CLAIMS

1. A culture method for causing differentiation of pluripotent mammalian cells, comprising culturing pluripotent mammalian cells to obtain a cell population at least partially differentiated into endoderm lineage cells, by using a cell culture chamber including a plurality of microchambers formed on a culture surface, the cell culture chamber having the culture surface formed of spaces in which a space structure of each of the microchambers has a height of 10 μm to 500 μm and a bottom area of 100 μm^2 to 1 mm^2.

2. The culture method for causing differentiation of pluripotent mammalian cells according to Claim 1, wherein the pluripotent mammalian cells are selected from the group consisting of embryonic stem cells (ES cells), induced pluripotent stem cells (iPS cells), teratocarcinoma cells, and sperm stem cells.

3. The culture method for causing differentiation of pluripotent mammalian cells according to Claim 1 or 2, wherein 1 to 3×10^5 pluripotent mammalian cells are seeded in one of the microchambers to obtain the cell population.

4. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 3, wherein the cell population is obtained by culturing the pluripotent mammalian cells in a culture medium including one kind or a mixture of two or more kinds selected from the group consisting of a TGF-β family, an FGF family, and a PI3-kinase signaling pathway inhibitor.

5. The culture method for causing differentiation of pluripotent mammalian cells according to Claim 4, wherein a member of the TGF-β family is one kind or a mixture of two or more kinds selected from the group consisting of Nodal, Activin A, Activin B, TGF-β, BMP2, and BMP4.

6. The culture method for causing differentiation of pluripotent mammalian cells according to Claim 4 or 5, wherein a member of the FGF family is one kind or a mixture of two or more kinds selected from the group consisting of b-FGF, FGF-4, and FGF-2.

7. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 4 to 6, wherein the PI3-kinase signaling pathway inhibitor is one kind or a mixture of two or more kinds selected from the group consisting of LY294002, rapamycin, wortmannin, lithium chloride, Akt inhibitor I, Akt inhibitor II, Akt inhibitor III, and NL-71-101.

8. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 7, wherein a cell population in which SOX17 is at least partially expressed and AFP is not expressed is obtained.

9. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 7, wherein a cell population in which SOX17 is at least partially expressed and Pdx-1 is not expressed is obtained.

10. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 7, wherein a cell population in which one of FoxA1 and FoxA2 is at least partially expressed and AFP is not expressed is obtained.

11. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 7, wherein a cell population in which one of FoxA1 and FoxA2 is at least partially expressed and Pdx-1 is not expressed is obtained.

12. A culture method for causing differentiation of pluripotent

mammalian cells, comprising culturing a cell population differentiated into endoderm lineage cells according to any one of Claims 1 to 11 in a culture medium including one kind or two or more kinds selected from the group consisting of FGF, BMP2, HGF, KGF, EGF, TGF-α, HB-EGF, VEGF, PDGF, DMSO, dexamethasone, oncostatin M, and insulin to obtain a second cell population partially including cells expressing one of albumin (ALB) and α-fetoprotein (AFP).

13. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 12, wherein the cell population is cultured in an atmosphere having an oxygen concentration of 4% or less.

14. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 13, wherein the cell culture chamber is a resin molding formed of one or a combination of two or more selected from the group consisting of acrylic resin, polylactic acid, polyglycolic acid, styrene resin, acrylic styrene copolymer resin, polycarbonate resin, polyester resin, polyvinyl alcohol resin, ethylene vinyl alcohol copolymer resin, thermoplastic elastomer, vinyl chloride resin, and silicon resin.

15. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 14, wherein in a portion corresponding to 50% or more of an upper portion of each side wall formed in a height direction of the space structure of the microchambers, an angle formed between the bottom and a side surface of each side wall is 80° to 90°.

16. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 15, wherein the bottom of each of the microchambers has a major axis that is in a range of 1 to 1.5 times greater than a minor axis of the bottom.

17. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 16, wherein a surface treatment is performed on a region in which the microchambers are formed.

18. The culture method for causing differentiation of pluripotent mammalian cells according to Claim 17, wherein the surface treatment includes coating with an inorganic substance.

19. The culture method for causing differentiation of pluripotent mammalian cells according to Claim 17 or 18, wherein the surface treatment includes coating with an extracellular matrix such as collagen or laminin.

20. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 17 to 19, wherein the surface treatment includes coating with a synthetic material.

21. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 17 to 20, wherein the surface treatment includes providing concave-convex on a bottom surface of each of the microchambers, the concave-convex having a diameter in a range of 1nm corresponding to a cell focal adhesion to 20μm corresponding to a cell.

CLAIMS

1. A culture method for causing differentiation of pluripotent mammalian cells, comprising using a cell culture chamber including a plurality of microchambers formed on a culture surface, the cell culture chamber having the culture surface formed of spaces in which a space structure of each of the microchambers has a height of 10 μm to 500 μm and a bottom area of 100 μm^2 to 0.1 mm^2, wherein

seeding pluripotent mammalian cells in the microchambers,

culturing the pluripotent mammalian cells and forming an aggregate of an embryoid body, and

obtaining a cell population at least partially differentiated into endoderm lineage cells from the aggregate,

the cell population being obtained by culturing the pluripotent mammalian cells in a culture medium including one kind or a mixture of two or more kinds selected from the group consisting of a TGF-β family, an FGF family, and a PI3-kinase signaling pathway inhibitor.

2. The culture method for causing differentiation of pluripotent mammalian cells according to Claim 1, wherein the pluripotent mammalian cells are selected from the group consisting of embryonic stem cells (ES cells), induced pluripotent stem cells (iPS cells), teratocarcinoma cells, and sperm stem cells.

3. The culture method for causing differentiation of pluripotent mammalian cells according to Claim 1 or 2, wherein 1 to 3×10^5 pluripotent mammalian cells are seeded in one of the microchambers to obtain the cell population.

4. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 3, wherein the cell population is obtained by culturing the pluripotent mammalian cells in a culture medium including at least one kind or a mixture of two or more kinds selected from the group consisting of a TGF-β family, and an FGF family.

5. The culture method for causing differentiation of pluripotent mammalian cells

according to any one of Claims 1 to 4, wherein a member of the TGF-β family is one kind or a mixture of two or more kinds selected from the group consisting of Nodal, Activin A, Activin B, TGF-β, BMP2, and BMP4.

6. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 5, wherein a member of the FGF family is one kind or a mixture of two or more kinds selected from the group consisting of b-FGF, FGF-4, and FGF-2.

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12. A culture method for causing differentiation of pluripotent mammalian cells, comprising culturing a cell population differentiated into endoderm lineage cells

according to any one of Claims 1 to 11 in a culture medium including one kind or two or more kinds selected from the group consisting of FGF, BMP2, HGF, KGF, EGF, TGF-α, HB-EGF, VEGF, PDGF, DMSO, dexamethasone, oncostatin M, and insulin, to obtain a second cell population partially including cells expressing one of albumin (ALB) and α-fetoprotein (AFP).

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15. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 14, wherein in a portion corresponding to 50% or more of an upper portion of each side wall formed in a height direction of the space structure of the microchambers, an angle formed between the bottom and a side surface of each side wall is 80° to 90°.

16. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 15, wherein the bottom of each of the microchambers has a major axis that is in a range of 1 to 1.5 times greater than a minor axis of the bottom.

17. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 16, wherein a surface treatment is performed on a region in which the microchambers are formed.

18. The culture method for causing differentiation of pluripotent mammalian cells according to Claim 17, wherein the surface treatment includes coating with an inorganic substance.

19. The culture method for causing differentiation of pluripotent mammalian cells according to Claim 17 or 18, wherein the surface treatment includes coating with an extracellular matrix such as collagen or laminin.

20. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 17 to 19, wherein the surface treatment includes coating with a synthetic material.

21. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 17 to 20, wherein the surface treatment includes providing concave and convex on a bottom surface of each of the microchambers, the concave and convex having a diameter in a range of 1 nm corresponding to a cell focal adhesion to 20 μm corresponding to a cell.

22. The culture method for causing differentiation of pluripotent mammalian cells according to any one of Claims 1 to 21, wherein

each of the microchambers comprises an opening plane of a square,

a length of one side of the square is 100 μm to 300 μm, and

a cell seeding density per an area of the culture surface is 5.0×10^3 to 5.0×10^5 cells/cm^2.