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*.

SUMMARY

[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 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.

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

(a) plating said human pluripotent stem cells at a density of less than 1 small colony/cm^2 on Matrigel-coated 6-well plates for at least 24 hours in a media comprising 80% DMEM/F12, 20% knockout serum replacement, L-glutamine, non-essential amino acids, beta-mercaptoethanol and FGF-2;

(b) contacting and incubating said human pluripotent stem cells from step (a) with a serum-free culture media comprising VEGF, BMP4 and a GSK3 inhibitor under hypoxic conditions to induce differentiation of said human pluripotent stem cells into cells of the mesoderm lineage;

(c) contacting and incubating said cells of the mesoderm lineage with a culture media comprising SCF, FGF-2, IL-3, IL-6, DKK1 and VEGF under hypoxic conditions until day 8 to differentiate the cells of the mesoderm lineage into cells of the hematopoietic cell lineage, and subsequently incubating the cells under normoxic conditions;

(d) maturing said cells of the hematopoietic cell lineage by contacting said cells with a culture media comprising SCF, FGF-2, IL-3 and IL-6 under normoxic conditions;

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

wherein the primitive-like macrophages express CD163, CD11b, HLA-DR, IBA-1, CXCR1 and CD14.

[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.

[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.

[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

Claims

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

(a) plating said human pluripotent stem cells at a density of less than 1 small colony/cm^2 on Matrigel-coated 6-well plates for at least 24 hours in a media comprising 80% DMEM/F12, 20% knockout serum replacement, L-glutamine, non-essential amino acids, beta-mercaptoethanol and FGF-2;

(b) contacting and incubating said human pluripotent stem cells from step (a) with a serum-free culture media comprising VEGF, BMP4 and a GSK3 inhibitor under hypoxic conditions to induce differentiation of said human pluripotent stem cells into cells of the mesoderm lineage;

(c) contacting and incubating said cells of the mesoderm lineage with a culture media comprising SCF, FGF-2, IL-3, IL-6, DKK1 and VEGF under hypoxic conditions until day 8 to differentiate the cells of the mesoderm lineage into cells of the hematopoietic cell lineage, and subsequently incubating the cells under normoxic conditions;

(d) maturing said cells of the hematopoietic cell lineage by contacting said cells with a culture media comprising SCF, FGF-2, IL-3 and IL-6 under normoxic conditions;

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

wherein the primitive-like macrophages express CD163, CD11b, HLA-DR, IBA-1, CXCR1 and CD14.

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 claim 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 contacted at step (b) of claim 1 with a differentiation media, comprising 1-20 ng/ml of BMP4, 5-100 ng/ml of

VEGF, up to 50 ng/ml of FGF-2, and 2-10 μM of CHIR99021; optionally wherein the cells are incubated with the media for about 2 days in an oxygen concentration of about 5%.

5. The method according to any one of claims 1 to 4, wherein the cells are further contacted at step (b) 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%.

6. The method according to any one of claims 1 to 5, wherein said cells are contacted at step (c) of claim 1 with a differentiation media comprising 5-100 ng/ml of VEGF; up to 50 ng/ml of FGF-2; up to 250 ng/ml of SCF; 30-500 ng/ml DKK1; up to 50 ng/ml of IL-6; and up to 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%.

7. The method according to any one of claims 1 to 6, wherein at step (d) of claim 1, the cells are matured by contact with a differentiation media comprising up to 50 ng/ml of FGF-2; up to 250 ng/ml of SCF; up to 50 ng/ml of IL-3 and up to 50 ng/ml of IL-6; optionally wherein the cells are incubated with the media for about 7 days in normoxic conditions.

8. The method according to any one of claims 1 to 7, wherein the cells are contacted and incubated at step (e) of claim 1 with a differentiation media comprising up to100 ng/ml M-CSF; optionally wherein the cells are incubated with the media for at least 6 days in normoxic conditions.

9. The method according to any one of claims 1 to 8, wherein the said method produces a yield of primitive-like macrophages of greater than $1X10^6$ /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.

10. The method of any one of claims 1 to 9, wherein said primitive-like macrophages are subsequently differentiated into a cell expressing a microglial marker, wherein the microglial marker is IBA-1.