

CLAIMS

What is claimed is:

1. A method of generating an induced pluripotent stem cell, wherein the method comprises expressing exogenous nucleic acids encoding the proteins OCT3/4, SOX2, KLF4, LIN28 and L-MYC and ~~at~~the p53-shRNA in a stem cell of the amniotic membrane of the umbilical cord under conditions suitable to reprogram the stem cell, wherein the stem cell of the amniotic membrane of the umbilical cord is subjected to transfection to transfer the exogenous nucleic acids into the stem cell, wherein the transfected stem cell is cultivated in a medium suitable for cell recovery, wherein the medium suitable for cell recovery contains a compound suppressing inflammatory response and enhancing cell survival, thereby generating the induced pluripotent stem cell.
2. The method of claim 1, wherein the stem cell of the amniotic membrane of the umbilical cord is a mesenchymal stem cell of the amniotic membrane of the umbilical cord or an epithelial stem cell of the amniotic membrane of the umbilical cord.
3. The method of claim 1 or 2, wherein the mesenchymal stem of the amniotic membrane of the umbilical cord is a mesenchymal stem cell population, wherein at least 90 % or more cells of the stem cell population express each of the following markers: CD73, CD90 and CD105.
4. The method of claim 3, wherein at least 90 % or more cells of the mesenchymal stem cell population lack expression of the following markers: CD34, CD45 and HLA-DR.
5. The method of any one of claims 3 or 4, wherein at least 91 % or more, 92 % or more, 93 % or more, 94 % or more, 95 % or more, 96 % or more, 97 % or more, 98 % or more 99 % or more cells of the mesenchymal stem cell population express each of CD73, CD90 and CD105 and lack expression of each of CD34, CD45 and HLA-DR.
6. The method of claim 1, wherein the exogenous nucleic acids encoding the proteins OCT3/4, SOX2, KLF4, LIN28 and L-MYC and the p53-shRNA are provided by one,

two or three vectors, wherein preferably a first vector encodes the protein OCT3/4 and the p53-shRNA, a second vector encodes the proteins SOX2 and KLF4 and a third vector encodes the proteins L-MYC and LIN28.

7. The method of any of claims 1 to 6, wherein the stem cell of the amniotic membrane of the umbilical cord is subjected to electroporation to transfer the exogenous nucleic acids into the stem cell.
8. The method of claim 9, wherein the mesenchymal stem cell of the amniotic membrane of the umbilical cord is subjected to electroporation with 1 pulse having a duration time of 15-25ms and a voltage of 1550-1650V, preferably to electroporation with 1 pulse having a duration time of 20ms and a voltage of 1600V.
9. The method of claim 8, wherein the ratio of the amount of vector plasmid DNA for each vector to the number of mesenchymal stem cells of the amniotic membrane of the umbilical cord (CLMC) subjected to electroporation is in the range of 1.5µg plasmid DNA to 1x10⁶ CLMC to of 2.5 µg plasmid DNA to 1x10⁶ CLMC, wherein the ratio is, for example, about 2.5 µg plasmid DNA : 1x10⁶ cells, about 2.25 µg plasmid DNA : 1x10⁶ cells, about 1.8 µg plasmid DNA: 1x10⁶ cells, about 1.7 µg plasmid DNA : 1x10⁶ cells, about 1.6 µg plasmid DNA : 1x10⁶ cells, about 1.5 µg plasmid DNA : 1x10⁶ cells, or preferably about 1.67 plasmid DNA : 1x10⁶ cells.
10. The method of claim 7, wherein the epithelial stem cell of the amniotic membrane of the umbilical cord is subjected to electroporation with 2 pulses having a duration time of 25-35ms and a voltage of 1300-1400V, preferably to electroporation with 2 pulses having a duration time of 30ms and a voltage of 1350V.
11. The method of claim 10, wherein the ratio of the amount of vector plasmid DNA for each vector to the number of epithelial stem cells of the amniotic membrane of the umbilical cells subjected to electroporation is in the range of about 1.5µg plasmid DNA to about 1x10⁶ cells to about 2.5µg plasmid DNA to about 1x10⁶ cells, wherein the ratio is, for example, about 1.5 µg plasmid DNA : 1x10⁶ cells, about 1.6 µg plasmid DNA : 1x10⁶ cells, about 1.7 µg plasmid DNA : 1x10⁶ cells, about 1.8 µg plasmid

DNA : 1×10^6 cells, about 1.9 μg plasmid DNA : 1×10^6 cells, about 2.0 μg plasmid DNA : 1×10^6 cells, about 2.5 μg plasmid DNA : 1×10^6 cells, preferably about 1.67 μg plasmid DNA : 1×10^6 cells.

12. The method of any of claims 1 to 11, wherein the medium suitable for cell recovery is a serum-free medium.
13. The method of claim 12, wherein the medium suitable for the recovery of a transfected mesenchymal stem cell of the amniotic membrane of the umbilical cord consists of 85 to 95 % (v/v) defined medium and 5 to 15 % (v/v) fetal bovine serum.
14. The medium of claim 13, wherein the medium suitable for the recovery of a transfected mesenchymal stem cell of the amniotic membrane of the umbilical cord consists of 90% (v/v) chemically defined medium and 10% (v/v) fetal bovine serum.
15. The medium of any of claims 14 or 15, wherein the medium contains ~~about~~ 85 to 95 % (v/v) CMRL 1066 and 5 to 15 % (v/v) FBS.
16. The method of any of claims 1 to 11, wherein the medium suitable for the recovery of a transfected epithelial stem cell of the amniotic membrane of the umbilical cord comprises Mammary Epithelial Basal Medium MCDB 170, EpiLife medium, DMEM (Dulbecco's modified eagle medium), F12 (Ham's F12 Medium) and FBS (Fetal Bovine Serum).
17. The method of claim 16, wherein the medium suitable for the recovery of a transfected epithelial stem cell of the amniotic membrane of the umbilical cord comprises Mammary Epithelial Basal Medium MCDB 170 in a final concentration of 10 to 30 % (v/v), EpiLife medium in a final concentration of 20 to 40 % (v/v), F12 in a final concentration of 5 to 15 % (v/v), DMEM in a final concentration of 30 to 45 % (v/v) and FBS in a final concentration of 0.1 to 2 % (v/v).
18. The method of claim 17, wherein the medium suitable for the recovery of a transfected epithelial stem cell of the amniotic membrane of the umbilical cord comprises

Mammary Epithelial Basal Medium MCDB 170 in a final concentration of 15 to 25 % (v/v), EpiLife medium in a final concentration of 25 to 35 % (v/v), F12 in a final concentration of 7.5 to 13 % (v/v), DMEM in a final concentration of 35 to 40 % (v/v) and FBS in a final concentration of 0.5 to 1.5 % (v/v).

19. The method of claim 18, wherein the medium suitable for the recovery of a transfected epithelial stem cell of the amniotic membrane of the umbilical cord comprises Mammary Epithelial Basal Medium MCDB 170 in a final concentration of 20 % (v/v), EpiLife medium in a final concentration of 30 % (v/v), F12 in a final concentration of 12.5 % (v/v), DMEM in a final concentration of 37.5 % (v/v) and FBS in a final concentration of 1.0 % (v/v).

20. The method of any of claims 16 to 19, wherein the medium suitable for the recovery of a transfected epithelial stem cell of the amniotic membrane of the umbilical cord is obtained by mixing to obtain a final volume of 1000 ml culture medium:

200 ml Mammary Epithelial Basal Medium MCDB 170

300ml EpiLife medium

250 ml DMEM

250 ml DMEM/F12

1% Fetal Bovine Serum.

~~21. The method of any of claims 16 to 20, wherein the medium suitable for the recovery of a transfected epithelial stem cell of the amniotic membrane of the umbilical cord comprises insulin in a final concentration of 1 to 7.5 µg/ml.~~

~~22. The method of any of claims 16 to 21, wherein the medium suitable for the recovery of a transfected epithelial stem cell of the amniotic membrane of the umbilical cord comprises human epidermal growth factor in a final concentration of 1 to 15ng/ml.~~

~~23. The method of any of claims 16 to 22, wherein the medium suitable for the recovery of a transfected epithelial stem cell of the amniotic membrane of the umbilical cord further comprises at least one of the following supplements: adenine, hydrocortisone, and 3,3',5-Triiodo L-thyronine sodium salt (T3).~~

~~24. The method of claim 23, wherein the medium suitable for the recovery of a transfected epithelial stem cell of the amniotic membrane of the umbilical cord comprises all three of adenine, hydrocortisone, and 3,3',5 Triiodo L-tyronine sodium salt (T3).~~

~~25. The method of any of claims 16 to 24, wherein the medium suitable for the recovery of a transfected epithelial stem cell of the amniotic membrane of the umbilical cord further comprises one of more Transforming Growth Factors (TGF).~~

~~26. The method of claim 25, wherein the medium comprises Transforming Growth Factor beta (TGF-beta) and/or transforming growth factor alpha.~~

~~27. The method of any of claims 16 to 26, wherein the medium suitable for the recovery of a transfected epithelial stem cell of the amniotic membrane of the umbilical cord further comprises Cholera Toxin from Vibrio cholerae.~~

218. The method of any of claims 1 to 207, wherein the compound is a glucocorticoid.

229. The method of claim 218, wherein the glucocorticoid is selected from the group consisting of prednisolone, methylprednisolone, dexamethasone, betamethasone, corticosterone and hydrocortisone.

2330. The method of claim 218 or 229, wherein the hydrocortisone concentration is 0.5μM to 2μM.

2431. The method of any one of claims 1 to 2330, wherein the cultivation is carried out in a coated cell culture vessel, wherein the cell culture vessel is preferably coated with a serum-derived substrate or a serum-free substrate.

~~32. The method of any one of claims 8 to 31, wherein the medium suitable for cell recovery is replaced with a mixture of two different cell culture media 1, 2 or 3 days after transfection, preferably 2 days after transfection, thereby yielding an induced pluripotent stem cell colony.~~

- ~~33. The method of claim 32, wherein the two different cell culture media are the medium suitable for cell recovery and a second cell culture medium.~~
- ~~34. The method of claim 32 or 33, wherein the two different cell culture media are mixed in a ratio of 1:1 (v/v) prepared by contacting 1 volume medium suitable for cell recovery to 1 volume second cell culture medium.~~
- ~~35. The method of claim 33 or 34, wherein the second cell culture medium is a maintenance medium for cultivation of induced pluripotent stem cells, wherein the medium is preferably selected from the group consisting of mTeSR1, StemMACS™ iPS-Brew XF, TeSRTM-E8, mTeSRTMPlus, TeSRTM2, mTeSRTM1, Corning® NutriStem® hPSC XF Medium, Essential 8 Medium, StemFlex, StemFit-Basic02 and PluriSTEM.~~
- ~~36. The method of any one of claims 32 to 35, wherein the mixture of cell culture media is replaced with the same mixture of cell culture media within 3, 4 or 5 days after transfection, preferably 4 days after transfection.~~
- ~~37. The method of any one of claims 32 to 36, wherein the mixture of cell culture media is replaced with the second cell culture medium within 5, 6 or 7 days after transfection, preferably 6 days after transfection.~~
- ~~38. The method of claim 37, wherein the second cell culture medium is changed daily or every second day, third day, preferably every second day.~~
- ~~39. The method of claim 37 or 438, wherein an induced pluripotent stem cell colony is selected when reaching a size of 0.5mm to 1.5mm in diameter, and the selected induced pluripotent stem colony is transferred to a coated cell culture vessel for cultivation and proliferation.~~
- ~~40. The method of claim 39, wherein the induced pluripotent stem cell colony is selected under bright field microscopy.~~

- ~~41. The method of claim 39 or 40, wherein the cell culture medium is changed daily or every second day, preferably every day.~~
- ~~42. The method of any one of claims 40 to 41, wherein the induced pluripotent stem cell colony is detached from the coated cell culture device when reaching a confluence of 50%.~~
- ~~43. The method of claim 42, wherein the induced pluripotent stem cell colony is detached with a reagent selected from the group consisting of dissociation reagent, a dispase or an EDTA solution.~~
- ~~44. The method of claim 42 or 43, wherein a cell population formed from the induced pluripotent stem cell colony is passaged when reaching 60-90% confluence, preferably when reaching 70-80% confluence.~~
- ~~45. The method of claim 44, wherein the cell population formed from the induced pluripotent stem cell colony is passaged in a ratio of 1:3 (v/v), wherein the passaging in a ratio of 1:3 (v/v) is performed by dividing 1 volume dissociated induced pluripotent stem cells into 2 volumes of dissociated induced pluripotent stem cells.~~
- ~~46. The method of claim 44 or 45, wherein the cell population formed from the induced pluripotent stem cell colony is dissociated with 0.5mM EDTA for passaging.~~
- ~~47. The method of claim 45 or 46, wherein the passaged cell population formed from the induced pluripotent stem cell colony is cultivated in a medium containing a substance enhancing the survival of the induced pluripotent stem cell.~~
- ~~48. The method of claim 47, wherein the substance enhancing the survival of the induced pluripotent stem cell colony is a ROCK inhibitor.~~
- ~~49. An induced pluripotent stem cell population obtained by the method as defined in any of claims 1 to 48.~~

~~50. A pharmaceutical composition comprising an induced pluripotent stem cell as defined in claim 49.~~

~~51. A method of differentiating an induced pluripotent stem cell as defined in claim 49 into a target cell, wherein the induced pluripotent stem cell is differentiated into the target cell under conditions suitable for differentiation, wherein the target cell is selected from the group consisting of an oligodendrocyte, a hematopoietic progenitor cell, a blood cell, a motor neuron, a cartilage cell, a muscle cell, a bone cell, a dental cell, a hair follicle cell, an inner ear hair cell, a skin cell, a melanocyte, an immune cell, an astrocyte, a reproductive cell, a corneal cell, an intestinal cell, a lung cell, a kidney cell, a stomach cell, a mesenteric cell, and a fat cell.~~

~~52. The method of claim 51, wherein the immune cell is selected from the group consisting of a T-lymphocyte, a B-lymphocyte, a microglia, and a natural killer cell.~~

~~53. The method of claim 51, wherein the induced pluripotent stem cell is cultivated in a medium adapted for proliferation and differentiation of the induced pluripotent stem cell into an oligodendrocyte.~~

~~54. An extracellular membranous vesicle produced by an induced pluripotent stem cell population as defined in claim 49.~~

~~55. The extracellular membranous vesicle of claim 54, wherein the vesicle is an exosome.~~

~~56. The use of an extracellular membranous vesicle as defined in claim 54 or 55 as delivery carrier of a therapeutic agent.~~

2557. A cell culture medium comprising Mammary Epithelial Basal Medium MCDB 170, EpiLife medium, DMEM (Dulbecco's modified eagle medium), F12 (Ham's F12 Medium) and FBS (Fetal Bovine Serum) wherein the medium comprises Mammary Epithelial Basal Medium MCDB 170 in a final concentration of 10 to 30 % (v/v), EpiLife medium in a final concentration of 20 to 40 % (v/v), F12 in a final

concentration of 5 to 15 % (v/v), DMEM in a final concentration of 30 to 45 % (v/v) and FBS in a final concentration of 0.1 to 2 % (v/v).

~~2658~~. The cell culture medium of claim ~~2557~~, wherein the medium comprises Mammary Epithelial Basal Medium MCDB 170 in a final concentration of 15 to 25 % (v/v), EpiLife medium in a final concentration of 25 to 35 % (v/v), F12 in a final concentration of 7.5 to 13 % (v/v), DMEM in a final concentration of 35 to 40 % (v/v) and FBS in a final concentration of 0.5 to 1.5 % (v/v).

~~2759~~. The cell culture medium of claim ~~2658~~, wherein the medium comprises Mammary Epithelial Basal Medium MCDB 170 in a final concentration of 20 % (v/v), EpiLife medium in a final concentration of 30 % (v/v), F12 in a final concentration of 12.5 (v/v), DMEM in a final concentration of 37.5 % (v/v) and FBS in a final concentration of 1.0 % (v/v).

~~2860~~. The cell culture medium of any of claims ~~2555~~ to ~~2759~~, wherein the medium is obtained by mixing to obtain a final volume of 1000 ml culture medium:
200 ml Mammary Epithelial Basal Medium MCDB 170,
300ml EpiLife medium,
250 ml DMEM,
250 ml DMEM/F12, and
1% Fetal Bovine Serum.

~~61. The cell culture medium of any of claims 55 to 60, wherein the medium comprises insulin in a final concentration of 1 to 7.5 µg/ml.~~

~~62. The cell culture medium of any of claims 55 to 61, wherein the medium comprises human epidermal growth factor (EGF) in a final concentration of 1 to 15ng/ml.~~

~~63. The cell culture medium of any of claims 55 to 62, wherein the medium suitable for the recovery of a transfected epithelial stem cell of the amniotic membrane of the umbilical cord comprises at least one of the following supplements: adenine, hydrocortisone, and 3,3',5-Triiodo L-thyronine sodium salt (T3).~~

- ~~64. The cell culture medium of claim 63, wherein the medium comprises all three of adenine, hydrocortisone, and 3,3',5-Triiodo L-thyronine sodium salt (T3).~~
- ~~65. The cell culture medium of claim 64, wherein the culture medium comprises adenine in a final concentration of 0.05 to 0.1 mM adenine, hydrocortisone in a final concentration of 0.1 to 0.5 μ M hydrocortisone and/or 3,3',5-Triiodo L-thyronine sodium salt (T3) in a final concentration of 0.1 to 5 ng/ml.~~
- ~~66. The cell culture medium of any of claims 55 to 65, wherein the medium comprises one of more Transforming Growth Factors (TGF).~~
- ~~67. The cell culture medium of claim 66, wherein the medium comprises Transforming Growth Factor beta 1 (TGF beta 1) in a final concentration of 0.1 to 5 ng/ml and/or transforming growth factor alpha (TGF alpha) in a final concentration of 1.0 to 10 ng/ml.~~
- ~~68. The medium of any of claims 55 to 67, wherein the medium comprises Cholera Toxin from *Vibrio cholerae* in a final concentration of 1×10^{-11} M to 1×10^{-10} M.~~