

CLAIMS

What is claimed is:

1. A method of manufacturing a population of human insulin producing cells, the method comprising the steps of:
 - (a) obtaining adult human tissue;
 - (b) processing said tissue to recover primary adult human cells;
 - (c) propagating and expanding said primary adult human cells to a predetermined number of cells;
 - (d) transdifferentiating said expanded cells, wherein said transdifferentiation comprises:
 - (1) infecting said expanded cells with at least one expression vector, said infecting occurring at a first time period,
 - (i) wherein said at least one expression vector comprises an adenoviral vector comprising a nucleic acid encoding a PDX-1 polypeptide and an adenoviral vector comprising a nucleic acid encoding a second human pancreatic transcription factor polypeptide, wherein said infecting with said adenoviral vectors occurs at the same time,
 - or
 - (ii) wherein said at least one expression vector comprises an adenoviral vector comprising a nucleic acid encoding a human PDX-1 polypeptide and a second pancreatic transcription factor polypeptide;
 - and
 - (2) infecting said expanded infected cells of (1) with an adenoviral vector comprising a nucleic acid encoding a MafA polypeptide, said infecting occurring at a second time period, wherein said second time period is after said first time period; and
 - (e) harvesting said transdifferentiated expanded cells;thereby manufacturing said population of human insulin producing cells,
wherein said adult human cells do not go through a stage wherein said cells comprise embryonic markers,

wherein said human insulin producing cells comprise short-term ectopic expression of PDX-1, NeuroD1 and MafA transcription factors following infection; expression and production of glucagon, an increased insulin content; an increased glucose regulated insulin secretion; an increased C-peptide secretion; or an increased endogenous Nkx6.1 transcription factor; or any combination thereof, compared with control non-transdifferentiated primary human adult cells,

and wherein said insulin producing cells do not express embryonic markers.

2. The method of claim 1, wherein said propagating and expanding comprises use of a bioreactor.
3. A method of manufacturing a population of human insulin producing cells, the method comprising the steps of:
 - (a) obtaining a human tissue;
 - (b) processing said tissue to recover a population of primary adult human cells;
 - (c) propagating and expanding said population of primary human cells to a predetermined number of cells, wherein said propagating comprises use of a bioreactor;
 - (d) transdifferentiating said expanded cell population, wherein said transdifferentiating comprises:
 - (1) infecting said expanded cell population with at least one expression vector, said infecting occurring at a first time period, wherein said at least one expression vector comprises an adenoviral vector comprising a nucleic acid encoding a PDX-1 polypeptide;
 - (2) infecting the cell population of step (1) with at least a further expression vector, said infecting occurring at a second time period, wherein said at least a further expression vector comprises an adenoviral vector comprising a second pancreatic transcription factor; and

(3) infecting the cell population of step (2) with at least another further expression vector, said infecting occurring at a third time period, wherein said at least another further expression vector comprises an adenoviral vector comprising a nucleic acid encoding a MafA polypeptide; and

(e) harvesting said transdifferentiated expanded cell population;

thereby manufacturing said population of human insulin producing cells,

wherein said adult human cells do not go through a stage wherein said cells comprise embryonic markers,

wherein said human insulin producing cells comprise short-term ectopic expression of PDX-1, NeuroD1 and MafA transcription factors following infection; expression and production of glucagon, an increased insulin content; an increased glucose regulated insulin secretion; an increased C-peptide secretion; or an increased endogenous Nkx6.1 transcription factor; or any combination thereof, compared with control non-transdifferentiated primary human adult cells,

and wherein said insulin producing cells do not express embryonic markers.

4. The method of any one of claims 1-3, wherein at step (a) said human tissue comprises
 - a. tissue obtained from a subject suffering from a pancreatic disorder or from insulin dependent diabetes; or
 - a. liver tissue, adipose tissue, bone marrow, skin, placenta, umbilical cord, Wharton's jelly, or umbilical cord blood;or any combination thereof.
5. The method of claims 1-4, wherein the primary human cells comprise endothelial cells, epithelial cells, fibroblasts, liver cells, bone marrow cells, muscle cells, spleen cells, kidney cells, blood cells, skin cells, pancreatic cells, pericentral liver cells, non-pancreatic cell, non-pancreatic beta cells, hepatocytes, hematopoietic stem cells, hepatic stem cells, mesenchymal stem cells, adipose tissue-derived mesenchymal stem cells, placental stem cells,

neural stem cells, or fetal liver cells.

6. The method of any one of claims 2-5,
 - a. wherein said bioreactor system comprises a single bioreactor or multiple bioreactors; or
 - b. wherein said bioreactor comprises a single use bioreactor, a multiple use bioreactor, a closed system bioreactor, or an open system bioreactor, or any combination thereof;or any combination thereof.
7. The method of any one of claims claim 2-6, wherein at step (d) said transdifferentiating of said expanded cells comprises transdifferentiation through a series of bioreactor systems.
8. The method of any one of claims 1-7, wherein said second pancreatic transcription factor is selected from NeuroD1 and Pax4.
9. The method of any one of claims 1-8, further comprising a step enriching said primary adult human cells for cells predisposed to transdifferentiation, wherein said enriching step is prior to said transdifferentiation or is concurrent with said transdifferentiation, or both.
10. The method of claim 9, wherein said predisposed cells comprise cells comprise:
 - (a) an active Wnt-signaling pathway;
 - (b) a capability of activating the glutamine synthetase response element (GSRE);
 - (c) increased expression of HOMER1, LAMP3, BMPR2, ITGA6, DCBLD2, THBS1, or VAMP4, or any combination thereof when said cells comprise liver cells;
 - (d) decreased expression of ABCB1, ITGA4, ABCB4, or PRNP, or any combination thereof when said cells comprise liver cells; or any combination thereof.

11. The method of any one of claims 1-9, further comprising a step increasing WNT signaling activation in said primary cells.
12. The method of claim 11, wherein said inducing comprises incubating said primary adult human cells with lithium.
13. The method of any one of claims 1-12, wherein said pre-determined number of cells comprises about 1 -2 billion cells.
14. The method of any one of claims 1-13, wherein at least 90% of said expanded cell population expresses CD73, CD90, CD105, or CD44, or any combination thereof prior to step (d).