combined with various surgical instruments necessary for implanting the filtration device into a subject.

3. Sequestration of Cells Associated with Inflammation

[0089] The device of the present invention should be configured to sequester leukocytes and/or platelets from a subject according to the claims. The device and its related systems and uses are not limited to a particular design or technique for facilitating inhibition of release of a pro-inflammatory substance from a leukocyte and/or deactivation of a leukocyte. 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. These terms encompass all types of samples obtained from humans and other animals, including but not limited to, body fluids such as urine, blood, serum, plasma, fecal matter, cerebrospinal fluid (CSF), semen, and saliva, as well as solid tissue. However, these examples are not to be construed as limiting the sample types applicable to the present invention. The term sample in the context of the present specification frequently refers to blood from a subject. The term "blood" refers to any aspect of the blood, for example, whole blood, treated blood, filtered blood, or any liquid derived from blood.

[0090] One or more passageways for flowing a biological sample, or one or more regions thereof, can be configured in any of a variety of ways to sequester leukocytes. If more than one passageway is used, they can be positioned in series and/or in parallel. One or more passageways may be contained within a cartridge, for example a disposable cartridge. A passageway or a passageway region can be defined by any

number of surfaces, for example, 1, 2, 3, 4, 5, 10, 20, 50, 100, or more surfaces. Examples of surfaces include, the interior walls of a device, such as cylindrical device walls and flat device walls, and/or the exterior surfaces of the hollow fibers described herein.

[0091] The surfaces that define a passageway or passageway region can be selected from a variety of forms that sequester leukocytes. For example, flat surfaces (e.g., sheets), curved surfaces (e.g., hollow tubes or fibers), patterned surfaces (e.g., z-folded sheets or dimpled surfaces), irregularly-shaped surfaces, or other configurations can be used in a passageway (or a region thereof) configured to sequester leukocytes. Any of these surfaces may include pores and be porous, selectively-porous, or semi-porous. For example, the surface can be 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 typically is 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 in a passageway or passageway region (that do not define the passageway) 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. These additional surfaces also can be selected from amongst the flat surfaces, curved surfaces, patterned surfaces, irregularly-shaped surfaces, and other configurations described above and the materials described below, and can have the enhancements described below.

[0092] Passageway surfaces or passageway region surfaces (e.g., the external surfaces of hollow fibers) that define and/or are part of a passageway or passageway region configured to sequester leukocytes are not limited to a particular type, kind or size, and may be made of any appropriate material. For example, a surface may be any biocompatible polymer comprising one or more of nylon, polyethylene, polyurethane, polyethylene terephthalate (PET), polytetrafluoroethylene (PTFE), polyarylethersulfone, 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 regenerated by the modified cupramonium process or by means of the viscose process, available from Terumo or Textikombinat (Pirna, GDR) respectively), polyacrylonitrile (PAN), polysulphone, 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 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. Surfaces may include silicon, for example, silicon nanofabricated membranes (see, e.g., U.S. Patent Publication No. 20040124147). Surfaces may include polysulphone 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. Cartridges comprising hollow fibers are not limited to particular dimensions (e.g., length, width, weight, or other dimension).

[0093] The passageway can include any combination of surfaces. For example, the surface(s) of a passageway or passageway region can include any combination of flat, curved, patterned, and/or irregularly shaped aspects. Moreover, a passageway or passageway region can be defined by or otherwise include surfaces of more than one material. Further, a passageway may include two or more regions. These different regions can have the same or different surfaces.

[0094] According to the invention, the device includes a housing containing hollow fibers. A passageway for blood is defined by the interior of the housing and the exterior of the hollow fibers. Leukocytes from the blood associate with a particular region within the passageway, specifically, with the exterior surface of the hollow fibers. Accordingly, 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 along the membrane. 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). 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.

[0095] The device of the present invention and its related systems may be 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. The device may be 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.

[0096] Any technique or combination of techniques that facilitates sequestration of the leukocytes can be used, including, for example, biological, chemical, mechanical and/or physical techniques. In particular, biological or chemical techniques for sequestration can be used. Such techniques include using tissues, cells, biomolecules (for example, proteins or nucleic acids), or small molecules to sequester leukocytes. When a leukocyte is activated, selectins are produced by the leukocyte. 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, 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 Peyers patch adhesion 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 fucosylated oligosaccharides and their precursors. 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.

[0097] In use, any of these biological or chemical materials may be bound to a surface of a device of the present invention (within a passageway of a SCID) to facilitate or enhance sequestration. Alternatively or in combination, any of these materials may be in solution in a device of the present invention. In this instance, the materials may sequester leukocytes in conjunction with additional techniques. For example, these materials may bind leukocytes in solution, agglomerating them to increase 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.

[0098] A device of the present invention may accomplish retention of leukocytes through control of mechanical forces. For example, leukocytes may be sequestered on the exterior surface of the hollow fibres

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). Useful shear forces between the flowing leukocytes and the sequestration surface(s) include a shear force of less than 1000 dynes/cm^2, or less than 500 dynes/cm^2, or less than 100 dynes/cm^2, or less than 10 dynes/cm^2, or less than 5 dynes/cm^2. Exemplary flow rates of blood through systems and devices according to the invention that are useful to achieve these shear forces include, 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.

[0099] A a device may physically retain the leukocytes on the exterior surface of the hollow fibres, by using surfaces such as membranes or filters or by exposing the leukocytes to increased passageway surface area, for example, a surface area greater than about 0.2 m^2, or from about 0.2 m^2 to about 2.0 m^2, or from about 0.5 m^2 to about 1.0 m^2, or about 0.7 m^2, so as to increase the amount of leukocytes that are sequestered and/or the time that a leukocyte is sequestered within the device.

[0100] A system can achieve sequestration by subjecting the leukocytes to a series of devices, for example, 2, 4, 10, 20, 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. The devices may be 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.

[0101] It should be understood that the sequestration techniques described herein also can apply to platelets. In the case of platelets, similar bioglocial, chemical, mechanical and/or physical techniques as described above may be used to sequester platelets. Agents used to sequester platelets may include one or more of glycoprotein Ibα (GPIbα), glycoprotein IIb (GPIIb), glycoprotein IIIa (GPIIIa), CD41, CD61, von Willebrand Factor, β_2-integrin macrophage antigen-1, selectins such as P-selectin, and a cell-adhesion molecule.

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

[0102] The device of the present invention may be configured to inhibit release of a pro-inflammatory substance from leukocytes and/or deactivate leukocytes, such as primed or 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 device is configured to provide an agent to the passageway, wherein the agent can inhibit release of a pro-inflammatory substance from a leukocyte or platelet, and/or deactivate a leukocyte or platelet A leukocyte inhibiting agent can be bound, covalently or noncovalently, to a surface of a passageway, for example, a hollow fiber. Additionally or alternatively, a leukocyte inhibiting agent can be infused into the device or system before, during, or after sequestration of the leukocytes, for example, at or near a membrane surface. As mentioned, the proof-of-concept SCID treated leukocytes with citrate, leading to increased subject survival.

[0103] 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. The leukocyte inhibiting agent may be 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 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 LHGGSPWPPC^{92}QYRGLTSPC^{39}K (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 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.

[0104] Any amount or concentration of leukocyte inhibiting agent can be used to inhibit the 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 from a leukocyte and/or deactivate a leukocyte sequestered within the same passageway or within an adjacent passageway. 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 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-inflammatory substance from the leukocytes and/or deactivate the leukocytes.

[0105] 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 the leukocyte. Moreover, the leukocyte can remain inhibited or deactivated for a period of time following sequestration. 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 a concentration of Ca_i (typically from about 0.20 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. The leukocyte can continue to become or remain inhibited or deactivated for a period of time following exposure to the leukocyte inhibiting agent.

[0106] 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 hours, from 1 to 7 days, one or more weeks, one or more months, or one year or more. The leukocyte inhibiting agent can be applied to the system before or during operation the system. The leukocyte inhibiting agent may be applied during operation of the system and the amount of leukocyte inhibiting agent applied to the system is monitored.

[0107] A leukocyte inhibiting agent can be titrated into the system (e.g., at a port **206** as shown in **Figures 2A-2D** and **3** or from a feed **435** and pump **436** as shown in **Figures 4B, 4C, 4E, and 4F).** 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 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. 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** and **3,** or from a feed **445** and pump **446** as shown in **Figures 4B, 4C, 4E,** and **4F),** to readjust the blood for reentry into the subject. For example, when a calcium chelating agent is used as the leukocyte inhibiting agent, calcium can be added back into the blood before reentry into the subject.

[0108] 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 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 **435** and pump **436).** 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 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;** the analogous location in the CPB situation is shown as a feed **445** and pump **446** in **Figures 4B, 4C, 4E,** and **4F).** The initial calcium infusion can be 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.

[0109] Exemplary citrate and calcium chloride titration protocols are shown in **Table 1** and in **Table 2,** respectively. The target Ca_i range in the SCID may be 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 changed to achieve the target Ca_i range in the SCID. The protocol for doing so is shown below, with infusion occurring at the infusion points described above.

TABLE 1

Citrate Infusion Titration Guidelines	
Circuit Ionized Ca^{2+} (between the SCID 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 2

Calcium Infusion Titration Guidelines	
Patient Ionized Ca^{2+} (drawn systemically front patient)	**Ca^{2+} Infusion (20 mg/mL $CaCl_2$) 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**

[0110] It should be understood that the deactivation techniques described herein also can apply to platelets. Agents used to deactivate a platelet and/or inhibit release of a pro-inflammatory substance from a platelet may 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-phenanthroline, and oxalic acid can deactivate a platelet and/or inhibit release of a pro-inflammatory substance from a platelet.

5. Indications

[0111] The device of the present invention can be used for treating and/or preventing a number of conditions that are associated with inflammation. 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 persistent inflammatory response with pathologic sequelae and/or (ii) infiltration of leukocytes, for example, mononuclear cells and neutrophils, leading to tissue destruction. Inflammatory conditions include primary inflammatory diseases arising within a subject and/or secondary inflammatory disorders arising as a response to a medical procedure. The device of the present invention can be used to treat any inflammatory condition for any subject. 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), a non-human primate, a rodent, and the like, which is to be the recipient of a particular diagnostic test or treatment.

[0112] Leukocytes, for example, neutrophils, are major contributors to the pathogenesis and progression of many clinical inflammatory conditions, including systemic inflammatory response syndrome (SIRS), sepsis, ischemia/reperfusion injury and acute respiratory distress syndrome (ARDS). Several different and diverse types of leukocytes exist; however, they are all produced and derived from a pluripotent cell in the bone marrow known as a hematopoietic stem cell.

[0113] 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 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.

[0114] 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 body's circulation and are derived from megakaryocytes.

[0115] The molecules that are primarily responsible for initiation of leukocyte and platelet 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.

[0116] Accordingly, the device of the present invention can be used to treat and/or prevent any inflammatory condition, including primary inflammatory diseases arising within a subject and/or secondary inflammatory disorders arising as a response to a medical procedure (e.g., dialysis or cardio-pulmonary bypass). Examples of applicable inflammatory conditions, including inflammatory diseases and/or disorders, include, but are not limited to, systemic inflammatory response syndrome (SIRS), cardiopulmonary bypass syndrome, acute respiratory distress syndrome (ARDS), sepsis, systemic lupus erythematosis, inflammatory bowel disease, pancreatitis, nephritis, multiple sclerosis, psoriasis, allograft rejection, asthma, chronic renal failure, cardiorenal syndrome, hepatorenal syndrome, and any acute organ failure from ischemic reperfusion injury to myocardium, central nervous system, liver, lungs, kidney, or pancreas.

[0117] Additional examples of inflammatory conditions include, but are not limited to, transplant (such as organ transplant, acute transplant, xenotransplant) or heterograft or homograft (such as is employed in burn treatment) rejection; ischemic or reperfusion injury such as ischemic or reperfusion injury incurred during harvest or organ transplantation, myocardial infarction or stroke; transplantation tolerance induction; arthritis (such as rheumatoid arthritis, psoriatic arthritis or osteoarthritis); respiratory and pulmonary diseases including but not limited to chronic obstructive pulmonary disease (COPD), emphysema, and bronchitis; ulcerative colitis and Crohn's disease; graft vs. host disease; T-cell mediated hypersensitivity diseases, including contact hypersensitivity, delayed-type hypersensitivity, and gluten-sensitive enteropathy (Celiac disease); contact dermatitis (including that due to poison ivy); Hashimoto's thyroiditis; Sjogren's syndrome; Autoimmune Hyperthyroidism, such as Graves' Disease; Addison's disease (autoimmune disease of the adrenal glands); Autoimmune polyglandular disease (also known as autoimmune polyglandular syndrome); autoimmune alopecia; pernicious anemia; vitiligo; autoimmune hypopituatarism; Guillain-Barre syndrome; other autoimmune diseases; glomerulonephritis; serum sickness; uticaria; allergic diseases such as respiratory allergies (hayfever, allergic rhinitis) or skin allergies; scleroderma; mycosis fungoides; acute inflammatory and respiratory responses (such as acute respiratory distress syndrome and ischemia/reperfusion injury); dermatomyositis; alopecia greata; chronic actinic dermatitis; eczema; Behcet's disease; Pustulosis palmoplanteris; Pyoderma gangrenum; Sezary's syndrome; atopic dermatitis; systemic sclerosis; morphea; trauma, such as trauma from a gun, knife, automobile accident, fall, or combat; and cell therapy, such as autologous, allogenic or xenogenic cell replacement. Additional inflammatory conditions are described elsewhere herein or are otherwise known in the art.

[0118] The device of the present invention may also be used to support the development and use of tissues

and organs *ex vivo.* For example, the present invention may be used to support organ harvesting procedures for transplantation, tissue engineering applications, ex vivo generation of organs, and the manufacture of and use of **b**io-microelectromechanical systems (MEMs).

EXAMPLES

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

[0119] This example describes a series of experiments used to evaluate the treatment of inflammation associated with the conditions of acute sepsis and acute renal failure.

(I) Background and Rationale

[0120] Leukocytes, especially neutrophils, are major contributors to the pathogenesis and progression of many clinical inflammatory disorders, including SIRS, sepsis, ischemia/reperfusion injury and acute respiratory distress syndrome (ARDS). A large number of therapeutic approaches are under investigation to limit the activation and tissue accumulation of leukocytes at sites of inflammation in order to minimize tissue destruction and disease progression. Severe sepsis with SIRS occurs in 200,000 patients annually in the U.S. with a mortality rate of 30-40%, even with use of intensive care units and broad spectrum antibiotics.

[0121] The origins of this research emanate from ongoing encouraging pre-clinical and clinical studies utilizing renal tubule progenitor cells in an extracorporeal device to treat acute renal failure (ARF). ARF arises from acute tubular necrosis (ATN) secondary to nephrotoxic and/or ischemic renal tubule cell injury in a cascade of events culminating in multi-organ failure and death. Mortality rates from ATN requiring renal replacement therapy range from 50 to 70 percent. This high mortality rate has persisted over the last several decades despite greater understanding of the pathophysiology of the disorder and improvements in hemodialysis and hemofiltration therapy.

[0122] The utilization of renal tubule progenitor cells as a therapy for these conditions was based upon the thesis that renal tubule cells play an important immunologic regulatory role in septic shock. Specifically, severe septic shock has been shown to result in acute tubular necrosis (ATN) and ARF within hours of bacteremia in a porcine model of septic shock. Thus, ARF develops early in the time course of septic shock, a time frame not appreciated clinically since it takes several days to observe a rise in blood urea nitrogen and serum creatinine after the acute insult. The loss of the kidney's immunoregulatory function in ARF and ATN results in a propensity to develop SIRS, sepsis, multiorgan failure and a high risk of death. A recent report has demonstrated a rise in sepsis events from 3.3% to nearly 60% in patients who develop ARF during the post-op course following open heart surgery.

[0123] The disorder of ARF, or ATN, may be especially amenable to therapy in conjunction with continuous hemofiltration techniques, since acute hemodialysis or hemofiltration alone has yet to reduce the mortality rate of ATN below 50 percent, despite advances in synthetic materials and extracorporeal circuits. ATN develops predominantly due to the injury and necrosis of renal proximal tubule cells. The early replacement of the functions of these cells during the episode of ATN, which develops concurrently with septic shock, may provide almost full renal replacement therapy in conjunction with hemofiltration. The addition of metabolic activity, such as ammoniagenesis and glutathione reclamation, endocrine activity, such as vitamin

D3 activation, and cytokine homeostasis may provide additional physiological replacement activities to change the current natural history of this disease progression.

[0124] One system used to test the effects of renal tubule progenitor cells on this condition consisted of a filtration device (a conventional high-flux hemofilter) followed in series by a renal assist device (RAD), generally as described in U.S. Patent No. 6,561,997. In those earlier experiments, a RAD referred to an extracorporeal system utilizing a standard hemofiltration cartridge containing human renal epithelial cells grown along the inner surface of the fibers. This arrangement allowed the filtrate to enter the internal compartments of the hollow fiber network, lined with renal tubule cells for regulated transport and metabolic function. Blood pumped out of the subject entered the fibers of the first hemofilter, where ultrafiltrate (UF) was formed and delivered into the lumens of the hollow fibers within the RAD downstream of the hemofilter. Processed UF exiting the RAD was collected and discarded as "urine." The filtered blood exiting the initial hemofilter entered the RAD through the extracapillary space (ECS) port and dispersed among the fibers of the device. Upon exiting the RAD, the processed blood was returned to the subject's body via a third pump. That extracorporeal blood circuit was based upon blood pump systems and blood tubing identical to those used for continuous or intermittent hemodialysis therapy in patients with renal failure.

[0125] *In vitro* studies of renal tubule progenitor cells in the RAD demonstrated that the cells retained differentiated active transport properties, differentiated metabolic activities and important endocrine processes. Additional studies showed that the RAD, when incorporated in series with a hemofiltration cartridge in an extracorporeal blood perfusion circuit, replaced filtration, transport, metabolic, and endocrine functions of the kidney in acutely uremic dogs. Furthermore, the RAD ameliorated endotoxin shock in acutely uremic animals.

[0126] To better understand the immunoregulatory role of renal tubule cell therapy, the tissue-specific consequences of sepsis with or without RAD therapy were evaluated with bronchoalveolar lavage (BAL). BAL specimens were used to assess pulmonary microvascular damage and inflammation in response to SIRS. Preliminary data detailed below demonstrated that renal cell therapy was associated with less protein leak from damaged blood vessels and less inflammation.

[0127] With this experimental model system, the role of renal cell therapy on systemic and tissue-specific inflammatory processes could be more carefully evaluated in a second series of evaluations. At the same time, in the clinical trials evaluating the RAD, a barrier to enrollment was the requirement for systemic anticoagulation with heparin to maintain blood perfusion of the extracorporeal blood lines and dialysis cartridges. Over the last decade, to relieve the requirement for systemic heparinization and better maintenance of blood perfusion in continuous renal replacement therapy (CRRT) circuits, regional anticoagulation with citrate as a calcium binder has become a standard therapeutic modality.

[0128] Thus, a comparison in pre-clinical animal models using sham non-cell cartridges and cell-containing cartridges was performed to confirm that citrate and low Ca_i levels in the blood circuit did not reduce the efficacy of renal tubule cell therapy observed with systemic heparin treatment. As detailed below, citrate anticoagulation in a two-cartridge system showed profound and unexpected results.

(II) Experiment A - Initial Experiment of the Animal Model

[0129] In the following example, an established reproducible model of SIRS in a porcine model of sepsis was employed. (See, e.g., Humes et al. (2003) Crit. Care Med. 31:2421-2428.)

Methods and Materials

[0130] Normal pigs (30-35 kg) were prepared by the introduction of appropriate catheters to assess cardiovascular parameters and treatment with continuous venovenous hemofiltration (CVVH). The pigs then received intraperitoneally 30 × 10^{10} bacteria/kg body weight of *E. coli.* Within 15 minutes after bacteria infusion, the animals were placed in a CVVH circuit with two cartridges, the first being a hemofilter and the second being a renal assist device (RAD) comprising porous, hollow fibers. For this experiment, the RAD refers to the device shown schematically in **Figure 7** in the circuit shown in **Figure 3.** In **Figure 7**, the RAD contains a plurality of membranes, which are hollow fibers **752** (only one is labeled for clarity). The luminal space within the fibers is called the intracapillary space ("ICS") **740.** The surrounding space is called the extracapillary space ("ECS") **742** within a housing **754** of the RAD. Blood containing activated leukocytes enters the ECS inlet **748,** moves into the ECS **742** surrounding the fibers **752,** and exits the RAD via the ECS outlet **750,** which enters into an outflow line. For this experiment, the hollow fibers **752** of the RAD are porous and contain allographic renal tubule cells, cultured in a monolayer on the lining of the lumen **740** of each fiber. The control was a sham-RAD that contained no renal tubule cells but was otherwise the same as the RAD.

[0131] As shown in **Figure 3,** blood exiting the animals was pumped into the fibers of the first hemofilter, where ultrafiltrate (UF) was formed and delivered into the ICS **740** within the RAD hollow fibers **752** downstream of the hemofilter. Processed UF exiting the RAD was collected and discarded as waste using a UF pump **304.** The filtered blood exiting the initial hemofilter entered the RAD through the extracapillary space (ECS) inlet **748** and dispersed among the fibers **752** of the device. Upon exiting the RAD via ECS outlet **750,** the processed blood was returned to the subject's body. The blood moved through the system via blood pumps **204** and **300** placed before and after the hemofiltration device and a third blood pump (not shown in **Figure 3**) placed between the RAD and the animal. Citrate or heparin was added to the system at **206** and, if necessary, a second agent (to prepare the blood for re-entry) was added at **258** before re-entry of the blood into the subject.

[0132] During the first hour following bacteria infusion, animals were resuscitated with volume consisting of 80 mL/kg of crystalloid and 80 mL/kg of colloid (Hepspan). At 15 minutes following bacteria infusion, animals received 100 mg/kg of the antibiotic Ceftriaxone to replicate the clinical situation. No animals received vasopressor or inotropic agents.

Results and Discussion

[0133] Blood pressure, cardiac output, heart rate, pulmonary capillary wedge pressure, systemic vascular resistance and renal blood flow were measured throughout the study. Using this model, it was shown that RAD treatment maintained better cardiovascular performance over controls as determined by cardiac output and renal blood flow. The improved renal blood flow was due to a lower renal vascular resistance in RAD animals compared to controls.

[0134] The improved cardiovascular parameters resulted in greater survival times. Control animals (treated with a sham-RAD, which has no renal tubule cells) all expired within 7 hours, whereas all RAD-treated animals survived greater than 7 hours. The RAD group survived 10 ± 2 hours compared to 5 ± 1 hour in the controls ($p < 0.02$). Plasma levels of interleukin (IL-6), a prognostic inflammatory indication in septic shock, as well as interferon-gamma (IFN-γ), an initiator of the cytokine inflammatory response, were lower in the RAD group compared to the control group.

[0135] The initial data demonstrated that the porcine model was a dependable model of acute septic shock and that RAD treatment improved cardiovascular performance associated with changes in cytokine profiles and resulted in a significant survival advantage. The initial data also demonstrated that RAD therapy can ameliorate the multiorgan dysfunction that arises in septic shock.

[0136] To improve the reproducibility of this model, the volume resuscitation protocol was increased from 100 mL/hour to 150 mL/hour immediately after the crystalloid/colloid bolus infusion at the time of bacteria administration. In addition to this improved resuscitation protocol, the tissue-specific consequences of sepsis with or without RAD therapy were evaluated with bronchoalveolar lavage (BAL) to better understand the immunoregulatory role of renal tubule cell therapy. BAL specimens were used to assess pulmonary microvascular damage and inflammation in response to SIRS. It was shown that renal cell therapy was associated with less protein leak from damaged blood vessels and less inflammation in BAL fluid samples as well as improvement of other cardiovascular effects of SIRS.

[0137] The above described refined animal model utilizing volume resuscitation was used in a series of studies to evaluate if the efficacy of RAD therapy under citrate regional anticoagulation was similar to that under systemic heparin anticoagulation. Thus, the comparison in pre-clinical animal models of sham RAD (non-cell) cartridges and RAD (cell-containing) cartridges was begun to assess whether citrate and low Ca_i levels in the blood circuit negatively affected the efficacy of renal tubule cell therapy observed with systemic heparin treatment.

[0138] Unexpectedly, the results showed that citrate anticoagulation using the RAD without renal cells (i.e., a SCID treated with citrate) was effective in ameliorating the lung damage from SIRS and was almost as effective in reducing the cardiovascular dysfunction and time to death from septic shock in this large animal model, as detailed below.

(III) Experiment B - Large Animal Model Comparison of Systems Utilizing or Lacking Renal Epithelial Cells

[0139] The improved porcine model of septic shock described above was used to assess the multi-organ effects of intervention with a renal assist device (RAD) versus a selective cytopheresis inhibitory device (SCID). In this experiment, both RAD and SCID refer to the device of **Figure 7** in the circuit of **Figure 3,** as described above. However, the RAD system contains porcine renal epithelial cells in the ICS **740** of the RAD **755** and receives heparin anticoagulation treatment. The SCID system contains no cells in the ICS **740** of the SCID **755** and receives citrate treatment (with no heparin). The following data were derived from a total of 14 animals. Seven animals were treated with sham controls, which were the RAD without porcine renal epithelial cells in the ICS and received heparin anticoagulation treatment, denoted as "Sham/Heparin" in **Figures 9-13.** Four animals were treated with a RAD that included porcine renal epithelial cells and systemic heparin therapy, denoted as "Cell RAD" in **Figures 9-13.** Three animals were treated with a SCID that included no cells in the ICS and received citrate regional anticoagulation, denoted as "Sham/Citrate" in **Figures 9-13.**

Observations of Cardiovascular Parameters

[0140] As demonstrated in **Figure 9,** the administration of bacteria described above into the peritoneal cavity induced a rapid, profound, and eventually fatal decline in mean arterial pressure (MAP) in all groups. The early data suggested that the SCID with citrate attenuated the effect on MAP compared to

sham/heparin control. The cardiac outputs (CO) are detailed for each group in **Figure 10.** The CO was substantially higher in the RAD group compared to the other groups. The citrate effect reached significance with more animals, although it was less pronounced than the RAD effect compared to the sham/heparin controls. A similar trend among the groups was observed in stroke volume as well.

[0141] As an approximate measure of systemic capillary leak induced with this septic course, the changes in hematocrit are shown in **Figure 11.** In **Figure 11,** the sham/heparin controls had a higher rate of increase with time, reflective of larger rates of volume loss from the intravascular compartment in the sham control group compared to both the RAD and SCID groups. These changes were associated with a substantial survival advantage in the RAD and SCID groups at this preliminary evaluation stage compared to sham/heparin group (see, **Figure 12**). The average survival times were 7.25 ± 0.26 hours for the sham/heparin group, 9.17 ± 0.51 hours for the SCID (sham/citrate) group, and 9.56 ± 0.84 hours for the RAD (with cells in the ICS space) group. These data indicated that the RAD (with cells in the ICS space), and unexpectedly, the SCID, both improve cardiac output, renal blood flow and survival times compared to the sham/heparin control.

Observations of Inflammatory Parameters

[0142] To investigate the effect of various therapeutic interventions with the porcine SIRS model, bronchoalveolar lavage (BAL) fluid was obtained at the time of death and evaluated for protein content as a parameter of microvascular damage, various inflammatory cytokines and the absolute number of polymorphonuclear cells (PMNs). As summarized in **Table 3,** preliminary data indicated that both RAD and SCID treatments resulted in less vascular damage and protein leak and less inflammatory cytokine release in the early phase of pulmonary involvement in SIRS. Levels of IL-6, IL-8 and tumor necrosis factor (TNF)-α were lower in the treatment interventions versus sham controls. Levels of IL-1 and IL-10 were not different. Absolute neutrophil counts in the sham controls were above 1000 cells/mL, and the RAD and SCID groups trended lower, although the n = 1 or 2 in each group.

TABLE 3

Protein and Cytokine Levels in Bronchoalveolar Lavage (BAL) Fluid from Pigs with Septic Shock					
	Protein (µg/mL)	TNF-α (pg/mL)	IL-1 (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)
Sham Control (n=6)	143 ± 11	21 ± 1	18 ± 2	63 ± 14	126 ± 42
RAD (n=3)	78 ± 10	18 ± 5	18 ± 5	32 ± 6	33 ± 10
SCD (n=2)	110 ± 12	13 ± 2	14 ± 8	33 ± 2	84 ± 62
Note: Mean ± SE. BAL performed at time of death.					

Observations of Leukocyte Sequestration

[0143] The hemodialysis literature suggests that blood circulation through the hollow fibers of a single cartridge results in a transient one-hour neutropenia response (see, e.g., Kaplow et al. (1968) JAMA 203:1135). To test whether blood flow through the extracapillary space of a second cartridge results in higher rates of adherence of circulating leukocytes, total white blood cell (WBC) counts and differentials in the septic animals were measured. The results are shown in **Figure 13.**

[0144] As shown in **Figure 13,** each group had a leukopenia response to the extracorporeal circuit, with a nadir developing at 2 hours and recovering at 7 hours. The average differential counts from baseline to 3 hours in these animals (n = 1-2 in each group, total = 5) are detailed in **Table 4.** All subsets of leukocytes declined, most prominently the neutrophils.

TABLE 4

	Total WBCs	Neutrophils	Lymphocytes	Monocytes
Baseline (hour 0)	15,696	6,422	6,792	306
Nadir (hour 3)	2,740	684	1,856	94
Note: Values are averages from 5 animals: 1 sham control, 2 RAD-treated, and 2 SCD-treated animals.				

[0145] To demonstrate the sequestration of leukocytes in the SCID, **Figures 14A-D** depicts the density of leukocyte adherence to the outer surfaces of the hollow fibers. These images demonstrate the sequestration of leukocytes in the SCID. Normal animals undergoing this treatment protocol do not drop their WBC below 9,000 during an 8-hour treatment course, suggesting that primed or activated leukocytes may be necessary to attach to the second membrane system.

[0146] These data confirm that the RAD improves cardiac output, renal blood flow (data not shown) and survival times compared to sham/heparin controls. Moreover, it was unexpected to find that the use of citrate in combination with a second, low shear force hollow fiber cartridge (i.e., a SCID) had a large anti-inflammatory effect, even though it contained no cells in the ICS.

Example 2. In vitro Studies of Leukocyte Sequestration and Inhibition and/or Deactivation

[0147] The experiment described in this example shows that leukocytes adhered to a dialysis membrane are inhibited and/or deactivated in the presence of citrate. In addition, other data have demonstrated that citrate anticoagulation abolishes degranulation of neutrophils (a calcium-dependent event) during hemodialysis of subjects with end stage renal disease (ESRD). To evaluate this process in more detail and expand it to other leukocyte populations and cytokine release, the following *in vitro* experiments were performed.

Methods and Materials

[0148] Leukocytes were isolated from normal healthy individuals using established methods. The leukocytes (10^6 cells per well) were placed into 12-well tissue culture plates containing 14 × 14 mm squares of polysulfone membranes (Fresenius, Walnut Creek, CA) and allowed to adhere for 60 minutes at 37 °C in RPMI media. The media was removed, cells washed with PBS, and the removed supernatants were analyzed for cell release. RPMI media with citrate (Ca_i = 0.25 mmol/L) or without citrate (Ca_i = 0.89 mmol/L) was used to achieve the Ca_i levels described in **Table 5** below. Each calcium condition also had media with or without lipopolysaccharide (LPS, 1 µg/mL) to activate the leukocytes.

[0149] The cells were exposed to these conditions for 60 seconds and removed from the media to assess release of lactoferrin (LF) and myeloperoxidase (MPO), proteins in exocytotic vesicles from neutrophils, and cytokines, IL-6 and IL-8, released from leukocytes. These compounds were assayed with commercially

available Elisa kits (R & D Systems, Cell Sciences, and EMD BioSciences).

Results and Discussion

[0150] The results are set forth in **Table 5.**

TABLE 5

	Lactoferrin (ng/mL/10^6 cells)		Myeloperoxidase (ng/mL/10^6 cells)		IL-6 (pg/mL/10^6 cells)		IL-8 (ng/mL/10^6 cells)	
	Baseline	Stimulated	Baseline	Stimulated	Baseline	Stimulated	Baseline	Stimulated
Normal Ca_i (0.89 mmol/L)	205	416	437	886	3.9	4.4	29.9	35.0
Citrate Ca_i (0.25 mmol/L)	221	187	268	270	3.3	2.9	25.8	19.4
Note: WBC isolations from two different normal controls; each condition analyzed in duplicate. Baseline was without LPS; stimulated condition was with LPS (1µg/mL).								

[0151] The citrate-containing media with low Ca_i had no increases in LF, MPO, IL-6, or IL-8, in contrast to the normal Ca_i media, which had substantial increases in these inflammatory proteins. These results demonstrate that the stimulation of leukocyte populations adhered to a dialysis membrane are inhibited and/or deactivated in the presence of citrate, which lowers the Ca_i level in the culture media. This low Ca_i level results in a change in cytosolic calcium levels to inhibit multiple inflammatory responses in leukocytes (e.g. release of a pro-inflammatory substance) and/or deactivate leukocytes.

Example 3. Treatment of Inflammation Associated with Acute Renal Failure (ARF) in Humans

[0152] The experiment described in this example shows the unexpected survival rates in human subjects treated with a SCID including hollow fiber tubes is in a system treated with citrate versus those patients treated with a similar device in a system treated with heparin. Specifically, in this experiment, SCID refers to a device of **Figure 7** in the circuit of **Figure 3.** No renal cells were included in the ICS of the SCID.

Background

[0153] The safety and efficacy of renal cell therapy on ten critically ill patients with ARF and multiorgan failure receiving continuous venovenous hemofiltration (CVVH) previously was investigated in Phase I/II trials (see, e.g., Humes et al. (2004) Kidney Int. 66(4):1578-1588). The predicted hospital mortality rates for these patients averaged greater than 85%. The devices used in the previously reported trial were seeded with human renal proximal tubule cells isolated from kidneys donated for cadaveric transplantation but found to be unsuitable for transplantation due to anatomic or fibrotic defects. The results of this clinical trial demonstrated that the experimental treatment could be delivered safely under study protocol guidelines for up to 24 hours when used in conjunction with CVVH. The clinical data indicated that the this system

exhibited and maintained viability, durability, and functionality in the clinical setting. Cardiovascular stability of the patients was maintained, and increased native kidney function, as determined by elevated urine outputs, temporally correlated with the treatment.

[0154] The system in the previous clinical investigation also demonstrated differentiated metabolic and endocrinologic activity. All but one treated patient with more than a 3-day follow-up showed improvement, as assessed by acute physiologic scores. Six of the 10 treated patients survived past 28 days with kidney function recovery, although mortality rates predicted for these 10 patients using the APACHE 3 scoring system were on average 85 percent. Plasma cytokine levels suggested that this cell therapy produced dynamic and individualized responses in patients depending on their unique pathophysiologic conditions.

[0155] The favorable Phase I/II trial results led to subsequent FDA-approved, randomized, controlled, Phase II investigations at 12-15 clinical sites to determine whether this cell therapy approach alters patient mortality. One Phase II study involved 58 patients, of whom 40 were randomized to RAD therapy and 18 made up a control group with comparable demographics and severity of illness by sequential organ failure assessment (SOFA) scores. The early results were as compelling as the Phase I/II results. Renal cell therapy improved the 28-day mortality rate from 61% in the conventional hemofiltration-treated control group to 34% in the cell-treated group (see, e.g., Tumlin et al. (2005) J. Am. Soc. Nephrol. 16:46A). This survival impact continued through the 90- and 180-day follow-up periods ($p < 0.04$), with the Cox proportional hazard ratio indicating that the risk of death was 50% of that observed in the conventional CRRT group. This survival advantage with renal cell therapy was observed for various etiologies of ARF and regardless of organ failure number (1 to 5+) or the presence of sepsis.

Method

[0156] An additional study was undertaken to evaluate a commercial cell manufacturing process and the addition of citrate regional anticoagulation. The results of these patient treatment groups were analyzed to compare the mortality rates in patients treated with a SCID (no cells in the ICS) with systemic heparin anticoagulation or citrate regional anticoagulation. The device used in these experiments is schematically shown in **Figure 7** in the circuit depicted in **Figure 3** as described above. However, for this experiment, the second blood line pump is between the SCID and the subject (not between the devices as shown in **Figure 3**).

Results

[0157] Table 6 shows that the SCID/citrate system yielded marked increases in survival rate at 28 days and between 90 to 180 days.

TABLE 6

	Survival							
	(N)	28 d	(N)	90-180 d	SOFA	OF	MOF	Sepsis
SCID/citrate	9/12	75%	8/12	67%	11.9	3.8	2.6	58%
SCID/heparin	6/12	50%	3/12	25%	12.3	4.1	2.65	58%
Note: SOFA = sequential organ failure assessment; OF = organ failure number; MOF = multiple organ failure number.								

[0158] **Figure 15** graphically shows the marked increase in survival rate between 0 and 180 days in patients treated with devices utilizing citrate ("SCID/citrate") instead of heparin ("SCID/heparin"). These survival differences occurred even though the patient groups had similar activity of disease as measured by SOFA scores and organ failure number. Neither group had cells in the ICS of the second cartridge of the system.

Discussion

[0159] This clinical impact was unexpected. These results provided unprecedented and surprising success in maximizing patient survival. Although these clinical data were derived from patients with ARF, it is contemplated that the observation will apply more generally, for example, to SIRS, ESRD and other inflammatory conditions. Further evaluation into potential mechanisms was accomplished with the histological assessment of non-cell cartridges in the citrate- and heparin-treated groups. Similar to data from the animal models described above, the citrate/SCID system had the external surfaces of the hollow fibers of the second cartridge covered with white blood cells on the blood side of this cartridge. Similar binding was seen in the heparin/SCID system.

Example 4. Treatment of Inflammation with a One-Device System

[0160] In some instances, it may be beneficial to use a treatment system using a single treatment device (i.e. a SCID without other treatment devices). As discussed previously, first treatment device (e.g., a hemofilter) may be used in an extracorporeal circuit that may activate leukocytes (in an unwanted fashion) in addition to performing its primary treatment function. The second treatment device in the series, the SCID, achieves adherence and systemic sequestration in the low-resistance compartment of the ECS (e.g., as shown in **Figures 2C** and **2D**). Thus, if the first treatment device is not needed to perform its primary function, it may be beneficial to remove it and reduce unwanted activation of leukocytes. In other such as sepsis, the circulating leukocytes may already be activated, and a single-device SCID system (e.g., as shown in **Figures 2A** and **2B**) with the blood flow through the low-resistance compartment of the ECS may be adequate for adherence and sequestration of leukocytes. Only a single pump on the bloodline is needed with this circuit, simplifying the therapeutic intervention.

[0161] This experiment evaluates the effectiveness of selective cytopheresis, as well as survival rate and the effect of diminishing and/or preventing an inflammatory response, in a subject (e.g., an animal or human patient having an inflammatory response or at risk for developing an inflammatory response). Specifically, this experiment compares a one-device system having only a SCID in the system with a two-device system having a SCID as well as other system components that treat blood, for example, one or more hemofiltration cartridges in the system. The one-device system can be particularly useful for subjects having or at risk of having conditions such as SIRS, in which leukocyte sequestration and, optionally, leukocyte deactivation and/or inhibition of release of a proinflammatory substance from a leukocyte, is a primary treatment objective for the extracorporcal circuit. A two-device (or multiple-device) system can be useful for subjects in need of more than one treatment using an extracorporeal circuit, for example, for a subject with acute renal failure who needs both kidney dialysis treatment and leukocyte sequestration and, optionally, leukocyte deactivation and/or inhibition of release of a proinflammatory substance from a leukocyte.

[0162] A first test system will include a single SCID as shown in one of **Figures 5** or **6** in the circuit of either **Figure 2A** or **2B,** respectively. A second test system will include a SCID as shown in one of **Figures 5** or **6** in the multi-device circuit of either **Figure 2C or 2D,** respectively. For both test systems, the SCID hollow

fiber cartridge or the entire system will contain citrate. For both systems, ultrafiltrate and cells will not be included in the ICS of the SCID.

[0163] Two groups of subjects (e.g., pigs) will be administered bacteria to induce sepsis and SIRS as described in Example 1 above. Each group will then be treated with one of the test systems and measurements such as those described in Example 1 will be taken. The measurements of the two groups will be compared. In addition to the one-device and two-device system configurations described, other configurations of the devices and systems containing those devices may also be tested.

[0164] It is anticipated that the magnitude of transient leukopenia and neutropenia will be comparable between the one-device and two-device systems. The relationship of WBC counts and influence on cardiovascular and pulmonary functional parameters, systemic and pulmonary inflammatory indicators, and change in leukocyte activation markers across the single- versus two-device systems will confirm whether the simpler single-device system or the two-device system is beneficial in situations not requiring a second treatment device, although it is anticipated that both single-device and two-device configurations will be effective.

Example 5. Comparison of Leukocyte Sequestration Surface Areas

[0165] This experiment evaluates the effectiveness of one or more SCID hollow fiber cartridges having different leukocyte sequestration surface areas in performing selective cytopheresis, to prevent an inflammatory response, and to enhance survival rates in test subjects. Several membrane sizes will be tested. Initial tests will include a comparison of SCID membranes with surface areas of about 0.7 m^2 and about 2.0 m^2, respectively. Additional test groups can include comparisons of membrane surface areas between about 0.7 m^2 and about 2.0 m^2 and/or membrane surface areas greater than about 2.0 m^2.

[0166] In one study, SCIDs having hollow fiber cartridges with various leukocyte sequestration surface areas, as described above, will be prepared. SCIDs of the general design of **Figure 5** will be placed in the circuits of **Figure 2A** or **2C,** or SCIDs of the general design of **Figure 6** will be placed in the circuits of **Figure 2B** or **2D.** Subjects (e.g., pigs) will be administered bacteria to induce sepsis and SIRS as described in Example 1 above. Groups of the subjects will then be treated with one or more of the systems described herein. For each system tested, at least two different SCID membrane surface area sized (e.g., 0.7 m^2 and 2.0 m^2) will be tested. Measurements such as those described in Example 1 will be taken, and the measurements from each of the groups will be compared.

[0167] In another study, subjects (e.g. pigs or calves) undergoing CPB will be studied. Treatment with CPB can cause organ dysfunction, including acute kidney injury (AKI) and acute lung injury (ALI). SCIDs having hollow fiber cartridges with various leukocyte sequestration surface areas, will be tested in a CPB circuit.

[0168] CPB will be performed on subjects as described in Examples 8 and 9 herein with SCIDs configured in circuits as shown in any of **Figures 4A-4F.** For each system, at least two different SCID membrane surface area sizes (e.g., 0.7 m^2 and 2.0 m^2) will be tested. Endpoint measurements will include those described herein, for example, in Example 1 or 8. In addition, the severity of CPB-induced AKI and CPB-induced ALI can be assessed as a function of SCID membrane sequestration surface area.

[0169] It is anticipated that increased membrane surface area will increase leukocyte binding and cause a longer time interval of the leukopenia induced with the SCID. Accordingly, it is anticipated that SCIDs with

larger sequestration surface areas (relative to smaller sequestration surface areas) will improve the effectiveness of selective cytopheresis (e.g., as measured by improved survival rate and/or improved effect of diminishing and/or preventing an inflammatory response) and will have greater beneficial effects on alleviating complications associated with CPB, such as organ injury associated with CPB (e.g., AKI and ALI).

Example 6. A Selective Cytopheresis Device in a Septic Shock Model with Acute Kidney Injury

[0170] The experiments described in this Example describe pre-clinical testing of one-pump and two-pump systems with a SCID and either citrate or heparin administration in a porcine model of septic shock with AKI. The experiments generally were directed to two assessments. First, the experiments assessed the efficacy of utilizing a SCID in a one-pump circuit (e.g., the SCID of **Figure 6** in the circuit of **Figure 2D**) versus a SCID in a two-pump circuit (e.g., the SCID of **Figure 7** in the circuit of **Figure 3**). "One-pump" or "two-pump" refers to the number of pumps on the blood line of a circuit as shown, for example, by pump **204** in **Figure 2D** (a one-pump system) or by pumps **204** and **300** in **Figure 3** (a two-pump system). An advantage to using a one-pump circuit is that existing dialysis equipment can be utilized without additional training or pump systems to deliver care at the bedside. In addition, the experiments assessed the mechanism of action of the SCID to sequester activated leukocytes and inhibit their activation state using citrate versus heparin.

Materials and Methods

[0171] To assess the efficacy of the SCID in a one-pump circuit versus a two-pump circuit, the following two test systems were prepared. First, a one-pump test system included the SCID of **Figure 6** in the circuit of **Figure 2D.** Second, a two-pump test system included the SCID of **Figure 7** in the circuit of **Figure 3.** Both test systems also included citrate or heparin and did not include cells in the ICS of the SCID.

[0172] The experiments in this example utilized the established porcine model of septic shock with associated AKI and multiorgan dysfunction, as described in Example 1. (See, e.g., Humes et al. (2003) Crit. Care Med. 31:2421-2428.) Briefly, two groups of subjects (pigs) were administered bacteria to induce sepsis and SIRS as described in Example 1 above. Each group then was treated with one of the one-pump or two-pump test systems. Each one-pump and two-pump system had two treatment subgroups, treatment with either citrate infusion or heparin infusion. Thus one group of subjects having sepsis and SIRS was treated with the one-pump system and with either citrate or heparin; the other group of subjects having sepsis and SIRS was treated with the two-pump system and with either citrate or heparin.

[0173] White blood cells, neutrophils, and platelets were measured to assess the relative efficacy of the one-pump and two-pump systems. In addition, to assess the mechanism of action for the sequestration and inhibition of activated leukocytes by the SCID with citrate versus heparin, several parameters were measured in systems that used either citrate or heparin. The assessed parameters included myeloperoxidase (MPO) and CD11b, which are indicators of neutrophil activation. For the measurement of CD11b, blood samples from animals were taken and a fluorescent antibody was added that binds to CD11b protein expressed on a leukocyte's cell surface. The white blood cells were separated into various subsets with cell sorting, and the neutrophils in the neutrophil gate were then analyzed by fluorescent intensity, which is proportional to the number of CD11b molecules on the surface that bound the fluorescent antibody. The entire neutrophil population was then analyzed, and the level of activation with CD11b expression was quantitatively assessed as mean fluorescent intensity (MFI). The assessed parameters also included animal survival.

Results

[0174] Figures 16A, 16B, and **17** show results of the effect of the one-pump and two-pump systems on leukocyte counts, neutrophil counts, and platelet counts. Because leukocyte sequestration **(Figure 16A),** neutrophil sequestration **(Figure 16B)** and platelet sequestration **(Figure 17)** were generally the same for citrate-treated and heparin-treated one-pump systems and for citrate-treated and heparin-treated two-pump systems, these figures display an average of the two one-pump subgroups as compare to an average of the two two-pump subgroups. **Figures 18-21** show the results of the citrate-treated or heparin-treated systems. Because the measured characteristic for **Figures 18-21** were generally the same for one-pump and two-pump systems treated citrate and for one-pump and two-pump systems treated with heparin, these figures display an average of the two citrate subgroups as compared to an average of the two heparin subgroups.

[0175] ***Two- pump versus one-pump test system comparison.*** To assess possible effects that pressure and/or flow differences between the one-pump and two-pump circuits might have on the sequestration of leukocytes in the SCIDs of the two test systems, white blood cell (WBC) and neutrophil counts in the systemic blood were examined. The results for the one-pump and two-pump systems relating to WBC and neutrophil counts are shown in **Figure 16A** and **Figure 16B,** respectively. As detailed in the Figures, no difference was observed in these parameters between the one-pump system (n=5) and two-pump system (n=5).

[0176] ***Platelet sequestration.*** The platelet count was also assessed for animals treated with either the one-pump or two-pump systems. As indicated in **Figure 17,** both the one-pump and the two-pump systems with the SCID showed decreased platelet counts for at least 9 hours following treatment with the SCID. These data indicate that systems having a SCID sequester platelets.

[0177] ***Neutrophil activation.*** Activated neutrophils release various enzymes in response to invading microbes or tissue injury to initiate tissue repair. The dominant enzyme released from neutrophil granules is myeloperoxidase (MPO). Accordingly, systemic levels of MPO were measured to indicate the level of neutrophil activation in subjects. **Figure 18** shows that the average MPO levels in animals treated with the SCID and citrate (SCID Mean; n=5) was lower than in animals treated with SCID and heparin (Heparin Mean; n=3). The level of neutrophil activation also was quantitated by measuring the expression of CD11b, a membrane protein responsible for binding onto the endothelium as a first step to exiting the circulation to a site of inflammation. As detailed in **Figure 19,** at hour 6 of sepsis induction, the MFI of neutrophils in the systemic circulation was dramatically increased in the animals treated with the SCID and heparin (Heparin (Systemic); n=4) compared to the animals treated with the SCID and citrate (Citrate (Systemic); n=4).

[0178] The analysis was further refined by assessing neutrophil MFI in the arterial and venous lines of the circuits to obtain an average across the whole circuit. Samples were taken simultaneously from the arterial line of the circuit where blood exits the subject into the bloodline and from the venous line of the circuit where blood exits the bloodline and re-enters the subject. The difference in MFI (arterial-venous) in the heparin group (n=4) and citrate group (n=4) was dramatically different at 3 and 6 hours, as shown in **Figure 20.** This data suggests that citrate infusion suppresses the level of neutrophil activation along the circuit, which can be indicative of less activated circulating neutrophils systemically for the same time periods.

[0179] ***Animal survival.*** The ultimate assessment of the efficacy of the SCID with citrate as compared to the SCID with heparin is the survival effect. As shown in **Figure 21,** a consistent survival time advantage was observed in the citrate group, as compared to the heparin group. The mean survival time for animals treated with the SCID with citrate was 8.38 +/- 0.64 hours (n=8), whereas the mean survival time for animals

treated with the SCID with heparin was 6.48 +/- 0.38 hours (n=11).

[0180] Additional assessments are contemplated. For example, data sets to evaluate the effect of the SCID with systemic heparinization versus regional citrate anticoagulation, or the effect of a one-pump or two-pump system can include: 1. cardiovascular parameters (heart rate; systolic, diastolic, and MAP; cardiac output; systemic vascular resistance, stroke volume; renal artery blood flow; central venous pressure; pulmonary capillary wedge pressure); 2. pulmonary parameters (pulmonary artery systolic and diastolic pressures, pulmonary, vascular resistance, arterial to alveolar O_2 gradient); 3. arterial blood gases (pO_2, pCO_2, pH, total CO_2); 4. complete blood counts (hematocrit (indirect measurement of capillary leak); WBC and Differential); 5. inflammatory indices (systemic serum levels of cytokines (IL-6, IL-8, IL-1, INF-γ, TNF-α)); and 6. pulmonary inflammation by BAL fluid parameters (protein content (vascular leak); total cell counts with differential; TNF-α, IL-6, IL-8, IL-1, INF-γ, neutrophil myeloperoxidase and elastase; alveolar macrophages from BAL fluid and baseline and stimulated levels of cytokines assessed after LPS challenge). In addition, SCID inflammatory parameters (serum levels from pre-hemofilter, pre-second cartridge and post-second cartridge of various cytokines (IL-6, IL-8, TNF-α, IL-1, INF-γ)) and neutrophil exocytotic compounds (myeloperoxidase, elastase and lactoferrin) can be measured to assess leukocyte activity, and simultaneous measurements of these elements also can be made in the UF pre-and post-second cartridge to correlate with the blood and UF compartments during the progression of treatment. Moreover, oxidative markers in serum and BAL fluid can be measured using gas chromatography and mass spectrometry to assess inflammation-induced oxidative stress in the various groups.

Conclusions

[0181] The data from the experiments confirm that an extracorporeal circuit that includes a SCID and citrate treatment can effectively sequester and inhibit the release of a pro-inflammatory substance from, or deactivate, a leukocyte. Specifically, these data show that leukocyte sequestration effects are similar between the one-pump and the two-pump circuits. In addition, the SCID and citrate treatment system diminished the level of neutrophil activation as compared to a SCID and heparin treatment system in a septic shock animal model. The efficacy of the SCID and citrate treatment system resulted in increased survival time in a lethal animal model of sepsis. Moreover, both the one-pump system and the two-pump system effectively sequestered platelets for at least nine hours. Based on this data, it is contemplated that sequestration of platelets and deactivating the platelets and/or inhibiting release of pro-inflammatory substances from the platelets may have beneficial effects similar to those achieved by sequestering leukocytes and deactivating the leukocytes and/or inhibiting release of pro-inflammatory substances from the leukocytes as described throughout the description and examples.

Example 7. Treatment of End Stage Renal Disease in Humans

[0182] The experiment described in this example is designed to evaluate survival rates in human subjects treated with an embodiment of the present invention, namely, a cartridge comprising a hollow fiber tube in a system treated with citrate versus a similar system treated with heparin. The system configuration in this experiment will be the SCID of one of **Figures 5** or **6** in the circuit of one of **Figures 2C** or **2D,** respectively, without cells in the ICS of the SCID. Methods and observations can include a comparison of the citrate versus heparin systems without additional renal cells in the SCID cartridge.

Background

[0183] One example of disease associated with a chronic pro-inflammatory state is end stage renal disease (ESRD). (see, e.g., Kimmel et al. (1998) Kidney Int. 54:236-244; Bologa et al. (1998) Am. J. Kidney Dis. 32:107-114; Zimmermann et al. (1999) Kidney Int. 55:648-658). Dialysis, the predominant therapy, is focused on small-molecule waste removal and fluid balance. However, it does not address the chronic inflammation associated with ESRD. In ESRD patients it is associated with severe morbidity and unacceptably high annual mortality rates of up to 21% (see, e.g., USRD System, USRDS 2001 Annual data report: Atlas of end-stage renal disease in the United States, 2001, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases: Bethesda. p. 561).

[0184] The life expectancy for patients with ESRD averages four to five years. Vascular degeneration, cardiovascular disease, poor blood pressure control, frequent infections, chronic fatigue, and bone degeneration impact significantly on the quality of life and generate high morbidity, frequent hospitalizations, and high costs. The dominant cause of mortality in ESRD patients is cardiovascular disease, accounting for nearly 50% of overall mortality in ESRD (see, e.g., USRD System, USRDS 2001 Annual data report: Atlas of end-stage renal disease in the United States, 2001, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases: Bethesda. p. 561), followed by infectious events.

[0185] ESRD patients develop a chronic inflammatory state that predisposes them to both cardiovascular disease as well as acute infectious complications. ESRD patients are more susceptible to infection despite adequate hemodialysis. Chronic hemodialysis induces a change in the pattern of cytokines equivalent to a chronic pro-inflammatory state (see, e.g., Himmelfarb et al. (2002) Kidney Int. 61(2):705-716; Himmelfarb et al. (2000) Kidney Int. 58(6):2571-2578), independent of membrane activation, inflammation, and clearance. These small proteins can be hemofiltered, but plasma levels are not changed due to increased rates of production (see, e.g., Kimmel *et al.* (1998) *supra;* Bologa *et al.* (1998) *supra;* Zimmermann *et al.* (1999) *supra;* Himmelfarb *et al.* (2002) *supra;* Himmelfarb *et al.* (2000) *supra).* Enhanced exposure to oxidative stress in ESRD patients undergoing hemodialysis further compromises the immune system and enhances susceptibility to infection (see, e.g., Himmelfarb *et al.* (2002) *supra;* Himmelfarb *et al.* (2000) *supra*).

[0186] Clinically, the chronic inflammatory state in ESRD patients is evident by elevated levels of CRP, an emerging clinical marker, along with elevated levels of pro-inflammatory cytokines, including IL-1, IL-6, and TNF-α (see, e.g., Kimmel *et al.* (1998) *supra;* Bologa *et al.* (1998) *supra;* Zimmermann *et al.* (1999) *supra).* All these parameters are associated with enhanced mortality in ESRD patients. Specifically, IL-6 has been identified as a single predictive factor closely correlated with mortality in hemodialysis patients. Each picogram per milliliter increase of IL-6 increases the relative mortality risk of cardiovascular disease by 4.4% (see, e.g., Bologa *et al.* (1998) *supra).* Indeed, growing evidence suggests that the pro-inflammatory state is due to the priming and activation of neutrophils in patients with ESRD (see, e.g., Sela et al. (2005) J. Am. Soc. Nephrol. 16:2431-2438).

Method

[0187] Patients with end-stage renal failure will have their blood treated with an extracorporeal circuit comprising a hemofiltration device, a SCID, and citrate or, as a control, a hemofiltration device, a SCID, and heparin (i.e., the SCID of one of **Figures 5** or **6** in the circuit of one of **Figure 2C** or **2D,** respectively, with citrate or with heparin treatment). The studies may also include within each SCID, renal tubule cells (so that the SCID also acts as a renal assist device or RAD). Blood will flow from the patient to the hemofiltration device, to the SCID, and back to the patient. Appropriate pumps and safety filters may also be included to facilitate flow of the blood back to the patient.

[0188] Data sets to evaluate the effect of the SCID with citrate or heparin will include SCID inflammatory parameters (serum levels from pre-hemofilter, pre-second cartridge and post-second cartridge of various cytokines (IL-6, IL-8, TNF-α, IL-1, INF-γ)) and neutrophil exocytotic compounds (myeloperoxidase, elastase and lactoferrin), which are measured to assess leukocyte activity across the various component cartridges within the SCID. If a circuit with a SCID and UF is used (e.g., the SCID of **Figure 7** in the circuit of **Figure 3**), simultaneous measurements of these elements will also be made in the UF pre-and post-second cartridge to correlate with the blood and UF compartments during the progression of treatment.

Results and Discussion

[0189] It is expected that ESRD patients whose blood is treated with the extracorporeal circuit comprising the SCID and citrate will show significantly better results as compared to ESRD patients treated with the extracorporeal circuit comprising the SCID and heparin. Specifically, it is expected that the pro-inflammatory markers will be lowered in patients receiving SCID with citrate treatment versus those receiving SCID with heparin treatment.

Example 8. A Selective Cytopheresis Device as Part of a Cardiopulmonary Bypass Circuit

[0190] The experiments described in this example employed a single hemofilter cartridge as the SCID (e.g., the SCID shown in **Figures 5** or **6**), which was connected to an extracorporeal circuit with blood flow (200 mL/minute) in a parallel circuit to a larger volume flow circuit. Citrate regional anticoagulation was used to improve both anticoagulation of this parallel circuit as well as a means to deactivate leukocytes, which were sequestered along the outer surface of the membranes within the SCID.

[0191] The protocol included an extracorporeal CPB circuit with a SCID in a calf model. The use of a SCID in each circuit had a temporal correlation to substantive falls in circulating leukocytes, predominantly neutrophils. This decline was sustained throughout the procedures without breakthrough of the sequestration effect. Circuit designs for easy incorporation of the SCID into the existing CPB circuits without safety issues are shown in **Figures 4B** and **4C.**

Background

[0192] Cardiac surgery advances have been absolutely dependent upon the techniques for CPB. Unfortunately, it has been recognized that a systemic inflammatory response occurs in association with CPB, resulting in multiple organ dysfunctions 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 ALI, AKI, bleeding disorders, altered liver function, neurologic dysfunction, and ultimately multiple organ failure (MOF).

[0193] Pulmonary dysfunction is very common after surgery requiring CPB. This acute lung injury can be mild, with postoperative dyspnea to fulminant ARDS. Nearly 20% of patients require mechanical ventilation for more than 48 hours following cardiac surgery requiring CPB. ARDS develops in approximately 1.5-2.0%

of CPB patients with a mortality rate exceeding 50%. Renal dysfunction with AKI is also a common occurrence in adult patients after CPB. Up to 40% of these patients develop rises in serum creatinine and BUN and in the 1-5% requiring dialytic support, the post-operative mortality rate approaches 80%.

[0194] The mechanisms responsible for multiple organ dysfunction following CPB are numerous, interrelated and complex, but growing evidence suggests a critical role in the activation of circulating blood leukocytes, especially the neutrophil, in the development of ARDS in CPB-induced post-pump syndrome. Increasing evidence supports that the acute lung injury in both ARDS and the post-pump syndrome is predominantly neutrophil mediated following PMN sequestration in the lungs. The sequestered and activated PMNs migrate into lung tissue, resulting in tissue injury and organ dysfunction. Therapeutic interventions described in the art that are directed toward leukocyte depletion during CPB have been evaluated both in pre-clinical animal models and early clinical studies. The results with leukocyte-depleting filters of the art have been inconsistent, with no reduction in circulating leukocyte counts during CPB but mild improvement of oxygen requirements. No significant clinical improvement was seen in patients undergoing elective coronary artery bypass graft (CABG) with a leukocyte-depleting filter of the art. In contrast, the systems, devices, and methods of the present invention will have beneficial effects, as described below. Depletion of leukocytes with a blood separator may improve postoperative lung gas exchange function.

Methods and Results

[0195] Surgery was performed on each of three calves, identified as SCID 102, SCID 103, and SCID 107. Each calf (approx. 100 kg) was placed under general anesthesia and connected to a CPB circuit in order to place a ventricular assist device (VAD). CPB was accomplished between 60-90 minutes with cardioplegia and aortic cross-clamping. The SCID was placed at the site depicted in either **Figure 4B** or **Figure 4C,** as identified below for each animal. Results of the three animals (SCID 102, SCID 103, and SCID 107) are summarized in **Figures 22A-22F** and **23A-23B.**

[0196] ***Surgery details and results.*** For SCID 102, the circuit was set up as in **Figure 4B,** with an F40 cartridge (Fresenius Medical Care, Germany) as the SCID in the circuit. As shown in **Figures 22A-22F,** declines were observed in leukocyte and platelet counts. As shown in **Figure 22E,** there was a decline in eosinophil count, which may be important in acute lung injury.

[0197] For SCID 103, the circuit was set up as in **Figure 4B,** with a HPH 1000 Hemoconcentrator (Minntech Therapeutic Technologies, Minneapolis, MN) as the SCID in the circuit. SCID treatment lasted 75 minutes, and an additional sample was taken 15 minutes following the end of SCID treatment. As shown in **Figures 22A-22E,** time-dependent declines in leukocytes were observed. The SCID was disconnected at 75 minutes with a dramatic rebound in neutrophils within 15 minutes. No clotting was observed.

[0198] For SCID 107, the circuit was set up as in **Figure 4C,** with HPH 1000 Hemoconcentrators (Minntech Therapeutic Technologies, Minneapolis, MN) used as each of the SCID and the hemofilter / hemoconcentrator in the circuit. CPB was initiated 15 minutes before the SCID was incorporated and SCID treatment lasted 45 minutes. An additional sample was taken 15 minutes following the end of SCID treatment. As shown in **Figures 22A-22F,** leukocyte and platelet numbers declined before incorporation of the SCID into the circuit, and except for monocytes, declined further with introduction of the SCID. In this surgery, pressure profiles were obtained and a UF flow of 50 mL/minute was demonstrated.

[0199] As shown in **Figures 23A** and **23B,** systemic Ca_i was maintained, and the SCID circuit Ca_i was in the target range. Of general note from these surgeries, no ultrafiltrate (UF) was observed with lower SCID pressures.

Conclusion

[0200] The experiments described in this Example suggest that incorporation of a SCID device into an extracorporeal circuit, such as a CPB circuit, can sequester leukocytes and platelets and enhance the likelihood of a successful clinical outcome during surgery.

Example 9. Treatment of Inflammation Associated with Cardiopulmonary Bypass-Induced Acute Lung Injury (ALI) and Acute Kidney Injury (AKI) in an Animal Model

[0201] As an extension of the experiments described in Example 8, the experiments described in this Example are designed to show efficacy of a device of the present invention to sequester leukocytes and inhibit their inflammatory action in the treatment of CPB-induced ALI and AKI.

[0202] Specifically, the aim of this Example entails optimizing a SCID protocol that effectively treats CPB-induced ALI or AKI. To achieve this goal, animals can be treated with any of the CPB circuits described in **Figures 4B, 4C, 4E,** or **4F,** each of which includes a SCID and citrate feed. Alternatively, CPB circuits described in **Figures 4A** or **4D** can be tested, each of which includes a SCID without citrate infusion. Moreover, a SCID used during CPB can be replaced with a fresh SCID while the treatment is occuring, and/or one or more SCIDs can be placed in series or in parallel in the "SCID" location of any of **Figures 4A-4F.**

[0203] A variety of porcine models have been reported in the literature to assess the mechanisms and therapeutic interventions of CPB-induced ALI. For example, it has been demonstrated in prior porcine models that demonstrable ALI can be incrementally induced with additive insults, which include the following: (1) the length of time for CPB from 60 to 120 minutes; (2) aortic cross clamping and cardiac cold cardioplegia producing ischemia/reperfusion injury; (3) cardiotomy suction with open reservoirs promoting activation of blood elements (leukocytes, platelets, and complement); and (4) endotoxin infusion post CPB promoting a SIRS response similar to that observed in patients due to detectable levels of endotoxin post CPB, presumably due to gastrointestinal barrier dysfunction following cardiac surgery and mild ischemia/reperfusion injury.

[0204] An established porcine protocol of CPB-induced ALI with significant changes in pulmonary function and molecular markers in bronchoalveolar (BAL) fluid within 3.5 hours following CPB and 2 hours post sequential lipopolysaccharide (LPS; 1 µg/kg over 60 minutes) has been reported. This reported protocol uses a femoral-femoral hypothermic bypass procedure followed by a 60-minute LPS infusion beginning 30 minutes after CPB was discontinued. Lung parameters were measured up to 2 hours following these sequential insults, with significant injury parameters observed. Other protocols could be developed to produce measurable ALI in 4 hours while being more reflective of clinical practice with CPB.

[0205] This example will use a clinically relevant model of ALI and AKI utilizing 60 minutes of CPB, aortic cross clamping and cardiac hypothermic cardioplegia as the baseline protocol, along with cardiotomy and cardiac suctioning during CPB into an open venous reservoir to promote incremental insults. If this is not sufficient to cause measurable ALI and AKI, then a 30-60-minute infusion of *E. Coli* LPS (0.5-1.0 µg/kg) beginning 30 minutes following completion of CPB will be added. The general approach to this CPB porcine model is detailed below.

CPB Protocol

[0206] In one exemplary protocol, Yorkshire pigs (30-35 kg) are premedicated with IM atropine (0.04 mg/kg), azaperone (4 mg/kg), and ketamine (25 mg/kg), and then anesthetized with 5 µg/kg of fentanyl and 5 mg/kg of thiopental. After intubation with an 8-mm endotracheal tube (Mallinckrodt Company, Mexico City, Mexico), the pigs are placed in the supine position. Anesthesia is maintained by continuous infusion of 5 mg/kg/hour of thiopental and 20 µg/kg/hour of fentanyl. Muscle relaxation is induced with 0.2 mg/kg of pancuronium followed by intermittent reinjections of 0.1 mg/kg to achieve optimal surgical and ventilatory conditions.

[0207] Ventilation is established using a volume cycle ventilator at 10 mL/kg total volume and an inspired oxygen fraction of 1 with no positive end expiratory pressure. Polyethylene monitoring lines are placed in the external jugular vein and the femoral artery and vein. Esophageal and rectal temperature probes are inserted. Median sternotomy is performed. A 16 or 20 mm Transonic perivascular flow probe is placed on the main pulmonary artery, and Millar microtip pressure transducers are placed in the pulmonary artery and left atrium. Prior to initiating CPB, baseline pulmonary artery pressure and flow rate and left atrial pressure readings are taken for determination of cardiac output. After systemic heparinization (300 U/kg), an 18F Medtronic DLP arterial cannula is placed in the ascending aorta and a 24F Medtronic DLP single-stage venous cannula is placed in the right atrium.

[0208] The CPB circuit is primed with 1,000 mL of lactated Ringer's solution and 25 mEq of $NaHCO_3$. The circuit consists of a Medtronic Biomedicus Centrifugal blood pump, a Medtronic Affinity hollow fiber oxygenator with integral heat exchanger, and a cardiotomy reservoir. A Medtronic Affinity 38-µm filter is placed in the arterial limb to capture particulate debris. The left ventricle is vented using a 12-Ga Medtronic standard aortic root cannula with vent line connected to a Sarns roller pump and the cardiotomy reservoir. Scavanged blood is salvaged with a cardiotomy suction catheter, also connected to the Sarns roller pump and the cardiotomy reservoir. Cardiopulmonary bypass is initiated, ventilation is discontinued, and systemic perfusion maintained at 2.4 L/min/m^2 body surface area. Moderate perfusion hypothermia (32°C rectal temperature) is used, and mean aortic pressure kept at 60-80 mmHg by modification of flow and intravenous phenylephrine infusion (0-2 µg/kg/min). The ascending aorta is cross clamped, and cardioplegia is delivered into the aortic root cannula at 7 degrees, consisting of the University of Michigan standard cardioplegia solution diluted with blood at a 4:1 ratio. The solution consists of citrate phosphate and dextrose (CPD), tromethamine, and potassium chloride. A total dose of 1 L of cardioplegia is delivered, and 500 mL is repeated every 20 minutes. Systemic rewarming is started after 40 minutes, and extracorporeal circulation discontinued after 60 minutes (clamping time 45 minutes). Prior to weaning from CPB, the lungs are inflated to 30-cm H_2O airway pressure for 10 seconds for three breaths, and the mechanical ventilation is resumed using the same ventilator settings. During weaning from CPB, an infusion of epinephrine (0-1 µg/kg/minute) is titrated to maintain aortic blood pressure within normal ranges. Within 30 minutes of discontinuation of extracorporeal circulation, the blood in the oxygenator is transferred back into the circulation, heparin is reversed by protamine (1 mg for 100 U heparin) and normothermic rectal temperature achieved. Physiologic measurements are recorded before and during CPB and for 4 hours after CPB.

Extracorporeal Circuit

[0209] With a porcine model of CPB with substantive changes reflective of ALI and AKI, the influence of the SCID in ameliorating organ injury can be directly tested. A single-cartridge SCID will be placed in a parallel circuit after the membrane oxygenator (as shown, for example, in **Figure 4F**). It is contemplated that the membrane oxygenator will activate circulating leukocytes, which are then sequestered in the SCID. Citrate

will be added to the regional SCID parallel blood circuit to lower blood ionized Ca_i to target levels, for example, about 0.2 to about 0.4 mM, with Ca^{2+}reinfusion at the end of the parallel circuit. Two groups of animals will be evaluated and compared. The first group will receive SCID and heparin anticoagulation, and the second group will receive SCID and citrate anticoagulation. Each group will have six animals, with initial analysis of the two groups after 3 animals from each group have been treated. Regional citrate anticoagulation will be achieved utilizing standard practice solutions and clinical protocols. Citrate acts as an anticoagulation agent by binding with calcium. The bound calcium is then unavailable to trigger clotting factors. Calcium is added to the bloodstream just before the blood is returned to the animal in order to restore systemic Ca_i levels that will allow adequate coagulation and cardiac function.

[0210] The current standard protocol used for continuous renal replacement therapy for citrate anticoagulation will be used. The ACD-A citrate IV solution (Baxter Healthcare) will be connected to a citrate infusion pump and the line to the SCID blood infusion port prior to the SCID. Calcium will be administered into the returned blood after the SCID via an infusion port to restore systemic calcium. Citrate infusion fluid rate (mL/hour) will be 1.5 times the blood flow rate (mL/minute) to achieve an Ca_i level pre-cartridge between 0.2 and 0.4 mmol/L.

[0211] The SCID blood flow rate is targeted to be 200 mL/minute and will be controlled with a pump placed in the blood circuit pre-SCID set at a flow rate of 200 mL/minute. Calcium chloride (20 mg/mL, 0.9% N.S.) will be infused into the blood line post-SCID to achieve an Ca_i level in the system (animal bloodstream values) between 0.9 and 1.2 mmol/L. Initial Ca^{2+} infusion rate is 10% of the citrate infusion rate. Ca_i levels will be evaluated in the arterial end of the CPB circuit prior to the pump system to reflect systemic Ca_i levels and in the venous end of the SCID parallel circuit. All Ca_i will be measured with an i-STAT® diagnostic device (Abbott Labs).

Measurement of Acute Lung Injury (ALI)

[0212] ***Pulmonary Function.*** ALI following CPB results in increases in alveolar-arterial oxygenation gradients, intrapulmonary shunt fraction, pulmonary compliance and pulmonary vascular resistance. These parameters will be measured every 30 minutes during the 4 hour post-CPB period.

[0213] ***Lung Tissue Analysis.*** ALI in the post-pump syndrome is associated with neutrophil accumulation in the lung and increases in interstitial fluid. Neutrophil aggregation will be assessed at the end of the research protocol by obtaining lung tissue from a segment not used for BAL. Samples of tissue will be used for myeloperoxidase tissue activity reflective of tissue neutrophil infiltration, histologic processing for semiquantitative neutrophil counts, and water weight in lung tissue, with the difference in weights prior to and after desiccation and expressed as percent of wet weight [(wet weight - dry weight) / wet weight].

[0214] ***BAL Fluid Analysis.*** BAL fluid is obtained by cannulation of the right middle lobe of the lung with three successive infusions of 20 mL of normal saline and gentle aspiration. The fluid is evaluated for protein content (reflective of microvascular injury) and cytokine concentration (IL-1, IL-6, IL-8, IL-10, IFN-γ, and TNF-α). Cell counts in the BAL fluid are determined after a cytospin with cytology staining to provide the total and percentage of various cell components, including epithelial, neutrophil, and macrophage/monocyte. Alveolar macrophages will be isolated, incubated overnight and their cytokine response to LPS evaluated the next day. Fluid levels of matrix-metalloproteinase-2 and -9, elastase, and myeloperoxidase are measured with well-established assays as a reflection of activated neutrophil-secreted products important in developing tissue injury.

Measurement of Acute Kidney Injury (AKI)

[0215] Recent clinical data have clearly demonstrated that neutrophil gelatinase-associated lipocalin (NGAL) is an early biomarker for AKI following CPB. The amount of NGAL in the urine and serum at 2 hours following CPB is a highly specific and sensitive predictive marker of AKI with subsequent increases in serum creatinine and BUN. Serum and urine will be collected at baseline, time of CPB discontinuance and q one hour after CPB in all animals. NGAL levels will be determined by a sensitive ELISA assay for pig. Differences in NGAL levels should reflect the degree of AKI in this animal model.

[0216] Serum chemistries will be measured with an automated chemical analyzer. Cytokine levels will be measured with commercial ELISA assay kits reactive to porcine cytokines: IL-1, IL-6, IL-8, IL-10, IFN-γ and TNF-α (R & D Systems). BAL fluid will be obtained for cell counts and cell-type distribution, protein as a measure of vascular leak, and cytokine levels, including IL-1, IL-6, IL-8, IL-10, IFN-γ and TNF-α

[0217] Cardiovascular and biochemical data will be analyzed by repeated-measures analysis of variance (ANOVA). Plasma levels of various moieties, and survival times will be compared utilizing Student's T-test, paired or non-paired as appropriate.

[0218] It is contemplated that animals receiving citrate regional anticoagulation in the CPB system that includes a SCID will have less pulmonary dysfunction, lung inflammation, and AKI as measured with NGAL. It is also contemplated that the degree of systemic WBC count with neutropenia and leukopenia will nadir at 3 hours but be of the same magnitude in both groups. It is also contemplated that the release of leukocytic inflammatory indices will be inhibited in the citrate versus heparin groups.

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patent documents cited in the description

- US6939407P [0001]
- US2002107469A1 [0011]
- US6561997B [0046] [0124]
- US20040124147A [0092]

Non-patent literature cited in the description

- **KANEIDER et al.**FEBS J, 2006, vol. 273, 4416-4424 [0005]
- **MAROSZYNSKA et al.**Ann. Transplant., 2000, vol. 5, 45-11 [0005]
- **GU et al.**J. Thorac. Cardiovasc. Surg., 1996, vol. 112, 494-500 [0009]
- **BOLLING et al.**J. Thorac. Cardiovasc. Surg., 1997, vol. 113, 1081-1090 [0009]
- **TANG et al.**Ann. Thorac. Surg., 2002, vol. 74, 372-377 [0009]
- **ALAOJA et al.**J. Thorac. Cardiovasc. Surg., 2006, vol. 132, 1339-1347 [0009]
- **KAPLOW et al.**JAMA, 1968, vol. 203, 1135- [0009] [0143]
- **HUMES et al.**Nat. Biotechnol., 1999, vol. 17, 451-455 [0011]
- **BUCHA et al.**Thromb. Res., 1990, vol. 60, 445-455 [0011]
- Dialyzers**SALEMMUJAIS**Dialysis TherapyHanley & Belfus, Inc.19930000 [0092]
- **TSCHESCHE et al.**J. Biol. Chem., 1994, vol. 269, 4830274-80 [0103]
- **HORL et al.**PNAS USA, 1990, vol. 87, 6353-57 [0103]
- **TAKASHI et al.**Am. J. Respirat. Cell and Molec. Biol., 2006, vol. 34, 647-652 [0103]
- **BALKE et al.**FEBS Letters, 1995, vol. 371, 300-302 [0103]
- **TOLWANI et al.**Clin. J. Am. Soc. Nephrol., 2006, vol. 1, 79-87 [0107]
- **HUMES et al.**Crit. Care Med., 2003, vol. 31, 2421-2428 [0129] [0172]
- **HUMES et al.**Kidney Int., 2004, vol. 66, 41578-1588 [0153]
- **TUMLIN et al.**J. Am. Soc. Nephrol., 2005, vol. 16, 46A- [0155]
- **KIMMEL et al.**Kidney Int., 1998, vol. 54, 236-244 [0183]
- **BOLOGA et al.**Am. J. Kidney Dis., 1998, vol. 32, 107-114 [0183]
- **ZIMMERMANN et al.**Kidney Int., 1999, vol. 55, 648-658 [0183]
- USRD System, USRDS 2001 Annual data report: Atlas of end-stage renal disease in the United StatesNational Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases20010000561- [0183] [0184]
- **HIMMELFARB et al.**Kidney Int., 2002, vol. 61, 2705-716 [0185]
- **HIMMELFARB et al.**Kidney Int., 2000, vol. 58, 62571-2578 [0185]
- **SELA et al.**J. Am. Soc. Nephrol., 2005, vol. 16, 2431-2438 [0186]

Patentkrav

1. Indretning, der kan anvendes til at sekvestrere aktiverede leukocytter og/eller blodplader fra en biologisk prøve, hvor indretningen omfatter et hus indeholdende hule fibre med et intrakapillært rum (ICS) og også omfatter en passage i form af et ekstrakapillært rum (ECS) defineret af husets indre og den ydre overflade af de hule fibre, et indløb til ECS og et udløb fra ECS, hvor indretningen er konfigureret til at tilvejebringe en tilstrækkelig lav forskydningskraft i passagen, således at aktiverede leukocytter og/eller blodplader fra den biologiske prøve vil klæbe til den ydre overflade af de hule fibre, når indretningen er i brug med den biologiske prøve, der pumpes gennem passagen; hvor de hule fibres ICS er afdækket i den ene ende.

2. Indretning ifølge krav 1, hvor de hule fibres ICS er afdækket i begge ender.

3. Indretning ifølge et hvilket som helst af de foregående krav, der er konfigureret således, at en forskydningskraft på mindre end 1000 dyn/cm^2 leveres i ECS, når den biologiske prøve pumpes gennem passagen.

4. Indretning ifølge et hvilket som helst af de foregående krav, der er konfigureret således, at en forskydningskraft på mindre end 100 dyn/cm^2 leveres i ECS, når den biologiske prøve pumpes gennem passagen.

5. Indretning ifølge et hvilket som helst af de foregående krav, der tillader aktiverede leukocytter og/eller blodplader fra den biologiske prøve at klæbe til den ydre overflade af de hule fibre, når den biologiske prøve pumpes gennem passagen med en strømningshastighed på fra ca. 100 ml/minut til ca. 500 ml/minut.

6. Indretning ifølge et hvilket som helst af de foregående krav, hvor de hule fibre er porøse og har porer, der tillader ultrafiltrat at passere ind i det intrakapillære rum, men som ikke tillader leukocytter eller blodplader fra den biologiske prøve at passere ind i det intrakapillære rum.

7. Indretning ifølge et hvilket som helst af de foregående krav, hvor fibrenes ydre overflader har et overfladeareal på mindst 0,2 m^2 som aktiverede leukocytter eller blodplader kan klæbe sig til.

8. Indretning ifølge et hvilket som helst af de foregående krav, der er konfigureret til at tilføre et middel til den nævnte passage; hvor midlet er i stand til at deaktivere aktiverede leukocytter eller blodplader eller til at hæmme leukocytter eller blodplader i at frigive et inflammatorisk stof.

9. Indretning ifølge krav 8, hvor midlet er et calciumchelateringsmiddel.

10. Indretning ifølge et hvilket som helst af de foregående krav, hvor den biologiske prøve er blod.

11. Indretning ifølge et hvilket som helst af de foregående krav, der har leukocytter og/eller blodplader, der er klæbet til den ydre overflade af de hule fibre.

DRAWINGS

Drawing

Figure 1

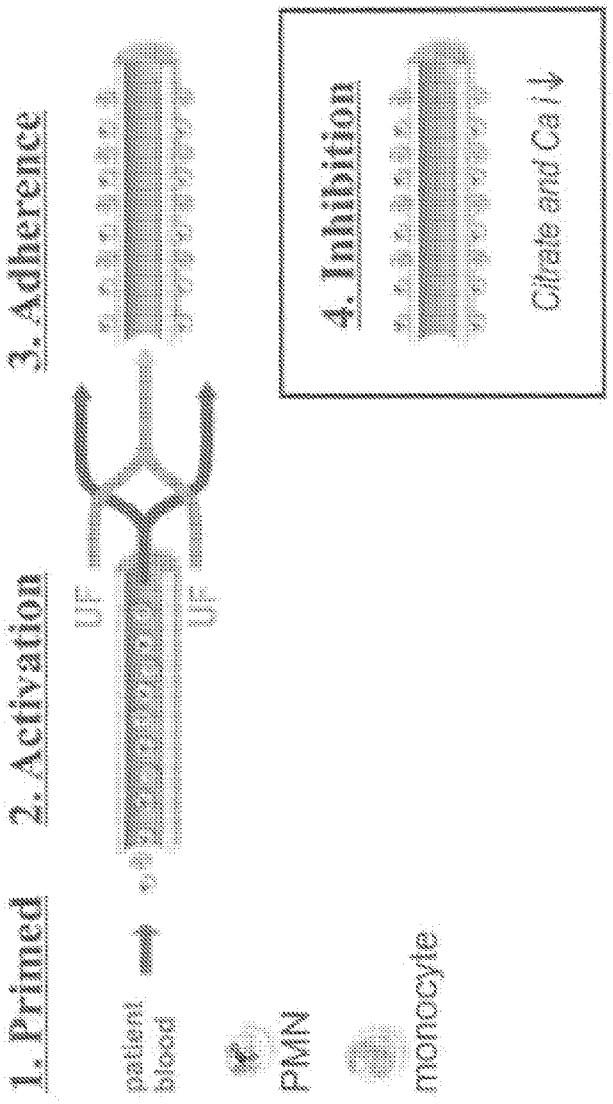


Figure 2A

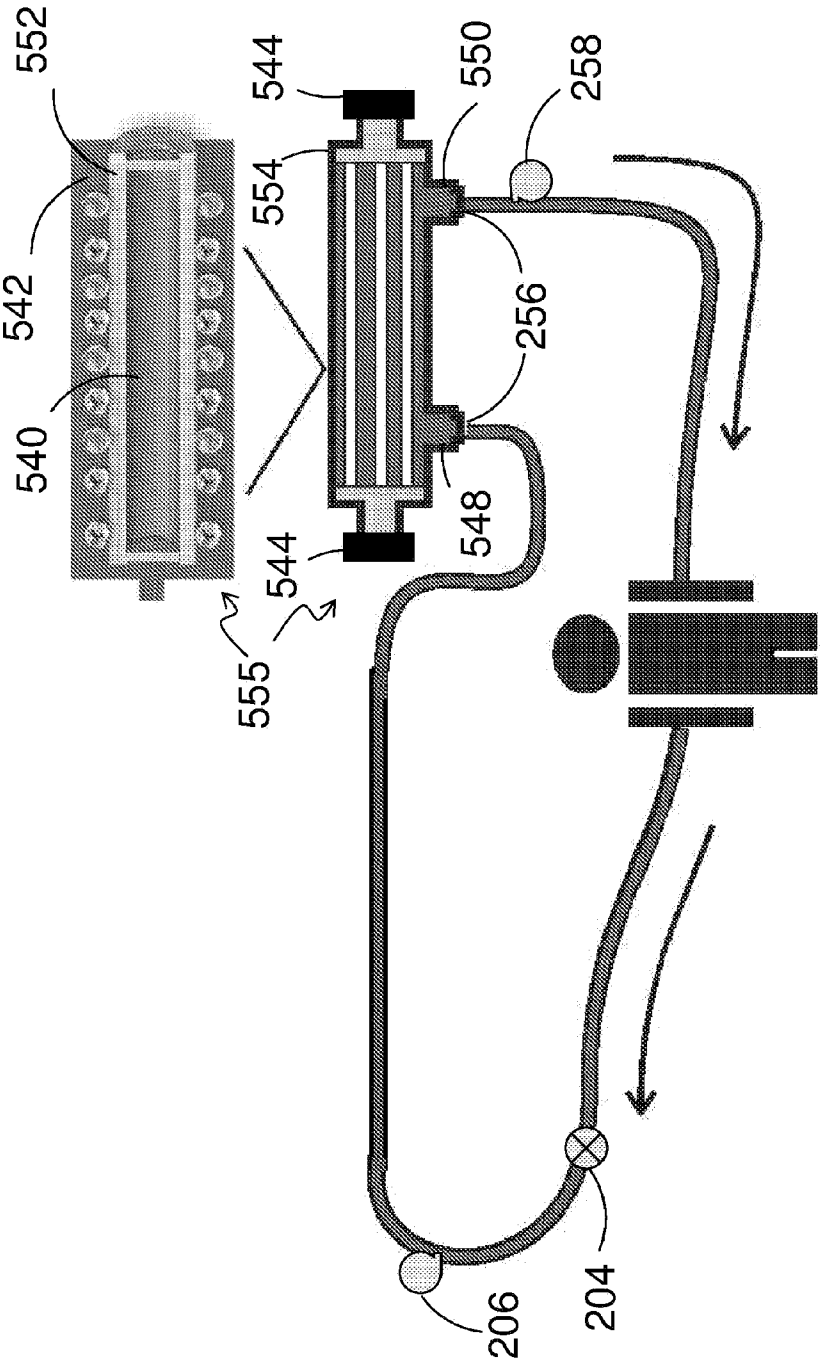


Figure 2B

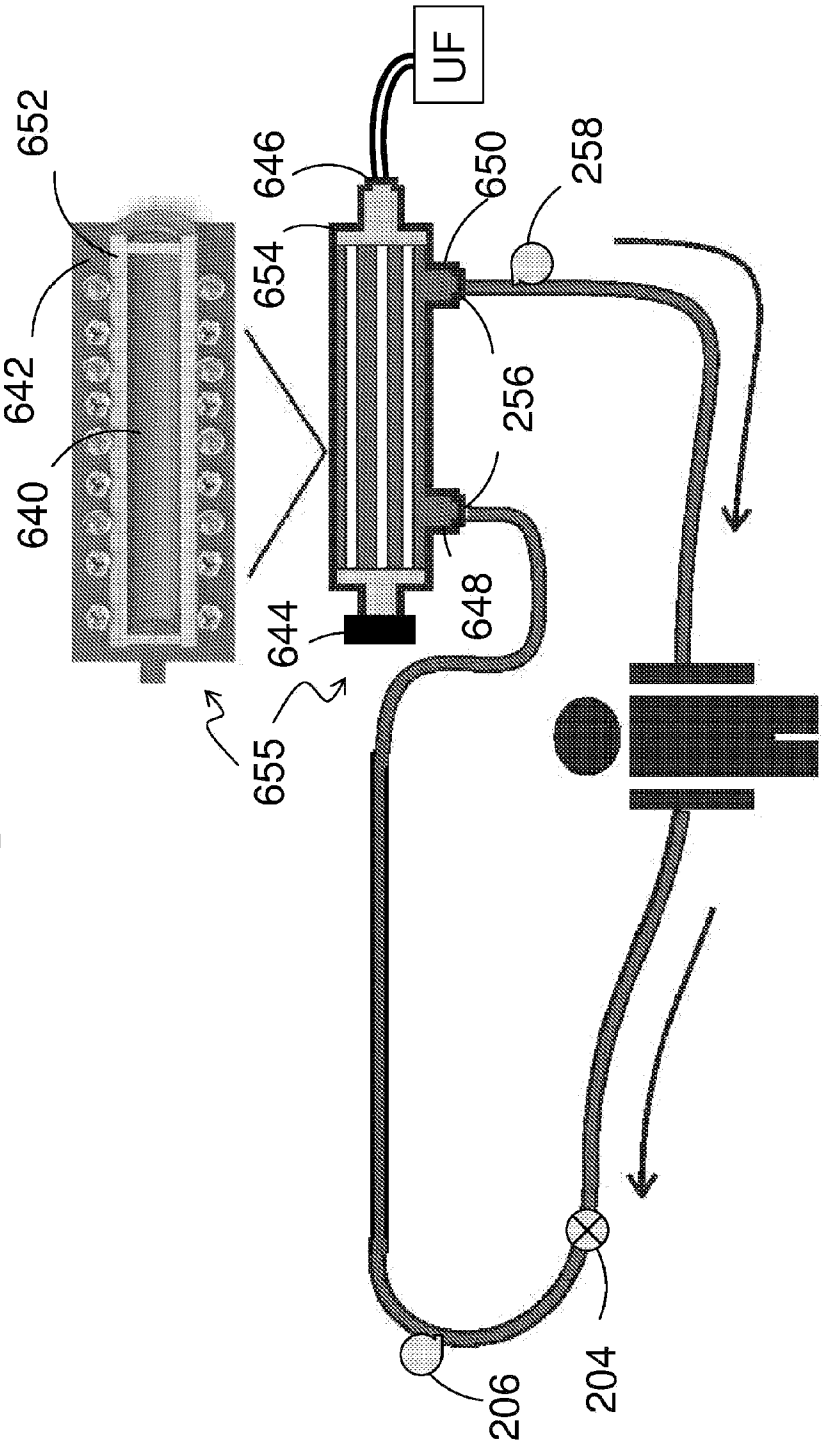


Figure 2C

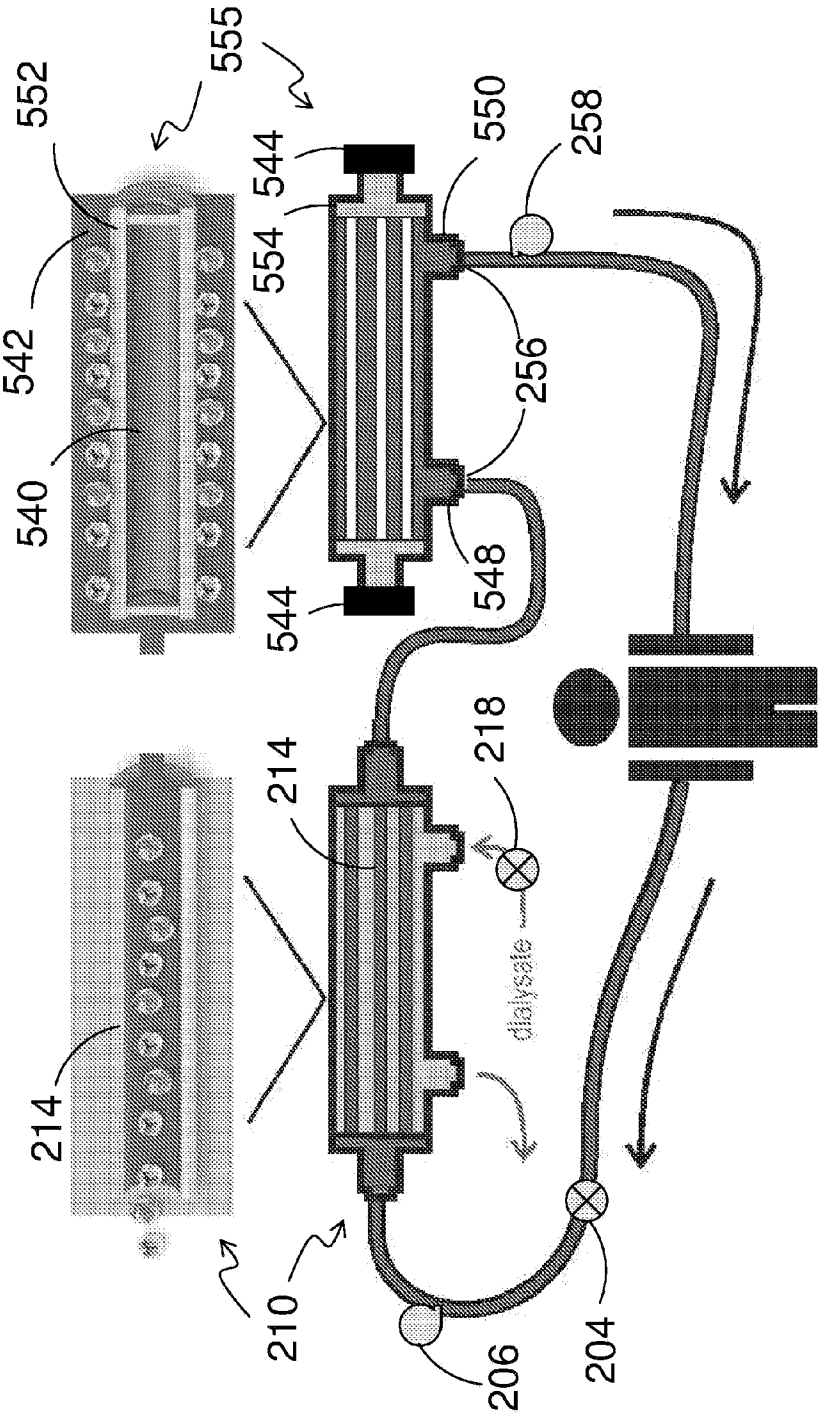

552
555
544
550
258
554
542
256
540
548
544
218
214
214
dialysate
210
206
204

Figure 2D

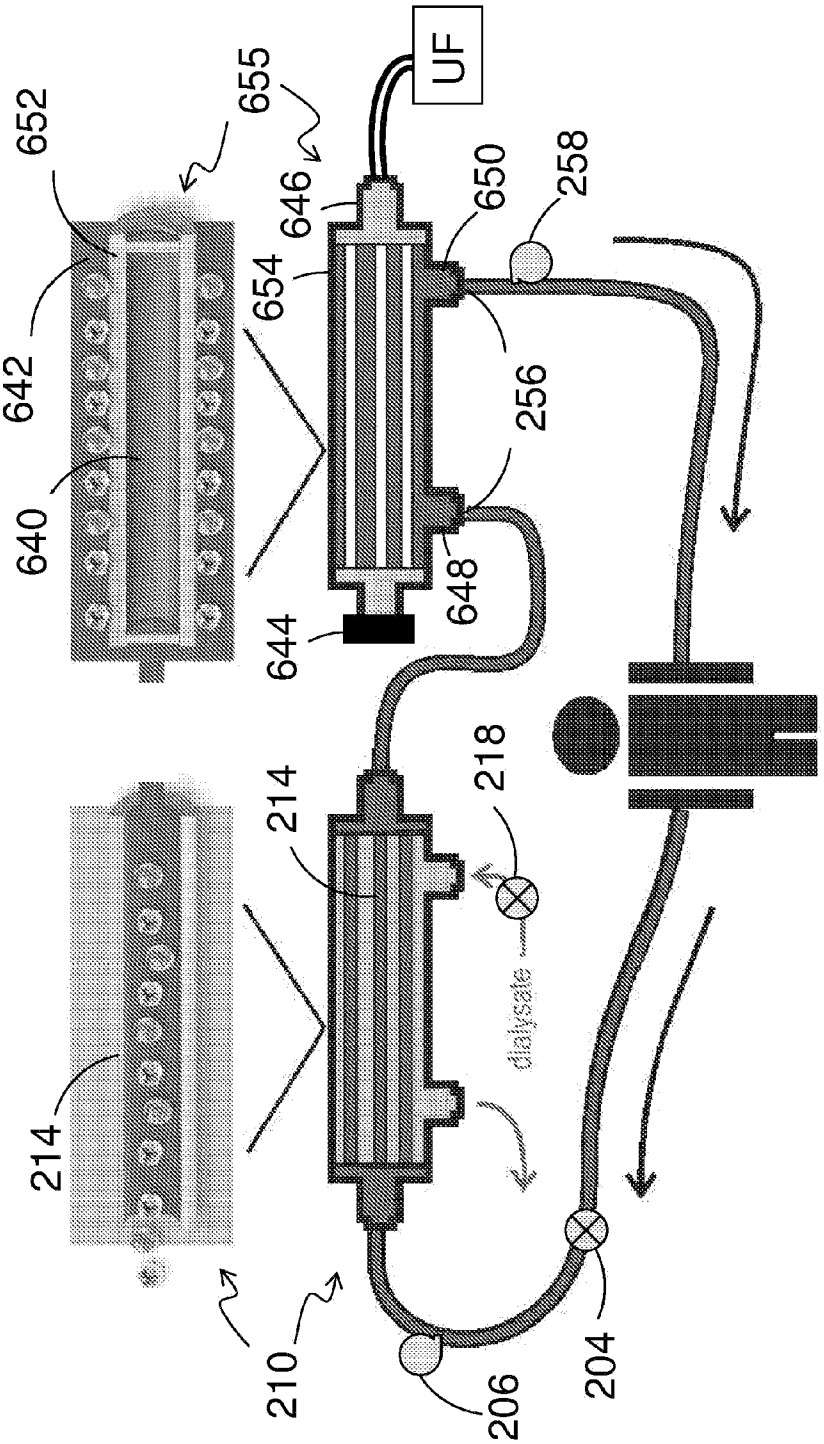

UF
655
652
646
650
258
654
642
256
640
648
644
218
214
dialysate
214
210
206
204

Figure 3

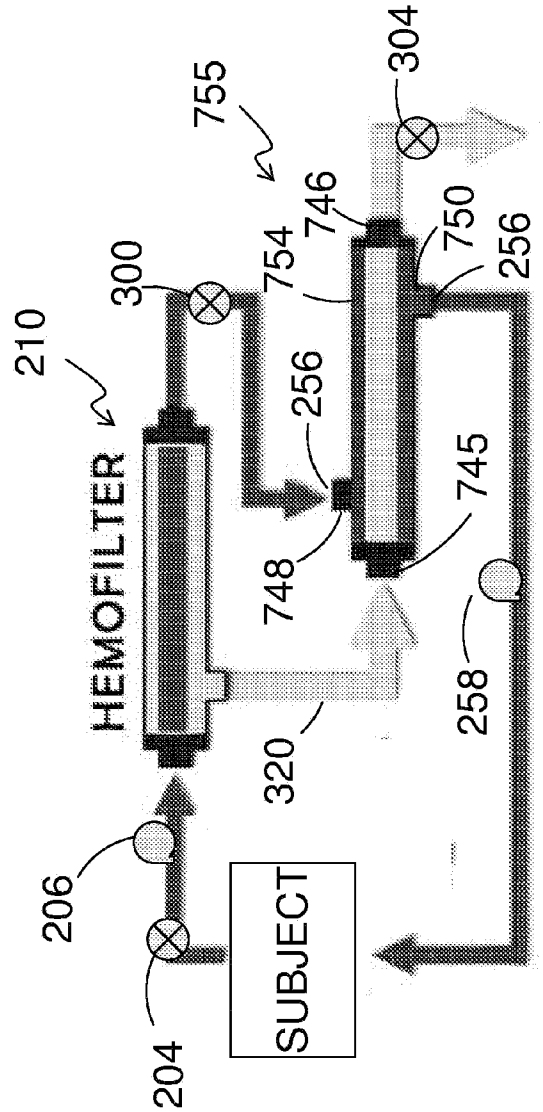

755
304
746
750
256
754
300
210
256
745
748
HEMOFILTER
258
320
206
SUBJECT
204

Figure 4A

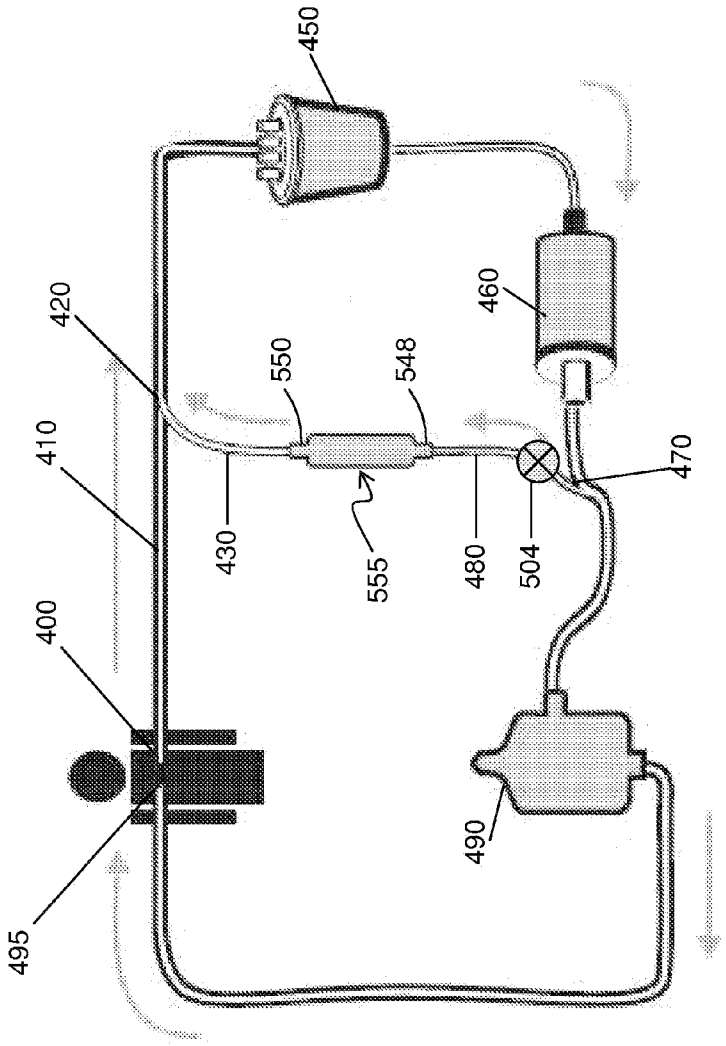


Figure 4B

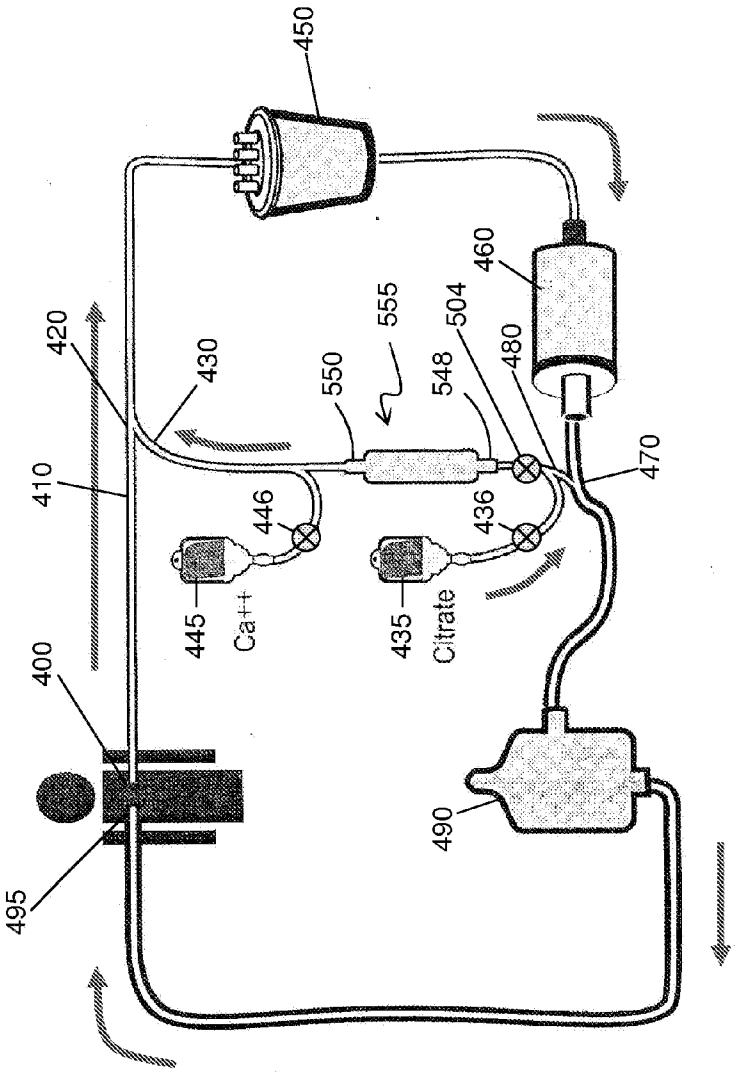

450
460
555
504
480
420
430
550
548
470
410
446
436
445
Ca++
435
Citrate
400
490
495

Figure 4C

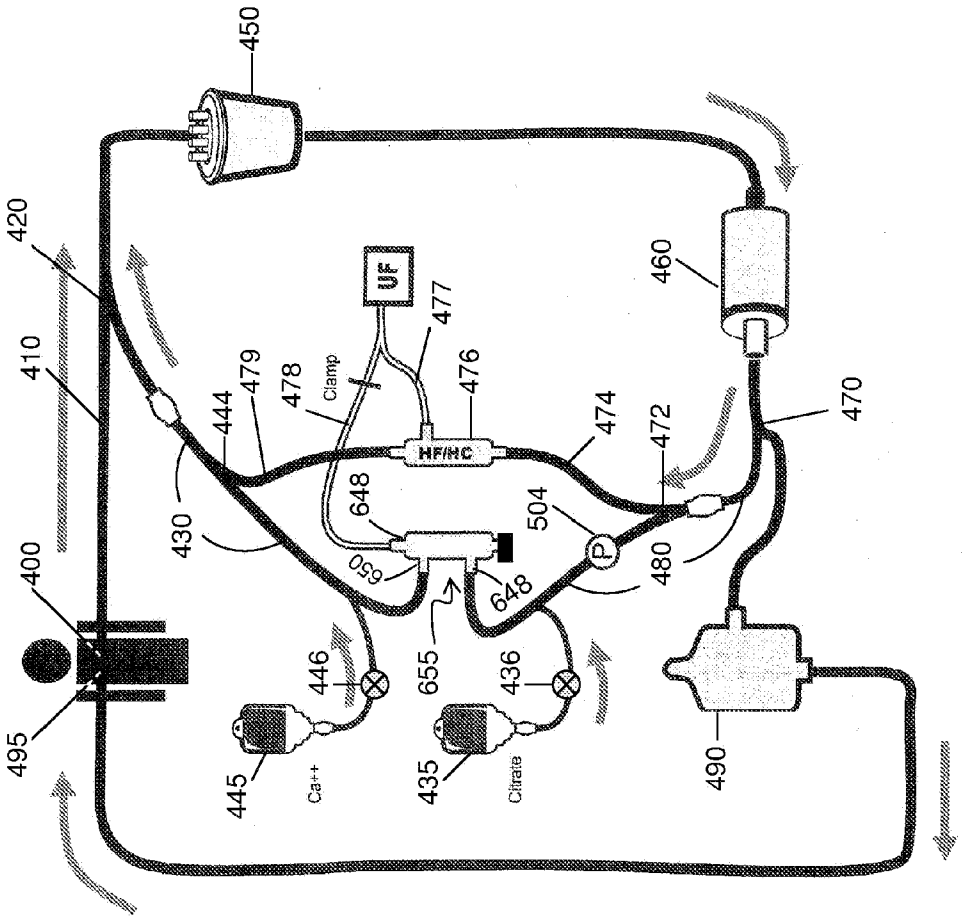

450
420
410
400
495
460
470
UF
477
476
479
478
Clamp
444
430
HF/HC
474
472
504
P
480
648
650
655
446
436
445
435
Ca++
Citrate
490

Figure 4D

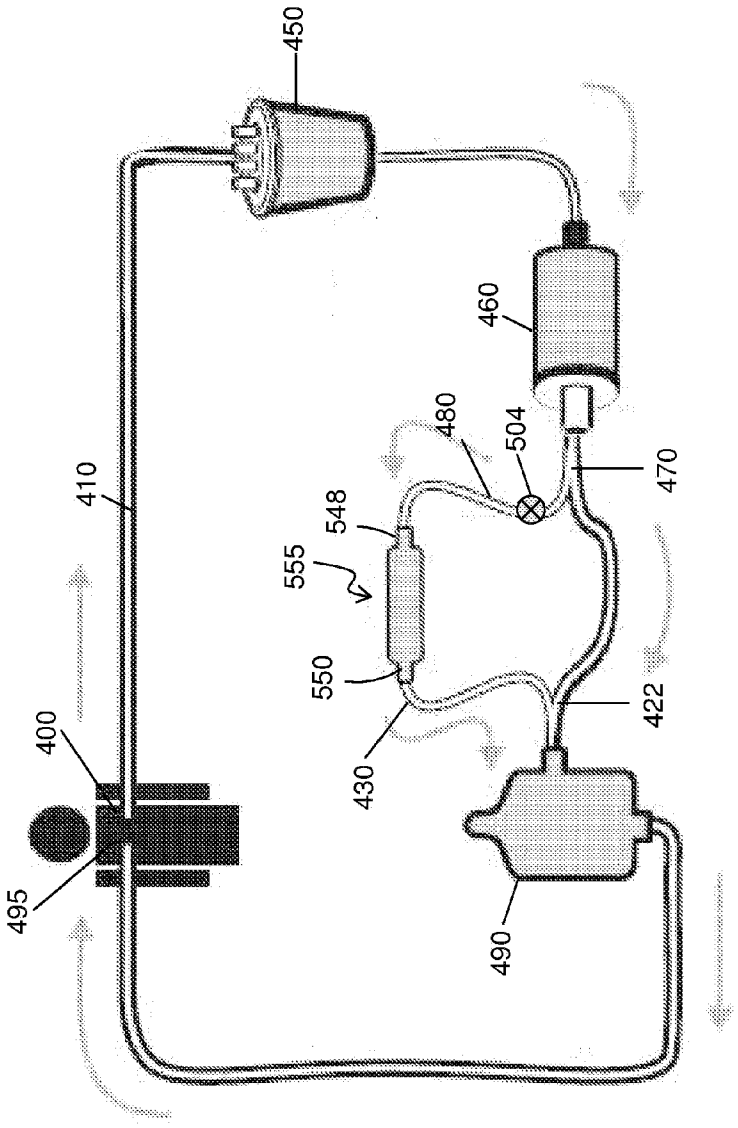


Figure 4E

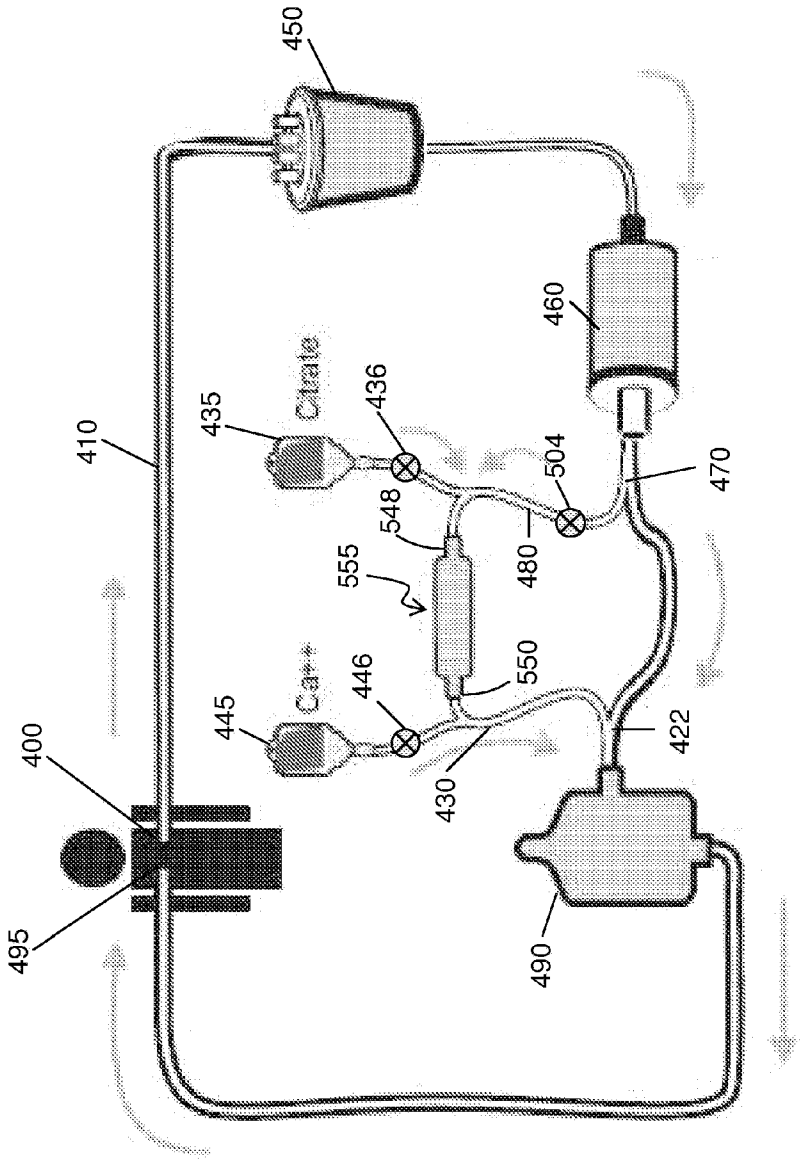

450
460
Citrate
435
436
410
504
470
548
480
555
Ca++
446
550
445
422
400
430
495
490

Figure 4F

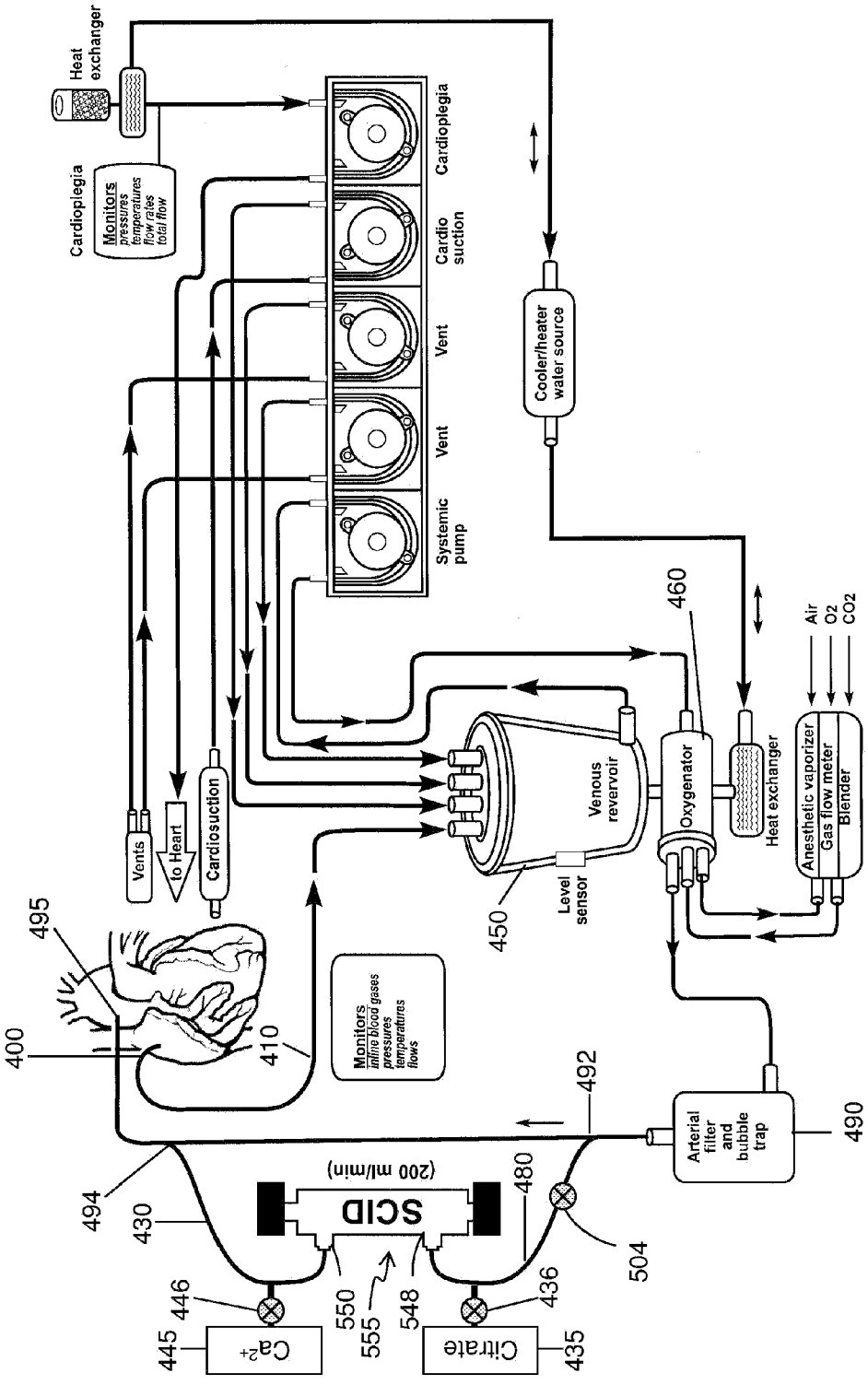

Heat exchanger
Cardioplegia
Monitors
pressures
temperatures
flow rates
total flow
Cardioplegia
Cardio suction
Vent
Vent
Systemic pump
Cooler/heater water source
460
Air
O2
CO2
Anesthetic vaporizer
Gas flow meter
Blender
Heat exchanger
Oxygenator
Venous revervoir
Level sensor
450
Vents
to Heart
Cardiosuction
495
400
410
Monitors
inline blood gases
pressures
temperatures
flows
492
Arterial filter and bubble trap
490
(200 ml/min)
SCID
494
430
480
504
446
445
Ca2+
550
555
548
436
Citrate
435

Figure 5

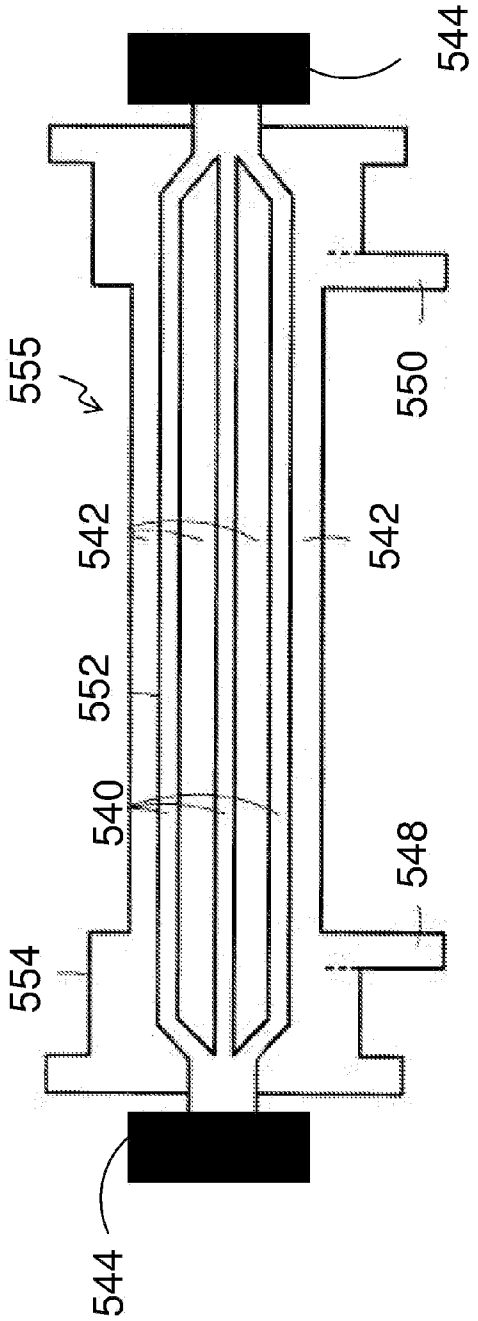


Figure 6

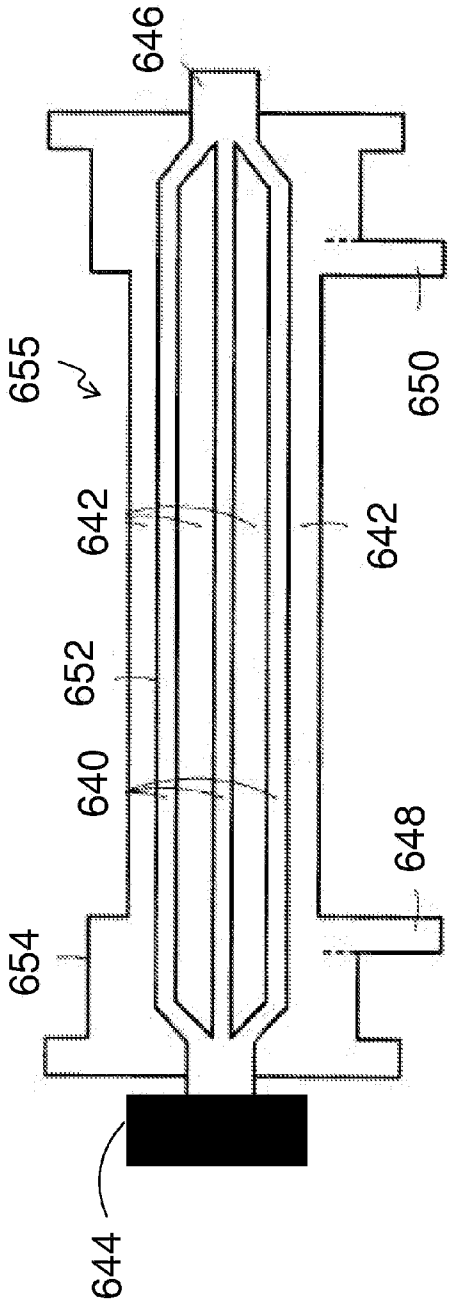


Figure 7

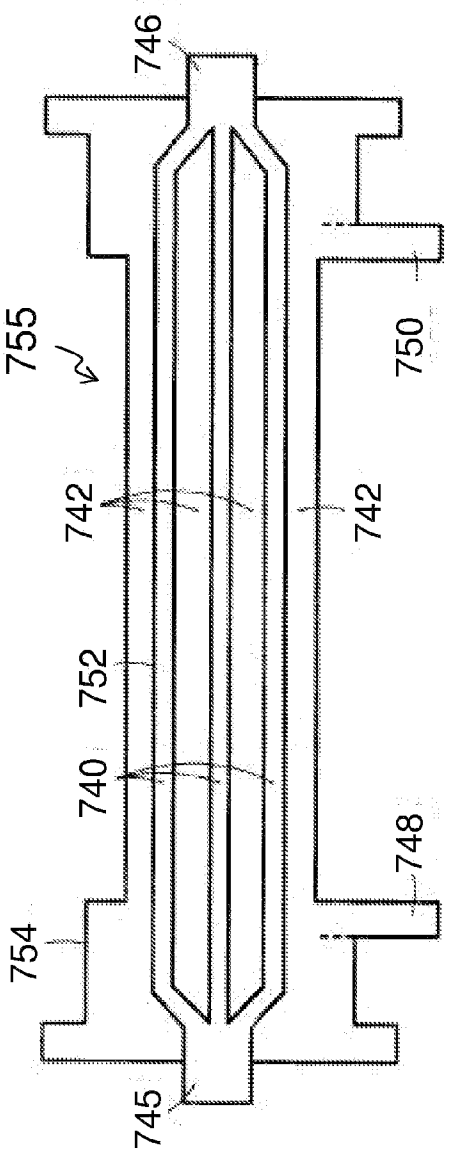


Figure 8
Monocartridge / Dual Fiber System

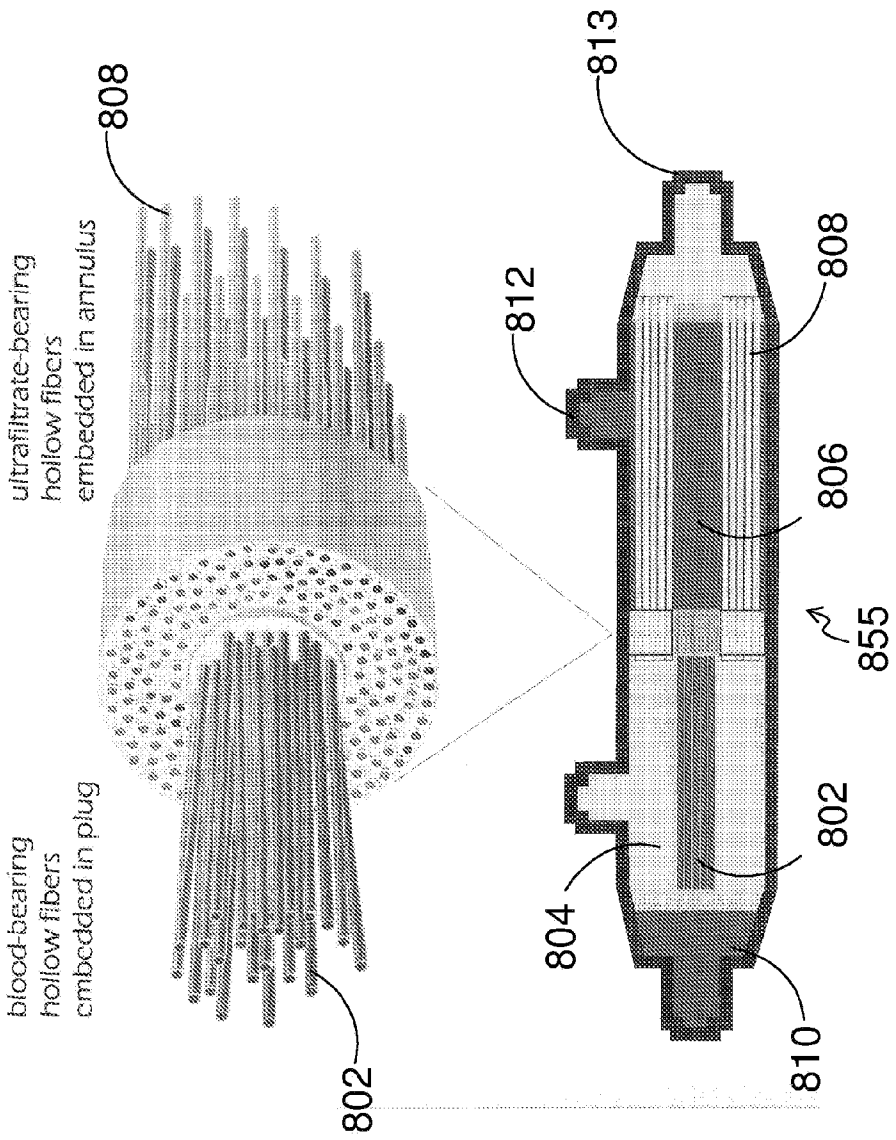


Figure 9

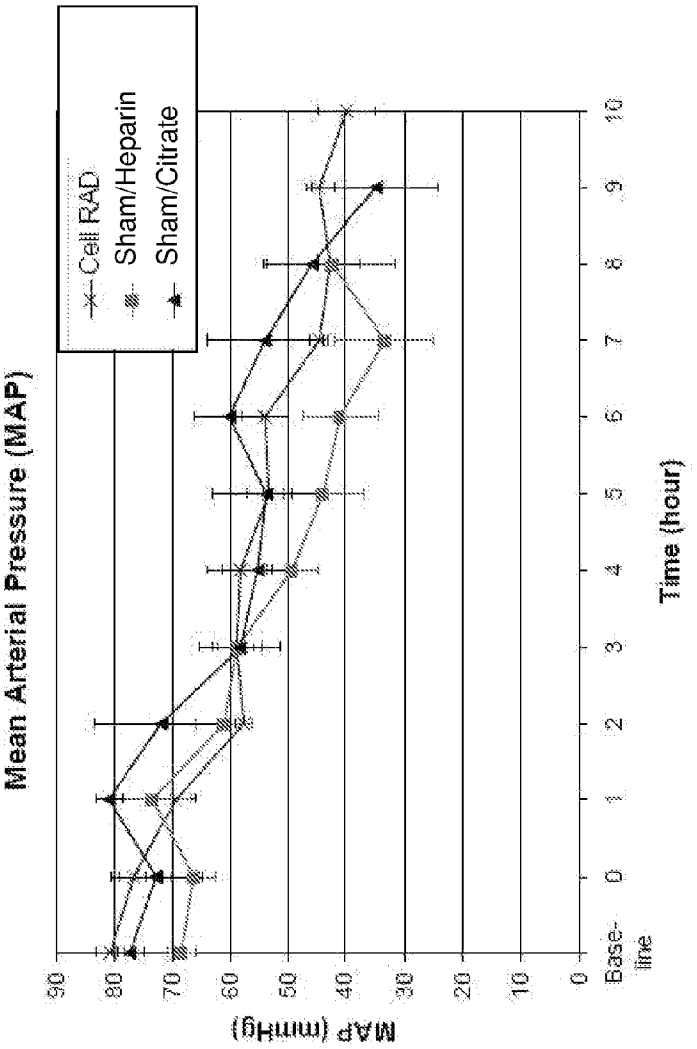

Mean Arterial Pressure (MAP)
Cell RAD
Sham/Heparin
Sham/Citrate
MAP (mmHg)
0
10
20
30
40
50
60
70
80
90
Base-line
0
1
2
3
4
5
6
7
8
9
10
Time (hour)

Figure 10

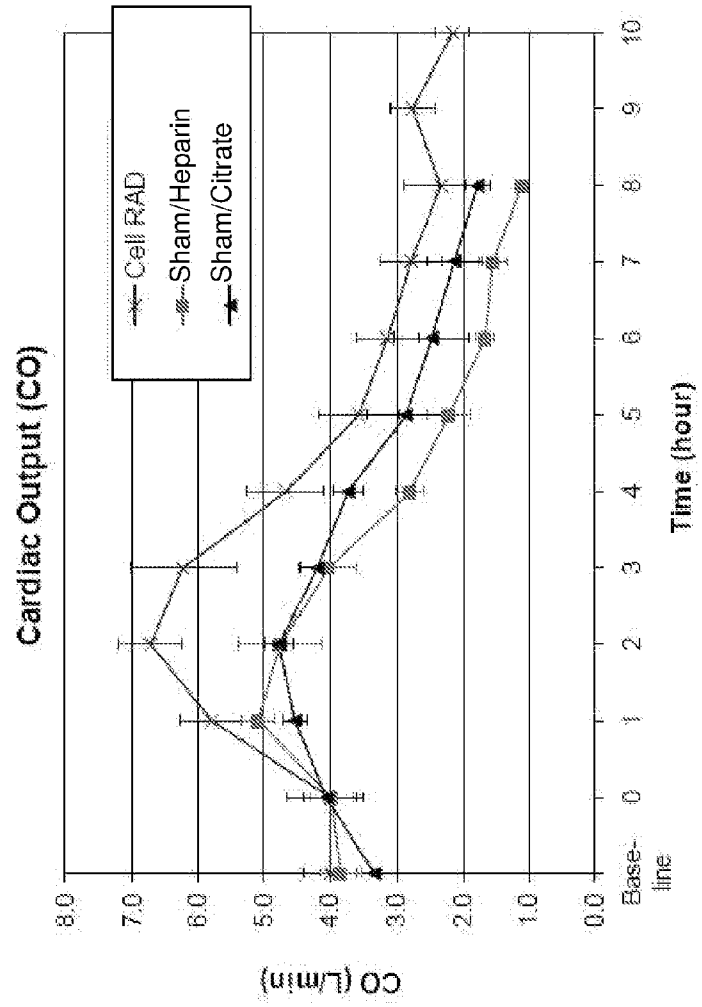

Cardiac Output (CO)
Cell RAD
Sham/Heparin
Sham/Citrate
CO (L/min)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Base-line
0
1
2
3
4
5
6
7
8
9
10
Time (hour)

Figure 11

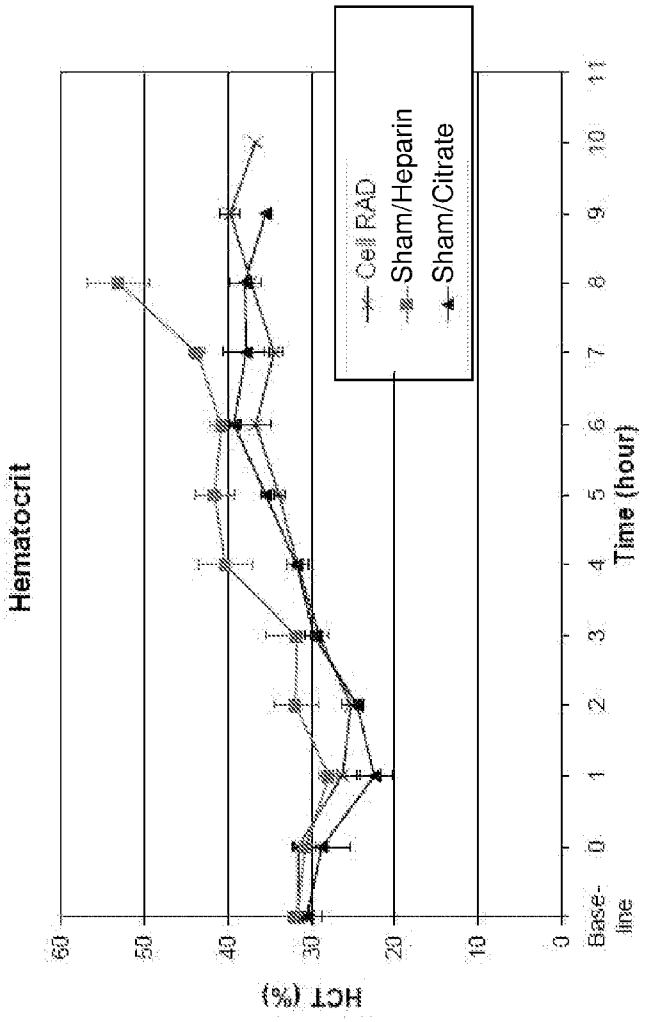

Hematocrit
HCT (%)
60
50
40
30
20
10
0
Base-line
0
1
2
3
4
5
6
7
8
9
10
11
Time (hour)
Cell RAD
Sham/Heparin
Sham/Citrate

Figure 12

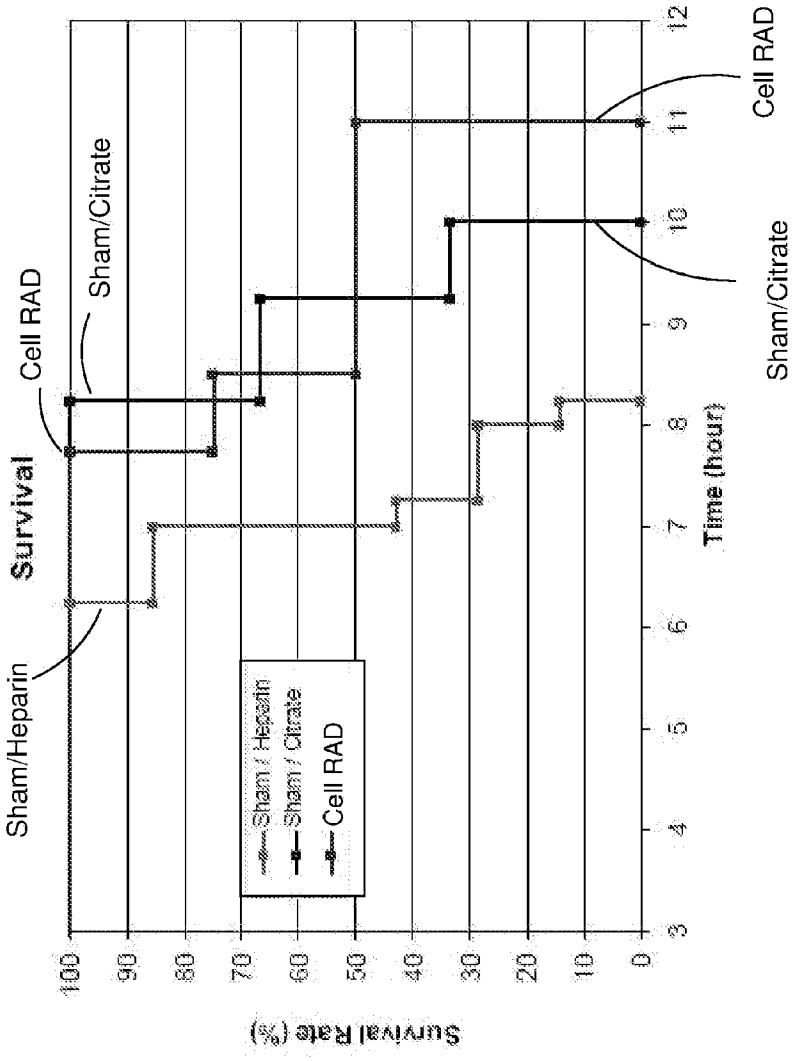

Survival
Sham/Heparin
Cell RAD
Sham/Citrate
Cell RAD
Sham/Citrate
Sham / Heparin
Sham / Citrate
Cell RAD
Survival Rate (%)
Time (hour)
100
90
80
70
60
50
40
30
20
10
0
3
4
5
6
7
8
9
10
11
12

Figure 13

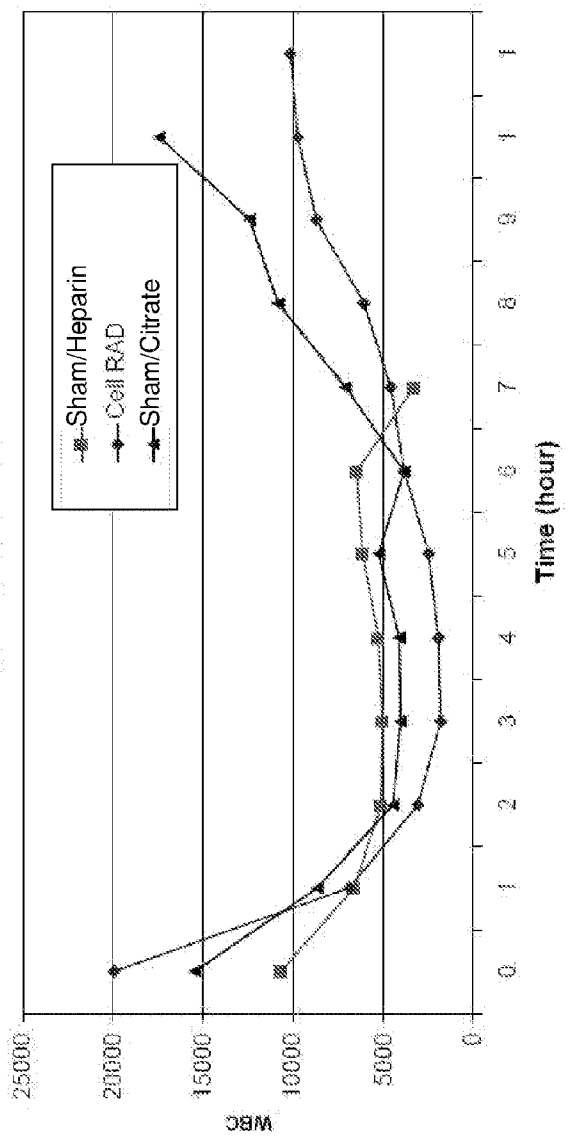

Sham/Heparin
Cell RAD
Sham/Citrate
WBC
25000
20000
15000
10000
5000
0
0
1
2
3
4
5
6
7
8
9
10
11
Time (hour)

Figure 14B

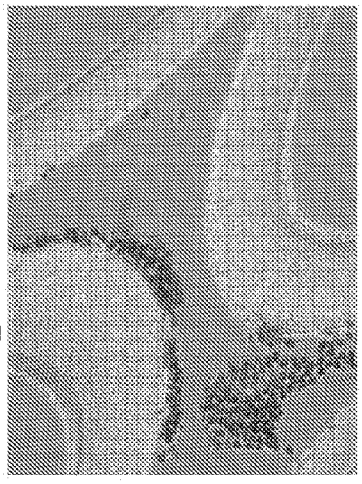


Figure 14D

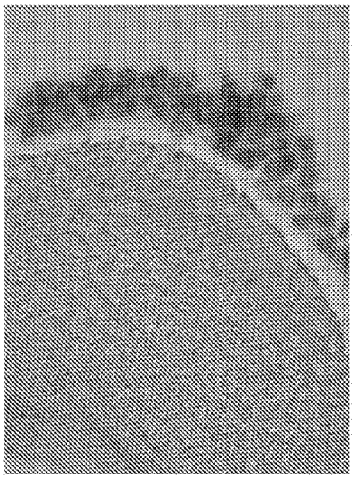


Figure 14A

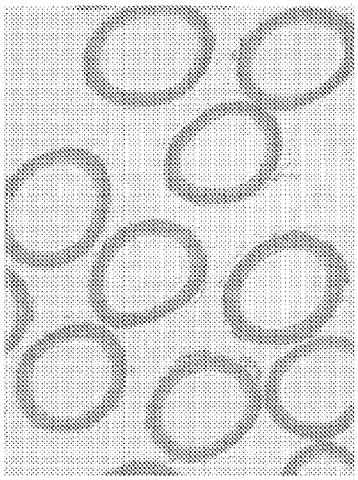


Figure 14C

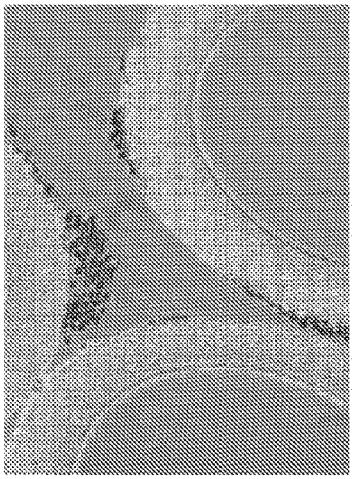


Figure 15

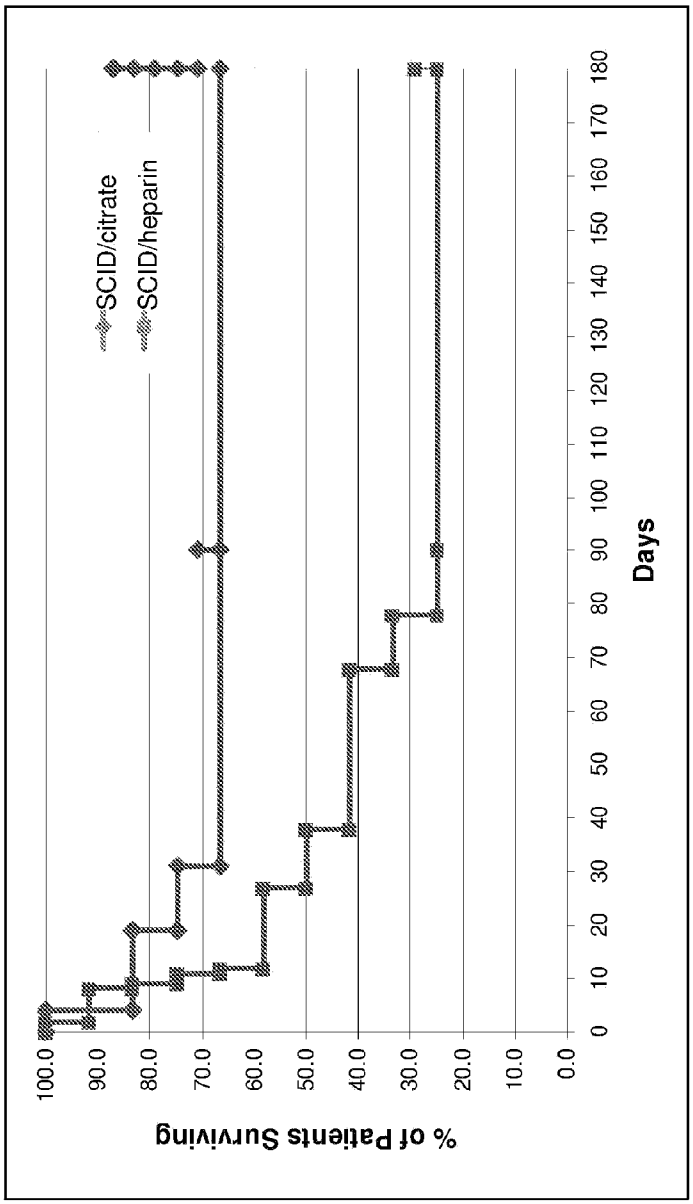

SCID/citrate
SCID/heparin
% of Patients Surviving
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
Days

Figure 16A

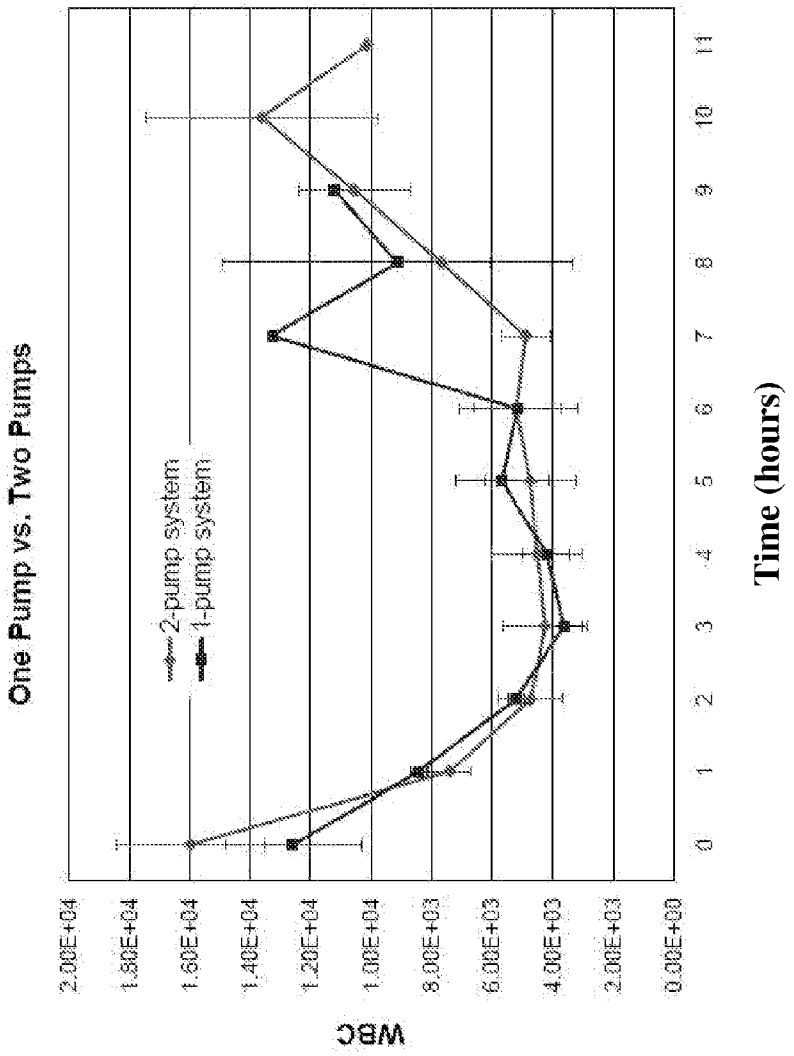

WBC
One Pump vs. Two Pumps
2-pump system
1-pump system
WBC
2.00E+04
1.80E+04
1.60E+04
1.40E+04
1.20E+04
1.00E+04
8.00E+03
6.00E+03
4.00E+03
2.00E+03
0.00E+00
0
1
2
3
4
5
6
7
8
9
10
11
Time (hours)

Figure 16B

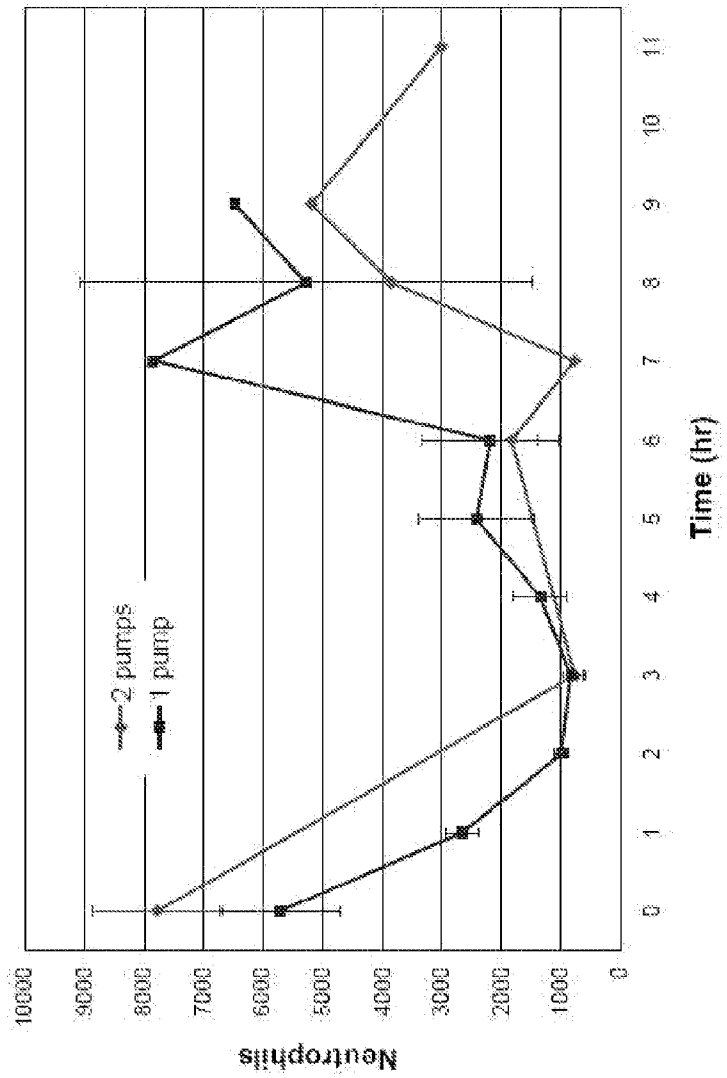

Neutrophils
One Pump vs. Two Pumps
2 pumps
1 pump
Neutrophils
Time (hr)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
1
2
3
4
5
6
7
8
9
10
11

Figure 17

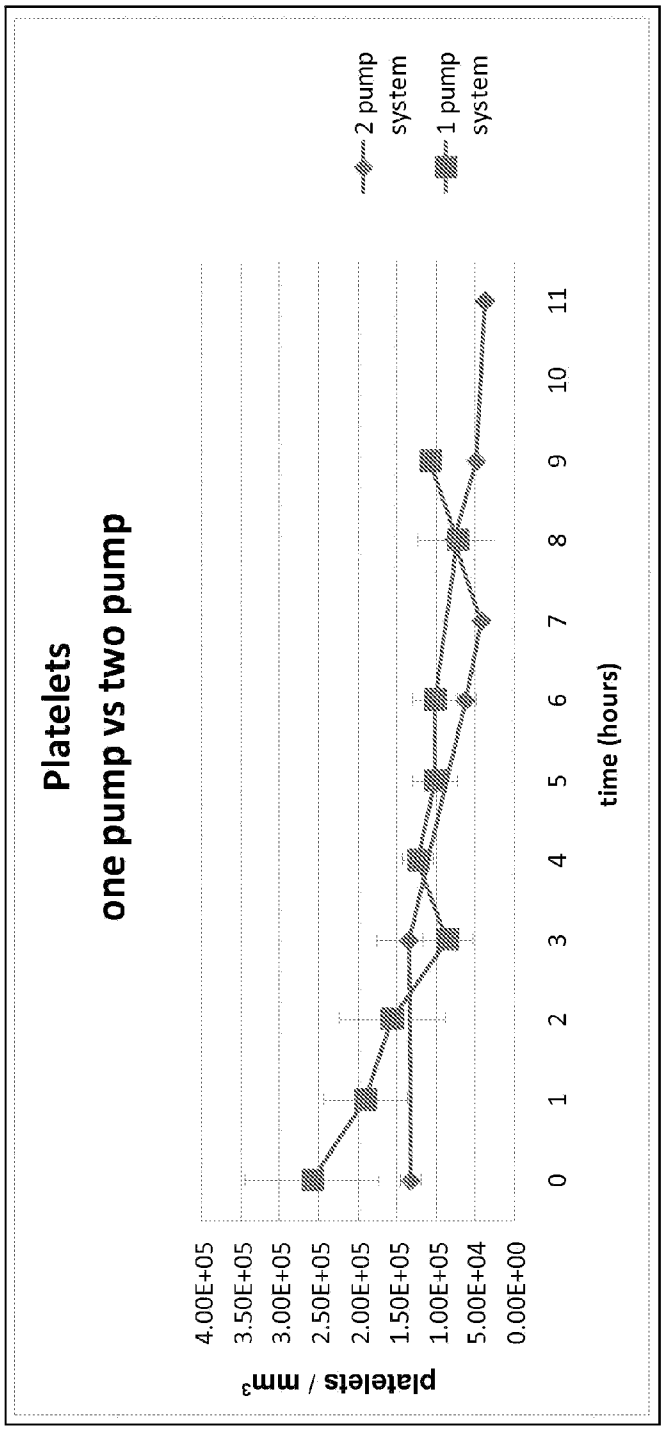

Platelets
one pump vs two pump
2 pump system
1 pump system
platelets / mm3
4.00E+05
3.50E+05
3.00E+05
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
0
1
2
3
4
5
6
7
8
9
10
11
time (hours)

Figure 18

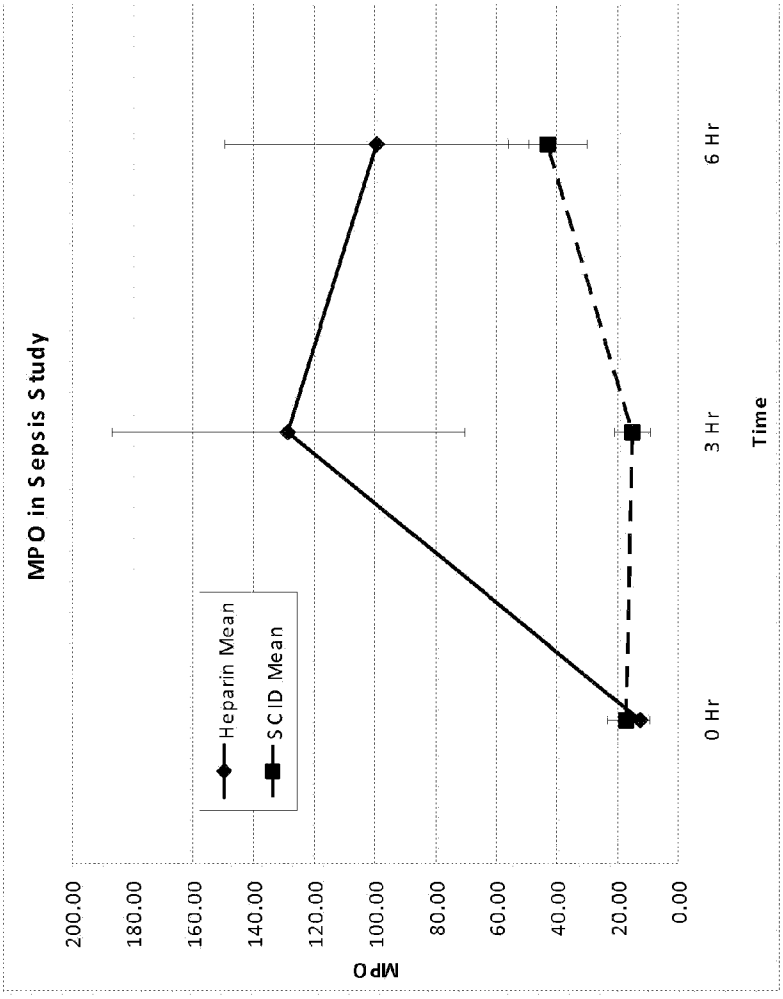

MPO in Sepsis Study
MPO
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Heparin Mean
SCID Mean
0 Hr
3 Hr
6 Hr
Time

Figure 19

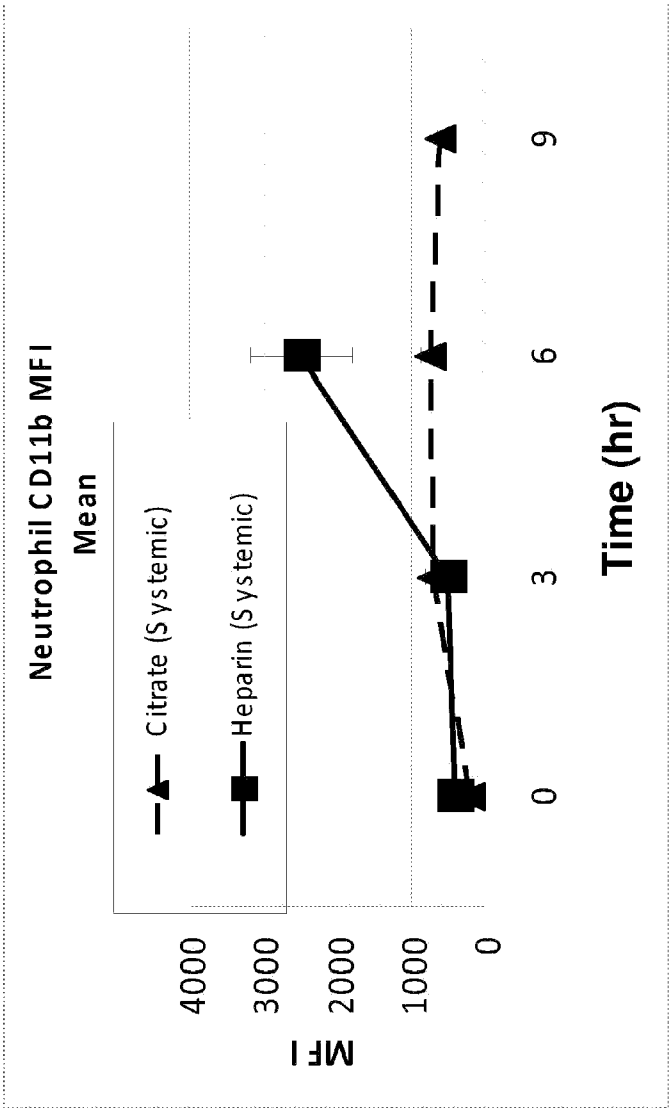

Neutrophil CD11b MFI
Mean
Citrate (Systemic)
Heparin (Systemic)
MFI
4000
3000
2000
1000
0
0
3
6
9
Time (hr)

Figure 20

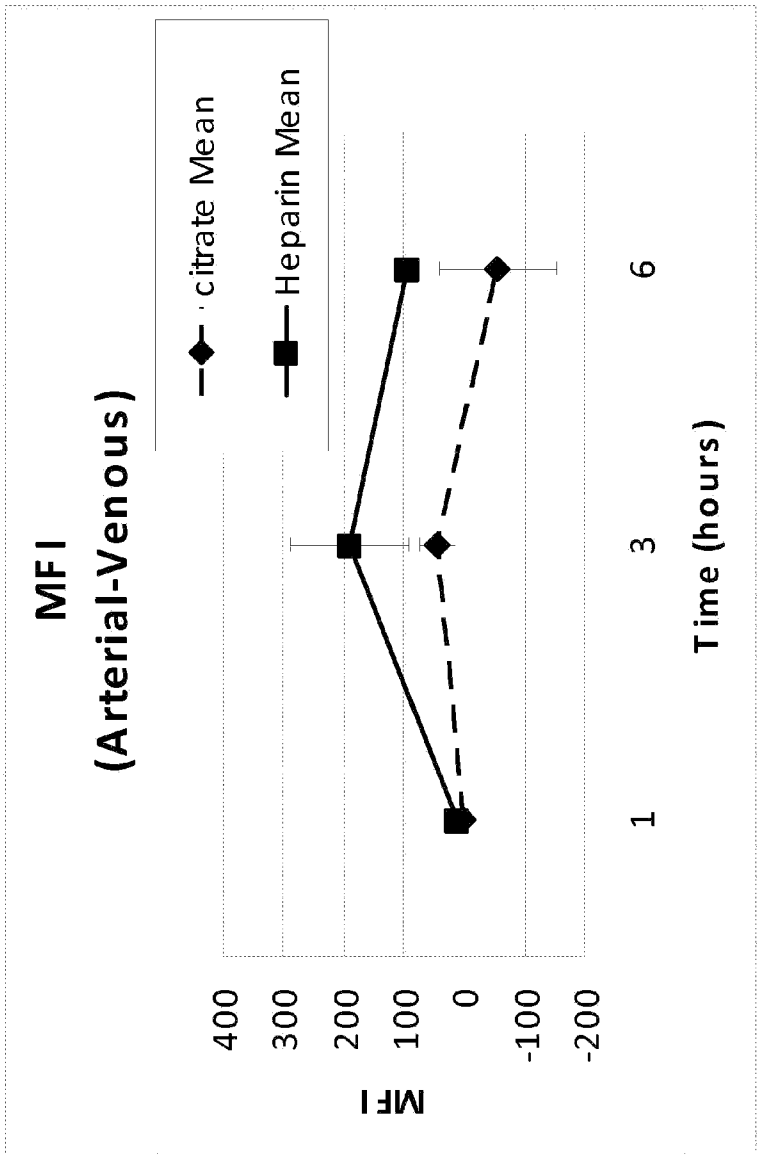

MFI
(Arterial-Venous)
citrate Mean
Heparin Mean
400
300
200
100
0
-100
-200
MFI
1
3
6
Time (hours)

Figure 21

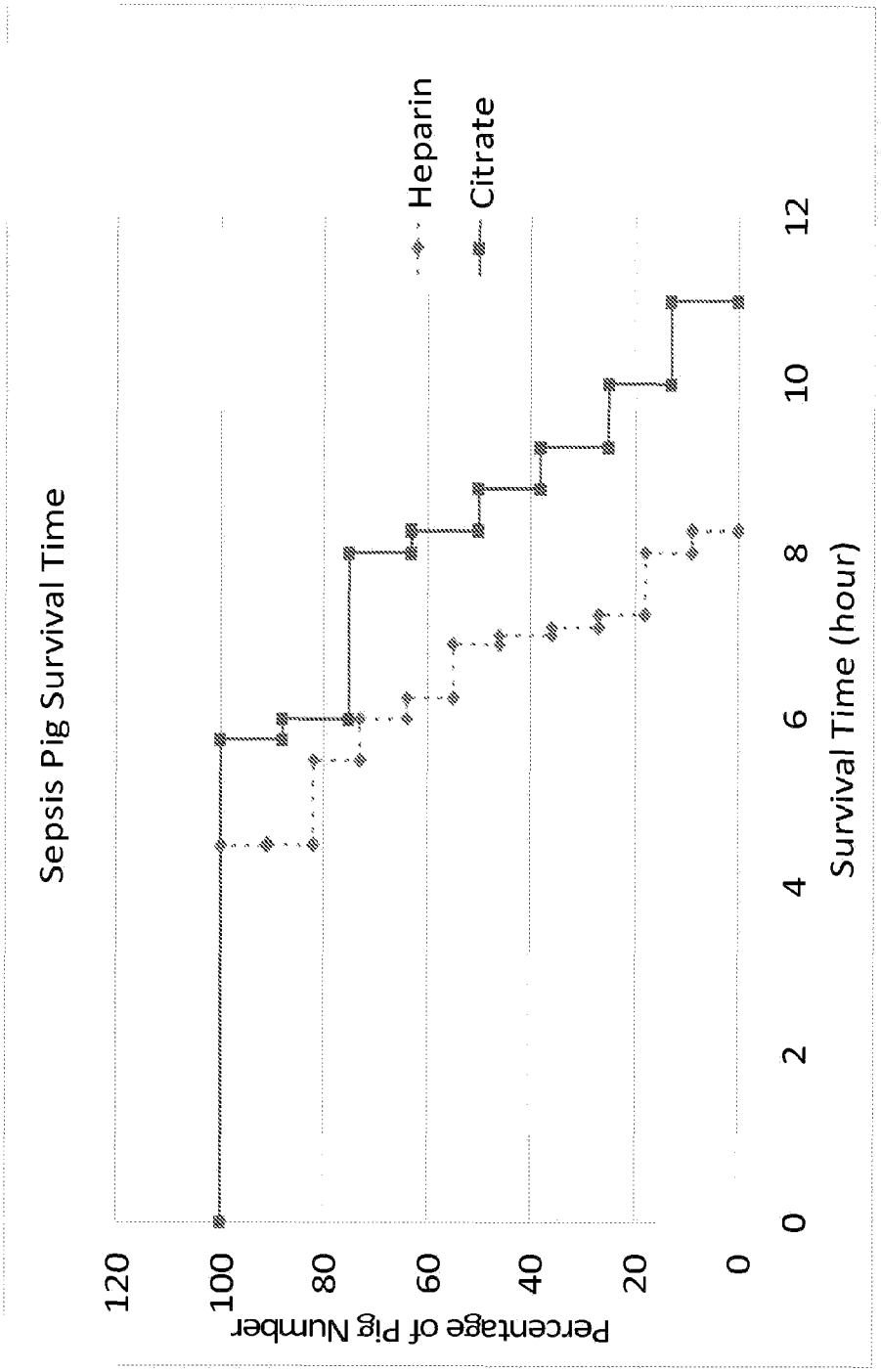

Sepsis Pig Survival Time
Heparin
Citrate
Percentage of Pig Number
120
100
80
60
40
20
0
0
2
4
6
8
10
12
Survival Time (hour)

Figure 22A

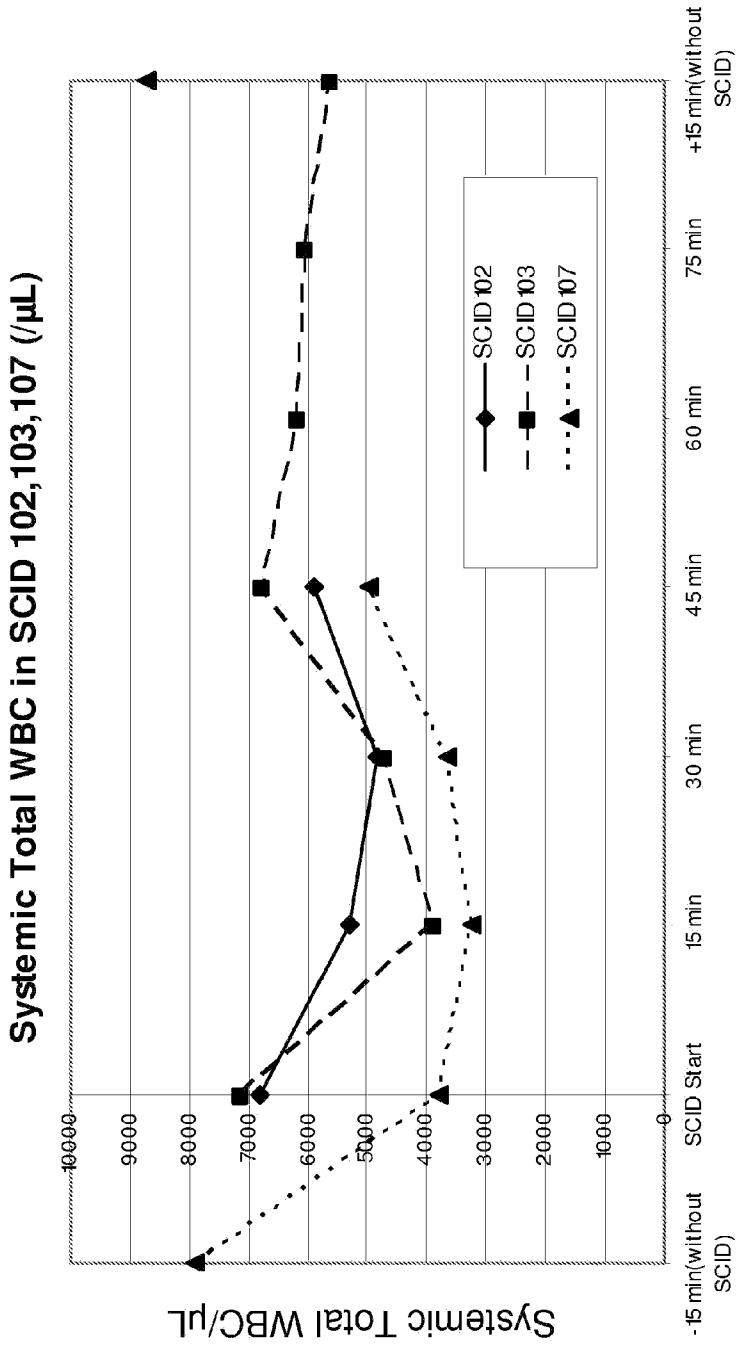

Systemic Total WBC in SCID 102,103,107 (/µL)
Systemic Total WBC/µL
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-15 min(without SCID)
SCID Start
15 min
30 min
45 min
60 min
75 min
+15 min(without SCID)
SCID 102
SCID 103
SCID 107

Figure 22B

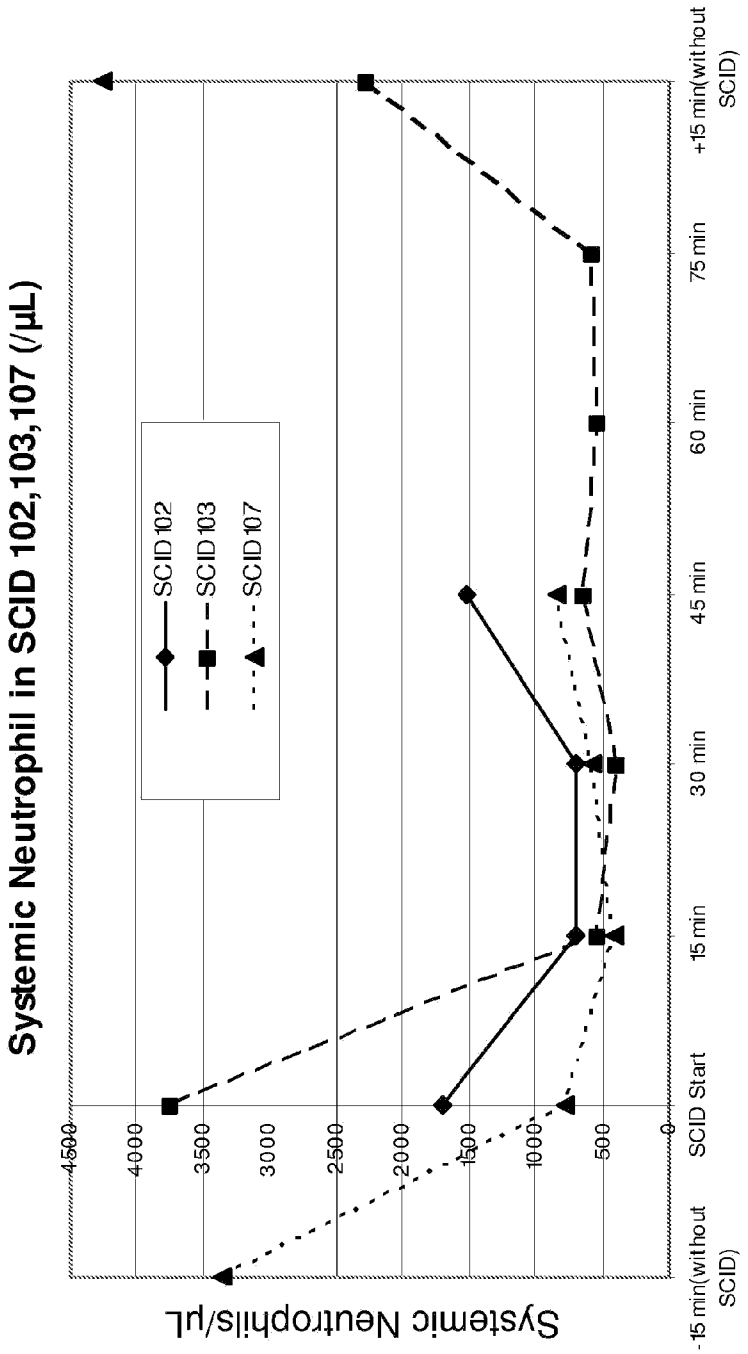

Systemic Neutrophil in SCID 102,103,107 (/μL)
SCID 102
SCID 103
SCID 107
Systemic Neutrophils/μL
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-15 min(without SCID)
SCID Start
15 min
30 min
45 min
60 min
75 min
+15 min(without SCID)

Figure 22C

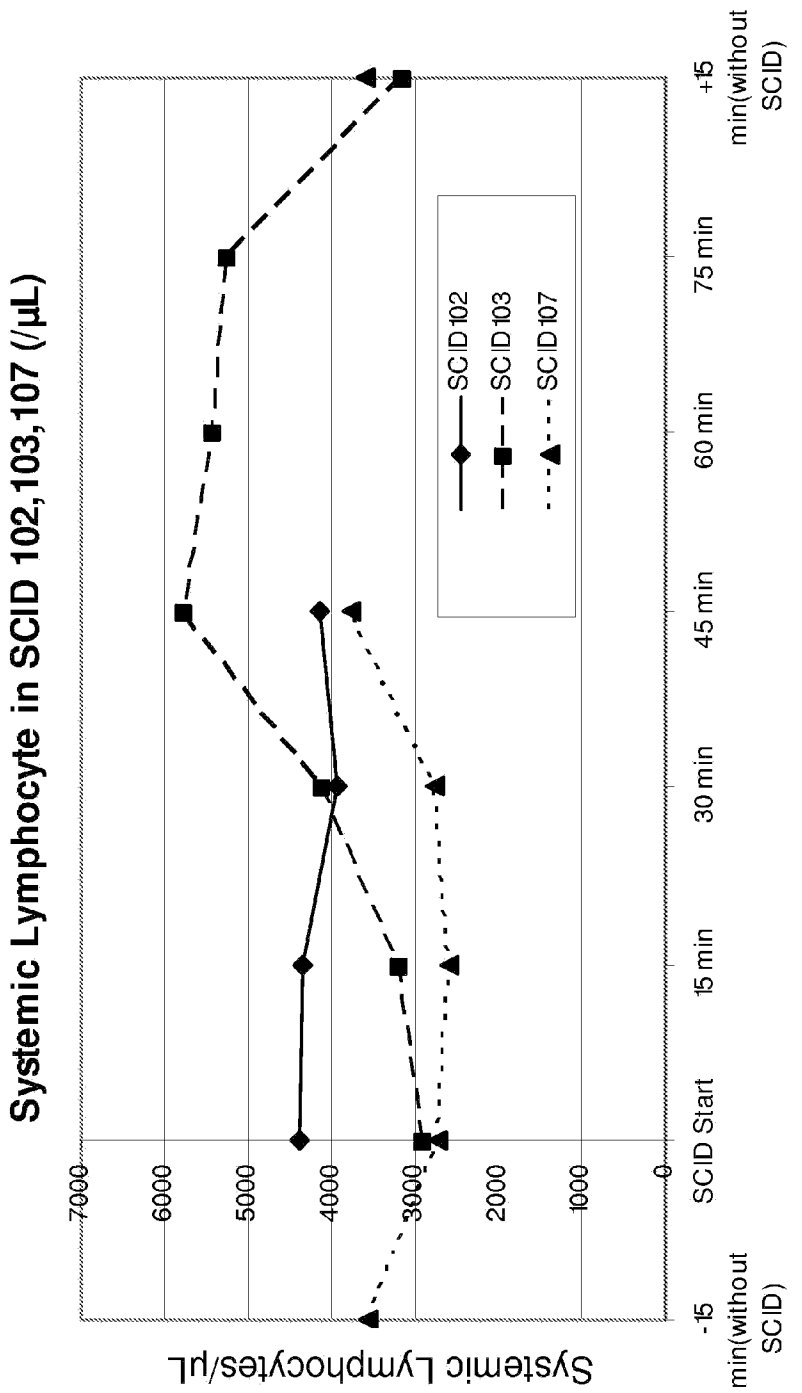

Systemic Lymphocyte in SCID 102,103,107 (/μL)
Systemic Lymphocytes/μL
7000
6000
5000
4000
3000
2000
1000
0
SCID 102
SCID 103
SCID 107
-15 min(without SCID)
SCID Start
15 min
30 min
45 min
60 min
75 min
+15 min(without SCID)

Figure 22D

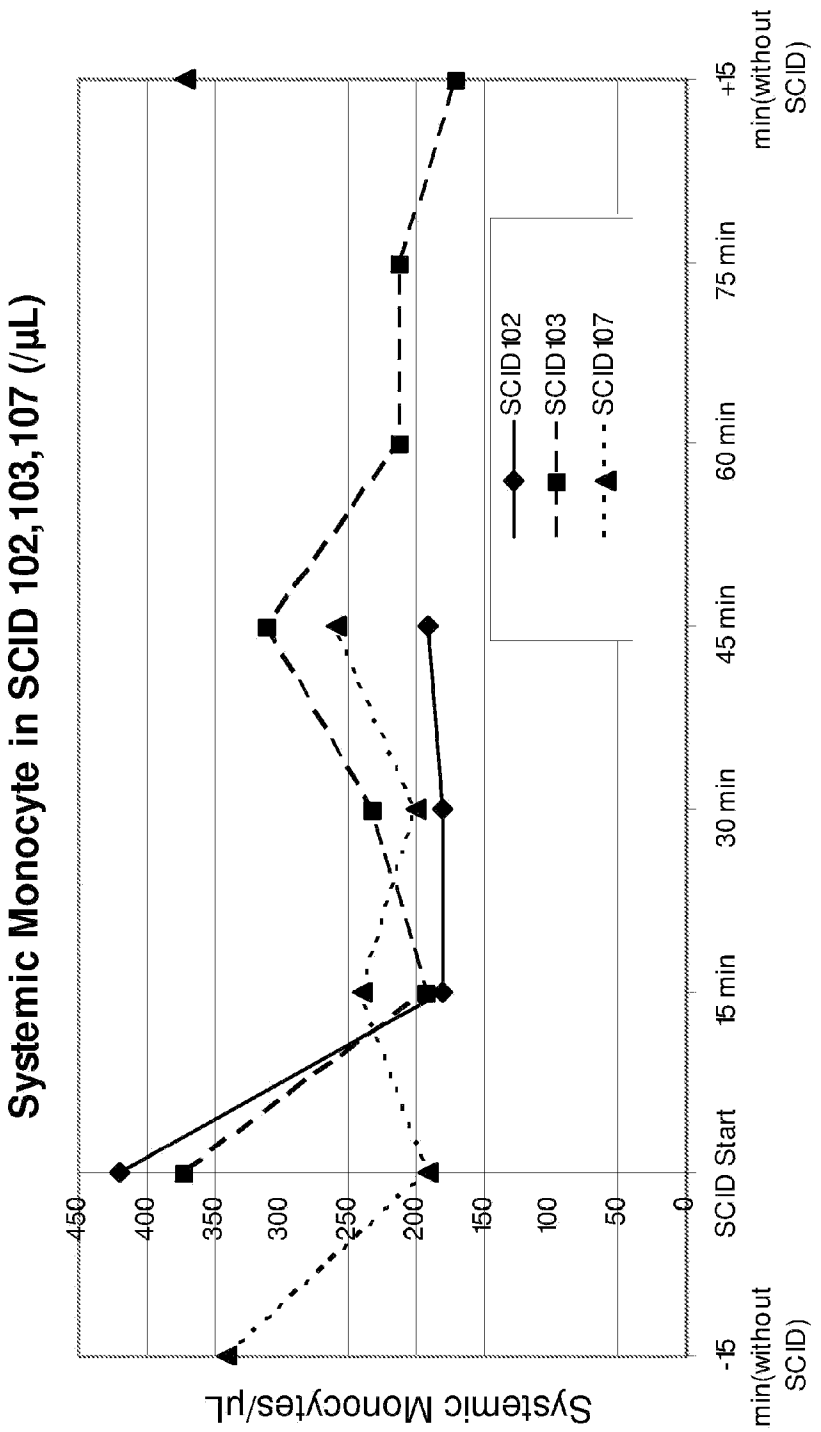

Systemic Monocyte in SCID 102,103,107 (/µL)
Systemic Monocytes/µL
450
400
350
300
250
200
150
100
50
0
-15 min(without SCID)
SCID Start
15 min
30 min
45 min
60 min
75 min
+15 min(without SCID)
SCID 102
SCID 103
SCID 107

Figure 22E

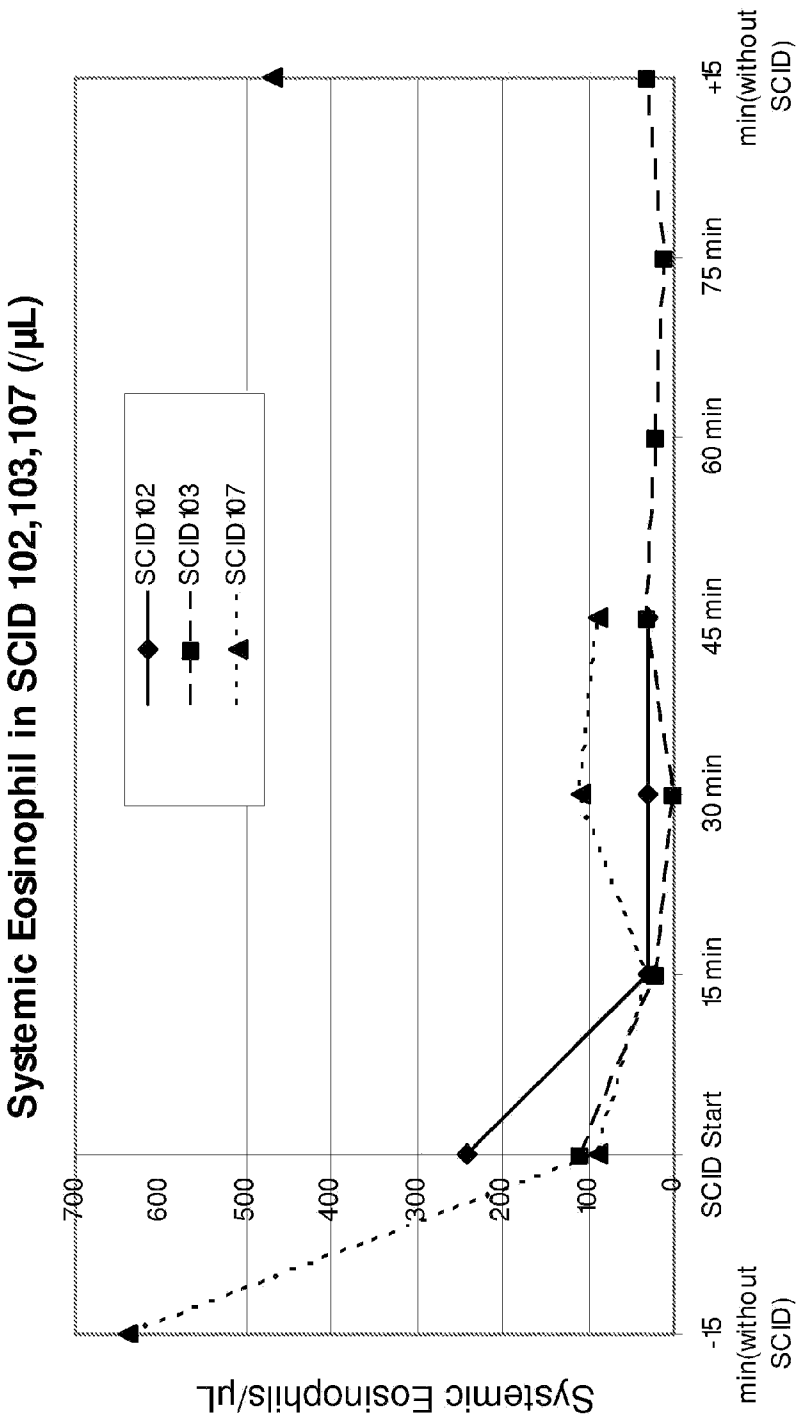

Systemic Eosinophil in SCID 102,103,107 (/μL)
SCID 102
SCID 103
SCID 107
Systemic Eosinophils/μL
700
600
500
400
300
200
100
0
-15 min(without SCID)
SCID Start
15 min
30 min
45 min
60 min
75 min
+15 min(without SCID)

Figure 22F

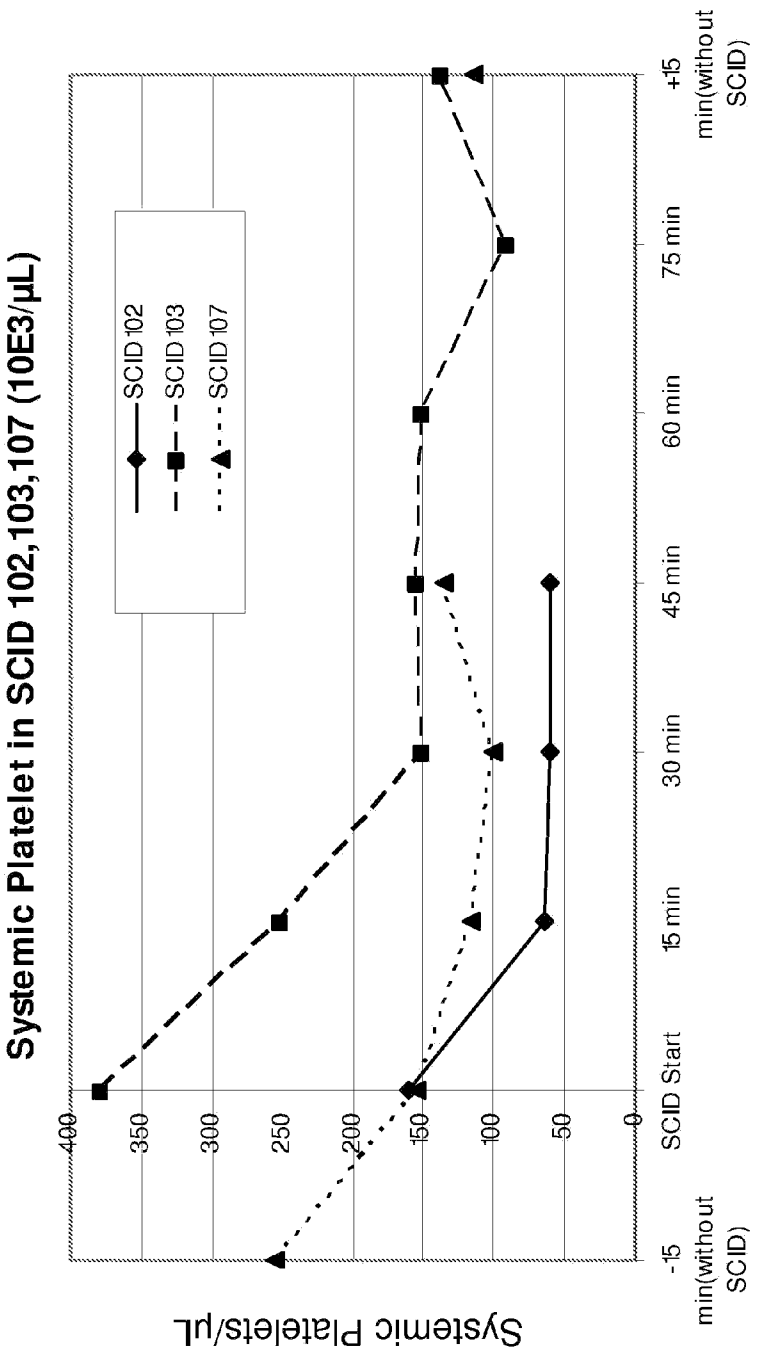

Systemic Platelet in SCID 102,103,107 (10E3/µL)
SCID 102
SCID 103
SCID 107
Systemic Platelets/µL
400
350
300
250
200
150
100
50
0
-15 min(without SCID)
SCID Start
15 min
30 min
45 min
60 min
75 min
+15 min(without SCID)

Figure 23A

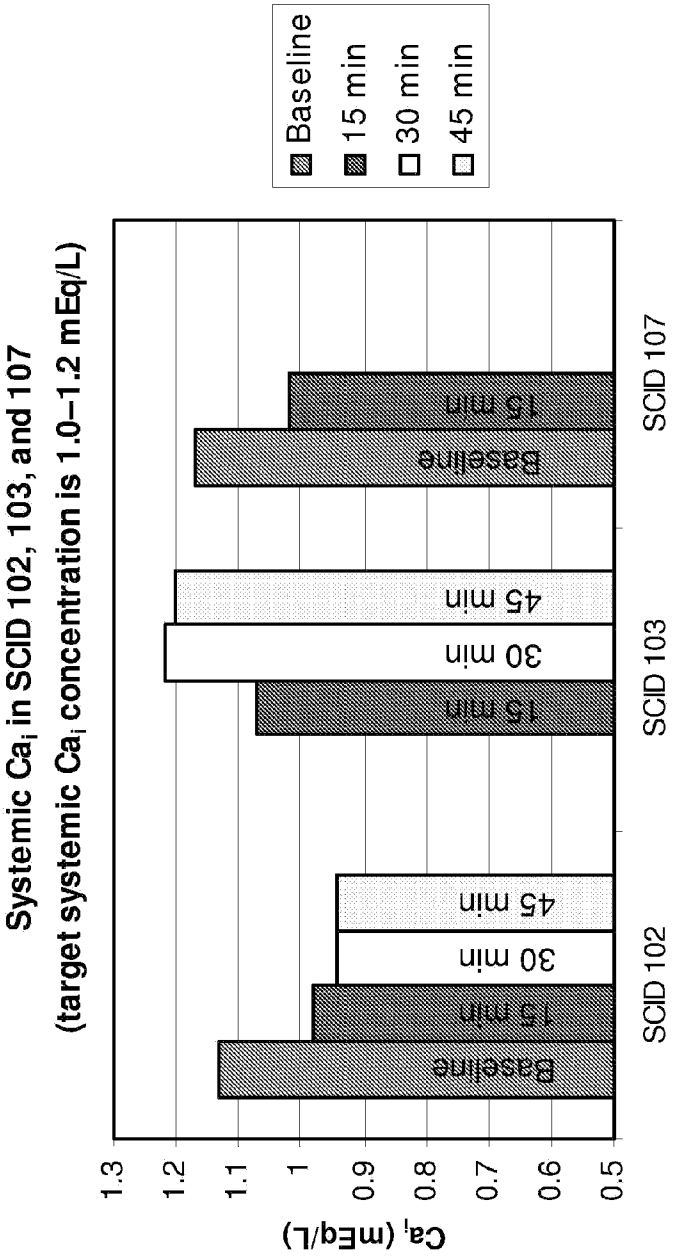
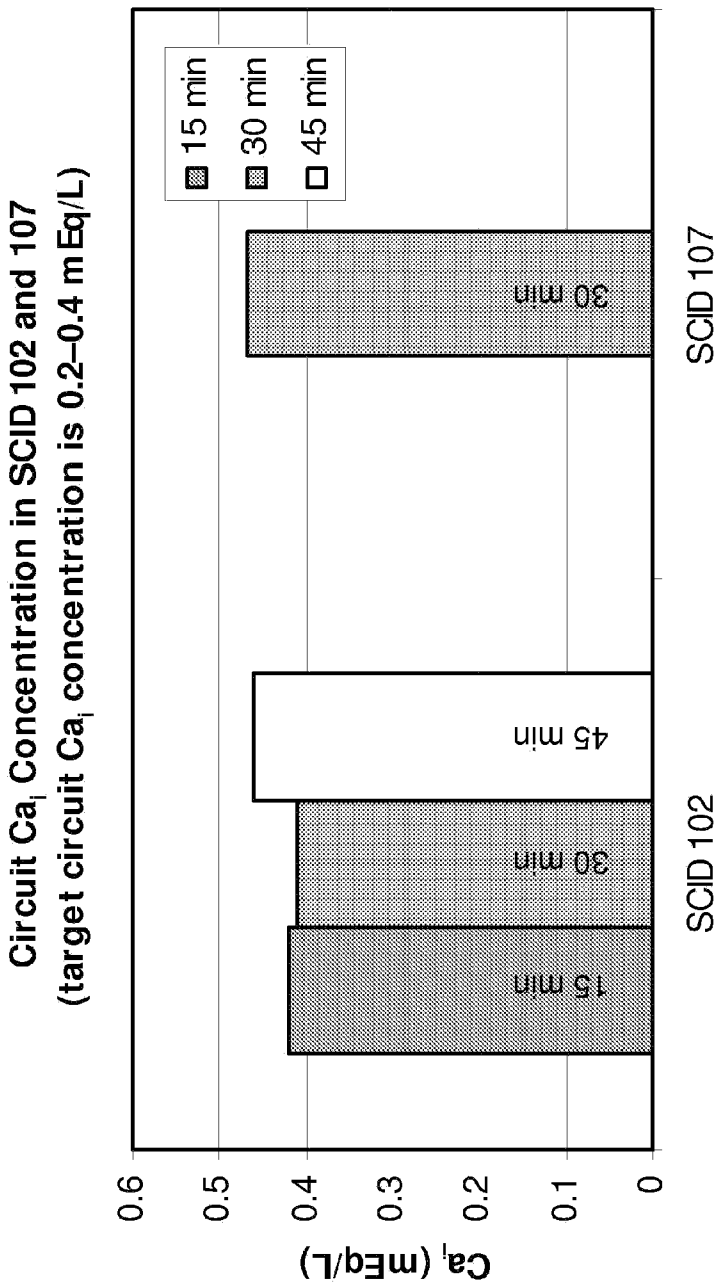

Systemic Ca$_i$ in SCID 102, 103, and 107
(target systemic Ca$_i$ concentration is 1.0–1.2 mEq/L)
Baseline
15 min
30 min
45 min
Ca$_i$ (mEq/L)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
SCID 102
SCID 103
SCID 107

Figure 23B


Circuit Ca_i Concentration in SCID 102 and 107
(target circuit Ca_i concentration is 0.2–0.4 mEq/L)
15 min
30 min
45 min
Ca_i (mEq/L)
0.6
0.5
0.4
0.3
0.2
0.1
0
15 min
30 min
45 min
30 min
SCID 102
SCID 107