Claims

1. A method of differentiating pluripotent cells comprising:

   a. treating pluripotent stem cells cultured in a planar adherent culture with a chelating agent or enzyme to release cell aggregates from the planar adherent culture;

   b. transferring the cell aggregates from the planar adherent culture to a dynamic suspension culture in the presence of a Rho-kinase inhibitor;

   c. diluting the culture using culture media and a Rho-kinase inhibitor to a concentration of cells from about 1 to about 1.5 million cells/ml;

   d. expanding the suspension of cell aggregates to generate pluripotent stem cell clusters; and

   e. differentiating the pluripotent stem cell clusters in the dynamic suspension culture system, wherein the step of differentiating comprises differentiating posterior foregut (Stage 3) cells into pancreatic endocrine precursor (Stage 4) cells in a medium supplemented with a Cyp26 inhibitor, and wherein the expanding comprises an environment that includes from about 0.1% to about 2% of bovine serum albumin.

2. The method claim 1, wherein the method increases the percentage of cells in G0/G1 phase of the cell cycle.

3. The method of claim 1 or claim 2, wherein the Rho kinase inhibitor in (i) step b., (ii) step. c, or (iii) step b. and step c. is Y-27632.

4. The method of any one of claims 1 to 3, wherein the pluripotent stem cells are adherent.

5. The method of any one of claims 1 to 4, wherein the pluripotent stem cells are human pluripotent stem cells.

6. The method of claim 5, wherein the human pluripotent stem cells are human embryonic stem cells.

7. The method of any one of claims 1 to 6, wherein the pluripotent stem cells are treated with a chelating agent.

8. The method of claim 7, wherein the chelating agent is EDTA.

9. The method of any one of claims 1 to 8, wherein the pluripotent stem cells are treated with an enzyme.

10. The method of claim 9, wherein the enzyme is a neutral protease or Accutase.

11. The method of any one of claims 1 to 10, wherein the treating in step a. does not completely disaggregate the cells to a single cell suspension.

12. The method of any one of claims 1 to 11, wherein the dynamic suspension culture system comprises microcarriers.

13. The method of any one of claims 1 to 12, wherein the medium supplemented with the Cyp26 inhibitor is further supplemented with an ALK5 inhibitor.

14. The method of claim 13, where the medium is further supplemented with a PKC activator.

15. The method of any one of claims 1 to 14, wherein the medium contains about 8 mM glucose.

16. The method of any one of claims 1 to 14, wherein the medium contains about 25 mM glucose.

17. The method of any one of claims 1 to 12, 15 or 16, wherein the medium supplemented with the Cyp26 inhibitor is further supplemented with SANT, TPPB and either LDN or SCIO.

18. The method of any one of claims 1 to 17, wherein the step of differentiating comprises differentiating the pluripotent stem cell clusters to definitive endoderm (Stage 1) cells in a culture medium supplemented with a cyclic aniline-pyridinotriazine and GDF8.

19. The method of claim 18, wherein the cyclic aniline-pyridinotriazine is 14-Prop-2-en-1-yl-3,5,7,14,17,23,27-heptaazatetracyclo[19.3.1.1~2,6 ~.1~8,12.~]heptacosa-1(25),2(27),3,5,8(26),9,11,21,23-non- aen-16-one.

20. The method of claim 19, wherein the medium is supplemented with 8mM glucose.

21. The method of any one of claims 18 to 20, wherein the method further comprises culturing the Stage 1 cells in a medium supplemented with FGF7 to differentiate the Stage 1 cells into Stage 2 cells.

22. The method of claim 21, wherein the method further comprises culturing the Stage 2 cells in a medium supplemented with FGF7, retinoic acid, SANT-1, TPPB and optionally LDN to differentiate the Stage 2 cells into Stage 3 cells.

23. The method of claim 22, wherein the medium is further supplemented with activin A.

24. The method of claim 22 or claim 23, wherein the method comprises differentiating the posterior foregut (Stage 3) cells into pancreatic endocrine precursor (Stage 4) cells in a medium supplemented with a Cyp26 inhibitor, an ALK5 inhibitor and a PKC activator.

25. The method of claim 22 or claim 23, wherein the method comprises differentiating the posterior foregut (Stage 3) cells into pancreatic endocrine precursor (Stage 4) cells in a medium supplemented with a Cyp26 inhibitor, SANT, TPPB and either LDN or SCIO.

26. The method of any one of claims 21 to 25, wherein the medium used for differentiation into Stage 1 cells, Stage 2 cells, Stage 3 cells, and Stage 4 cells is supplemented with about 8mM glucose.

27. The method of any one of claims 21 to 25, wherein the medium used for differentiation into Stage 3 cells and Stage 4 cells is supplemented with about 25 mM glucose.