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(54) Title: METHODS FOR MAKING CYTOKINE COMPOSITIONS FROM TISSUES USING NON-CENTRIFUGAL METHODS

(57) Abstract: Apparatus and methods for generating a solution rich in interleukin-1 receptor antagonist from a tissue comprising cytokine-producing cells. The apparatus can include a filter used with a separation system. The separation system can include a float.

METHODS FOR MAKING CYTOKINE COMPOSITIONS FROM TISSUES USING NON-CENTRIFUGAL METHODS

INTRODUCTION

[0001] The present technology relates to methods of treating inflammatory disorders, including osteoarthritis. In particular, methods comprise use of solutions comprising cytokines, including such solutions derived from blood fractions obtained by non-centrifugal methods.

[0002] Inflammation is a complex cellular and biochemical process that occurs in the affected blood vessels and adjacent tissues in response to an injury or abnormal stimulation caused by a physical, chemical, or biologic agent, such as a pathogen, allergen or irritant. The inflammatory process includes local reactions and resulting morphologic changes in tissue; the destruction or removal of the causative agent; and the responses that lead to repair and healing. In most instances, inflammation is a beneficial and transient process, which subsides as the body attacks and overcomes an infectious or other harmful agent. However, in some instances, inflammation can be chronic self-perpetuating process, for example, as part of an ongoing degenerative process (such as arthritis) or autoimmune disease, leading to destruction of tissue. Chronic inflammation is associated with a variety of disorders, including rheumatoid arthritis, atherosclerosis, ischemic heart disease, periodontitis, colitis, and some cancers.

[0003] An inflammatory response consists of a cascade of biochemical events, involving the local vascular system and immune system, and various cells within the injured tissue. The process involves the release of numerous cell-derived mediators, including histamine, interferon-gamma, interleukin-8, leukotriene, nitric oxide, prostaglandins, tumor necrosis factor-alpha, and interleukin-1. In particular, interleukin-1 (IL-1) includes a family of cytokines that can stimulate lymphocytes and macrophages, activate phagocytes, increase prostaglandin production, contribute to degeneration of bone joints, increase bone marrow cell proliferation, and are involved in many chronic inflammatory conditions. IL-1 can be generated by macrophages, monocytes, and dendritic cells, and can be part of the inflammatory response against infection.

[0004] The mode of action of IL-1 can be mediated by interleukin-1 receptor antagonist protein (IL-1ra; also known as "IRAP"). IL-1ra binds to the same receptor on the cell surface as IL-1, and thus prevents IL-1 from sending a signal to that cell. IL-1ra

is secreted from white blood cells, including monocytes, macrophages, neutrophils, polymorphonuclear cells (PMNs), and other cells, and can modulate a variety of IL-1 related immune and inflammatory responses, as described by Arend WP, Malyak M, Guthridge CJ, Gabay C (1998) "Interleukin-1 receptor antagonist: role in biology" Annu. Rev. Immunol. 16: 27-55. Production of IL-1ra is stimulated by several substances including adherent immunoglobulin G (IgG), other cytokines, and bacterial or viral components. IL-1ra, as well as other cytokines such as soluble tumor necrosis factor receptor 1 (sTNF-R1), soluble tumor necrosis factor receptor 2 (sTNF-R2) and (soluble interleukin receptor II (sIL-1RII), is an important natural anti-inflammatory protein in arthritis, colitis, and granulomatous pulmonary disease.

[0005] IL-1ra can be used in the treatment of rheumatoid arthritis, an autoimmune disease in which IL-1 plays a key role, reducing inflammation and cartilage degradation associated with the disease. For example, Kineret™ (anakinra) is a recombinant, non-glycosylated form of IL-1ra (Amgen Manufacturing, Ltd., Thousand Oaks, California). Various recombinant interleukin-1 inhibitors and methods of treatment are described in U.S. Patent No. 6,599,873, Sommer et al., issued July 29, 2003; U.S. Patent No. 5,075,222, Hannum et al., issued December 24, 1991; and U.S. Patent Application Publication No. 2005/0197293, Mellis et al., published September 8, 2005. In addition, methods for producing IL-1ra from body fluids, including the use of autologous fluids, are described in U.S. Patent No. 6,623,472, Reinecke et al., issued September 23, 2003; U.S. Patent No. 6,713,246, Reinecke et al., issued March 30, 2004; and U.S. Patent No. 6,759,188, Reinecke et al., issued July 6, 2004.

[0006] Many such treatments for inflammation are known in the art. Therapies known in the art may be directed to removal of the underlying irritant or agent causing the inflammatory reaction, or by mediating one or more aspects of the inflammatory response. Examples include glucocorticoid steroids (such as hydrocortisone, cortisone, prednisone, and beclomethasone), non-steroidal anti-inflammatory drugs (such as aspirin, ibuprofen and naproxen), and immune selective anti-inflammatories. However, many such treatments present side effects, particularly during chronic administration, or have pharmacologic characteristics that limit their use. For example, while compositions and methods using IL-1ra are known in the art, they may be associated with issues regarding stability and half-life of IL-1ra as well as the

amount and rate of IL-1ra provided. Moreover, many treatments do nothing to address the underlying causes of the inflammatory process. Accordingly, improved methods of treating inflammation are needed, offering one or more of improved efficacy, reduced side effects, and improved dosing characteristics.

SUMMARY

[0007] The present technology provides methods for generating solutions rich in anti-inflammatory cytokines for use in treatment of inflammation and other disorders mediated by interleukin-1 and tumor necrosis factor-alpha. Methods for generating such solutions include contacting a liquid volume of cytokine-producing cells with a solid extraction material, removing the solid extraction material from the liquid, and freeze drying the liquid. The liquid volume of cytokine-producing cells is obtained from whole blood by non-centrifugal methods, such as filtration, antibody binding, and electrophoretic methods. Thus, the present technology provides methods for generating a solution rich in interleukin-1 receptor antagonist (IL-1ra) comprising:

(a) obtaining a cytokine cell suspension, such as cytokine-producing cells, by separating cytokine-producing cells from a tissue comprising cytokine-producing cells using a non-centrifugal process;

(b) contacting the cytokine cell suspension with a solid extraction material; and

(c) separating the liquid from the solid extraction material to obtain the solution rich in IL-1ra.

The tissue comprising cytokine-producing cells may be selected from the group consisting of whole blood, bone marrow aspirate, adipose tissue, fractions thereof, and mixtures thereof.

[0008] In various embodiments, the anti-inflammatory cytokine composition comprises

(i) interleukin-1 receptor antagonist (IL-1ra) at a concentration of at least about 10,000 pg/ml;

(ii) soluble Tumor Necrosis Factor Receptor 1 (sTNF-R1) at a concentration of at least about 1,200 pg/ml; and

(iii) a protein selected from the group consisting of sTNF-RII, IGF-I, EGF, HGF, PDGF-AB, PDGF-BB, VEGF, TGF-β1, and sIL-1RII, and mixtures thereof, wherein the concentration of the protein in the composition is greater than the concentration of the protein in normal blood.

In some embodiments, the compositions additionally comprise white blood cells, platelets, or combinations thereof. The present technology also provides methods of treating a condition mediated by interleukin-1 receptor, such as inflammation, in a human or other mammalian subject, comprising topical administration of a composition of the present technology.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Figs. 1A-1D are a plan view of a device for making a suspension of white blood cells, before (Fig. 1A) and after (Fig. 1B) centrifugation, and during withdrawal (Figs. 1C and 1D); and

[0010] Figs. 2A and 2B show a device for activating a sample to generate anti-inflammatory cytokines, before (Fig. 2A) and after (Fig. 2B) centrifugation.

[0011] Corresponding reference numerals indicate corresponding parts throughout the several views of the drawings. It should be noted that the figures set forth herein are intended to exemplify the general characteristics of materials, compositions, devices, and methods among those of the present technology, for the purpose of the description of certain embodiments. These figures may not precisely reflect the characteristics of any given embodiment, and are not necessarily intended to fully define or limit specific embodiments within the scope of this technology.

DESCRIPTION

[0012] The following description of technology is merely exemplary in nature of the composition, manufacture and use of one or more inventions, and is not intended to limit the scope, application, or uses of any specific invention claimed in this application or in such other applications as may be filed claiming priority to this application, or patents issuing therefrom. A non-limiting discussion of terms and phrases intended to aid understanding of the present technology is provided at the end of this Detailed Description.

[0013] The present technology relates to compositions, methods of making compositions, and methods of using compositions for the treatment of inflammatory disorders, and other disorders mediated by interleukin-1. In general, such compositions are made by a process comprising:

(a) obtaining a cytokine cell suspension (a "cytokine cell suspension," as discussed further below) by separating cytokine-producing cells from a tissue comprising cytokine-producing cells using a non-centrifugal process; and

(b) fractionating the liquid to produce a protein solution comprising one or more proteins, such as interleukin-1 receptor antagonist.

Protein Compositions

[0014] The present technology provides methods for treating inflammatory disorders other disorders mediated by interleukin-1 in humans or other mammalian subjects using compositions (herein referred to as "Protein Solutions") comprising proteins dissolved, suspended or otherwise carried for delivery to a mammalian subject in a physiologically-acceptable medium. In various embodiments, such compositions comprise proteins (e.g., cytokines) that are native to whole blood in normal mammal subjects. Such compositions may also contain viable cells, including platelets, white blood cells, and combinations thereof.

[0015] In various embodiments, the Protein Solution comprises at least two proteins selected from the group consisting of IL-1ra, sTNF-RI, sTNF-RII (soluble tumor necrosis factor-receptor 2), IGF-I (insulin-like growth factor 1), EGF (epidermal growth factor), HGF (hepatocyte growth factor), PDGF-AB (platelet-derived growth factor AB), PDGF-BB (platelet-derived growth factor BB), VEGF (vascular endothelial growth factor), TGF-β1 (transforming growth factor- β1, and sIL-1RII (soluble interleukin receptor II), wherein the concentration of each protein in the composition is greater than the concentration of the protein in normal blood. While the concentration of every such protein in the composition may be greater than its respective concentrations in in normal blood, it is not necessary that the concentration of more than two of the proteins be greater than their respective concentrations in normal blood.

[0016] In various embodiments, the platelet-rich protein solution comprises the following components.

Table 1. Protein Solution Exemplary Protein Components

Component	Composition Concentration	Normal Whole Blood Concentration
plasma proteins (total)	about 80 mg/ml or greater about 100 mg/ml or greater about 200 mg/ml or greater about 250 mg/ml or greater	about 67 mg/ml
albumin	about 60 mg/ml or greater about 100 mg/ml of greater	about 56 mg/ml
fibrinogen	about 3.2 mg/ml or greater about 4 mg/ml or greater	about 2.9 mg/ml
IL-1ra	about 10,000 pg/ml or greater about 25,000 pg/ml or greater about 30,000 pg/ml or greater from about 25,000 to about 110,000 pg/ml from about 25,000 to about 40,000 pg/ml	about 4200 pg/ml
sTNF-RI	about 1,200 pg/ml or greater about 1,800 pg/ml or greater about 3,000 pg/ml or greater	about 630 pg/ml
sTNF-RII	about 3,000 pg/ml or greater about 5,000 pg/ml or greater about 7,000 pg/ml or greater about 9,000 pg/ml or greater	about 1200 pg/ml

sIL-1RII	about 15,000 pg/ml or greater about 20,000 pg/ml or greater about 25,000 pg/ml or greater	about 11,800 pg/ml
Growth factors		
EGF	about 800 pg/ml or greater about 1,000 pg/ml or greater about 1,200 pg/ml or greater	about 250 pg/ml
HGF	about 1,000 pg/ml or greater about 2,500 pg/ml or greater about 2,800 pg/ml or greater about 3,000 pg/ml or greater	about 500 pg/ml
PDGF-AB	about 35,000 pg/ml or greater about 50,000 pg/ml or greater about 70,000 pg/ml or greater	about 6,000 pg/ml
PDGF-BB	about 10,000 pg/ml or greater about 15,000 pg/ml or greater about 20,000 pg/ml or greater	about 1,500 pg/ml
TGF-β1	about 100,000 pg/ml or greater about 150,000 pg/ml or greater about 190,000 pg/ml or greater	about 10,000 pg/ml
IGF-1	about 130,000 pg/ml or greater about 150,000 pg/ml or greater about 160,000 pg/ml or greater	about 70,000 pg/ml
VEGF	about 500 pg/ml or greater	about 150 pg/ml

	about 600 pg/ml or greater about 800 pg/ml or greater	

[0017] Protein concentrations can be measured using the methods known in the art. For example, Quantikine Human Immunoassays (R&D Systems, Inc., Minneapolis, MN) may be used to assay for IL-1ra, IL-1β, IL-8, sTNF-RI, TNF-α, IL-6, sTNF-RII, IL-10, IL-13, and IL-4, according to the manufacturer's instructions. Immunoassays may be performed for hepatocyte growth factor and soluble IL-1RII.

[0018] The composition further preferably comprises viable white blood cells, lysed white blood cells, or both. In a preferred composition, the Protein Solution comprises monocytes, granulocytes, and platelets. In various embodiments, a Protein Solution comprises the following components.

Table 2. Protein Solution Exemplary Cellular Components

Component	Composition Concentration	Normal Whole Blood Concentration
white blood cells	at least about 15 k/ µl at least about 30 k/ µl from about 30 to about 60 k/ µl from about 40 to about 50 k/ µl	6.5 k/µl
red blood cells	less than about 3 M/ µl less than about 2 M/ µl less than about 2.5 M/ µl	4.5 M/ µl
platelets	at least about 400 k/ µl at least about 800 k/ µl at least about 1,000 k/ µl	240 k/ µl
neutrophils	at least about 5 k/ µl at least about 10 k/ µl at least about 12 k/ µl	3.7 k/ µl

monocytes	at least about 1 k/ µl at least about 2 k/ µl at least about 3 k/ µl	0.5 k/ µl
lymphocytes	at least about 5 k/ µl at least about 10 k/ µl at least about 20 k/ µl	2 k/ µl
eosinophiles	at least about 0.15 k/ µl at least about 0.18 k/ µl	0.1 k/ µl
basophils	at least about 0.2 k/ µl at least about 0.4 k/ µl at least about 0.6 k/ µl	0.1 k/ µl

[0019] It will be understood that this concentration is species specific. Further, it is understood that concentrations may vary among individual subjects. Thus, in methods comprising production of a Protein Solution from the blood or other tissue containing cytokine-producing cells, the concentration of proteins and cells in the Protein Solution may vary from those recited above; the values recited above are mean values for concentrations as may be seen in a population of subjects.

[0020] In various embodiments, the concentration of one or more of the proteins or other components in the Protein Solution is greater than the concentration of the component in normal blood. (Compositions with such higher concentrations of components are said to be "rich" in such components.) As referred to herein, the concentration of a component in "normal" blood or other tissue is the concentration found in the general population of mammalian subjects from which the tissue is obtained, e.g., in normal whole blood. In methods wherein the anti-inflammatory cytokine composition is derived from tissue from a specific subject, the "normal"

concentration of a protein or cell may be the concentration in the blood of that individual before processing is performed to derive the protein or cell.

[0021] Thus, in various embodiments, the concentration of one or more components of the Protein Solution is greater than about 1.5 times, about 2 times, or about 3 times, greater than the concentration of the component in normal blood. For example, components may have greater concentrations in the compositions, relative to normal (whole) blood, as follows:

- IL-1ra, at a concentration that is at least about 2.5, or at least about 3 or at least about 5, times greater;
- sTNF-RI, at a concentration that is at least about 2, or at least about 2.5 or at least about 3, times greater;
- sTNF-RII, at a concentration that is at least about 2, or at least about 2.5 or at least about 3, times greater;
- sIL-1RII, at a concentration that is at least about 1.5, or at least about 1.8 or at least about 2, times greater;
- EGF, at a concentration that is at least about 2, or at least about 3 or at least about 5, times greater;
- HGF, at a concentration that is at least about 2, or at least about 3 or at least about 4, times greater;
- PDGF-AB, at a concentration that is at least about 2, or at least about 3 or at least about 5, times greater;
- PDGF-BB, at a concentration that is at least about 2, or at least about 3 or at least about 5, times greater;
- TGF-β1, at a concentration that is at least about 3, or at least about 4 or at least about 6, times greater;
- IGF-1, at a concentration that is at least about 1.2, or at least about 1.4 or at least about 1.5, times greater;
- VEGF, at a concentration that is at least about 2, or at least about 2.5 or at least about 3, times greater;
- white blood cells, at a concentration that is at least about 2, or at least about 3 or at least about 4, times greater;

- platelets, at a concentration that is at least about 2, or at least about 3 or at least 4, times greater;
- neutrophils, at a concentration that is at least 1.5, or at least 2 or at least 3, times greater;
- monocytes, at a concentration that is at least 3, or at least 4 or at least 6, times greater;
- lymphocytes, at a concentration that is at least 5, or at least 8 or at least 10, times greater; and
- basophils, at a concentration that is at least 2, or at least 4 or at least 6, times greater

Also, the concentration of erythrocytes in the Protein Solution is preferably at least half, or at least a third, of the concentration of erythrocytes in normal blood.

[0022] For example, a Protein Solution may comprise:

(a) at least about 10,000 pg/ml IL1-ra;

(b) at least about 1,200 pg/ml sTNF-RI; and

(c) a protein selected from the group consisting of sTNF-RII, IGF-I, EGF, HGF, PDGF-AB, PDGF-BB, VEGF, TGF-β1, and sIL-1RII, and mixtures thereof, wherein the protein has a concentration higher than the protein's baseline concentration in normal blood.

[0023] In another example, a Protein Solution comprises:

(a) interleukin-1 receptor antagonist (IL-1ra), at a concentration at least 3 times greater than the concentration of IL-1ra in normal blood;

(b) soluble tissue necrosis factor-r1 (sTNF-r1), at a concentration at least 2 times greater than the concentration of IL-1ra in normal blood;

(c) white blood cells at a concentration at least 2 times greater than the concentration of white blood cells in normal blood; and

(d) platelets, at a concentration at least 2 times greater than the concentration of platelets in normal blood.

[0024] In some embodiments, the concentration of IL-1ra in the Protein Solution is preferably at least 5,000, or at least 10,000, times greater than the concentration of interleukin-1α in the Protein Solution. The ratio of IL-1ra:interleukin-1β (IL-1 β) concentrations is preferably at least 100. In some embodiments, the

concentration of IL-1ra in the Protein Solution is preferably at least 1500, or at least 8000, times greater than the concentration of IL-1 β in the Protein Solution. The ratio of sIL-1RII:interleukin-1β (IL-1 β) concentrations is preferably greater than 1. In some embodiments, the sIL-1RII in the Protein Solution is preferably at least 2000, or at least 45000, times greater the concentration of interleukin-1β in the Protein Solution.

[0025] The present technology provides Protein Solutions wherein one or more components of the Protein Solution are obtained from non-autologous sources, such as through recombinant or synthetic methods, or by isolation from allogeneic sources (i.e., from subjects of the same species as the subject to whom the solution is to be administered) or xenogeneic sources (i.e., from animal sources other than the species to whom the solution is to be administered). In some embodiments, the Protein Solutions consists, or consists essentially, of such allogeneic components. However, in various embodiments, the Protein Solution comprises one or more components (e.g., platelets) derived from the subject to whom the solution is to be administered in a treatment methods according to this technology. Such components are, accordingly, "autologous." In some embodiments, the Protein Solution comprises mixtures of autologous and allogeneic components.

Methods of Making Protein Solutions

[0026] As discussed above, the Protein Solution is made by fractionating a liquid comprising cytokine-producing cells, to produce a protein solution comprising cytokines, such as IL1-ra. In various embodiments, Protein Solutions are made by derivation of one or more components from tissue comprising cytokine-producing cells. As referred to herein, a "cytokine producing tissue" is a tissue obtained from a mammalian subject, comprising cells that are capable of producing cytokines. Such cells include white blood cells, adipose stromal cells, bone marrow stromal cells, and combinations thereof. It is understood that white blood cells include monocytes, lymphocytes, and granulocytes such as neutrophils, eosinophils, and basophils. White blood cells useful in the methods of this technology preferably include monocytes and neutrophils. Cytokine producing tissues among those useful herein include blood, adipose tissue, bone marrow, and fractions thereof, as further discussed below.

[0027] Blood useful herein includes whole blood, plasma, platelet-rich plasma, platelet-poor plasma, and blood clots. In a preferred embodiment, methods of the present technology use platelet-rich plasma (PRP), containing white blood cells and platelets, comprising the buffy coat layer created by sedimentation of whole blood. Adipose tissue useful herein includes any fat tissue, including white and brown adipose tissue, which may be derived from subcutaneous, omental/visceral, mammary, gonadal, or other adipose tissue sites. Bone marrow useful herein includes red marrow and yellow marrow. In a preferred embodiment, bone marrow is bone marrow concentrate, obtained from the red marrow of long bones, comprising hematopoietic and mesenchymal stems cells. As discussed above, compositions of the present technology may be made from blood, adipose, and bone marrow tissue from allogeneic sources, relative to the subject to be treated according to methods of this technology. Compositions may also be made from combinations of allogeneic and autologous tissues.

[0028] In some embodiments, methods comprise fractionating a liquid (a "cytokine cell suspension.") comprising cells capable of producing cytokines, such as IL1-ra and sTNF-R1. As discussed above, such cells include white blood cells, adipose stromal cells, bone marrow stromal cells, and combinations thereof. In some embodiments, the cytokine cell suspension is a liquid comprising white blood cells. It should be understood that the cytokine cell suspension comprises cells and an extra-cellular liquid, regardless of the relative proportions of the cells and liquid. In some embodiments, the suspension may comprise primarily cells, with liquid being present as only a minor component, essentially wetting the cells. In some embodiments, the liquid may comprise two phases, consisting of a phase primarily consisting of liquid and a phase primarily consisting of cells, forming a suspension of cells in the liquid only upon agitation or other mixing.

Obtaining a Cytokine Cell Suspension

[0029] Obtaining cytokine-producing cells may be performed directly in some methods of this technology, whereby a health care practitioner or other individual performs isolation, processing, or other processes for creating the liquid, in a procedure that includes the contacting and isolating steps. In some embodiments, the processes for creating the liquid are performed contemporaneously with the contacting and isolating

steps, as part of a point-of-care procedure, as discussed further herein. Alternatively, obtaining the liquid may be indirect, involving only the acquisition of the liquid for use in the contacting and isolating steps, wherein the processing to create the liquid has previously been performed by another party.

[0030] As discussed above, the methods of this technology comprise obtaining a liquid comprising cytokine-producing cells by non-centrifugal processing of a tissue containing cytokine-producing cells (e.g., white blood cells), obtained from a mammalian subject. As referred to herein, a "non-centrifugal method" comprises a process for obtaining tissue fractions comprising cytokine-producing cells from tissue without use of a centrifuge. In some embodiments, methods are "non-gravimetric," wherein , based on physical, chemical or physicochemical properties of the cells other than density, wherein the concentration of white blood cells in the fraction are higher than the concentration of white blood cells in the tissue. Such non-gravimetric methods are, in particular, distinguished from methods wherein a white blood cell fraction is created by centrifugation of whole blood or other tissue. In some embodiments, the non-centrifugal method comprises a process solely based on such properties of white blood cells other than density.

[0031] In some embodiments, non-centrifugal methods useful herein include obtaining a volume of cytokine-producing cells and suspending the volume in a suitable medium for subsequent processing in the methods of this technology. In other embodiments, a fraction of cytokine-producing cells is isolated from tissue, wherein the fraction comprises cytokine-producing cells in the medium suitable for subsequent processing. The medium may be constituted so as to maintain viability of the cytokine-producing cells, including physiological saline and serum. Thus, in various embodiments, methods comprise:

(a) mixing a volume of cytokine-producing cells with a liquid medium to form a cytokine cell suspension;

(b) contacting the cytokine cell suspension a solid extraction material; and

(c) separating liquid from the polyacrylamide beads to obtain a solution rich in IL-1ra.

[0032] As further discussed below, the volume of cytokine-producing cells may be made by a process comprising one or more of:

(a) expressing a tissue comprising cytokine-producing cells through a hollow fiber apparatus, forming a liquid volume comprising cytokine-producing cells;

(b) separation of cytokine-producing cells from whole blood using a density gradient aid;

(c) electrophoresis; and

(d) binding of cytokine-producing cells to antibody conjugated beads.

[0033] A cytokine cell suspension may be prepared by admixing cells with a suitable liquid, using methods known in the art. For example, white blood cells may be isolated from whole blood by lysing red blood cells or by centrifugation of whole blood utilizing a density gradient aid. An example of a suitable density gradient aid is Ficoll-Paque™ Plus (GE Healthcare Bio-Sciences, Piscataway, New Jersey, USA), which comprises a hydrophilic polysaccharide which aggregates red blood cells. For example, in such a method, whole blood may be diluted 1:1 with an appropriate buffer, such as phosphate buffered saline (PBS), in a container. A volume of Ficoll-Paque is added to a centrifuge tube. The whole blood:PBS mixture is then gently layered on top of the Ficoll-Paque. Care should be taken to ensure the Ficoll- Paque and whole blood:PBS layers do not mix. The centrifuge tube containing layers of Ficoll-Paque and whole blood:PBS is centrifuged to separate blood components. Centrifugation can be from about 25 to about 35 minutes at about 400 x g. After centrifugation, the blood components are present in separate layers. The top most layer comprises plasma. Directly below the plasma is a buffy coat, which contains white blood cells. Directly below the buffy coat is the Ficoll-Paque, and below the Ficoll-Paque are the red blood cells. The buffy coat, containing white blood cells, such as mononuclear cells, is collected and placed in a sterile centrifuge tube. Optionally, the white blood cells can be washed one or more times by suspending the cells in PBS, pelleting the cells by centrifugation, and removing the supernatant. After the cells are washed, they can be re-suspended in an appropriate buffer, such as PBS, or in plasma.

[0034] In some cases, a density gradient may be used to further separate mononuclear and polymorphonuclear cells. White blood cells may also be prepared from whole blood using filtration; an example includes the Acelere™ MNC Harvest System (Pall Life Sciences, Ann Arbor, Michigan, USA). White blood cells can also be

obtained from bone marrow. The white blood cells may be then suspended in a suitable medium, such as plasma, so as to maintain their viability.

[0035] Methods for isolating a liquid comprising white blood cell from a tissue include filtration, antibody binding, and electrophoretic methods. Filtrations methods include size exclusion filters, such as hollow fiber arrays, including such filters known in the art. A commercially available filter is LeukoCatch syringe filters are described by Okuzaki, et al., BMC Clinical Pathology 2011, 11:9, incorporated herein by reference. The LeukoCatch comprises one or more Leukosorb® B Medium filters (Pall Corp, Port Washington, NY) sandwiched between plastic stoppers and set at the bottom of a 10 ml syringe. Leukocytes, but not red blood cells are captured by the filter. Therefore, a LeukoCatch syringe comprising a plunger can be used to aspirate a sample of whole blood, wherein the leukocytes are captured by the filter. Pushing the plunger down expels the red blood cells from the syringe, but not the leukocytes, which are retained in the filter. The leukocytes can be washed by aspirating phosphate buffered saline (PBS) by pulling the plunger up, and expelling the PBS by pushing the plunger down. Washing can be repeated one or more times. The leukocytes are then eluted by pulling elution buffer into the syringe, and then expelling the elution buffer, now containing leukocytes, into a clean and sterile container. Elution buffer can comprise 10 mM Tris-HCL (pH 7.5), 100 mM NaCl, 1% Triton X-100, 1 mM EDTA (pH 8.0), 0.1 mg/mL PMSF, 1 mM Aprotinin, 0.001 mg/ml leupeptin, 0.001 mg/ml pepstatin A, 1 mM NaF, 1 mM Na_3VO_4, and 10 mM β-glycerophosphate.

[0036] Another non-centrifugal method for generating a liquid volume comprising cytokine-producing cells comprises inducing electrical fields on electrically charged plates to separate cells based on their affinity for different electrical charges. In one such method as described by Wang, et al., Anal Chem. 2000 February 15; 72(4): 832-839 dielectrophoretic field-flow-fractionation (DEP-FFF) can be used to fractionate leukocytes from blood. Leukocytes have different dielectric properties than erythrocytes. Microelectrodes produce DEP forces that levitate blood cells in a thin chamber comprising a top plate and a bottom plate separated by a spacer. When the cells reach an equilibrium height based on their dielectric properties, a carrier fluid moves through the chamber. Cells at different heights travel through the chamber at different velocities, resulting in their separation. For example, whole blood can be diluted 1:1000 in a

sucrose buffer. When placed in the thin chamber, a DEP field of about 10 kHz can be applied for DEP-FFF. With a flow rate of about 0.5 ml/min, leukocytes can be enriched 35-fold.

[0037] Yet another non-centrifugal method for generating a liquid volume comprising cytokine-producing cells comprises the use of magnetic particles, such as magnetic beads, that specifically bind to cytokine-producing cells. The magnetic particle can be coupled to a molecule that specifically binds to mononuclear leukocytes. The molecule and be a ligand or an antibody. For example, U.S. Patent No. 7,867,765, by Faustman, et al., issued January 11, 2011 describes magnetic beads conjugated to proteins that bind specifically to white blood cell surface proteins. Antibody or ligand conjugated beads are added to a container containing a sample comprising cytokine-producing cells. The antibody or ligand binds to cytokine-producing cells. After incubating for a preselected period of time, a magnet is placed or held on the outside of the container. While the magnet is placed or held on the outside of the container, the container is tilted to decant contents that are not attracted to the magnet. The content that is attracted to the magnet, white blood cell conjugated magnetic beads, can be washed one or more times with an appropriate buffer, such as PBS. Optionally, the magnetic beads can be separated from the cytokine-producing cells. Finally, the mononuclear cytokine-producing cells can be resuspended in an appropriate buffer, for example PBS or plasma.

[0038] In some embodiments, obtaining cytokine cell suspension comprises filtering blood to isolate white blood cells, and suspending the white blood cells in plasma to form a white blood cell suspension. A device for creating such whole blood cell suspension is shown in Figure 1A. Collecting white blood cells (leukocytes) from a whole blood sample can be performed using filtration methods, as discussed above. For example, as illustrated in Figure 1A, a filter 220 can be placed at the end of a syringe 200 in which a volume of whole blood is placed. The volume of whole blood can be passed through the filter 220, such as by forcing the blood via a plunger 202 in the syringe 200. Additionally, a centrifuge or gravity system can be used to force the volume of whole blood through the filter 220. White blood cells are collected in the filter 220 while the remaining components of the whole blood, such as red blood cells and plasma, pass through the filter 220. Collecting the separated the white blood cells can then be

performed by backwashing the filter 220 with a selected fluid, such as plasma, saline, or other selected fluid. The backwashed material can be backwashed into a collection container, such as the syringe 200, after the whole blood is expressed through the filter in a first direction.

[0039] According to various embodiments, therefore, the syringe 200 can have a volume of whole blood positioned therein. The filter 220 can filter and collect white blood cells from whole blood volume, as the whole blood volume is forced (e.g. expressed) through the filter 220. The filter 220 can be positioned at or on the end of the syringe 200. Once the syringe 200 that included the volume of whole blood has expressed the whole blood through the filter 220, the syringe 200 and the filter 220 can be interconnected with a fluid source for backwashing the filter 220. The plunger 202 can then be moved in a second or direction to fill the syringe 200 to pass the backwashing fluid, such as saline or plasma, through the filter 220 to fill the syringe with the backwashed material from the filter, including the white blood cells.

[0040] In addition to expressing the whole blood through the syringe and filter into a separate container, and backwashing with a backwashing material from a different source, various components or fractions of the whole blood sample can also be used to backwash the filter. For example, if whole blood is passed through the filter in a first direction, the remaining plasma and/or buffy coat in the whole blood can be used to backwash the filter. Collection of fractionation of the remaining whole blood portions into plasma and/or buffy coat and/or red blood cells can be performed for the various devices such as those disclosed in U.S. Patent No. 7,845,499, Higgins et al., issued December 7, 2010; and U.S. Patent Application Publication No. 2011/0192804, Landrigan et al., published August 11, 2011. For example, with reference to Figure 1A, a syringe 200 can be filled with an anti-coagulated whole blood sample that is expressed through a filter 220 into a fractionation vessel or tube 230. The fractionation tube 230 can include a buoy system 240 similar to that disclosed in U.S. Patent Application Publication No. 2011/0192804. The buoy system 240 can include a first buoy member 242 that is moveable or fixed relative to a second buoy member 244 with a third buoy member 246. The buoy system 240 can have a connection port or valve or opening 250 that is connectable or permanently connectable with an extraction and/or filling tube with the input port 260. The input port 260 can also be used as an extraction port from the

tube 230. Additionally, a separate or plasma extraction port 270 can also be provided into the separation tube 230. An extraction or straw tube 272 can be interconnected with the plasma extraction port 270.

[0041] Once the separation tube 230 is filled with the whole blood after it has passed through the white blood filter 220, the tube 230 can be centrifuged to separate the filtered whole blood into selected fractions, such as a red blood cells fraction 300, a buffy coat 310, and a plasma fraction 320. The buoy system 240 can be used to separate the three fractions 300, 310, and 320 for withdrawal from the fractionation tube 230. The fractionated filtered blood, as illustrated in Figure 1B, can allow for withdrawal of the selected fraction from a single separation tube 230 based upon the selected fraction desired for further processing and/or application. Additionally, as discussed above, the filter 220 can include the white blood cells, or a majority or selected portion of the white blood cells, from the whole blood sample included in the syringe 200. The syringe 200 can then be used to withdraw a selected or multiple fractions from the separation tube 230 by connecting with either the input port 260 or a collection tube 350 connected with the port, valve, or opening 250 of the buoy system 240, as illustrated in Figure 1C and/or connecting with the plasma extraction port 270 to withdraw material through the plasma extraction tube 272, as illustrated in Figure 1D.

[0042] It is understood that the filter 220 can be positioned between the syringe 200 and the respective ports 260, 270 such that the material withdrawn from the separation tube 230 will backwash the filter 220.

[0043] As discussed above, the fractions within the separation tube 230 can be based upon separation of the filtered whole blood sample positioned within the tube 230. According to various embodiments, the factions can include at least a red blood cell fraction 300, a buffy coat fraction as the fraction 310 and the plasma fraction as the fraction 320. Accordingly, according to various embodiments, the buffy coat fraction 310 can be used to backwash the filter 220, as illustrated in Figure 1B. Alternatively, or in addition to the buffy coat backwash, the plasma fraction 320 can be used to backwash the filter 220, as illustrated in Figure 1B. It is understood that either or both of the fractions, including the buffy coat fraction 310 and/or the plasma fraction 320 can be used to backwash the filter 220. Accordingly, the separation tube 230 can be used to separate a filtered whole blood sample into selected fractions to backwash the filter 220

for various purposes. It is further understood that additional materials can be used to backwash the filter either initially, intermediately, or following a backwash with the separated whole blood, such as with saline. Accordingly, it is understood that the filter 220 can be backwashed to remove the filtered white blood cells from the filter 220 for use as discussed further herein.

Contacting With a Solid Extraction Material

[0044] In some embodiments, fractionating a cytokine cell suspension comprises contacting the liquid with a solid extraction material. Such processes comprise:

(a) obtaining a cytokine cell suspension;

(b) contacting the liquid with a solid extraction material; and

(c) isolating a protein-containing liquid from the solid extraction material.

[0045] Accordingly, in various embodiments, the cytokine cell suspension is incubated or otherwise contacted with a solid extraction material to produce a protein-containing liquid. This liquid is then isolated from the solid extraction material, as a Protein Solution of the present technology. Without limiting the scope, mechanism or function of the present technology, solid extraction materials useful herein concentrate cytokines or other proteins in the liquid volume of cytokine-producing cells and may, in some embodiments, activate, stimulate or otherwise increase production of cytokines, including IL-1ra. Thus, in some embodiments, methods comprising activating a cytokine cell suspension with a solid extraction material.

[0046] The solid extraction material can include various materials that provide a particular surface area to contact the cells. The solid extraction material may be a continuous material or may be discontinuous and comprise a plurality of separate particles. For example, the solid extraction material may be in the form of a plurality of beads, fibers, powder, a porous material, or a surface of a container comprising the liquid containing the cells. The solid extraction material may comprise geometric forms having various cross-sectional shapes, such as spherical, oval, or polygonal, among others. The solid extraction material can also comprise a continuous porous network, similar to a sponge, or can include a plurality of individual porous particles. The solid extraction

material may also provide a larger surface area by being porous in comparison to a non-porous material.

[0047] In some embodiments, the solid extraction material includes particles having a large aspect ratio, for example, where the particles are needle-like in shape. The solid extraction material may also be formed as long fibers and may be or take a form similar to glass wool.

[0048] In some cases, the solid extraction material can comprise the internal walls of a container holding the cytokine cell suspension. For example, the solid extraction material may comprise the lumen of a syringe that contains the cytokine cell suspension. Other containers include tubes, such as centrifuge tubes, or a blood fractionation device or concentrator assembly as described elsewhere herein.

[0049] Where the solid extraction material is a continuous material, such as a porous sponge-like material, the solid extraction material can be used in an amount sufficient to absorb or adsorb or include substantially the entire liquid volume of cytokine-producing cells within the pores or interstices of the solid extraction material. Where the solid extraction material is a discontinuous material, such as a plurality of particles, the solid extraction material can be combined with the liquid containing the cells to form a slurry-like composition. The slurry can vary in consistency from paste-like, having a high-solids fraction, to a readily flowable slurry having a low-solids fraction.

[0050] The solid extraction material can provide a large surface area with which to contact the cells. However, in some cases, the solid extraction material can be further treated to increase its surface area, for example, by physically or chemically etching or eroding the surface of the solid extraction material. With respect to chemical etching, a corrosive agent can be used to modify the surface of the solid extraction material depending on the nature of the material. The modified surface may be produced by employing an alkali or an acid, for example chromosulphonic acid, in particular about 20% to about 80% in strength, preferably about 50% chromosulphonic acid. The solid extraction material can be incubated with the corrosive agent for about 5 min to about 30 min in order to chemically etch the surface and increase the surface area. The solid extraction material can then be washed to remove the corrosive agent. For example, the solid extraction material can include the internal walls of a container for holding the

cytokine cell suspension where the internal walls are etched to subsequently increase the surface area in contact with the liquid.

[0051] Various polymers, metals, ceramics, and glasses can be used as the solid extraction material. In some embodiments, the solid extraction material comprises a hygroscopic material. Examples of suitable solid extraction material materials include glasses, minerals, polymers, metals, and polysaccharides. Minerals include corundum and quartz. Polymers include polystyrene, polyethylene, polyvinyl chloride, polypropylene, and polyacrylamide. Metals include titanium. Polysaccharides include dextran and agarose. A preferred solid extraction material comprises, or consists essentially of, polyacrylamide, as further described below.

[0052] The solid extraction material may comprise, for example, continuous solid extraction material of glass or a plurality of glass particles, glass wool, a continuous solid extraction material of metal such as titanium, a plurality of metal beads, metal powder, and combinations thereof. A continuous solid extraction material of metal can include a block or other three-dimensional shape formed of porous metal or metal alloys with an open cell structure. The solid extraction material may include various beads or particles of various sizes including substantially spherical beads. Beads include polystyrene beads, polyacrylamide beads, glass beads, metal (e.g., titanium) beads, or any other appropriate beads. Beads may be any size appropriate for the container and the amount of cytokine cell suspension being used. In some instances, bead sizes can range from about 0.001 millimeters to about 3 millimeters in diameter. Where the bead size is sufficiently small, the beads can appear more like a powder.

[0053] Polyacrylamide beads used as the solid extraction material can be formed by polymerizing acrylamide monomer using controlled and standardized protocols as known in the art to produce relatively uniform beads formed of polyacrylamide gel. In general, polyacrylamide is formed by polymerizing acrylamide with a suitable bifunctional crosslinking agent, most commonly N,N'-methylenebisacrylamide (bisacrylamide). Gel polymerization is usually initiated with ammonium persulfate and the reaction rate is accelerated by the addition of a catalyst, such as N,N,N',N'-tetramethylethylenediamine (TEMED). In various embodiments, polyacrylamide beads comprise 0.5 micromole of carboxyl groups per milliliter of beads, imparting a slight anionic character (negative charge). The beads are also typically

resistant to changes in pH, and are stable in many aqueous and organic solutions. By adjusting the total acrylamide concentration, the polyacrylamide gel can be formed in a wide range of pore sizes. Moreover, the polyacrylamide beads can be formed in many sizes and can have relatively uniform size distributions. Bead size may range from several micrometers in diameter to several millimeters in diameter. For example, various types of Bio-Gel™ P polyacrylamide gel beads (Bio-Rad Laboratories, Hercules, California, USA) have particle sizes ranging from less than about 45 μm up to about 180 μm. Polyacrylamide beads are also available from SNF Floerger (Riceboro, Georgia, USA), Pierce Biotechnology, Inc. (Rockford, Illinois, USA), and Polymers, Inc. (Fayetteville, Arkansas, USA).

[0054] Once polymerized, polyacrylamide beads can be dried and stored in a powder-like form. The dry beads are insoluble in water but can swell considerably upon being rehydrated. Rehydration returns the polyacrylamide beads to a gel consistency that can be from about two to about three times the dry state size. Thus, dry polyacrylamide beads (i.e., desiccating polyacrylamide beads) may be used to absorb a portion of a liquid volume, including solutes smaller than the bead pore size, and can serve to concentrate IL-1ra and other proteins produced by the cytokine-producing cells. For example, combining dry polyacrylamide beads with the blood and/or platelet-rich plasma in step 230 activates production of IL-1ra by the cytokine-producing cells and also reduces the total liquid volume as the dry beads rehydrate and swell.

[0055] Without limiting the scope, mechanism or function of the present technology, it has been discovered that surface contact with the solid extraction material can activate the cells and the solid extraction material can, in some cases, assist in the separation and concentration of the resulting Protein Solution rich in cytokines, including IL-1ra. For example, in the case of a porous solid extraction material, a portion of the liquid comprising the cells can enter the pores and remain therein. Cells in the liquid may contact this additional surface area. In some embodiments, the pores are too small for the cells to enter, but a portion of the liquid can enter the pores. Liquid can be removed from the solid extraction material and pores by centrifuging, for example.

[0056] The solid extraction material is preferably sterilized, using techniques among known in the art, in order to prevent contamination of the cytokine cell suspension. For example, heat and pressure sterilization methods, such as autoclaving,

may be used depending on the particular composition of the solid extraction material. Alternative methods, such as chemical sterilization or irradiation, can be used where the solid extraction material may be adversely affected by the autoclaving process.

[0057] In some embodiments, the cytokine cell suspension is incubated with solid extraction material for a time effective to remove a portion of the liquid. The incubation may be carried out over a period from about 30 seconds to about 72 hours and may be carried out at a temperature from about 20°C to about 41°C. For example, the incubation may be from about one minute to about 48 hours, from about 2 minutes to about 24 hours, from about 5 minutes to about 12 hours, from about 10 minutes to about 6 hours, from about 15 minutes to about 5 hours, from about 30 minutes to about 4 hours, from about 1 hour to about 3 hours, or from about 2 hours to about 2.5 hours. In some embodiments, the incubation is conducted at about 37°C. In some embodiments the liquid is not incubated, but is contacted with the solid extraction material for only so long as necessary to perform subsequent processing. The contacting may occur at ambient conditions, e.g., at a temperature of about 20-25°C.

[0058] In some embodiments, the cytokine cell suspension and the solid extraction material are agitated to more thoroughly mix these components during contact. The agitation may be accomplished by inverting, shaking, rocking, stirring, or vortexing the liquid and solid extraction material. Agitation may increase contact of the cells within the liquid with the solid extraction material. Agitation may be performed once, repeated multiple times, repeated periodically, or may be continuous. The liquid comprising the cells and the solid extraction material may also be agitated while the liquid is stimulated with an electromagnetic field, as described below. Additional aspects and features relating to producing protein-rich solutions using polyacrylamide beads and other solid extraction materials are described in: U.S. Patent Application Publication No. 2009/0220482, Higgins et al., published September 3, 2009; U.S. Patent Application Publication No. 2010/0055087, Higgins et al., published March 4, 2010; U.S. Patent Application Publication 2011/0052561, Hoeppner, published March 3, 2011; International Publication WO 2012/030593, Higgins et al., published March 8, 2012; and U.S. Patent Application Publication 2012/0172836, Higgins et al., published July 5, 2012.

[0059] Contacting of the liquid containing cytokine-producing cells with the solid extraction material may be performed using a suitable container or other apparatus to effect the contact. Contacting may be performed in a continuous process wherein a flow of the liquid is passed over or through the solid extraction material, or the liquid and solid extraction material may be contained in a vessel. As discussed above, the vessel may comprise the solid extraction material, or may merely serve as a container holding the beads or other forms of the material. Containers useful in the present technology include those known in the art, such as the Plasmax™ Plus Plasma Concentrator, commercially available from Biomet Biologics, LLC (Warsaw, Indiana, USA) and may include those devices and methods of use as described in U.S. Patent No. 7,553,413, Dorian et al., issued June 30, 2009; and U.S. Patent No. 7,694,828, Swift et al., issued April 13, 2010.

[0060] Such a device is shown in Figures 2A and 2B, for exemplary use with a polyacrylamide gel bead solid extraction material. The device 400 has an upper chamber 405 and a lower chamber 410. The upper chamber 405 has an end wall 415 through which the agitator stem 420 of a gel bead agitator 425 extends. The device 400 also has an inlet port 430 that extends through the end wall 415 and into the upper chamber 405. The device 400 also includes an outlet port 435 that communicates with a plasma concentrate conduit 440. The floor of upper chamber 405 includes a filter 445, the upper surface of which supports desiccated concentrating polyacrylamide beads 450.

[0061] During use, a fluid 455 containing cytokine-producing cells and, optionally, platelets is injected to the upper chamber 405 via the inlet port 430 and mixed with the polyacrylamide beads 450. The fluid 455 and polyacrylamide beads 450 may be mixed by rotating the agitator stem 420 and the gel bead agitator 425, to help mix the fluid 455 and beads 450. The mixed fluid 455 and polyacrylamide beads 450 are then incubated for the desired time at the desired temperature. The device 400 is then centrifuged so that liquid passes to the lower chamber 410 while the polyacrylamide beads 450 are retained by a filter 445, thereby separating the polyacrylamide beads 150 from the resulting solution 460 of IL-1ra and other proteins that collects in the lower chamber 410. The solution 460 may be removed from the device via outlet port 435.

[0062] The cytokine cell suspension can be stimulated with an electromagnetic field, before or during the contacting of the liquid with a solid extraction

material. Thus, in some embodiments, stimulation of the liquid comprising the cells can be performed prior to contacting the liquid and the solid extraction material. However, it is preferred that at least a portion of the contacting step and at least a portion of the stimulating step overlap in time such that the liquid comprising the cells is concurrently in contact with the solid extraction material and stimulated with the electromagnetic field.

[0063] Stimulating the cytokine cell suspension with an electromagnetic field may involve various forms of electromagnetic stimulation, such as a pulsed electromagnetic field or a capacitively coupled electromagnetic field. In some embodiments, the liquid is stimulated using a power source coupled to a stimulation coil. The current passing through the coil produces a pulsing magnetic field which induces in the liquid a pulsing electric field. The coil may partially surround the liquid as it is held within a container, such as a tube or syringe. The coil may be integrated into to the container holding the cytokine cell suspension or may be removable. For example, a plastic tube can be formed with an integrated coil or the coil can be temporarily coupled to the container or placed within the container; for example, the tube can be configured so that the coil can be snapped onto the container. The power source can be coupled to the coil as needed to perform the stimulating step.

[0064] Stimulation of the liquid with an electromagnetic field may also include placing at least two electrodes across the liquid. Electrical energy may then be applied to the electrodes so as to capacitively couple the electrodes and generate the electromagnetic field there between. The electromagnetic field is therefore able to pass through the liquid so as to increase the rate and/or amount of cytokine production. In other embodiments, electrodes can be used to produce a direct current or one or more coils can be used to produce a pulsed electromagnetic field.

[0065] The strength of the electromagnetic field during stimulation can be at least about 0.5 microvolts per centimeter, whether produced by direct current, capacitively coupled current, or pulsed electromagnetic field. In the case of a direct current electrode, the amplitude of the current can be from about 1 to about 200 microamperes, and in some embodiments, the amplitude may be from about 20 to about 100 microamperes. In still further embodiments, the current may be about 20, about 60,

or about 100 microamperes. It should be understood, however, that the amplitude of the current may be of other suitable magnitudes.

[0066] The electromagnetic field applied during the stimulating step may be constant or vary over time. For example, a sinusoidal time varying electromagnetic field can be applied using the electrodes placed across the liquid. Such a sinusoidal time varying electromagnetic field can have a peak voltage across the electrodes from about 1 volt to about 10 volts, and in some embodiments, the peak voltage can be about 5 volts. The corresponding electric field produced can have an amplitude of from about 0.1 millivolt per centimeter (mV/cm) to about 100 mV/cm, and in some embodiments can be about 20 mV/cm. The sinusoidal time varying electric field may have a frequency of from about 1,000 Hz to about 200,000 Hz, and in some embodiments the frequency may be about 60,000 Hz.

[0067] The electromagnetic field applied to the liquid may also be a pulsed electromagnetic field. The pulsed electromagnetic field can be induced using an external coil and a pulse generator. In this regard, a pulsed electromagnetic field may have a pulse duration of from about 10 microseconds per pulse to about 2000 microseconds per pulse. The pulse duration in one embodiment can be about 225 microseconds. The pulses may include electromagnetic bursts, in which a burst can comprise from 1 pulse to about 200 pulses. Alternatively, the electromagnetic field may have bursts that comprise from about 10 pulses to about 30 pulses. In this regard, in one embodiment each burst may comprise about 20 pulses.

[0068] The frequency at which bursts in the pulsed electromagnetic are applied may vary. In this regard, bursts can be repeated at a frequency of from about 1 Hz to about 100 Hz in some embodiments, and can be repeated at a frequency of about 10 Hz to about 20 Hz in other embodiments. Furthermore, bursts can repeat at a frequency of about 1.5 Hz, about 15 Hz or about 76 Hz. A burst can have a duration from about 10 microseconds up to about 40,000 microseconds. In this regard, a burst can have a duration of about 4.5 milliseconds.

[0069] Suitable devices for generating a capacitively coupled electromagnetic field include SpinalPak® spinal stimulator (EBI, L.P., Parsippany, New Jersey) or a DC stimulation device such as an SpF® XL IIb spinal fusion stimulator (EBI, L.P., Parsippany, New Jersey). Pulsed electromagnetic fields can be produced using various

known methods and apparatuses, such as using a single coil or a pair of Helmholtz coils. For example, a suitable apparatus includes the EBI Bone Healing System® Model 2001 (EBI, L.P., Parsippany, New Jersey) and the BTBS stimulation coil. With respect to direct current, an electric field may be generated using any known device for generating a direct current electric field, such as for example, the Osteogen™ implantable bone growth stimulator (EBI, L.P., Parsippany, New Jersey). Other suitable devices for generating electromagnetic fields may be used.

[0070] Electromagnetic stimulation of the cytokine cell suspension can be continued and/or repeated as desired with respect to contacting the liquid and the solid extraction material. It should be understood, however, that the step of stimulating the liquid with an electromagnetic field includes fields other than, or in addition to, electric or electromagnetic fields associated with ambient conditions (such the electromagnetic fields generated by casual exposure to radios, telephones, desktop computers or similar devices).

[0071] In some embodiments, both the contacting and stimulating steps as shown in Figure 1 are performed in less than about 1 hour. The contacting and stimulating steps can also be performed at temperatures ranging from about 20°C to about 37°C. In a preferred embodiment, the temperature of the cytokine cell suspension is kept at about 37°C during the contacting and stimulating steps. One or both of the contacting and stimulating steps are typically performed ex vivo.

Therapeutic Compositions

[0072] The present technology also provides compositions comprising a Protein Solution and a second component comprising active materials, physiological carriers, and combinations thereof. In some embodiments, compositions comprise a safe and effective amount of the Protein Solution and a safe and effective amount of a second active. A "safe and effective" amount of a component is an amount that is sufficient to have the desired therapeutic effect in the human or other mammalian subject, without undue adverse side effects (such as toxicity, irritation, or allergic response), commensurate with a reasonable benefit/risk ratio when used in the manner of this technology. The specific safe and effective amount of the component will, obviously, vary with such factors as the particular condition being treated, the physical condition of

the patient, the nature of concurrent therapy (if any), the specific components used, the specific route of administration and dosage form, the carrier (if any) employed, and the desired dosage regimen.

[0073] Active materials among those useful herein include biologics and pharmaceutical actives. Biologics include blood fractions, such as PRP, blood products, and concentrated bone marrow aspirate (cBMA).

[0074] Accordingly, in some embodiments, the present technology provides compositions comprising a safe and effective amount of a Protein Solution and a safe and effective amount of cBMA. An autologous therapeutic composition comprises APS and cBMA in an APS:cBMA ratio of about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9 or about 1:10. Alternatively, the APS:cBMA ratio can be about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1 or about 10:1. cBMA can include hematopoietic, stem cells, stromal stem cells, mesenchymal stem cells, endothelial progenitor cells, red blood cells, cytokine-producing cells, fibroblasts, reticulocytes, adipose cells, or endothelial cells.

[0075] In some embodiments, the cBMA and Protein Solution may be produced simultaneously. Thus, in reference to Figure 1 and the processes described above, bone marrow aspirate may be added to the whole blood, prior to or during the contacting with a solid extraction material; such a process involves operation of both steps 115 and 130. For example, bone marrow aspirate may be added to whole blood prior or during isolation of platelet-rich plasma in step 120. Such methods include those described in U.S. Application Publication No. 2006/0278588, Woodell-May, published December 14, 2006.

[0076] Pharmaceutical actives among those useful herein include herein include organic molecules, proteins, peptides, peptidomimetics, nucleic acids, nucleoproteins, antisense molecules, polysaccharides, glycoproteins, lipoproteins, carbohydrates and polysaccharides, botanical extracts, and synthetic and biologically engineered analogs thereof, living cells (other than cytokine-producing cells stromal cells) such as chondrocytes, bone marrow cells, viruses and virus particles, natural extracts, and combinations thereof. Specific non-limiting examples of bioactive materials include hormones, antibiotics and other anti-infective agents, hematopoietics, thrombopoietics, antiviral agents, antitumor agents (chemotherapeutic agents),

antipyretics, analgesics, anti-inflammatory agents, antiallergy agents, vasodilators, cytokines, growth factors, gene regulators, vitamins, minerals and other nutritionals, nutraceuticals and combinations thereof. In particular, actives include bronchodilators (such as albuterol, levabuterol, irbuterol, ipatropium, salmeterol, and formoterol), glucocorticosteroids (such as mometasone, fluticasone, budesonide, and beclomethosone), antibiotics, antivirals, and combinations thereof. In some embodiments, compositions may comprise growth factors in addition to those present in the Protein Solution, such Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor Beta (TGF-β), Insulin-Like Growth Factor (IGF), Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF), Vascular Endothelial Growth Factor (VEGF), and Bone Morphogenetic Proteins (BMPs).

[0077] In some embodiments, Protein Solutions comprise one or more cytokines from synthetic or recombinant sources. In particular, by way of example, a Protein Solution may comprise interleukin-1 receptor antagonist (IL-1ra) that is synthetic or recombinant, or isolated from autologous, allogeneic or xenogeneic blood or other biologic sources, aside from the methods described above. For example, Kineret™ (anakinra) is a recombinant, non-glycosylated form of IL-1ra, sold by Amgen Manufacturing, Ltd. (Thousand Oaks, California). Various recombinant interleukin-1 inhibitors and methods of treatment are described in U.S. Patent No. 6,599,873, Sommer et al., issued July 29, 2003; U.S. Patent No. 5,075,222, Hannum et al., issued December 24, 1991; and U.S. Application Publication No. 2005/0197293, Mellis et al., published September 8, 2005. In addition, methods for producing IL-1ra from body fluids, including the use of autologous fluids, are described in U.S. Patent No. 6,623,472, Reinecke et al., issued September 23, 2003; U.S. Patent No. 6,713,246, Reinecke et al., issued March 30, 2004; and U.S. Patent No. 6,759,188, Reinecke et al., issued July 6, 2004. When an allogeneic anti-inflammatory cytokine composition is to be generated, multiple sources of IL-1ra from multiple subjects may be pooled together.

[0078] In some embodiments, Protein Solutions comprise one or more cytokines derived from a tissue comprising cytokine-producing cells, by contacting a cytokine cell suspension with a solid extraction material, wherein the liquid is isolated from a tissue using centrifugation or other gravimetric methods. Liquids comprising cytokine-producing cells include blood, adipose tissue, bone marrow, and fractions

thereof, such as platelet-rich plasma. Solid extraction materials include those described above. Devices for making blood fractions by centrifugation of whole blood are described in U.S. Patent No. 7,992,725, Leach et al., issued August 9, 2011; U.S. Patent No. 7,374,678, Leach, issued May 20, 2008; U.S. Patent No. 7,179,391, Leach et al., issued February 20, 2007; U.S. Patent No. 7,992,725, Leach et al., issued August 9, 2011; U.S. Patent No. 7,806,276, Leach et al., issued October 5, 2010; and U.S. Patent No. 8,048,297, Leach et al., issued November 1, 2011. Methods for making solutions rich in cytokines are described in U.S. Patent Application Publication No. 2009/0220482, Higgins et al., published September 3, 2009; U.S. Patent Application Publication No. 2010/0055087, Higgins et al., published March 4, 2010; U.S. Patent Application Publication 2011/0052561, Hoeppner, published March 3, 2011; International Publication WO 2012/030593, Higgins et al., published March 8, 2012; and U.S. Patent Application Publication 2012/0172836, Higgins et al., published July 5, 2012.

[0079] Other methods for producing components of Protein Solutions useful herein are described in the following co-filed applications, the disclosures of which are incorporated by reference herein. U.S. Patent Application Serial Number 13/840,562, Binder et al., Methods and Non-Immunogenic Compositions for Treating Inflammatory Diseases; U.S. Patent Application Serial Number 13/841,083, Landrigan, et al., Treatment of Inflammatory Respiratory Disease Using Protein Solutions; U.S. Patent Application Serial Number 13/837,005, Woodell-May et al., Methods and Acellular Compositions for Treating Inflammatory Disorders; U.S. Patent Application Serial Number 13/837,480, O'Shaughnessey, et al., Treatment of Pain Using Protein Solutions; U.S. Patent Application Serial Number 13/840,129, Matusuka, et al., Treatment of Collagen Defects Using Protein Solutions; and U.S. Patent Application Serial Number 13/841,103, Landrigan, et al., Treatment of Peripheral Vascular Disease Using Protein Solutions, all of which are incorporated by reference herein.

[0080] The compositions may comprise a carrier material, in addition to any liquid comprising the Protein Solution. It should be understood that in various embodiments of the present technology, methods of treatment employ the Protein Solution as comprised and made above, without further carrier, by direct injection or other application to the site of treatment. However, in other embodiments, an additional carrier material may be used for such reasons as for ease of administration, to facilitate

administration using a particular delivery device, enhancing activity, an increasing the length of time the Protein Solution remains at the site of administration. Carriers among those useful herein include saline, hyaluronic acid, collagen, buffers (such as Hank's Buffer), cell culture media, blood products (such as PRP and platelet poor plasma), and mixtures thereof.

[0081] Protein Solutions, and compositions comprising Protein Solutions may be sterilized prior to administration, by any suitable method. For example, a Protein Solution may be sterilized by including a sterile filter to process the product made by the processes described above. In some embodiments, an antibiotic may be included in the solid extraction material during the contacting step described above, or may be added at one or more of the various steps in the methods and treatments described herein. Alternatively, or in addition, the Protein Solution may be produced asceptically.

[0082] Protein Solutions and compositions comprising Protein Solutions may also be lyophilized (freeze drying, or cryodesiccation) after production, using methods among those known in the art. Thus, the Protein Solution can be lyophilized after it is isolated from the solid extraction material. When freeze dried, the anti-inflammatory cytokine composition can be hydrated with a suitable media 170, at a time before administration or at a time of administration. Hydration may be accomplished by mixing the composition with a solution including saline, buffers, blood, blood fractions, bone marrow aspirate, concentrated bone marrow aspirate, and combinations thereof.

[0083] In some embodiments, a cryopreservative storage solution is be added to the Protein Solution, to provide stability for subsequent storage at reduced temperatures. Suitable storage solutions include those in the art, such as glycerol and dimethylsulfoxide (DMSO). The composition may be stored at reduced temperatures, such as from about 1 °C to about 6 °C. In some embodiments, the composition is stored under liquid nitrogen, at about – 80 °C. Preferably, the cryopreservative storage solution is removed from the Protein Solution prior to administration to a mammalian subject. Removal of the storage solution may be performed by methods including those known in the art for processing stored blood comprising cryopreservatives. Washing may be performed using a wash solution, such as saline. In such embodiments, the blood type of the subject to be treated may be matched to the blood type of the donor from whom the cytokine cell suspension was obtained.

Methods of Treatment

[0084] The present technology provides methods for the treatment of an inflammatory disorder or other disorder mediated by IL1-ra in a human or other mammalian subject, comprising administration of a Protein Solution of the present technology to the subject. Methods include treating an inflammatory disorder using a Protein Solution that is made by contacting a cytokine cell suspension with a solid extraction material, wherein the liquid is whole blood, bone marrow aspirate, adipose tissue, or fractions thereof.

[0085] Such diseases may be characterized by elevated neutrophil counts. Without limiting the mechanism, utility or function of the present technology, the methods and treatments of this technology mediate the effects of interleukin-1 and its role in the inflammation cascade. As generally discussed above, interleukin-1 (IL-1) includes a family of cytokines that can stimulate lymphocytes, neutrophils, and macrophages, activate phagocytes, increase airway fibrosis, promote lymphocyte nodules in the airways, increase production of both MMP-9 and MMP-12, and are involved in many chronic inflammatory conditions. IL-1 can be generated by macrophages, monocytes, and dendritic cells, and can be part of the inflammatory response against infection. See, Lappalainen et al., "Interleukin-1β Causes Pulmonary Inflammation, Emphysema, and Airway Remodeling in the Adult Murine Lung" American Journal of Respiratory Cell and Molecular Biology, vol. 32, no. 4, pages 311-318 (April 2005).

[0086] The mode of action of IL-1 can be mediated by IL-1ra. IL-1ra binds to the same receptor on the cell surface as IL-1, and thus prevents IL-1 from sending a signal to that cell. IL-1ra is secreted from cytokine-producing cells, including monocytes, macrophages, neutrophils, polymorphonuclear cells (PMNs), and other cells, and can modulate a variety of IL-1 related immune and inflammatory responses, as described by Arend WP, Malyak M, Guthridge CJ, Gabay C (1998) "Interleukin-1 receptor antagonist: role in biology" Annu. Rev. Immunol. 16: 27-55. Production of IL-1ra is stimulated by several substances including adherent immunoglobulin G (IgG), other cytokines, and bacterial or viral components. Likewise, the mode of action of TNF-α can be mediated by sTNF-RI and sTNF-RII, which prevent TNF-α from binding to membrane bound TNF-RI and/or TNF-RII.

[0087] Examples of inflammatory disorders treated by the methods of this technology include rheumatoid arthritis, osteoarthritis, osteolytis, tendonitis, synovitis, peripheral vascular disease, and inflammatory respiratory diseases (such as chronic obstructive pulmonary disease, fibrosis, emphysema, acute respiratory distress syndrome, and pneumonia). Treatment methods also include the prevention, reduction or elimination of pain associated with various disorders, such as pain associated with traumatic injury, muscle strain, arthritis (rheumatoid arthritis and osteoarthritis), synovitis, sacroiliac joint disorders, back disorders, post-surgical injections, tendon injections, sports medicine procedure (for example, ACL repair, MCL repair, BTB repair, patella repair, or cartilage repair), contusions, muscle strains, post traumatic osteoarthritis. Methods also include stimulation of chondrocyte production at the site of a collagen defect, such as defects at joints associated with arthritis, injuries or surgical procedures.

[0088] In some embodiments, methods of the present technology comprise administration of a Protein Solution to the site of a tissue defect to prevent or treat a disorder associated with IL-1ra. As referred to herein, such "tissue defects" include any condition involving tissue which is inadequate for physiological or cosmetic purposes. Examples of such defects include those that are congenital, those that result from or are symptomatic of disease, disorder, or trauma, and those that are consequent to surgical or other medical procedures. Embodiments include treatment for vascular, bone, skin, nerve, and organ tissue defects. Examples of such defects include those resulting from osteoporosis, spinal fixation procedures, hip and other joint replacement procedures, chronic wounds, fractures, sclerosis of tissues and muscles, and spinal cord or other nerve injury. In various embodiments, the compositions and methods of this invention may be used in methods associated with the repair bone or cartilage defects.

[0089] In various embodiments, methods are for the treatment of inflammatory disorders in a human. In other embodiments, treatment is for non-human mammals, such as companion, working, and sports animals. For example, such methods of this technology may be used for the treatment of inflammatory disorders in horses.

[0090] The present technology also provides point-of-care methods for making a Protein Solution. As referred to herein, a "point-of-care method" wherein the processes of the present technology are performed at a time proximate to the

administration of the Protein Solution to the subject being treated. Such methods may be performed at a location proximate, such as in the same room (for example, bed side) or otherwise immediately adjacent, to the mammalian subject to be transfused with the RBCs. In various embodiments, a "proximate time" may be, for example, within 12 hours, within 8 hours, within 2 hours, within 1 hour or within 30 minutes of administration of the Protein Solution to the subject.

[0091] In some embodiments, the Protein Solution is administered with a concomitant therapy. Such therapies include, for example, the administration of pharmaceutical actives or biologics, as described above. In some embodiments, concomitant therapies are administered concurrently with a Protein Solution. For example, methods may comprise administration of a Protein Solution with a safe and effective amount of an active selected from the group consisting of glucocorticosteroids, non-steroidal anti-inflammatories, antibiotics, antivirals, and combinations thereof.

[0092] In some embodiments, methods comprise administration of a Protein Solution with concentrated bone marrow aspirate, as described above. For example, cBMA and a Protein Solution may be administered concomitantly. Accordingly, in some embodiments, the present technology provides compositions comprising a safe and effective amount of a Protein Solution and a safe and effective amount of cBMA. An autologous therapeutic composition comprises APS and cBMA in an APS:cBMA ratio of about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9 or about 1:10. Alternatively, the APS:cBMA ratio can be about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1 or about 10:1. cBMA can include hematopoietic, stem cells, stromal stem cells, mesenchymal stem cells, endothelial progenitor cells, red blood cells, white blood cells, fibroblasts, reticulocytes, adipose cells, or endothelial cells. Methods for producing cBMA among those useful herein are described in U.S. Application Publication No. 2006/0278588, Woodell-May, published December 14, 2006.

[0093] Methods of the present technology generally comprise administration of a Protein Solution to the site of inflammation in a mammalian subject. Administration of the Protein Solution can be performed with any suitable device, including such devices known in the art for topical delivery of compositions to the muscle, joint, vascular, lung or other tissue. For example, topical delivery for treatment of

inflammation or pain associated with joint disorders may comprise injection of a Protein Solution at or near the joint. Treatment for inflammatory respiratory diseases may comprise delivery of a Protein Solution by endotracheal tubes, inhalers and nebulizers.

Non-limiting Discussion of Terminology

[0094] The headings (such as "Introduction" and "Summary") and sub-headings used herein are intended only for general organization of topics within the present disclosure, and are not intended to limit the disclosure of the technology or any aspect thereof. In particular, subject matter disclosed in the "Introduction" may include novel technology and may not constitute a recitation of prior art. Subject matter disclosed in the "Summary" is not an exhaustive or complete disclosure of the entire scope of the technology or any embodiments thereof. Classification or discussion of a material within a section of this specification as having a particular utility is made for convenience, and no inference should be drawn that the material must necessarily or solely function in accordance with its classification herein when it is used in any given composition.

[0095] The disclosure of all references, including published and pending patents and patent applications, cited in this disclosure are incorporated by reference herein.

[0096] The description and specific examples, while indicating embodiments of the technology, are intended for purposes of illustration only and are not intended to limit the scope of the technology. Equivalent changes, modifications and variations of specific embodiments, materials, compositions and methods may be made within the scope of the present technology, with substantially similar results. Moreover, recitation of multiple embodiments having stated features is not intended to exclude other embodiments having additional features, or other embodiments incorporating different combinations of the stated features. Specific examples are provided for illustrative purposes of how to make and use the compositions and methods of this technology and, unless explicitly stated otherwise, are not intended to be a representation that given embodiments of this technology have, or have not, been made or tested.

[0097] As used herein, the words "prefer" or "preferable" refer to embodiments of the technology that afford certain benefits, under certain circumstances.

However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the technology.

[0098] As used herein, the word "include," and its variants, is intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that may also be useful in the materials, compositions, devices, and methods of this technology. Similarly, the terms "can" and "may" and their variants are intended to be non-limiting, such that recitation that an embodiment can or may comprise certain elements or features does not exclude other embodiments of the present technology that do not contain those elements or features.

[0099] Although the open-ended term "comprising," as a synonym of non-restrictive terms such as including, containing, or having, is used herein to describe and claim embodiments of the present technology, embodiments may alternatively be described using more limiting terms such as "consisting of" or "consisting essentially of." Thus, for any given embodiment reciting materials, components or process steps, the present technology also specifically includes embodiments consisting of, or consisting essentially of, such materials, components or processes excluding additional materials, components or processes (for consisting of) and excluding additional materials, components or processes affecting the significant properties of the embodiment (for consisting essentially of), even though such additional materials, components or processes are not explicitly recited in this application. For example, recitation of a composition or process reciting elements A, B and C specifically envisions embodiments consisting of, and consisting essentially of, A, B and C, excluding an element D that may be recited in the art, even though element D is not explicitly described as being excluded herein. Further, as used herein the term "consisting essentially of" recited materials or components envisions embodiments "consisting of" the recited materials or components.

[00100] A" and "an" as used herein indicate "at least one" of the item is present; a plurality of such items may be present, when possible. "About" when applied to values indicates that the calculation or the measurement allows some slight imprecision in the value (with some approach to exactness in the value; approximately or

reasonably close to the value; nearly). If, for some reason, the imprecision provided by "about" is not otherwise understood in the art with this ordinary meaning, then "about" as used herein indicates at least variations that may arise from ordinary methods of measuring or using such parameters.

[00101] As referred to herein, ranges are, unless specified otherwise, inclusive of endpoints and include disclosure of all distinct values and further divided ranges within the entire range. Thus, for example, a range of "from A to B" or "from about A to about B" is inclusive of A and of B. Disclosure of values and ranges of values for specific parameters (such as temperatures, molecular weights, weight percentages, etc.) are not exclusive of other values and ranges of values useful herein. It is envisioned that two or more specific exemplified values for a given parameter may define endpoints for a range of values that may be claimed for the parameter. For example, if Parameter X is exemplified herein to have value A and also exemplified to have value Z, it is envisioned that Parameter X may have a range of values from about A to about Z. Similarly, it is envisioned that disclosure of two or more ranges of values for a parameter (whether such ranges are nested, overlapping or distinct) subsume all possible combination of ranges for the value that might be claimed using endpoints of the disclosed ranges. For example, if Parameter X is exemplified herein to have values in the range of 1–10, or 2–9, or 3–8, it is also envisioned that Parameter X may have other ranges of values including 1–9, 1–8, 1–3, 1–2, 2–10, 2–8, 2–3, 3–10, and 3–9.

What is claimed is:

1. A method for generating a solution rich in interleukin-1 receptor antagonist (IL-1ra) comprising:
   (a) obtaining a liquid volume comprising cytokine producing cells by separating cytokine-producing cells from a tissue comprising cytokine-producing cells using a non-centrifugal process;
   (b) contacting the liquid volume comprising cytokine-producing cells with a solid extraction material; and
   (c) separating the liquid volume from the solid extraction material to obtain the solution rich in IL-1ra.

2. The method according to Claim 1, wherein the tissue comprising cytokine-producing cells is selected from the group consisting of whole blood, bone marrow aspirate, adipose tissue, fractions thereof, and mixtures thereof.

3. The method according to Claim 1, wherein the non-centrifugal process comprises:
   (i) mixing a tissue or tissue fraction comprising cytokine-producing cells with magnetic beads that are coupled to an antibody operable to bind mononuclear leukocytes; and
   (ii) collecting mononuclear leukocytes bound by the antibody for use as the liquid comprising concentrated cytokine-producing cells.

4. The method according to Claim 1, wherein the non-centrifugal process comprises passing a tissue or tissue fraction comprising cytokine-producing cells through a size exclusion filter.

5. The method according to Claim 4, wherein the non-centrifugal process comprises collecting anticoagulated whole blood in a syringe, and expressing the anticoagulated blood through a hollow fiber apparatus.

6. The method according to Claim 1, wherein the solution rich in IL-1ra further comprises one or more proteins selected from the group consisting of, sTNF-RI, sTNF-RII, IGF-I, EGF, HGF, PDGF-AB, PDGF-BB, VEGF, TGF-β1, and sIL-1 RII.

7. The method according to Claim 1, wherein the solution rich in IL-1ra comprises:
   (i) interleukin-1 receptor antagonist (IL-1ra) at a concentration of at least about 10,000 pg/ml;
   (ii) soluble Tumor Necrosis Factor Receptor 1 (sTNF-R1) at a concentration of at least about 1,200 pg/ml; and
   (iii) a protein selected from the group consisting of sTNF-RII, IGF-I, EGF, HGF, PDGF-AB, PDGF-BB, VEGF, TGF-β1, and sIL-1RII, and mixtures thereof, wherein the concentration of the protein in the composition is greater than the concentration of the protein in normal blood.

8. The method according to Claim 1, wherein the solid extraction material is selected from the group consisting of corundum, quartz, titanium, dextran, agarose, polyacrylamide, polystyrene, polyethylene, polyvinyl chloride, polypropylene, and combinations thereof.

9. The method according to Claim 8, wherein the solid extraction material comprises polyacrylamide.

10. The method according to Claim 1, wherein the solid extraction material comprises a form selected from the group consisting of a bead, fiber, powder, porous material, and combinations thereof.

11. The method according to Claim 10, wherein the surface of the solid extraction material is etched to increase its surface area.

12. The method according to Claim 1, wherein the contacting is from about 30 minutes to about 2 hours.

13. The method according to Claim 1, wherein the contacting comprises subjecting the liquid to an electromagnetic field.

14. The method according to Claim 13, wherein the electromagnetic field comprises a pulsed electromagnetic field or a capacitively coupled electromagnetic field.

15. A method for treating a disorder mediated by interleukin-1 (IL-1) in a mammalian subject, the method comprising administering a solution rich in interleukin-1 receptor antagonist (IL-1ra) made according to Claim 1 to the subject.

16. The method according to Claim 15, wherein the disorder is arthritis, osteolysis, tendonitis, synovitis, pain, traumatic injury, muscle strain, or an inflammatory respiratory disease.

17. The method according to Claim 15, wherein the solution is administered with a concomitant therapy selected from the group consisting of hyaluronic acid injections, collagen carriers, steroids, microspheres, off loader braces with and without electrical stimulation, analgesics, and mixtures thereof.

18. The method according to Claim 15, wherein the tissue comprising cytokine-producing cells is autologous to the subject.

19. The method according to Claim 15, wherein the mammalian subject is human.

20. The method according to Claim 15, wherein the mammalian subject is a companion, working, or sports animal.

21. A method for treating a disorder mediated by interleukin-1 (IL-1) in a mammalian subject, the method comprising:

(a) mixing a volume of cytokine-producing cells with a liquid medium to form a cytokine cell suspension, wherein the cytokine-producing cells are selected from the group consisting of white blood cells, adipose stromal cells and mesenchymal stromal cells, and mixtures thereof;

(b) contacting the cytokine cell suspension with polyacrylamide beads for from about 30 minutes to about 2 hours; and

(c) separating liquid from the polyacrylamide beads to obtain a solution rich in IL-1ra.

22. The method according to Claim 21, wherein the volume of cytokine-producing cells is made by a process comprising one or more of:

(a) expressing a tissue comprising cytokine-producing cells through a hollow fiber apparatus, forming a liquid volume comprising cytokine-producing cells;

(b) separation of cytokine-producing cells from whole blood using a density gradient aid;

(c) electrophoresis; and

(d) binding of cytokine-producing cells to antibody conjugated beads.

23. The method according to Claim 22, wherein the tissue comprising cytokine-producing cells is selected from the group consisting of whole blood, bone marrow aspirate, adipose tissue, fractions thereof, and mixtures thereof.

24. The method according to Claim 23, wherein the tissue comprising cytokine-producing cells is autologous with the subject.

25. The method according to Claim 21, wherein the solution rich in IL-1ra has an IL-1ra concentration of at least about 10,000 pg/ml.

26. The method according to Claim 25, wherein the solution rich in IL-1ra further comprises at least one protein selected from the group consisting of sTNF-RII, IGF-I, EGF, HGF, PDGF-AB, PDGF-BB, VEGF, TGF-β1, and sIL-1RII,

wherein the concentration of the protein is greater than the concentration of the protein in normal blood.

27. A system to allow for separation of a component of a multiple component material, comprising:
a filter configured to filter a first component from the multiple component material and to allow a remaining multiple component material to pass;
a separation system to receive the remaining multiple component material, including
a separation container;
a buoy system positioned within the separation container;
wherein a second component of the remaining multiple component material is operable to be separated from a third component of the remaining multiple component material within the separation container by the buoy system.

28. The system of Claim 27, wherein the multiple component material is whole blood and the first component is white blood cells.

29. The system of Claim 28, wherein the second component is a buffy coat.

30. The system of Claim 29, wherein the third fraction is plasma.

31. The system of Claim 29, further comprising:
a withdrawal container connected to the filter;
wherein the separation system further includes a withdrawal port;
wherein the withdrawal container is configured to be connected to the withdrawal port and draw the buffy coat from the separation container through the withdrawal port and through the filter into the withdrawal container.

32. The system of Claim 31, wherein the first component includes cytokine-producing cells;

wherein the buffy coat is operable to separate the cytokine-producing cells from the filter to obtain a solution rich in IL-1ra.

33. The system of Claim 31, wherein the buoy system includes:
a first buoy member and a second buoy member, wherein the buffy coat is contained between the first buoy member and the second buoy member after formation of the second fraction and the third fraction.

34. The system of Claim 33, wherein the first buoy member and the second buoy member are moveable relative to one another.

35. A system to allow for separation of a component of a multiple component material, comprising:
a filter configured to filter a white blood cell component from the multiple component material and to allow a remaining multiple component material to pass;
a separation system to receive the remaining multiple component material, including
a separation container defining an internal volume;
a buoy system positioned in the internal volume of the separation container, wherein the buoy system includes a first buoy member and a second buoy member;
wherein a second component of the remaining multiple component material is operable to be separated from a third component of the remaining multiple component material within the separation container;
wherein the second component is able to be positioned between the first buoy member and the second buoy member;
wherein the third component is able to be positioned between the first buoy member and an end of the separation container.

36. The system of Claim 35, further comprising:
a withdrawal container configured to connect to the separation system to withdraw at least one of the second component or the third component from the separation system.

37. The system of Claim 36, wherein the withdrawal container is connected to the filter.

38. The system of Claim 37, wherein the filter is backwashed with the withdrawn at least first component or the second component.

39. The system of Claim 38, wherein the first buoy member is moveable relative to the second buoy member.

40. The system of Claim 39, wherein the filter is a size exclusion hollow fiber array.

41. A method of separating a component of a multiple component material, comprising:
passing the multiple component material through a filter configured to filter a white blood cell component from the multiple component material and allowing a remaining multiple component material to pass;
passing the remaining multiple component material into a separation container defining an internal volume of a separation system;
operating the separation system to separate at least a second component from a third component within the separation container; and
withdrawing at least one of the second component or the third component from the separation container through the filter to backwash the filter.

42. The method of Claim 41, further comprising:
forming at least one of the second component or the third component between a first buoy member and a second buoy member.

43. The method of Claim 42, further comprising:
moving the first buoy member relative to the second buoy member.

44. The method of Claim 41, wherein operating the separation system to separate at least the second component from the third component within the separation container includes centrifuging the separation container including the remaining multiple component material.

45. The method of Claim 44, wherein withdrawing at least one of the second component or the third component includes withdrawing at least one of a buffy coat fraction or a plasma fraction from the separation system.

46. The method of Claim 45, further comprising:
obtaining a solution rich in IL-1ra by withdrawing at least one of the buffy coat fraction or the plasma fraction from the separation system through the filter.

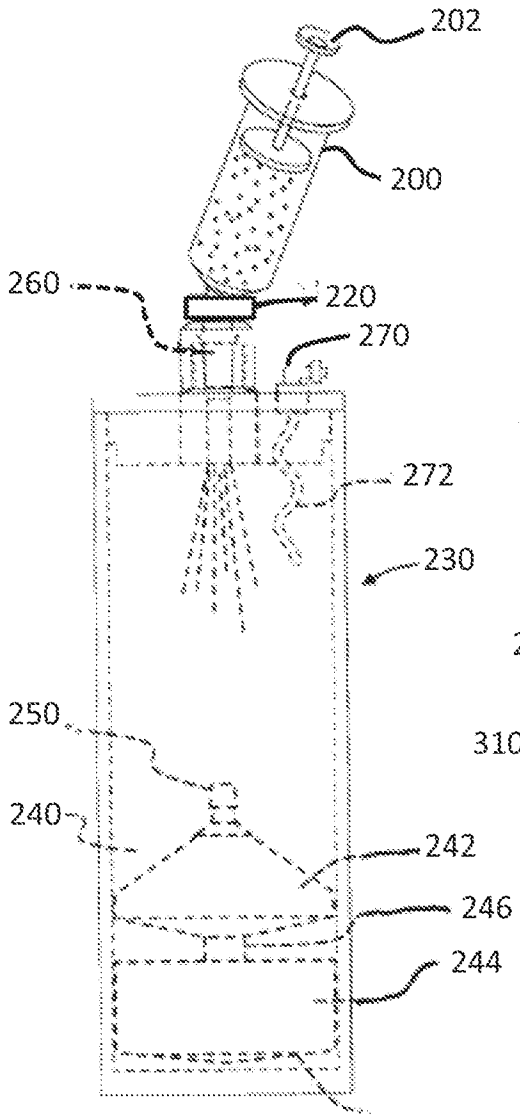

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Fig. 1A

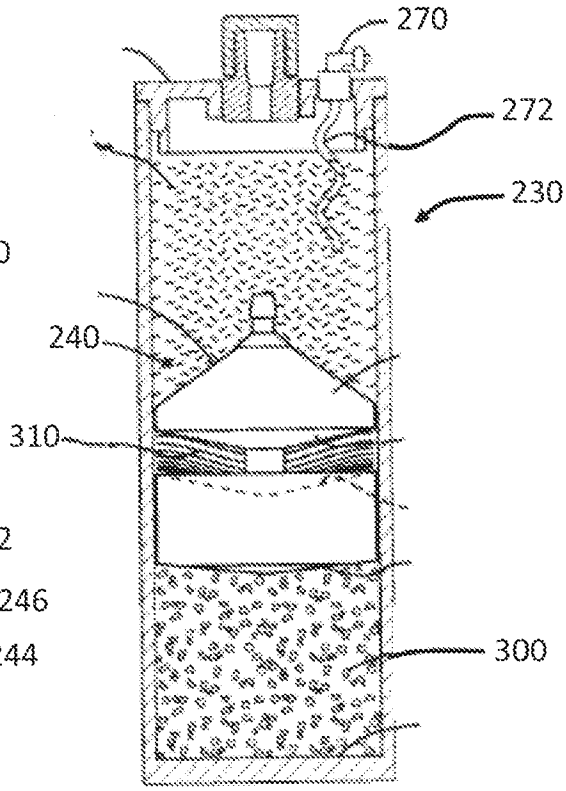

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Fig. 1B

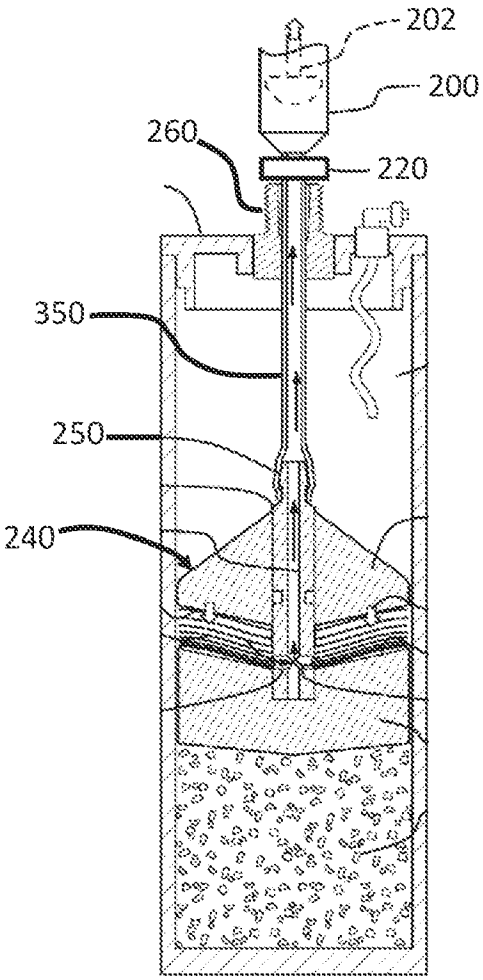


Fig. 1C

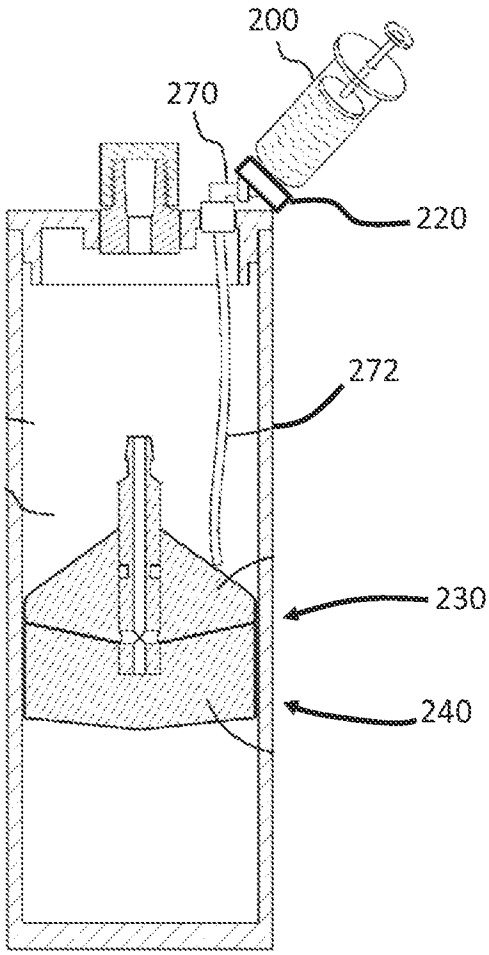


Fig. 1D

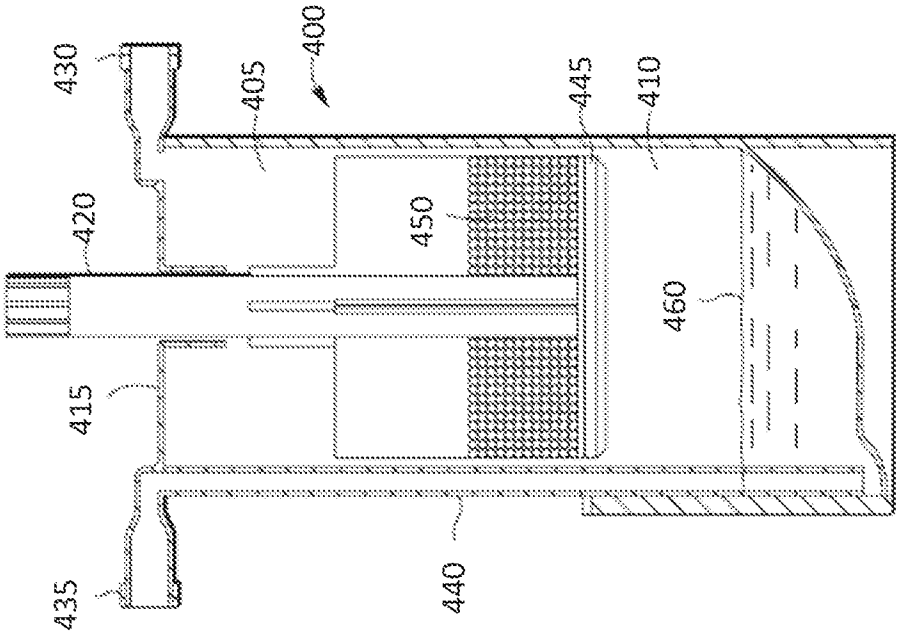

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Fig. 2B

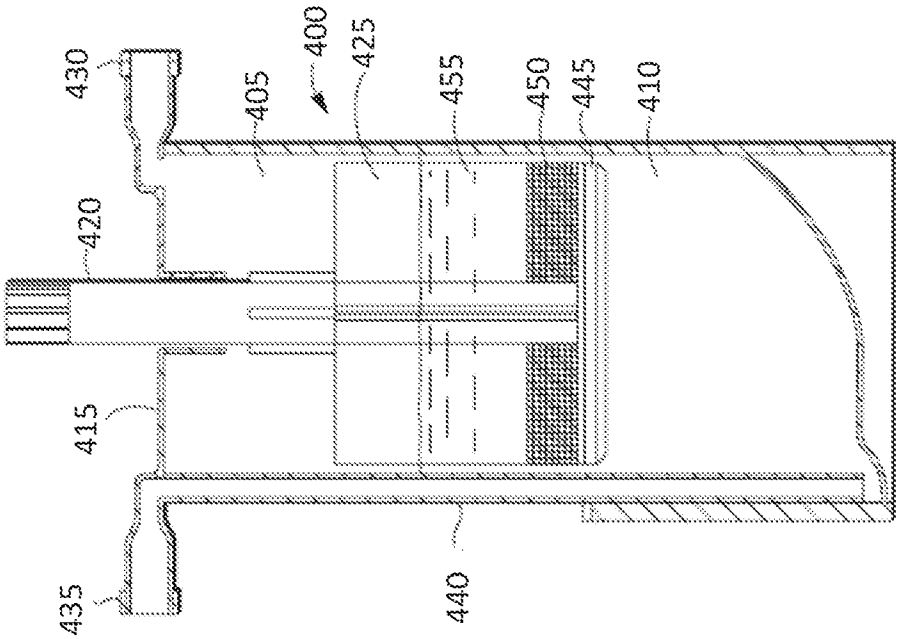

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Fig. 2A