Claims

What is claimed is:

1. A method of producing cells with neuron functions from stem cells, which comprises the following steps:

1) culturing the obtained stem cells in a medium with high serum content;

2) transferring the cells cultured in the first step into a medium having low serum content and containing cell growth factors and neurotrophic factors;

3) transferring the cells cultured in the second step into a differentiation medium and continue to culture the cells;

4) transferring the cells cultured in the third step into a medium containing nerve cell maturation factors and continue to culture the cells;

5) transferring the cells cultured in the fourth step into a medium containing nerve factors and continue to culture the cells; and

the produced cells with neuron functions are nerve cells expressing neuron specific enolase (NSE) and neurofilament protein-M (NF-M), and secreting neurotrophic factor (NT) and brain derived neurotrophic factor (BDNF),~~.~~

~~2. The method according to claim 1,~~ wherein the stem cells are human derived mesenchymal stem cells~~.~~;

wherein the medium used in Step 1) is DMEM supplemented with 15-25% of serum; and the culture time of the cell is 8-24 hours;

wherein the medium used in Step 2) is an initial differentiation medium, comprising a DMEM/F12 mixture medium supplemented with 0.1-0.5% of serum and comprises equal content of N2 mixed cofactor, 5-15 ng/ml of fibroblast growth factor (FGF) and 5-15ng/ml of endothelial growth factor (EGF) and the culture time of the cells is 8-24 hours;

wherein the differentiation medium used in Step 3) comprises a DMEM/F12 mixture medium supplemented with 0.1-0.5% serum, and contains equal content of N2 mixed cofactor and 50-500 μg/ml of total proteins from embryonic brain tissue, and the culture time of the cells is 3-15 days;

wherein the medium used in Step 4) is a DMEM/F12 mixture medium supplemented with 0.1-0.5% of serum, and contains equal content of N2 mixed cofactor, 10-50 nM of retinoic acid (RA) and 100-1000ng/mL of Sonic hedgehog (Shh), and the culture time of the cells is 2-5 days;

wherein the medium used in Step 5) is a DMEM/F12 mixture medium supplemented with 5-15% of serum, and contains 5-20 ng/ml of brain-derived neurotrophic factor (BDNF), 10-50 ng/ml of glial cell-derived neurotrophic factor (GDNF) and 20-50 ng/ml of insulinoid growth factor (IGF), and the culture time of the cells is 5-10 days.

~~3~~2. The method according to claim ~~2~~1, wherein the stem cells are placental mesenchymal stem cells, bone marrow mesenchymal stem cells or adipose mesesnchymal stem cells.

~~4~~3. The method according to claim ~~3~~2, wherein the stem cells are placental mesenchymal stem cells.

~~5. The method according to any one of claims 1-4, wherein the medium used in Step 1) is DMEM supplemented with 15-25% of serum; and the culture time of the cell is 8-24 hours.~~

~~6~~4. The method according to claim ~~5~~1, wherein in step 1) the serum is 20% of FBS.

~~7~~5. The method according to claim ~~5~~1, wherein in step 1) the culture time of the cell is 16 hours.

~~8. The method according to any one of claims 1 to 5, wherein the medium used in Step 2) is an initial differentiation medium, which is a DMEM/F12 mixture medium supplemented with 0.1-0.5% of serum and comprises equal content of N2 mixed cofactor, 5-15 ng/ml of fibroblast growth factor (FGF) and 5-15ng/ml of endothelial growth factor (EGF) and the culture time of the cells is 8-24 hours.~~

~~9~~6. The method according to claim ~~8~~1, wherein in step 2) said initial differentiation medium is a DMFM/F12 mixture medium supplemented with 0.2% of FBS, and contains 1% of N2 mixed cofactor, 2 μg/ml heparin, 1% MEM, 10 ng/ml of bFGF (basic FGF) and 10 ng/ml of EGF.

~~10~~7. The method according to claim ~~8~~1, wherein in step 2) the culture time of the cells is 16 hours.

~~11. The method according to any one of claims 1-5, wherein the medium used in Step 3) is a differentiation medium, which is a DMEM/F12 mixture medium supplemented with 0.1-0.5% serum, and contains equal content of N2 mixed cofactor and 50-500 μg/ml of embryonic brain protein, and the culture time of the cells is 3-15 days.~~

~~12~~8. The method according to claim ~~1~~1, wherein in step 3) the differentiation medium is a DMEM/F12 mixture medium supplemented with 0.2% of FBS, and contains 1% of N2 mixed cofactor, 2 μg/ml heparin, 1% MEM and 200 μg/ml central nervous system (CNS) protein.

~~13~~9. The method according to claim 1~~1~~, wherein in step 3) the culture time of the cells is 6 days.

~~14. The method according to any one of claims 1-5, wherein the medium used in Step 4) is a DMEM/F12 mixture medium supplemented with 0.1-0.5% of serum, and contains equal content of N2 mixed cofactor, 10-50 nM of retinoic acid (RA) and 100-1000ng/mL of Sonic hedgehog (Shh), and the culture time of the cells is 2-5 days.~~

~~15~~10. The method according to claim 14, wherein in step 4) said medium is a DMEM/F12 mixture medium supplemented with 0.2% of FBS, and contains 1% of N2 mixed cofactor, 2 μg/ml of heparin, 20nM of RA, and 500 ng/ml of Shh.

~~16~~11. The method according to claim 14, wherein in step 4) the culture time of the cells is 3 days.

~~17. The method according to any one of claims 1-5, wherein the medium used in Step 5) is a DMEM/F12 mixture medium supplemented with 5-15% of serum, and contains 5-20 ng/ml of brain-derived neurotrophic factor (BDNF), 10-50 ng/ml of glial cell-derived neurotrophic factor (GDNF) and 20-50 ng/ml of insulinoid growth factor (IGF), and the culture time of the cells is 5-10 days.~~

~~18~~12. The method according to claim 1~~7~~, wherein in step 5) the medium is a DMEM/F12 mixture medium supplemented with 10% FBS, and contains 10 ng/ml of BDNF, 20 ng/ml of GDNF and 50 ng/ml of IGF-II.

~~19~~13. The method according to claim 1~~7~~, wherein in step 5) culture time of the cells is 7 days.

~~20~~14. The method according to any one of claims 1-~~5~~3, wherein the serum is selected from human umbilical cord blood serum and/or fetal bovine serum; and the condition of the incubator is 5% carbon dioxide and 95% air.

~~21~~15. Cells with neuron functions as prepared by the method of any one of claims 1-~~20~~14, wherein the cells are nerve cells expressing neuron specific enolase (NSE) and neurofilament protein-M (NF-M) , and secreting neurotrophic factor (NT) and brain derived neurotrophic factor (BDNF).

~~22~~16. The cells according to claim ~~21~~15, wherein the cells are nerve cells expressing neuron

specific enolase (NSE), glial fibrillary acidic protein (GFAP) , growth related protein factor (GAP-43) and neurofilament protein-M (NF-M) , and secreting neurotrophic factor-3 (NT-3), neurotrophic factor-4 (NT-4) and brain derived neurotrophic factor (BDNF) .

~~23~~17. Use of the method in any one of claims 1-~~20~~14 or the cells with neuron functions in any one of claims 21-22 in the preparation of medicines for treating injuries with the loss of neuron function.

~~24~~18. The use according to claim ~~23~~17, wherein the cells with neuron functions are nerve cells expressing neuron specific enolase (NSE), neurofilament protein-M (NF-M), and secreting neurotrophic factor (NT) and brain derived neurotrophic factor(BDNF).

~~25~~19. The use according to claim ~~23~~17, wherein the injuries with the loss of neuron function are Parkinson's disease and spinal cord injury.

~~26~~20. Use of the method in any one of claims 1-~~20~~14 or the cells with neuron functions in any one of claims ~~21-22~~15-16 in the manufacture of a medicament for treatment of injuries with the loss of neuron function.

~~27~~21. The use according to claim 26, wherein the injuries are Parkinson's disease and spinal cord injury.

~~28~~22. The cells according to claim ~~21~~15 or ~~22~~16 for use in treating injuries with the loss of neuron function by transplanting the cells into an injury site of a patient with neurological diseases, and the neurological diseases includes, Parkinson's disease and spinal cord injury.

~~29~~23. The cells for use according to claim ~~28~~22, wherein the cells with neuron functions express neuron specific enolase (NSE) and neurofilament protein-M (NF-M), and secret neurotrophic factor (NT) and brain derived neurotrophic factor(BDNF).

Claims

What is claimed is:

1. A method of producing cells with neuron functions from stem cells, which comprises the following steps:
1) culturing the obtained stem cells in a medium with high serum content;
2) transferring the cells cultured in the first step into a medium having low serum content and containing cell growth factors and neurotrophic factors;
3) transferring the cells cultured in the second step into a differentiation medium and continue to culture the cells;
4) transferring the cells cultured in the third step into a medium containing nerve cell maturation factors and continue to culture the cells;
5) transferring the cells cultured in the fourth step into a medium containing nerve factors and continue to culture the cells; and
the produced cells with neuron functions are nerve cells expressing neuron specific enolase (NSE) and neurofilament protein-M (NF-M), and secreting neurotrophic factor (NT) and brain derived neurotrophic factor (BDNF),
wherein the stem cells are human derived mesenchymal stem cells;
wherein the medium used in Step 1) is DMEM supplemented with 15-25% of serum; and the culture time of the cell is 8-24 hours;
wherein the medium used in Step 2) is an initial differentiation medium, comprising a DMEM/F12 mixture medium supplemented with 0.1-0.5% of serum and comprises equal content of N2 mixed cofactor, 5-15 ng/ml of fibroblast growth factor (FGF) and 5-15ng/ml of endothelial growth factor (EGF) and the culture time of the cells is 8-24 hours;
wherein the differentiation medium used in Step 3) comprises a DMEM/F12 mixture medium supplemented with 0.1-0.5% serum, and contains equal content of N2 mixed cofactor and 50-500 μg/ml of total proteins from embryonic brain tissue, and the culture time of the cells is 3-15 days;
wherein the medium used in Step 4) is a DMEM/F12 mixture medium supplemented with 0.1-0.5% of serum, and contains equal content of N2 mixed cofactor, 10-50 nM of retinoic acid (RA) and 100-1000ng/mL of Sonic hedgehog (Shh), and the culture time of the cells is 2-5 days;
wherein the medium used in Step 5) is a DMEM/F12 mixture medium supplemented with 5-15% of serum, and contains 5-20 ng/ml of brain-derived neurotrophic factor (BDNF), 10-50 ng/ml of glial cell-derived neurotrophic factor (GDNF) and 20-50 ng/ml of insulinoid growth factor (IGF), and the culture time of the cells is 5-10 days.

2. The method according to claim 1, wherein the stem cells are placental mesenchymal stem

cells, bone marrow mesenchymal stem cells or adipose mesesnchymal stem cells.

3. The method according to claim 2, wherein the stem cells are placental mesenchymal stem cells.

4. The method according to claim 1, wherein in step 1) the serum is 20% of FBS.

5. The method according to claim 1, wherein in step 1) the culture time of the cell is 16 hours.

6. The method according to claim 1, wherein in step 2) said initial differentiation medium is a DMFM/F12 mixture medium supplemented with 0.2% of FBS, and contains 1% of N2 mixed cofactor, 2 μg/ml heparin, 1% MEM, 10 ng/ml of bFGF (basic FGF) and 10 ng/ml of EGF.

7. The method according to claim 1, wherein in step 2) the culture time of the cells is 16 hours.

8. The method according to claim 1, wherein in step 3) the differentiation medium is a DMEM/F12 mixture medium supplemented with 0.2% of FBS, and contains 1% of N2 mixed cofactor, 2 μg/ml heparin, 1% MEM and 200 μg/ml central nervous system (CNS) protein.

9. The method according to claim 1, wherein in step 3) the culture time of the cells is 6 days.

10. The method according to claim 1, wherein in step 4) said medium is a DMEM/F12 mixture medium supplemented with 0.2% of FBS, and contains 1% of N2 mixed cofactor, 2 μg/ml of heparin, 20nM of RA, and 500 ng/ml of Shh.

11. The method according to claim 1, wherein in step 4) the culture time of the cells is 3 days.

12. The method according to claim 1, wherein in step 5) the medium is a DMEM/F12 mixture medium supplemented with 10% FBS, and contains 10 ng/ml of BDNF, 20 ng/ml of GDNF and 50 ng/ml of IGF-II.

13. The method according to claim 1, wherein in step 5) culture time of the cells is 7 days.

14. The method according to any one of claims 1-3, wherein the serum is selected from human umbilical cord blood serum and/or fetal bovine serum; and the condition of the incubator is 5% carbon dioxide and 95% air.

15. Cells with neuron functions as prepared by the method of any one of claims 1-14, wherein the cells are nerve cells expressing neuron specific enolase (NSE) and neurofilament protein-M (NF-M) , and secreting neurotrophic factor (NT) and brain derived neurotrophic factor (BDNF).

16. The cells according to claim 15, wherein the cells are nerve cells expressing neuron specific enolase (NSE), glial fibrillary acidic protein (GFAP) , growth related protein factor (GAP-43) and neurofilament protein-M (NF-M) , and secreting neurotrophic factor-3 (NT-3), neurotrophic factor-4 (NT-4) and brain derived neurotrophic factor (BDNF) .

17. Use of the method in any one of claims 1-14 or the cells with neuron functions in any one of claims 21-22 in the preparation of medicines for treating injuries with the loss of neuron function.

18. The use according to claim 17, wherein the cells with neuron functions are nerve cells expressing neuron specific enolase (NSE), neurofilament protein-M (NF-M), and secreting neurotrophic factor (NT) and brain derived neurotrophic factor(BDNF).

19. The use according to claim 17, wherein the injuries with the loss of neuron function are Parkinson's disease and spinal cord injury.

20. Use of the method in any one of claims 1-14 or the cells with neuron functions in any one of claims 15-16 in the manufacture of a medicament for treatment of injuries with the loss of neuron function.

21. The use according to claim 26, wherein the injuries are Parkinson's disease and spinal cord injury.

22. The cells according to claim 15 or 16 for use in treating injuries with the loss of neuron function by transplanting the cells into an injury site of a patient with neurological diseases, and the neurological diseases includes, Parkinson's disease and spinal cord injury.

23. The cells for use according to claim 22, wherein the cells with neuron functions express neuron specific enolase (NSE) and neurofilament protein-M (NF-M), and secret neurotrophic factor (NT) and brain derived neurotrophic factor(BDNF).