various physiological conditions. Complications from wounds may result in increased morbidity and mortality (Doshi, 2008).

[0008] Conventional wound treatments include surgical debridement, antibiotic therapies and various dressings (Moran, 2008; Fonder, 2008). Wounds resistant to conventional treatment are also referred to as refractory wounds. These wounds lead to a decrease in quality of life and can result in increased morbidity and mortality. Thus, a need continues to exist for effective compositions and methods for wound healing.

SUMMARY OF THE INVENTION

[0008a] In one aspect, there is provided a substantially cell-free activated leukocyte-conditioned supernatant (ALCS), wherein the ALCS is made by:

incubating human leukocytes under conditions of time and temperature to activate the leukocytes, wherein the activated leukocytes comprise activated granulocytes, activated monocytes, and activated lymphocytes;

subjecting the activated leukocytes to hypo-osmotic shock;

adding to the activated leukocytes subjected to the hypo-osmotic shock to a physiologically acceptable solution containing inorganic ions, organic osmolytes, or a combination thereof in an amount which restores isotonicity;

separating the activated leukocytes from the resulting isotonic solution;

mixing the separated activated leukocytes with serum to form an incubation composition;

incubating the incubation composition (i) at room temperature for 8 to 72 hours; or (ii) at about 37°C for 3 to 5 hours; and

separating the activated leukocytes and other cellular matter from the incubation composition to produce a substantially cell-free ALCS.

[0008b] In one aspect, there is provided the use of the ALCS as defined above, in the manufacture of a medicament for treating a wound or inhibiting the onset of infection in a wound.

[0008c] In one aspect, there is provided a method of making a substantially cell-free ALCS activated leukocyte-conditioned supernatant (ALCS), comprising:

incubating human leukocytes under conditions of time and temperature to activate the leukocytes;

subjecting the leukocytes to hypo-osmotic shock;

adding to the leukocytes subjected to the hypo-osmotic shock to a physiologically acceptable solution containing inorganic ions, organic osmolytes, or a combination thereof in an amount which restores isotonicity;

separating the leukocytes from the resulting isotonic solution;

mixing the separated leukocytes with serum to form an incubation composition;

incubating the incubation composition (i) at room temperature for 8 to 72 hours; or (ii) at about 37°C for 3 to 5 hours; and

separating the leukocytes and other cellular matter from the incubation composition to produce a substantially cell-free ALCS.

[0008d] In one aspect, there is provided an article of manufacture comprising the ALCS as defined above, and a dressing, scaffold, or matrix.

[0009] One aspect of the present invention is directed to a method for making a substantially cell-free supernatant conditioned by activated leukocytes-activated leukocyte conditioned supernatant ("ALCS"). In some embodiments, the method includes the steps of a) incubating human leukocytes under conditions of time and temperature (e.g., for about 90 minutes to about 24 hours at a temperature from about room temperature to about 37°C, and in some embodiments is for about 8 to about 20 hours at room temperature) to activate the leukocytes (also referred to herein as the first incubation); b) subjecting the leukocytes to hypo-osmotic shock (e.g., by contacting the leukocytes with a physiologically acceptable aqueous solution such as distilled water); c) adding to the leukocytes of step b, a physiologically acceptable salt solution in an amount effective to restore isotonicity; d) mixing the leukocytes of step c with a medium such as serum (which may be obtained from the same or a different blood sample as the leukocytes) to form an incubation composition; e) incubating the composition under conditions of time and temperature to condition the medium, thus producing the ALCS (also referred to herein as the second incubation); and f) separating at least some of the leukocytes and other cellular matter from the incubation composition of e), so as to produce a substantially cell-free ALCS.

[0010] In another embodiment, the invention is directed to a method for making a substantially cell-free, activated leukocyte-conditioned supernatant (ALCS), comprising the steps of: a) incubating human leukocytes under conditions of time and temperature to activate the leukocytes; b) subjecting the leukocytes to hypo-osmotic

WHAT IS CLAIMED IS:

1. A substantially cell-free activated leukocyte-conditioned supernatant (ALCS), wherein the ALCS is made by:

(a) obtaining activated leukocytes; contacting the activated leukocytes with an incubation medium to form an incubation composition; incubating the incubation composition at a temperature and time sufficient to increase the concentration of at least one cytokine or growth factor in the incubation composition; and removing leukocytes from the incubation composition to produce a substantially cell-free ALCS activated leukocyte-conditioned supernatant (ALCS), or

(b) incubating human leukocytes under conditions of time and temperature to activate the leukocytes; subjecting the leukocytes to hypo-osmotic shock; adding to the leukocytes subjected to the hypo-osmotic shock to a salt solution in an amount which restores isotonicity; separating the leukocytes from the resulting isotonic solution; mixing the separated leukocytes with serum to form an incubation composition; incubating the incubation composition under conditions of time and temperature to increase the concentration of at least one cytokine or growth factor in the incubation composition; and separating the leukocytes and other cellular matter from the incubation composition to produce a substantially cell-free ALCS.

2. A substantially cell-free activated leukocyte-conditioned supernatant (ALCS) comprising at least about 1000pg/mL human IL-8, at least about 10-20pg/mL human IL-6, and at least about 20pg/mL human TNFalpha.

3. The use of the ALCS according to any one of claims 1 or 2 in the manufacture of a medicament for the treatment of a wound or the inhibition of the onset of infection in a wound;

wherein optionally:

(i) the wound is a decubital ulcer, a pressure ulcer, a lower extremity ulcer, tendonitis, a deep sternal wound, a post-operative wound, a refractory post-operative wound of the trunk area, a wound to the great saphenous vein following harvesting of the great saphenous vein, a venous ulcer, a burn wound, a battlefield wound, or an anal fissure;

(ii) the wound is a lower extremity ulcer in a diabetic patient;

(iii) the wound is a venous ulcer, pressure ulcer, or post-operative ulcer;

(iv) the wound is caused by trauma;

(v) the wound is caused by surgery;

(vi) the ALCS is injected into the wound;

(vii) the ALCS is injected into tissue surrounding the wound;

(viii) the wound is an open wound and the ALCS is applied into the open wound;

(ix) the wound is an open wound and the ALCS is applied to the open wound via a dressing, wherein the dressing is optionally a dry dressing, a barrier dressing, or a bioactive dressing;

(x) the ALCS is derived from an autologous blood sample; and/or

(xi) or the ALCS is derived from an allogeneic blood sample.

4. A method of making a substantially cell-free ALCS activated leukocyte-conditioned supernatant (ALCS), comprising:

(a) obtaining activated leukocytes; contacting the activated leukocytes with an incubation medium to form an incubation composition; incubating the incubation composition at a temperature and time sufficient to increase the concentration of at least one cytokine or growth factor in the incubation composition; and removing leukocytes from the incubation composition to produce a substantially cell-free ALCS activated leukocyte-conditioned supernatant (ALCS); or

(b) incubating human leukocytes under conditions of time and temperature to activate the leukocytes; subjecting the leukocytes to hypo-osmotic shock; adding to the leukocytes subjected to the hypo-osmotic shock to a salt solution in an amount which restores isotonicity; separating the leukocytes from the resulting isotonic solution; mixing the separated leukocytes with serum to form an incubation composition; incubating the incubation composition under conditions of time and temperature to increase the concentration of at least one cytokine or growth factor in the incubation composition; and separating the leukocytes and other cellular matter from the incubation composition to produce a substantially cell-free ALCS.

5. The method of claim 4, wherein the incubation of the human leukocytes to activate the leukocytes:

(i) occurs at a temperature of about 12°C to about 28°C for a time ranging from about 8 to about 20 hours;

(ii) occurs at a temperature of about 18°C to about 24°C for a time ranging from about 8 to about 12 hours;

(iii) occurs at a temperature of 12°C to about 28°C for a time ranging from about 90 minutes upwards of 2, 3, 4, 5, 6, 7, 8, or 12 to about 20 hours;

(iv) occurs at a temperature up to about 37°C and for a time ranging from 5 hours to about 24 hours; and/or

(v) is conducted in a container to which leukocytes adhere and/or which contains a scaffold comprising leukocyte agonists or adhesion molecules.

6. The method of claim 4, wherein the adding a salt solution in an amount which restores isotonicity to the leukocytes subjected to the hypo-osmotic shock comprises contacting the leukocytes with water or a hypotonic salt solution for about 25 to about 45 seconds.

7. The method of claim 4, wherein:

(i) the leukocytes and the serum are obtained from the same donor;

(ii) the leukocytes and the serum are obtained from different donors;

(iii) the serum of is obtained by contacting a separate sample of human plasma with a coagulating agent and removing coagulated solids, wherein optionally:

(a) the plasma and the leukocytes are obtained from the same donor,

(b) the plasma and the leukocytes are obtained from different donors,

(c) the contacting of the human plasma with the coagulating agent is

(d) conducted substantially contemporaneously with the incubating of the human leukocytes under conditions of time and temperature to activate the leukocytes,

(e) the plasma is obtained from an AB+ donor, and/or

(f) the coagulating agent is $CaCl_2$;

(iv) the serum is frozen prior to contacting leukocytes; and/or

(v) the method further comprises freezing, lyophilizing or freeze-drying the ALCS.

8. The method of claim 4, wherein the incubation composition is incubated:

(i) at room temperature for a time of about 8 to about 18 hours up to 72 hours; or

(ii) at about 37°C for a time of about 1 to about 5 hours.

9. The method of claim 4, further comprising mixing and the leukocytes separated from the incubation composition with a second serum to form a second incubation composition; incubating the second incubation composition under conditions of time and temperature to increase the concentration of at least one cytokine or growth factor in the incubation composition; and separating the leukocytes and other cellular matter from the second incubation composition, so as to produce a second sample of the substantially cell-free ALCS.

10. An article of manufacture comprising the ALCS of claim 1 or 2, and a dressing, scaffold, or matrix; wherein optionally:

(i) the dressing is gauze, a bandage, a non-adhesive mesh, a membrane, foils, foam, or a tissue adhesive;

(ii) the dressing is a moisture-keeping barrier optionally chosen from a paste, a cream, an ointment, a nonpermeable or semi-permeable membrane or foil, a hydrocolloid, a hydrogel, or combinations thereof;

(iii) the dressing is an antimicrobial dressing;

(iv) the scaffold or matrix is a solid before implantation; and/or

(v) the scaffold or matrix is a gel that solidifies following implantation.

WHAT IS CLAIMED IS:

1. A substantially cell-free activated leukocyte-conditioned supernatant (ALCS), wherein the ALCS is made by:

incubating human leukocytes under conditions of time and temperature to activate the leukocytes, wherein the activated leukocytes comprise activated granulocytes, activated monocytes, and activated lymphocytes;

subjecting the activated leukocytes to hypo-osmotic shock;

adding to the activated leukocytes subjected to the hypo-osmotic shock to a physiologically acceptable solution containing inorganic ions, organic osmolytes, or a combination thereof in an amount which restores isotonicity;

separating the activated leukocytes from the resulting isotonic solution;

mixing the separated activated leukocytes with serum to form an incubation composition;

incubating the incubation composition (i) at room temperature for 8 to 72 hours; or (ii) at about 37°C for 3 to 5 hours; and

separating the activated leukocytes and other cellular matter from the incubation composition to produce a substantially cell-free ALCS.

2. The substantially cell-free ALCS of claim 1, wherein the ALCS comprises at least about 1000pg/mL human IL-8, at least about 10-20pg/mL human IL-6, and at least about 20pg/mL human TNFalpha.

3. Use of the ALCS of any one of claims 1 or 2, in the manufacture of a medicament for treating a wound or inhibiting the onset of infection in a wound.

4. A method of making a substantially cell-free ALCS activated leukocyte-conditioned supernatant (ALCS), comprising:

incubating human leukocytes under conditions of time and temperature to activate the leukocytes;

subjecting the leukocytes to hypo-osmotic shock;

adding to the leukocytes subjected to the hypo-osmotic shock to a physiologically acceptable solution containing inorganic ions, organic osmolytes, or a combination thereof in an amount which restores isotonicity;

separating the leukocytes from the resulting isotonic solution;

mixing the separated leukocytes with serum to form an incubation composition;

incubating the incubation composition (i) at room temperature for 8 to 72 hours; or (ii) at about 37°C for 3 to 5 hours; and

separating the leukocytes and other cellular matter from the incubation composition to produce a substantially cell-free ALCS.

5. The method of claim 4, wherein the incubation of the human leukocytes to activate the leukocytes:

(i) occurs at a temperature of about 12°C to about 28°C for a time ranging from about 8 to about 20 hours;

(ii) occurs at a temperature of about 18°C to about 24°C for a time ranging from about 8 to about 12 hours;

(iii) occurs at a temperature of 12°C to about 28°C for a time ranging from about 90 minutes upwards of 2, 3, 4, 5, 6, 7, 8, or 12 to about 20 hours; or

(iv) occurs at a temperature up to about 37°C and for a time ranging from 5 hours to about 24 hours.

6. The method of claim 4, wherein the subjecting the leukocytes to hypo-osmotic shock comprises contacting the leukocytes with water or a hypotonic salt solution for about 25 to about 45 seconds.

7. The method of claim 4, wherein:

(i) the leukocytes and the serum are obtained from the same donor;

(ii) the leukocytes and the serum are obtained from different donors;

(iii) the serum of is obtained by contacting a separate sample of human plasma with a coagulating agent and removing coagulated solids;

(iv) the serum is obtained by contacting a separate sample of human plasma with a coagulating agent and removing coagulated solids, wherein:

(a) the plasma and the leukocytes are obtained from the same donor,

(b) the plasma and the leukocytes are obtained from different donors,

(c) the contacting of the human plasma with the coagulating agent is conducted substantially contemporaneously with the incubating of the human leukocytes under conditions of time and temperature to activate the leukocytes,

(e) the plasma is obtained from an AB+ donor, and/or

(f) the coagulating agent is $CaCl_2$;

(v) the serum is frozen prior to contacting leukocytes; and/or

(vi) the method further comprises freezing, lyophilizing, or freeze-drying the ALCS.

8. The use of claim 4, further comprising mixing and the leukocytes separated from the incubation composition with a second serum to form a second incubation composition; incubating the second incubation composition under conditions of time and temperature to increase the concentration of at least one cytokine or growth factor in the incubation composition; and separating the leukocytes and other cellular matter from the second incubation composition, so as to produce a second sample of the substantially cell-free ALCS.

9. An article of manufacture comprising the ALCS of claim 1 or 2, and a dressing, scaffold, or matrix.

10. The article of manufacture of claim 9, wherein:

(i) the dressing is gauze, a bandage, a non-adhesive mesh, a membrane, foils, foam, or a tissue adhesive;
(ii) the dressing is a moisture-keeping barrier;
(iii) the dressing is a moisture keeping barrier selected from the group comprising a paste, a cream, an ointment, a nonpermeable or semi-permeable membrane or foil, a hydrocolloid, a hydrogel, and combinations thereof;
(iii) the dressing is an antimicrobial dressing;
(iv) the scaffold or matrix is a solid before implantation; and/or
(v) the scaffold or matrix is a gel that solidifies following implantation.

11. The method of claim 3, wherein:

(i) the wound is a decubital ulcer, a pressure ulcer, a lower extremity ulcer, tendonitis, a deep sternal wound, a post-operative wound, a refractory post-operative wound of the trunk area, a wound to the great saphenous vein following harvesting of the great saphenous vein, a venous ulcer, a burn wound, a battlefield wound, or an anal fissure;

(ii) the wound is a lower extremity ulcer in a diabetic patient;

(iii) the wound is a venous ulcer, pressure ulcer, or post-operative ulcer;

(iv) the wound is caused by trauma;

(v) the wound is caused by surgery;

(vi) the ALCS is injected into the wound;

(vii) the ALCS is injected into tissue surrounding the wound;

(viii) the wound is an open wound and the ALCS is applied into the open wound;

(ix) the wound is an open wound and the ALCS is applied to the open wound via a dressing,

(x) the wound is an open wound and the ALCS is applied to the open wound via a dry dressing, a barrier dressing, or a bioactive dressing;

(xi) the ALCS is derived from an autologous blood sample; and/or

(xii) the ALCS is derived from an allogeneic blood sample.

12. The method of claim 4, wherein the incubation of the human leukocytes to activate the leukocytes is conducted in a container to which leukocytes adhere and/or which contains a scaffold comprising leukocyte agonists or adhesion molecules.