CLAIMS

What is claimed is:

1. An in vitro differentiated population of pancreatic endoderm cells obtained from the stepwise differentiation of pluripotent cells, wherein cells at each step of differentiation are cultured in medium comprising 5 mM to 20 mM glucose.

2. The differentiated population of pancreatic endoderm cells of claim 1, wherein greater than 30% of the differentiated pancreatic endoderm cells are PDX-1+ NKX6.1+, SOX2-, and CDX2-.

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

4. The differentiated population of pancreatic endoderm cells of claims 1 to 3, wherein the stepwise differentiation comprises culturing undifferentiated human embryonic stem cells in medium further supplemented with a TGF-B ligand.

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

6. The differentiated population of pancreatic endoderm cells of claim 1, wherein the stepwise differentiation comprises culturing definitive endoderm cells in medium further supplemented with a FGF ligand.

7. The differentiated population of pancreatic endoderm cells of claims 1 to 4, wherein the stepwise differentiation comprises culturing gut tube cells in medium further supplemented with a shh inhibitor, a FGF ligand, a PKC activator, a TGF-B ligand, a retinoid, and a gradient of a BMP inhibitor.

8. The differentiated population of pancreatic endoderm cells of claim 5, wherein the stepwise differentiation comprises culturing posterior foregut cells in medium further supplemented with a PKC activator, a shh inhibitor, a retinoid, and a BMP inhibitor.

9. The differentiated population of pancreatic endoderm cells of claims 1 to 6, wherein the stepwise differentiation comprises culturing cells in medium further supplemented with ascorbic acid.

10. An in vitro method for the stepwise differentiation of pluripotent cells into a population of cells of the pancreatic endoderm lineage, which comprises culturing the cells at each stage of differentiation in medium comprising 5 mM to 20 mM glucose.

11. The in vitro method of claim 10, further comprising differentiating the pluripotent cells into definitive endoderm (DE) cells by culturing the pluripotent cells in medium supplemented with a TGF-B ligand and a WNT activator.

12. The in vitro method of claim 11, further comprising differentiating the DE cells into gut tube cells by culturing the DE cells in medium supplemented with a FGF ligand.

13. The in vitro method of claim 12, further comprising differentiating the gut tube cells into posterior foregut endoderm cells by culturing the gut tube cells in medium supplemented with a shh inhibitor, a FGF ligand, a PKC activator, a TGF-B ligand, a retinoid, and a BMP inhibitor.

14. The in vitro method of claim 13, further comprising differentiating the posterior foregut endoderm cells into pancreatic foregut cells by culturing the posterior foregut endoderm cells in medium supplemented with a PKC activator, a shh inhibitor, a retinoid, and a BMP inhibitor.

15. The in vitro method of claim 14, further comprising differentiating the pancreatic foregut cells into pancreatic endoderm cells by culturing the pancreatic foregut cells in medium supplemented with a shh inhibitor, a TGF-B inhibitor, and a retinoid.

16. The in vitro method of claim 15, further comprising differentiating the pancreatic endoderm cells into a pancreatic beta cell population.

17. The in vitro method of claims 10 to 16, wherein in at least one step the medium is further supplemented with ascorbic acid.

18. The in vitro method of claims 10 to 17, wherein greater than 10% of the cells in the differentiated population are single hormonal insulin positive cells.

19. The in vitro method of claim 18, wherein greater than 30% of pancreatic endoderm cells in culture are PDX-1+, NKX6.1+, SOX2-, and CDX2-.

20. An in vitro method for differentiating human embryonic stem cells into pancreatic beta cells comprising:

a) culturing undifferentiated human embryonic stem cells in medium supplemented with glucose, a TGF-B ligand, and a WNT activator, to generate a population of definite endoderm (DE) cells;
b) culturing the DE cells in medium supplemented with glucose, and a FGF ligand to generate a population of gut tube cells;
c) culturing the gut tube cells in medium supplemented with glucose, a shh inhibitor, a FGF ligand, a PKC activator, a TGF-B ligand, a retinoid, and a gradient of a BMP inhibitor to generate a population of posterior foregut endoderm cells expressing PDX-1 and SOX2;
d) culturing the posterior foregut cells in medium supplemented with glucose, a PKC activator, a shh inhibitor, a retinoid, and a BMP inhibitor to generate a population of pancreatic foregut cells expressing PDX-1 and NKX6.1, and expressing lower level of SOX2 as compared to the posterior foregut cells;
e) culturing the pancreatic foregut cells in medium supplemented with glucose, a shh inhibitor, a TGF-B inhibitor, and a retinoid to obtain a population of pancreatic endoderm cells expressing PDX-1, a higher level of NKX6.1, and a lower level of SOX2 as compared to pancreatic foregut cells; and
f) differentiating the pancreatic endoderm cells into a pancreatic beta cell population.

21. The method of claim 20, wherein the pancreatic beta cell population is PDX-1 +, NKX6.1+, SOX2-, and CDX2-.

22. The method of claim 20 or 21, wherein in at least one step the medium is further supplemented with ascorbic acid.

23. The method of claim 22, wherein the pancreatic beta cells are single hormonal insulin-producing cells which are also NKX6.1+ and PDX-1+.

CLAIMS

1. An *in vitro* method for producing a population of pancreatic cells comprising the steps of:
a) culturing undifferentiated human pluripotent stem cells in a medium supplemented with 5 mM to 20 mM glucose, a TGF-β ligand, and a WNT activator, to generate a population of definite endoderm (DE) cells;

b) culturing the DE cells in a medium supplemented with 5 mM to 20 mM glucose, and a FGF ligand to generate a population of gut tube cells;

c) culturing the gut tube cells in medium supplemented with 5 mM to 20 mM glucose, a shh inhibitor, a FGF ligand, a PKC activator, a TGF-β ligand, a retinoid, and a gradient of a BMP inhibitor to generate a population of posterior foregut endoderm cells expressing PDX-1 and SOX2;

d) culturing the posterior foregut cells in medium supplemented with 5 mM to 20 mM glucose, a PKC activator, a shh inhibitor, a retinoid, and a BMP inhibitor to generate a population of pancreatic foregut cells expressing PDX-1 and NKX6.1, and expressing lower level of SOX2 as compared to the posterior foregut cells;

e) culturing the pancreatic foregut cells in medium supplemented with 5 mM to 20 mM glucose, a shh inhibitor, a TGF-β inhibitor, ascorbic acid and a retinoid to obtain a population of cells,

wherein said population comprises:
(i) greater than 30% of pancreatic endoderm cells that are PDX-1+, NKX6.1+, SOX2- and CDX2-, and

(ii) greater than 10% of single hormonal insulin positive cells.

2. An *in vitro* method for differentiating human pluripotent stem cells comprising the steps of:

a) culturing human pluripotent stem cells in a medium supplemented with 5 mM to 20 mM glucose, a TGF-β ligand, and a WNT activator, to generate a population of definite endoderm (DE) cells;

b) culturing the DE cells in a medium supplemented with 5 mM to 20 mM glucose, and a FGF ligand to generate a population of gut tube cells;

c) culturing the gut tube cells in medium supplemented with 5 mM to 20 mM glucose, a shh inhibitor, a FGF ligand, a PKC activator, a TGF-β ligand, a retinoid, and a gradient of a BMP inhibitor to generate a population of posterior foregut endoderm cells expressing PDX-1 and SOX2;

d) culturing the posterior foregut cells in medium supplemented with 5 mM to 20 mM glucose, a PKC activator, a shh inhibitor, a retinoid, and a BMP inhibitor to generate a population of pancreatic foregut cells expressing PDX-1 and NKX6.1, and expressing lower level of SOX2 as compared to the posterior foregut cells;

e) culturing the pancreatic foregut cells in medium supplemented with 5 mM to 20 mM glucose, a shh inhibitor, a TGF-β inhibitor, and a retinoid to obtain a population of pancreatic endoderm cells expressing PDX-1, a higher level of NKX6.1, and a lower level of SOX2 as compared to pancreatic foregut cells; and

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

3. The method of claim 2, wherein in at least one step the medium is further supplemented with ascorbic acid.

4. The method of claim 3, wherein the pancreatic β cells are single hormonal insulin-producing cells which are also NKX6.1+ and PDX-1+.

5. The method of claim 2, wherein the pancreatic β-cell population is PDX-1+, NKX6.1+, SOX2-, and CDX2-.