CLAIMS

What is claimed is:

1. A method of culturing pluripotent stem cells comprising seeding the pluripotent stem cells on a surface at a seeding density of from about 0.8 x 10^5 cells/cm^2 to about 3.0 x 10^5 cells/cm^2.

2. The method of claim 1, wherein the pluripotent stem cells are embryonic stem cells.

3. The method of claim 2, wherein the embryonic stem cells are human embryonic stem cells.

4. The method of claim 1, wherein the pluripotent stem cells are seeded on a surface comprising Matrigel™.

5. A method of differentiating pluripotent stem cells comprising seeding the pluripotent stem cells on a surface at a density of from about 0.8 x 10^5 cells/cm^2 to about 3.0 x 10^5 cells/cm^2; and differentiating the pluripotent stem cells to cells expressing markers indicative of definitive endoderm.

6. The method of claim 5, wherein the pluripotent stem cells are embryonic stem cells.

7. The method of claim 6, wherein the embryonic stem cells are human embryonic stem cells.

8. The method of claim 5, wherein the surface where the pluripotent stem cells are seeded comprises Matrigel™.

9. The method of claim 5, wherein the cells expressing markers indicative of definitive endoderm are human.

10. A method of obtaining cells expressing markers indicative of definitive endoderm comprising differentiating pluripotent stem cells into cells expressing markers indicative of definitive endoderm, wherein the pluripotent stem cells have been seeded on a surface at a density of from about 0.8 x 10^5 cells/cm^2 to about 3.0 x 10^5 cells/cm^2.

11. The method of claim 10, wherein the pluripotent stem cells are embryonic stem cells.

12. The method of claim 11, wherein the embryonic stem cells are human embryonic stem cells.

13. The method of claim 10, wherein the surface where the pluripotent stem cells are seeded comprises Matrigel™.

14. The method of claim 10, wherein the cells expressing markers indicative of definitive endoderm are human.

15. A method of differentiating cells expressing markers indicative of definitive endoderm comprising seeding pluripotent stem cells on a first surface at a seeding density sufficient to maximize differentiation efficiency of the pluripotent stem cells; differentiating the pluripotent stem cells into cells expressing markers indicative of definitive endoderm; seeding the cells expressing markers indicative of definitive endoderm at a seeding density sufficient to maximize differentiation efficiency of the cells expressing markers indicative of definitive endoderm; and differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endoderm.

16. The method of claim 15, wherein the pluripotent stem cells are seeded on the first surface at a seeding density of from about 0.8×10^5 cells/cm^2 to about 3.0×10^5 cells/cm^2.

17. The method of claim 15, wherein the cells expressing markers indicative of definitive endoderm are seeded on the second surface at a seeding density of from about 1.5×10^5 cells/cm^2 to about 5.0×10^5 cells/cm^2.

18. The method of claim 15, wherein the pluripotent stem cells are embryonic stem cells.

19. The method of claim 18, wherein the embryonic stem cells are human embryonic stem cells.

20. The method of claim 15, wherein the first surface comprises Matrigel™.

21. The method of claim 15, wherein the second surface comprises Matrigel™.

22. The method of claim 15, wherein the first surface and the second surface are the same surface.

23. The method of claim 15, wherein the cells expressing markers indicative of definitive endoderm are human.

24. The method of claim 15, wherein the cells expressing markers indicative of pancreatic endoderm are human.

25. A method of differentiating cells expressing markers indicative of definitive endoderm comprising differentiating pluripotent stem cells into cells expressing markers indicative of definitive endoderm; and differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endoderm; wherein the pluripotent stem cells have been seeded on a surface at a seeding density of from about 0.8 x 10^5 cells/cm^2 to about 3.0 x 10^5 cells/cm^2.

26. The method of claim 25, wherein the pluripotent stem cells are embryonic stem cells.

27. The method of claim 26, wherein the embryonic stem cells are human embryonic stem cells.

28. The method of claim 25, wherein the surface comprises Matrigel™.

29. The method of claim 25, wherein the cells expressing markers indicative of definitive endoderm are human.

30. The method of claim 25, wherein the cells expressing markers indicative of pancreatic endoderm are human.

31. A method of obtaining cells expressing markers indicative of pancreatic endoderm comprising:
    a) seeding pluripotent stem cells on a surface;
    b) differentiating the pluripotent stem cells into cells expressing markers indicative of definitive endoderm; and
    c) differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endoderm.

32. The method of claim 31, wherein the pluripotent stem cells are seeded on a surface at a seeding density of from about 0.8 x 10^5 cells/cm^2 to about 3.0 x 10^5 cells/cm^2.

33. The method of claim 31, further comprising the step of seeding the cells expressing markers indicative of definitive endoderm at a seeding density of from about 1.5 x 10^5 cells/cm^2 to about 5.0 x 10^5 cells/cm^2.

34. The method of claim 31, wherein the pluripotent stem cells are embryonic stem cells.

35. The method of claim 34, wherein the embryonic stem cells are human embryonic stem cells.

36. The method of claim 31, wherein the surface comprises Matrigel™.

37. The method of claim 31, wherein the cells expressing markers indicative of definitive endoderm are human.

38. The method of claim 31, wherein the cells expressing markers indicative of pancreatic endoderm are human.

39. A method of obtaining cells expressing markers indicative of pancreatic endocrine comprising:
    a) seeding pluripotent stem cells on a surface;
    b) differentiating the pluripotent stem cells into cells expressing markers indicative of definitive endoderm; and
    c) differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endocrine.

40. The method of claim 39, wherein the pluripotent stem cells are seeded at a seeding density of from about 0.8 x 10^5 cells/cm^2 to about 3.0 x 10^5 cells/cm^2.

41. The method of claim 39, further comprising the step of seeding the cells expressing markers indicative of definitive endoderm at a seeding density of from about 1.5 x 10^5 cells/cm^2 to about 5.0 x 10^5 cells/cm^2.

42. The method of claim 39, wherein the pluripotent stem cells are embryonic stem cells.

43. The method of claim 40, wherein the embryonic stem cells are human embryonic stem cells.

44. The method of claim 39, wherein the surface comprises Matrigel™.

45. The method of claim 39, wherein the cells expressing markers indicative of definitive endoderm are human.

46. The method of claim 39, wherein the cells expressing markers indicative of pancreatic endoderm are human.

47. A method of differentiating cells expressing markers indicative of definitive endoderm comprising seeding cells expressing markers indicative of definitive endoderm on a surface at a seeding density of from about 1.5 x 10^5 cells/cm^2 to about 5.0 x 10^5 cells/cm^2; and differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endoderm.

48. The method of claim 47, wherein the cells expressing markers indicative of definitive endoderm are human.

49. The method of claim 47, wherein the cells expressing markers indicative of pancreatic endoderm are human.

50. A method of differentiating cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endocrine comprising seeding cells expressing markers indicative of definitive endoderm on a surface at a seeding density of from about 1.5 x 10^5 cells/cm^2 to about 5.0 x 10^5 cells/cm^2; and differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endocrine.

51. The method of claim 50, wherein the cells expressing markers indicative of definitive endoderm are human.

52. The method of claim 50, wherein the cells expressing markers indicative of pancreatic endoderm are human.

53. A population of cells differentiated in vitro from human embryonic stem cells, showing a drop in expression of at least one marker selected from PDX-1, NKX6.1, NGN3, NKX2.2, NeuroD, and insulin, and upregulation of ZIC1 and CDX2 when compared to human

embryonic stem cells; and wherein the human embryonic stem cells are seeded on a surface at a seeding density of less than 5 x 10^5 cells/cm^2.

Claims

1. A method of culturing pluripotent stem cells comprising seeding the pluripotent stem cells on a surface at a seeding density of from 0.8 x 10^5 cells/cm^2 to 3.0 x 10^5 cells/cm^2.

2. The method of claim 1, wherein the pluripotent stem cells are embryonic stem cells.

3. The method of claim 2, wherein the embryonic stem cells are human embryonic stem cells.

4. The method of claim 1, wherein the pluripotent stem cells are seeded on a surface comprising Matrigel™.

5. A method of differentiating pluripotent stem cells comprising seeding the pluripotent stem cells on a surface at a density of from 0.8 x 10^5 cells/cm^2 to 3.0 x 10^5 cells/cm^2; and differentiating the pluripotent stem cells to cells expressing markers indicative of definitive endoderm.

6. The method of claim 5, wherein the pluripotent stem cells are embryonic stem cells.

7. The method of claim 6, wherein the embryonic stem cells are human embryonic stem cells.

8. The method of claim 5, wherein the surface where the pluripotent stem cells are seeded comprises Matrigel™.

9. The method of claim 5, wherein the cells expressing markers indicative of definitive endoderm are human.

10. A method of obtaining cells expressing markers indicative of definitive endoderm comprising differentiating pluripotent stem cells into cells expressing markers indicative of definitive endoderm, wherein the pluripotent stem cells have been seeded on a surface at a density of from 0.8 x 10^5 cells/cm^2 to 3.0 x 10^5 cells/cm^2.

11. The method of claim 10, wherein the pluripotent stem cells are embryonic stem cells.

12. The method of claim 11, wherein the embryonic stem cells are human embryonic stem cells.

13. The method of claim 10, wherein the surface where the pluripotent stem cells are seeded comprises Matrigel™.

14. The method of claim 10, wherein the cells expressing markers indicative of definitive endoderm are human.

15. A method of differentiating cells expressing markers indicative of definitive endoderm comprising seeding pluripotent stem cells on a first surface at a seeding density of from 0.8×10^5 cells/cm^2 to 3.0×10^5 cells/cm^2; differentiating the pluripotent stem cells into cells expressing markers indicative of definitive endoderm; seeding the cells expressing markers indicative of definitive endoderm on a second surface; and differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endoderm.

16. A method of differentiating cells expressing markers indicative of definitive endoderm comprising seeding pluripotent stem cells on a first surface; differentiating the pluripotent stem cells into cells expressing markers indicative of definitive endoderm; seeding the cells expressing markers indicative of definitive endoderm on a second surface at a seeding density of from 1.5×10^5 cells/cm^2 to 5.0×10^5 cells/cm^2; and differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endoderm.

17. A method of differentiating cells expressing markers indicative of definitive endoderm comprising differentiating pluripotent stem cells into cells expressing markers indicative of definitive endoderm; and differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endoderm; wherein the pluripotent stem cells have been seeded on a surface at a seeding density of from 0.8×10^5 cells/cm^2 to 3.0×10^5 cells/cm^2.

18. The method of claim 17, wherein the pluripotent stem cells are embryonic stem cells.

19. The method of claim 18, wherein the embryonic stem cells are human embryonic stem cells.

20. The method of claim 17, wherein the surface comprises Matrigel™.

21. The method of claim 17, wherein the cells expressing markers indicative of definitive endoderm are human.

22. The method of claim 17, wherein the cells expressing markers indicative of pancreatic endoderm are human.

23. A method of obtaining cells expressing markers indicative of pancreatic endoderm comprising:

    a. seeding pluripotent stem cells on a surface at a seeding density of from 0.8×10^5 cells/cm^2 to 3.0×10^5 cells/cm^2;

    b. differentiating the pluripotent stem cells into cells expressing markers indicative of definitive endoderm; and

    c. differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endoderm.

24. A method of obtaining cells expressing markers indicative of pancreatic endoderm comprising:

    a. seeding pluripotent stem cells on a surface;

    b. differentiating the pluripotent stem cells into cells expressing markers indicative of definitive endoderm;

    c. seeding the cells expressing markers indicative of definitive endoderm at a seeding density of from 1.5×10^5 cells/cm^2 to 5.0×10^5 cells/cm^2; and

    d. differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endoderm.

25. A method of obtaining cells expressing markers indicative of pancreatic endocrine comprising:

a. seeding pluripotent stem cells on a surface at a seeding density of from 0.8 x 10^5 cells/cm^2 to 3.0 x 10^5 cells/cm^2;

b. differentiating the pluripotent stem cells into cells expressing markers indicative of definitive endoderm; and

c. differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endocrine.

26. A method of obtaining cells expressing markers indicative of pancreatic endocrine comprising:

a. seeding pluripotent stem cells on a surface;

b. differentiating the pluripotent stem cells into cells expressing markers indicative of definitive endoderm

c. seeding the cells expressing markers indicative of definitive endoderm at a seeding density of from 1.5 x 10^5 cells/cm^2 to 5.0 x 10^5 cells/cm^2; and

d. differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endocrine.

27. A method of differentiating cells expressing markers indicative of definitive endoderm comprising seeding cells expressing markers indicative of definitive endoderm on a surface at a seeding density of from 1.5 x 10^5 cells/cm^2 to 5.0 x 10^5 cells/cm^2; and differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endoderm.

28 The method of claim 27, wherein the cells expressing markers indicative of definitive endoderm are human.

29. The method of claim 27, wherein the cells expressing markers indicative of pancreatic endoderm are human.

30. A method of differentiating cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endocrine comprising seeding cells expressing markers indicative of definitive endoderm on a surface at a seeding density of

from 1.5 x 10^5 cells/cm^2 to 5.0 x 10^5 cells/cm^2; and differentiating the cells expressing markers indicative of definitive endoderm into cells expressing markers indicative of pancreatic endocrine.

31. The method of claim 30, wherein the cells expressing markers indicative of definitive endoderm are human.

32. The method of claim 30, wherein the cells expressing markers indicative of pancreatic endocrine are human.

33. A population of cells differentiated in vitro from human embryonic stem cells, showing a drop in expression of at least one marker selected from PDX-1, NKX6.1, NGN3, NKX2.2, NeuroD, and insulin, and upregulation of ZIC1 and CDX2 when compared to human embryonic stem cells; and wherein the human embryonic stem cells are seeded on a surface at a seeding density of less than 5 x 10^5 cells/cm^2.