

protocols for hematopoietic differentiation of human cells that can affect reproducibility and yields. Accordingly, there is a need to provide a method for hematopoietic differentiation that overcomes, or at least ameliorates, one or more of the disadvantages described above to recapitulate the embryonic origin of macrophages *in vitro*.

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SUMMARY OF THE INVENTION

[0006] In one aspect, there is provided 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
10 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;
- 15 (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.

[0006a] In one aspect, there is provided a method for culturing primitive-like
20 macrophages from pluripotent stem cells wherein the method comprises:

- (a) contacting and incubating said pluripotent stem cells with a serum-free culture media comprising a GSK3 inhibitor to induce differentiation of said pluripotent stem cells into cells of the mesoderm lineage;
- (b) contacting and incubating said cells of the mesoderm lineage with a culture media
25 comprising DKK1 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
30 primitive-like macrophages.

[0007] In one aspect, there is provided a kit when used in the method as defined herein, comprising a GSK3 inhibitor and DKK1 with instructions for use.

[0008] In one aspect, there is provided a primitive-like macrophage obtained by the method as described herein.

5 [0009] In one aspect, there is provided a use of the primitive-like macrophage as described herein 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.

[0010] In one aspect, there is provided a use of the microglial cell as described herein for developing *in vitro* disease models, wherein the disease is a neurodegenerative disease.

10 [0011] In one aspect, there is provided a use of the primitive-like macrophage as described herein for screening compounds to treat a disease, wherein the disease is a neurodegenerative disease or a metabolic disease.

[0012] In one aspect, there is provided a use of the microglial cell as described herein for screening compounds to treat a disease, wherein the disease is a neurodegenerative disease.

[0013] In one aspect, there is provided a use of the primitive-like macrophage as described herein for in the manufacture of a medicament for wound healing and tissue regeneration.

[0014] In one aspect, there is provided a use of the primitive-like macrophage as described herein 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.

[0015] In one aspect, there is provided 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.

[0015a] In one aspect, there is provided a use of the primitive-like macrophages obtained by the method described herein 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.

[0015b] In one aspect, there is provided a use of the microglial cell obtained by the method described herein 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.

[0015c] In one aspect, there is provided a use of the primitive-like macrophages obtained by the method described herein in the manufacture of a medicament for wound healing and tissue regeneration.

- 5 [0015d] In one aspect, there is provided a use of the primitive-like macrophages obtained by the method described herein in the manufacture of a medicament 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.

10 **BRIEF DESCRIPTION OF THE DRAWINGS**

[0016] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0017] **Fig. 1** shows a flow diagram of the method of the invention.

- 15 [0018] **Fig 2** shows the timeline for the addition of growth factors used in the method of the invention.

[0019] **Fig. 3** shows the flow cytometry analysis of macrophages differentiated from a) H1 ES cells, b) patient-derived WT iPSCs and c) patient-derived mutant iPSCs: Cells are sequentially gated on their FSC/SSC profile, then live cells (DAPI-), then hematopoietic cell
20 (CD45+) and then singlets (SSC-A and SSC-W gate). Profile of expression of common macrophage markers are shown: CD14, HLA-DR, CD11b and CD163. Macrophages

Claims

1. A method for culturing primitive-like macrophages from pluripotent stem cells wherein the method comprises:
 - (a) contacting and incubating said pluripotent stem cells with a serum-free culture media comprising a GSK3 inhibitor to induce differentiation of said pluripotent stem cells into cells of the mesoderm lineage;
 - (b) contacting and incubating said cells of the mesoderm lineage with a culture media comprising DKK1 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.
2. The method according to claim 1, wherein the pluripotent stem cells are selected from embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC); optionally wherein the pluripotent stem cells are human H1 embryonic stem cells (ESC); optionally wherein the pluripotent 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 pluripotent 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 pluripotent 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 pluripotent 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 pluripotent 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 2-10 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; 30-500 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 up to 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. The method of any one of claims 1 to 10, wherein said primitive-like macrophages are subsequently differentiated into microglial cells, alveolar macrophages, Kupffer cells, Langerhans cells, or other tissue-specific macrophages.
13. Use of the primitive-like macrophages obtained by the method of any one of claims 1-10 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.
14. Use of the microglial cell obtained by the method of claim 12 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.

15. The use according to claims 13 or 14, 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.

16. Use of the primitive-like macrophages obtained by the method of any one of claims 1-10 in the manufacture of a medicament for wound healing and tissue regeneration.

17. Use of the primitive-like macrophages obtained by the method of any one of claims 1-10 in the manufacture of a medicament 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.