# CLAIMS

[Claim 1]

A method for producing a cell aggregate including glial progenitor cells and no feeder cell and no xenogeneic-cell-derived component derived from feeder cells, comprising:

(1) a step of subjecting feeder-free pluripotent stem cells to suspension culture in an embryoid-body-forming culture medium containing one or more SMAD signaling inhibitors and one or more Wnt signaling activators in the absence of feeder cells for 5 days to 10 days, to form a cell aggregate;

(2) a step of subjecting the cell aggregate obtained in (1) to suspension culture in an embryoid-body-forming culture medium containing retinoic acid but no SHH signaling activator;

(3) a step of subjecting the cell aggregate obtained in (2) to suspension culture in an embryoid-body-forming culture medium or neuron-and-glia-proliferating culture medium containing retinoic acid and one or more SHH signaling activators; and

(4) a step of subjecting the cell aggregate obtained in (3) to suspension culture in a neuron-and-glia-proliferating culture medium containing no retinoic acid but containing one or more SHH signaling activators,

and optionally further comprising:

(5) a step of subjecting the cell aggregate obtained in (4) to suspension culture in a neuron-and-glia-proliferating culture medium containing neither retinoic acid nor an SHH signaling activator.

[Claim 2]

The production method according to claim 1, wherein, in step (1), pluripotent stem cells are cultured by using a culture vessel having a plurality of pores uniform in shape.

[Claim 3]

The production method according to claim 1 or 2, wherein, in step (1), step (1) is sustained until a cell aggregate satisfying at least one of the following conditions:

1) having an RNA expression level of at least one of SOX1, PAX6, HES4, and HES5 increased by 100 times or more;

2) having an RNA expression level of OCT3/4 decreased by 200 times or more; and

3) having an RNA expression level of NANOG decreased by 400 times or more,

as compared with the cell aggregate at initiation of step (1), is obtained, and step (2) is then initiated.

[Claim 4]

The production method according to any one of claims 1 to 3, wherein, in step (2), step (2) is sustained until a cell aggregate satisfying at least one of the following conditions:

1) having an RNA expression level of at least one of ASCL1, DCX, HEY1, ZBTB20, βIII tubulin, ELAVL3, and SLIT1 increased by 5 times or more; and
2) having an RNA expression level of at least one of HOXB3, HOXA4, HOXB4, HOXB6, and HOXB8 increased by 5 times or more,
as compared with the cell aggregate at initiation of step (2), is obtained, and step (3) is then initiated.

[Claim 5]

The production method according to any one of claims 1 to 4, wherein step (2) is performed for 4 days to 11 days.

[Claim 6]

The production method according to any one of claims 1 to 5, wherein an oxygen concentration is 3% to 10% in steps (1) and (2).

[Claim 7]

The production method according to any one of claims 1 to 6, wherein, in step (3), step (3) is sustained until a cell aggregate satisfying at least one of the following conditions:
1) having an RNA expression level of at least one of HEY2, NKX6.2, and NKX2.2 increased by 5 times or more; and
2) having an RNA expression level of OLIG1 and/or OLIG2 increased by 10 times or more,
as compared with the cell aggregate at initiation of step (3), is obtained, and step (4) is then initiated.

[Claim 8]

The production method according to any one of claims 1 to 7, wherein step (3) is performed for 4 days to 11 days.

[Claim 9]

The production method according to any one of claims 1 to 8, wherein, in step (4), step (4) is sustained until a cell aggregate satisfying at least one of the following conditions:
1) having an RNA expression level of at least one of NFIA, NFIB, SLC1A3, S100B, and FABP7 increased by 10 times or more; and
2) having an RNA expression level of PAX6 decreased by 5 times or more,
as compared with the cell aggregate at initiation of step (4), is obtained, and step (5) is then initiated.

[Claim 10]

The production method according to any one of claims 1 to 9, wherein step (4) is performed for 4 days or more.

[Claim 11]

The production method according to any one of claims 1 to 10, wherein, in step (4), the cell aggregate obtained in step (3) is dispersed at initiation of step (4), and the dispersed cells are then subjected to suspension culture, to form a cell aggregate again.

[Claim 12]

The production method according to any one of claims 1 to 11, comprising step (5), wherein, in step (5), the cell aggregate obtained in step (4) is dispersed at initiation of step (5), and the dispersed cells are then subjected to suspension culture for 5 days to 100 days, to form a cell aggregate again.

[Claim 13]

The production method according to any one of claims 1 to 12, comprising step (5), wherein, in step (5), step (5) is sustained until one or more markers selected from an O4 antigen, NG2, OLIG2, PDGFRα, SOX10, SPON1, FAM181B, TIMP4, SOX6, GRIK3, LHFPL3, KLF9, an A2B5 antigen, CNP, and PLP are expressed.

[Claim 14]

The production method according to any one of claims 1 to 12, comprising step (5), wherein, in step (5), step (5) is sustained until one or more proteins selected from the group consisting of SPARCL1, MIF, MCP-1, IL-8, SCF, M-CSF, HGF, GRO-α, LIF, IFN-γ, and TRAIL are detected in the medium for culture of the cell aggregate.

[Claim 15]

The production method according to any one of claims 1 to 14, wherein the SMAD signaling inhibitors are a TGFβ inhibitor and a BMP inhibitor.

[Claim 16]

The production method according to claim 15, wherein the TGFβ inhibitor is one or more selected from the group consisting of SB431542, A83-01, SB202190, SB505124, NPC30345, SD093, SD908, SD208, LY2109761, LY364947, LY580276, Galunisertib (LY2157299), LY3200882, SB525334, GW788388, RepSox, and Lefty-1.

[Claim 17]

The production method according to claim 15 or 16, wherein the BMP inhibitor is one or more selected from the group consisting of Noggin, LDN-193189, LDN-212854, Dorsomorphin, K02288, Chordin, and Follistatin.

[Claim 18]

The production method according to any one of claims 1 to 17, wherein the Wnt signaling activators are one or more selected from the group consisting of a GSK3β inhibitor, Wnt3a, a Wnt agonist, Dkk, and R-Spondin.

[Claim 19]

The production method according to any one of claims 1 to 17, wherein the Wnt signaling activators are one or more selected from the group consisting of CHIR99021, BIO, Kenpaullone, SB216763, and L803-mts.

[Claim 20]

The production method according to any one of claims 1 to 19, wherein the SHH signaling activators are one or more selected from the group consisting of Purmorphamine, SAG, SHH protein, and an SHH fragment.

[Claim 21]

The production method according to any one of claims 1 to 20, wherein the pluripotent stem cells are induced pluripotent stem cells.

[Claim 22]

The production method according to any one of claims 1 to 20, wherein the pluripotent stem cells are human induced pluripotent stem cells.

[Claim 23]

The production method according to any one of claims 1 to 22, wherein the cell aggregate including glial progenitor cells has the following characteristics:

(a) including oligodendrocyte progenitor cells, astrocyte progenitor cells, and neural progenitor cells;

(b) expressing a spinal region marker, wherein the spinal region marker is selected from the group consisting of HOXB3, HOXB4, HOXB6, and HOXD8; and

(c) including no feeder cell and containing no xenogeneic-cell-derived component derived from feeder cells.

[Claim 24]

A method for producing a cell population including oligodendrocytes, astrocytes, and neural cells, comprising a step of culturing a cell aggregate including glial progenitor cells with use of a maturation culture medium for 5 days to 60 days, wherein the cell aggregate is produced by the production method according to any one of claims 1 to 23.

[Claim 25]

The production method according to claim 24, wherein the cell population including oligodendrocytes, astrocytes, and neural cells includes:

(i) cells expressing one or more markers selected from the group consisting of an O4 antigen, GalC, MBP, APC, GSTπ, CNP, PLP, OLIG2, SOX10, PDGFRα, and NG2;

(ii) cells expressing one or more markers selected from the group consisting of βIII tubulin, MAP2, and ELAVL3; and

(iii) cells expressing one or more markers selected from the group consisting of SLC1A3, S100B, AQP4, GFAP, and NG2.

[Claim 26]

The production method according to claim 24 or 25, wherein the maturation culture medium is a culture medium containing at least one of T3, NT-3, and LIF.

[Claim 27]

The production method according to claim 26, wherein the maturation culture medium further contains CNTF.

[Claim 28]

A cell aggregate comprising glial progenitor cells and no feeder cell and no xenogeneic-cell-derived component derived from feeder cells, obtained by the production method according to any one of claims 1 to 23.

[Claim 29]

A cell population comprising oligodendrocytes, astrocytes, and neural cells,

obtained by the production method according to any one of claims 24 to 27.

[Claim 30]

The cell aggregate according to claim 28, for use in treating a demyelinating disease or disease based on or involving glial cell dysfunction.

[Claim 31]

The cell aggregate or cell population according to claim 30, wherein the demyelinating disease or disease based on or involving glial cell dysfunction is acute-phase, subacute-phase, or chronic-phase spinal cord injury.

[Claim 32]

A method for evaluating a toxicity or drug efficacy of a test substance, comprising contacting the test substance with the cell aggregate according to claim 28 and detecting or quantifying an influence of the test substance on the cell aggregate or the cell population.