Claims

1. A method for culturing isolated corneal endothelial cells comprising the steps of:

   (i) ~~contacting~~maintaining isolated corneal endothelial cells which have not been transformed to immortalized cells with a maintenance medium comprising Human Endothelial-Serum Free Medium (Human Endothelial-SFM) and serum to stabilize the corneal endothelial cells;

   (ii) c~~ontacting~~ulturing the isolated stabilized corneal endothelial cells with ~~a proliferative~~M2 or M4 medium to propagate and/or expand corneal endothelial cells; and

   (iii) ~~contacting~~maintaining the propagated and/or expanded corneal endothelial cells with said maintenance medium to substantially preserve the morphology and characteristics of the corneal endothelial cells.

~~2. The method according to claim 1, wherein step (ii) comprises culturing the isolated corneal endothelial cells in the proliferative medium.~~

~~3~~2. The method according to claim 1 ~~or 2~~, wherein step (iii) is performed before the isolated corneal endothelial cells differentiate or change morphology and/or characteristics.

~~4~~3. The method according ~~to any one of the preceding~~ claim~~s~~ 1 or 2 wherein step (iii) comprises replacing the ~~proliferative~~ M2 or M4 medium with the maintenance medium.

~~5~~4. The method according to any one of the preceding claims wherein step (iii) comprises culturing the propagated and/or expanded corneal endothelial cells in the maintenance medium.

~~6~~5. The method according to any one of claims 1 to ~~4~~3, wherein step (iii) comprises passaging the propagated and/or expanded corneal endothelial cells in the maintenance medium.

~~7. The method according to any one of the preceding claims, wherein the proliferative medium comprises a supplemented basal medium.~~

~~8. The method according to any one of the preceding claims, wherein the proliferative medium comprises a basal medium supplemented with serum.~~

~~9. The method according to any one of the preceding claims, wherein the proliferative medium comprises a basal medium supplemented with at least 2% serum.~~

~~10. The method according to any one of the preceding claims, wherein the proliferative medium comprises a basal medium supplemented with 5% serum.~~

~~11. The method according to any one of claims 7 to 10, wherein the proliferative medium comprises a basal medium supplemented with at least one supplement selected from the group consisting of amino acids, antimicrobials, cholera toxin, chondroitin, Dimethyl sulphoxide (DMSO), Endothelial Cell Growth Supplement (ECGS), growth factors, insulin, steroids, minerals, pituitary extract, transferrin and vitamins.~~

~~12. The method according to any one of the preceding claims, wherein the maintenance medium comprises a basal medium.~~

~~13. The method according to claim 12, wherein the maintenance medium comprises a basal medium supplemented with serum.~~

~~14~~6. The method according to any one of the preceding claims ~~13~~, wherein the maintenance medium comprises ~~a basal medium supplemented with~~ at least 2% serum.

~~15~~7. The method according to any one the preceding claim~~s 13 or 14~~, wherein maintenance medium comprises~~ a basal medium supplemented with~~ 2.5% or 5% serum.

~~16~~8. The method according to any one of the preceding claims, wherein maintenance medium is supplemented with at least one antimicrobial.