Claims

What is claimed is:

~~1. An isolated neuronal stem cell, wherein the cell is differentiated from a parthenogenetically activated oocyte produced by a method comprising:~~

~~a) parthenogenetically activating a human oocyte, wherein activating comprises:~~

~~i) contacting the oocyte with an ionophore at high O_2 tension, and~~

~~ii) contacting the oocyte with a serine-threonine kinase inhibitor under low O_2 tension;~~

~~b) cultivating the activated oocyte of (a) at low O_2 tension until blastocyst formation;~~

~~c) transferring the blastocyst to a layer of feeder cells, and culturing the transferred blastocyst under high O_2 tension;~~

~~d) mechanically isolating an inner cell mass (ICM) from trophectoderm of the blastocyst of (c); and~~

~~e) culturing the cells of the ICM of (d) on a layer of feeder cells,~~

~~wherein culturing (e) is carried out under high O_2 tension, thereby producing human stem cells, wherein the human stem cells are further cultured in neuronal induction media and maintained in neuronal proliferation media.~~

~~2. The neuronal stem cell of claim 1, wherein the cell is histocompatible with the oocyte donor.~~

~~3. The neuronal stem cell of claim 2, wherein the cell is histocompatible with a population group based on a matching haplotype.~~

~~4. The neuronal stem cell of claim 1, wherein the cell has a different pattern of zygosity from an ESC.~~

~~5. The neuronal stem cell of claim 1, wherein the cell contains only the maternal genome.~~

~~6. The neuronal stem cell of claim 1, wherein the cell is histocompatible with the oocyte donor, has a different pattern of zygosity from an ESC and contains only the maternal genome.~~

~~7. The neuronal stem cell of claim 1, wherein the cell is transplantable to humans.~~

~~8. The neuronal stem cell of claim 1, wherein the cell is undifferentiated, partially differentiated or fully differentiated.~~

~~9. The neuronal stem cell of claim 1, wherein the cell can be differentiated into a neuronal cell.~~

~~10. The neuronal stem cell of claim 9, wherein the cell can be differentiated into a neuronal cell selected from the group consisting of: a neuron, a glial cell, an oligodendrocyte and an astrocyte.~~

~~11. The differentiated neuronal cell of claim 10, wherein the cell is a neuron.~~

~~12. The neuron of claim 11, wherein the neuron is selected from the group consisting of: a cholinergic neuron, a GABAergic neuron, a glutamatergic neuron, a dopaminergic neuron and a serotonergic neuron.~~

~~13. The neuron of claim 12, wherein the neuron is a dopaminergic neuron.~~

~~14. The neuronal stem cell of claim 1, wherein the cell expresses neural markers selected from the group consisting of: SOX2, Nestin, Mushashi-1, TUBB3, MAP2, FOXO4, GFAP, CD113 and CD15.~~

~~15~~1. A method ~~for~~ of producing neuronal stem cells, the method comprising:

a) parthenogenetically activating a human oocyte, wherein activating comprises:

i) contacting the oocyte with an ionophore at high O_2 tension, and

ii) contacting the oocyte with a serine-threonine kinase inhibitor under low O_2 tension;

b) cultivating the activated oocyte of (a) at low O_2 tension until blastocyst formation;

c) transferring the blastocyst to a layer of feeder cells, and culturing the transferred blastocyst under high O_2 tension;

d) mechanically isolating an inner cell mass (ICM) from trophectoderm of the blastocyst of (c);

e) culturing the cells of the ICM of (d) on a layer of feeder cells,

wherein culturing (e) is carried out under high O_2 tension, thereby producing human stem cells;

f) growing parthenogenetically derived human stem cells on a feeder layer of fibroblast cells for at least 2 days;

g) growing parthenogenetically derived human stem cells on a petri dish without fibroblast feeder layer for at least 1 day;

h) culturing the cells in a neuronal induction media;

i) obtaining a single cell suspension of the cells from (h); and

j) culturing the single cells from step (i) on a petri dish with no fibroblast feeder layer in a neuronal proliferation media.

~~16~~2. The method of claim ~~15~~1, wherein the neuronal induction media comprises:

a) Penicillin-Streptomycin-Amphotericin Solution

b) DMEM/F12;

c) MEM Non-Essential Amino Acids Solution;

d) L-Glutamine;

e) N2 Supplement; and

f) bFGF.

~~17. The method of claim 15, wherein the neuronal proliferation media comprises:~~

~~a) Penicillin-Streptomycin-Amphotericin;~~

~~b) DMEM/F12;~~

~~c) GlutaMAX™-I;~~

~~d) StemPro® Neural Supplement;~~

~~e) bFGF; and~~

~~f) EGF.~~

~~18~~3. The method of claim ~~15~~1, wherein the petri dish is coated with ~~CELLstart~~ an extracellular matrix substrate.

~~19. A neuronal stem cell produced by the method of claim 15.~~

~~20~~4. The method of claim ~~15~~1, wherein a neuroepithelial rosette forms in about 1-2 weeks.

~~21. Isolated neuronal stem cells derived using the method comprising:~~

~~a) parthenogenetically activating a human oocyte, wherein activating comprises:~~

~~i) contacting the oocyte with an ionophore at high O_2 tension, and~~

~~ii) contacting the oocyte with a serine-threonine kinase inhibitor under low O_2 tension;~~

~~b) cultivating the activated oocyte of (a) at low O_2 tension until blastocyst formation;~~

~~c) transferring the blastocyst to a layer of feeder~~

~~transferred blastocyst under high O_2 tension;~~

~~d) mechanically isolating an inner cell mass (IC~~

~~blastocyst of (c);~~

~~e) culturing the cells of the ICM of (d) on a laye~~

~~wherein culturing (e) is carried out under high O_2 tensio~~

~~stem cells;~~

~~f) growing parthenogenetically derived human s~~

~~fibroblast cells for at least 2 days;~~

~~g) growing parthenogenetically derived human s~~

~~fibroblast feeder layer for at least 1 day;~~

~~h) culturing the cells in a neuronal induction me~~

i) obtaining a single cell suspension of the cells

~~31. The cells of claim 21, wherein the cell contains only the maternal genome.~~

~~32. The neuronal stem cell of claim 21, wherein the cell is histocompatible with the oocyte donor, has a different pattern of zygosity from an ESC and contains only the maternal genome.~~

~~33. The neuronal stem cell of claim 21, wherein the cell is transplantable to humans.~~

~~34. A method of differentiating neuronal stem cells, the method comprising culturing neuronal stem cells in neuronal differentiation media.~~

~~35. The method of claim 34, wherein the neuronal differentiation media comprises:~~

~~a) Penicillin-Streptomycin-Amphotericin;~~

~~b) DMEM/F12;~~

~~c) GlutaMAX™ I; and~~

~~d) StemPro® Neural Supplement.~~

~~36. The method of claim 34 wherein the neuronal stem cells are differentiated into a neuronal cell selected from the group consisting of: a neuron, a glial cell, an oligodendrocyte and an astrocyte.~~

~~37. The differentiated neuronal cell produced by the method of claim 36, wherein the cell is a neuron.~~

~~38. The neuron of claim 37, wherein the neuron is selected from the group consisting of: a cholinergic neuron, a GABAergic neuron, a glutamatergic neuron, a dopaminergic neuron and a serotonergic neuron.~~

~~39. The neuron of claim 38, wherein the neuron is a dopaminergic neuron.~~

~~40. Differentiated cells produced by the method of claim 34.~~

~~41. The cells produced by the method of claim 34, wherein the cells are differentiated from a parthenogenetically activated oocyte.~~

~~42~~5. A method ~~for~~ of producing neuronal stem cells comprising:

a) parthenogenetically activating a human oocyte, wherein activating comprises:

i) contacting the oocyte with an ionophore at high O_2 tension, and

ii) contacting the oocyte with a serine-threonine kinase inhibitor under low O_2 tension;

b) cultivating the activated oocyte of (a) at low O_2 tension until blastocyst formation;

c) transferring the blastocyst to a layer of feeder cells, and culturing the transferred blastocyst under high O_2 tension;

d) mechanically isolating an inner cell mass (ICM) from trophectoderm of the blastocyst of (c);

e) culturing the cells of the ICM of (d) on a layer of feeder cells,

wherein culturing (e) is carried out under high O_2 tension, thereby producing human stem cells;

f) cultivation of human stem cells in feeder-free conditions;

g) exposure of said cells to neuronal induction medium;

h) mechanical isolation of partially differentiated cells; and

i) further expansion and maintenance of said cells until maturation.

436. The method of claim-425, wherein the neuronal induction media comprises:

a) Penicillin-Streptomycin-Amphotericin Solution;

b) DMEM/F12;

c) MEM Non-Essential Amino Acids Solution;

d) L-Glutamine;

e) N2 Supplement; and

f) bFGF.

~~44. The method of claim 42, wherein the neuronal proliferation media comprises:~~

~~a) Penicillin-Streptomycin-Amphotericin;~~

~~b) DMEM/F12;~~

~~c) GlutaMAX™-I;~~

~~d) StemPro® Neural Supplement;~~

~~e) bFGF; and~~

~~f) EGF.~~

457. The method of claim 425, wherein feeder-free conditions utilize the ECM substrate are selected from the group consisting of: laminin, gelatin, fibronectin and a combination thereof.

~~46. The neuronal stem cell produced by the method of claim 42.~~

478. The method of claim 425, wherein a neuroepithelial rosette forms after 1-2 weeks.

~~48. Isolated neuronal stem cells derived using the method comprising:~~

a) parthenogenetically activating a human oocyte, wherein activating comprises:

i) contacting the oocyte with an ionophore at high O_2 tension, and

ii) contacting the oocyte with a serine-threonine kinase inhibitor under low O_2 tension;

b) cultivating the activated oocyte of (a) at low O_2 tension until blastocyst formation;

c) transferring the blastocyst to a layer of feeder cells, and culturing the transferred blastocyst under high O_2 tension;

d) mechanically isolating an inner cell mass (ICM) from trophectoderm of the blastocyst of step (c);

e) culturing the cells of the ICM of (d) on a layer of feeder cells, wherein culturing (e) is carried out under high O_2 tension, thereby producing human stem cells;

f) cultivation of the human stem cells in feeder-free conditions;

g) exposure of said cells to neuronal induction medium;

h) mechanical isolation of partially differentiated cells; and

i) further expansion and maintenance of said cells until maturation.

49. The cells of claim 48, wherein the cells express neural stem cell markers selected from the group consisting of: SOXB1 family NES, MSH-1, CXCR4, CCND1, LHX2, PAX6 and GAP43.

50. The cells of claim 48, wherein the neuronal stem cells maintain the neuronal phenotype for at least 30 passages.

51. The cells of claim 48, wherein the neuronal stem cells can differentiate into neuronal cells.

52. The cells of claim 51, wherein the neuronal cells are selected from the group consisting of neurons, astrocytes and oligodendrocytes.

53. A method of differentiating neuronal stem cells, the method comprising culturing neuronal stem cells in neuronal differentiation media.

54. The method of claim 53, wherein the neuronal differentiation media comprises:

a) Penicillin-Streptomycin-Amphotericin;

b) DMEM/F12;

c) GlutaMAX™-I; and

~~d) StemPro® Neural Supplement.~~

~~55. The method of claim 53 wherein the neuronal stem cells are differentiated into a neuronal cell selected from the group consisting of: a neuron, an oligodendrocyte and an astrocyte.~~

~~56. The differentiated cells produced by the method of claim 53.~~

~~57. The cells of claim 53, wherein the cells are differentiated from a parthenogenetically activated oocyte produced by the method comprising:~~

~~a) parthenogenetically activating a human oocyte, wherein activating comprises: i) contacting the oocyte with an ionophore at high O_2 tension, and~~

~~ii) contacting the oocyte with a serine threonine kinase inhibitor under low O_2 tension;~~

~~b) cultivating the activated oocyte of (a) at low O_2 tension until blastocyst formation;~~

~~c) transferring the blastocyst to a layer of feeder cells, and culturing the transferred blastocyst under high O_2 tension;~~

~~d) mechanically isolating an inner cell mass (ICM) from trophectoderm of the blastocyst of step (c); and~~

~~e) culturing the cells of the ICM of (d) on a layer of feeder cells,~~

~~wherein culturing (e) is carried out under high O_2 tension, thereby producing human stem cells.~~

Claims

What is claimed is:

1. A method of producing neuronal stem cells, the method comprising:

a) parthenogenetically activating a human oocyte, wherein activating comprises:

i) contacting the oocyte with an ionophore at high O_2 tension, and

ii) contacting the oocyte with a serine-threonine kinase inhibitor under low O_2 tension;

b) cultivating the activated oocyte of (a) at low O_2 tension until blastocyst formation;

c) transferring the blastocyst to a layer of feeder cells, and culturing the transferred blastocyst under high O_2 tension;

d) mechanically isolating an inner cell mass (ICM) from trophectoderm of the blastocyst of (c);

e) culturing the cells of the ICM of (d) on a layer of feeder cells,

wherein culturing (e) is carried out under high O_2 tension, thereby producing human stem cells;

f) growing parthenogenetically derived human stem cells on a feeder layer of fibroblast cells for at least 2 days;

g) growing parthenogenetically derived human stem cells on a petri dish without fibroblast feeder layer for at least 1 day;

h) culturing the cells in a neuronal induction media;

i) obtaining a single cell suspension of the cells from (h); and

j) culturing the single cells from step (i) on a petri dish with no fibroblast feeder layer in a neuronal proliferation media.

2. The method of claim 1, wherein the neuronal induction media comprises:

a) Penicillin-Streptomycin-Amphotericin Solution

b) DMEM/F12;

c) MEM Non-Essential Amino Acids Solution;

d) L-Glutamine;

e) N2 Supplement; and

f) bFGF.

3. The method of claim 1, wherein the petri dish is coated with an extracellular matrix substrate.

4. The method of claim 1, wherein a neuroepithelial rosette forms in about 1-2 weeks.

5. A method of producing neuronal stem cells comprising:

a) parthenogenetically activating a human oocyte, wherein activating comprises:

i) contacting the oocyte with an ionophore at high O_2 tension, and

ii) contacting the oocyte with a serine-threonine kinase inhibitor under low O_2 tension;

b) cultivating the activated oocyte of (a) at low O_2 tension until blastocyst formation;

c) transferring the blastocyst to a layer of feeder cells, and culturing the transferred blastocyst under high O_2 tension;

d) mechanically isolating an inner cell mass (ICM) from trophectoderm of the blastocyst of (c);

e) culturing the cells of the ICM of (d) on a layer of feeder cells,

wherein culturing (e) is carried out under high O_2 tension, thereby producing human stem cells;

f) cultivation of human stem cells in feeder-free conditions;

g) exposure of said cells to neuronal induction medium;

h) mechanical isolation of partially differentiated cells; and

i) further expansion and maintenance of said cells until maturation.

6. The method of claim 5, wherein the neuronal induction media comprises:

a) Penicillin-Streptomycin-Amphotericin Solution;

b) DMEM/F12;

c) MEM Non-Essential Amino Acids Solution;

d) L-Glutamine;

e) N2 Supplement; and

f) bFGF.

7. The method of claim 5, wherein feeder-free conditions utilize the ECM substrate are selected from the group consisting of: laminin, gelatin, fibronectin and a combination thereof.

8. The method of claim 5, wherein a neuroepithelial rosette forms after 1-2 weeks.