MICROCARRIERS FOR PLURIPOTENT STEM CELL CULTURE

FIELD

The present invention relates to the fields of cell biology, molecular biology and biotechnology. More particularly, the invention relates to a method of culturing stem cells on particulate carriers.

BACKGROUND

Stem cells, unlike differentiated cells have the capacity to divide and either self-renew or differentiate into phenotypically and functionally different daughter cells (Keller, Genes Dev. 2005;19:1129-1155; Wobus and Boheler, Physiol Rev. 2005;85:635-678; Wiles, Methods in Enzymology. 1993;225:900-918; Choi et al, Methods Mol Med. 2005;105:359-368).

Human embryonic stem cells (hESC) are pluripotent cells with the capability of differentiating into a variety of stem cell types. The pluripotency of stem cells such as embryonic stem cells (ESCs) and their ability to differentiate into cells from all three germ layers makes these an ideal source of cells for regenerative therapy for many diseases and tissue injuries (Keller, Genes Dev. 2005;19:1129-1155; Wobus and Boheler, Physiol Rev. 2005;85:635-678).

Expansion of stem cells to large quantities, requiring one or more passages, is a pre-requisite for cell therapy.

Currently, stem cells (including human embryonic stem cells, hESC) which grow as colonies are routinely maintained on plastic culture surfaces in 2 dimensional (2D) growth. Expansion to larger quantities on 2D culture would necessitate the use of large surface areas. The manual nature of passaging the cells by repeated pipetting or enzymatic treatment to break up these 2D colonies to smaller sizes would become impractical. Preparing numerous

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Claims

1. A method of culturing human embryonic stem cells, or human induced pluripotent stem cells, in suspension culture in vitro, the method comprising:

(i) attaching human embryonic stem cells or human induced pluripotent stem cells to a plurality of microcarriers to form microcarrier-stem cell complexes, wherein the surface of the microcarriers is coated in a matrix comprising an extracellular matrix component;

(ii) culturing the microcarrier-stem cell complexes in suspension culture;

(iii) passaging the cultured cells from (ii); and

(iv) repeating steps (i)-(iii) through at least 7 passages,

wherein stem cells in the culture after step (iv) are pluripotent.

2. The method of claim 1 wherein steps (i)-(iii) are repeated through at least 10 passages.

3. The method of claim 1 or 2 wherein the microcarriers are rod-shaped.

4. The method of any one of claims 1 to 3 wherein the matrix comprises one or more of Matrigel™ (BD Biosciences), hyaluronic acid, laminin, fibronectin, vitronectin, collagen, elastin, heparan sulphate, dextran, dextran sulphate, chondroitin sulphate.

5. The method of any one of claims 1 to 3 wherein the matrix comprises a mixture of laminin, collagen I, heparan sulfate proteoglycans, and entactin 1.

6. The method of any one of claims 1 to 5 wherein the number of stem cells is expanded between each passage.

7. The method of any one of claims 1 to 6 wherein the microcarrier comprises or consists of one or more of cellulose, dextran, hydroxylated methacrylate, collagen, gelatin, polystyrene, plastic, glass, ceramic, silicone.

8. The method of any one of claims 1 to 6 wherein the microcarrier is a macroporous or microporous carboseed microcarrier.

9. The method of any one of claims 1 to 8 wherein the microcarrier is coupled with protamine or polylysine.

10. The method of any one of claims 1 to 9 wherein the microcarrier is positively charged.

11. The method of any one of claims 1 to 10 wherein the microcarrier has a positive surface charge.

12. The method of any one of claims 1 to 11 wherein the microcarrier is hydrophilic.

13. The method of any one of claims 1, 2 or 4 to 12 wherein the microcarriers have a substantially spherical shape.

14. The method of any one of claims 1 to 13 wherein in step (ii) the stem cells are cultured for a period of time sufficient to expand the number of stem cells in the culture.

15. The method of any one of claims 1 to 14 wherein in each repeat cycle the stem cells of step (i) are obtained from the passaged cells of step (iii) of the preceding repeat cycle.

16. The method of any one of claims 1 to 15 wherein in step (iv), steps (i)-(iii) are repeated through one of: at least 8 passages, at least 9 passages, at least 10 passages, at least 11 passages, at least 12 passages, at least 13 passages, at least 14 passages, at least 15 passages, at least 16 passages, at least 17 passages, at least 18 passages, at least 19 passages, at least 20 passages, at least 21 passages, at least 22 passages, at least 23 passages, at least 24 passages, at least 25 passages, at least 30 passages, at least 40 passages, at least 50 passages, at least 60 passages, at least 70 passages, at least 80 passages, at least 90 passages, at least 100 passages.

17. The method of any one of claims 1 to 16 wherein for at least 60% of the cycles of steps (i)-(iii) the microcarriers are coated in a matrix.

18. The method of any one of claims 1 to 17 wherein in cycles of steps (i)-(iii) the microcarriers are coated in the same matrix.

19. The method of any one of claims 1 to 18 wherein the matrix is different or absent in first and second consecutive cycles of steps (i)-(iii).

20. The method of any one of claims 1 to 19 wherein after step (iv) at least 60% of the stem cells in the culture are pluripotent.

21. The method of any one of claims 1 to 20 wherein after step (iv) at least 60% of the stem cells in the culture express one, two, three or all of Oct4, SSEA4, TRA-1-60 and Mab84.

22. The method of any one of claims 1 to 21 wherein the method comprises culturing the stem cells in serum free media, or stem cell conditioned media, or feeder cell free conditions.

23. The method of any one of claims 1 to 22 wherein feeder cells are also attached to the microcarriers.

24. The method of any one of claims 1 to 22 wherein the culture further comprises feeder cells attached to microcarriers which are different to the microcarriers to which the stem cells are attached.

25. Pluripotent stem cells obtained by the method of any one of claims 1 to 24.

26. The method of any one of claims 1 to 24 further comprising the step of inducing differentiation of the stem cells obtained after step (iv).

27. The method of claim 26 wherein the method comprises placing the microcarrier-stem cell complexes under conditions which induce the differentiation of the stem cells.

28. The method of any one of claims 1 to 24 wherein after step (iv) the method comprises the step of separating stem cells from the microcarriers and culturing the separated stem cells in non-microcarrier culture under conditions which induce differentiation of the stem cells.

29. The method of any one of claims 1 to 24 further comprising the differentiation of pluripotent stem cells, comprising:

(v) attaching pluripotent stem cells obtained after step (iv) to a plurality of second microcarriers to form microcarrier-stem cell complexes, wherein the surface of the second microcarriers is coated in a second matrix or is uncoated; and

(vi) culturing the microcarrier-stem cell complexes from (v) in suspension culture under conditions that induce the differentiation of the stem cells.

30. The method of claim 29 wherein the first and second matrix are the same.

31. The method of claim 30 wherein the first and second matrix are different.

32. The method of any one of claims 29 to 31 wherein the first and second microcarriers are the same.

33. The method of any one of claims 29 to 31 wherein the first and second microcarriers are different.

34. The method of any one of claims 29 to 33 wherein the method further comprises:

(vii) attaching differentiated stem cells obtained from step (vi) to a plurality of third microcarriers to form microcarrier-stem cell complexes, wherein the surface of the third microcarriers is coated in a third matrix or is uncoated; and

(viii) culturing the microcarrier-stem cell complexes from (vii) in suspension culture under conditions that induce the further differentiation of the differentiated stem cells.

35. The method of claim 24 wherein the third matrix is different to the first and second matrix.

36. The method of claim 34 wherein the third matrix is the same as one of the first and second matrix.

37. The method of any one of claims 34 to 36 wherein the third microcarriers are different to the first and second microcarriers.

38. The method of any one of claims 34 to 36 wherein the third microcarriers are the same as one of the first and second microcarriers.

39. A differentiated cell obtained by the method of any one of claims 36 to 38.

40. The method of any one of claims 26 to 38 wherein the differentiated cells are cultured to form an embryoid body.

41. An embryoid body obtained by the method of claim 40.

42. A method of culturing stem cells in suspension culture in vitro, the method comprising:

(i) attaching stem cells to a plurality of microcarriers to form microcarrier-stem cell complexes, wherein the surface of the microcarriers is coated in Matrigel™;

(ii) culturing the microcarrier-stem cell complexes in suspension culture;

(iii) passaging the cultured cells from (ii); and

(iv) repeating steps (i)-(iii) through at least 7 passages,

wherein stem cells in the culture after step (iv) are pluripotent, wherein the culture is free of feeder cells, wherein the number of stem cells is expanded between each passage and wherein the stem cells are human or primate embryonic stem cells or human or primate induced pluripotent stem cells.

43. A method of culturing and differentiating human embryonic stem cells, or human induced pluripotent stem cells, in vitro, the method comprising:

(i) attaching human embryonic stem cells or human induced pluripotent stem cells to a plurality of first microcarriers to form microcarrier-stem cell complexes, wherein the surface of the first microcarriers is coated in a first matrix comprising an extracellular matrix component;

(ii) culturing the microcarrier-stem cell complexes in suspension culture;

(iii) passaging the cultured cells from (ii); and

(iv) repeating steps (i)-(iii) through at least 7 passages,

wherein stem cells in the culture after step (iv) are pluripotent, the method further comprising:

(v) attaching pluripotent stem cells obtained after step (iv) to a plurality of second microcarriers to form microcarrier-stem cell complexes, wherein the surface of the second microcarriers is coated in a second matrix or is uncoated; and

(vi) culturing the microcarrier-stem cell complexes from (v) in suspension culture under conditions that induce the differentiation of the stem cells.

~~44. The method of claim 43 wherein the microcarriers are rod-shaped.~~

45. The method of claim 43 or 44 wherein the first and second matrix are the same.

46. The method of claim 43 or 44 wherein the first and second matrix are different.

47. The method of any one of claims 43 to 46 wherein the first and second microcarriers are the same.

48. The method of any one of claims 43 to 46 wherein the first and second microcarriers are different.

49. The method of any one of claims 43 to 48 wherein the method further comprises:

(vii) attaching differentiated stem cells obtained from step (vi) to a plurality of third microcarriers to form microcarrier-stem cell complexes, wherein the surface of the third microcarriers is coated in a third matrix or is uncoated; and

(viii) culturing the microcarrier-stem cell complexes from (vii) in suspension culture under conditions that induce the further differentiation of the differentiated stem cells.

50. The method of claim 49 wherein the third matrix is different to the first and second matrix.

51. The method of claim 49 wherein the third matrix is the same as one of the first and second matrix.

52. The method of any one of claims 49 to 51 wherein the third microcarriers are different to the first and second microcarriers.

53. The method of any one of claims 49 to 41 wherein the third microcarriers are the same as one of the first and second microcarriers.

54. A method of differentiating stem cells in vitro, comprising attaching pluripotent human embryonic stem cells, or human induced pluripotent stem cells to a plurality of microcarriers to form microcarrier-stem cell complexes, wherein the surface of the microcarriers is coated in a matrix comprising an extracellular matrix component, and culturing the microcarrier-stem cell complexes in suspension culture under conditions that induce the differentiation of the stem cells.

55. The method of claim 54 wherein the microcarriers are rod-shaped.

56. The method of claim 54 or 55 wherein the matrix comprises one or more of laminin, fibronectin, vitronectin, Matrigel™ (BD Biosciences), hyaluronic acid, collagen, elastin, heparan sulphate, dextran, dextran sulphate, chondroitin sulphate.

57. The method of any one of claims 54 or 55 wherein the matrix comprises a mixture of laminin, collagen I, heparan sulfate proteoglycans, and entactin 1.

58. The method of any one of claims 54 to 57 wherein the microcarrier comprises or consists of one or more of cellulose, dextran, hydroxylated methacrylate, collagen, gelatin, polystyrene, plastic, glass, ceramic, silicone.

59. The method of any one of claims 54 to 57 wherein the microcarrier is a macroporous or microporous carboseed microcarrier.

60. The method of any one of claims 54 to 59 wherein the microcarrier is coupled with protamine or polylysine.

61. The method of any one of claims 54 to 60 wherein the microcarrier is positively charged.

62. The method of any one of claims 54 to 61 wherein the microcarrier has a positive surface charge.

63. The method of any one of claims 54 to 62 wherein the microcarrier is hydrophilic.

64. The method of any one of claims 54 or 55 to 63 wherein the microcarriers have a substantially spherical shape.

65. A method of culturing human multipotent stem cells in suspension culture in vitro, the method comprising:

(i) attaching human multipotent stem cells to a plurality of microcarriers to form microcarrier-stem cell complexes;

(ii) culturing the microcarrier-stem cell complexes in suspension culture;

(iii) passaging the cultured cells from (ii); and

(iv) repeating steps (i)-(iii) through at least 2 passages,

wherein stem cells in the culture after step (iv) are multipotent.

66. The method of claim 65 wherein in (i) the surface of the microcarriers is coated in a matrix.

67. Multipotent stem cells obtained by the method of claim 65 or 66.

68. The method of claim 65 or 66 further comprising the step of inducing differentiation of the stem cells obtained after step (iv).

69. The method of claim 68 wherein the method comprises placing the microcarrier-stem cell complexes under conditions which induce the differentiation of the stem cells.

70. The method of any one of claims 65 to 69 wherein after step (iv) the method comprises the step of separating stem cells from the microcarriers and culturing the separated stem cells in non-microcarrier culture under conditions which induce differentiation of the stem cells.

71. The method of claim 65 further comprising the differentiation of multipotent stem cells, comprising:

(v) attaching multipotent stem cells obtained after step (iv) to a plurality of second microcarriers to form microcarrier-stem cell complexes, wherein the surface of the second microcarriers is coated in a second matrix or is uncoated; and

(vi) culturing the microcarrier-stem cell complexes from (v) in suspension culture under conditions that induce the differentiation of the stem cells.

72. A differentiated cell obtained by the method of any one of claims 65 to 71.

73. A method of culturing and differentiating human multipotent stem cells in vitro, the method comprising:

(i) attaching human multipotent stem cells to a plurality of first microcarriers to form microcarrier-stem cell complexes;

(ii) culturing the microcarrier-stem cell complexes in suspension culture;

(iii) passaging the cultured cells from (ii); and

(iv) repeating steps (i) (iii) through at least 2 passages,

wherein stem cells in the culture after step (iv) are multipotent, the method further comprising:

(v) attaching multipotent stem cells obtained after step (iv) to a plurality of second microcarriers to form microcarrier-stem cell complexes, wherein the surface of the second microcarriers is coated in a second matrix or is uncoated; and

(vi) culturing the microcarrier-stem cell complexes from (v) in suspension culture under conditions that induce the differentiation of the stem cells.

74. The method of claim 73 wherein in (i) the surface of the microcarriers is coated in a first matrix.

75. A method of differentiating stem cells in vitro, comprising attaching human multipotent stem cells to a plurality of microcarriers to form microcarrier-stem cell complexes, wherein the surface of the microcarriers is coated in a matrix or is uncoated, and culturing the microcarrier-stem cell complexes in suspension culture under conditions that induce the differentiation of the stem cells.

76. The method of any one of claims 65 to 75 wherein the stem cells are adult stem cells, or multipotent stem cells derived from pluripotent stem cells.

77. The method of any one of claims 65 to 76 wherein the microcarriers are rod-shaped.

78. The method of any one of claims 65 to 77 wherein the matrix comprises an extracellular matrix component.

79. The method of any one of claims 65 to 77 wherein the matrix comprises one or more of Matrigel™ (BD Biosciences), hyaluronic acid, laminin, fibronectin, vitronectin, collagen, elastin, heparan sulphate, dextran, dextran sulphate, chondroitin sulphate.

80. The method of any one of claims 65 to 77 wherein the matrix comprises a mixture of laminin, collagen I, heparan sulfate proteoglycans, and entactin 1.

81. The method of any one of claims 65 to 80 wherein the microcarrier comprises or consists of one or more of cellulose, dextran, hydroxylated methacrylate, collagen, gelatin, polystyrene, plastic, glass, ceramic, silicone.

82. The method of any one of claims 65 to 80 wherein the microcarrier is a macroporous or microporous carboseed microcarrier.

83. The method of any one of claims 65 to 82 wherein the microcarrier is positively charged.

84. The method of any one of claims 65 to 83 wherein the microcarrier has a positive surface charge.

85. The method of any one of claims 65 to 84 wherein in step (ii) the stem cells are cultured for a period of time sufficient to expand the number of stem cells in the culture.

86. The method of any one of claims 65 to 85 wherein in each repeat cycle the stem cells of step (i) are obtained from the passaged cells of step (iii) of the preceding repeat cycle.

87. The method of any one of claims 65 to 86 wherein in step (iv), steps (i)-(iii) are repeated through one of: at least 3 passages, at least 4 passages, at least 5 passages, at least 6 passages, at least 7 passages, at least 8 passages, at least 9 passages, at least 10 passages, at least 11 passages, at least 12 passages, at least 13 passages, at least 14 passages, at least 15 passages, at least 16 passages, at least 17 passages, at least 18 passages, at least 19 passages, at least 20 passages, at least 21 passages, at least 22 passages, at least 23 passages, at least 24 passages, at least 25 passages, at least 30 passages, at least 40 passages, at least 50 passages, at least 60 passages, at least 70 passages, at least 80 passages, at least 90 passages, at least 100 passages.

88. A microcarrier suitable for use in the growth and/or differentiation of pluripotent or multipotent cells in in vitro suspension culture, wherein the microcarrier comprises one or more of cellulose, dextran, hydroxylated methacrylate, or collagen, and wherein the microcarrier has an elongate shape and has a longest dimension of less than about 2000μm and a shortest dimension of more than about 10μm, and wherein the surface of the microcarrier is coated in a matrix comprising an extracellular matrix component, and wherein one or a plurality of human pluripotent or human multipotent cells are attached to the matrix coating.

~~89. The microcarrier of claim 88 wherein the microcarrier is rod-shaped.~~

90. The microcarrier of claim 88 or 89 wherein the matrix coating comprises one or more of MatrigelTM (BD Biosciences), hyaluronic acid, laminin, or fibronectin.

91. The microcarrier of any one of claims 88 to 90 wherein the cells are pluripotent cells.

92. The microcarrier of any one of claims 88 to 91 wherein the pluripotent cells are primate or human embryonic stem cells, or induced pluripotent stem cells.

93. The microcarrier of any one of claims 88 to 92 wherein the microcarrier is positively charged.

94. The microcarrier of any one of claims 88 to 93 wherein the microcarrier has a positive surface charge.

95 The microcarrier of any one of claims 88 to 94 having a longest dimension of between 50μm and 400μm.

96. An aggregate comprising two or more microcarriers having pluripotent or multipotent cells attached thereto, each according to any one of claims 88 to 95.

97. Use of a microcarrier according to any one of claims 88 to 95 in the culture of human pluripotent or multipotent cells in vitro to generate new cells having pluripotent or multipotent status.

98. Use of a microcarrier according to any one or claims 88 to 95 in the in vitro differentiation of human pluripotent or multipotent cells.

99. A method of culturing human pluripotent or multipotent cells in vitro to generate new cells having pluripotent or multipotent status, the method comprising culturing a microcarrier according to any one or claims 88 to 95 under conditions suitable for the generation of new cells having pluripotent or multipotent status.

100. A method of differentiating human pluripotent or multipotent cells in vitro, the method comprising culturing a microcarrier according to any one or claims 88 to 95 under conditions that induce the differentiation of the pluripotent or multipotent cells.

Claims:

1. A method of culturing human embryonic stem cells, or human induced pluripotent stem cells, in suspension culture *in vitro*, the method comprising:

(i) attaching human embryonic stem cells or human induced pluripotent stem cells to a plurality of microcarriers to form microcarrier-stem cell complexes, wherein the surface of the microcarriers is coated in a matrix comprising an extracellular matrix component;

(ii) culturing the microcarrier-stem cell complexes in suspension culture;

(iii) passaging the cultured cells from (ii); and

(iv) repeating steps (i)-(iii) through at least 7 passages,

wherein stem cells in the culture after step (iv) are pluripotent, and wherein the matrix comprises one or more of Matrigel™ (BD Biosciences), hyaluronic acid, laminin, fibronectin, vitronectin, heparan sulphate and chondroitin sulphate, or a mixture of laminin, collagen I, heparan sulfate proteoglycans, and entactin 1.

2. The method of claim 1 wherein the microcarriers are rod-shaped or have a substantially spherical shape.

3. The method of any one of claims 1 or 2 wherein the number of stem cells is expanded between each passage.

4. The method of any one of claims 1 or 2 wherein the microcarrier comprises or consists of one or more of cellulose, dextran, hydroxylated methacrylate, collagen, gelatin, polystyrene, plastic, glass, ceramic, silicone, or wherein the microcarrier is a macroporous or microporous carboseed microcarrier.

5. The method of any one of claims 1 to 4 wherein the microcarrier is coupled with protamine or polylysine.

6. The method of any one of claims 1 to 5 wherein the microcarrier is positively charged.

7. The method of any one of claims 1 to 6 wherein in step (ii) the stem cells are cultured for a period of time sufficient to expand the number of stem cells in the culture.

8. The method of any one of claims 1 to 7 wherein in each repeat cycle the stem cells of step (i) are obtained from the passaged cells of step (iii) of the preceding repeat cycle.

9. The method of any one of claims 1 to 8 wherein in step (iv), steps (i)-(iii) are repeated through one of: at least 8 passages, at least 9 passages, at least 10 passages, at least 11 passages, at least 12 passages, at least 13 passages, at least 14 passages, at least 15 passages, at least 16 passages, at least 17 passages, at least 18 passages, at least 19 passages, at least 20 passages, at least 21 passages, at least 22 passages, at least 23 passages, at least 24 passages, at least 25 passages, at least 30 passages, at least 40 passages, at least 50 passages, at least 60 passages, at least 70 passages, at least 80 passages, at least 90 passages, at least 100 passages.

10. The method of any one of claims 1 to 9 wherein in cycles of steps (i)-(iii) the microcarriers are coated in the same matrix.

11. The method of any one of claims 1 to 10 wherein after step (iv) at least 60% of the stem cells in the culture are pluripotent.

12. The method of any one of claims 1 to 11 wherein after step (iv) at least 60% of the stem cells in the culture express one, two, three or all of Oct4, SSEA4, TRA-1-60 and Mab84.

13. The method of any one of claims 1 to 12 wherein the method comprises culturing the stem cells in serum free media, or stem cell conditioned media, or feeder cell free conditions.

14. The method of any one of claims 1 to 13 wherein feeder cells are also attached to the microcarriers.

15. The method of any one of claims 1 to 14 wherein the culture further comprises feeder cells attached to microcarriers which are different to the microcarriers to which the stem cells are attached.

16. The method of any one of claims 1 to 15 further comprising the step of inducing differentiation of the stem cells obtained after step (iv).

17. The method of claim 16 wherein the method comprises placing the microcarrier-stem cell complexes under conditions which induce the differentiation of the stem cells.

18. The method of any one of claims 1 to 17 wherein after step (iv) the method comprises the step of separating stem cells from the microcarriers and culturing the separated stem cells in non-microcarrier culture under conditions which induce differentiation of the stem cells.

19. The method of any one of claims 1 to 18 further comprising the differentiation of pluripotent stem cells, comprising:

(v) attaching pluripotent stem cells obtained after step (iv) to a plurality of second microcarriers to form microcarrier-stem cell complexes, wherein the surface of the second microcarriers is coated in a second matrix or is uncoated; and

(vi) culturing the microcarrier-stem cell complexes from (v) in suspension culture under conditions that induce the differentiation of the stem cells.

20. The method of claim 19 wherein the method further comprises:

(vii) attaching differentiated stem cells obtained from step (vi) to a plurality of third microcarriers to form microcarrier-stem cell complexes, wherein the surface of the third microcarriers is coated in a third matrix or is uncoated; and

(viii) culturing the microcarrier-stem cell complexes from (vii) in suspension culture under conditions that induce the further differentiation of the differentiated stem cells.

21. The method of any one of claims 16 to 20 wherein the differentiated cells are cultured to form an embryoid body.

22. A method of culturing stem cells in suspension culture *in vitro*, the method comprising:

(i) attaching stem cells to a plurality of microcarriers to form microcarrier-stem cell complexes, wherein the surface of the microcarriers is coated in Matrigel™;

(ii) culturing the microcarrier-stem cell complexes in suspension culture;

(iii) passaging the cultured cells from (ii); and

(iv) repeating steps (i)-(iii) through at least 7 passages,

wherein stem cells in the culture after step (iv) are pluripotent, wherein the culture is free of feeder cells, wherein the number of stem cells is expanded between each passage and wherein the stem cells are human or primate embryonic stem cells or human or primate induced pluripotent stem cells.

Claims:

1. A method of culturing human embryonic stem cells, or human induced pluripotent stem cells, in suspension culture *in vitro*, the method comprising:

(i) attaching human embryonic stem cells or human induced pluripotent stem cells to a plurality of microcarriers to form microcarrier-stem cell complexes, wherein the surface of the microcarriers is coated in a matrix comprising an extracellular matrix component;

(ii) culturing the microcarrier-stem cell complexes in suspension culture;

(iii) passaging the cultured cells from (ii); and

(iv) repeating steps (i)-(iii) through at least 7 passages,

wherein stem cells in the culture after step (iv) are pluripotent, and wherein the matrix comprises one or more of Matrigel™ (BD Biosciences), hyaluronic acid, laminin, fibronectin, vitronectin, heparan sulphate and chondroitin sulphate, or a mixture of laminin, collagen I, heparan sulfate proteoglycans, and entactin 1.

2. The method of claim 1 wherein the microcarriers are rod-shaped or have a substantially spherical shape.

3. The method of any one of claims 1 or 2 wherein the number of stem cells is expanded between each passage.

4. The method of any one of claims 1 or 2 wherein the microcarrier comprises or consists of one or more of cellulose, dextran, hydroxylated methacrylate, collagen, gelatin, polystyrene, plastic, glass, ceramic, silicone, or wherein the microcarrier is a macroporous or microporous carboseed microcarrier.

5. The method of any one of claims 1 to 4 wherein the microcarrier is coupled with protamine or polylysine.

6. The method of any one of claims 1 to 5 wherein the microcarrier is positively charged.

7. The method of any one of claims 1 to 6 wherein in step (ii) the stem cells are cultured for a period of time sufficient to expand the number of stem cells in the culture.

8. The method of any one of claims 1 to 7 wherein in each repeat cycle the stem cells of step (i) are obtained from the passaged cells of step (iii) of the preceding repeat cycle.

9. The method of any one of claims 1 to 8 wherein in step (iv), steps (i)-(iii) are repeated through one of: at least 8 passages, at least 9 passages, at least 10 passages, at least 11 passages, at least 12 passages, at least 13 passages, at least 14 passages, at least 15 passages, at least 16 passages, at least 17 passages, at least 18 passages, at least 19 passages, at least 20 passages, at least 21 passages, at least 22 passages, at least 23 passages, at least 24 passages, at least 25 passages, at least 30 passages, at least 40 passages, at least 50 passages, at least 60 passages, at least 70 passages, at least 80 passages, at least 90 passages, at least 100 passages.

10. The method of any one of claims 1 to 9 wherein in cycles of steps (i)-(iii) the microcarriers are coated in the same matrix.

11. The method of any one of claims 1 to 10 wherein after step (iv) at least 60% of the stem cells in the culture are pluripotent.

12. The method of any one of claims 1 to 11 wherein after step (iv) at least 60% of the stem cells in the culture express one, two, three or all of Oct4, SSEA4, TRA-1-60 and Mab84.

13. The method of any one of claims 1 to 12 wherein the method comprises culturing the stem cells in serum free media, or stem cell conditioned media, or feeder cell free conditions.

14. The method of any one of claims 1 to 13 wherein feeder cells are also attached to the microcarriers.

15. The method of any one of claims 1 to 14 wherein the culture further comprises feeder cells attached to microcarriers which are different to the microcarriers to which the stem cells are attached.

16. The method of any one of claims 1 to 15 further comprising the step of inducing differentiation of the stem cells obtained after step (iv).

17. The method of claim 16 wherein the method comprises placing the microcarrier-stem cell complexes under conditions which induce the differentiation of the stem cells.

18. The method of any one of claims 1 to 17 wherein after step (iv) the method comprises the step of separating stem cells from the microcarriers and culturing the separated stem cells in non-microcarrier culture under conditions which induce differentiation of the stem cells.

19. The method of any one of claims 1 to 18 further comprising the differentiation of pluripotent stem cells, comprising:

(v) attaching pluripotent stem cells obtained after step (iv) to a plurality of second microcarriers to form microcarrier-stem cell complexes, wherein the surface of the second microcarriers is coated in a second matrix or is uncoated; and

(vi) culturing the microcarrier-stem cell complexes from (v) in suspension culture under conditions that induce the differentiation of the stem cells.

20. The method of claim 19 wherein the method further comprises:

(vii) attaching differentiated stem cells obtained from step (vi) to a plurality of third microcarriers to form microcarrier-stem cell complexes, wherein the surface of the third microcarriers is coated in a third matrix or is uncoated; and

(viii) culturing the microcarrier-stem cell complexes from (vii) in suspension culture under conditions that induce the further differentiation of the differentiated stem cells.

21. The method of any one of claims 16 to 20 wherein the differentiated cells are cultured to form an embryoid body.

22. A method of culturing stem cells in suspension culture *in vitro*, the method comprising:

(i) attaching stem cells to a plurality of microcarriers to form microcarrier-stem cell complexes, wherein the surface of the microcarriers is coated in Matrigel™;

(ii) culturing the microcarrier-stem cell complexes in suspension culture;

(iii) passaging the cultured cells from (ii); and

(iv) repeating steps (i)-(iii) through at least 7 passages,

wherein stem cells in the culture after step (iv) are pluripotent, wherein the culture is free of feeder cells, wherein the number of stem cells is expanded between each passage and wherein the stem cells are human or primate embryonic stem cells or human or primate induced pluripotent stem cells.