and (B) is that after the treatment.

Fig. 16 is the graph showing a score (NIHSS) change, which reflects the conditions of the patient in treatment period.

Fig. 17 is the photograph showing the function recovery state of the patient. (A) is the photograph showing the recovery status of a hand function, and (B) is that showing the retrieve status of physique.

Fig. 18 is the result of the time-dependent change after Mini Mental State (Mini Mental State: MMS) or Hasegawa style test were performed for both of an administration group and non-administration group. Fig. 18(A) shows the result of the non-administration group, and Fig. 18(B) shows that of the administration group.

Fig. 19 is the photograph showing the treatment results against the refractory skin diseases. Fig. 19(A) shows the status before treatment start, and Fig. 19(B) shows that of the treatment finish.

Mode for Carrying Out the Invention

[0023]

The present invention is explained in detail in below.

In order to obtain the immortalized stem cell of the present invention, firstly, the stem cell is isolated from the cell group consisting of a mammal mesenchymal cell, early generated embryo, and somatic cells except the mesenchymal cell. As the mammal animal, it is preferable to be selected from the group consisting of human, swine, equine, and monkey, because the cells obtained from the mammal are genetically similar to the human cells and are not dangerous for infectious disease.

In the present specification, the terms, “mesenchymal cell”, is defined as the cells having differentiation ability into the cells belonging to the mesenchymal such as osteoblast, adipocyte, muscle cell, cartilage cell, and the like. As the specific mesenchymal cell, the dental pulp cell, bone marrow cell, umbilical cell and adipocyte of the above-mentioned animals. Also, the terms, “early-generated embryo”, is defined as the embryo in the early stage

by the blastocyst, namely, it is in the progressed stage than the fertilized egg, and necessary for establishing ES cell. The terms, “somatic cell”, are defined as the general term of the cell except a germ cell among those being composed of a living body.

[0024]

Furthermore, the terms, “dental pulp cell”, is one of stem cells included in the nerve for the teeth, and has the regeneration ability. Since it is protected by hard material, teeth, does not permeate UV light or radioactive ray, the genes in them is not easily damaged. The terms, “bone marrow cell”, are defined as the general term for the cells obtained from bone-marrow aspirate, and the bone marrow cell includes a leukocyte series cell such as a myeloblast cell, an erythroblast series cell, a megakaryocyte cell, and plasma cells and the like.

In the present specification, the terms, “umbilical cell”, are that exists in the umbilical cord, which binds the embryo and a placenta. It is included in the umbilical cord, and also includes in umbilical blood. The umbilical cord includes the umbilical blood which enriches hematopoietic stem cells.

[0025]

As the genes introduced into the stem cells as described above, there are mentioned such as hTERT, bmi-1, E6, E7, Oct3/4, Sox2, Klf4, c-Myc, INK4a, and the like. The gene, hTERT, is a gene for telomere repair enzyme; bmi-1 is the gene of Bmi-1, which is one of proteins being composed of polycomb group complex. Here, Bmi-1 is necessary for maintaining the hematopoietic stem cells with the effect to increase the stem cell by enhancing the activity.

Both E6 and E7 are early genes of either HPV-16 or HPV-18. Also, Oct3/4 is the gene that cooperates with Sox2 to enhance the transcription of the target gene. Klf4 (Kruppel type transcription factor 4) regulates the genes relating to the cell division and the embryogenesis, and it relates to the gastrointestinal system cancer as the tumor suppressor.

Sox2 is belonging to SRY-related HMG box gene family, and it is known as the gene that related to the maintenance of undifferentiated functions

and (B) is that after the treatment.

Fig. 16 is the graph showing a score (NIHSS) change, which reflects the conditions of the patient in treatment period.

Fig. 17 is the photograph showing the function recovery state of the patient. (A) is the photograph showing the recovery status of a hand function, and (B) is that showing the retrieve status of physique.

Fig. 18 is the result of the time-dependent change after Mini Mental State (Mini Mental State: MMS) or Hasegawa style test were performed for both of an administration group and non-administration group. Fig. 18(A) shows the result of the non-administration group, and Fig. 18(B) shows that of the administration group.

Fig. 19 is the photograph showing the treatment results against the refractory skin diseases. Fig. 19(A) shows the status before treatment start, and Fig. 19(B) shows that of the treatment finish.

Mode for Carrying Out the Invention

[0023]

The present invention is explained in detail in below.

In order to obtain the immortalized stem cell of the present invention, firstly, the stem cell is isolated from the cell group consisting of a mammal mesenchymal cell, early generated embryo, and somatic cells except the mesenchymal cell. As the mammal animal, it is preferable to be selected from the group consisting of human, swine, equine, and monkey, because the cells obtained from the mammal are genetically similar to the human cells and are not dangerous for infectious disease.

In the present specification, the terms, "mesenchymal cell", is defined as the cells having differentiation ability into the cells belonging to the mesenchymal such as osteoblast, adipocyte, muscle cell, cartilage cell, and the like. As the specific mesenchymal cell, the dental pulp cell, bone marrow cell, umbilical cell and adipocyte of the above-mentioned animals.

The terms, “somatic cell”, are defined as the general term of the cell except a germ cell among those being composed of a living body.

[0024]

Furthermore, the terms, “dental pulp cell”, is one of stem cells included in the nerve for the teeth, and has the regeneration ability. Since it is protected by hard material, teeth, does not permeate UV light or radioactive ray, the genes in them is not easily damaged. The terms, “bone marrow cell”, are defined as the general term for the cells obtained from bone-marrow aspirate, and the bone marrow cell includes a leukocyte series cell such as a myeloblast cell, an erythroblast series cell, a megakaryocyte cell, and plasma cells and the like.

In the present specification, the terms, “umbilical cell”, are that exists in the umbilical cord, which binds the embryo and a placenta. It is included in the umbilical cord, and also includes in umbilical blood. The umbilical cord includes the umbilical blood which enriches hematopoietic stem cells.

[0025]

As the genes introduced into the stem cells as described above, there are mentioned such as hTERT, bmi-1, E6, E7, Oct3/4, Sox2, K1f4, c-Myc, INK4a, and the like. The gene, hTERT, is a gene for telomere repair enzyme; bmi-1 is the gene of Bmi-1, which is one of proteins being composed of polycomb group complex. Here, Bmi-1 is necessary for maintaining the hematopoietic stem cells with the effect to increase the stem cell by enhancing the activity.

Both E6 and E7 are early genes of either HPV-16 or HPV-18. Also, Oct3/4 is the gene that cooperates with Sox2 to enhance the transcription of the target gene. K1f4 (Krupple type transcription factor 4) regulates the genes relating to the cell division and the embryogenesis, and it relates to the gastrointestinal system cancer as the tumor suppressor.

Sox2 is belonging to SRY-related HMG box gene family, and it is known as the gene that related to the maintenance of undifferentiated functions

CLAIMS

1. An immortalized stem cell produced by isolating a stem cell from mammal cells selected from the group consisting of a dental pulp stem cell, a bone marrow stem cell, a umbilical stem cell, an adipose stem cell, an early-developed embryo, and a somatic cell other than mesenchymal stem cell; performing a primary culture of said stem cell to obtain said primarily cultured stem cells; transfecting 4 genes selected from the group consisting of hTERT, bmi-1, E6, E7, Oct3/4, Sox2, Klf4, c-Myc, and p16INK4a into said primarily cultured stem cell to produce gene-transduced cells; and selecting an immortalized stem cell from said gene-transduced cells, which has both abilities for telomere repairing and cell dividing at least 200 times, by using STRO-1 expression as an index.

2. (Deleted)

3. (Amended) The immortalized stem cell according to the claim 1, wherein said stem cell is any one of teeth selected from the group consisting of an exfoliated dens deciduous tooth, an exfoliated dens permanence tooth, a removed deciduous tooth, and a removed dens permanence tooth.

4. The immortalized stem cell according to the claim 1, wherein said early-developed embryo is an embryo of blastoderm.

5. (Amended) The immortalized stem cell according to any one of the claims 1, 3 and 4, wherein said mammal is selected from the group consisting of human, swine, equine and monkey.

6. (Deleted)

7. (Deleted)

8. (Amended) The immortalized stem cell according to any one of the claims 1 and 3 to 5, wherein at least 40 % of the cells are STRO-1 expression cells and they have neonatal bone quantity production ability equal to that of a primary cultured cell at population doubling times of 20.

9. (Amended) The immortalized stem cell according to the any one of the claims 1, 3 to 5 and 8, wherein said immortalized stem cell secretes at least IGF-1, VEGF, TGF-β1 and HGF into a culture supernatant.

10. (Amended) A pharmaceutical composition comprising said culture supernatant of said immortalized stem cell according to any one of the claims 1, 3 to 5, 8 and 9.

11. A pharmaceutical preparation for restoring damaged tissue comprising said pharmaceutical composition according to the claim 10.

12. The pharmaceutical preparation for restoring damaged tissue according to the claim 11, wherein a dosage form of said pharmaceutical preparation for restoring damaged tissue is any one of that selected from the group consisting of powder form, liquid form, gel form, spray form and percutaneous system form.

13. (Amended) The pharmaceutical preparation for restoring damaged tissue according to the claim 11 or 12, wherein said damaged tissue is any one of that selected from the group consisting of a damaged tissue with ulcer or bedsore, a damaged brain tissue by cell degeneration, a brain tissue of which is partially lost by a surgical operation, a brain tissue damaged by traumatic brain disease, a damaged brain tissue with inflammatory

brain disease, a damaged bone tissue, a damaged periodontal tissue, and a damaged tissue by refractory skin disease.

14. The pharmaceutical preparation for restoring damaged tissue according to the claim 13, wherein said cell degeneration is caused by the disease selected from the group consisting of Alzheimer disease, Parkinson's disease, cognitive impairment, schizophrenia, depression disease, cerebral hypoxia, amyotrophic lateral sclerosis, cerebral infarction, cerebellar degeneration, diabetes, and hepatitis.

15. The pharmaceutical preparation for restoring damaged tissue according to the claim 13, wherein said traumatic brain disease is caused by a traffic accident or fall accident.

16. The pharmaceutical preparation for restoring damaged tissue according to the claim 13, wherein said inflammatory encephalopathy is caused by a disease selected from the group consisting of encephalitis encephalopathy, epileptic, Jakob disease, and polio.

17. (Deleted)

18. The pharmaceutical preparation for restoring damaged tissue according to the claim 13, wherein said refractory skin disease is atopic dermatitis.

19. (Amended) The pharmaceutical preparation for restoring damaged tissue according to any one of the claims 11 to 16, wherein the content of the culture supernatant of the preparation is 50 to 500 % (w/v), when the supernatant produced by the immortalized stem cell of any one of the claims 1, 3 to 5, 8 and 9 is 100 %.

20. (Amended) A method for producing an immortalized stem cell comprising the steps of:

isolating a stem cell from mammal cells selected from the group consisting of a

dental pulp stem cell, a bone marrow stem cell, a umbilical stem cell, and an adipose stem cell, an early-developed embryo, and a somatic cell other than said mesenchymal cell;

culturing said stem cell primarily to obtain a primarily cultured cell;

transfecting 4 genes selected from the group consisting of hTERT, bmi-1, E6, E7, Oct3/4, Sox2, Klf4, c-Myc and p16INK4a, into said primarily cultured cell to produce a gene-transduced cell; and

selecting the dental pulp stem cell from said gene-transduced cell, which has both abilities for telomere repairing and cell dividing at least 200 times, by using STRO-1 expression amount and bone regeneration ability at a cell doubling time of 20 as indexes.

21. (Deleted)

22. The method for producing an immortalized stem cell according to the claim 20, wherein the dental pulp cell is any one of teeth selected from the group consisting of an exfoliated dens deciduous tooth, an exfoliated dens permanence tooth, a removed dens deciduous tooth, and a removed dens permanence tooth.

23. (Amended) The method for producing an immortalized stem cell according to the claim 20, wherein the primary embryo is an embryo of blastodisc stage.

24. (Amended) The method for producing an immortalized stem cell according to any one of the claims 20 to 22, wherein said mammal is any one of animals selected from the group consisting of human, swine, equine, and monkey.

25. (Deleted)

26. (Deleted)

CLAIMS

1. An immortalized stem cell produced by: isolating a stem cell from mammal cells selected from the group consisting of a dental pulp stem cell, a bone marrow stem cell, a umbilical stem cell, an adipose stem cell, an early-developed embryo, and a somatic cell other than mesenchymal stem cell; performing a primary culture of said stem cell to obtain said primarily cultured stem cells; transfecting 4 genes selected from the group consisting of hTERT, bmi-1, E6, E7, Oct3/4, Sox2, Klf4, c-Myc, and p16INK4a into said primarily cultured stem cell to produce gene-transduced cells; and selecting an immortalized stem cell from said gene-transduced cells, which has both abilities for telomere repairing and cell dividing at least 200 times, by using STRO-1 expression amount and bone regeneration ability at a cell doubling time of 20 as indexes.

2. The immortalized stem cell according to the claim 1, wherein said stem cell is any one of a tooth selected from the group consisting of an exfoliated dens deciduous tooth, an exfoliated dens permanence tooth, a removed deciduous tooth, and a removed dens permanence tooth.

3. The immortalized stem cell according to any one of the claims 1, wherein said mammal is selected from the group consisting of human, swine, equine and monkey.

4. The immortalized stem cell according to any one of the claims 1 to 3, wherein at least 40 % of the cells is STRO-1 expression cell and they have neonatal bone quantity production ability equal to that of a primary cultured cell at population doubling times of 20.

5. The immortalized stem cell according to the any one of the claims 4, wherein said immortalized stem cell secretes at least IGF-1, VEGF, TGF-β1 and HGF into a culture

supernatant.

6. A pharmaceutical composition comprising said culture supernatant of said immortalized stem cell according to the claim 1 to 5.

7. A pharmaceutical preparation for restoring damaged tissue comprising said pharmaceutical composition according to the claim 6.

8. The pharmaceutical preparation for restoring damaged tissue according to the claim 7, wherein a dosage form of said pharmaceutical preparation for restoring damaged tissue is any one of that selected from the group consisting of powder form, liquid form, gel form, spray form and percutaneous system form.

9. The pharmaceutical preparation for restoring damaged tissue according to the claim 7, wherein said damaged tissue is any one of that selected from the group consisting of a damaged tissue with ulcer or bedsore, a damaged brain tissue by cell degeneration, a brain tissue damaged by traumatic brain disease, a damaged brain tissue with inflammatory brain disease, a damaged bone tissue, a damaged periodontal tissue, and a damaged tissue by refractory skin disease.

10. The pharmaceutical preparation for restoring damaged tissue according to the claim 9, wherein said cell degeneration is caused by the disease selected from the group consisting of Alzheimer disease, Parkinson’s disease, cerebral infarction, cerebellar degeneration, diabetes, and hepatitis.

11. The pharmaceutical preparation for restoring damaged tissue according to the claim 9, wherein said traumatic brain disease is caused by a traffic accident or fall accident.

12. The pharmaceutical preparation for restoring damaged tissue according to the claim 9, wherein said inflammatory brain disease is a disease selected from the group consisting of encephalitis encephalopathy, epileptic, Jakob disease, and polio.

13. The pharmaceutical preparation for restoring damaged tissue according to the claim 9, wherein said refractory skin disease is atopic dermatitis.

14. The pharmaceutical preparation for restoring damaged tissue according to the claims 6, the content of the culture supernatant of said immortalized stem cell in the reparation is 50 to 500 % (w/v), when the culture supernatant originally produced by the immortalized stem cell is 100 %; wherein

the immortalized stem cell secretes at least IGF-1, VFGF, TGF-β1, and HGF into the culture supernatant, and it is produced by using a method comprising the steps of:

isolating a stem cell from mammal cells selected from the group consisting of a dental pulp stem cell, a bone marrow stem cell, a umbilical stem cell, an adipose stem cell, an early-developed embryo, and a somatic cell other than mesenchymal stem cell;

performing a primary culture of said stem cell to obtain said primarily cultured stem cells;

transfecting 4 genes selected from the group consisting of hTERT, bmi-1, E6, E7, Oct3/4, Sox2, Klf4, c-Myc, and p16INK4a into said primarily cultured stem cell to produce gene-transduced cells; and

selecting an immortalized stem cell from said gene-transduced cells, which has both abilities for telomere repairing and cell dividing at least 200 times, by using STRO-1 expression amount and bone regeneration ability at a cell doubling time of 20 as an index.

15. A method for producing an immortalized stem cell comprising the steps of:

isolating a stem cell from mammal cells selected from the group consisting of a dental pulp stem cell, a bone marrow stem cell, a umbilical stem cell, and an adipose stem cell, an early-developed embryo, and a somatic cell other than said mesenchymal cell;

culturing said stem cell as primary culture to obtain a primarily cultured cell;

transfecting 4 genes selected from the group consisting of hTERT, bmi-1, E6, E7, Oct3/4, Sox2, Klf4, c-Myc and p16INK4a, into said primarily cultured cell to produce a gene-transduced cell; and

selecting the dental pulp stem cell from said gene-transduced cell, which has both abilities for telomere repairing and cell dividing at least 200 times, by using STRO-1 expression amount and bone regeneration ability at a cell doubling time of 20 as indexes

16. The method for producing an immortalized stem cell according to the claim 15, wherein the dental pulp cell is any one of the teeth selected from the group consisting of exfoliated dens deciduous, exfoliated dens permanence, removed dens deciduous, and removed dens permanence.

17. The method for producing an immortalized stem cell according to the claim 15, wherein the mammal is any one of the animal selected from the group consisting of human, swine, equine, and monkey.