

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

[9a] 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 cell clusters, dissociating the 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.

[9b] 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 cell clusters, and subculturing the collected 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.

[9c] In another aspect, the present invention provides a composition comprising a population of RPE cells produced by the method as described herein.

[9d] In another aspect, the present invention provides a pharmaceutical composition comprising a population of RPE cells produced by the method as described herein and a pharmaceutically acceptable carrier.

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

[9f] In another aspect, the present invention provides the composition as described herein or 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