

Solution to Problem

[0009]

The present inventors have conducted intensive studies for solving the above problems, and have found that when human iPS cells are cultured in a differentiation medium containing KSR and the like without using serum or feeder cells, the cells autonomously differentiate in about 4 weeks and form a (multi-zone) colony consisting of concentric zones of different ectodermal cell lineage. In addition, it has been found that ocular surface ectodermal lineage cells including epithelial stem cells can be separated from the resultant colony, and that corneal epithelial progenitor cells are differentiation-induced, isolated and further maturation-cultured to provide stratified corneal epithelial cells. The resultant corneal epithelial cells can be recovered in sheet form and transplanted and have been confirmed to function as corneal epithelium in vitro and in vivo like corneal epithelial cells derived from somatic cells.

[0010]

The present disclosure has been accomplished based on the above findings, and relates to the following (1) to (15):

(1) A method for producing a colony consisting of concentric zones of different ectodermal cell lineage,

comprising subjecting pluripotent stem cells (particularly, induced pluripotent stem cells) to two-dimensional culture in a serum-free medium without using feeder cells;

(1a) In an aspect, there is provided a method for producing a colony consisting of concentric zones of different ectodermal cell lineages, comprising subjecting pluripotent stem cells to two-dimensional culture in a serum-free medium without using feeder cells, by autonomous differentiation of the pluripotent stem cells, wherein the colony comprises zones of neuroectodermal lineage cells, neural crest lineage cells/optic cup lineage cells, ocular surface ectodermal lineage cells, and surface ectodermal lineage cells, respectively;

(2) The method according to (1) above, wherein the concentric zones of different ectodermal cell lineage are formed by autonomous differentiation of the pluripotent stem cells;

(3) The method according to (1) or (2) above, wherein the serum-free medium does not contain 0.5 nM or more of BMP4 (bone morphogenetic protein 4), transforming growth factor, or activin;

(4) The method according to (3) above, wherein the serum-free medium further does not contain at least one selected from the group consisting of high concentration retinoic acid, a BMP inhibitor, a TGF β inhibitor, and Noggin;

(5) The method according to any one of (1) to (4) above, wherein the pluripotent stem cells are cultured using a vessel coated with one or more selected from the group consisting of collagen, fibronectin, laminin or laminin fragments, vitronectin, basement membrane matrices, gelatin, hyaluronic acid, polylysine, vitronectin, and hyaluronic acid;

(6) The method according to any one of (1) to (5) above, wherein the colony comprises zones of neuroectodermal lineage cells, neural crest lineage

cells/optic cup lineage cells, ocular surface ectodermal lineage cells, and surface ectodermal lineage cells, respectively;

(7) A method for producing eye-related cells, comprising the steps of:

producing a colony consisting of concentric zones of different ectodermal cell lineage by the method according to any one of (1) to (6) above,

separating cells contained in a particular zone from the colony, and

inducing the differentiation of the cells into eye-related cells;

(7a) In another aspect, there is provided a method for producing eye-related cells, comprising the steps of: producing a colony consisting of concentric zones of different ectodermal cell lineages by the method as defined herein, separating cells contained in a particular zone from the colony, and inducing the differentiation of the cells into eye-related cells;

(8) The method according to (7) above, wherein the eye-related cells are any one selected from the group consisting of corneal epithelial cells, retinal pigment epithelial cells, neuroretinal cells, conjunctival epithelial cells, limbal epithelial cells, corneal endothelial cells, keratocytes, iris stromal cells, scleral cells, iris pigment epithelial cells, ciliary epithelial cells, optic nerve cells, sublimbal

fibroblasts, subconjunctival fibroblasts, lacrimal gland cells, meibomian gland cells, goblet cells, lens epithelial cells, and eyelid epithelial cells;

(9) A method for producing corneal epithelial cells, comprising the steps of:

producing a colony consisting of concentric zones of different ectodermal cell lineage by the method according to any one of (1) to (6) above,

culturing the colony in a medium containing a growth factor,

separating cells contained in a zone of ocular surface ectodermal lineage cells and culturing the cells in a medium containing KGF, a Rock inhibitor, and a serum substitute to induce differentiation into corneal epithelial progenitor cells, and

isolating the corneal epithelial progenitor cells and inducing differentiation into corneal epithelial cells;

(9a) In another aspect, there is provided a method for producing corneal epithelial cells, comprising the steps of: producing a colony consisting of concentric zones of different ectodermal cell lineages by the method as defined herein, culturing the colony in a medium containing a growth factor, separating cells contained in a zone of ocular surface ectodermal lineage cells and culturing the cells in a medium containing KGF, a Rock inhibitor, and a serum substitute to induce differentiation into corneal epithelial progenitor cells, and isolating the corneal epithelial progenitor cells and inducing differentiation into corneal epithelial cells;

(10) The method according to (9) above, wherein the corneal epithelial cells are isolated using the presence or absence of expression of one or more markers selected from the group consisting of ITG β 4, SSEA4, TRA-1-60, and CD200, preferably ITG β 4 positivity and SSEA4 positivity, more preferably TRA-1-60 or CD200 negativity and ITG β 4 positivity and SSEA4 positivity, still more preferably CD200 negativity and ITG β 4 positivity and SSEA4 positivity, as an index;

(11) Corneal epithelial cells produced by the method according to (9) or (10) above, the corneal epithelial cells being K12-, pax6-, and p63-positive;

(12) A method for producing a corneal cell sheet, comprising a step of producing corneal epithelial cells

by the method according to (9) or (10) above and a step of further subjecting the resultant corneal epithelial cells to stratification culture;

(12a) In another aspect, there is provided a method for producing a corneal cell sheet, comprising the steps of producing corneal epithelial cells by the method as defined herein and further subjecting the resultant corneal epithelial cells to stratification culture;

(13) A colony consisting of concentric zones of different ectodermal cell lineage derived from pluripotent stem cells (particularly, induced pluripotent stem cells);

(13a) In another aspect, there is provided a colony consisting of concentric zones of different ectodermal cell lineages derived from pluripotent stem cells, wherein the colony comprises zones of neuroectodermal lineage cells, neural crest lineage cells/optic cup lineage cells, ocular surface ectodermal lineage cells, and surface ectodermal lineage cells, respectively;

(14) The colony according to (13) above, produced by the method according to any one of (1) to (6) above;

(15) A stratified corneal epithelial cell sheet derived from pluripotent stem cells (particularly, induced pluripotent stem cells), wherein the corneal epithelial cells are K12-, pax6-, and MUC16-positive; and

(16) Corneal epithelial cells derived from pluripotent stem cells (particularly, induced pluripotent

stem cells) produced by separating and culturing cells contained in a zone of ocular surface ectodermal lineage cells in the colony according to (13) or (14), the corneal epithelial cells being K12-, pax6-, and p63-positive.

Advantageous Effects of Invention

[0011]

A method has heretofore been reported for inducing epithelial cells from human iPS cells; however, stratified functional cells have not been prepared which hold markers specific for corneal epithelial cells, such

Claims

[Claim 1]

A method for producing a colony consisting of concentric zones of different ectodermal cell lineages, comprising subjecting pluripotent stem cells to two-dimensional culture in a serum-free medium without using feeder cells, by autonomous differentiation of the pluripotent stem cells,

wherein the colony comprises zones of neuroectodermal lineage cells, neural crest lineage cells/optic cup lineage cells, ocular surface ectodermal lineage cells, and surface ectodermal lineage cells, respectively.

[Claim 2]

The method according to claim 1, wherein the serum-free medium does not contain 0.5 nM or more of BMP4 (bone morphogenetic protein 4), transforming growth factor, or activin.

[Claim 3]

The method according to claim 2, wherein the serum-free medium further does not contain at least one selected from the group consisting of high concentration

retinoic acid, a BMP inhibitor, a TGF β inhibitor, and Noggin.

[Claim 4]

The method according to any one of claims 1 to 3, wherein the pluripotent stem cells are cultured using a

vessel coated with one or more selected from the group consisting of collagen, fibronectin, laminin or laminin fragments, vitronectin, basement membrane matrices, gelatin, hyaluronic acid, and polylysine=.

[Claim 5]

A method for producing eye-related cells, comprising the steps of:

producing a colony consisting of concentric zones of different ectodermal cell lineages by the method according to any one of claims 1 to 4,

separating cells contained in a particular zone from the colony, and

inducing the differentiation of the cells into eye-related cells.

[Claim 6]

The method according to claim 5, wherein the eye-related cells are any one selected from the group consisting of corneal epithelial cells, retinal pigment epithelial cells, neuroretinal cells, conjunctival epithelial cells, limbal epithelial cells, corneal

endothelial cells, keratocytes, iris stromal cells, scleral cells, iris pigment epithelial cells, ciliary epithelial cells, optic nerve cells, sublimbal fibroblasts, subconjunctival fibroblasts, lacrimal gland cells, meibomian gland cells, goblet cells, lens epithelial cells, and eyelid epithelial cells.

[Claim 7]

A method for producing corneal epithelial cells, comprising the steps of:

producing a colony consisting of concentric zones of different ectodermal cell lineages by the method according to any one of claims 1 to 4,

culturing the colony in a medium containing a growth factor,

separating cells contained in a zone of ocular surface ectodermal lineage cells and culturing the cells in a medium containing KGF, a Rock inhibitor, and a serum substitute to induce differentiation into corneal epithelial progenitor cells, and

isolating the corneal epithelial progenitor cells and inducing differentiation into corneal epithelial cells.

[Claim 8]

The method according to claim 7, wherein the corneal epithelial cells are isolated using the presence or absence of expression of one or more markers selected

from the group consisting of ITG β 4, SSEA4, TRA-1-60, and CD200 as an index.

[Claim 9]

A method for producing a corneal cell sheet, comprising the steps of producing corneal epithelial cells by the method according to claim 7 or 8 and further subjecting the resultant corneal epithelial cells to stratification culture.

[Claim 10]

A colony consisting of concentric zones of different ectodermal cell lineages derived from pluripotent stem cells, wherein the colony comprises zones of neuroectodermal lineage cells, neural crest lineage cells/optic cup lineage cells, ocular surface ectodermal lineage cells, and surface ectodermal lineage cells, respectively.