CLAIMS

1. A method of culturing cells for use in therapy comprising the steps of:

(i) culturing a sample of cells in a first cell culture medium comprising a first serum, first serum substitute and/or first serum fraction for sufficient time to enable the cells to achieve a desired level of expansion; and

(ii) prior to harvesting cells for use in therapy said first cell culture medium is replaced with a second cell culture medium comprising a second serum, a second serum substitute and/or second serum fraction which serum or serum fraction has the same species origin as the cells being cultured or the second serum substitute replicates the same species origin and the cells are cultured for less than 48 hours before harvesting for use.

2. A method of culturing cells for use in therapy comprising the steps of:

(i) culturing a sample of cells in a first cell culture medium comprising a first serum, first serum substitute and/or first serum fraction for sufficient time to enable the cells to achieve a desired level of expansion; and

(ii) prior to harvesting cells for use in therapy said first cell culture medium is replaced with a second cell culture medium comprising a second serum, a second serum substitute and/or second serum fraction which serum or serum fraction has the same species origin as the cells being cultured or the second serum substitute replicates the same species origin and the cells are cultured for between about 24 hours and about 36 hours before the cells are harvested for use in cell therapy.

3. A method according to claim 1 or 2, wherein the first serum, first serum substitute and/or first serum fraction is selected from the group consisting of fetal calf serum (FCS), heat inactivated fetal calf serum (HIFCS), platelet lysate, serum albumin and growth factors.

4. A method according to any one of claims 1 to 3, wherein the sample of cells comprises a cell selected from the group consisting of a hepatic cell, a hemopoietic cell, a fibroblast, an adipose cell, a mesenchymal stromal cell, a cardiac cell, an endothelial cell, an epithelial cell, a neuronal cell, a glial cell, an endocrine cell, or progenitors thereof.

5. A method according to claim 4, wherein the cell is a mesenchymal stromal cell (MSC), optionally a mesenchymal progenitor cell (MPC).

6. A method according to any one of claims 1 to 5, wherein the sample of cells are isolated from a human, a cameline, an equine, a canine or a feline.

7. A method according to any one of claims 1 to 6, wherein the first medium in step (i) comprises a base medium selected from the group consisting of DMEM (high or low glucose), Eagle's basal medium (MEM), Ham's F10 medium (F10), Ham's F-12 medium (F12), Iscove's modified Dulbecco's medium, M-199, Mesenchymal Stem Cell Growth Medium (MSCGM), Liebovitz's L-15 medium, MCDB, DMEM/F12, RPMI 1640, advanced DMEM (Gibco), DMEM/MCDB201 (Sigma), and CELL-GRO FREE or a combination thereof, preferably the base medium is DMEM.

8. A method according to claim 7, wherein the base medium is supplemented with heat inactivated fetal calf serum (HIFCS).

9. A method according to any one of claims 1 to 8, wherein the sample of cells in step (i) comprises between 1.0×10^3 cells per cm^2 of culture vessel and 1.0×10^{10} cells per cm^2 of culture vessel.

10. A method according to any one of claims 1 to 9, wherein the sample of cells in step (i) are cultured until the desired level of expansion of the cells has been reached which is approximately 70-85% confluent.

11. A method according to any one of claims 1 to 10, wherein the second medium in step (ii) comprises a base medium selected from the group consisting of DMEM (high or low glucose), Eagle's basal medium (MEM), Ham's F10 medium (F10), Ham's F-12 medium (F12), Iscove's modified Dulbecco's medium, M-199, Mesenchymal Stem Cell Growth Medium (MSCGM), Liebovitz's L-15 medium, MCDB, DMEM/F12, RPMI 1640, advanced DMEM (Gibco), DMEM/MCDB201 (Sigma), and CELL-GRO FREE or a combination thereof, optionally the base medium is DMEM phenol red-free.

12. A method according to claim 11, wherein the base medium is supplemented with human serum or human serum albumin, optionally the human serum or human serum albumin is about 2% v/v.

13. A method of culturing human mesenchymal stromal cells for use in cell therapy comprising the steps of:

(i) culturing mesenchymal stromal cells in a first cell culture medium comprising DMEM supplemented with heat inactivated fetal calf serum (HIFCS) until said cells reach about 70-85% confluence; and

(ii) prior to harvesting cells for use in therapy said first cell culture medium is removed and replaced with a second cell culture medium comprising DMEM phenol red-free

and about 2% human serum albumin and the cells are then cultured for a further about 24 hours to about 36 hours.

14. A method of culturing human mesenchymal stromal cells for use in cell therapy comprising the steps of:

(i) culturing between 1.0×10^3 cells per cm^2 of culture vessel and 1.0×10^{10} cells per cm^2 of culture vessel of mesenchymal stromal cells in a first cell culture medium comprising DMEM supplemented with heat inactivated fetal calf serum (HIFCS) until said cells reach about 70-85% confluence; and

(ii) prior to harvesting cells for use in therapy said first cell culture medium is removed and replaced with a second cell culture medium comprising DMEM phenol red-free and about 2% human serum albumin and the cells are then cultured for a further about 24 hours to about 36 hours.

15. A method for preparing cells for allogeneic cell therapy in a patient comprising the steps of:

(i) providing a sample of cells from a healthy donor;

(ii) culturing said sample of cells in a first cell culture medium comprising a first serum, first serum substitute and/or first serum fraction for sufficient time to enable the cells to achieve a desired level of expansion; and

(iii) prior to harvesting cells for use in therapy said first cell culture medium is replaced with a second cell culture medium comprising a second serum, a second serum substitute and/or second serum fraction which serum or serum fraction has the same species origin as the cells being cultured or the second serum substitute replicates the same species origin and the cells are cultured for about 24 hours to about 36 hours; and

(iv) harvesting said cells.

16. A method according to claim 15, wherein the first serum, first serum substitute and/or first serum fraction is selected from the group consisting of fetal calf serum (FCS), heat inactivated fetal calf serum (HIFCS), platelet lysate, serum albumin and growth factors.

17. A method according to claim 15 or 16, wherein the sample of cells comprises a cell selected from the group consisting of a hepatic cell, a hemopoietic cell, a fibroblast, a mesenchymal stromal cell, a cardiac cell, an endothelial cell, an epithelial cell, a neuronal cell, a glial cell, and an endocrine cell, or progenitors thereof.

18. A method according to any one of claims 15 to 17, wherein the first medium in step (ii) comprises a base medium selected from the group consisting of DMEM (high or low

glucose), Eagle's basal medium (MEM), Ham's F10 medium (F10), Ham's F-12 medium (F12), Iscove's modified Dulbecco's medium, M-199, Mesenchymal Stem Cell Growth Medium (MSCGM), Liebovitz's L-15 medium, MCDB, DMEM/F12, RPMI 1640, advanced DMEM (Gibco), DMEM/MCDB201 (Sigma), and CELL-GRO FREE or a combination thereof, optionally the base medium is DMEM.

19. A method according to any one of claims 15 to 18, wherein the second medium in step (iii) comprises the base medium DMEM phenol red-free and is supplemented with human serum or human serum albumin, optionally the human serum or human serum albumin is about 2%.

20. A composition for use in cell therapy comprising cells produced by a method according to any one of claims 1 to 19, wherein said cells have increased expression of one or more cell surface markers selected from the group consisting of CD54, CD61, CD140A and CD201 compared to cells grown in media containing fetal calf serum.

21. A composition according to claim 20, wherein the cells are suspended in a syringe or in a collapsible container.

22. A composition according to claim 20 or 21, wherein the cells are suspended in a media suitable for therapeutic administration to a patient.

23. A kit comprising:

(i) a composition according to any one of claims 20 to 22, wherein said composition is contained within a syringe or collapsible container; and

(ii) instructions for use.

24. Use of human mesenchymal stromal cells in the manufacture of a medicament useful in cell therapy, wherein said human mesenchymal stromal cells are produced by:

(i) culturing said mesenchymal stromal cells in a first cell culture medium comprising DMEM supplemented with heat inactivated fetal calf serum (HIFCS) until said cells reach about 70-85% confluence;

(ii) prior to harvesting cells for use in therapy said first cell culture medium is removed and replaced with a second cell culture medium comprising DMEM phenol red-free and about 2% human serum albumin and the cells are then cultured for less than 48 hours; and

(iii) harvesting said mesenchymal stromal cells in a pharmaceutically acceptable medium, diluent or carrier suitable for therapeutic administration to a patient.

25. An implantable medical device comprising a composition according to any one of claims 20 to 22.

The claims defining the invention are as follows:

1. A method of culturing cells for use in therapy comprising the steps of:

(i) culturing a sample of cells in a first cell culture medium comprising a first serum, first serum substitute and/or first serum fraction for sufficient time to enable the cells to achieve a desired level of expansion; and

(ii) prior to harvesting cells for use in therapy said first cell culture medium is replaced with a second cell culture medium comprising a second serum, a second serum substitute and/or second serum fraction which serum or serum fraction has the same species origin as the cells being cultured or the second serum substitute replicates the same species origin and the cells are cultured for less than 48 hours before harvesting for use.

2. A method of culturing cells for use in therapy comprising the steps of:

(i) culturing a sample of cells in a first cell culture medium comprising a first serum, first serum substitute and/or first serum fraction for sufficient time to enable the cells to achieve a desired level of expansion; and

(ii) prior to harvesting cells for use in therapy said first cell culture medium is replaced with a second cell culture medium comprising a second serum, a second serum substitute and/or second serum fraction which serum or serum fraction has the same species origin as the cells being cultured or the second serum substitute replicates the same species origin and the cells are cultured for between about 24 hours and about 36 hours before the cells are harvested for use in cell therapy.

3. A method according to claim 2, wherein the first serum, first serum substitute and/or first serum fraction is selected from the group consisting of fetal calf serum (FCS), heat inactivated fetal calf serum (HIFCS), platelet lysate, serum albumin and growth factors.

4. A method according to claim 2 or 3, wherein the sample of cells comprises a cell selected from the group consisting of a hepatic cell, a hemopoietic cell, a fibroblast, an adipose cell, a mesenchymal stromal cell, a cardiac cell, an endothelial cell, an epithelial cell, a neuronal cell, a glial cell, an endocrine cell, or progenitors thereof.

5. A method according to claim 4, wherein the cell is a mesenchymal stromal cell (MSC).

6. A method according to claim 5, wherein the mesenchymal stromal cell is a mesenchymal progenitor cell (MPC).

7. A method according to any one of claims 2 to 6, wherein the sample of cells are isolated from a human, a cameline, an equine, a canine and a feline.

8. A method according to any one of claims 2 to 7, wherein the first medium in step (i) comprises a base medium selected from the group consisting of DMEM (high or low glucose), Eagle's basal medium (MEM), Ham's F10 medium (F10), Ham's F-12 medium (F12), Iscove's modified Dulbecco's medium, M-199, Mesenchymal Stem Cell Growth Medium (MSCGM), Liebovitz's L-15 medium, MCDB, DMEM/F12, RPMI 1640, advanced DMEM (Gibco), DMEM/MCDB201 (Sigma), and CELL-GRO FREE or a combination thereof.

9. A method according to claim 8, wherein the base medium is DMEM.

10. A method according to claim 8 or 9, wherein the base medium is supplemented with heat inactivated fetal calf serum (HIFCS).

11. A method according to any one of claims 2 to 10, wherein the sample of cells in step (i) comprises between 1.0 x 10^3cells per cm^2 of culture vessel and 1.0 x 10^{10} cells per cm^2 of culture vessel.

12. A method according to claim 11, wherein the sample of cells in step (i) comprises between 1.0 x 10^3cells per cm^2 of culture vessel and 1.0 x 10^7 cells per cm^2 of culture vessel.

13. A method according to any one of claims 2 to 12, wherein the sample of cells in step (i) are cultured until the desired level of expansion of the cells has been reached which is approximately 70-85% confluent.

14. A method according to any one of claims 2 to 13, wherein the second medium in step (ii) comprises a base medium selected from the group consisting of DMEM (high or low glucose), Eagle's basal medium (MEM), Ham's F10 medium (F10), Ham's F-12 medium (F12), Iscove's modified Dulbecco's medium, M-199, Mesenchymal Stem Cell Growth Medium (MSCGM), Liebovitz's L-15 medium, MCDB, DMEM/F12, RPMI 1640, advanced DMEM (Gibco), DMEM/MCDB201 (Sigma), and CELL-GRO FREE or a combination thereof.

15. A method according to claim 14, wherein the base medium is DMEM phenol red-free.

16. A method according to claim 15, wherein the base medium is supplemented with human serum or human serum albumin.

17. A method according to claim 16, wherein the human serum or human serum albumin is about 2% v/v.

18. A method of culturing human mesenchymal stromal cells for use in cell therapy comprising the steps of:

(i) culturing mesenchymal stromal cells in a first cell culture medium comprising DMEM supplemented with heat inactivated fetal calf serum (HIFCS) until said cells reach about 70-85% confluence; and

(ii) prior to harvesting cells for use in therapy said first cell culture medium is removed and replaced with a second cell culture medium comprising DMEM phenol red-free and about 2% human serum albumin and the cells are then cultured for a further about 24 hours to about 36 hours.

19. A method of culturing human mesenchymal stromal cells for use in cell therapy comprising the steps of:

(i) culturing between 1.0×10^3 cells per cm^2 of culture vessel and 1.0×10^{10} cells per cm^2 of culture vessel of mesenchymal stromal cells in a first cell culture medium comprising DMEM supplemented with heat inactivated fetal calf serum (HIFCS) until said cells reach about 70-85% confluence; and

(ii) prior to harvesting cells for use in therapy said first cell culture medium is removed and replaced with a second cell culture medium comprising DMEM phenol red-free and about 2% human serum albumin and the cells are then cultured for a further about 24 hours to about 36 hours.

20. A method for preparing cells for allogeneic cell therapy in a patient comprising the steps of:

(i) providing a sample of cells from a healthy donor;

(ii) culturing said sample of cells in a first cell culture medium comprising a first serum, first serum substitute and/or first serum fraction for sufficient time to enable the cells to achieve a desired level of expansion; and

(ii) prior to harvesting cells for use in therapy said first cell culture medium is replaced with a second cell culture medium comprising a second serum, a second serum substitute and/or second serum fraction which serum or serum fraction has the same species origin as the cells being cultured or the second serum substitute replicates the same species origin and the cells are cultured for about 24 hours to about 36 hours; and

(iv) harvesting said cells.

21. A method according to claim 20, wherein the healthy donor has less than or equal to a 50% tissue match to the patient.

22. A method according to claim 20, wherein the first serum, first serum substitute and/or first serum fraction is selected from the group consisting of fetal calf serum (FCS), heat inactivated fetal calf serum (HIFCS), platelet lysate, serum albumin and growth factors.

23. A method according to any one of claims 20 to 22, wherein the sample of cells comprises a cell selected from the group consisting of a hepatic cell, a hemopoietic cell, a fibroblast, a mesenchymal stromal cell, a cardiac cell, an endothelial cell, an epithelial cell, a neuronal cell, a glial cell, an endocrine cell, or progenitors thereof.

24. A method according to any one of claims 20 to 23, wherein the sample of cells are isolated from a human, a cameline, an equine, a canine and a feline.

25. A method according to any one of claims 20 to 24, wherein the first medium in step (ii) comprises a base medium selected from the group consisting of DMEM (high or low glucose), Eagle's basal medium (MEM), Ham's F10 medium (F10), Ham's F-12 medium (F12), Iscove's modified Dulbecco's medium, M-199, Mesenchymal Stem Cell Growth Medium (MSCGM), Liebovitz's L-15 medium, MCDB, DMEM/F12, RPMI 1640, advanced DMEM (Gibco), DMEM/MCDB201 (Sigma), and CELL-GRO FREE or a combination thereof.

26. A method according to claim 25, wherein the base medium is DMEM.

27. A method according to any one of claims 20 to 26, wherein the second medium in step (iii) comprises the base medium DMEM phenol red-free and is supplemented with human serum or human serum albumin.

28. A method according to claim 27, wherein the human serum or human serum albumin is about 2%.

29. A composition for use in cell therapy comprising cells produced by a method according to any one of claims 2 to 28, wherein said cells have increased expression of one or more cell surface markers selected from the group consisting of CD54, CD61, CD140A and CD201 compared to cells grown in media containing fetal calf serum.

30. A composition according to claim 28 or 29, wherein the cells are suspended in a syringe.

31. A composition according to claim 28 or 29, wherein the cells are suspended in a collapsible container.

32. A composition according to any one of claims 28 to 32, wherein the cells are suspended in a media suitable for therapeutic administration to a patient.

33. A kit comprising:

(i) a composition according to claims 28 or 29, wherein said composition is contained within a syringe; and

(ii) instructions for use.

34. A kit comprising:

(i) a composition according to claims 28 or 29, wherein said composition is contained within a collapsible container; and

(ii) instructions for use.

35. A method of cell therapy comprising:

(i) providing a sample of cells from a healthy donor;

(ii) culturing said sample of cells in a first cell culture medium comprising a first serum, first serum substitute and/or first serum fraction for sufficient time to enable the cells to achieve a desired level of expansion; and

(ii) prior to harvesting cells for use in therapy said first cell culture medium is replaced with a second cell culture medium comprising a second serum, a second serum substitute and/or second serum fraction which serum or serum fraction has the same species origin as the cells being cultured or the second serum substitute replicates the same species origin and the cells are cultured for less than 48 hours;

(iv) harvesting said cells; and

(v) implanting or administering said cells to a patient in need thereof.

36. Use of human mesenchymal stromal cells in the manufacture of a medicament useful in cell therapy, wherein said human mesenchymal stromal cells are produced by:

(i) culturing said mesenchymal stromal cells in a first cell culture medium comprising DMEM supplemented with heat inactivated fetal calf serum (HIFCS) until said cells reach about 70-85% confluence;

(ii) prior to harvesting cells for use in therapy said first cell culture medium is removed and replaced with a second cell culture medium comprising DMEM phenol red-free and about 2% human serum albumin and the cells are then cultured for less than 48 hours; and

(iii) harvesting said mesenchymal stromal cells in a pharmaceutically acceptable medium, diluent or carrier suitable for therapeutic administration to a patient.

37. An implantable medical device comprising a composition according to claim 29.