

CLAIMS:

1. A method of culturing mesenchymal lineage precursor or stem cells in a three dimensional culture, the method comprising culturing a population of mesenchymal lineage precursor or stem cells on an adherent material in a cell culture medium, wherein the mesenchymal lineage precursor or stem cells are attached to the adherent material and, wherein the cell culture medium is animal serum free.
2. The method of claim 1, wherein the cell culture medium comprises platelet derived growth factor (PDGF) and fibroblast growth factor 2 (FGF2).
3. The method of claim 2, wherein the cell culture medium also comprises EGF.
4. The method according to any one of claims 1 to 3, wherein the mesenchymal lineage precursor or stem cells are cultured in a bioreactor.
5. The method according to any one of claims 1 to 4, wherein the adherent material is a microcarrier.
6. The method of claim 5, wherein the microcarrier has a degradable core.
7. The method of claim 5 or claim 6, wherein the microcarrier has a carbohydrate polymer or glycoprotein core.
8. The method according to any one of claims 1 to 7, wherein the adherent material or microcarrier is coated with a glycoprotein.
9. The method of claim 7 or claim 8, wherein the glycoprotein is a collagen or vitronectin.
10. The method of claim 9, wherein the vitronectin is human vitronectin or a synthetic mimetic thereof.
11. The method according to any one of claims 7 to 10, wherein the glycoprotein is synthetic.

12. The method of claim 11, wherein the carbohydrate polymer is linked in a calcium dependent manner.
13. The method according to any one of claims 1 to 12, wherein the culture medium comprises 0.5 g/L to 5 g/L of microcarrier.
14. The method according to any one of claims 5 to 13, wherein the microcarrier is a porous microcarrier.
15. The method of claim 14, wherein the microcarrier is a macroporous microcarrier.
16. The method according to any one of claims 1 to 15, wherein the culture medium is free of animal components.
17. The method according to any one of claims 1 to 16, wherein about 70% of medium is replaced every 24 hours of culture.
18. The method according to any one of claims 1 to 17, further comprising dissociating the mesenchymal lineage precursor or stem cells from the adherent material by contacting them with a dissociating agent.
19. The method of claim 17 or claim 18, further comprising vibrating the adherent material for a period of time at a frequency and amplitude sufficient to release the mesenchymal lineage precursor or stem cells from the adherent material.
20. The method according to any one of claims 1 to 19, further comprising degrading the adherent material.
21. The method according to claim 20, wherein the adherent material is degraded by adding an enzyme to the culture medium.
22. The method according to any one of claims 1 to 21, wherein mesenchymal lineage precursor or stem cells are seeded at between 5,000 and 20,000 cells/ml.

23. The method according to any one of claims 1 to 21, wherein mesenchymal lineage precursor or stem cells are seeded at 10,000 cells/ml.
24. The method according to any one of claims 1 to 23, wherein mesenchymal lineage precursor or stem cells have been culture expanded from a master cell bank.
25. The method of claim 24, wherein the mesenchymal lineage precursor or stem cells have been culture expanded from a master cell bank in a two dimensional culture format.
26. The method according to any one of claims 1 to 25, further comprising recovering the cells from the culture medium and cryopreserving the recovered cells.
27. The method of claim 26, wherein the recovered cells are washed and concentrated prior to cryopreservation.
28. The method according to any one of claims 1 to 27, wherein the mesenchymal lineage precursor or stem cells are cultured in a three dimensional culture for at least 6 days, preferably between 5 and 8 days, more preferably 7 days.
29. The method according to any one of claims 4 to 28, wherein the bioreactor is a stirred tank and/or packed bed bioreactor.
30. A composition comprising a population of mesenchymal lineage precursor or stem cells and cell culture medium, wherein the cell culture medium is animal serum free and comprises an adherent material, PDGF and FGF2, and wherein the mesenchymal lineage precursor or stem cells are attached to the adherent material.
31. The composition of claim 30, wherein the adherent material is as defined in any one of claims 5 to 15.

32. The method according to any one of claims 1 to 29 or the composition according to claims 30 or 31, wherein the mesenchymal lineage precursor or stem cells are mesenchymal precursor cells or mesenchymal stem cells.
33. The method according to any one of claims 1 to 29 or 32 or, the composition according to claim 30 or 31, wherein the PDGF in the culture medium is PDGF-BB.
34. The method according to any one of claims 1 to 29 or 32 or 33 or, the composition according to any one of claims 30 to 32, wherein the culture medium:
 - comprises between 3.0 ng/ml and 120 ng/ml of PDGF-BB;
 - comprises between 2 pg/ml and 6 ng/ml of FGF2;
 - comprises less than 0.8 ng/ml of FGF2;
 - further comprises EGF.
35. The method according to any one of claims 1 to 29 or 32 to 34 or, the composition according to any one of claims 30 to 34, wherein the culture medium further comprises between 0.08 ng/ml and 7 ng/ml of EGF.
36. The method according to any one of claims 1 to 29 or 32 to 35 or, the composition according to any one of claims 30 to 35, wherein the culture medium comprises alpha-minimal essential medium or fetal bovine serum free expansion medium.
37. The method according to any one of claims 1 to 29, 32 to 36 or, the composition according to any one of claims 30 to 36, wherein the culture medium maintains the stem cells in an undifferentiated state.
38. A method of culturing stem cells in a bioreactor, the method comprising culturing a population of mesenchymal lineage precursor or stem cells in a bioreactor comprising cell culture medium, wherein the cell culture medium is animal serum free and comprises platelet derived growth factor (PDGF) and fibroblast growth factor 2 (FGF2) and optionally EGF.