

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, in which the mesenchymal lineage precursor or stem cells are attached to the adherent material, in which the adherent material is a porous microcarrier coated with a glycoprotein and in which the cell culture medium is animal serum free.
2. A method according to Claim 1, in which the cell culture medium comprises platelet derived growth factor (PDGF-BB) and fibroblast growth factor 2 (FGF2), preferably in which the cell culture medium also comprises EGF.
3. A method according to Claim 1 or 2, in which the mesenchymal lineage precursor or stem cells are cultured in a bioreactor, preferably in which the bioreactor is a stirred tank and/or packed bed bioreactor.
4. A method according to Claim 1, 2 or 3, in which the microcarrier has a degradable core.
5. A method according to any of Claims 1 to 4, in which the microcarrier has a carbohydrate polymer or glycoprotein core, preferably in which the carbohydrate polymer is linked in a calcium dependent manner.
6. A method according to any of Claims 1 to 4, in which the glycoprotein is a collagen or vitronectin, more preferably in which the glycoprotein is synthetic.
7. A method according to Claim 6, in which the vitronectin is human vitronectin or a synthetic mimetic thereof.
8. A method according to any preceding claim, in which the culture medium comprises 0.5 g/L to 5 g/L of microcarrier.
9. A method according to any of Claims 4 to 8, in which the microcarrier is a macroporous microcarrier.

10. A method according to any preceding claim, in which the culture medium is free of animal components, preferably in which the culture medium maintains the cells in an undifferentiated state.
11. A method according to any preceding claim, in which:
 - about 70% of medium is replaced every 24 hours of culture; and/or
 - the method further comprises dissociating the mesenchymal lineage precursor or stem cells from the adherent material, preferably by contacting them with a dissociating agent, more preferably by 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, more preferably by degrading the adherent material, optionally by adding an enzyme to the culture medium; and/or
 - 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.
12. A method according to any preceding claim, in which mesenchymal lineage precursor or stem cells are seeded at between 5,000 and 20,000 cells/ml, preferably at 10,000 cells/ml.
13. A method according to any preceding claim, in which mesenchymal lineage precursor or stem cells have been culture expanded from a master cell bank, preferably in which the mesenchymal lineage precursor or stem cells have been culture expanded from a master cell bank in a two dimensional culture format.
14. A method according to any preceding claim, further comprising recovering the cells from the culture medium and cryopreserving the recovered cells, preferably in which the recovered cells are washed and concentrated prior to cryopreservation.
15. A method according to any preceding claim, in which the mesenchymal lineage precursor or stem cells are mesenchymal precursor cells or mesenchymal stem cells.