

CLAIMS

1. A method for inducing differentiation into conjunctival epithelial cells, conjunctival goblet cells, and conjunctival
5 epithelial stem and progenitor cells, the method comprising

(1) obtaining colonies of a self-formed ectodermal autonomous multi-zone (SEAM) cell population thereby

(a) seeding pluripotent stem cells at a density of 300 to 700 cell/cm² onto a plate coated with laminin 511E8, culturing
10 the pluripotent stem cells for 8 to 10 days in maintenance culture, and

(b) culturing the pluripotent stem cells in two dimensions for 4 weeks in serum-free animal cell culture medium to autonomously induce differentiation of the pluripotent stem
15 cells; and

(2) culturing the colonies of SEAM cell population in a conjunctival differentiation medium for 3 to 5 weeks, and then inducing differentiation by culturing the colonies of SEAM cell population in a conjunctival epithelial maintenance medium for
20 2 to 6 weeks, thereby capable of isolating cells from the SEAM cell population using the expression levels of ITGβ4, SSEA-4, and CD200 as indicators,

wherein the conjunctival differentiation medium contains 0.1 to 100 ng/mL an epidermal growth factor (EGF) signaling
25 activator and Rock inhibitor,

wherein the conjunctival epithelial maintenance medium contains 0.1 to 100 ng/mL EGF signaling activator, Rock inhibitor and serum replacement, and

wherein the EGF signaling activator is selected from

epidermal growth factor, transforming growth factor α (TGF α) and amphi-regulin (AR).

2. A method for producing conjunctival epithelial cells,
5 conjunctival goblet cells, and conjunctival epithelial stem and progenitor cells, the method comprising

(1) obtaining colonies of a self-formed ectodermal autonomous multi-zone (SEAM) cell population thereby

(a) seeding pluripotent stem cells at a density of 300 to
10 700 cell/cm² onto a plate coated with laminin 511E8, culturing the pluripotent stem cells for 8 to 10 days in maintenance culture, and

(b) culturing the pluripotent stem cells in two dimensions for 4 weeks in serum-free animal cell culture medium to
15 autonomously induce differentiation of the pluripotent stem cells; and

(2) culturing the colonies of SEAM cell population in a conjunctival differentiation medium for 3 to 5 weeks, and then inducing differentiation by culturing the colonies of SEAM cell
20 population in a conjunctival epithelial maintenance medium for 2 to 6 weeks,

wherein the conjunctival differentiation medium contains 0.1 to 100 ng/mL an epidermal growth factor (EGF) signaling activator and Rock inhibitor,

25 wherein the conjunctival epithelial maintenance medium contains 0.1 to 100 ng/mL EGF signaling activator, Rock inhibitor and serum replacement, and wherein the EGF signaling activator is selected from epidermal growth factor, transforming growth factor α (TGF α) and

amphi-regulin (AR); and (3) isolating cells from the SEAM cell population using the expression levels of ITG β 4, SSEA-4, and CD200 as indicators as determined with antibodies thereagainst.

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3. The method according to claim 2, wherein the isolated cells are CD200-negative cells.

4. The method according to claim 2 or 3, wherein the isolated
10 cells are weakly SSEA-4-positive and ITG β 4-positive cells.

5. The method according to claim 4, wherein the isolated cells are conjunctival epithelial stem and progenitor cells.

15 6. The method according to claim 2 or 3, wherein the isolated cells are selected from weakly SSEA-4-positive and ITG β 4-negative cells and SSEA-4-positive and ITG β 4-negative cells.

20 7. The method according to claim 6, wherein the isolated cells are conjunctival epithelial cells and/or conjunctival goblet cells.

8. A method for producing a conjunctival epithelial cell sheet,
25 the method comprising the steps of:

producing conjunctival epithelial stem and progenitor cells by the method of any one of claims 2 to 5, and

culturing the conjunctival epithelial stem and progenitor cells in a medium containing keratinocyte growth factor (KGF).

9. A method for producing a conjunctival epithelial cell sheet,
the method comprising the steps of:

producing conjunctival epithelial stem and progenitor
5 cells by the method of any one of claims 2 to 5, and
culturing the conjunctival epithelial stem and progenitor
cells in a medium containing a fibroblast growth factor receptor
2b (FGFR2b) activator.

10 10. A method for screening for a drug for a conjunctiva-related
disease, the method comprising the steps of:

producing conjunctival epithelial cells, conjunctival
goblet cells, and/or conjunctival epithelial stem and
progenitor cells by the method of any one of claims 2 to 7, and
15 culturing the conjunctival epithelial cells, the
conjunctival goblet cells, and/or the conjunctival epithelial
stem and progenitor cells.