of the present invention, the PAX6+/MITF+ RPE progenitor cells may be obtained from a population of pluripotent stem cells.

[9a] In one aspect, the present invention provides a method for producing a population of retinal epithelium (RPE) cells, the method comprising: (i) obtaining RPE progenitor cells and fractionating the RPE progenitor cells to collect cell clusters of PAX6+/MITF+ RPE progenitor cells and dissociating the PAX6+/MITF+ RPE progenitor cell clusters into single PAX6+/MITF+ RPE progenitor cells; (ii) culturing the single PAX6+/MITF+ RPE progenitor cells in a differentiation medium such that the PAX6+/MITF+ RPE progenitor cells differentiate to RPE cells; and (iii) harvesting the RPE cells produced in step (ii); thereby producing a population of RPE cells.

[9b] In another aspect, the present invention provides a method for producing a population of retinal epithelium (RPE) cells, the method comprising: (i) obtaining RPE progenitor cells and fractionating the RPE progenitor cells to collect cell clusters of PAX6+/MITF+ RPE progenitor cells, (ii) culturing the PAX6+/MITF+ RPE progenitor cell clusters in a differentiation medium such that the PAX6+/MITF+ RPE progenitor cells differentiate to RPE cells; and (iii) harvesting the RPE cells produced in step (ii); thereby producing a population of RPE cells.

[9c] In another aspect, the present invention provides a method for producing a population of retinal epithelium (RPE) cells, the method comprising: (i) obtaining RPE progenitor cells and fractionating the RPE progenitor cells to collect cell clusters of PAX6+/MITF+ RPE progenitor cells and dissociating the PAX6+/MITF+ RPE progenitor cell clusters into single PAX6+/MITF+ RPE progenitor cells; (ii) culturing the single PAX6+/MITF+ RPE progenitor cells in a differentiation medium such that the PAX6+/MITF+ RPE progenitor cells differentiate to RPE cells; and (iii) harvesting and cryopreserving the RPE cells produced in step (ii); wherein the RPE cells are expanded by passaging at least one time, thereby producing a population of RPE cells.

[9d] In another aspect, the present invention provides a method for producing a population of retinal epithelium (RPE) cells, the method comprising: (i) obtaining RPE progenitor cells and fractionating the RPE progenitor cells to collect cell clusters of PAX6+/MITF+ RPE progenitor cells, (ii) culturing the PAX6+/MITF+ RPE progenitor cell clusters in a differentiation medium such that the PAX6+/MITF+ RPE progenitor cells differentiate to RPE cells; and (iii) harvesting and cryopreserving the RPE cells produced in step (ii); wherein the RPE cells are expanded by passaging at least one time, thereby producing a population of RPE cells.

[9e] In another aspect, the present invention provides a method for producing a population of retinal epithelium (RPE) cells, the method comprising: (i) culturing a population of pluripotent stem cells in a first differentiation medium, such that the pluripotent stem cells differentiate into RPE progenitor cells; (ii) dissociating the RPE progenitor cells, fractionating the RPE progenitor cells to collect RPE progenitor cell clusters, dissociating the collected RPE progenitor cell clusters into single RPE progenitor cells, and subculturing the RPE progenitor single cells in a second differentiation medium such that the RPE progenitor cells differentiate to RPE cells; and (iii) harvesting the RPE cells produced in step (ii) thereby producing a population of RPE cells.

[9f] In another aspect, the present invention provides a method for producing a population of retinal epithelium (RPE) cells, the method comprising: (i) culturing a population of pluripotent stem cells in a first differentiation medium, such that the pluripotent stem cells differentiate into RPE progenitor cells; (ii) dissociating the RPE progenitor cells, fractionating the RPE progenitor cells to collect RPE progenitor cell clusters, and subculturing the collected RPE progenitor cell clusters in a second differentiation medium such that the RPE progenitor cells differentiate to RPE cells; and (iii) harvesting the RPE cells produced in step (ii) thereby producing a population of RPE cells.

[9g] In another aspect, the present invention provides a method for producing a population of retinal epithelium (RPE) cells, the method comprising: (i) culturing a population of pluripotent stem cells in a first differentiation medium, such that the pluripotent stem cells differentiate into RPE progenitor cells; (ii) dissociating the RPE progenitor cells, fractionating the RPE progenitor cells to collect RPE progenitor cell clusters, dissociating the collected RPE progenitor cell clusters into single RPE progenitor cells, and subculturing the single RPE progenitor cells in a second differentiation medium such that the RPE progenitor cells differentiate to RPE cells; and (iii) harvesting and cryopreserving the RPE cells produced in step (ii); wherein the RPE cells are expanded by passaging at least one time, thereby producing a population of RPE cells.

[9h] In another aspect, the present invention provides a method for producing a population of retinal epithelium (RPE) cells, the method comprising: (i) culturing a population of pluripotent stem cells in a first differentiation medium, such that the pluripotent stem cells differentiate into RPE progenitor cells; (ii) dissociating the RPE progenitor cells, fractionating the RPE progenitor cells to collect RPE progenitor cell clusters, and subculturing the collected RPE progenitor cell clusters in a second differentiation medium such that the RPE progenitor cells differentiate to RPE cells; and (iii) harvesting and

cryopreserving the RPE cells produced in step (ii); wherein the RPE cells are expanded by passaging at least one time, thereby producing a population of RPE cells.

[9i] In another aspect, the present invention provides a pharmaceutical composition comprising a population of at least 1 x 10^4 RPE cells produced by the method as described herein and a pharmaceutically acceptable carrier, wherein the RPE cells express one or more markers selected from the group consisting of RPE65, CRALBP, PEDF, Bestrophin, MITF, OTX2, PAX2, PAX6, premelanosome protein (PMEL or gp-100), tyrosinase, and ZO1.

[9j] In another aspect, the present invention provides use of the pharmaceutical composition as described herein in the manufacture of a medicament for the treatment of a patient with a retinal disease, wherein an effective amount of the pharmaceutical composition of as described herein is to be administered to the patient.

[9k] In another aspect, the present invention provides the pharmaceutical composition as described herein for use in therapy.

[10] In an aspect, the present invention provides a method for producing a population of retinal epithelium (RPE) cells, the method comprising: (i) culturing a population of pluripotent stem cells in a first differentiation medium, such that the cells differentiate into RPE progenitor cells; (ii) dissociating the RPE progenitor cells, fractionating the cells to collect RPE progenitor cell clusters, dissociating the RPE progenitor cell clusters into single cells, and subculturing the single cells in a second differentiation medium such that the cells differentiate to RPE cells; and (iii) harvesting the RPE cells produced in step (ii); thereby producing a population of RPE cells. In another aspect, the present invention provides a method for producing a population of retinal epithelium (RPE) cells, the method comprising: (i) culturing a population of pluripotent stem cells in a first differentiation medium, such that the cells differentiate into RPE progenitor cells; (ii) dissociating the RPE progenitor cells, fractionating the cells to collect RPE progenitor cell clusters, and subculturing the collected RPE progenitor cell clusters in a second differentiation medium such that the cells differentiate to RPE cells; and (iii) harvesting the RPE cells produced in step (ii) thereby producing a population of RPE cells. In an embodiment of the present invention, the RPE progenitor cells are positive for PAX6/MITF. In another embodiment, prior to step (i), the pluripotent stem cells are cultured on feeder cells in a medium that supports pluripotency. In a further embodiment, prior to step (i), the pluripotent stem cells are cultured feeder-free in a

medium that supports pluripotency. In an embodiment, the medium that supports pluripotency is supplemented with bFGF.

[11] The methods may further comprise harvesting the RPE cells produced in step (ii) in any of the methods described by dissociating the RPE cells, fractionating the RPE cells to collect RPE cell clusters, dissociating the RPE cell clusters into single RPE cells, and culturing the single RPE cells. In another embodiment, the method may further comprise harvesting the RPE cells produced in step (ii) in any of the methods described by dissociating the RPE cells, collecting RPE cell clusters, and selectively picking RPE cell clusters. The method may additionally comprise dissociating the selectively picked RPE cell clusters into single RPE cells and culturing the single RPE cells.

[12] In any of the embodiments of the present invention, the method may further comprise expanding the RPE cells. The RPE cells may be expanded by culturing the cells in maintenance media supplemented with FGF. In an embodiment, the RPE cells are cultured in maintenance medium comprising FGF during the first 1, 2, or 3 days of RPE proliferation at

CLAIMS:

1. A method for producing a population of retinal epithelium (RPE) cells, the method comprising:

(i) obtaining RPE progenitor cells and fractionating the RPE progenitor cells to collect cell clusters of PAX6+/MITF+ RPE progenitor cells and dissociating the PAX6+/MITF+ RPE progenitor cell clusters into single PAX6+/MITF+ RPE progenitor cells;

(ii) culturing the single PAX6+/MITF+ RPE progenitor cells in a differentiation medium such that the PAX6+/MITF+ RPE progenitor cells differentiate to RPE cells; and

(iii) harvesting the RPE cells produced in step (ii);

thereby producing a population of RPE cells.

2. A method for producing a population of retinal epithelium (RPE) cells, the method comprising:

(i) obtaining RPE progenitor cells and fractionating the RPE progenitor cells to collect cell clusters of PAX6+/MITF+ RPE progenitor cells,

(ii) culturing the PAX6+/MITF+ RPE progenitor cell clusters in a differentiation medium such that the PAX6+/MITF+ RPE progenitor cells differentiate to RPE cells; and

(iii) harvesting the RPE cells produced in step (ii);

thereby producing a population of RPE cells.

3. A method for producing a population of retinal epithelium (RPE) cells, the method comprising:

(i) obtaining RPE progenitor cells and fractionating the RPE progenitor cells to collect cell clusters of PAX6+/MITF+ RPE progenitor cells and dissociating the PAX6+/MITF+ RPE progenitor cell clusters into single PAX6+/MITF+ RPE progenitor cells;

(ii) culturing the single PAX6+/MITF+ RPE progenitor cells in a differentiation medium such that the PAX6+/MITF+ RPE progenitor cells differentiate to RPE cells; and

(iii) harvesting and cryopreserving the RPE cells produced in step (ii);

wherein the RPE cells are expanded by passaging at least one time,

thereby producing a population of RPE cells.

4. A method for producing a population of retinal epithelium (RPE) cells, the method comprising:

(i) obtaining RPE progenitor cells and fractionating the RPE progenitor cells to collect cell clusters of PAX6+/MITF+ RPE progenitor cells,

(ii) culturing the PAX6+/MITF+ RPE progenitor cell clusters in a differentiation medium such that the PAX6+/MITF+ RPE progenitor cells differentiate to RPE cells; and

(iii) harvesting and cryopreserving the RPE cells produced in step (ii);

wherein the RPE cells are expanded by passaging at least one time,

thereby producing a population of RPE cells.

5. The method of any one of claims 1-4, wherein step (iii) further comprises harvesting the RPE cells produced in step (ii) by dissociating the RPE cells, fractionating the RPE cells, collecting RPE cell clusters, dissociating the RPE cell clusters into single RPE cells, and culturing the single RPE cells.

6. The method of any one of claims 1-5, wherein the RPE progenitor cells are obtained from a population of pluripotent stem cells.

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

8. The method of any one of claims 1-7, wherein the RPE cells are expanded by passaging two times.

9. The method of any one of claims 1-7, wherein the RPE cells are expanded by passaging three times.

10. The method of claim 8 or 9, wherein the RPE cells are expanded by culturing the cells in maintenance media supplemented with FGF.

11. The method of claim 10, wherein the maintenance medium comprises FGF during the first 1, 2, or 3 days of RPE proliferation at each passage, followed by culturing the RPE cells in maintenance media lacking FGF.

12. The method of claim 10 or 11, wherein FGF is added before confluence.

13. The method of any one of claims 1-12, wherein the differentiation medium further comprises heparin and/or ROCK inhibitor.

14. The method any one of claims 1, 3 and 5-13, wherein any one of the dissociation steps is carried out by treating the cells with a dissociation reagent.

15. The method of claim 14, wherein the dissociation reagent is selected from the group consisting of collagenase, accutase, chelator*e.g.*, trypsin, dispase, or any combinations thereof.

16. The method of claim 15, wherein the collagenase is collagenase I or collagenase IV; and/or the chelator is an EDTA based dissociation solution.

17. The method of any one of claims 3-16, wherein the cells are cryopreserved in a medium comprising one or more cryopreservative selected from the group consisting of DMSO (dimethyl sulfoxide), ethylene glycol, glycerol, 2-methyl-2-4-pentanediol (MPD), propylene glycol, and sucrose.

18. The method of any one of claims 6-17, wherein the population of pluripotent stem cells is embryoid bodies.

19. The method of any one of claims 1-18, wherein the cells are cultured on feeder cells.

20. The method of any one claims 1-18, wherein the cells are cultured under feeder-free conditions.

21. The method of any one of claims 1-20, wherein the cells are cultured in a non-adherent culture.

22. The method of any one of claims 1-20, wherein the cells are cultured in an adherent culture.

23. The method of any one of claims 1-22, wherein the differentiation medium is embryoid body differentiation medium (EBDM).

24. The method of any one of claims 1-22, wherein the differentiation medium comprises one or more differentiation agents selected from the group consisting of nicotinamide, a transforming factor-β (TGFβ) superfamily member (*e.g.*, activin A, activin B, and activin AB), nodal, anti-mullerian hormone (AMH), bone morphogenetic proteins (BMP) (*e.g.*, BMP2, BMP3, BMP4, BMP5, BMP6, and BMP7, growth and differentiation factors (GDF)), WNT pathway inhibitor (*e.g.*, CKI-7, DKK1), a TGF pathway inhibitor (*e.g.*, LDN193189, Noggin), a BMP pathway inhibitor (*e.g.*, SB431542), a sonic hedgehog signal inhibitor, a bFGF inhibitor, and a MEK inhibitor (*e.g.*, PD0325901).

25. The method of claim 24, wherein the differentiation medium comprises nicotinamide.

26. The method of claims 24 or 25, wherein the differentiation medium comprises activin.

27. The method of any one of claims 1-26, wherein the cell clusters of PAX6+/MITF+ RPE progenitor cells are greater than or equal to 40 μm and less than or equal to 200 μm in size.

28. The method of any one of claims 1-27, wherein the cell clusters of PAX6+/MITF+ RPE progenitor cells are greater than or equal to 40 μm and less than or equal to 100 μm in size.

29. The method of any one of claims 1-28, wherein in step (ii), the PAX6+/MITF+ RPE progenitor cells are cultured on an extracellular matrix selected from the group consisting of laminin or a fragment thereof, fibronectin, vitronectin, Matrigel, CellStart, collagen, and gelatin.

30. The method of claim 29, wherein the extracellular matrix is laminin or a fragment thereof.

31. The method of claim 30, wherein the laminin is selected from the group consisting of laminin-521 and laminin-511.

32. The method of claim 31, wherein the laminin is a recombinant laminin-511 E8 fragment.

33. The method of any one of claims 1-32, wherein the duration of culturing in step (ii) is 1 week to 8 weeks.

34. The method of any one of claims 1-32, wherein the duration of culturing in step (ii) is at least about 3 weeks.

35. The method of any one of claims 1-32, wherein the duration of culturing in step (ii) is about 6 weeks.

36. The method of any one of claims 5-35, wherein the RPE cell clusters are greater than or equal to 40 μm and less than or equal to 200 μm in size.

37. The method of claim 36, wherein the RPE cell clusters are greater than or equal to 40 μm and less than or equal to 100 μm in size.

38. The method of any one of claims 5-37, wherein the single RPE cells are cultured in a medium that supports RPE growth or differentiation.

39. The method of claim 38, wherein the single RPE cells are cultured on an extracellular matrix selected from the group consisting of laminin or a fragment thereof, fibronectin, vitronectin, Matrigel, CellStart, collagen, and gelatin.

40. The method of claim 39, wherein the extracellular matrix is gelatin.

41. The method of claim 39, wherein the extracellular matrix is laminin or a fragment thereof.

42. The method of any one of claims 1-41, wherein the population of RPE cells are at least 75% pure, at least 80% pure, at least 90% pure, at least 95% pure, at least 96% pure, at least 97% pure, at least 98% pure, or at least 99% pure.

43. The method of any one of claims 1-42, wherein the RPE cells are human RPE cells.

44. A method for producing a population of retinal epithelium (RPE) cells, the method comprising:

(i) culturing a population of pluripotent stem cells in a first differentiation medium, such that the pluripotent stem cells differentiate into RPE progenitor cells;

(ii) dissociating the RPE progenitor cells, fractionating the RPE progenitor cells to collect RPE progenitor cell clusters, dissociating the collected RPE progenitor cell clusters into single RPE progenitor cells, and subculturing the single RPE progenitor cells in a second differentiation medium such that the RPE progenitor cells differentiate to RPE cells; and

(iii) harvesting the RPE cells produced in step (ii)

thereby producing a population of RPE cells.

45. A method for producing a population of retinal epithelium (RPE) cells, the method comprising:

(i) culturing a population of pluripotent stem cells in a first differentiation medium, such that the pluripotent stem cells differentiate into RPE progenitor cells;

(ii) dissociating the RPE progenitor cells, fractionating the RPE progenitor cells to collect RPE progenitor cell clusters, and subculturing the collected RPE progenitor cell clusters in a second differentiation medium such that the RPE progenitor cells differentiate to RPE cells; and

(iii) harvesting the RPE cells produced in step (ii)

thereby producing a population of RPE cells.

46. A method for producing a population of retinal epithelium (RPE) cells, the method comprising:

(i) culturing a population of pluripotent stem cells in a first differentiation medium, such that the pluripotent stem cells differentiate into RPE progenitor cells;

(ii) dissociating the RPE progenitor cells, fractionating the RPE progenitor cells to collect RPE progenitor cell clusters, dissociating the collected RPE progenitor cell clusters into single RPE progenitor cells, and subculturing the single RPE progenitor cells in a second differentiation medium such that the RPE progenitor cells differentiate to RPE cells; and

(iii) harvesting and cryopreserving the RPE cells produced in step (ii);

wherein the RPE cells are expanded by passaging at least one time,

thereby producing a population of RPE cells.

47. A method for producing a population of retinal epithelium (RPE) cells, the method comprising:

(i) culturing a population of pluripotent stem cells in a first differentiation medium, such that the pluripotent stem cells differentiate into RPE progenitor cells;

(ii) dissociating the RPE progenitor cells, fractionating the RPE progenitor cells to collect RPE progenitor cell clusters, and subculturing the collected RPE progenitor cell clusters in a second differentiation medium such that the RPE progenitor cells differentiate to RPE cells; and

(iii) harvesting and cryopreserving the RPE cells produced in step (ii);

wherein the RPE cells are expanded by passaging at least one time,

thereby producing a population of RPE cells.

48. The method of any one of claims 44-47, wherein step (iii) further comprises harvesting the RPE cells produced in step (ii) by dissociating the RPE cells, fractionating the RPE cells to collect RPE cell clusters, dissociating the RPE cell clusters into single RPE cells, and culturing the single RPE cells.

.

49. The method of any one of claims 44-48, wherein the RPE progenitor cells are positive for PAX6/MITF.

50. The method of any one of claims 44-49, wherein the RPE cells are expanded by passaging two times.

51. The method of any one of claims 44-49, wherein the RPE cells are expanded by passaging three times.

52. The method of claim 50 or 51, wherein the RPE cells are expanded by culturing the cells in maintenance media supplemented with FGF.

53. The method of claim 52, wherein the maintenance medium comprises FGF during the first 1, 2, or 3 days of RPE proliferation at each passage, followed by culturing the RPE cells in maintenance media lacking FGF.

54. The method of claim 52 or 53, wherein FGF is added before confluence.

55. The method of any one of claims 44-54, wherein the first and/or second differentiation medium further comprises heparin and/or ROCK inhibitor.

56. The method any one of claims 44-55, wherein any one of the dissociation steps is carried out by treating the cells with a dissociation reagent.

57. The method of claim 56, wherein the dissociation reagent is selected from the group consisting of collagenase, accutase, chelator, trypsin, dispase, or any combinations thereof.

58. The method of claim 57, wherein the collagenase is collagenase I or collagenase IV; and/or the chelator is an EDTA based dissociation solution.

59. The method of any one of claims 46-58 wherein the cells are cryopreserved in a medium comprising one or more cryopreservative selected from the group consisting of DMSO (dimethyl sulfoxide), ethylene glycol, glycerol, 2-methyl-2-4-pentanediol (MPD), propylene glycol, and sucrose.

60. The method of any one of claims 44-59, wherein the pluripotent stem cells are human embryonic stem cells.

61. The method of any one of claims 44-59, wherein the pluripotent stem cells are human induced pluripotent stem cells.

62. The method of any one claims 44-61, wherein the population of pluripotent stem cells is embryoid bodies.

63. The method of any one of claims 44-62, wherein prior to step (i), the pluripotent stem cells are cultured on feeder cells in a medium that supports pluripotency.

64. The method of any one of claims 44-62, wherein prior to step (i), the pluripotent stem cells are cultured feeder-free in a medium that supports pluripotency.

65. The method of claim 63 or 64, wherein the medium that supports pluripotency is supplemented with bFGF.

66. The method of any one of claims 44-65, wherein step (i), (ii), and/or (iii) is performed in a non-adherent culture.

67. The method of any one of claims 44-65, wherein step (i), (ii), and/or (iii) is performed in an adherent culture.

68. The method of any one of claims 44-67, wherein the first and second differentiation medium are the same medium.

69. The method of any one of claims 44-67, wherein the first and second differentiation medium are different media.

70. The method of any one of claims 44-68, wherein the first and second differentiation medium is embryoid body differentiation medium (EBDM).

71. The method of any one of claims 44-69, wherein the first differentiation medium comprises one or more differentiation agents selected from the group consisting of nicotinamide, a transforming factor-β (TGFβ) superfamily member (*e.g.*, activin A, activin B, and activin AB), nodal, anti-mullerian hormone (AMH), bone morphogenetic proteins (BMP) (*e.g.*, BMP2, BMP3, BMP4, BMP5, BMP6, and BMP7, growth and differentiation factors (GDF)), WNT pathway inhibitor (*e.g.*, CKI-7, DKK1), a TGF pathway inhibitor (*e.g.*, LDN193189, Noggin), a BMP pathway inhibitor (*e.g.*, SB431542), a sonic hedgehog signal inhibitor, a bFGF inhibitor, and a MEK inhibitor (*e.g.*, PD0325901).

72. The method of any one of claims 44-69, wherein the second differentiation medium comprises one or more differentiation agents selected from the group consisting of nicotinamide, a transforming factor-β (TGFβ) superfamily member (*e.g.*, activin A, activin B, and activin AB), nodal, anti-mullerian hormone (AMH), bone morphogenetic proteins (BMP) (*e.g.*, BMP2, BMP3, BMP4, BMP5, BMP6, and BMP7, growth and differentiation factors (GDF)), WNT pathway inhibitor (*e.g.*, CKI-7, DKK1), a TGF pathway inhibitor (*e.g.*, LDN193189, Noggin), a

BMP pathway inhibitor (*e.g.*, SB431542), a sonic hedgehog signal inhibitor, a bFGF inhibitor, and a MEK inhibitor (*e.g.*, PD0325901).

73. The method of claim 71 or 72, wherein the first differentiation medium comprises nicotinamide.

74. The method of any one of claims 71-73, wherein the second differentiation medium comprises activin.

75. The method of any one claims 44-74, wherein the duration of culturing in step (i) is 1 weeks to 12 weeks.

76. The method of any one of claims 44-74, wherein the duration of culturing in step (i) is at least about 3 weeks.

77. The method of any one of claims 44-76, wherein the duration of culturing in step (i) is 6 to 10 weeks.

78. The method of any one of claims 44-77, wherein the RPE progenitor cell clusters collected in step (ii) are greater than or equal to 40 μm and less than or equal to 200 μm in size.

79. The method of any one of claims 44-78, wherein the RPE progenitor cell clusters collected in step (ii) are greater than or equal to 40 μm and less than or equal to 100 μm in size.

80. The method of any one of claims 44-79, wherein in step (ii), the RPE progenitor cells are subcultured on an extracellular matrix selected from the group consisting of laminin, fibronectin, vitronectin, Matrigel, CellStart, collagen, and gelatin.

81. The method of claim 80, wherein the extracellular matrix comprises laminin or a fragment thereof.

82. The method of claim 81, wherein the laminin or fragment thereof is selected from the group consisting of laminin-521 and laminin-511.

83. The method of any one of claims 44-82, wherein the duration of subculturing in step (ii) is 1 week to 8 weeks.

84. The method of any one claims 44-82, wherein the duration of subculturing in step (ii) is at least about 3 weeks.

85. The method of any one of claims 44-84, wherein the duration of subculturing in step (ii) is about 6 weeks.

86. The method of any one of claims 48-85, wherein the RPE cell clusters are greater than or equal to 40 μm and less than or equal to 200 μm in size.

87. The method of claim 86, wherein the RPE cell clusters are greater than or equal to 40 μm and less than or equal to 100 μm in size.

88. The method of any one of claims 48-87, wherein the single RPE cells are cultured in a medium that supports RPE growth or differentiation.

89. The method of claim 88, wherein the single RPE cells are cultured on an extracellular matrix selected from the group consisting of laminin or a fragment thereof, fibronectin, vitronectin, Matrigel, CellStart, collagen, and gelatin.

90. The method of claim 89, wherein the extracellular matrix is gelatin.

91. The method of claim 89, wherein the extracellular matrix is laminin or a fragment thereof.

92. The method of any one of claims 44-91, wherein the population of RPE cells are at least 75% pure, at least 80% pure, at least 90% pure, at least 95% pure, at least 96% pure, at least 97% pure, at least 98% pure, or at least 99% pure.

93. The method of any one of claims 44-92, wherein the RPE cells are human RPE cells.

94. The method of any of claims 1-93, wherein the RPE cells express one or more markers selected from the group consisting of RPE65, CRALBP, PEDF, Bestrophin, MITF, OTX2, PAX2, PAX6, premelanosome protein (PMEL or gp-100), tyrosinase, and ZO1.

95. The method of any one of claims 1-94, wherein the RPE cells express Bestrophin, PMEL, CRALBP, MITF, PAX6, and ZO1.

96. The method of any one of claims 1-94, wherein the RPE cells express Bestrophin, PAX6, MITF, and RPE65.

97. The method of any one of claims 1-94, wherein the RPE cells express MITF and at least one marker selected from the group consisting of Bestrophin and PAX6.

98. The method of any one of claims 1-97, wherein the expression in the RPE cells of one or more stem cell markers selected from the group consisting of OCT4, NANOG, Rex- 1, alkaline phosphatase, SOX2, TDGF- 1, DPPA-2, DPPA-4, stage specific embryonic antigen (SSEA)-3 and SSEA-4, tumor rejection antigen (TRA)-l -60 and TRA-1-80 is down-regulated compared to their expression in pluripotent stem cells.

99. The method of any one of claims 1-98, wherein the expression in the RPE cells of OCT4, SSEA4, TRA-1-81, and alkaline phosphatase is down-regulated compared to their expression in pluripotent stem cells.

100. The method of any one of claims 1-98, wherein the expression in the RPE cells of OCT4, NANOG, and SOX2 is down-regulated compared to their expression in pluripotent stem cells.

101. A pharmaceutical composition comprising a population of at least 1 x 10^4 RPE cells produced by the method of any one of claims 1-100, and a pharmaceutically acceptable carrier, wherein the RPE cells express one or more markers selected from the group consisting of RPE65, CRALBP, PEDF, Bestrophin, MITF, OTX2, PAX2, PAX6, premelanosome protein (PMEL or gp-100), tyrosinase, and ZO1.

102. Use of the pharmaceutical composition of claim 101 in the manufacture of a medicament for the treatment of a patient with a retinal disease, wherein an effective amount of the the pharmaceutical composition of claim 102 is to be administered to the patient.

103. The use of claim 102, wherein the retinal disease is selected from the group consisting of retinal degeneration, choroideremia, diabetic retinopathy, age-related macular degeneration (AMD) (dry or wet), retinal detachment, retinitis pigmentosa, Stargardt's Disease, Angioid streaks, Myopic Macular Degeneration, geographic atrophy secondary to AMD, and glaucoma.

104. The pharmaceutical composition of claim 101 for use in therapy.