

Claims

~~1. A method for producing a cell mass comprising 1) neural cells or neural tissue and 2) nonneural epithelial tissue, comprising the following steps (1) and (2):
(1) a first step of dispersing pluripotent stem cells into single cells and suspension-culturing the dispersed pluripotent stem cells for about 12 hr to 48 hr to form a cell aggregate in the presence of a non-canonical Wnt signal transduction pathway inhibiting substance,
(2) a second step of suspension-culturing the aggregate obtained in the first step in the presence of a BMP signal transduction pathway activating substance, thereby obtaining a cell mass comprising 1) neural cells or neural tissue and 2) nonneural epithelial tissue.~~

21. A method for producing a cell mass ~~comprising 1) neural cells or neural tissue and 2) nonneural epithelial tissue,~~ the method comprising the following steps (a), (1) and (2):

(a) ~~(a)~~ step a of maintenance-culturing pluripotent stem cells in the absence of feeder cells and in a medium containing 1) a TGF β family signal transduction pathway inhibiting substance selected from the group consisting of Alk5/TGF β R1 inhibitors such as SB431542 , SB505124, SB525334, LY2157299, LY2109761, GW788388, LY364947, SD-208, EW-7197, A 83-01, RepSox, and SMAD3 inhibitors such as SIS3 and/or a Sonic hedgehog signal transduction pathway activating substance selected from the group consisting of proteins belonging to the Hedgehog family (e.g., Shh, Ihh), Shh receptor, Shh receptor agonist, Smo agonist, Purmorphamine, GSA-10, Hh-Ag1.5, 20(S)-Hydroxycholesterol and SAG (Smoothened Agonist; N-Methyl-N' - (3-pyridinylbenzyl)-N' - (3-chlorobenzo[b]thiophene-2-carbonyl)-1,4-

diaminocyclohexane), and 2) a factor for maintaining an undifferentiated state,

(1) a first step of dispersing pluripotent stem cells into single cells and suspension-culturing the dispersed pluripotent stem cells, which were maintenance-cultured in step a, for about 12 hr to 48 hr to form a cell aggregate in the presence of a non-canonical Wnt signal transduction pathway inhibiting substance selected from the group consisting of IWP-2, IWP-3, IWP-4, IWP-L6, IWP-12, LGK-974, ETC-159, GNF-6231 and Wnt-C59,

(2) a second step of suspension-culturing the aggregate obtained in the first step in the presence of a BMP signal transduction pathway activating substance selected from the group consisting of BMP2, BMP4, BMP7 and GDF7, thereby obtaining ~~a the cell mass comprising 1) neural cells or neural tissue and 2) nonneural epithelial tissue,~~

wherein the cell mass comprises 1) neural cells or neural tissue and 2) nonneural epithelial tissue, not less than 30% of the surface of the 1) neural cells or neural tissue is coated with 2) nonneural epithelial tissue, and wherein a space having a distance between the 1) neural cells or neural tissue and the 2) nonneural epithelial tissue on the outer side of not less than 30 μ m is formed in at least a part of the surface region of the 1) neural cells or neural tissue coated with the 2) nonneural epithelial tissue.

2. The production method according to claim 1, wherein the BMP signal transduction pathway activating substance in the second step has a concentration corresponding to BMP signal transduction activity of SAG at 0.1 nM - 1.5 nM.

3. The production method according to claim 1 or 2, wherein the BMP signal transduction pathway activating substance

comprises BMP4 in a concentration of from 0.1 nM to 1.5 nM
~~non-canonical Wnt signal transduction pathway inhibiting substance is a PORCN inhibitor.~~

~~4. The production method according to claim 3, wherein the PORCN inhibitor is at least one kind of compound selected from the group consisting of IWP-2, IWP-3, IWP-4, IWP-L6, IWP-12, LCK-974, ETC-159, CNF-6231 and Wnt-C59.~~

54. The production method according to any one of claims 1 to 43, wherein the culturing in the step (1) and/or the step (2) is performed in the further presence of a TGF β signal transduction pathway inhibiting substance selected from the group consisting of Alk5/TGF β R1 inhibitors such as SB431542, SB505124, SB525334, LY2157299, LY2109761, GW788388, LY364947, SD-208, EW-7197, A 83-01, RepSox, and SMAD3 inhibitors such as SIS3.

~~6. The production method according to any one of claims 1 to 5, wherein in the cell mass comprising 1) neural cells or neural tissue and 2) nonneural epithelial tissue, not less than 30% of the surface of the 1) neural cells or neural tissue is coated with 2) nonneural epithelial tissue, and wherein a space having a distance between the 1) neural cells or neural tissue and the 2) nonneural epithelial tissue on the outer side of not less than 30 μ m is formed in at least a part of the surface region of the 1) neural cells or neural tissue coated with the 2) nonneural epithelial tissue.~~

75. A cell mass comprising 1) neural cells or neural tissue and 2) nonneural epithelial tissue obtained by the production method according to any one of claims 1 to 64, wherein not less than 30% of the surface of the 1) neural cells or neural tissue is coated with 2) nonneural epithelial tissue, and wherein

a space having a distance between the 1) neural cells or neural tissue and the 2) nonneural epithelial tissue on the outer side of not less than 30 μm is formed in at least a part of the surface region of the 1) neural cells or neural tissue coated with the 2) nonneural epithelial tissue.

~~8. A method for producing a nonneural epithelial tissue sheet, comprising the following steps (1) - (4):~~

~~(1) a first step of dispersing pluripotent stem cells into single cells and suspension-culturing the dispersed pluripotent stem cells for about 12 hr to 48 hr to form a cell aggregate in the presence of a non-canonical Wnt signal transduction pathway inhibiting substance,~~
~~(2) a second step of suspension-culturing the aggregate obtained in the first step in the presence of a BMP signal transduction pathway activating substance, thereby obtaining a cell mass comprising 1) neural cells or neural tissue and 2) nonneural epithelial tissue,~~
~~(3) a third step of collecting 2) nonneural epithelial tissue from the cell mass obtained in the second step,~~
~~(4) a fourth step of dispersing the 2) nonneural epithelial tissue obtained in the third step and culturing same on a flat plane, thereby obtaining a nonneural epithelial tissue sheet.~~

96. A method for producing a nonneural epithelial tissue sheet, comprising the following step (a) and the following steps (1) - (4):

(a) step a of maintenance-culturing pluripotent stem cells in the absence of feeder cells and in a medium containing 1) a TGF β family signal transduction pathway inhibiting substance selected from the group consisting of Alk5/TGF β R1 inhibitors such as SB431542, SB505124, SB525334, LY2157299, LY2109761, GW788388, LY364947, SD-208, EW-7197, A 83-01, RepSox, and SMAD3 inhibitors such as SIS3 and/or a Sonic

hedgehog signal transduction pathway activating substance selected from the group consisting of proteins belonging to the Hedgehog family (e.g., Shh, Ihh), Shh receptor, Shh receptor agonist, Smo agonist, Purmorphamine, GSA-10, Hh-Agl.5, 20(S)-Hydroxycholesterol and SAG (Smoothened Agonist; N-Methyl-N'-(3-pyridinylbenzyl)-N'-(3-chlorobenzo[b]thiophene-2-carbonyl)-1,4-diaminocyclohexane),

and 2) a factor for maintaining an undifferentiated state,

(1) a first step of dispersing pluripotent stem cells into single cells and dispersed suspension-culturing the pluripotent stem cells, which were maintenance-cultured in step a, for about 12 hr to 48 hr to form a cell aggregate in the presence of a non-canonical Wnt signal transduction pathway inhibiting substance selected from the group consisting of IWP-2, IWP-3, IWP-4, IWP-L6, IWP-12, LGK-974, ETC-159, GNF-6231 and Wnt-C59,

(2) a second step of suspension-culturing the aggregate obtained in the first step in the presence of a BMP signal transduction pathway activating substance selected from the group consisting of BMP2, BMP4, BMP7 and GDF7, thereby obtaining a cell mass comprising 1) neural cells or neural tissue and 2) nonneural epithelial tissue,

wherein not less than 30% of the surface of the 1) neural cells or neural tissue is coated with 2) nonneural epithelial tissue, and wherein a space having a distance between the 1) neural cells or neural tissue and the 2) nonneural epithelial tissue on the outer side of not less than 30 μm is formed in at least a part of the surface region of the 1) neural cells or neural tissue coated with the 2) nonneural epithelial tissue,

(3) a third step of collecting 2) nonneural epithelial tissue from the cell mass obtained in the second step,

(4) a fourth step of dispersing the 2) nonneural epithelial tissue obtained in the third step and culturing same on a flat plane, thereby obtaining a nonneural epithelial tissue sheet.

~~107~~. The production method according to claim ~~68~~ or ~~9~~, wherein the 2) nonneural epithelial tissue is cornea or a precursor tissue thereof.

8. The production method according to claim 6 or 7, wherein the BMP signal transduction pathway activating substance comprises BMP4 in a concentration of from 0.1 nM to 1.5 nM.

~~11. A nonneural epithelial tissue sheet obtained by the production method according to any one of claims 8 to 10.~~

~~129~~. A cell mass comprising 1) neural cells or neural tissue and 2) nonneural epithelial tissue, wherein not less than 30% of the surface of the 1) neural cells or neural tissue is coated with 2) nonneural epithelial tissue, and wherein a space having a distance between the 1) neural cells or neural tissue and the 2) nonneural epithelial tissue on the outer side of not less than 30 µm is formed in at least a part of the surface region of the 1) neural cells or neural tissue coated with the 2) nonneural epithelial tissue.

~~1310~~. The cell mass according to claim ~~129~~, wherein the 2) nonneural epithelial tissue is cornea or precursor tissue thereof.

~~1411~~. The cell mass according to claim ~~12-9~~ or ~~1310~~, wherein the 1) neural cells or neural tissue are/is central nervous system cells or tissue or precursor tissue thereof.

~~1512~~. The cell mass according to claim ~~1411~~, wherein the central nervous system cell or tissue is retina.

~~1613~~. A method for evaluating toxicity or efficacy of a test substance, comprising a step of bringing the cell mass

comprising 1) neural cells or neural tissue and 2) nonneural epithelial tissue according to any one of claims ~~12~~9 to ~~15~~12, or a cell mass comprising 1) neural cells or neural tissue and 2) nonneural epithelial tissue obtained by the production method according to any one of claims 1 to ~~6~~4 into contact with a test substance, and

a step of detecting an influence of the test substance on the cells or tissue.

~~17~~14. A method for evaluating toxicity or efficacy of a test substance, comprising a step of bringing a nonneural epithelial tissue sheet obtained by the method according to any one of claims ~~8~~6 to ~~10~~8 into contact with a test substance, and

a step of detecting an influence of the test substance on the nonneural epithelial tissue sheet.

~~18~~15. A therapeutic drug for a disease due to a disorder of a sensory organ, comprising a cell mass obtained by the method according to any one of claims 1 to ~~6~~4, or a nonneural epithelial tissue sheet obtained by the method according to any one of claims ~~8~~6 to ~~10~~8.