Claims

1. An in vitro differentiated population of pancreatic endoderm cells obtained from the stepwise differentiation of pluripotent cells, wherein cells at each stage of differentiation are cultured in medium comprising 8 mM to 20 mM glucose, wherein greater than 30% of the differentiated pancreatic endoderm cells are PDX-1 + NKX6.1 +, SOX2-, and CDX2-, and

wherein the stepwise differentiation comprises culturing gut tube cells in a medium further supplemented with SANT-1, FGF 7, TPB, activin A, retinoic acid, and LDN-193189.

2. The differentiated pancreatic endoderm cell population of claim 1, wherein greater than 10% of the cells in the differentiated population are single hormonal insulin positive cells.

3. The differentiated pancreatic endoderm cell population of claim 1 or 2, wherein the stepwise differentiation comprises culturing undifferentiated human embryonic stem cells in medium further supplemented with activin A.

4. The differentiated pancreatic endoderm cell population of claim 1 or 2, wherein the stepwise differentiation comprises culturing undifferentiated human embryonic stem cells in medium further supplemented with a WNT activator.

5. The differentiated pancreatic endoderm cell population of claim 1, wherein the stepwise differentiation comprises culturing definitive endoderm (DE) cells in medium further supplemented with FGF 7.

6. The differentiated pancreatic endoderm cell population of claim 4, wherein the stepwise differentiation comprises culturing posterior foregut cells in medium further supplemented with TPB, SANT-1, retinoic acid, and LDN-193189.

7. The differentiated pancreatic endoderm cell population of claim 1 or 2, wherein the stepwise differentiation comprises culturing cells in medium further supplemented with ascorbic acid.

8. An in vitro method, wherein the in vitro method stepwise differentiates human pluripotent stem cells into a pancreatic endoderm lineage cell population,

Comprises culturing cells in each differentiation stage in a medium containing 8 mM to 20 mM glucose, and

Comprises differentiating the human pluripotent stem cells into definitive endoderm (DE) cells by culturing human pluripotent stem cells in a medium supplemented with activin A and WNT activators;

Differentiating the DE cells into gut tube cells by culturing the DE cells in a medium supplemented with FGF 7; and

Differentiating the gut tube cells into posterior foregut by culturing gut tube cells in a medium supplemented with SANT-1, FGF 7, TPB, activin A, retinoic acid, and LDN-193189, wherein over 30% of the pancreatic endoderm cell population is PDX-1+, NKX6.1+, SOX2- and CDX2-.

9. The in vitro method of claim 8, further comprising differentiating the posterior foregut cells into pancreatic foregut cells by culturing the posterior foregut cells in a medium supplemented with TPB, SANT-1, retinoic acid, and LDN-193189.

10. The in vitro method of claim 9, further comprising differentiating the pancreatic foregut cells into pancreatic endoderm cells by culturing the pancreatic foregut cells in a medium supplemented with SANT-1, ALK5 inhibitor, and retinoic acid.

11. The in vitro method of claim 10, further comprising differentiating pancreatic endoderm cells into a pancreatic beta cell population.

12. The in vitro method of any one of claims 8 to 11, wherein the medium is further supplemented with ascorbic acid in one or more stages.

13. The in vitro method of any one of claims 8 to 11, wherein greater than 10% of cells in the differentiated population are single hormonal insulin positive cells.

14. An in vitro method for differentiating human pluripotent stem cells into pancreatic beta cells, wherein the in vitro method differentiates human pluripotent stem cells into pancreatic beta cells, and comprises

a) generating a definitive endoderm (DE) cell population by culturing undifferentiated human pluripotent stem cells in a medium supplemented with 8 mM to 20 mM glucose, activin A, and WNT activator;

b) generating a gut tube cell population by culturing the DE cells in a medium supplemented with 8 mM to 20 mM glucose and FGF 7;

c) generating a posterior foregut cell population expressing PDX-1 and SOX2 by culturing the gut tube cells in a medium supplemented with 8 mM to 20 mM glucose, SANT-1, FGF 7, TPB, activin A, retinoic acid, and LDN-193189;

d) generating a pancreatic foregut cell population expressing PDX-1 and NKX6.1 and expressing a lower level of SOX2 compared to the posterior foregut cells by culturing the posterior foregut cells in a medium supplemented with 8 mM to 20 mM glucose, TPB, SANT-1, retinoic acid, and LDN-193189;

e) obtaining a pancreatic endoderm cell population expressing PDX-1 and a higher level of NKX6.1 and a lower level of SOX2 compared to the pancreatic foregut cells by culturing the pancreatic foregut cells in a medium supplemented with 8 mM to 20 mM glucose, SANT-1, ALK5 inhibitor, and retinoic acid; and

f) differentiating the pancreatic endoderm cells into a pancreatic beta cell population.

15. The in vitro method of claim 14, wherein the pancreatic beta cell population is PDX-1+, NKX6.1+, SOX2- and CDX2-.

16. The in vitro method of claim 14 or 15, wherein the medium is further supplemented with ascorbic acid in one or more stages.

17. The in vitro method of claim 16, wherein the pancreatic beta cells are single hormone-producing insulin cells, which are also NKX6.1+ and PDX-1+.