

## CLAIMS

1. A method of producing corneal endothelial cell (CEC)-like cells from pluripotent stem cells, comprising
  - a) culturing the pluripotent stem cells in the presence of at least one transforming growth factor beta (TGF-beta) inhibitor, at least one Wntless-related integration site (Wnt) activator and retinoic acid, and
  - b) culturing cells from step a) in the presence of at least one TGF-beta inhibitor and at least one Wnt activator, but in the absence of or gradually decreasing concentration of retinoic acid,
- thereby producing CEC-like cells.
2. The method according to claim 1, wherein the TGF-beta inhibitor is selected from the group consisting of SB431542 and SB505124.
3. The method according to claim 1 or 2, wherein the Wnt activator is selected from the group consisting of glycogen synthase kinase 3 (GSK3) inhibitors and proteins of R-spondin family.
4. The method according to claim 3, wherein the GSK3 inhibitor is CHIR99021.
5. The method according to any one of claims 1-4, wherein the retinoic acid is all-trans-retinoic acid (ATRA).
6. The method according to any one of claims 1-5, wherein the duration of step a) is from 3 to 10 days, preferably from 3 to 5 days.
7. The method according to any one of claims 1-6, wherein the duration of step b) is from 1 to 20 days, preferably from 1 to 10 days.
8. The method according to claim 2, wherein the concentration of SB431542 or SB505124 is from 1  $\mu$ M to 100  $\mu$ M, preferably from 1 to 30  $\mu$ M, more preferably from 5  $\mu$ M to 15  $\mu$ M, and still more preferably 10  $\mu$ M.
9. The method according to claim 4, wherein the concentration of CHIR99021 is from about 1  $\mu$ M to about 15  $\mu$ M, preferably from about 1  $\mu$ M to about 10  $\mu$ M, more preferably from about 1  $\mu$ M to about 5  $\mu$ M, and still more preferably about 4  $\mu$ M.
10. The method according to any one of claims 1-9, wherein the concentration of retinoic acid in step a) is 10  $\mu$ M.
11. The method according to any one of claims 1-10, wherein the concentration of retinoic acid is gradually decreased to the concentration of 0 – 1  $\mu$ M in step b).

12. The method according to any one of claims 1-11, wherein the pluripotent stem cells are selected from the group consisting of induced pluripotent stem cells and embryonic stem cells, with the proviso that if human embryonic stem cells are used, the method does not involve destruction of human embryos.
- 5 13. The method according to any one of claims 1-12, wherein the cells are cultured on a cell culture substrate coated with one or more ECM proteins selected from the group consisting of laminins, collagens, vitronectin, fibronectin, nidogens, proteoglycans, and E-cadherin, and isoforms, fragments, and peptide sequences thereof.
14. The method according to claim 13, wherein the ECM protein is laminin-521.
- 10 15. The method according to any one of claims 1-14, wherein the CEC-like cells express one or more markers selected from the group consisting of zonula occludens-1 (ZO-1), ATPase Na<sup>+</sup>/K<sup>+</sup> transporting subunit alpha 1 (ATP1A1), solute carrier family 4 member 4 (SLC4A4), CD166, aquaporin 1 (AQP1), paired like homeodomain 2 (PITX2), and forkhead box C1 (FOXC1).
- 15 16. The method according to any one of claims 1-15, wherein CEC-like cells for use in the treatment of corneal endothelial dysfunction or for use in drug development are obtainable.
17. The method according to claim 16, wherein the cells are human CEC-like cells.
18. Use of TGF-beta inhibitor, Wnt activator and retinoic acid, in combination at the  
20 same time, for inducing CEC-like cells from pluripotent stem cells.
19. A method for the manufacture of a preparation comprising CEC-like cells and a solution, carrier, adjuvant and/or excipient, preferably a pharmaceutically acceptable solution, carrier, adjuvant and/or excipient, the method comprising a step of producing the CEC-like cells in accordance with any one of claims 1-17.
- 25 20. The method according to claim 19, wherein the CEC-like cells provided as a cell monolayer.
21. The method according to claim 19 or 20, wherein the preparation is a pharmaceutical preparation for use in the treatment of corneal epithelial dysfunction.