

WHAT IS CLAIMED IS:

1. A method of producing mammalian insulin-secreting cells, comprising:
 - a. culturing mammalian stem cells in adhesion, thereby allowing the mammalian stem cells to spontaneously form three-dimensional structures; and
 - b. culturing of the three-dimensional structures in suspension;wherein the culturing steps comprise at least a 20-day exposure to retinoic acid and cyclopamine, and do not comprise exposing the stem cells of three-dimensional structures to Wnt3A.
2. The method of claim 1, wherein the mammalian stem cells are human stem cells.
3. The method of claim 1, wherein the mammalian stem cells are non-human primate stem cells.
4. The method of claim 1, wherein the mammalian stem cells were derived from a cell line.
5. A method of producing insulin-secreting cells, comprising:
 - a. culturing mammalian stem cells on an adhesive substrate in a first medium comprising Activin-A and Wortmannin, wherein the mammalian stem cells are not exposed to Wnt3a;
 - b. further culturing the cells in at least one additional medium comprising retinoic acid and cyclopamine; and
 - c. transferring the cells to a suspension culture when the cells form three-dimensional cell structures;wherein the cells are exposed to retinoic acid and cyclopamine for at least 20 days.
6. The method of claim 5, wherein the mammalian stem cells form three-dimensional structures when cultured on the adhesive substrate.
7. The method of claim 5, wherein the mammalian stem cells are human stem cells.

8. The method of claim 5, wherein the mammalian stem cells are non-human primate stem cells.
9. A method of producing mammalian insulin-secreting cells, comprising
 - a. culturing mammalian stem cells in a first medium comprising an endoderm-inducing factor, thereby differentiating the mammalian stem cells into endoderm cells; and
 - b. culturing the endoderm cells from (a) in a second medium comprising an endocrine-inducing factor, thereby differentiating the endoderm cells into endocrine cells;wherein the mammalian stem cells were not exposed to keratinocyte growth factors (KGF) prior to differentiation into endoderm cells.
10. The method of claim 9, wherein the mammalian stem cells are human stem cells.
11. The method of claim 9, wherein the mammalian stem cells are non-human primate stem cells.
12. The method of any one of claims 9-11, wherein the endoderm-inducing factor comprises Activin-A.
13. The method of any one of claims 9-12, wherein the first medium comprises Wortmannin.
14. The method of any one of claims 9-13, wherein the first medium does not comprise Wnt3A.
15. The method of any one of claims 9-14, wherein the cells are cultured in the first medium for 1-3 days.
16. The method any one of claims 9-15, wherein the endocrine-inducing factor comprises retinoic acid and cylopamine.
17. The method of any one of claims 9-16, wherein the second medium comprises noggin.
18. The method of any one of claims 9-17, wherein the second medium comprises KGF.

19. The method of any one of claims 9-18, wherein the cells are cultured in the second medium for 1-4 days.
20. The method of any one of claims 9-19, further comprising culturing the endocrine cells in a third medium comprising KGF, thereby differentiating the endocrine cells into pancreatic progenitor cells.
21. The method of claim 20, wherein the third medium comprises noggin and epidermal growth factor (EGF).
22. The method of claim 20 or claim 21, wherein the third medium comprises retinoic acid and cyclopamine.
23. The method of any one of claims 20-22, wherein the cells are cultured in the third medium for 1-4 days.
24. The method of claim 20, further comprising culturing the pancreatic progenitor cells in a fourth medium comprising noggin, EGF, γ -secretase inhibitor XXI, and Alk5i II.
25. The method of claim 24, wherein the fourth medium comprises retinoic acid and cyclopamine.
26. The method of claim 24 or claim 25, wherein the cells are cultured in the fourth medium for 1-4 days.
27. The method of claim 24, further comprising culturing the pancreatic progenitors in a fifth medium comprising Alk5i II, and retinoic acid.
28. The method of claim 27, wherein the fifth medium comprises cyclopamine.
29. The method of claim 27 or claim 28, wherein the cells are cultured in the fifth medium for 1-5 days.
30. The method of claim 27, further comprising culturing the pancreatic progenitors in a sixth medium comprising Alk5i II, nicotinamide, and insulin-like growth factor (IGF)-I.

31. The method of claim 30, wherein the sixth medium comprises retinoic acid and cyclopamine.
32. The method of claim 30 or claim 31, wherein the cells are cultured in the sixth medium for 1-9 days.
33. The method of any one of claims 9-32, wherein the mammalian stem cells are derived from pancreatic primary tissue.
34. The method of any one of claims 9-32, wherein the mammalian stem cells are human embryonic stem cells.
35. The method of any one of claims 9-32, wherein the mammalian stem cells are induced pluripotent stem cells.
36. The method of any one of claims 9-32, wherein the mammalian stem cells are reprogrammed cells that are not pluripotent.
37. The method of claim 36, wherein the reprogrammed cells are derived from pancreatic primary tissue.
38. The method of claim 36 or claim 37, wherein the reprogrammed cells are reprogrammed by expressing reprogramming genes without incorporating the reprogramming genes into the genome of the cell.
39. The method of claim 38, wherein the reprogramming genes are encoded on at least one episomal expression plasmid.
40. The method of claim 38 or claim 39, wherein the reprogramming genes comprise Oct4, Sox2, Klf4, and L-Myc.
41. The method of any one of claims 9-40, wherein the cells are cultured for 30 days or less.
42. A method of producing insulin-secreting cells, comprising
 - a. culturing human stem cells in a first medium comprising Activin-A and Wortmannin, thereby differentiating the human stem cells into endoderm cells,

wherein the human stem cells were not exposed to keratinocyte growth factors (KGF) prior to differentiation into endoderm cells; and

- b. culturing the endoderm cells from (a) in a second medium comprising retinoic acid and cyclopamine, thereby differentiating the endoderm cells into endocrine cells;
 - c. culturing the endocrine cells from (b) in a third medium comprising KGF, noggin, and EGF, thereby differentiating the endocrine cells into pancreatic progenitor cells;
 - d. culturing the pancreatic progenitor cells from (c) in a fourth medium comprising noggin, EGF, γ -secretase inhibitor XXI, and Alk5i II, thereby differentiating the pancreatic progenitor cells into insulin-producing cells.
43. The method of claim 42, wherein the second culture medium further comprises KGF.
44. The method of claim 42 or claim 43, wherein the third and fourth mediums further comprise retinoic acid and cyclopamine.
45. The method of any one of claims 42-44, wherein the insulin producing cells from (d) are further cultured in a fifth medium comprising Alk5I II and retinoic acid, and optionally cyclopamine.
46. The method of claim 45, further comprising further culturing the cells in a sixth medium comprising Alk5i II, nicotinamine, IGF-I, and optionally retinoic acid and cyclopamine.
47. The method of any one of claims 42-46, wherein the total culture time is less than 30 days.
48. The method of any one of claims 42-47, wherein the human stem cells are derived from pancreatic primary tissue.
49. A cell-based composition for treating diabetes, comprising a population of surrogate pancreatic cells and a suitable carrier for implantation into a human subject in need thereof, wherein at least 66% of the cells are insulin-producing pancreatic cells.

50. The cell-based composition of claim 49, wherein at least 66% of the surrogate pancreatic cells express NeuroD1.
51. The cell-based composition of claim 49 or claim 50, wherein at least 68% of the surrogate pancreatic cells express Nkx6.1.
52. The cell-based composition of any one of claims 49-51, wherein the insulin-producing pancreatic cells were derived according to a method comprising
- a. culturing a population of human stem cells on an adhesive substrate in a first medium comprising an endoderm-inducing factor, wherein the mammalian stem cells are not exposed to Wnt3a;
 - b. further culturing the cells in in at least one additional medium comprising retinoic acid and cyclopamine
 - c. transferring the cells to a suspension culture when the cells form three-dimensional cell structures;
- wherein the cells are exposed to retinoic acid and cyclopamine for at least 20 days,
wherein the cells are exposed to retinoic acid and cyclopamine for at least 20 days.
53. The cell-based composition of claim 52, wherein the endoderm-inducing factor comprises Activin-A and Wortmannin.
54. The cell-based composition of any one of claims 49-53, wherein the human stem cells are derived from pancreatic primary tissue.
55. The cell-based composition of any one of claims 49-53, wherein the human stem cells are human embryonic stem cells.
56. The cell-based composition of any one of claims 49-53, wherein the human stem cells are induced pluripotent stem cells.
57. The cell-based composition of any one of claims 49-53, wherein the human stem cells are reprogrammed cells that are not pluripotent.

58. The cell-based composition of claim 57, wherein the reprogrammed cells are reprogrammed by expressing reprogramming genes without incorporating the reprogramming genes into the genome of the cell.
59. The cell-based composition of claim 58, wherein the reprogramming genes are encoded on at least one episomal expression plasmid.
60. The cell-based composition of claim 59, wherein the reprogramming genes comprise Oct4, Sox2, Klf4, and L-Myc.
61. The cell-based composition of any one of claims 49-60, wherein the carrier comprises a macro-capsule.
62. The cell-based composition of claim 61, wherein the macro-capsule comprises alginate, cellulose sulfate, glucomannan, or a combination thereof.
63. A method of identifying undifferentiated cells that preferentially differentiate into an endodermal lineage, comprising assessing expression of BHMT2 and NAP1L1 in an undifferentiated cell and identifying the cell as having a preference for differentiating into an endodermal lineage if BHMT2 expression is down-regulated relative to a control cell and NAP1L1 expression is up-regulated relative to a control cell.
64. The method of claim 63 further comprising assessing the expression of Cox7A1 and HSPB2 in the undifferentiated cell and identifying the cell as having a preference for differentiating into an endodermal lineage if both Cox7A1 and HSPB2 expression are down-regulated relative to a control cell.
65. The method of claims 63 or 64, wherein the control cell is a pluripotent cell that does not exhibit preferential differentiation to the endodermal lineage or a substantial inability to differentiate to the mesodermal lineage.
66. The method of claim 63, wherein BHMT2 expression is down-regulated at least 2 logs relative to the control cell and NAP1L1 expression is up-regulated at least 2 logs relative to the control cell.

67. The method of claim 66, wherein both Cox7A1 and HSPB2 expression are down-regulated at least 2 logs relative to the control cell.
68. The method of claims 63 or 64 further comprising assessing expression of GLIS2, CCDC58, MTX3 and C7orf29.
69. The method of claim 68, wherein GLIS2, CCDC58, and MTX3 expression is up-regulated relative to the control cell and C7orf29 expression is down-regulated relative to the control cell.
70. The method of claims 63 or 64, wherein the level of expression is assessed by Q-PCR.
71. The method of claims 63 or 64, wherein the level of expression is assessed by microarray analysis.