Claims

1. A method for producing neural cells or a neural tissue, comprising the following steps (1) - (3):

(1) a first step of culturing pluripotent stem cells in the absence of feeder cells and in a medium comprising 1) a Sonic hedgehog signal transduction pathway activating substance, and 2) a factor for maintaining undifferentiated state, wherein pluripotent-like state of the pluripotent stem cells is maintained throughout step (1),

(2) a second step of culturing the cells obtained in the first step in suspension to form a cell aggregate, and

(3) a third step of culturing the aggregate obtained in the second step in suspension in the presence of (a) a TGFβ family signal transduction pathway inhibiting substance and/or a Wnt signal transduction pathway inhibiting substance or (b) a BMP signal transduction pathway activating substance, as a differentiation-inducing factor to obtain an aggregate containing neural cells or a neural tissue, wherein the differentiation-inducing factor is added to the medium on or before day 9 after the start of the second step; and,

wherein the Sonic hedgehog signal transduction pathway activating substance is selected from a protein belonging to the Hedgehog family, Shh receptor agonist, Purmorphamine(9-cyclohexyl-N-[4-(4-morpholinyl)phenyl]-2-(1-naphthalenyloxy)-9H-purin-6-amine) and SAG(N-methyl-N'-(3-pyridinylbenzyl)-N'-(3-chlorobenzo[b]thiophene-2-carbonyl)-1,4-diaminocyclohexane); and,

wherein the BMP signal transduction pathway activating substance is selected from BMP2, BMP4, BMP7, GDF7 and anti-BMP receptor antibody; and

wherein the TGFβ family signal transduction pathway inhibiting substance is selected from Lefty, SB431542, A-83-01, LY-364947, SB-505124, Dorsomorphin and LDN193189; and,

wherein the Wnt signal transduction pathway inhibiting substance is selected from CKI-1, D4476, IWP-2 and IWR-1-endo.

2. The production method according to claim 1, wherein, in the second step, the cells obtained in the first step are dispersed, and the dispersed cells are cultured in suspension.

3. The production method according to claim 1 or 2, wherein the factor for maintaining undifferentiated state is one or more proteins selected from the group consisting of bFGF, FGF4, FGF8, EGF, Nodal, Activin A, Activin B, TGFβ1 and TGFβ2.

4. The production method according to claim 3, wherein the factor for maintaining undifferentiated state is bFGF.

5. The production method according to any of claims 1 to 4, wherein, in the second step, the cells are cultured in suspension in a serum-free medium containing a Sonic hedgehog signal transduction pathway activating substance, wherein the Sonic hedgehog signal transduction pathway activating substance is selected from a protein belonging to the Hedgehog family, Shh receptor agonist, Purmorphamine(9-cyclohexyl-N-[4-(4-morpholinyl)phenyl]-2-(1-naphthalenyloxy)-9H-purin-6-amine) and SAG(N-methyl-N'-(3-pyridinylbenzyl)-N'-(3-chlorobenzo[b]thiophene-2-carbonyl)-1,4-diaminocyclohexane).

6. The production method according to claim 1 to 5, wherein the BMP signal transduction pathway activating substance is BMP4.

7. A method for producing retinal cells or a retinal tissue, comprising the following steps (1) - (3):
(1) a first step of culturing pluripotent stem cells in the absence of feeder cells and in a medium comprising 1) a TGFβ family signal transduction pathway inhibiting substance and/or

a Sonic hedgehog signal transduction pathway activating substance, and 2) a factor for maintaining undifferentiated state, wherein pluripotent-like state of pluripotent stem cells is maintained throughout step (1)

(2) a second step of culturing the cells obtained in the first step in suspension to form a cell aggregate, and

(3) a third step of culturing the aggregate obtained in the second step in suspension in the presence of BMP signal transduction pathway activating substance to obtain an aggregate containing retinal cells or a retinal tissue, wherein the BMP signal transduction pathway activating substance is added to the medium between day 1 and day 9 after the start of the second step; and

wherein a TGFβ family signal transduction pathway inhibiting substance is selected from Lefty, SB431542, A-83-01, LY-364947, SB-505124, Dorsomorphin and LDN193189; and

wherein the Sonic hedgehog signal transduction pathway activating substance is selected from a protein belonging to the Hedgehog family, Shh receptor agonist, Purmorphamine(9-cyclohexyl-N-[4-(4-morpholinyl)phenyl]-2-(1-naphthalenyloxy)-9H-purin-6-amine) and SAG(N-methyl-N'-(3-pyridinylbenzyl)-N'-(3-chlorobenzo[b]thiophene-2-carbonyl)-1,4-diaminocyclohexane); and

wherein the BMP signal transduction pathway activating substance is selected from BMP2, BMP4, BMP7, GDF7 and anti-BMP receptor antibody.

8. The production method according to claim 7, wherein, in the second step, the cells obtained in the first step are dispersed, and the dispersed cells are cultured in suspension.

9. The production method according to claim 7 or 8, wherein the factor for maintaining undifferentiated state is one or more proteins selected from the group consisting of bFGF, FGF4, FGF8,

EGF, Nodal, Activin A, Activin B, TGFβ1 and TGFβ2.

10. The production method according to claim 9, wherein the factor for maintaining undifferentiated state is bFGF.

11. The production method according to claim 7, wherein the BMP signal transduction pathway activating substance is BMP4.

12. The production method according to any of claims 7 to 11, wherein the aggregate obtained in the third step comprises one or more cells selected from the group consisting of retinal progenitor cell, neural retinal progenitor cell, photoreceptor precursor cell, photoreceptor cell, rod photoreceptor cell, cone photoreceptor cell, horizontal cell, amacrine cell, interneuron, ganglion cell, retinal pigment epithelial cell, and ciliary marginal zone cell.

13. The production method according to any of claims 7 to 12, wherein the medium in the second step contains a Sonic hedgehog signal transduction pathway activating substance; and wherein the Sonic hedgehog signal transduction pathway activating substance is selected from a protein belonging to the Hedgehog family, Shh receptor agonist, Purmorphamine(9-cyclohexyl-N-[4-(4-morpholinyl)phenyl]-2-(1-naphthalenyloxy)-9H-purin-6-amine) and SAG(N-methyl-N'-(3-pyridinylbenzyl)-N'-(3-chlorobenzo[b]thiophene-2-carbonyl)-1,4-diaminocyclohexane).

14. The production method according to claim 13, wherein the pluripotent stem cells are human pluripotent stem cells, the Sonic hedgehog signal transduction pathway activating substance in the medium in the second step has a concentration corresponding to Sonic hedgehog signal transduction activity of SAG at 10 nM - 700 nM, and the retinal tissue is a human retinal tissue.

15. The production method according to any of claims 7 to 13 , wherein, in the third step, the aggregate is cultured in a medium containing a Sonic hedgehog signal transduction pathway activating substance at a concentration not more than a concentration corresponding to Sonic hedgehog signal transduction activity of SAG at 700 nM.

16. The production method according to any of claims 1 to 5, wherein in the third step the aggregate obtained in the second step in suspension is cultured in the presence of a TGFβ family signal transduction pathway inhibiting substance and/or a Wnt signal transduction pathway inhibiting substance, wherein the TGFβ family signal transduction pathway inhibiting substance is selected from Lefty, SB431542, A-83-01, LY-364947, SB-505124, Dorsomorphin and LDN193189; and,
wherein the Wnt signal transduction pathway inhibiting substance is selected from CKI-1, D4476, IWP-2 and IWR-1-endo.

17. The production method according to any of claims 1 to 6, and 16, wherein the neural cells or the neural tissue is a cerebral cells or a cerebral tissue.

18. The production method according to any of claims 1 to 17, wherein a period for the culturing in the first step is 0.5 hr - 144 hr.

19. The production method according to any of claims 1 to 18, wherein the first step is performed by an adhesion culturing method.

20. The production method according to any of claims 1 to 19, wherein, in the third step, the differentiation-inducing factor is added to the medium between day 3 and day 6 after the start

of the second step.

21. The production method according to any of claims 1 to 20, wherein pluripotent-like state of pluripotent stem cells is the state wherein the cells express Oct3/4.

22. The production method according to any of claims 1 to 13 and 15 to 21, wherein the pluripotent stem cells are primate pluripotent stem cells.

23. The production method according to any of claims 1 to 13 and 15 to 22, wherein the pluripotent stem cells are human pluripotent stem cells.

24. The production method according to any of claims 1 to 23, wherein the pluripotent stem cells are induced pluripotent stem cells.

25. The production method according to any of claims 1 to 24, wherein uniformed aggregates are formed in the second step.

26. The production method according to any of claims 1 to 25, wherein the suspension culturing is performed in the absence of a basement membrane preparation.

27. A method for producing a neural retina comprising:

producing a cell aggregate comprising a retinal tissue by the production method according to any one of claims 7 to 15 and 18 to 25 and

cutting out the retinal tissue from the cell aggregate.