population to be differentiated as functional human corneal endothelial cells had been decided, which was also a timing when the proliferation viability thereof was still high, and then preserve this cell population, then by harvesting cells at the same timing as this and repeatedly performing subculturing and culturing, it was possible to achieve a large-scale culturing of functional human corneal endothelial cells in a far shorter time period than is possible via the conventional technology, and thereby additionally established a method of culturing of functional human corneal endothelial cells on a large scale.

In this manner, the inventors of the present disclosure discovered that if cells were destined to become functional human corneal endothelial cells by being cultured under appropriate culturing conditions, then by harvesting those cells in an immature state and either preserving or subculturing them, it was possible to obtain the desired target functional human corneal endothelial cells (exhibiting enhanced viability and reduced contamination), and thereby enabled the present embodiments disclosed herein to be completed for the first time.

[Means for Solving the Problem]

[0010]

The present disclosure refers to subject matter as defined in the attached set of claims as examples. In other words, the storage method of human corneal endothelial cells and/or human corneal endothelial precursor cells according to the present disclosure is as exemplified in a non-limiting manner as follows.

[Item 1] A storage method of human corneal endothelial cells and/or human corneal endothelial precursor cells characterized in that human corneal endothelial cells and/or human corneal endothelial precursor cells that have been cultured using a culture medium that contains a ROCK inhibitor, and in which either epidermal growth factor

(a) ~ (d) given below have been met, and are subcultured.

(a) During a period from immediately after a morphology of the human corneal endothelial cells and/or human corneal endothelial precursor cells has shifted from a spindle fibroblast-like shape having irregular elongated projections to a tessellated shape whose major axis-minor axis ratio is close to 1, until immediately before boundaries between the cells become indistinct.

(b) During a period from when an expression level of CD44 becomes equal to or less than half a maximum value observed after the most recent subculturing until this expression level reaches a plateau.

(c) When the cell density of the human corneal endothelial cells and/or human corneal endothelial precursor cells is not less than 900 cells/mm^2 and not more than 2500 cells/mm^2.

(d) When the number of culturing days since the most recent subculturing is not less than 4 days and not more than 14 days.

[Summary]

[0011a]

In one aspect, the present disclosure refers to a storage methodof human corneal endothelial cells and/or human corneal endothelial precursor cells characterized in that human corneal endothelial cells and/or human corneal endothelial precursor cells that have been cultured using a culture medium that contains a ROCK inhibitor, and in which the content of epidermal growth factor (EGF) is less than a concentration that will cause a transformation are harvested at a timing when any one of or a plurality of the conditions (a) ~ (d) given below have been met, and are placed in a suspension state and then preserved:

(a) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, during a period from immediately after a morphology of the human corneal endothelial cells and/or human corneal endothelial precursor cells has shifted from a spindle-shaped to a polygonal shape or an elliptical shape whose major axis-minor axis ratio is close to 1, until immediately before boundaries between the cells become indistinct;

(b) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, during a period from when an expression level of CD44 becomes equal to or less than half a maximum value observed after the most recent subculturing until this expression level reaches a plateau;

(c) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, when the cell density of the human corneal endothelial cells and/or human corneal endothelial precursor cells is not less than 900 cells/mm^2 and not more than 2500 cells/mm^2; and

(d) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, when the number of culturing days since the most recent subculturing is not less than 4 days and not more than 14 days.

[0011b]

In another aspect, the present disclosure refers to a cell population of human corneal endothelial cells, obtained by culturing and/or performing differentiation and maturation on the human corneal endothelial cells and/or the human corneal endothelial precursor cells after these have been preserved using the storage method as described herein cultured and/or differentiated and matured using a culture medium that contains a ROCK inhibitor and in which the content of epidermal growth factor (EGF) is less than a concentration that will cause a transformation, satisfies any one of or a plurality of the

following conditions (1) ~ (8):

(1) no fibroblast, foreign matter, discoloration, or other abnormality is found upon an external visual inspection being performed using phase-contrast images;

(2) the cell survival rate is 70% or more in Trypan blue dye exclusion assay;

(3) PDGF-BB concentration of the cell supernatant is 100 pg/mL or more in a purity test performed via an ELISA;

(4) results of a purity test of the cells performed via a FACS satisfies all of the following ranges: $CD166^{+} > 99\%$; $CD24^{+} < 5\%$; $CD44^{high} < 5\%$; $CD44^{neg\sim low} > 90\%$; $CD90^{+} < 5\%$;

(5) the percentage content of effector cells provided with human corneal endothelium function is greater than 90%, wherein the effector cell is a cell having the same type of corneal endothelial characteristics as human corneal endothelial cells when present in the human body;

(6) the pumping function Na^{+}/K^{+} ATPase is positive;

(7) the barrier function ZO-1 is positive; and

(8) the human corneal endothelial cell density (ECD) is 1500 cells /mm^2 or greater.

[0011c]

In yet another aspect, the present disclosure refers to a Cell population obtained by culturing human corneal endothelial cells and/or human corneal endothelial precursor cells preserved using the storage method described in any one of Claims 1 to 6 cultured and/or differentiated and matured using a culture medium that contains a ROCK inhibitor and in which the content of epidermal growth factor (EGF) is less than a concentration that will cause a transformation, wherein results of a purity test performed via a FACS of the cell population satisfy all of the following ranges: $CD166^{+} > 99\%$; $CD24^{+} < 5\%$;

$CD44^{high}$ < 5%; $CD44^{neg\sim low}$ > 90%; $CD90^{+}$ < 5%.

[0011d]

In a further aspect, the present disclosure refers to a suspension solution of cell population obtained by culturing human corneal endothelial cells and/or human corneal endothelial precursor cells preserved using the storage method described herein cultured and/or differentiated and matured using a culture medium that contains a ROCK inhibitor and in which the content of epidermal growth factor (EGF) is less than a concentration that will cause a transformation, wherein results of a purity test performed via a FACS of the cell population satisfy all of the following ranges: $CD166^{+}$ > 99%; $CD24^{+}$ < 5%; $CD44^{high}$ < 5%; $CD44^{neg\sim low}$ > 90%; $CD90^{+}$ < 5%.

[0011e]

In another further aspect, the present disclosure refers to a suspension solution preparation method for preparing a suspension solution of human corneal endothelial cells and/or human corneal endothelial precursor cells characterized in that human corneal endothelial cells and/or human corneal endothelial precursor cells that have been cultured using a culture medium that contains a ROCK inhibitor, and in which the content of epidermal growth factor (EGF) is less than a concentration that will cause a transformation are harvested at a timing when any one of or a plurality of the conditions (a) ~ (d) given below have been met, and are placed in a suspension state:

(a) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, during a period from immediately after a morphology of the human corneal endothelial cells and/or human corneal endothelial precursor cells has shifted from a spindle fibroblast-like shape having irregular elongated projections to a tessellated shape whose major axis-minor axis ratio including the projections is close to 1, until immediately before boundaries between the cells become indistinct;

(b) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, during a period from when an expression level of CD44 becomes equal to or less than half a maximum value observed after the most recent subculturing until this expression level reaches a plateau;

(c) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, when the cell density of the human corneal endothelial cells and/or human corneal endothelial precursor cells is not less than 900 cells/mm^2 and not more than 2500 cells/mm^2; and

(d) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, when the number of culturing days since the most recent subculturing is not less than 4 days and not more than 14 days.

[0011f]

In yet another aspect, the present disclosure refers to a culturing method for culturing human corneal endothelial cells and/or human corneal endothelial precursor cells characterized in that human corneal endothelial cells and/or human corneal endothelial precursor cells that have been cultured using a culture medium that contains a ROCK inhibitor, and in which the content of epidermal growth factor (EGF) is less than a concentration that will cause a transformation are harvested at a timing when any one of or a plurality of the conditions (a) ~ (d) given below have been met, and are subcultured:

(a) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, during a period from immediately after a morphology of the human corneal endothelial cells and/or human corneal endothelial precursor cells has shifted from a spindle fibroblast-like shape having irregular elongated projections to a tessellated shape whose major axis-minor axis ratio is close to 1, until immediately before boundaries between the cells become indistinct;

(b) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, during a period from when an expression level of CD44 becomes equal to or less than half a maximum value observed after the most recent subculturing until this expression level reaches a plateau;

(c) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, when the cell density of the human corneal endothelial cells and/or human corneal endothelial precursor cells is not less than 900 cells/mm^2 and not more than 2500 cells/mm^2; and

(d) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, when the number of culturing days since the most recent subculturing is not less than 4 days and not more than 14 days.

[0011g]

In yet a further aspect, the present disclosure refers to a method for the preservation of cultured human corneal endothelial cells and/or human corneal endothelial precursor cells, the method comprising,

obtaining a plurality of human corneal endothelial cells and/or human corneal endothelial precursor cells;

separating at least a portion of the plurality of cells to generate a cell population for evaluation and a cell population for preservation;

evaluating at least a portion of the cell population for evaluation for one or more of the following criteria:

(a) a morphology of the human corneal endothelial cells and/or human corneal endothelial precursor cells to identify cells that have shifted from spindle-shaped to either of (i) a polygonal shape or (ii) an elliptical shape whose major axis-minor axis ratio is close to 1 and still have distinct boundaries between the cells if the cells are seeded at a

cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2;

(b) an expression level of CD44 to identify cells expressing a consistent level of CD44 after previously expressing CD44 at a level equal to or less than half a maximum value observed after an immediately prior subculturing if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2;

(c) cell density to identify when the cell density of the human corneal endothelial cells and/or human corneal endothelial precursor cells is not less than 900 cells/mm^2 and not more than 2500 cells/mm^2 if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2; and

(d) a time period between the evaluation and an immediately prior subculturing to determine if the time is not less than 4 days and not more than 14 days if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2; and

placing at least a portion of the cells from the cell population for preservation in a storage media when the cell population for evaluation meets one or more of the evaluation criteria of (a), (b), (c), or (d).

[0011h]

In one aspect, the present disclosure refers to a method for processing cultured human corneal endothelial cells and/or human corneal endothelial precursor cells, the method comprising,

culturing a plurality of human corneal endothelial cells and/or human corneal endothelial precursor cells in a culture vessel comprising a culture media;

separating at least a portion of the plurality of cells to generate a cell population for evaluation and a remainder cell population;

evaluating at least a portion of the cell population for evaluation for one or more

of the following criteria:

(a) a morphology of the human corneal endothelial cells and/or human corneal endothelial precursor cells to identify cells that have shifted from spindle-shaped to either of (i) a polygonal shape or (ii) an elliptical shape whose major axis-minor axis ratio is close to 1 and still have distinct boundaries between the cells if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2;

(b) an expression level of CD44 to identify cells expressing a consistent level of CD44 after previously expressing CD44 at a level equal to or less than half a maximum value observed after an immediately prior subculturing if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2;

(c) cell density to identify when the cell density of the human corneal endothelial cells and/or human corneal endothelial precursor cells is not less than 900 cells/mm^2 and not more than 2500 cells/mm^2 if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2; and

(d) a time period between the evaluation and an immediately prior subculturing to determine if the time is not less than 4 days and not more than 14 days if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2; and

placing at least a portion of the cells from the remainder cell population for preservation in a storage media when the cell population for evaluation meets one or more of the evaluation criteria of (a), (b), (c), or (d); or

placing at least a portion of the cells from the remainder cell population in a culture vessel comprising a culture media when the cell population for evaluation fails to meet each of the evaluation criteria of (a), (b), (c), or (d), and culturing the remainder cell population to meet one or more of the evaluation criteria of (a), (b), (c), or (d).

[0011i]

In another aspect, the present disclosure refers to a method as described herein, further comprising removing the preserved cells from preservation and returning them to a culture vessel for additional culturing, wherein the cells subjected to additional culturing meet one or more of the following criteria:

(1) no fibroblast, foreign matter, discoloration, or other abnormality is found upon an external visual inspection being performed using phase-contrast images;

(2) the cell survival rate is 70% or more in Trypan blue dye exclusion assay;

(3) PDGF-BB concentration of the cell supernatant is 100 pg/mL or more in a purity test performed via an ELISA;

(4) the percentage content of effector cells provided with human corneal endothelium function is greater than 90%, wherein the effector cell is a cell having the same type of corneal endothelial characteristics as human corneal endothelial cells when present in the human body;

(5) the pumping function of Na^+/K^+ ATPase is positive;

(6) the barrier function of ZO-1 is positive; and

(7) the human corneal endothelial cell density (ECD) is 1500 cells /mm^2 or greater.

[0011j]

In yet another aspect, the present disclosure refers to a method as described herein, wherein cells that are removed from the preservation state are suitable for delivery to the eye of a subject to treat an ocular disease or injury.

[0011k]

In a further aspect, the present disclosure refers to a method as described herein, wherein cells that are removed from the preservation state are suitable for preparation of

a medicament for delivery to the eye of a subject to treat an ocular disease or injury.

[0011l]

In another further aspect, the present disclosure refers to a method as described herein, wherein cells that are removed from the preservation state are suitable for use in the treatment of an ocular disease or injury.

[Effects of the Invention]

[0012]

According to the present disclosure, human corneal endothelial cells and/or human corneal endothelial precursor cells from which it is possible to obtain a cell population, whose functional human corneal endothelial cell percentage content is made higher than about 90% by culturing the cells again after they have been preserved, is able to be preserved for a period of at least several days, while a high survival rate of the cells is maintained.

As a result, a cell population whose functional human corneal endothelial cell percentage content is made higher than about 90% by culturing the cells again is able to be supplied to an international market as a regenerative medical product.

Furthermore, it is possible to create a master cell bank using cryopreservation,

What is claimed is:

1. A storage method of human corneal endothelial cells and/or human corneal endothelial precursor cells characterized in that human corneal endothelial cells and/or human corneal endothelial precursor cells that have been cultured using a culture medium that contains a ROCK inhibitor, and in which the content of epidermal growth factor (EGF) is less than a concentration that will cause a transformation are harvested at a timing when any one of or a plurality of the conditions (a) ~ (d) given below have been met, and are placed in a suspension state and then preserved:

(a) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, during a period from immediately after a morphology of the human corneal endothelial cells and/or human corneal endothelial precursor cells has shifted from a spindle-shaped to a polygonal shape or an elliptical shape whose major axis-minor axis ratio is close to 1, until immediately before boundaries between the cells become indistinct;

(b) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, during a period from when an expression level of CD44 becomes equal to or less than half a maximum value observed after the most recent subculturing until this expression level reaches a plateau;

(c) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, when the cell density of the human corneal endothelial cells and/or human corneal endothelial precursor cells is not less than 900 cells/mm^2 and not more than 2500 cells/mm^2; and

(d) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, when the number of culturing days since the most recent

subculturing is not less than 4 days and not more than 14 days.

2. The storage method according to Claim 1, wherein the human corneal endothelial cells and/or human corneal endothelial precursor cells are preserved at a temperature of not more than 10 degrees centigrade.

3. The storage method according to Claim 1, wherein the human corneal endothelial cells and/or human corneal endothelial precursor cells are preserved at a temperature of not more than -30 degrees centigrade.

4. The storage method according to any one of Claims 1 to 3, wherein the human corneal endothelial cells and/or human corneal endothelial precursor cells are preserved for 24 hours or longer after being placed in a suspension state.

5. The storage method according to any one of Claims 1 to 4, wherein the transformation includes endothelial-mesenchymal transitions.

6. The storage method according to any one of Claims 1 to 5, wherein the human corneal endothelial cells and/or human corneal endothelial precursor cells are created using, as a source, cells selected from a group consisting of corneal endothelial cell-derived cells, pluripotent stem cells, mesenchymal stem cells, corneal endothelial precursor cells harvested from a corneal endothelium, cells harvested from a corneal endothelium, and corneal endothelial precursor cells and corneal endothelial-like cells produced via a direct programing method.

7. A cell population of human corneal endothelial cells, obtained by culturing and/or performing differentiation and maturation on the human corneal endothelial cells and/or the human corneal endothelial precursor cells after these have been preserved using the storage method according to any one of Claims 1 to 6 cultured and/or differentiated and matured using a culture medium that contains a ROCK inhibitor and in which the content of epidermal growth factor (EGF) is less than a concentration that will

cause a transformation, satisfies any one of or a plurality of the following conditions (1) ~ (8):

(1) no fibroblast, foreign matter, discoloration, or other abnormality is found upon an external visual inspection being performed using phase-contrast images;

(2) the cell survival rate is 70% or more in Trypan blue dye exclusion assay;

(3) PDGF-BB concentration of the cell supernatant is 100 pg/mL or more in a purity test performed via an ELISA;

(4) results of a purity test of the cells performed via a FACS satisfies all of the following ranges:

$CD166^+ > 99\%$

$CD24^+ < 5\%$

$CD44^{high} < 5\%$

$CD44^{neg\sim low} > 90\%$

$CD90^+ < 5\%$;

(5) the percentage content of effector cells provided with human corneal endothelium function is greater than 90%, wherein the effector cell is a cell having the same type of corneal endothelial characteristics as human corneal endothelial cells when present in the human body;

(6) the pumping function Na^+/K^+ ATPase is positive;

(7) the barrier function ZO-1 is positive; and

(8) the human corneal endothelial cell density (ECD) is 1500 cells /mm^2 or greater.

8. The cell population according to Claim 7, wherein the effector cells are cells in which an endothelial-mesenchymal transition has not occurred.

9. The cell population according to Claim 7 or 8, wherein the effector cells are

cells that either express a functional protein that is required to demonstrate corneal endothelial functional properties, or are cells in which an inhibitor protein that inhibits the corneal endothelial functional properties is not expressed or in which any expression of an inhibitor protein is reduced,

wherein the functional protein is selected from a group consisting of Na^+/K^+ ATPase, ZO-1, a sodium/ hydrogen exchanger 1 (NHE1), aquaporin 1 (AQP-1), and carbonic anhydrase 5B (CA5B), and

wherein the inhibitor protein includes at least one or more metabolism-related enzymes selected from a group containing

ATP citrate lyase (ACLY), aconitase 1 (ACO1), isocitrate dehydrogenase 1 (IDH1), malate dehydrogenase 1 (MDH1), malic enzyme 1 (ME1), ACSS2, acetyl-CoA acetyltransferase 2 (ACAT2), and lactate dehydrogenase (LDH).

10. The cell population according to Claim 9, wherein the functional protein further includes any one or a plurality of metabolism-related enzymes selected from a group containing citrate synthase (CS), aconitase 2 (ACO2), isocitrate dehydrogenase 2 (IDH2), malate dehydrogenase 2 (MDH2), malic enzyme 3 (ME3), ACSS1, acetyl-CoA acetyltransferase 1 (ACAT1), pyruvate dehydrogenase (PDH), BCAT2, and branched chain keto acid dehydrogenase (BCKDH2).

11. Cell population obtained by culturing human corneal endothelial cells and/or human corneal endothelial precursor cells preserved using the storage method described in any one of Claims 1 to 6 cultured and/or differentiated and matured using a culture medium that contains a ROCK inhibitor and in which the content of epidermal growth factor (EGF) is less than a concentration that will cause a transformation, wherein results of a purity test performed via a FACS of the cell population satisfy all of the following ranges:

$CD166^{+}$ > 99%

$CD24^{+}$ < 5%

$CD44^{high}$ < 5%

$CD44^{neg\sim low}$ > 90%

$CD90^{+}$ < 5%.

12. A suspension solution of cell population obtained by culturing human corneal endothelial cells and/or human corneal endothelial precursor cells preserved using the storage method described in any one of Claims 1 to 6 cultured and/or differentiated and matured using a culture medium that contains a ROCK inhibitor and in which the content of epidermal growth factor (EGF) is less than a concentration that will cause a transformation, wherein results of a purity test performed via a FACS of the cell population satisfy all of the following ranges:

$CD166^{+}$ > 99%

$CD24^{+}$ < 5%

$CD44^{high}$ < 5%

$CD44^{neg\sim low}$ > 90%

$CD90^{+}$ < 5%.

13. A suspension solution preparation method for preparing a suspension solution of human corneal endothelial cells and/or human corneal endothelial precursor cells characterized in that human corneal endothelial cells and/or human corneal endothelial precursor cells that have been cultured using a culture medium that contains a ROCK inhibitor, and in which the content of epidermal growth factor (EGF) is less than a concentration that will cause a transformation are harvested at a timing when any one of or a plurality of the conditions (a) ~ (d) given below have been met, and are placed in a suspension state:

(a) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, during a period from immediately after a morphology of the human corneal endothelial cells and/or human corneal endothelial precursor cells has shifted from a spindle fibroblast-like shape having irregular elongated projections to a tessellated shape whose major axis-minor axis ratio including the projections is close to 1, until immediately before boundaries between the cells become indistinct;

(b) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, during a period from when an expression level of CD44 becomes equal to or less than half a maximum value observed after the most recent subculturing until this expression level reaches a plateau;

(c) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, when the cell density of the human corneal endothelial cells and/or human corneal endothelial precursor cells is not less than 900 cells/mm^2 and not more than 2500 cells/mm^2; and

(d) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, when the number of culturing days since the most recent subculturing is not less than 4 days and not more than 14 days.

14. A culturing method for culturing human corneal endothelial cells and/or human corneal endothelial precursor cells characterized in that human corneal endothelial cells and/or human corneal endothelial precursor cells that have been cultured using a culture medium that contains a ROCK inhibitor, and in which the content of epidermal growth factor (EGF) is less than a concentration that will cause a transformation are harvested at a timing when any one of or a plurality of the conditions (a) ~ (d) given below have been met, and are subcultured:

(a) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not

more than 500 cells/mm^2, during a period from immediately after a morphology of the human corneal endothelial cells and/or human corneal endothelial precursor cells has shifted from a spindle fibroblast-like shape having irregular elongated projections to a tessellated shape whose major axis-minor axis ratio is close to 1, until immediately before boundaries between the cells become indistinct;

(b) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, during a period from when an expression level of CD44 becomes equal to or less than half a maximum value observed after the most recent subculturing until this expression level reaches a plateau;

(c) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, when the cell density of the human corneal endothelial cells and/or human corneal endothelial precursor cells is not less than 900 cells/mm^2 and not more than 2500 cells/mm^2; and

(d) if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2, when the number of culturing days since the most recent subculturing is not less than 4 days and not more than 14 days.

15. A method for the preservation of cultured human corneal endothelial cells and/or human corneal endothelial precursor cells, the method comprising,

obtaining a plurality of human corneal endothelial cells and/or human corneal endothelial precursor cells;

separating at least a portion of the plurality of cells to generate a cell population for evaluation and a cell population for preservation;

evaluating at least a portion of the cell population for evaluation for one or more of the following criteria:

(a) a morphology of the human corneal endothelial cells and/or human corneal

endothelial precursor cells to identify cells that have shifted from spindle-shaped to either of (i) a polygonal shape or (ii) an elliptical shape whose major axis-minor axis ratio is close to 1 and still have distinct boundaries between the cells if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2;

(b) an expression level of CD44 to identify cells expressing a consistent level of CD44 after previously expressing CD44 at a level equal to or less than half a maximum value observed after an immediately prior subculturing if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2;

(c) cell density to identify when the cell density of the human corneal endothelial cells and/or human corneal endothelial precursor cells is not less than 900 cells/mm^2 and not more than 2500 cells/mm^2 if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2; and

(d) a time period between the evaluation and an immediately prior subculturing to determine if the time is not less than 4 days and not more than 14 days if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2; and

placing at least a portion of the cells from the cell population for preservation in a storage media when the cell population for evaluation meets one or more of the evaluation criteria of (a), (b), (c), or (d).

16. The method of Claim 15, wherein the human corneal endothelial cells and/or human corneal endothelial precursor cells are preserved at a temperature of 10 degrees centigrade or less.

17. The method of Claim 15or 16, wherein the human corneal endothelial cells and/or human corneal endothelial precursor cells are preserved for at least 24 hours.

18. The method according to any one of Claims 15to 17, wherein the human

corneal endothelial cells and/or human corneal endothelial precursor cells are created using, as a source, cells selected from a group consisting of corneal endothelial cell-derived cells, pluripotent stem cells, mesenchymal stem cells, corneal endothelial precursor cells harvested from a corneal endothelium, cells harvested from a corneal endothelium, and corneal endothelial precursor cells and corneal endothelial-like cells produced via a direct programing method.

19. A method for processing cultured human corneal endothelial cells and/or human corneal endothelial precursor cells, the method comprising,

culturing a plurality of human corneal endothelial cells and/or human corneal endothelial precursor cells in a culture vessel comprising a culture media;

separating at least a portion of the plurality of cells to generate a cell population for evaluation and a remainder cell population;

evaluating at least a portion of the cell population for evaluation for one or more of the following criteria:

(a) a morphology of the human corneal endothelial cells and/or human corneal endothelial precursor cells to identify cells that have shifted from spindle-shaped to either of (i) a polygonal shape or (ii) an elliptical shape whose major axis-minor axis ratio is close to 1 and still have distinct boundaries between the cells if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2;

(b) an expression level of CD44 to identify cells expressing a consistent level of CD44 after previously expressing CD44 at a level equal to or less than half a maximum value observed after an immediately prior subculturing if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2;

(c) cell density to identify when the cell density of the human corneal endothelial cells and/or human corneal endothelial precursor cells is not less than 900

cells/mm^2 and not more than 2500 cells/mm^2 if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2; and

(d) a time period between the evaluation and an immediately prior subculturing to determine if the time is not less than 4 days and not more than 14 days if the cells are seeded at a cell density of not less than 300 cells/mm^2 and not more than 500 cells/mm^2; and

placing at least a portion of the cells from the remainder cell population for preservation in a storage media when the cell population for evaluation meets one or more of the evaluation criteria of (a), (b), (c), or (d); or

placing at least a portion of the cells from the remainder cell population in a culture vessel comprising a culture media when the cell population for evaluation fails to meet each of the evaluation criteria of (a), (b), (c), or (d), and culturing the remainder cell population to meet one or more of the evaluation criteria of (a), (b), (c), or (d).

20. The method of Claim 19, wherein the culture media comprises a ROCK inhibitor, and wherein the culture media comprises a concentration of epidermal growth factor (EGF) below a threshold concentration that will cause a transformation of the cells.

21. The method of Claim 19 or 20, wherein the culture media comprises a ROCK inhibitor, and wherein the culture media comprises a concentration of epidermal growth factor (EGF) of less than 2 ng/mL.

22. The method of Claim 21, wherein the culture media does not include EGF.

23. The method according to any one of Claims 19 to 22, wherein the human corneal endothelial cells and/or human corneal endothelial precursor cells are created using, as a source, cells selected from a group consisting of corneal endothelial cell-derived cells, pluripotent stem cells, mesenchymal stem cells, corneal endothelial

precursor cells harvested from a corneal endothelium, cells harvested from a corneal endothelium, and corneal endothelial precursor cells and corneal endothelial-like cells produced via a direct programing method.

24. A method according to any one of Claim 15 to 23, further comprising removing the preserved cells from preservation and returning them to a culture vessel for additional culturing, wherein the cells subjected to additional culturing meet one or more of the following criteria:

(1) no fibroblast, foreign matter, discoloration, or other abnormality is found upon an external visual inspection being performed using phase-contrast images;

(2) the cell survival rate is 70% or more in Trypan blue dye exclusion assay;

(3) PDGF-BB concentration of the cell supernatant is 100 pg/mL or more in a purity test performed via an ELISA;

(4) the percentage content of effector cells provided with human corneal endothelium function is greater than 90%, wherein the effector cell is a cell having the same type of corneal endothelial characteristics as human corneal endothelial cells when present in the human body;

(5) the pumping function of Na^+/K^+ ATPase is positive;

(6) the barrier function of ZO-1 is positive; and

(7) the human corneal endothelial cell density (ECD) is 1500 cells /mm^2 or greater.

25. A method according to any one of Claims 15 to 23, wherein cells that are removed from the preservation state are suitable for delivery to the eye of a subject to treat an ocular disease or injury.

26. A method according to any one of Claims 15 to 23, wherein cells that are removed from the preservation state are suitable for preparation of a medicament for

delivery to the eye of a subject to treat an ocular disease or injury.

27. A method according to any one of Claims 15 to 23, wherein cells that are removed from the preservation state are suitable for use in the treatment of an ocular disease or injury.

28. The method of any one of Claims 25 to 27, wherein the ocular disease is a corneal disease or injury.