

Claims

1. A method for culturing primitive-like macrophages from stem cells wherein the method comprises:

(a) contacting and incubating said stem cells with a serum-free culture media comprising a GSK3 inhibitor to induce differentiation of said stem cells into cells of the mesoderm lineage;

(b) contacting and incubating said cells of the mesoderm lineage with a culture media comprising DKK1 to differentiate the cells of the mesoderm lineage into cells of the hematopoietic cell lineage;

(c) maturing said cells of the hematopoietic cell lineage;

(d) contacting and incubating said mature cells of the hematopoietic cell lineage with a culture media comprising M-CSF to drive the differentiation of said hematopoietic cells into primitive-like macrophages.

2. The method according to claim 1, wherein the stem cells are selected from embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC); optionally wherein the stem cells are human H1 embryonic stem cells (ESC); optionally wherein the stem cells are human induced pluripotent stem cells (iPSC).

3. The method according to claims 1 or 2, wherein the GSK3 inhibitor is 6- bromoindirubin-3'-oxime (BIO), CHIR-99021, SB216763, CHIR-98014, TWS119, IM-12, 1-Azakenpaullone, AR-A014418, SB415286, AZD1080, AZD2858, indirubin, A 1070722, TCS 2002, Tideglusib, or any derivatives thereof.

4. The method according to any one of claims 1 to 3, wherein the stem cells are plated at a density of less than 1 small colony/cm² on Matrigel-coated 6-well plates at least 24 hours prior to step (a) of claim 1; optionally wherein the stem cells are incubated with a media comprising 80% DMEM/F12, 20% knockout serum replacement, L-glutamine, non-essential amino acids, beta-mercaptoethanol and FGF-2, prior to step (a) of claim 1; optionally wherein the stem cells are incubated for about 1 day in said media.
5. The method according to any one of claims 1 to 4, wherein the stem cells are contacted at step (a) of claim 1 with a differentiation media, comprising 1-20 ng/ml of BMP4, 5-100 ng/ml of VEGF, 0-50 ng/ml of FGF-2, and 0-10 μ M of CHIR99021; optionally wherein the differentiation media is StemPro® 34 from Invitrogen; optionally wherein the cells are incubated with the media for about 2 days in an oxygen concentration of about 5%.
6. The method according to any one of claims 1 to 5, wherein the cells are further contacted at step (a) with 1-20 ng/ml of BMP4 and 5-100 ng/ml of VEGF; optionally wherein the cells are incubated for about 2 days with said media in an oxygen concentration of about 5%.
7. The method according to any one of claims 1 to 6, wherein said cells are contacted at step (b) of claim 1 with a differentiation media comprising 5-100 ng/ml of VEGF; 0- 50 ng/ml of FGF-2; 0-250 ng/ml of SCF; 0-500 ng/ml DKK1; 0-50 ng/ml of IL-6; and 0-50 ng/ml of IL-3; optionally wherein said cells are incubated for about 3 days with said media in an oxygen concentration of about 5%.

8. The method according to any one of claims 1 to 7, wherein at step (c) of claim 1, the cells are matured by contact with a differentiation media comprising 0-50 ng/ml of FGF-2; 0-250 ng/ml of SCF; 0-50 ng/ml of IL-3 and 0-50 ng/ml of IL-6; optionally wherein the cells are incubated with the media for 10 about 7 days in normoxic conditions.
9. The method according to any one of claims 1 to 8, wherein the cells are contacted and incubated at step (d) of claim 1 with a differentiation media comprising 0-100 ng/ml M-CSF; optionally wherein the cells are incubated with the media for at least 6 days in normoxic conditions.
10. The method according to any one of claims 1 to 9, wherein the said method produces a yield of primitive-like macrophages of greater than 1×10^6 /well in a 6-well culture plate between 21 to 25 days of culture; optionally wherein said method further comprises isolating said primitive-like macrophages using FACS sorting or magnetic separation.
11. A kit when used in the method of any one of claims 1 to 10, comprising a GSK3 inhibitor and DKK1 with instructions for use.
12. A primitive-like macrophage obtained by the method of any one of claims 1-10.

13. The primitive-like macrophage of claim 12, wherein said primitive-like macrophage is subsequently differentiated into a microglial cell, an alveolar macrophage, a Kupffer cell, a Langerhans cell, or other tissue-specific macrophages.

14. Use of the primitive-like macrophage according to claim 12 for developing *in vitro* disease models, wherein the disease is a neurodegenerative disease, a metabolic disease, a respiratory disease, a cardiovascular disease, a connective tissue disease, cancer or an inflammatory disease or for screening compounds to treat a disease, wherein the disease is a neurodegenerative disease or a metabolic disease.

15. Use of the microglial cell according to claim 13 for developing *in vitro* disease models, wherein the disease is a neurodegenerative disease; or for screening compounds to treat a disease, wherein the disease is a neurodegenerative disease.

16. The use according to claims 14 or 15, wherein the neurodegenerative disease is Alzheimer's disease, Huntington's disease or Rett syndrome; optionally wherein the metabolic disease is obesity or diabetes; optionally wherein the respiratory disease is pulmonary alveolar proteinosis; optionally wherein the cardiovascular disease is atherosclerosis; optionally wherein the connective tissue disease is fibrosis; optionally wherein the inflammatory disease is arthritis, experimental autoimmune encephalomyelitis, multiple sclerosis or inflammatory bowel disease.

17. Use of the primitive-like macrophage according to claim 12 in the manufacture of a medicament for wound healing and tissue regeneration.

18. Use of the primitive-like macrophage according to claim 12 for delivering a cargo molecule into a tissue, wherein the cargo molecule is an immunomodulatory cytokine or chemokine, or an enzyme that activates pro-drugs.

19. A method for culturing primitive-like macrophages from murine stem cells wherein the method comprises:

(a) contacting and incubating said stem cells with a serum-free culture media comprising FGF2 and BMP4 to induce differentiation of said stem cells into cells of the mesoderm lineage;

(b) contacting and incubating said cells of the mesoderm lineage with a culture media comprising FGF2, BMP4, Activin A and VEGF to differentiate the cells of the mesoderm lineage into cells of the hematopoietic cell lineage;

(c) maturing said cells of the hematopoietic cell lineage;

(d) contacting and incubating said mature cells of the hematopoietic cell lineage with a culture media comprising M-CSF to drive the differentiation of said hematopoietic cells into primitive-like macrophages.