[Claims]

[Claim 1]
A method of manufacturing a functional human corneal endothelial cell capable of eliciting a human corneal function when injected into an anterior chamber of a human eye, the method comprising
proliferating and/or differentiating or maturing a corneal endothelial progenitor cell in the presence of a Rho-associated protein kinase (ROCK) inhibitor, and (i) in the absence of epidermal growth factor (EGF) or (ii) in the presence of EGF at a concentration of less than 5 ng/mL.

[Claim 2]
The method according to claim 1, comprising proliferating and/or differentiating or maturing the corneal endothelial progenitor cell in the absence of EGF.

[Claim 3]
The method according to claim 1, comprising proliferating and/or differentiating or maturing the corneal endothelial progenitor cell in the presence of EGF at a concentration of less than 5 ng/mL.

[Claim 4]
The method according to claim 3, comprising proliferating and/or differentiating or maturing the corneal endothelial progenitor cell in the presence of EGF at a concentration of less than 1 ng/mL.

[Claim 5]
The method according to claim 3 or 4, comprising proliferating and/or differentiating or maturing the corneal endothelial progenitor cell in the presence of EGF for no more than the initial 7 days of each passage of the proliferation and/or differentiation or maturation.

[Claim 6]
The method according to any of claims 1-5, further comprising, before the proliferating and/or differentiating or maturing the corneal endothelial progenitor cell, dedifferentiating a human corneal endothelial tissue-derived cell to obtain the corneal endothelial progenitor cell.

[Claim 7]
The method according to any one of claims 1-5, wherein the human corneal endothelial progenitor cell is made from a corneal endothelial tissue-derived cell, a pluripotent stem cell, a mesenchymal stem cell, a cell collected form a corneal endothelium, or a corneal endothelial precursor cell or a corneal endothelial-like cell made by a direct programming method.

[Claim 8]

A method of producing a functional human corneal endothelial cell capable of eliciting a human corneal function when infused into an anterior chamber of a human eye, the method comprising:

a) obtaining a human corneal endothelial progenitor cell by culturing donor corneal endothelial cells in a medium; and

b) further subculturing the human corneal endothelial progenitor cell in the medium to produce the functional human corneal endothelial cell;

wherein the medium comprises a Rho-associated protein kinase (ROCK) inhibitor, and (i) contains no epidermal growth factor (EGF), (ii) comprises less than 5 ng/ml EGF, or (iii) comprises EGF for no more than the initial 7 days of each passage.

[Claim 9]

The method according to claim 8, wherein the medium comprises no EGF.

[Claim 10]

The method according to claim 8, wherein the medium comprises less than 5 ng/mL EGF.

[Claim 11]

The method according to claim 10, wherein the medium comprises less than 1 ng/mL EGF.

[Claim 12]

The method according to any one of claims 1-11, wherein the ROCK inhibitor is Y-27632.

[Claim 13]

The method according to any one of claims 1-12, comprising confirming that the cell

(i) mitochondria-dependent oxidative phosphorylation is increased in mitochondria of the functional human corneal endothelial cell;

(ii) acetyl-CoA expression is not increased in the cytoplasm or nucleus of the functional human corneal endothelial cell; and

(iii) epigenetic multigene expression through histone acetylation by acetyl-CoA is not induced in the functional human corneal endothelial cell.

[Claim 14]
The method according to any one of claims 1-13, comprising confirming that one or more metabolism-related enzymes selected from the group consisting of 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), branched chain amino acid transaminase 2(BCAT2), and branched-chain ketoacid dehydrogenase 2 (BCKDH2) are expressed in mitochondria of the functional human corneal endothelial cell.

[Claim 15]
The method according to any one of claims 1-14, comprising confirming that ATP citrate lyase (ACLY), aconitase 1 (ACO1), isocitrate dehydrogenase 1 (IDH1), malate dehydrogenase 1 (MDH1), malic enzyme 1 (ME1), acetyl-CoA synthetase 2 (ACSS2), acetyl-CoA acetyltransferase 2 (ACAT2), and/or lactate dehydrogenase (LDH) is not expressed, in the functional human corneal endothelial cell.

[Claim 16]
The method according to any one of claims 1-15, comprising confirming the expression of an ion channel and/or a monocarboxylic acid transporter leading to a corneal endothelia cell functional property in the functional human corneal endothelial cell.

[Claim 17]
The method according to any one of claims 1-16, comprising confirming the expression of sodium/hydrogen exchanger 1 (NHE1) and/or aquaporin 1 (AQP-1) in the functional human corneal endothelial cell.

[Claim 18]
The method according to any one of claims 1-17, comprising confirming increased expression of bicarbonic anhydrase 5B (CA5B) functional human corneal endothelial cell.

[Claim 19]
The method according to any one of claims 1-18, comprising confirming that
(i) a metabolic enzyme related to a TCA cycleis not present in the cytoplasm or nucleus of the functional human corneal endothelial cell; or
(ii) a metabolic enzyme related to a TCA cycle is organelle-selectively localized in mitochondria of the functional human corneal endothelial cell.

[Claim 20]
A functional cultured human corneal endothelial cell capable of eliciting a human corneal endothelium functional property when infused into an anterior chamber of a human eye,

wherein the cell expresses one or more of Na+/K+ ATPase α-1 (ATP1A1), anion exchange protein 2 (AE2), tight junction protein 1 (ZO-1), electrogenic sodium bicarbonate cotransporter 1 (NBCe1), sodium/hydrogen exchanger 1 (NHE1), monocarboxylate transporter 4 (MCT4), solute carrier family 4 member 11 (SLC4A11), solute carrier family 44 member 1 (SLC44A1), solute carrier family 2 member 13 (SLC2A13), solute carrier family 12 member 1 (SLC12A1), solute carrier family 25 member 42 (SLC25A42), solute carrier family 25 member 18 (SLC25A18), solute carrier family 4 member 1 adaptor protein (SLC4A1AP), or solute carrier family 16 member 2 (SLC16A2).

[Claim 21]
The functional cultured human corneal endothelial cell according to claim 20, which expresses one or more of ATP1A1, AE2, ZO-1, NBCe1, NHE1, MCT4, SLC4A11, or SLC25A42.

[Claim 22]
The functional cultured human corneal endothelial cell according to claim 20, which expresses one or more of SLC4A11, SLC44A1, SLC2A13, SLC12A1, SLC25A42, SLC25A18, SLC4A1AP, or SLC16A2.

[Claim 23]
The functional cultured human corneal endothelial cell according to any one of claims 20-22 wherein one or more metabolism-related enzymes selected from the group consisting of citrate synthase (CS), aconitase 2 (ACO2), isocitrate dehydrogenase 2 (IDH2), malate dehydrogenase 2 (MDH2), malic enzyme 3 (ME3), acetyl-CoA synthetase 1 (ACSS1), acetyl-CoA acetyltransferase 1 (ACAT1), pyruvate dehydrogenase (PDH), branched chain amino acid transaminase 2 (BCAT2), and branched-chain ketoacid dehydrogenase 2 (BCKDH2) are expressed in mitochondria of the functional cultured human corneal endothelial cell.

[Claim 24]
The functional cultured human corneal endothelial cell according to any one of claims 20-23, wherein ATP citrate lyase (ACLY), aconitase 1 (ACO1), isocitrate dehydrogenase 1 (IDH1), malate dehydrogenase 1 (MDH1), malic enzyme 1 (ME1), acetyl-CoA synthetase 2 (ACSS2), acetyl-CoA acetyltransferase 2 (ACAT2), and/or lactate dehydrogenase (LDH) is not expressed or is not substantially expressed in the functional cultured human corneal endothelial cell.

[Claim 25]
The functional cultured human corneal endothelial cell according to any one of claims 20-24, wherein expression of sodium/hydrogen exchanger 1 (NHE1) and/or aquaporin 1 (AQP-1) is increased in the functional cultured human corneal endothelial cell.

[Claim 26]
The functional cultured human corneal endothelial cell according to any one of claims 20-25, wherein expression of bicarbonic anhydrase 5B (CA5B) is increased in the functional cultured human corneal endothelial cell.

[Claim 27]
A cell population comprising a functional cultured human corneal endothelial cell according to any one of claims 20-26.