A SCALABLE METHOD FOR PRODUCING RETINAL PIGMENT EPITHELIUM (RPE) CELLS

FIELD OF INVENTION

[001] The present disclosure relates to the field of cell culture techniques and cell differentiation, and methods thereof, and particularly to a cell culture method for producing retinal pigment epithelium (RPE) cells from induced pluripotent stem cells (iPSCs).

BACKGROUND OF THE INVENTION

[002] Macular degeneration also known as age-related macular degeneration (AMD) is a foremost cause of blindness in people over the age of 60, worldwide. Clinically, this disease starts with distortion in central vision, which is caused due to a damage in the macula, and eventually leads to legal blindness. Vision loss has a significant impact on quality of life and incurs a substantial cost to the economy. Furthermore, AMD is a complex and progressive neurodegenerative disorder that triggers visual impairment due to the loss of retinal pigmented epithelium (RPE) and the light-sensitive photoreceptors, which they support, protect, and provide nutrition. Currently, there is no curative treatment for the most common form of this disease, i.e., dry AMD. Therefore, there is an immediate need to develop therapeutic strategies to address the loss of RPE cells and provide a cure for AMD.

SUMMARY

[003] In an aspect of the present disclosure, there is provided a method for obtaining RPE (retinal pigment epithelium) cells from iPSCs (induced pluripotent stem cells) comprising the steps of: (a) generating embryoid bodies from a culture of iPSCs, wherein the embryoid bodies are in non-adherent suspension culture, (b) plating the embryoid bodies on a culture dish coated with a suitable extracellular matrix in differentiation induction media (DIM) and culturing for 6-8 days to obtain neuroectoderm lineage, wherein the DIM comprises at least one WNT pathway inhibitor and at least two SMAD pathway inhibitors, (c) culturing the neuroectoderm lineage for 11-22 days in differentiation propagation media (DPM)

for rosette formation, wherein the DPM does not comprise any inhibitor, (d) culturing the rosettes of step (c) in Retinal Pigment Epithelium Maturation Media (RPEMM) for 23-45 days to facilitate retinal progenitor cells formation, and (e) plating the retinal progenitor cells of step (d) on a culture dish coated with suitable extracellular matrix and culturing for 47-75 days in RPEMM to obtain RPE cells.

[004] In an aspect of the present disclosure, there is provided a retinal pigment epithelium cell, or a population thereof, produced by the method as disclosed herein.

[005] In an aspect of the present disclosure, there is provided a pharmaceutical composition comprising the retinal pigment epithelium cells produced by the method as disclosed herein; and pharmaceutically acceptable carriers.

[006] In an aspect of the present disclosure, there is provided a method of treating a retinal degeneration disease in a subject comprising: administering the composition as disclosed herein to the subject. In another aspect, the present disclosure refers to the use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating retinal degeneration disease. In yet another aspect, the present disclosure refers to the use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a retinal degeneration disease in a subject, wherein the medicament is to be administered to the subject.

[007] These and other features, aspects, and advantages of the present subject matter will be better understood with reference to the following description. This summary is provided to introduce a selection of concepts in a simplified form. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter.

BRIEF DESCRIPTION OF DRAWINGS

[008] The following drawings form a part of the present specification and are included to further illustrate aspects of the present disclosure. The disclosure may be better understood by reference to the drawings in combination with the detailed description of the specific embodiments presented herein.

[009] Figure 1 depicts the Differentiation of RPE, A) Embryoid body formation from iPSCs and their specification towards retinal fate through neuroectoderm induction; B) Formation of rosette-like structures and epithelial clusters indicating the onset of retinal differentiation; C) Retinal progenitors showing fate commitment towards RPE cells with the onset of pigmentation; D) RPE cultures with increase in pigmentation levels and typical hexagonal morphology; E) RPE culture plate

I/We Claim:

1. A method for obtaining RPE (retinal pigment epithelium) cells from iPSCs (induced pluripotent stem cells) comprising the steps of:
   (a) generating embryoid bodies from a culture of iPSCs, wherein the embryoid bodies are in non-adherent suspension culture,
   (b) plating the embryoid bodies on a culture dish coated with a suitable extracellular matrix in differentiation induction media (DIM) and culturing for 6-8 days to obtain neuroectoderm lineage, wherein the DIM comprises at least one WNT pathway inhibitor and at least two SMAD pathway inhibitors,
   (c) culturing the neuroectoderm lineage for 11-22 days in differentiation propagation media (DPM) for rosette formation, wherein the DPM does not comprise any inhibitor,
   (d) culturing the rosettes of step (c) in Retinal Pigment Epithelium Maturation Media (RPEMM) for 23-45 days to facilitate retinal progenitor cells formation, and
   (e) plating the retinal progenitor cells of step (d) on a culture dish coated with suitable extracellular matrix and culturing for 47-75 days in RPEMM to obtain RPE cells.
2. The method as claimed in claim 1, wherein the method further comprises enriching the RPE cells, comprising:
   a) enzymatically dissociating the RPE cells from the extracellular matrix to obtain a single cell suspension of RPEs; and
   b) plating the single cell suspension of RPEs of step (a) on a culture dish coated with suitable extracellular matrix and culturing for 75-100 days in RPEMM.
3. The method as claimed in claim 1, wherein the culture of iPSCs has a confluency in the range of 80%-90%.
4. The method as claimed in claim 1, wherein generating the embryoid bodies in step (a) comprises:

(a) culturing the iPSCs for 24 hours in growth media to form embryoid bodies, wherein the growth media comprises an expansion media and a ROCK inhibitor, and

(b) gradually contacting the developing embryoid bodies from the growth media to the DIM by (i) culturing the developing embryoid bodies in a media composition comprising the expansion media and DIM in a ratio of 3:1 for 24 hours, (ii) culturing the developing embryoid bodies obtained in step (i) in a media composition comprising the expansion media and DIM in a ratio of 1:1 for 24 hours, and (iii) culturing the embryoid bodies obtained in step (ii) in DIM for 24 hours followed by plating the embryoid bodies on a culture dish.

5. The method as claimed in claim 1, wherein the rosette formation in step (c) comprises:

(i) maintaining the neuroectoderm lineage in DIM for 24 hours;

(ii) maintaining the neuroectoderm lineage obtained in step (i) in a media composition comprising DIM and DPM in a ratio of 1:1 for 24-48 hours; and

(iii) culturing the neuroectoderm lineage obtained from step (ii) in DPM for 24 hours for facilitating rosette formation.

6. The method as claimed in claim 1, wherein the retinal progenitor cells formation in step (d) comprises:

(i) maintaining the rosettes in DPM for 24 hours;

(j) maintaining the rosettes obtained in step (i) in a media composition comprising DPM and RPEMM in a ratio of 1:1 for 24 hours; and

(k) culturing the rosettes obtained from step (j) in RPEMM for 24 hours for facilitating the formation of retinal progenitor cells.

7. The method as claimed in claim 1 or claim 2, wherein the at least one suitable extracellular matrix is selected from matrigel, laminin, vitronectin, fibronectin, collagen, poly-L-lysine, poly-L-ornithine, or combinations thereof.

8. The method as claimed in claim 1, wherein the at least one WNT pathway inhibitor is selected from 4-(1,3,3a,4,7,7a-Hexahydro-1,3-dioxo-4,7-methano-2H-isoindol-2-yl)-N-8-quinolinyl-Benzamide, 5-(Phenylsulfonyl)-N-piperidin-4-yl-2(trifluoromethyl)benzene sulfonamide, 2-(2′,3-Dimethyl-[2,4′-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl) acetamide, 2-(4-(2-methyl pyridin-4-yl) phenyl)-N-(4-(pyridin-3-yl)phenyl)acetamide, 8-Tetrahydro-2-[4-(trifluoro methyl)phenyl]-4H-thio pyrano[4,3-d]pyrimidin-4-one, or combinations thereof.

9. The method as claimed in claim 1, wherein the at least two SMAD pathway inhibitors are selected from 4-[4-(1,3-benzodioxol-5-yl)-5-pyridin-2-yl-1H-imidazol-2-yl]benzamide, 4-(6-(4-(piperazin-1-yl)phenyl)pyrazolo[1,5-a]pyrimidin-3-yl)quinoline, 3-[(1R)-1-(2,6-dichloro-3-fluorophenyl)ethoxy]-5-(1-piperidin-4-ylpyrazol-4-yl)pyridin-2-amine, 5-chloro-2-N-[2-methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]phenyl]-4-N-(2-propan-2-ylsulfonylphenyl)pyrimidine-2,4-diamine, 9-ethyl-6,6-dimethyl-8-(4-morpholin-4-ylpiperidin-1-yl)-11-oxo-5H-benzo[b]carbazole-3-carbonitrile, 5-chloro-2-N-(5-methyl-4-piperidin-4-yl-2-propan-2-yloxyphenyl)-4-N-(2-propan-2-ylsulfonylphenyl) pyrimidine-2,4-diamine, or combinations thereof.

10. The method as claimed in claim 4, wherein the ROCK inhibitor is (1R,4r)-4-((R)-1-aminoethyl)-N-(pyridin-4-yl)cyclohexanecarboxamide.

11. The method as claimed in claim 2, wherein the enzymatic dissociation of RPE cells in step (a) is carried out using an enzyme selected from the group consisting of Accutase, Tryple select, TrypLE, Gentle Cell Dissociation Reagent (GCDR), and Dispase.

12. A retinal pigment epithelium cell, or a population thereof, produced by the method as claimed in claim 1.

13. A pharmaceutical composition comprising the retinal pigment epithelium cell or population thereof as claimed in claim 12; and pharmaceutically acceptable carriers.

14. Use of the pharmaceutical composition as claimed in claim 13 in the manufacture of a medicament for treating retinal degeneration disease.

15. The use of claim 14, wherein the retinal degeneration disease is selected from a group consisting of age-related macular degeneration, and retinal diseases associated with early and late-stage photoreceptor degeneration.

16. Use of the pharmaceutical composition as claimed in claim 13 in the manufacture of a medicament for treating a retinal degeneration disease in a subject, wherein the medicament is to be administered to the subject.