SUMMARY

[0005]

The present disclosure aims to provide a novel method for producing astrocytes from neural progenitor cells. The present disclosure also aims to provide a method for selectively culturing astrocytes from a cell population comprising neurons and astrocytes, by utilization of the property of astrocytes to adhere to uncoated culture vessels.

[0006]

As a result of intensive study to solve the above problem, the present inventors first discovered that, by utilizing the property of astrocytes to adhere to uncoated culture vessels, differentiation into astrocytes can be highly efficiently induced from neural progenitor cells prepared without carrying out a selection step, thereby completed the present disclosure.

[0007]

The present disclosure provides a method for producing astrocytes, comprising the steps:

(1) culturing neural progenitor cells in a culture medium comprising a neurotrophic factor;

(2) dissociating the cells obtained in the step (1); and

(3) subjecting the cells obtained in the step (2) to adherent culture in a culture medium comprising a neurotrophic factor using an uncoated culture vessel.

There is also provided the method as described above, wherein said neurotrophic factor is a factor selected from the group consisting of GDNF, BDNF and NT-3.

There is also provided as described above, further comprising the steps:

(4) dissociating the cells obtained in the step (3); and

(5) subjecting the cells obtained in the step (4) to adherent culture in a culture medium that does not contain a factor selected from the group consisting of GDNF, BDNF and NT-3 using an uncoated culture vessel.

In one aspect of the present disclosure, there is provided a method for producing astrocytes, comprising the steps:

(1) culturing neural progenitor cells in a culture medium comprising GDNF, BDNF and NT-3 using a coated culture vessel;

(2) dissociating the cells obtained in the step (1);

(3) subjecting the cells obtained in the step (2) to adherent culture in a culture medium comprising GDNF, BDNF and NT-3 using an uncoated culture vessel.

(4) dissociating the cells obtained in the step (3); and

(5) subjecting the cells obtained in the step (4) to adherent culture in a culture medium that does not contain a factor selected from the group consisting of GDNF, BDNF and NT-3 using an uncoated culture vessel.

There is also provided the method as described above, further comprising repeating, at least twice, dissociating the cells obtained in the step (5) and then subjecting the dissociated cells to adherent culture in a culture medium that does not contain a factor selected from the group consisting of GDNF, BDNF and NT-3 using an uncoated culture vessel.

There is also provided the method as described above, further comprising:

(6) dissociating the cells obtained in the step (5); and

(7) subjecting the cells obtained in the step (6) to adherent culture in a culture medium that does not contain a factor selected from the group consisting of GDNF, BDNF and NT-3 using a gelatin-coated culture vessel.

There is also provided the method as described above, wherein the culture is carried out for not less than 66 days in the step (1).

There is also provided the method as described above, wherein the culture is carried out for not less than 30 days in the step (3).

There is also provided the method as described above, wherein the culture is carried out for not less than 20 days in the step (5).

There is also provided the method as described above, wherein said neural progenitor cells are cells produced by culturing pluripotent stem cells in a culture medium comprising a BMP inhibitor and a TGFβ inhibitor.

There is also provided the method as

described above, wherein said BMP inhibitor is Dorsomorphin and said TGFβ inhibitor is SB431542.

There is also provided the method as described above, wherein said step of producing neural progenitor cells from pluripotent stem cells comprises forming an embryoid body/bodies and then subjecting the embryoid body/bodies to adherent culture.

There is also provided the method as described above, wherein said neural progenitor cells are human neural progenitor cells.

There is also provided an astrocyte produced by the method as described above.

There is also provided a method for selectively culturing astrocytes in a cell population comprising neurons and astrocytes, comprising:

(I) dissociating the cell population; and

(II) culturing the cells obtained in the step (I) using an uncoated culture vessel.

There is also provided the method as described above, wherein, in the step (II), the cells obtained in the step (I) are subjected to adherent culture in a culture medium comprising a neurotrophic factor.

There is also provided the method as described above, further comprising:

(III) dissociating the cells obtained in the step (II); and

(IV) subjecting the cells obtained in the step (III) to adherent culture in a

culture medium that does not contain a factor selected from the group consisting of GDNF, BDNF and NT-3 using an uncoated culture vessel.

In one aspect of the present disclosure, there is provided a method for selectively culturing astrocytes in a cell population comprising neurons and astrocytes, comprising:

(I) dissociating the cell population;

(II) subjecting the cells obtained in the step (I) to adherent culture in a culture medium comprising GDNF, BDNF and NT-3 using an uncoated culture vessel;

(III) dissociating the cells obtained in the step (II); and

(IV) subjecting the cells obtained in the step (III) to adherent culture in a culture medium that does not contain a factor selected from the group consisting of GDNF, BDNF and NT-3 using an uncoated culture vessel.

There is also provided the method as described above, further comprising repeating, at least twice, dissociating the cells obtained in the step (IV) and then subjecting the dissociated cells to adherent culture

in a culture medium that does not contain a factor selected from the group consisting of GDNF, BDNF and NT-3 using an uncoated culture vessel.

There is also provided the method as described above, wherein the culture is carried out for not less than 30 days in the step (II).

There is also provided the method as described above, wherein the culture is carried out for not less than 20 days in the step (IV).

There is also provided the method as described above, wherein said cell population comprising neurons and astrocytes are produced from pluripotent stem cells by:

(i) culturing pluripotent stem cells in a culture medium comprising a BMP inhibitor and a TGFβ inhibitor; and

(ii) culturing the cells obtained in the step (i) in a culture medium comprising a neurotrophic factor.

There is also provided the method as described above, wherein the step (i) comprises forming an embryoid body/bodies from pluripotent stem cells in a culture medium comprising a BMP inhibitor and a TGFβ inhibitor, and then subjecting the embryoid body/bodies to adherent culture.

There is also provided the method as described above, wherein said BMP inhibitor is Dorsomorphin and said TGFβ inhibitor is SB431542.

There is also provided the method as described above, wherein said neurotrophic factor is a factor selected from the group consisting of GDNF, BDNF and NT-3.

There is also provided the method as described above, wherein the culture is carried out for not less than 66 days in the step (ii).

There is also provided the method as described above, wherein said cell population comprising neurons and astrocytes is derived from human.

[0008]

By the method described in the present disclosure, astrocytes can be produced from neural progenitor cells. Further, using the obtained astrocytes, therapeutic agents for diseases such as astrocytoma, epilepsy, Alexander disease and neurodegeretative diseases can be developed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009]

Fig. 1 shows a scheme for producing astrocytes from pluripotent stem cells.

Fig. 2 shows the results (photographs) obtained by hierarchical cluster analysis of the gene expression pattern of each type of cells using a microarray.

Fig. 3 shows immunostaining images (photographs) of astrocytes obtained by differentiation induction of each type of human iPS cells. In this figure, the blue areas indicate images of nuclei stained with DAPI, and the red areas indicate stained images produced by a GFAP antibody.

Fig. 4 shows the content of GFAP-positive cells among astrocytes produced by differentiation induction of each type of human iPS cells.

Fig. 5 shows the capacity of fibroblasts (left panel), or astrocytes obtained by differentiation induction of human iPS cells (right panel), to take up glutamate in the medium. The amount of glutamate decreased with time from an initial concentration of 250 μM represents the uptake of glutamate into the cells.

EMBODIMENTS FOR CARRYING OUT THE INVENTION

[0010]

The present invention is described below in detail.

[0011]

CLAIMS

1. A method for producing astrocytes, comprising the steps:

(1) culturing neural progenitor cells in a culture medium comprising GDNF, BDNF and NT-3 using a coated culture vessel;

(2) dissociating the cells obtained in the step (1);

(3) subjecting the cells obtained in the step (2) to adherent culture in a culture medium comprising GDNF, BDNF and NT-3 using an uncoated culture vessel.

(4) dissociating the cells obtained in the step (3); and

(5) subjecting the cells obtained in the step (4) to adherent culture in a culture medium that does not contain a factor selected from the group consisting of GDNF, BDNF and NT-3 using an uncoated culture vessel.

2. The method according to claim 1, further comprising repeating, at least twice, dissociating the cells obtained in the step (5) and then subjecting the dissociated cells to adherent culture in a culture medium that does not contain a factor selected from the group consisting of GDNF, BDNF and NT-3 using an uncoated culture vessel.

3. The method according to claim 1 or 2, further comprising the steps:

(6) dissociating the cells obtained in the step (5); and

(7) subjecting the cells obtained in the step (6) to adherent culture in a culture medium that does not contain a factor selected from the group consisting of GDNF, BDNF and NT-3 using a gelatin-coated culture vessel.

4. The method according to any one of claims 1 to 3, wherein the culture is carried out for not less than 66 days in the step (1).

5. The method according to any one of claims 1 to 4, wherein the culture is carried out for not less than 30 days in the step (3).

6. The method according to any one of claims 1 to 5, wherein the culture is carried out for not less than 20 days in the step (5).

7. The method according to any one of claims 1 to 6, wherein said neural progenitor cells are cells produced by culturing pluripotent stem cells in a culture medium comprising a BMP inhibitor and a TGFβ inhibitor.

8. The method according to claim 7, wherein said BMP inhibitor is Dorsomorphin and said TGFβ inhibitor is SB431542.

9. The method according to claim 7 or 8, wherein said step of producing neural progenitor cells from pluripotent stem cells comprises forming an embryoid body/bodies and then subjecting the embryoid body/bodies to adherent culture.

10. The method according to any one of claims 1 to 9, wherein said neural progenitor cells are human neural progenitor cells.

11. A method for selectively culturing astrocytes in a cell population comprising neurons and astrocytes, comprising:

(I) dissociating the cell population;

(II) subjecting the cells obtained in the step (I) to adherent culture in a culture medium comprising GDNF, BDNF and NT-3 using an uncoated culture vessel;

(III) dissociating the cells obtained in the step (II); and

(IV) subjecting the cells obtained in the step (III) to adherent culture in a culture medium that does not contain a factor selected from the group consisting of GDNF, BDNF and NT-3 using an uncoated culture vessel.

12. The method according to claim 11, further comprising repeating, at least twice, dissociating the cells obtained in the step (IV) and then subjecting the dissociated cells to adherent culture in a culture medium that does not contain a

factor selected from the group consisting of GDNF, BDNF and NT-3 using an uncoated culture vessel.

13. The method according to claim 11 or 12, wherein the culture is carried out for not less than 30 days in the step (II).

14. The method according to any one of claims 11 to 13, wherein the culture is carried out for not less than 20 days in the step (IV).

15. The method according to any one of claims 11 to 14, wherein said cell population comprising neurons and astrocytes are produced from pluripotent stem cells by:

(i) culturing pluripotent stem cells in a culture medium comprising a BMP inhibitor and a TGFβ inhibitor; and

(ii) culturing the cells obtained in the step (i) in a culture medium comprising GDNF, BDNF and NT-3.

16. The method according to claim 15, wherein the step (i) comprises forming an embryoid body/bodies from pluripotent stem cells in a culture medium comprising a BMP inhibitor and a TGFβ inhibitor, and then subjecting the embryoid body/bodies to adherent culture.

17. The method according to claim 15 or 16, wherein said BMP inhibitor is Dorsomorphin and said TGFβ inhibitor is SB431542.

18. The method according to any one of claims 15 to 17, wherein the step (ii) is carried out using an uncoated culture vessel.

19. The method according to any one of claims 15 to 18, wherein the culture is carried out for not less than 66 days in the step (ii).

20. The method according to any one of claims 11 to 19, wherein said cell population comprising neurons and astrocytes is derived from human.