CLAIMS

What is claimed is:

1. A method for producing cells expressing markers characteristic of pancreatic endocrine cells from pluripotent stem cells, comprising the steps of:

    a. culturing pluripotent stem cells;

    b. differentiating the pluripotent stem cells into cells expressing markers characteristic of pancreatic foregut precursor cells; and

    c. differentiating the cells expressing markers characteristic of pancreatic foregut precursor cells into cells expressing markers characteristic of pancreatic endocrine cells by treatment with at least one medium supplemented with an ALK5 inhibitor, or a thyroid hormone selected from triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine and mixtures thereof, or both ALK5 inhibitor and thyroid hormone, and culturing at the air-liquid interface.

2. The method of claim 1, wherein the cells expressing markers characteristic of pancreatic endocrine cells are positive for NKX6.1, PDX1, and HB9.

3. The method of claim 1, wherein the cells expressing markers characteristic of pancreatic endocrine cells co-express NKX6.1 and chromogranin-A.

4. The method of claim 3, wherein at least thirty percent of the resulting cells co-express NKX6.1 and chromogranin-A.

5. The method of claim 1, wherein the cells expressing markers characteristic of pancreatic endocrine cells co-express NKX6.1 and insulin.

6. The method of claim 5, wherein at least thirty percent of the resulting cells co-express NKX6.1 and insulin.

7. The method of claim 1, wherein the method further comprises differentiating cells expressing markers characteristic of foregut endoderm cells into cells expressing markers characteristic of pancreatic foregut precursor cells by treatment with a medium supplemented with an ALK5 inhibitor, or a thyroid hormone selected from triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine and mixtures thereof, or both an ALK5 inhibitor and thyroid hormone in a planar culture.

8. The method of claim 7, wherein the method further comprises differentiating the cells expressing markers characteristic of pancreatic foregut precursor cells into cells expressing markers characteristic of pancreatic endoderm/endocrine precursor cells by treatment with a medium supplemented with an ALK5 inhibitor, or a thyroid hormone selected from triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine and mixtures thereof, or both an ALK5 inhibitor and thyroid hormone, and culturing at the air-liquid interface.

9. The method of claim 1, wherein the method comprises treatment with a medium supplemented with triiodothyronine and an ALK5 inhibitor.

10. The method of claim 1, wherein the method further comprises culturing the cells on a porous substrate.

11. The method of claim 10, wherein the porous substrate is uncoated.

12. The method of claim 1, wherein said ALK5 inhibitor is selected from the group consisting of: ALK5 inhibitor II, ALK5i, SD208, TGF-B inhibitor SB431542, ITD-1, LY2109761, A83-01, LY2157299, TGF-β receptor inh V, TGF-β receptor inh I, TGF-β receptor inh I TGF-β receptor inh IV, TGF-β receptor inh VII, TGF-β receptor inh VIII, TGF-β receptor inh II, TGF-β receptor inh VI, TGF-β receptor inh III.

13. The method of claim 12, wherein said ALK5 inhibitor is ALK5 inhibitor II.

14. The method of claim 1, wherein the method further comprises treating cells expressing markers characteristic of pancreatic endoderm/pancreatic endocrine precursor cells with a medium supplemented with an ALK5 inhibitor, or a thyroid hormone selected from

triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine and mixtures thereof, or both ALK5 inhibitor and thyroid hormone.

15. The method of claim 14, wherein the thyroid hormone is triiodothyronine and the ALK5 inhibitor is ALK5 inhibitor II.

16. The method of claim 15, wherein the medium is further supplemented with one or more of retinoic acid, ascorbic acid, SANT-1 or LDN-193189.

17. The method of claim 1, wherein the method increases the expression of pancreatic hormones.

18. The method of claim 16, wherein said the method reduces expression of PTF1a, SOX9, CDX2, ZIC1 and SOX2.

19. The method of claim 1, wherein the method increases the number of NXK6.1 positive cells that co-express insulin, chromogranin-A or both chromogranin-A and insulin.

20. The method of claim 1, wherein the pluripotent stem cells are of non-embryonic origins.

21. The method of claim 1, wherein the cells expressing markers characteristic of pancreatic endocrine cells express markers characteristic of β cells.

22. A method of producing cells expressing markers characteristic of pancreatic endocrine cells comprising differentiating cells expressing markers characteristic of foregut endoderm cells into cells expressing markers characteristic of pancreatic endocrine cells by treatment with at least one medium supplemented with an ALK5 inhibitor, or a thyroid hormone selected from triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine and mixtures thereof, or both ALK5 inhibitor and thyroid hormone, while culturing at the air-liquid interface.

23. The method of claim 22, wherein only cells expressing markers characteristic of pancreatic foregut precursor cells, pancreatic endoderm cells and pancreatic endocrine precursor cells are cultured at the air-liquid interface.

24. The method of claim 22, wherein the cells expressing markers characteristic of pancreatic endocrine cells are positive for NKX6.1, PDX1, and HB9.

25. The method of claim 22, wherein the cells expressing markers characteristic of pancreatic endocrine cells are single hormone positive cells.

26. The method of claim 25, wherein the cells expressing markers characteristic of pancreatic endocrine cells co-express NKX6.1 and chromogranin-A.

27. The method of claim 25, wherein the cells expressing markers characteristic of pancreatic endocrine cells co-express NKX6.1 and insulin.

28. The method of claim 22, wherein the method further comprises culturing the cells on a porous substrate.

29. The method of claim 28, wherein the cells are in a sheet or aggregate clusters on top of the porous substrate.

30. The method of claim 22, wherein the method comprises treating cells expressing markers characteristic of pancreatic endoderm/pancreatic endocrine precursor cells with a medium supplemented with an ALK5 inhibitor, or a thyroid hormone selected from triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine and mixtures thereof, or both ALK5 inhibitor and thyroid hormone.

31. The method of claim 30, wherein the thyroid hormone is triiodothyronine and the ALK5 inhibitor is ALK5 inhibitor II.

32. The method of claim 31, wherein the medium is further supplemented with one or more of retinoic acid, ascorbic acid, SANT-1 or LDN-193189.

33. The method of claim 22, wherein the cells expressing markers characteristic of pancreatic endocrine cells express markers characteristic of β cells.

34. A method of inducing PDX1, NKX6.1, and HB9 expression in cells derived from pluripotent stem cells comprising:

a. culturing pluripotent stem cells;

b. differentiating the pluripotent stem cells into cells expressing markers characteristic of pancreatic foregut precursor cells; and

c. differentiating the cells expressing markers characteristic of pancreatic foregut precursor cells into cells expressing PDX1, NKX6.1, and HB9 by treatment with a medium supplemented with an ALK5 inhibitor, or a thyroid hormone selected from triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine and mixtures thereof, or both ALK5 inhibitor and thyroid hormone, and culturing at the air-liquid interface.

35. The method of claim 34, wherein the pluripotent stem cells are of non-embryonic origins.

36. The method of claim 34, wherein the method further comprises culturing cells expressing markers characteristic of pancreatic endoderm cells or pancreatic endocrine precursor cells in a medium supplemented with triiodothyronine and an ALK5 inhibitor at the air-liquid interface.

37. The method of claim 36, wherein the ALK5 inhibitor is ALK5 inhibitor II.

38. The method of claim 34, wherein the medium is further supplemented with one or more of retinoic acid, ascorbic acid, SANT-1 or LDN-193189.

39. An in vitro cell culture for differentiating cells at an air-liquid interface comprising:

a. a culture vessel;

b. a volume of differentiation medium within said vessel sufficient to fill only a portion of the volume of said vessel;

c. air within said vessel that fills a portion of said vessel adjoining said medium;

d. a porous substrate located at the interface between said medium and said air; and

e. cells derived from pluripotent stem cells disposed upon the surface of said substrate such that said medium contacts only a portion of the surface of said cells; and

wherein said cells derived from pluripotent stem cells express markers characteristic of foregut endoderm cells.

40. An in vitro cell culture for differentiating cells at an air-liquid interface comprising:

a. a culture vessel;

b. a volume of differentiation medium within said vessel sufficient to fill only a portion of the volume of said vessel;

c. air within said vessel that fills a portion of said vessel adjoining said medium;

d. a porous substrate located at the interface between said medium and said air; and

e. cells derived from pluripotent stem cells disposed upon the surface of said substrate such that said medium contacts only a portion of the surface of said cells; and

wherein said cells derived from pluripotent stem cells express markers characteristic of pancreatic foregut precursor cells.

41. An in vitro cell culture for differentiating cells at an air-liquid interface comprising:

a. a culture vessel;

b. a volume of differentiation medium within said vessel sufficient to fill only a portion of the volume of said vessel;

c. air within said vessel that fills a portion of said vessel adjoining said medium;

d. a porous substrate located at the interface between said medium and said air; and

e. cells derived from pluripotent stem cells disposed upon the surface of said substrate such that said medium contacts only a portion of the surface of said cells; and

wherein said differentiation medium comprises a growth medium supplemented with an ALK5 inhibitor, or a thyroid hormone selected from the group consisting of triiodothyronine, thyroxine, triiodothyronine analogues, thyroxine analogues and mixtures thereof, or both an ALK5 inhibitor and thyroid hormone; and

wherein said growth medium is selected from MCDB131 and BLAR media.

42. The cell culture of claim 41, wherein the growth medium is BLAR medium.

43. The cell culture of claim 42, wherein said growth medium is supplemented with triiodothyronine and ALK5 inhibitor II.

44. The cell culture of claim 39, wherein said differentiation medium comprises a growth medium supplemented with a growth factor selected from FGF7, FGF10 and mixtures thereof, a PKC activator selected from TPB, PDBu, PMA and ILV, and a BMP Receptor Inhibitor selected from LDN-193189, Noggin and Chordin.

45. The cell culture of claim 44, wherein said growth medium is selected from MCDB131 and BLAR media.

46. The cell culture of claim 45, wherein the growth medium is BLAR medium.

47. The cell culture of claim 46, wherein said growth medium is supplemented with FGF7, TPB and LDN-193189.

48. The cell culture of claim 40, wherein said differentiation medium comprises a growth medium supplemented with an ALK5 inhibitor, and a BMP inhibitor selected from the group consisting of LDN-193189, Noggin or Chordin.

49. The cell culture of claim 48, wherein said growth medium is selected from MCDB131 and BLAR media.

50. The cell culture of claim 49, wherein the growth medium is BLAR medium.

51. The cell culture of claim 50, wherein said growth medium is supplemented with LDN-193189 and ALK5 inhibitor II.

52. An in vitro cell culture for differentiating cells at an air-liquid interface comprising:

a. a culture vessel;

b. a volume of differentiation medium within said vessel sufficient to fill only a portion of the volume of said vessel;

c. air within said vessel that fills a portion of said vessel adjoining said medium;

d. a porous substrate located at the interface between said medium and said air; and

e. cells derived from pluripotent stem cells disposed upon the surface of said substrate such that said medium contacts only a portion of the surface of said cells; and

wherein said cells derived from pluripotent stem cells express markers characteristic of pancreatic endoderm cells;

wherein said differentiation medium comprises a growth medium supplemented with an ALK5 inhibitor, or a thyroid hormone selected from the group consisting of triiodothyronine, thyroxine, triiodothyronine analogues, thyroxine analogues and mixtures thereof, or both an ALK5 inhibitor and thyroid hormone; and

wherein said growth medium is selected from MCDB131 and BLAR media.

53. The cell culture of claim 52, wherein the growth medium is BLAR medium.

54. The cell culture of claim 53, wherein said growth medium is supplemented with triiodothyronine and ALK5 inhibitor II.

55. An in vitro cell culture for differentiating cells at an air-liquid interface comprising:

a. a culture vessel;

b. a volume of differentiation medium within said vessel sufficient to fill only a portion of the volume of said vessel;

c. air within said vessel that fills a portion of said vessel adjoining said medium;

d. a porous substrate located at the interface between said medium and said air; and

e. cells derived from pluripotent stem cells disposed upon the surface of said substrate such that said medium contacts only a portion of the surface of said cells; and

wherein said cells derived from pluripotent stem cells express markers characteristic of pancreatic endocrine precursor cells;

wherein said differentiation medium comprises a growth medium supplemented with an ALK5 inhibitor,or a thyroid hormone selected from the group consisting of triiodothyronine, thyroxine, triiodothyronine analogues, thyroxine analogues and mixtures thereof, or both an ALK5 inhibitor and thyroid hormone; and

wherein said growth medium is selected from MCDB131 and BLAR media.

56. The cell culture of claim 55, wherein the growth medium is BLAR medium.

57. The cell culture of claim 56, wherein said growth medium is supplemented with triiodothyronine and ALK5 inhibitor II.

58. The cell culture of claim 43, wherein the growth medium is further supplemented with one or more of:

a. a smoothened receptor inhibitor selected from MRT10 or cyclopamine;

b. a SHH signaling pathway antagonist selected from SANT-1 or HPI-1;

c. a BMP Receptor Inhibitor selected from LDN-193189, Noggin or Chordin;

d. a PKC activator selected from TPB, PDBu, PMA, and ILV;

e. a fibroblast growth factor selected from FGF7 or FGF10;

f. retinoic acid;

g. ascorbic acid;

h. heparin; and

i. zinc sulfate.

59. The cell culture of claim 51 wherein, the media is further supplemented with one or more of:

a. a smoothened receptor inhibitor selected from MRT10 or cyclopamine;

b. a SHH signaling pathway antagonist selected from SANT-1 or HPI-1;

c. a thyroid hormone selected from the group consisting of triiodothyronine, thyroxine, triiodothyronine analogues, thyroxine analogues and mixtures thereof;

d. a PKC activator selected from TPB, PDBu, PMA, and ILV;

e. a fibroblast growth factor selected from FGF7 or FGF10;

f. retinoic acid;

g. ascorbic acid;

h. heparin; and

i. zinc sulfate.

60. The cell culture of claim 54, wherein the media is further supplemented with one or more of:

    a. a smoothened receptor inhibitor selected from MRT10 or cyclopamine;

    b. a SHH signaling pathway antagonist selected from SANT-1 or HPI-1;

    c. a BMP Receptor Inhibitor selected from LDN-193189, Noggin or Chordin;

    d. a PKC activator selected from TPB, PDBu, PMA, and ILV;

    e. a fibroblast growth factor selected from FGF7 or FGF10;

    f. retinoic acid;

    g. ascorbic acid;

    h. heparin; and

    i. zinc sulfate.

61. The cell culture of claim 57, wherein the media is further supplemented with one or more of:

    a. a smoothened receptor inhibitor selected from MRT10 or cyclopamine;

    b. a SHH signaling pathway antagonist selected from SANT-1 or HPI-1;

    c. a BMP Receptor Inhibitor selected from LDN-193189, Noggin or Chordin;

    d. a PKC activator selected from TPB, PDBu, PMA, and ILV;

    e. a fibroblast growth factor selected from FGF7 or FGF10;

    f. retinoic acid;

    g. ascorbic acid;

    h. heparin; and

i. zinc sulfate.

62. The cell culture of claim 58, wherein said medium is further supplemented with retinoic acid, ascorbic acid and SANT-1.

63. The cell culture of claim 59, wherein said medium is further supplemented with retinoic acid, heparin, SANT-1 and zinc sulfate.

64. The cell culture of claim 60, wherein said medium is further supplemented with LDN-193189, retinoic acid, ascorbic acid, and SANT-1.

65. The cell culture of claim 61, wherein said medium is further supplemented with LDN-193189, SANT-1, and heparin.

66. The cell culture of claim 61 wherein, at least about thirty percent of the resulting cells express NKX6.1 and insulin.

67. The cell culture of claim 61 wherein, at least about thirty percent of the resulting cells express NKX6.1 and chromogranin-A.

68. A medium useful for inducing differentiation in cells derived from pluripotent stem cells comprising a growth medium supplemented with:

a. an ALK5 inhibitor selected from the group consisting of: ALK5 inhibitor II, ALK5i, SD208, TGF-B inhibitor SB431542, ITD-1, LY2109761, A83-01, LY2157299, TGF-β receptor inh V, TGF-β receptor inh I, TGF-β receptor inh I TGF-β receptor inh IV, TGF-β receptor inh VII, TGF-β receptor inh VIII, TGF-β receptor inh II, TGF-β receptor inh VI, TGF-β receptor inh III; and

b. a BMP Receptor Inhibitor selected from LDN-193189, Noggin or Chordin; and

further comprising an SHH signaling pathway antagonist selected form SANT-1 or HPI-1.

69. The medium of claim 68, further comprising retinoic acid.

70. The medium of claim 69, comprising ALK5 inhibitor II, SANT-1, LDN-193189 and retinoic acid.

71. The medium of claim 70, further comprising one or more supplements selected from:

a. a smoothened receptor inhibitor selected from MRT10 or cyclopamine;

b. a thyroid hormone selected from the group consisting of triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine and mixtures thereof;

c. ascorbic acid;

d. heparin; and

e. zinc sulfate.

72. The differentiation medium of claim 70, wherein said growth medium is selected from MCDB-131 and BLAR media.

73. The differentiation media of claim 71, wherein said growth medium is supplemented with ALK5 inhibitor II, SANT-1, LDN-193189, heparin, zinc sulfate and retinoic acid.

74. A medium useful for inducing differentiation in cells derived from pluripotent stem cells comprising a growth medium supplemented with:

a. an ALK5 inhibitor selected from the group consisting of: ALK5 inhibitor II, ALK5i, SD208, TGF-B inhibitor SB431542, ITD-1, LY2109761, A83-01, LY2157299, TGF-β receptor inh V, TGF-β receptor inh I, TGF-β receptor inh I TGF-β receptor inh IV, TGF-β receptor inh VII, TGF-β receptor inh VIII, TGF-β receptor inh II, TGF-β receptor inh VI, TGF-β receptor inh III;

b. a thyroid hormone selected from the group consisting of triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine and mixtures thereof; and

c. a SHH signaling pathway antagonist selected from SANT-1 or HPI-1.

75. The medium of claim 74, further comprising retinoic acid.

76. The medium of claim 75, further comprising ascorbic acid.

77. The medium of claim 74, further comprising one or more supplements selected from:

    a. a BMP Receptor Inhibitor selected from LDN-193189, Noggin or Chordin;

    b. heparin; and

    c. zinc sulfate.

78. The differentiation medium of claim 77, wherein said growth medium is selected from MCDB-131 and BLAR media.

79. The differentiation medium of claim 78, wherein said growth medium is supplemented with ALK5 inhibitor II, LDN-139189, zinc sulfate, triiodothyronine, SANT-1 and heparin.

80. An *in vitro* cell culture comprising a population of differentiated pluripotent stem cells expressing markers characteristic of pancreatic endocrine cells wherein at least thirty percent of said differentiated cells express NKX6.1 and chromagranin.

81. A method of screening compounds for effect on pancreatic hormone production comprising culturing cells according to the method of claim 1, in the presence of a test compound and measuring the effect of said test compound on the production of pancreatic hormones.

82. The screening method of claim 81, wherein said pancreatic hormone is insulin.