SUMMARY

[0007] In one aspect, there is provided an *in vitro* cell culture comprising: human cells, wherein at least 10% of said human cells are immature beta cells that express insulin (INS) and NKX6.1 and chromogranin A (CHGA); and an effective amount of Y-27632 to promote survival of the immature beta cells in the cell culture. In another aspect, there is provided a cryopreserved *in vitro* cell culture comprising: human cells, wherein the human cells comprise unipotent human immature beta cell suspension aggregates and wherein at least 10% of cells in the aggregates are human immature beta cells that express INS and NKX6.1 and CHGA, and a cryopreservation agent. In another aspect, there is provided a cryopreserved *in vitro* cell culture comprising: human cells, wherein at least 10% of said human cells are immature beta cells that express INS and NKX6.1 and CHGA; and a cryopreservation agent. In a further aspect, there is provided a cryopreserved *in vitro* cell culture comprising: human cells, wherein at least 50% of said human cells are immature beta cells that express INS and NKX6.1 and CHGA; and a cryopreservation agent. In one example, an in vitro method of producing a cell population including human PDX1 positive pancreatic endoderm cells is provided. The method includes contacting a cell population including definitive endoderm lineage cells with a TGFβ superfamily growth factor and a Wnt family member, thereby generating the cell population including PDX1 positive pancreatic endoderm cells. In examples, the TGFβ superfamily growth factor is selected from the group consisting of Nodal, Activin A, Activin B, BMP2, BMP4, GDF8, GDF-10, GDF-11 and GDF-15. In examples, the method further includes contacting the cell population including definitive endoderm lineage cells with an ERBB receptor kinase activating agent. In examples, the TGFβ superfamily growth factor is Activin A. In examples, the Wnt family member is Wnt3a. In examples, the ERBB receptor kinase activating agent is Heregulin.

[0008] In examples, the cell population including PDX1 positive pancreatic endoderm cells is contacted with at least 25 ng/mL of the TGFβ superfamily growth factor. In examples, the cell population including PDX1 positive pancreatic endoderm cells is contacted with at least 50 ng/mL of the TGFβ superfamily growth factor. In examples, the cell population including PDX1 positive pancreatic endoderm cells is contacted with at least 75 ng/mL of the TGFβ superfamily growth factor.

[00036] One example provides a composition and method for differentiating pluripotent stem cells *in vitro* to PEC cultures comprising minimal cells committed to the endocrine lineage (CHGA+). In one example, the cells committed to the endocrine lineage are characterized by the expression of CHGA. In one example, less than 50%, preferably less than 40%, 30%, more preferably less than 20%, 10%, 5%, 2%, 1% of the cells in the cells in the PEC culture are cells committed to the endocrine lineage (CHGA+).

[00037] One example provides a composition and method for differentiating pluripotent stem cells *in vitro* to substantially PEC cultures and further differentiating the PEC culture to endocrine cells *in vitro*. In one example, the endocrine cells express CHGA. In one example, the endocrine cells can produce insulin *in vitro*. In one example, the *in vitro* endocrine insulin secreting cells may produce insulin in response to glucose stimulation. In one example, more than 10% preferably more than 20%, 30%, 40% and more preferably more than 50%, 60%, 70%, 80%, 90%, 95%, 98% or 100% of the cells in the cells population are endocrine cells.

[00038] One example provides a method for differentiating pluripotent stem cells *in vitro* to endocrine cells comprising the steps of: (a) obtaining a cell population comprising PEC (b) contacting the cell population in step (a) with growth factors selected from the group consisting of a bone morphogenetic protein (BMP), a hedgehog inhibitor, Platelet-derived growth factor (PDGF), member of the fibroblast growth factor family, a retinoid, insulin growth factor 1 and 2 (IGF1 and IGF2) and nicotinamide thereby generating endocrine cells. In one example the endocrine cells express CHGA. In one example, the endocrine cells are immature insulin-producing beta-cells *in vitro*. In one example, the endocrine cells are loaded into an implantable semipermeable encapsulation device, mature and are capable of producing insulin in response to glucose stimulation *in vivo*. In one example, more than 10% preferably more than 20%, 30%, 40% and more preferably more than 50%, 60%, 70%, 80%, 90%, 95%, 98% or 100% of the cells in the cell population are endocrine cells. In one example, SSH is added at 10g/mL to 100ng/mL. In one example, PDGF is added at 10ng/mL to 100ng/mL. In one example, BMP is added at 10g/mL to 20ng/mL. In one example, FGF2 is added at 2ng/mL to 20ng/mL. In one example, IGF1 or IGF2 are added at 25ng/mL to 100ng/mL. In one example, the above compounds are added in combination with each other. In one example the member of the fibroblast growth factor family is FGF2. In one example the retinoid is retinoic acid and/or retinoic acid analogs such as TTNPB or TT3, Insulin-like growth factor I (IGF-I or IGF1) and -II (IGF-II or IGF2). In one example the bone morphogenetic protein is BMP4. In one example the hedgehog inhibitor is sonic Hedgehog,

CLAIMS

What is claimed is:

1. An *in vitro* cell culture comprising:

human cells, wherein at least 10% of said human cells are immature beta cells that express insulin (INS) and NKX6.1 and Chromogranin A (CHGA); and

an effective amount of Y-27632 to promote survival of the immature beta cells in the cell culture.

2. The cell culture of claim 1, wherein the cell culture is in a semipermeable encapsulation device.

3. The cell culture of claim 1, wherein said human immature beta cells do not substantially express NGN3.

4. A cryopreserved *in vitro* cell culture comprising:

human cells, wherein the human cells comprise unipotent human immature beta cell suspension aggregates and wherein at least 10% of cells in the aggregates are human immature beta cells that express INS and NKX6.1 and CHGA, and

a cryopreservation agent.

5. The cell culture of claim 4, wherein the unipotent human immature beta cell suspension aggregates are in a semipermeable encapsulation device.

6. The cell culture of claim 4, wherein the suspension aggregates are about 50 to 200 microns in size.

7. A cryopreserved *in vitro* cell culture comprising:

human cells, wherein at least 10% of said human cells are immature beta cells that express INS and NKX6.1 and CHGA; and

a cryopreservation agent.

8. The cell culture of claim 7, wherein said human immature beta cells do not substantially express NGN3.

9. The cell culture of claim 7, wherein the immature beta cells are in a semipermeable encapsulation device.

10. The cell culture of claim 7, wherein the immature beta cells are in a perforated encapsulation device.

11. The cell culture of claim 7, wherein said human immature beta cells further co-expresses pancreatic-duodenal homeobox factor-1 (PDX1).

12. The cell culture of claim 7, wherein said human immature beta cells are capable of maturing to mature beta cells.

13. The cell culture of claim 7, wherein said human immature beta cells further expresses MAFB.

14. The cell culture of claim 7, further comprising Chromogranin A(CHGA)-negative cells.

15. The cell culture of claim 7, further comprising a compound selected from the group consisting of bone morphogenetic protein, sonic hedgehog, platelet-derived growth factor, a member of the FGF superfamily, retinoic acid, retinoic acid analogs, insulin-like growth factor, a vitamin B3 derivative, and Nicotinamide.

16. The cell culture of claim 7, further comprising a matrix.

17. A cryopreserved *in vitro* cell culture comprising:

human cells, wherein at least 50% of said human cells are immature beta cells that express INS and NKX6.1 and CHGA; and

a cryopreservation agent.

18. The cell culture of claim 17, wherein the immature beta cells are in a semipermeable encapsulation device.