

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 a 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 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 1 to 20, wherein the compound is a glucocorticoid.
22. The method of claim 21, wherein the glucocorticoid is selected from the group consisting of prednisolone, methylprednisolone, dexamethasone, betamethasone, corticosterone and hydrocortisone.
23. The method of claim 21 or 22, wherein the hydrocortisone concentration is 0.5 μ M to 2 μ M.
24. The method of any one of claims 1 to 23, 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.

25. 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).
26. The cell culture medium of claim 25, 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).
27. The cell culture medium of claim 26, 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).
28. The cell culture medium of any of claims 25 to 27, 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.