

Claims

1. A method for inducing stem cells, comprising inducing stem cells from somatic cells floating-cultured in a gel medium.
2. The method for inducing stem cells according to claim 1, wherein the gel medium is not stirred.
3. The method for inducing stem cells according to claim 1 or 2, wherein the gel medium is gelled with deacetylated gellan gum.
4. The method for inducing stem cells according to any one of claims 1 to 3, wherein the gel medium is free from a growth factor.
5. The method for inducing stem cells according to any one of claims 1 to 3, wherein the gel medium contains a growth factor at a concentration of 40% by weight or lower.
6. The method for inducing stem cells according to any one of claims 1 to 3, wherein the gel medium is free from bFGF.
7. The method for inducing stem cells according to any one of claims 1 to 6, wherein the gel medium comprises a human ES/iPS culture medium.
8. A floating culture method for stem cells, comprising floating-culturing stem cells in a gel medium without a growth factor.
9. A floating culture method for stem cells, comprising floating-culturing stem cells in a gel medium with a growth factor at a concentration of 40% by weight or lower.
10. A floating culture method for stem cells, comprising floating-culturing stem cells in a gel medium without bFGF.
11. A floating culture method for stem cells, comprising floating-culturing stem cells in a gel medium with bFGF at a concentration of 40 µg/L or lower.
12. The floating culture method for stem cells according to any one of claims 8 to 11, wherein the gel medium is not stirred.
13. The floating culture method for stem cells according to any one of claims 8 to 12, wherein the gel medium is gelled with deacetylated gellan gum.
14. The floating culture method for stem cells according to any one of claims 8 to 13, wherein the gel medium contains a ROCK inhibitor.
15. The floating culture method for stem cells according to

- any one of claims 8 to 12, wherein the concentration of the stem cells in the gel medium is 0.1×10^5 cells/mL or higher.
16. The floating culture method for stem cells according to any one of claims 8 to 13, further comprising, before the floating culture, dissociating the stem cells into single cells, and placing the stem cells dissociated into single cells in the gel medium.
 17. The floating culture method for stem cells according to claim 16, wherein in the floating culture, the single cells form colonies while maintaining their clonality.
 18. The floating culture method for stem cells according to any one of claims 11 to 15, further comprising, before the floating culture, hanging drop-culturing the stem cells using a grating plate to form colonies, and placing the formed colonies in the gel medium.
 19. The floating culture method for stem cells according to any one of claims 8 to 18, wherein the stem cells proliferate while maintaining their undifferentiated states.
 20. The floating culture method for stem cells according to any one of claims 8 to 19, wherein the gel medium comprises a human ES/iPS culture medium.
 21. A floating culture vessel for stem cells comprising:
 - a dialysis membrane which accommodates stem cells and a medium; and
 - a container which accommodates the dialysis membrane, wherein a medium is placed in the container.
 22. The floating culture vessel for stem cells according to claim 21, wherein a molecular weight cutoff of the dialysis membrane is 0.1 kDa or larger.
 23. The floating culture vessel for stem cells according to claim 21 or 22, wherein the dialysis membrane is made of at least one member selected from cellulose ester, cellulose ester derivatives, regenerated cellulose, and cellulose acetate.
 24. A floating culture method for stem cells comprising:
 - placing stem cells and a gel medium in a dialysis membrane;
 - placing the dialysis membrane in a container;
 - placing a gel medium in the container; and
 - floating-culturing the stem cells in the gel medium in the dialysis membrane.
 25. The floating culture method for stem cells according to claim 24, wherein a molecular weight cutoff of the

- dialysis membrane is 0.1 kDa or larger.
26. The floating culture method for stem cells according to claim 24 or 25, wherein the dialysis membrane is made of at least one member selected from cellulose ester, cellulose ester derivatives, regenerated cellulose, and cellulose acetate.
 27. The floating culture method for stem cells according to any one of claims 24 to 26, wherein the gel medium is supplemented with a ROCK inhibitor.
 28. The floating culture method for stem cells according to any one of claims 24 to 27, wherein the gel medium is not stirred.
 29. The floating culture method for stem cells according to any one of claims 24 to 28, wherein the gel medium is a medium gelled with deacetylated gellan gum.
 30. The floating culture method for stem cells according to any one of claims 24 to 29, wherein the gel medium is free from a growth factor.
 31. The floating culture method for stem cells according to any one of claims 24 to 29, wherein the gel medium contains a growth factor at a concentration of 40% by weight or lower.
 32. The floating culture method for stem cells according to any one of claims 24 to 29, wherein the gel medium is free from bFGF.
 33. The floating culture method for stem cells according to any one of claims 24 to 32, wherein the concentration of the stem cells in the gel medium is 0.1×10^5 cells/mL or higher.
 34. The floating culture method for stem cells according to any one of claims 24 to 33, further comprising, before the floating culture, dissociating the stem cells into single cells, and placing the stem cells dissociated into single cells in the gel medium.
 35. The floating culture method for stem cells according to claim 34, wherein in the floating culture, the single cells form colonies while maintaining their clonality.
 36. The floating culture method for stem cells according to any one of claims 24 to 35, further comprising, before the floating culture, hanging drop-culturing the stem cells using a grating plate to form colonies, and placing the formed colonies in the gel medium.
 37. The floating culture method for stem cells according to any one of claims 24 to 36, wherein the stem cells proliferate while maintaining their undifferentiated

states.

38. The floating culture method for stem cells according to any one of claims 24 to 37, further comprising replacing the gel medium in the container with a fresh gel medium.
39. The floating culture method for stem cells according to any one of claims 24 to 38, further comprising supplementing the gel medium in the container with a fresh gel medium.
40. The floating culture method for stem cells according to any one of claims 24 to 39, wherein the gel medium in the dialysis membrane is not replaced.
41. The floating culture method for stem cells according to any one of claims 24 to 40, wherein the gel medium comprises a human ES/iPS culture medium.
42. A method for inducing stem cells by floating, comprising:
placing somatic cells and a gel medium in a dialysis membrane;
placing the dialysis membrane in a container;
placing a gel medium in the container; and
inducing stem cells from the somatic cells floating in the gel medium in the dialysis membrane.
43. The method for inducing stem cells by floating according to claim 42, wherein a molecular weight cutoff of the dialysis membrane is 0.1 kDa or larger.
44. The method for inducing stem cells by floating according to claim 42 or 43, wherein the dialysis membrane is made of at least one member selected from cellulose ester, cellulose ester derivatives, regenerated cellulose, and cellulose acetate.
45. The method for inducing stem cells by floating according to any one of claims 42 to 44, wherein the gel medium is not stirred.
46. The method for inducing stem cells by floating according to any one of claims 42 to 45, wherein the gel medium is gelled with deacetylated gellan gum.
47. The method for inducing stem cells by floating according to any one of claims 42 to 46, wherein the gel medium is free from a growth factor.
48. The method for inducing stem cells by floating according to any one of claims 42 to 47, wherein the gel medium is free from bFGF.
49. The method for inducing stem cells by floating according to any one of claims 42 to 48, further comprising, before the floating culture,
dissociating the somatic cells into single cells, and
placing the somatic cells dissociated into single cells in the gel medium.
50. The method for inducing stem cells by floating according

- to claim 49, wherein in the floating culture, the single cells form colonies while maintaining their clonality.
51. The method for inducing stem cells by floating according to any one of claims 42 to 50, further comprising replacing the gel medium in the container with a fresh gel medium.
52. The method for inducing stem cells by floating according to any one of claims 42 to 51, further comprising supplementing the gel medium in the container with a fresh gel medium.
53. The method for inducing stem cells by floating according to any one of claims 42 to 52, wherein the gel medium in the dialysis membrane ~~tube~~ is not replaced.
54. The method for inducing stem cells by floating according to any one of claims 42 to 53, wherein the gel medium comprises a human ES/iPS culture medium.
55. A method for producing induced pluripotent stem cells, comprising:
preparing somatic cells; and
transferring reprogramming factor RNAs into the somatic cells by a lipofection method.
56. The method for producing induced pluripotent stem cells according to claim 55, wherein the somatic cells are blood cells.
57. The method for producing induced pluripotent stem cells according to claim 56, wherein the blood cells are monocytes.
58. The method for producing induced pluripotent stem cells according to claim 56, wherein the blood cells are hematopoietic stem/progenitor cells.
59. The method for producing induced pluripotent stem cells according to any one of claims 56 to 58, wherein the blood cells are CD34-positive.
60. The method for producing induced pluripotent stem cells according to any one of claims 56 to 59, wherein the blood cells are blood cells separated on condition that the cells are CD34-positive.
61. The method for producing induced pluripotent stem cells according to claim 56 or 57, wherein the blood cells are CD3- positive.
62. The method for producing induced pluripotent stem cells according to any one of claims 56, 57, and 61, wherein the blood cells are blood cells separated on condition that the cells are CD3-positive.
63. The method for producing induced pluripotent stem cells according to any one of claims 55 to 62, wherein the reprogramming factor RNAs comprise *Oct3/4* mRNA, *Sox2* mRNA,

Klf4 mRNA, and *c-Myc* mRNA.

64. The method for producing induced pluripotent stem cells according to claim 63, wherein the reprogramming factor RNAs further comprise at least one member selected from the group consisting of *GLIS1* mRNA, *FOXH1* mRNA, *L-MYC* mRNA, and *p53-dn* mRNA.
65. The method for producing induced pluripotent stem cells according to claim 63 or 64, wherein the reprogramming factor RNAs further comprise *LIN28A* mRNA or *LIN28B* mRNA.
66. The method for producing induced pluripotent stem cells according to any one of claims 55 to 65, wherein an siRNA lipofection reagent or an mRNA lipofection reagent is used in the lipofection with the reprogramming factor RNAs.
67. The method for producing induced pluripotent stem cells according to any one of claims 55 to 66, wherein at least one member selected from Lipofectamine(R) RNAiMAX transfection reagent, Lipofectamine(R) MessengerMAX transfection reagent, Stemfect(R) RNA transfection reagent, and ReproRNA(R) transfection reagent is used in the lipofection with the reprogramming factor RNAs.
68. The method for producing induced pluripotent stem cells according to any one of claims 56 to 62, wherein the number of the blood cells for the lipofection with the reprogramming factor RNAs is 1 to 1×10^8 .
69. The method for producing induced pluripotent stem cells according to any one of claims 56 to 62 and 68, wherein the amounts of the reprogramming factor RNAs for the lipofection with the reprogramming factor RNAs are 5 ng to 50 μ g per run.
70. The method for producing induced pluripotent stem cells according to any one of claims 56 to 62, 68, and 69, wherein the amount of the lipofection reagent for the lipofection with the reprogramming factor RNAs is 0.1 μ L to 500 μ L per run.
71. The method for producing induced pluripotent stem cells according to any one of claims 56 to 62 and 68 to 70, wherein the lipofection with the reprogramming factor RNAs is performed for 0.1 hours or longer and 24 hours or shorter per run.

- 72.The method for producing induced pluripotent stem cells according to any one of claims 56 to 62 and 68 to 71, wherein the lipofection with the reprogramming factor RNAs is performed a plurality of times.
- 73.The method for producing induced pluripotent stem cells according to any one of claims 56 to 72, wherein the medium used for the lipofection with the reprogramming factor RNAs is Opti-MEM(R).
- 74.The method according to claim 57, further comprising separating the monocytes from blood using a filter.
- 75.A method for producing particular somatic cells from animal cells, comprising:
preparing animal cells; and
transferring an inducer RNA into the animal cells by lipofection to differentiate the animal cells into somatic cells.
- 76.The method for producing particular somatic cells from animal cells according to claim 75, wherein the animal cells are stem cells.
- 77.The method for producing particular somatic cells from animal cells according to claim 76, wherein the stem cells are induced pluripotent stem cells.
- 78.The method for producing particular somatic cells from animal cells according to claim 76 or 77, wherein the stem cells are iPS cells.
- 79.The method for producing particular somatic cells from animal cells according to claim 77, wherein the stem cells are embryonic stem cells.
- 80.The method for producing particular somatic cells from animal cells according to claim 75, wherein the animal cells are human fibroblasts.
- 81.The method for producing particular somatic cells from animal cells according to claim 75, wherein the animal cells are blood cells.
- 82.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 81, wherein the inducer RNA comprises an mRNA corresponding to a drug resistance gene.
- 83.The method for producing particular somatic cells from animal cells according to claim 82, further comprising selecting cells that exhibit the drug resistance after the lipofection.
- 84.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 83, wherein the inducer RNA comprises an mRNA corresponding to a puromycin resistance gene.
- 85.The method for producing particular somatic cells from animal cells according to claim 84, further comprising

- selecting cells that exhibit puromycin resistance after the lipofection.
- 86.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 85, wherein the somatic cells are neuronal cells.
- 87.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 86, wherein the inducer RNA comprises *Ngn2* mRNA.
- 88.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 87, wherein the induced neuronal cells are *Ngn2*-positive.
- 89.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 88, wherein the induced neuronal cells are β -III Tubulin-, MAP2-, PSA-NCAM-, or vGlut-positive.
- 90.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 89, wherein MessengerMAX(R) is used in the lipofection with the inducer RNA.
- 91.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 90, wherein the number of the cells for the lipofection with the inducer RNA is 1×10^4 to 1×10^8 .
- 92.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 91, wherein the amount of the inducer RNA for the lipofection with the inducer RNA is 200 ng to 5000 ng per run.
- 93.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 92, wherein the amount of the lipofection reagent for the lipofection with the inducer RNA is 0.1 μ L to 100 μ L per run.
- 94.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 93, wherein the medium used for the lipofection with the inducer RNA is Opti-MEM(R).
- 95.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 94, wherein the animal cells are differentiated into the somatic cells within ten days from the lipofection with the inducer RNA.
- 96.The method for producing particular somatic cells from animal cells according to any one of claims 75 to 95,

- wherein the transfer of the inducer RNA into the animal cells by lipofection is repeated a plurality of times.
97. The method for producing particular somatic cells from animal cells according to any one of claims 75 to 96, wherein the animal cells are cultured on a substrate coated with basement membrane matrix.
98. The method for producing particular somatic cells from animal cells according to any one of claims 75 to 97, wherein the animal cells are cultured in a medium with B18R.
99. The method for producing particular somatic cells from animal cells according to any one of claims 75 to 97, wherein the animal cells are cultured in a medium without B18R.