

# Cultivation and characterisation of human peripheral cornea derived endothelial cells

## Authors

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## Abstract

To confirm that human corneal rims left over from DALK/DSEK/PK surgeries could be useful sources for ex vivo endothelial cell expansion. Human corneal rims remaining from DALK/DSEK/PK surgeries were utilized (1:1 sex ratio, age 63+20 years, endothelial cell density >2,500 cells/mm<sup>2</sup>). The time from death to use varied between 3 days and 1.5 months. Endothelial cells isolated using a two-step, peel-and-digest method, whereby the Descemet's membrane and endothelial cells were peeled off under a dissecting microscope, followed by digestion in collagenase. The isolated cells were suspended in TrypLE prior to plating onto FNC-coated tissue culture plates. The cells were then cultured in Ham's F12:M199 (1:1) media supplemented with, ascorbic acid, transferrin, sodium selenite and bFGF. Characterisation of the cultured cells was performed by RT-qPCR and immunofluorescence staining accordingly. The number of isolated endothelial cells was repeatedly low (< 20,000

cells). However, improved techniques allowed to reduce stromal cell contamination. It was observed that endothelial cell proliferation was improved when the culture surface area was reduced. Furthermore, typical endothelial cobble stone morphology was observed when the cell density was high. Cell morphology and growth showed notable difference related to donor age and preservation time. ZO-1, Na/K-ATPase and PITX2 were used to confirm the endothelial phenotype. Preserved human corneal rims can be utilized for ex vivo expansion of corneal endothelial cells but further optimization is needed.