maturation. Methods to generate brain organoids have generated inconsistent results in terms of the emergence of microglia in these organoids. This has impeded microglia research aiming to decipher the role of human microglia during the early stages of brain development.

[0005] There is therefore a need to develop methods for generating brain organoids that comprise microglia cells.

SUMMARY

[0005a] In one aspect, provided herein is a method for generating a microglia-sufficient brain organoid comprising the step of incubating primitive-like macrophage cells with a brain organoid that is between 15 to 30 days old from the pluripotent state in cerebral organoid medium comprising CSF-1 in a low attachment cell culture vessel to generate microglia cells.

[0006] In one aspect, there is provided a method for generating a microglia-sufficient brain organoid comprising the step of incubating primitive-like macrophage cells with a brain organoid that is between about 15 to about 30 days old in cerebral organoid medium comprising CSF-1 in a low attachment cell culture vessel to generate microglia cells.

[0007] In another aspect, there is provided a microglia-sufficient brain organoid obtained by the method as described herein.

[0008] In another aspect, there is provided a method for screening a compound that targets microglia function comprising the steps of contacting the microglia-sufficient brain organoid as described herein with said compound and analysing the microglia cell for a predetermined trait.

[0009] In another aspect, there is provided a method for screening a compound to treat a neurodegenerative disease comprising the step of contacting the microglial-sufficient brain organoid as described herein with said compound and analysing the microglia cell for a predetermined trait.

[0010] In another aspect, there is provided a kit when used in the method as described herein, comprising cerebral organoid medium comprising CSF-1 together with instructions for use.

[0011] In another aspect, there is provided a method of isolating one or more predetermined populations of cells from a microglia-sufficient brain organoid comprising the steps of:

a) incubating the microglia-sufficient brain organoid in a digestion solution at 37°C for about 30 minutes;

b) physically agitating the microglia-sufficient brain organoid from step a);

c) subjecting the microglia-sufficient brain organoid from step b) to heat treatment at 1400rpm for 10 minutes at 37°C;

Claims

1. A method for generating a microglia-sufficient brain organoid comprising the step of incubating primitive-like macrophage cells with a brain organoid that is between 15 to 30 days old from the pluripotent state in cerebral organoid medium comprising CSF-1 in a low attachment cell culture vessel to generate microglia cells.

2. The method of claim 1, wherein the brain organoid is about 26 days old.

3. The method of claim 1 or 2, wherein the cerebral organoid medium comprises 25-100 ng/ml CSF-1.

4. The method of any one of claims 1 to 3, wherein 150,000 or less primitive-like macrophages are incubated with the brain organoid.

5. The method of any one of claims 1 to 4, wherein the primitive-like macrophage cells and brain organoid are incubated together for at least 1 week.

6. The method of claim 5, further comprising the step of dissociating cells of the microglia-sufficient brain organoid and isolating one or more predetermined populations of cells.

7. The method of claim 6, wherein the one or more predetermined populations of cells are isolated using fluorescence activated cell sorting (FACS) or magnetic activated cell sorting (MACS).

8. The method of claim 1, wherein the primitive-like macrophage cells are generated from a first population of stem cells by:

i) incubating said stem cells in a culture medium comprising a GSK3 inhibitor, BMP4 and VEGF to differentiate said stem cells into cells of the mesoderm lineage;

ii) incubating said cells of the mesoderm lineage in a culture medium comprising FGF-2 to differentiate said cells of the mesoderm lineage into hemangioblast cells;

iii) incubating said hemangioblast cells in a culture medium comprising VEGF and FGF-2;

iv) incubating said hemangioblast cells in a culture medium comprising DKK1, SCF, FGF2, IL3 and IL6 to differentiate said hemangioblast cells to hematopoietic cells;

v) incubating said hematopoietic cells in a culture medium comprising SCF, FGF-2, IL-3 and IL-6 to induce maturation of said hematopoietic cells;

vi) incubating said matured hematopoietic cells in a culture medium comprising CSF-1 to differentiate said matured hematopoietic cells to primitive-like macrophage cells.

9. The method of claim 8, wherein the primitive-like macrophages are generated over a period of 26 days.

10. The method of claim 1, wherein the brain organoid is generated from a second population of stem cells by:

i) incubating said second population of stem cells in a low attachment cell culture vessel to form an embryoid body (EB);

ii) incubating said embryoid body in neural induction medium to differentiate said embryoid body into an organoid comprising neuroectoderm cells;

iii) embedding the organoid comprising neuroectoderm cells in Matrigel and incubating the organoid comprising neuroectoderm cells in cerebral organoid medium containing N2 and B27 without vitamin A to differentiate said organoid comprising neuroectoderm cells to an organoid comprising neural epithelial cells;

iv) incubating the organoid comprising neural epithelial cells with cerebral organoid medium containing N2 and B27 with vitamin A to differentiate said organoid comprising neural epithelial cells to said brain organoid.

11. The method of claim 10, wherein the brain organoid is generated over a period of about 26 days.

12. The method of any one of claims 8-11, wherein the first and second populations of stem cells are embryonic stem cells (ESC), induced pluripotent stem cells (iPSC) or combinations thereof.

13. A microglia-sufficient brain organoid obtained by the method of any one of claims 1 to 12.

14. A method for screening a compound that targets microglia function comprising the steps of contacting the microglia-sufficient brain organoid of claim 13 with said compound and analysing the microglia cell for a predetermined trait.

15. A method for screening a compound to treat a neurodegenerative disease comprising the step of contacting the microglial-sufficient brain organoid of claim 13 with said compound and analysing the microglia cell for a predetermined trait.

16. A kit when used in the method of any one of claims 1 to 12, comprising cerebral organoid medium comprising CSF-1 together with instructions for use.

17. The kit when used in the method according to claim 16, further comprising:

a) a first culture medium comprising a GSK3 inhibitor, BMP4 and VEGF;

b) a second culture medium comprising BMP4, VEGF and FGF-2;

c) a third culture medium comprising VEGF and FGF-2;

d) a fourth culture medium comprising VEGF, DKK1, SCF, FGF-2, IL-3 and IL-6;

e) a fifth culture medium comprising FGF, IL6, IL3 and SCF; and

f) a sixth culture medium comprising CSF-1;

to generate primitive-like macrophage cells from a first population of stem cells; and

g) a neural induction medium;

h) a cerebral organoid medium containing N2 and B27 without vitamin A; and

i) a cerebral organoid medium containing N2 and B27 with vitamin A;

to generate brain organoids from a second population of stem cells.

18. The method of claim 1, further comprising the step of isolating one or more predetermined populations of cells from the microglia-sufficient brain organoid comprising the steps of:

a) incubating the microglia-sufficient brain organoid in a digestion solution at 37°C for about 30 minutes;

b) physically agitating the microglia-sufficient brain organoid from step a);

c) subjecting the microglia-sufficient brain organoid from step b) to heat treatment with shaking at 1400 rpm for 10 minutes at 37°C;

d) physically agitating the microglia-sufficient brain organoid from step c);

e) incubating the microglia-sufficient brain organoid from step d) at room temperature to allow debris to settle;

f) removing the digestion solution;

g) isolating the one or more predetermined populations of cells from the digestion solution.

19. The method of claim 18, wherein steps c) to f) are repeated at least two more times.

20. The method of claims 18 or 19, wherein the digestion solution is filtered and centrifuged prior to step g).