CLAIMS

1. A sterile pharmaceutical composition for repairing a vascular injury in a subject in need thereof, wherein the vascular injury is an acute myocardial infarction resulting from underlying diseases and the subject is a revascularized subject, the sterile pharmaceutical composition comprising:

(a) a therapeutically effective amount of a sterile chemotactic hematopoietic stem cell product, the chemotactic hematopoietic stem cell product comprising a nonexpanded, isolated population of autologous mononuclear cells enriched for CD34+ cells, which further contains a subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity; and

(b) a stabilizing amount of serum, wherein the stabilizing amount of serum is at least 10% (v/v),

wherein the pharmaceutical composition is further characterized as having the following properties for at least 24 hours following acquisition of the chemotactic hematopoietic stem cell product when tested in vitro after passage through a catheter:

(i) at least 70% of the cells are CD34+ cells;

(ii) retains the CXCR-4-mediated chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity;

(iii) the cells are at least 70% viable; and

(iv) is able to form hematopoietic colonies in vitro.

2. The sterile pharmaceutical composition of claim 1, wherein the pharmaceutical composition is further characterized as having the following properties for at least 24 hours following acquisition of the chemotactic hematopoietic stem cell product when tested in vitro after passage through a catheter: (v) retains at least 2% of the CXCR-4-mediated chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity.

3. The sterile pharmaceutical composition according to claim 1, wherein the pharmaceutical composition is further characterized as having the following properties

for at least 48 hours following acquisition of the chemotactic hematopoietic stem cell product when tested in vitro after passage through a catheter:

(i) is capable of forming hematopoietic colonies in vitro; and

(ii) retains at least 2% of the CXCR-4-mediated chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity.

4. The sterile pharmaceutical composition according to claim 1, wherein the pharmaceutical composition is further characterized as having the following properties for at least 72 hours following acquisition of the chemotactic hematopoietic stem cell product when tested in vitro after passage through a catheter:

(i) is capable of forming hematopoietic colonies in vitro; and

(ii) retains at least 2% of the CXCR-4-mediated chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity.

5. The sterile pharmaceutical composition according to claim 1, wherein the chemotactic hematopoietic stem cell product is purified from cellular components of a bone marrow aspirate acquired from the subject.

6. The sterile pharmaceutical composition according to claim 1, wherein the therapeutically effective amount of the chemotactic hematopoietic stem cell product comprises at least 0.1 x 10^6 potent CD34+/CXCR-4+ cells that have CXCR-4 mediated chemotactic activity.

7. The sterile pharmaceutical composition according to claim 1, wherein the composition is formulated for intravascular administration through a catheter to an infarct-related artery.

8. The sterile pharmaceutical composition according to claim 7, wherein the catheter is a flow control catheter.

9. The sterile pharmaceutical composition according to claim 7, wherein the catheter is a balloon catheter.

10. The sterile pharmaceutical composition according to claim 7, wherein the catheter has an internal diameter of at least about 0.36 mm.

11. The sterile pharmaceutical composition according to claim 1, wherein the composition is formulated for administration through a catheter into the myocardium.

12. The sterile pharmaceutical composition according to claim 1, wherein the composition further includes (c) at least one compatible active agent.

13. The sterile pharmaceutical composition according to claim 1, wherein sterility of the chemotactic hematopoietic stem cell product is confirmed by a method comprising:

(a) centrifuging the hematopoietic stem cell product to form (i) a separated cell product comprising a pellet comprising the isolated population of mononuclear cells enriched for CD34+ cells, and (ii) a supernatant;

(b) removing the supernatant from the separated cell product without disturbing the cell pellet of the separated cell product; and

(c) analyzing the sterility of the supernatant of the separated cell product, thereby determining the sterility of the cell pellet of the separated cell product without depleting the chemotactic hematopoietic stem cell product.

14. A method of preparing the sterile pharmaceutical composition of claim 1, comprising:

(a) acquiring a chemotactic hematopoietic stem cell preparation comprising CD34+ cells and a subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity from a subject under sterile conditions;

(b) optionally transporting the preparation of (a) to a processing facility;

(c) sterilely purifying CD34+ cells from the preparation of (a) so as to yield a chemotactic hematopoietic stem cell product comprising an isolated population of CD34+ mononuclear cells which contains a subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity, wherein purifying of step (c) is initiated within about 12 hours to about 24 hours of completion of the acquiring step (a);

(d) confirming sterility of the chemotactic hematopoietic stem cell product;

(e) sterilely formulating the sterile chemotactic hematopoietic stem cell product to form a pharmaceutical composition, wherein the formulating comprises combining the chemotactic hematopoietic stem cell product with at least 20% (v/v) serum;

(f) confirming sterility of the pharmaceutical composition without exhausting the chemotactic hematopoietic stem cell product in the composition;

(g) releasing the sterile pharmaceutical composition as eligible for infusion into a subject, wherein releasing step (g) proceeds only if the sterile pharmaceutical composition is to be infused into the subject within about 48 hours of completion of acquiring step (a);

(h) preloading a therapeutically effective amount of the pharmaceutical composition into an intravascular delivery apparatus; and

(i) optionally transporting the delivery apparatus containing the therapeutically effective amount of the sterile pharmaceutical composition to a cardiac catheterization facility for intravascular infusion into the subject.

15. The method according to claim 14, further comprising the step of storing the acquired preparation between steps (a) and (b).

16. The method according to claim 14, wherein step (a) acquiring the chemotactic hematopoietic stem cell preparation comprises a mini-bone marrow harvesting technique.

17. The method according to claim 16, wherein the mini-bone marrow harvesting technique comprises:

(i) preloading harvesting syringes with heparin prior to harvesting bone marrow from the subject;

(ii) aspirating the bone marrow from a left posterior iliac crest and a right posterior iliac crest of the subject using the harvesting syringes and a mini-bone marrow harvest technique to form harvested bone marrow; and

(iii) infusing the harvested bone marrow into a collecting bag.

18. The method according to claim 14, wherein step (b) transporting step comprises:

(i) placing the acquired chemotactic hematopoietic stem cell preparation in a collection bag;

(ii) placing the collection bag in a secondary bag;

(iii) preparing an open shipping container;

(iv) placing the secondary bag containing the collection bag in the open shipping container;

(v) sealing the shipping container; and

(vi) shipping the shipping container to the processing facility.

19. The method according to claim 14, wherein step (f) confirming the sterility of the pharmaceutical composition comprises:

(i) centrifuging the pharmaceutical composition to form (a') a separated cell product comprising a cell pellet comprising the isolated population of CD34+ mononuclear which contain a subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity cells and (b') a supernatant;

(ii) sterilely removing the supernatant from the separated cell product without disturbing the cell pellet of the separated cell product; and

(iii) analyzing whether the supernatant of the separated cell product is contaminated by a microbe; thereby determining the sterility of the cell pellet of the separated cell product, without depleting the stem cell product.

20. The method according to claim 14, wherein the therapeutically effective amount of the pharmaceutical composition of step (h) comprises at least 0.5 x 10^6 CD34+/CXCR-4+ potent cells that have CXCR-4 mediated chemotactic activity.

21. The method according to claim 14, wherein the intravascular delivery apparatus preloaded in step (h) comprises:

(i) an infusion syringe attached to a sterile four-way stopcock;

(ii) a flushing syringe attached to the sterile four-way stopcock containing a flushing solution, and

(iii) a catheter attached to the delivery apparatus by the sterile four-way stopcock for delivery of the pharmaceutical composition to the subject.

22. The method according to claim 21, wherein the catheter is a flow control catheter.

23. The method according to claim 21, wherein the catheter is a balloon dilatation catheter.

24. The method according to claim 21, wherein the catheter has an internal diameter of at least about 0.36 mm.

25. Use of a sterile pharmaceutical composition comprising a chemotactic hematopoietic stem cell product in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of a vascular injury, wherein the vascular injury is an acute myocardial infarction resulting from underlying disease, the pharmaceutical composition comprising: a therapeutically effective amount of a sterile isolated autologous chemotactic hematopoietic stem cell product, the chemotactic hematopoietic stem cell product comprising

(a) a nonexpanded, isolated population of autologous mononuclear cells enriched for CD34+ cells, which further contains a subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity; and

(b) a stabilizing amount of serum, wherein the stabilizing amount of serum is at least 10% (v/v),

wherein the pharmaceutical composition is formulated for administration to the subject parenterally through a catheter; and

wherein the sterile pharmaceutical composition is further characterized as having the following properties for at least 24 hours following acquisition of the chemotactic hematopoietic stem cell product when tested in vitro after passage through a catheter:

(i) at least 70% of the cells are CD34+ cells;

(ii) retains the CXCR-4-mediated chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity;

(iii) the cells are at least 70% viable; and

(iv) is able to form hematopoietic colonies in vitro.

26. The use according to claim 25, wherein the sterile pharmaceutical composition is further characterized as having the following properties for at least 24 hours following acquisition of the chemotactic hematopoietic stem cell product when tested in vitro after passage through a catheter: (v) retains at least 2% of the chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity.

27. The use according to claim 25, wherein the sterile pharmaceutical composition is further characterized as having the following properties for at least 48 hours following acquisition of the chemotactic hematopoietic stem cell product when tested in vitro after passage through a catheter: (i) is capable of forming hematopoietic colonies in vitro; and (ii) retains at least 2% of the CXCR-4-mediated chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity.

28. The use according to claim 25, wherein the sterile pharmaceutical composition is further characterized as having the following properties for at least 72 hours following acquisition of the chemotactic hematopoietic stem cell product when tested in vitro after passage through a catheter: (i) is capable of forming hematopoietic colonies in vitro; and (ii) retains at least 2% of the chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity.

29. The use according to claim 25, wherein the therapeutically effective amount of the chemotactic hematopoietic stem cell product comprises at least 0.5×10^6 potent CD34+/CXCR-4+ cells that have CXCR-4 mediated chemotactic activity.

30. The use according to claim 25, wherein the step of parenterally administering comprises delivering the sterile pharmaceutical composition intravascularly to an infarct-related artery through a catheter, and wherein the catheter is a component of an intravascular delivery apparatus.

31. The use according to claim 30, wherein the intravascular delivery apparatus comprises (i) an infusion syringe attached to a sterile four-way stopcock, wherein the infusion syringe contains the pharmaceutical composition; (ii) a flushing syringe attached to the sterile four-way stopcock containing a flushing solution; and (iii) a catheter

attached to the delivery apparatus by the sterile four-way stopcock for delivery of the sterile pharmaceutical composition to the subject.

32. The use according to claim 25, wherein the catheter is a flow control catheter.

33. The use according to claim 25, wherein the catheter is a balloon dilatation catheter.

34. The use according to claim 25, wherein the catheter has an internal diameter of at least about 0.36 mm.

35. The use according to claim 25, wherein the specific time interval is further defines as being at least 5 days post-infarction and less than 14 days post-infarction.

36. The use according to claim 25, wherein the sterile pharmaceutical composition is administered through the catheter into the myocardium.

37. The use according to claim 25, wherein the sterile pharmaceutical composition further comprises (c) at least one compatible active agent.

38. The use according to claim 37, wherein the at least one compatible active agent is selected from the group consisting of an angiotension converting enzyme inhibitor, a beta-blocker, a diuretic, an anti-arrhythmic agent, an anti-anginal agent, an anticoagulant agent, a vasoactive agent, an anticoagulant agent, a fibrinolytic agent, and a hypercholesterolemic agent.

CLAIMS

1. A sterile pharmaceutical composition for treating a vascular injury in a subject in need thereof, wherein the vascular injury is an acute myocardial infarction resulting from underlying diseases and the subject is a revascularized subject, the pharmaceutical composition being prepared by a process comprising:

(a) sterilely purifying a population of nucleated cells comprising CD34+ cells obtained from the subject under sterile conditions to produce enriched CD34+ cells, wherein the enriched CD34+ cells, which further contain a subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4 mediated chemotactic activity, are a chemotactic hematopoietic stem cell product,

(b) confirming that, for at least 24 hours after obtaining the population of nucleated cells comprising CD34+ cells from the subject, the subpopulation of potent CD34+/CXCR-4+ cells, when tested in vitro after passage through a catheter of internal diameter of at least 0.36 mm diameter:

(i) has CXCR-4 mediated chemotactic activity;

(ii) can form hematopoietic colonies; and

(iii) is at last 70% viable;

the sterile pharmaceutical composition comprising:

(1) a therapeutically effective amount of ~~a~~ the sterile chemotactic hematopoietic stem cell product obtained from the subject and comprising the isolated population of nucleated cells enriched for CD34+ cells, which further contains a subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity; wherein the therapeutic amount of the chemotactic hematopoietic stem cell product comprises at least 0.5 x 10^6 potent CD34+/CXCR-4+ cells that have CXCR-4 mediated chemotactic activity and is effective to treat the vascular injury; and

(2) a stabilizing amount of serum, wherein the stabilizing amount of serum is at least 10% (v/v), and is effective to retain the CXCR-4 mediated chemotactic activity and hematopoietic colony forming activity of the subpopulation of CD34+/CXCR-4+ cells;

wherein the pharmaceutical composition is formulated for administration to the subject parenterally through a catheter.

2. The sterile pharmaceutical composition of claim 1, wherein for at least 24 hours following acquisition of the population of nucleated cells when tested in vitro after passage through a catheter:

(iv) the composition retain at least 2% of the CXCR-4-mediated chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity.

3. The sterile pharmaceutical composition according to claim 1, wherein for at least 48 hours following acquisition of the population of nucleated cells when tested in vitro after passage through a catheter:

(a) the cells in the composition are capable of forming hematopoietic colonies in vitro; and

(b) the composition retains at least 2% of the CXCR-4-mediated chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity.

4. The sterile pharmaceutical composition according to claim 1, wherein for at least 72 hours following acquisition of the population of nucleated cells when tested in vitro after passage through a catheter:

(a) the cells in the composition are ~~is~~ capable of forming hematopoietic colonies in vitro; and

(b) the composition retains at least 2% of the CXCR-4-mediated chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity.

5. The sterile pharmaceutical composition according to claim 1, wherein the population of nucleated cells comprising CD34+ cells is derived from bone marrow.

6. The sterile pharmaceutical composition according to claim 1, wherein the composition is formulated for intravascular administration through the catheter to an infarct-related artery.

7. The sterile pharmaceutical composition according to claim 6, wherein the catheter is a flow control catheter.

8. The sterile pharmaceutical composition according to claim 6, wherein the catheter is a balloon catheter.

9. The sterile pharmaceutical composition according to claim 1, wherein the composition is formulated for administration through the catheter into the myocardium.

10. The sterile pharmaceutical composition according to claim 1, wherein the composition further includes (c) at least one compatible active agent.

11. The sterile pharmaceutical composition according to claim 1, wherein sterility of the chemotactic hematopoietic stem cell product is confirmed by a method comprising:

(a) centrifuging the hematopoietic stem cell product to form (i) a separated cell product comprising a pellet comprising the isolated population of mononuclear cells enriched for CD34+ cells, and (ii) a supernatant;

(b) removing the supernatant from the separated cell product without disturbing the cell pellet of the separated cell product; and

(c) analyzing the sterility of the supernatant of the separated cell product, thereby determining the sterility of the cell pellet of the separated cell product without depleting the chemotactic hematopoietic stem cell product.

12. The sterile pharmaceutical composition according to claim 1, wherein the process for preparing the pharmaceutical composition further comprises:

(c) assessing sterility of the chemotactic hematopoietic stem cell product by the steps of:

(i) centrifuging the hematopoietic stem cell product to form a separated cell product comprising a cell pellet comprising the enriched population of isolated CD34+ cells and a supernatant;

(ii) sterilely removing the supernatant of the separated cell product without disturbing the cell pellet of the separated cell product; and

(iii) analyzing whether the supernatant of the separated cell product is contaminated by a microbe, thereby determining the sterility of the cell pellet of the separated cell product without depleting the chemotactic hematopoietic stem cell product;

(d) confirming sterility of the pharmaceutical composition without exhausting the chemotactic hematopoietic stem cell product in the composition;

(e) releasing the sterile pharmaceutical composition as eligible for infusion into a the subject, wherein releasing step (e) proceeds only if the sterile pharmaceutical composition is to be

infused into the subject within at least about 48 hours of acquisition of the population of nucleated cells from the subject; and

(f) optionally preloading the therapeutic amount of the pharmaceutical composition into an intravascular delivery apparatus.

13. The method according to claim 12, further comprising the step of storing the mononuclear cells prior to purifying step (a).

14. The method according to claim 12, wherein the intravascular delivery apparatus preloaded in step (f) comprises:

(i) an infusion syringe attached to a sterile four-way stopcock;

(ii) a flushing syringe attached to the sterile four-way stopcock containing a flushing solution; and

(iii) a catheter attached to the delivery apparatus by the sterile four-way stopcock for delivery of the pharmaceutical composition to the subject.

15. The method according to claim 14, wherein the catheter is a flow control catheter.

16. The method according to claim 14, wherein the catheter is a balloon dilatation catheter.

17. Use of a sterile pharmaceutical composition comprising a chemotactic hematopoietic stem cell product in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of a vascular injury, wherein the vascular injury is an acute myocardial infarction resulting from underlying disease, the pharmaceutical composition being prepared by a process comprising:

(a) sterilely purifying a population of nucleated cells comprising CD34+ cells obtained from the subject under sterile conditions to produce enriched CD34+ cells, wherein the enriched CD34+ cells, which further contain a subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4 mediated chemotactic activity, are a chemotactic hematopoietic stem cell product;

(b) confirming that, for at least 24 hours after obtaining the population of nucleated cells comprising CD34+ cells from the subject, the subpopulation of potent CD34+/CXCR-4+ cells, when tested in vitro after passage through a catheter of internal diameter of at least 0.36 mm:

(i) has CXCR-4 mediated chemotactic activity;

(ii) can form hematopoietic colonies; and

(iii) is at least 70% viable;

the pharmaceutical composition comprising:

(1) a therapeutic amount of the sterile isolated autologous chemotactic hematopoietic stem cell product obtained from the subject and comprising the isolated population of nucleated cells enriched for CD34+ cells, which further contains a subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity, wherein the therapeutic amount of the chemotactic hematopoietic stem cell product comprises at least 0.5 x 10^6 potent CD34+/CXCR-4+ cells that have CXCR-4 mediated chemotactic activity and is effective to treat the vascular injury; and

(2) a stabilizing amount of serum, wherein the stabilizing amount of serum is at least 10%-(v/v), and is effective to retain the CXCR-4 mediated chemotactic activity and hematopoietic colony forming activity of the subpopulation of CD34+/CXCR-4+ cells;

wherein the pharmaceutical composition is formulated for administration to the subject parenterally through a catheter.

18. The use according to claim 17, wherein for at least 24 hours following acquisition of the population of nucleated cells when tested in vitro after passage through a catheter:

(iv) the composition retains at least 2% of the chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity.

19. The use according to claim 17, wherein for at least 48 hours following acquisition of the population of nucleated cells when tested in vitro after passage through a catheter:

(a) the cells in the composition are ~~is~~ capable of forming hematopoietic colonies in vitro; and

(b) the composition retains at least 2% of the CXCR-4-mediated chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity.

20. The use according to claim 17, wherein for at least 72 hours following acquisition of the population of nucleated cells when tested in vitro after passage through a catheter:

(a) the cells in the composition are capable of forming hematopoietic colonies in vitro; and

(b) the composition retains at least 2% of the chemotactic activity of the subpopulation of potent CD34+/CXCR-4+ cells that have CXCR-4-mediated chemotactic activity.

21. The use according to claim 17, wherein the catheter is a component of an intravascular delivery apparatus.

22. The use according to claim 21, wherein the intravascular delivery apparatus comprises (i) an infusion syringe attached to a sterile four-way stopcock, wherein the infusion syringe contains the pharmaceutical composition; (ii) a flushing syringe attached to the sterile four-way stopcock containing a flushing solution; and (iii) a catheter attached to the delivery apparatus by the sterile four-way stopcock for delivery of the sterile pharmaceutical composition to the subject.

23. The use according to claim 17, wherein the catheter is a flow control catheter.

24. The use according to claim 17, wherein the catheter is a balloon dilatation catheter.

25. The use according to claim 17, wherein the sterile pharmaceutical composition is administered through the catheter into the myocardium.

26. The use according to claim 17, wherein the sterile pharmaceutical composition further comprises (c) at least one compatible active agent.

27. The use according to claim 26, wherein the at least one compatible active agent is selected from the group consisting of an angiotensin converting enzyme inhibitor, a beta-blocker, a diuretic, an anti-arrhythmic agent, an anti-anginal agent, an anticoagulant agent, a vasoactive agent, an anticoagulant agent, a fibrinolytic agent, and a hypercholesterolemic agent.