Claims

1. A method of differentiating neural cells, the method comprising:

(a) performing a first incubation comprising culturing pluripotent stem cells in a non-adherent culture vessel under conditions to produce a cellular spheroid, wherein beginning at the initiation of the first incubation (day 0) the cells are exposed to (i) an inhibitor of TGF-β/activating-Nodal signaling; (ii) at least one activator of Sonic Hedgehog (SHH) signaling; (iii) an inhibitor of bone morphogenetic protein (BMP) signaling; and (iv) an inhibitor of glycogen synthase kinase 3β (GSK3β) signaling; and

(b) performing a second incubation comprising culturing cells of the spheroid in a substrate-coated culture vessel under conditions to neurally differentiate the cells.

2. The method of claim 1, wherein the second incubation begins on about day 7.

3. The method of claim 1 or claim 2, wherein the cells are exposed:

(i) to the inhibitor of TGF-β/activating-Nodal signaling up to a day at or before day 7 (ii) to the inhibitor of TGF-β/activating-Nodal beginning at day 0 and through day 6, inclusive of each day;

(iii) to the at least one activator of SHH signaling up to a day at or before day 7; (iv) to the at least one activator of SHH signaling beginning at day 0 and through day 6, inclusive of each day; (v) to the inhibitor of BMP signaling up to a day at or before day 11; (vi) to the inhibitor of BMP signaling beginning at day 0 and through day 10, inclusive of each day; (vii) to the inhibitor of GSK3β signaling up to a day at or before day 13; (viii) to the inhibitor of GSK3b signaling beginning at day 0 and through day 12, inclusive of each day;

(ix) to transforming growth factor beta-3 (TGFβ3) (collectively, "BAGCT") and an inhibitor of Notch signaling beginning on day 11; and/or

(x) to BAGCT and the inhibitor of Notch signaling beginning at day 11 and until harvest of the neurally differentiated cells, optionally until day 18, optionally until day 25.

4. The method of any one of claims 1-3, wherein culturing the cells under conditions to neurally differentiate the cells comprises exposing the cells to (i) brain-derived neurotrophic factor (BDNF); (ii) ascorbic acid; (iii) glial cell-derived neurotrophic factor (GDNF); (iv) dibutyryl cyclic AMP (dbcAMP); (v) transforming growth factor beta-3 (TGFβ3) (collectively, "BAGCT"); and (vi) an inhibitor of Notch signaling.

5. The method of claim 1, wherein:

(a) beginning at the initiation of the first incubation day (day 0), the cells are exposed to (i) an inhibitor of TGF-β/activating-Nodal signaling through day 6, each day inclusive; (ii) at least one activator of Sonic Hedgehog (SHH) signaling through day 6, each day inclusive; (iii) an inhibitor of bone morphogenetic protein (BMP) signaling through day 6, each day inclusive; and (iv) an inhibitor of glycogen synthase kinase 3β (GSK3β) signaling through day 6, each day inclusive; and

(b) the performing a second incubation begins on day 7, and comprises culturing the cells in a culture vessel coated with a substrate selected from among: laminin, collagen, entactin, heparin sulfate proteoglycans, and combinations thereof, wherein beginning on day 7, the cells are exposed to (i) an inhibitor of BMP signaling and (ii) an inhibitor of GSK3β signaling; and beginning on day 11, the cells are exposed to (i) brain-derived neurotrophic factor (BDNF); (ii) ascorbic acid; (iii) glial cell-derived neurotrophic factor (GDNF); (iv) dibutyryl cyclic AMP (dbcAMP); (v) transforming growth factor beta-3 (TGFβ3) (collectively, "BAGCT"); and (vi) an inhibitor of Notch signaling.

6. The method of any one of claims 1-5, further comprising harvesting the neurally differentiated cells, optionally wherein the harvesting is carried out at about day 16 or later, wherein the harvesting is carried out between about day 16 and about day 30, wherein the harvesting is carried out between day 18 and day 25, and/or wherein the harvesting is carried out at or about at day 18 or about at day 25.

7. The method of any one of claims 6, wherein the neurally differentiated cells are determined dopaminergic neuron progenitor cells, optionally wherein the dopaminergic neuron progenitor cells are capable of innervating host tissue upon transplantation into a subject.

8. The method of any one of claims 1-7, wherein, prior to performing the second incubation, the spheroid is dissociated to produce a cell suspension, and cells of the cell suspension are cultured in the substrate-coated culture vessel, optionally wherein the dissociating is carried out at a time when the spheroid cells express at least one of PAX6 and OTX2, and/or wherein the dissociating is carried out on about day 7, and/or wherein the spheroid is dissociated by enzymatic dissociation.

9. The method of any one of claims 1-**Error! Reference source not found.**, wherein the inhibitor of TGF-β/activating-Nodal signaling is SB431542;

wherein the at least one activator of SHH signaling is SHH or purmorphamine; and/or wherein the at least one activator of SHH signaling comprises two activators of SHH signaling selected from SHH protein and purmorphamine; and/or wherein the inhibitor of BMP signaling is LDN193189, and/or wherein the inhibitor of GSK3β signaling is CHIR99021; and/or wherein the inhibitor of Notch signaling is DAPT.

10. The method of any one of claims 1-**Error! Reference source not found.**, wherein the culturing in the first incubation and/or the second incubation is carried out in media comprising serum or a serum replacement, or wherein the cells are cultured in the absence of serum for the duration of culture.

11. The method of any one of claims 1-10, wherein the cells are exposed to an inhibitor of Rho-associated protein kinase (ROCK) signaling on day 0, day 7, day 16, and/or day 20, optionally wherein the ROCK inhibitor is Y-27632.

12. The method of any one of claims 1-11, wherein at least about 50% of the media is replaced daily or every other day, or every third day.

13. The method of any one of claims 6-12, further comprising formulating the harvested cells with a cryoprotectant, optionally further comprising cryopreserving the harvested cells, and optionally thawing the cells prior to use.

14. The method of any one of claims 1-13, wherein the pluripotent stem cells are embryonic stem (ES) cells, induced pluripotent stem cells (iPSCs), or a combination thereof.

15. The method of any one of claims 1-14, wherein the pluripotent stem cells are human induced pluripotent stem cells, and/or wherein the pluripotent stem cells are autologous to a subject to be treated with the neurally differentiated cells or allogeneic to a subject to be treated with the neurally differentiated cells.

16. The method of any one of claims 1-15, wherein the pluripotent stem cells are hypoimmunogenic, optionally wherein the pluripotent stem cells are engineered to (a) remove genes encoding one or more of polymorphic HLA-A/-B/-C and HLA class II molecules; and (b) to provide genes encoding one or more of PD-L1, HLA-G, and CD47, optionally into a AAVS1 safe harbor locus.

17. A therapeutic composition produced by the method of any of claims 1-16.

18. The therapeutic composition of claim 17, wherein cells of the composition express EN1 and CORIN and less than 10% of the total cells in the composition express TH.

19. A therapeutic composition comprising determined dopamine neuron progenitor cells (DDPCs) derived from pluripotent stem cells, wherein cells in the composition express EN1 and CORIN and less than 10% of the total cells in the composition express TH.

20. A therapeutic composition comprising determined dopamine neuron progenitor cells (DDPCs) derived from pluripotent stem cells, wherein the therapeutic composition exhibits a ratio of counts per million (CPM) *TH* to CPM *GAPDH* of less than about 2.5×10^{-2}.

21. A therapeutic composition comprising determined dopamine neuron progenitor cells (DDPCs) derived from pluripotent stem cells, wherein the therapeutic composition exhibits one or more of:

(a) a ratio of counts per million (CPM) *NEUROG1* to CPM *GAPDH* of greater than about 2×10^{-5};

(b) a ratio of CPM *EDN3* to CPM *GAPDH* of greater than about 5×10^{-4};

(c) a ratio of CPM *HES1* to CPM *GAPDH* of greater than about 4×10^{-3};

(d) a ratio of CPM *PSRC1* to CPM *GAPDH* of greater than about 8×10^{-3};

(e) a ratio of CPM *NEK6* to CPM *GAPDH* of greater than about 2×10^{-3};

(f) a ratio of CPM *IQGAP3* to CPM *GAPDH* of greater than about 1 x 10^{-2};

(g) a ratio of CPM *USP44* to CPM *GAPDH* of greater than about 8 x 10^{-4};

(h) a ratio of CPM *CEP55* to CPM *GAPDH* of greater than about 3 x 10^{-3};

(i) a ratio of CPM *KIF20A* to CPM *GAPDH* of greater than about 2 x 10^{-2};

(j) a ratio of CPM *AURKA* to CPM *GAPDH* of greater than about 2 x 10^{-2};

(k) a ratio of CPM *CALCA* to CPM *GAPDH* of less than about 1 x 10^{-3};

(l) a ratio of CPM *GLRA2* to CPM *GAPDH* of less than about 1 x 10^{-3};

(m) a ratio of CPM *MAPT* to CPM *GAPDH* of less than about 1 x 10^{-1};

(n) a ratio of CPM *CAMK2B* to CPM *GAPDH* of less than about 5 x 10^{-2};

(o) a ratio of CPM *SYT13* to CPM *GAPDH* of less than about 1 x 10^{-2};

(p) a ratio of CPM *LHFPL4* to CPM *GAPDH* of less than about 1 x 10^{-3};

(q) a ratio of CPM *RET* to CPM *GAPDH* of less than about 5 x 10^{-3};

(r) a ratio of CPM *KCND3* to CPM *GAPDH* of less than about 1 x 10^{-2};

(s) a ratio of CPM *NSG2* to CPM *GAPDH* of less than about 5 x 10^{-2}; and

(t) a ratio of CPM *SNAP25* to CPM *GAPDH* of less than about 2x 10^{-2},

optionally wherein the therapeutic composition exhibits between two and twenty of (a)-(t).

22. The therapeutic composition of any one of claims 17 and 19-20, wherein cells in the therapeutic composition express EN1 and CORIN.

23. The therapeutic composition of any one of claims 16-21, wherein the therapeutic composition exhibits:

(a) a ratio of counts per million (CPM) *EN1* to CPM *GAPDH* of greater than about 1 x 10^{-4}; and/or

(b) a ratio of CPM *CORIN* to CPM *GAPDH* of greater than about 2 x 10^{-2}; and/or

(c) a ratio of counts per million (CPM) *TH* to CPM *GAPDH* of less than about 3 x 10^{-2}.

24. The therapeutic composition of any one of claims16 and 20-22, wherein cells in the composition express TH.

25. The therapeutic composition of any one of claims 17 and 19-23, wherein less than 10% of the total cells in the composition express TH.

26. The therapeutic composition of any one of claims 16-24, wherein the determined cells are capable of engrafting and innervating other cells in vivo; and/or wherein the determined cells are capable of producing dopamine, optionally wherein the determined cells do not produce or do not substantially produce norepinephrine; and/or wherein the therapeutic composition comprises a cryoprotectant; and/or wherein the composition is for use in treatment of a neurodegenerative disease or condition in a subject.

27. The therapeutic composition of any one of claims 16-25, wherein the composition is for use in treatment of a neurodegenerative disease or condition in a subject, wherein the neurodegenerative disease or condition comprises a loss of dopaminergic neurons, optionally wherein the neurodegenerative disease or condition comprises a loss of dopaminergic neurons in the SN or in the SNc, or wherein the neurodegenerative disease or condition is Parkinson's disease.

28. The therapeutic composition according to any one of claims 16-26, for use in a method of treatment, comprising administering to a subject in need thereof a therapeutically effective amount of the therapeutic composition, optionally wherein the subject has a neurodegenerative disease or condition, wherein the subject has a neurodegenerative disease or condition comprising the loss of dopaminergic neurons, wherein the subject has Parkinsonism, and/or wherein the subject has Parkinson's disease, optionally idiopathic Parkinson's disease.

29. The therapeutic composition for use according to claim 27, wherein the administering comprises delivering cells of a composition by stereotactic injection, wherein the administering comprises delivering cells of a composition through a catheter, wherein the cells are delivered to the striatum of the subject, wherein the cells of the therapeutic composition are autologous to the subjector allogeneic to the subject, and/or wherein the cells of the therapeutic composition are hypoimmunogenic, optionally wherein the cells of the therapeutic composition are engineered to (a) remove genes encoding one or more of polymorphic HLA-A/-B/-C and HLA class II molecules; and (b) to

provide genes encoding one or more of PD-L1, HLA-G, and CD47, optionally into a AAVS1 safe harbor locus.