

Patent Literature 1: International Publication No. WO
2012/073238

Patent Literature 2: International Publication No. WO
2010/134619

Patent Literature 3: International Publication No.
W02012/144582

Non Patent Literature

[0007]

Non Patent Literature 1: Guenou, H., et al., The Lancet
374, (2009) p1745-1753.

Non Patent Literature 2: Metallo, C.M., et al., Stem
cells (2008) 26, 372-380.

Non Patent Literature 3: R. Hayashi et al., Volume 7,
Issue 9, e45435, PLOS ONE, 2012

Summary

Technical Problem

[0008]

An object of the present invention is to provide a method for inducing the differentiation of pluripotent stem cells (particularly, induced pluripotent stem cells) into an ocular surface epithelial lineage, such as corneal epithelium or conjunctival epithelium, without using feeder cells or serum and to provide a stratified, functional cultured corneal epithelial cell sheet from pluripotent stem cells (particularly, induced pluripotent stem cells).

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, wherein the medium contains serum replacement, wherein the pluripotent stem cells are cultured using a vessel coated with an effective amount of laminin or laminin fragments without receiving an external stimulus from a differentiation inducer or a differentiation induction promoter;

(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 example, 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

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 an epithelial cell differentiation medium containing one or more selected from the group consisting of KGF, bFGF and Rock inhibitors, 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 further culturing the corneal epithelial progenitor

cells to induce differentiation into corneal epithelial cells and identifying K12-positive cells as 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 yet 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 and further culturing the resultant corneal epithelial cells in the epithelial cell differentiation medium containing one or more selected from the group consisting of KGF, bFGF and Rock inhibitors;

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

(13a) In yet 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;

(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;

(16a) In yet another aspect, there is provided a method for producing corneal epithelial progenitor 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 differentiation medium for epithelium, containing a serum free medium usable for the culture of an epithelial cell and growth factors selected from the group consisting of KGF, a Rock inhibitor, bFGF, and a serum substitute, separating ocular surface epithelial stem cells and culturing the cells in a medium for corneal epithelium culture containing a serum free medium usable for the culture of an epithelial cell and KGF, a Rock inhibitor, and a serum substitute to induce differentiation into corneal epithelial progenitor cells;

(16b) In yet another aspect, there is provided a method for producing conjunctival 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 differentiation medium for epithelium, containing a serum free medium usable for the culture of an epithelial cell and growth factors selected from the group consisting of KGF, a Rock inhibitor, bFGF, and a serum substitute, separating ocular surface epithelial stem cells and culturing the cells in a medium for corneal epithelium culture containing a serum free medium usable for the culture of an epithelial cell and KGF, a Rock inhibitor, and a serum substitute to induce differentiation into corneal epithelial progenitor cells, continuing culturing the cells in the corneal epithelium culture medium, and separating PAX6 positive, K13-positive, K12-negative cells from among cells isolated as SSEA4-negative, ITGB4-positive cells as conjunctival epithelial cells;

(16c) In yet another aspect, there is provided a method for producing retinal pigment 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 differentiation medium for epithelium, containing a serum free medium usable for the culture of an epithelial cell and growth factors selected from the group consisting of

KGF, a Rock inhibitor, bFGF, and a serum substitute, and separating, as retinal pigment epithelial cells, cobblestone-like epithelial cells with pigment contained in a zone of neuroectodermal lineage cells and/or a zone of neural crest lineage cells/optic cup lineage; and

(16d) In yet another aspect, there is provided a method for producing neural crest 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 differentiation medium for epithelium, containing a serum free medium usable for the culture of an epithelial cell and growth factors selected from the group consisting of KGF, a Rock inhibitor, bFGF, and a serum substitute, and separating cells contained in a zone of neural crest lineage cells/optic cup lineage and isolating the cell using a neural crest cell marker.

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

as keratin 12, pax6, and MUC16. The epithelial cells obtained by the method of the present disclosure express these markers, are transplantable, and function as corneal epithelial cells in vitro and in vivo like corneal epithelial cells derived from somatic cells. The method of the present disclosure does not use animal-derived feeder cells or serum; thus, the cells obtained are highly safe and suited for clinical application.

In addition, not only corneal epithelial cells but also various cells constituting the eye, such as conjunctival epithelial cells, retinal cells, and lacrimal gland cells, can be prepared from the colony consisting of concentric zones of different ectodermal cell lineage obtained by the method of the present disclosure.

Brief Description of Drawings

[0012]

[Figure 1] Figure 1 is a diagram showing a scheme of a method for inducing differentiation from human iPS cells to ocular surface epithelial/corneal epithelial cells.

[Figure 2] Figure 2 is a series of photographs showing a process of inducing differentiation from human iPS cells to ocular surface epithelial/corneal epithelial cells in which Figure 2A is a series of photographs at day 0, day 5, day 10, day 15, day 20, and day 25, and Figure 2B is a photograph at 6 weeks after.

[0072]

In addition, a pharmaceutically acceptable organic solvent, polyvinyl alcohol, polyvinylpyrrolidone, carboxyvinyl polymer, carboxymethylcellulose sodium, sodium polyacrylate, alginate sodium, water-soluble dextran, carboxymethyl starch sodium, pectin, methylcellulose, ethylcellulose, xanthan gum, gum Arabic, casein, agar, polyethylene glycol, diglycerine, glycerin, propylene glycol, vaseline, paraffin, stearyl alcohol, stearic acid, mannitol, sorbitol, or lactose, a pharmaceutically acceptable surfactant, a buffer, an emulsifier, a suspension agent, a soothing agent, a stabilizer, or the like may be contained, if necessary.

[0073]

An actual additive is selected from among the above substances alone or in combination; however, it is not intended to be limited thereto.

[0074]

Examples of a disease to be a target for the cell medicine of the present invention include Stevens-Johnson syndrome, ocular pemphigoid, thermal/chemical injury, aniridia, Salzmann corneal degeneration, idiopathic

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,

wherein the medium contains serum replacement,

wherein the pluripotent stem cells are cultured using a vessel coated with an effective amount of laminin or laminin fragments without receiving an external stimulus from a differentiation inducer or a differentiation induction promoter.

[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]

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 3,

culturing the colony in an epithelial cell differentiation medium containing one or more selected from the group consisting of KGF, bFGF and Rock inhibitors,

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 further culturing the corneal epithelial progenitor cells to induce differentiation into corneal epithelial cells and identifying K12-positive cells as corneal epithelial cells.

[Claim 5]

The method according to claim 4, 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 6]

A method for producing a corneal cell sheet, comprising the steps of producing corneal epithelial cells by the method according to claim 4 or 5 and further subjecting the resultant corneal epithelial cells to stratification culture and further culturing the resultant corneal epithelial cells in the epithelial cell differentiation medium containing one or more selected from the group consisting of KGF, bFGF and Rock inhibitors.

[Claim 7]

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.

[Claim 8]

A method for producing corneal epithelial progenitor 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 3,

culturing the colony in a differentiation medium for epithelium, containing a serum free medium usable for the culture of an epithelial cell and growth factors selected from the group consisting of KGF, a Rock inhibitor, bFGF, and a serum substitute,

separating ocular surface epithelial stem cells and culturing the cells in a medium for corneal epithelium culture containing a serum free medium usable for the culture of an epithelial cell and KGF, a Rock inhibitor, and a serum substitute to induce differentiation into corneal epithelial progenitor cells.

[Claim 9]

The method according to claim 8, further comprising isolating the corneal epithelial progenitor cells 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 10]

The method according to claim 8, further comprising isolating TRA-1-60 negative, ITGB4 positive and SSEA4 positive cells as corneal epithelial progenitor cells.

[Claim 11]

The method according to claim 8, further comprising isolating CD200 negative, ITGB4 positive and SSEA4 positive cells as corneal epithelial progenitor cells.

[Claim 12]

A method for producing conjunctival 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 3,

culturing the colony in a differentiation medium for epithelium, containing a serum free medium usable for the culture of an epithelial cell and growth factors selected from the group consisting of KGF, a Rock inhibitor, bFGF, and a serum substitute,

separating ocular surface epithelial stem cells and culturing the cells in a medium for corneal epithelium culture containing a serum free medium usable for the culture of an epithelial cell and KGF, a Rock inhibitor, and a serum substitute to induce differentiation into corneal epithelial progenitor cells,

continuing culturing the cells in the corneal epithelium culture medium, and separating PAX6 positive, K13-positive, K12-negative cells from among cells isolated as SSEA4-negative, ITGB4-positive cells as conjunctival epithelial cells.

[Claim 13]

A method for producing retinal pigment 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 3,

culturing the colony in a differentiation medium for epithelium, containing a serum free medium usable for the culture of an epithelial cell and growth factors selected from the group consisting of KGF, a Rock inhibitor, bFGF, and a serum substitute, and

separating, as retinal pigment epithelial cells, cobblestone-like epithelial cells with pigment contained in a zone of neuroectodermal lineage cells and/or a zone of neural crest lineage cells/optic cup lineage.

[Claim 14]

A method for producing neural crest 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 3,

culturing the colony in a differentiation medium for epithelium, containing a serum free medium usable for the culture of an epithelial cell and growth factors selected from the group consisting of KGF, a Rock inhibitor, bFGF, and a serum substitute, and

separating cells contained in a zone of neural crest lineage cells/optic cup lineage and isolating the cell using a neural crest cell marker.