

CLAIMS

1. A method of manufacturing a population of human insulin producing cells, the method comprising the steps of:
 - (a) obtaining adult human liver tissue;
 - (b) processing said liver tissue to recover primary adult human liver cells;
 - (c) propagating and expanding said primary adult human liver cells to a predetermined number of cells;
 - (d) transdifferentiating said expanded cells, wherein said transdifferentiating 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 expression vector comprising an adenoviral vector comprising a nucleic acid encoding a second pancreatic transcription factor polypeptide selected from NeuroD1 and Pax4, 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 PDX-1 polypeptide and a second pancreatic transcription factor polypeptide selected from NeuroD1 and Pax4;
 - 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 having an increased insulin content, or increased glucose regulated insulin secretion, or increased glucose regulated C-peptide secretion, or any combination thereof, compared with control non-transdifferentiated liver cells.

2. The method of claim 1, wherein said population of human insulin producing cells expresses increased endogenous Nkx6.1 compared with control non-transdifferentiated liver cells.
3. The method of claim 1, wherein said adult human liver cells express cell surface markers comprising CD73, CD90, CD105, or CD44 or any combination thereof.
4. The method of claim 1, wherein at step (a) said liver tissue is obtained from a subject suffering from pancreatitis or from insulin dependent diabetes.
5. The method of claim 1, wherein at step (c) said propagating and expanding said liver cells comprises expansion through a series of sub-cultivation steps up to a production bioreactor system, or at step (d) said transdifferentiating of said expanded cells comprises transdifferentiation occurring in a series of bioreactor systems, or a combination thereof.
6. The method of claim 5, wherein said bioreactor system comprises a single bioreactor or multiple bioreactors.
7. The method of claim 5, 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.
8. The method of claim 1, further comprising a step of enriching said primary adult human liver cells for cells predisposed to transdifferentiation, wherein said enriching step is prior to said transdifferentiating step (d).
9. The method of claim 8, wherein said predisposed cells comprise pericentral liver cells.
10. The method of claim 8, said method further comprising incubating said primary adult human liver cells with lithium.
11. The method of claim 10, wherein said incubating is prior to transdifferentiation or at

the same time as transdifferentiation.

12. The method of claim 8, wherein said predisposed cells comprise cells comprising:
 - (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;
 - (d) decreased expression of ABCB1, ITGA4, ABCB4, or PRNP, or any combination thereof; or
any combination thereof.
13. The method of claim 1, wherein said pre-determined number of cells comprises at least 1 billion cells.
14. A population of human insulin producing cells manufactured by a method comprising the steps of:
 - (a) obtaining adult human liver tissue;
 - (b) processing said liver tissue to recover primary adult human liver cells;
 - (c) propagating and expanding said primary adult human liver cells to a predetermined number of cells;
 - (d) transdifferentiating said expanded cells, wherein said transdifferentiating 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 expression vector comprising an adenoviral vector comprising a nucleic acid encoding a second pancreatic transcription factor polypeptide selected from NeuroD1 and Pax4, 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 PDX-1 polypeptide and a second pancreatic transcription factor polypeptide selected from NeuroD1

and Pax4;

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 culture;

wherein said population of human insulin producing cells have an increased insulin content or increased glucose stimulated insulin secretion, or increased glucose regulated C-peptide secretion, or any combination thereof, compared with control non-transdifferentiated liver cells.

15. The population of human insulin producing cells of claim 14, wherein said population of human insulin producing cells expresses increased Nkx6.1 compared with control non-transdifferentiated liver cells.
16. The population of human insulin producing cells of claim 14, wherein said adult human primary liver cells express cell surface markers comprising CD73, CD90, CD105, or CD44, or any combination thereof.
17. The population of human insulin producing cells of claim 14, for use in a cell-based therapy for a patient suffering from pancreatitis or from insulin dependent diabetes.
18. The population of human insulin producing cells of claim 17, wherein said cells are autologous or allogeneic with said patient.
19. A composition comprising the population of human insulin producing cells of claim 14, and a pharmaceutically acceptable carrier.
20. The population of human insulin producing cells of claim 14, further comprising a step enriching said primary adult human liver cells for cells predisposed to transdifferentiation, wherein said enriching step is prior to said transdifferentiating step (d).
21. The population of human insulin producing cells of claim 20, wherein said

predisposed cells comprise pericentral liver cells.

22. The population of human insulin producing cells of claim 20, said method further comprising incubating said primary adult human liver cells with lithium.
23. The population of human insulin producing cells of claim 20, wherein said predisposed cells comprise cells comprising:
 - (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;
 - (d) decreased expression of ABCB1, ITGA4, ABCB4, or PRNP, or any combination thereof; orany combination thereof.
24. The population of human insulin producing cells of claim 20, wherein at step (c) said propagating and expanding said liver cells comprises expansion through a series of sub-cultivation steps up to a production bioreactor system, or at step (d) said transdifferentiating of said expanded cells comprises transdifferentiation occurring in a series of bioreactor systems, or a combination thereof.
25. The population of human insulin producing cells of claim 24, wherein said bioreactor system comprises a single bioreactor or multiple bioreactors.
26. The population of human insulin producing cells of claim 24, 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.