# Investigating umbilical cord cells for repair of the damaged corneal endothelium

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## Background

- The **cornea** is the outermost transparent layer of the eye. The **corneal endothelium** maintains corneal transparency and vision (Fig. 1), by action of the **corneal endothelial cells (CECs)**.[1]
- When CECs are damaged, as seen in Fuchs endothelial corneal dystrophy **(FECD)** (Fig. 2), vision is lost.[2,3]
- The only long-term treatment option for FECD is corneal transplantation, however, access to transplantation is limited due to a global shortage of donor corneal tissue.[3]
- **Human umbilical vein endothelial cells (HUVECs)** isolated from the umbilical cord are an attractive option for a CEC replacement therapy, owing to their endothelial origin and progenitor cell properties.

**Research aim:** To develop a CEC replacement therapy using HUVECs.

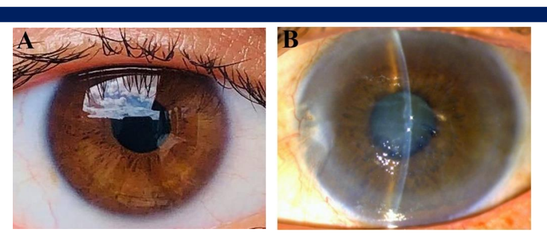



Figure 1: Healthy cornea (A) and FECD cornea (B).

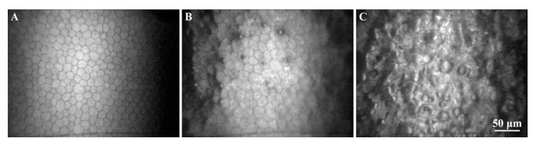
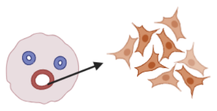



Figure 2: Healthy CECs (A), early FECD (B), and late-stage FECD (C).

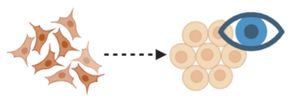
## Methods

**Isolation and characterisation**



Cells isolated from the umbilical vein and characterised using morphology, ICC (immunocytochemistry), and flow cytometry.

**Differentiation and characterisation**



HUVECs differentiated into CECs using a CEC conditioned medium. Differentiation characterised using morphology, polymerase chain reaction (PCR), and ICC.

**Functional testing**

Functional properties of CEC-like cells evaluated using electric cell-substrate impedance sensing (ECIS), dye-leak assay, and scratch assay.

## Results: Isolation and characterisation of HUVECs

Isolated cells (n = 20) displayed a cobblestone morphology in culture (Fig. 3), and showed expression of HUVEC positive markers CD31 and CD146, as assessed by ICC and flow cytometry (Fig. 4 & 5).

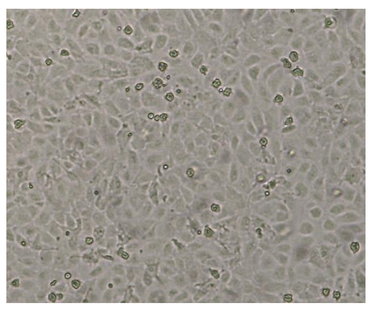


Figure 3: Cultured HUVECs.

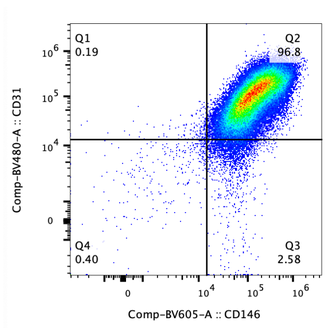



Figure 4: Flow cytometry plot of CD31 vs CD146 expression by HUVECs.

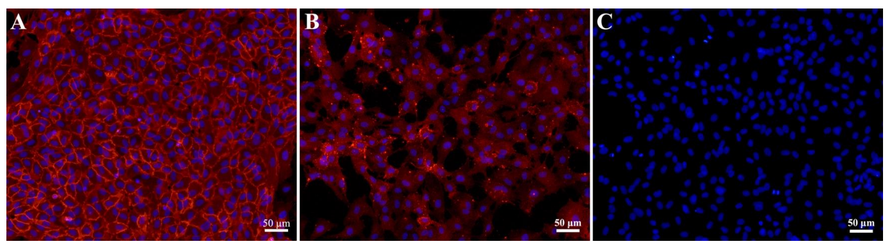



Figure 5: HUVECs immuno labelled with CD31 (A), CD146 (B), and secondary antibody only control (C).

## Results: Differentiating HUVECs → CECs

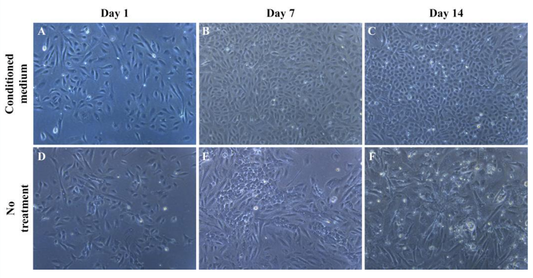



Figure 6: CM differentiated cells (top panel) and no treatment cells (bottom panel) at day 1, 7, and 14 of culture.

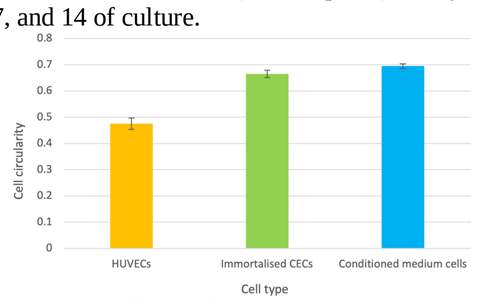



Figure 7: Cell circularity measurements of no treatment HUVECs, immortalised CECs, and CM differentiated cells at day 14.

HUVECs (n = 10) were differentiated into CEC-like cells using a conditioned medium **(CM)** from an immortalised CEC cell line.

- Differentiated cells displayed a polygonal morphology (Fig. 6).
- Differentiated cells had higher cell circularity than no treatment and immortalised CECs (Fig. 7).
- Differentiated cells showed higher gene and protein expression of CEC markers in comparison to no treatment cells (Fig. 8 & 9).
- Differentiated cells formed tight junctions, as seen by ZO1 expression along cell membranes (Fig. 8C).

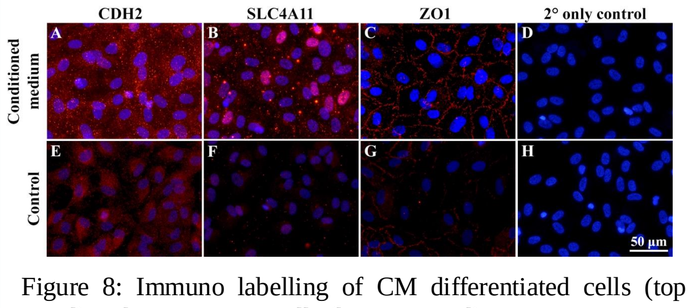



Figure 8: Immuno labelling of CM differentiated cells (top panel) and no treatment cells (bottom panel).

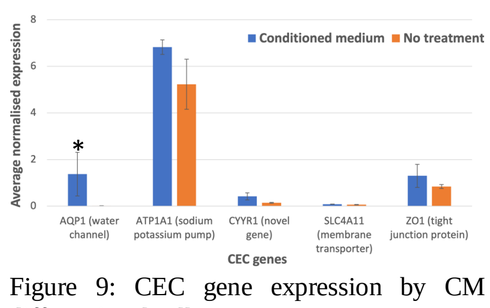



Figure 9: CEC gene expression by CM differentiated cells vs no treatment.

## Results: Functional testing of differentiated cells

Differentiated CEC-like cells showed high electrical resistance, in comparison to control HUVECs and immortalised CECs, indicating the presence of tight junctions between adjoining cells which are resisting the flow of current (Fig. 6A). In addition, the dye-leak assay showed reduced leakage of FITC-dextran across the cell membrane of differentiated CEC-like cells, providing further evidence for their barrier properties (Fig. 6B).

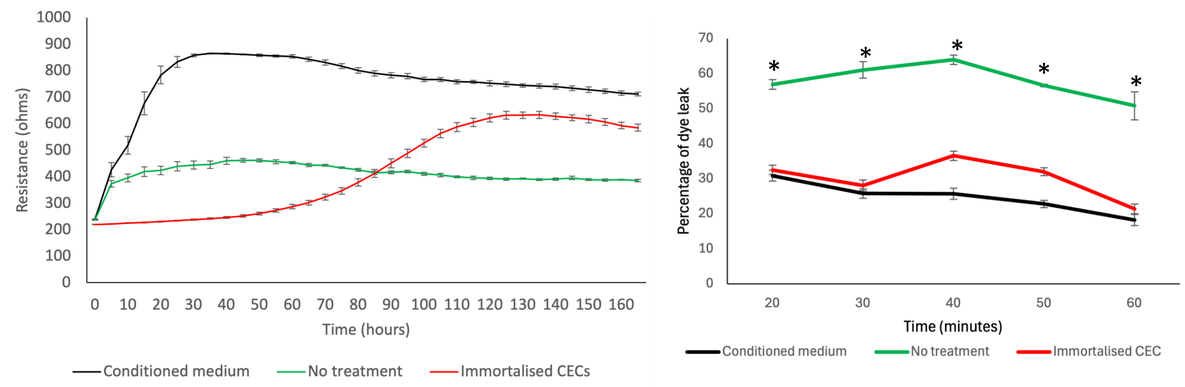



Figure 6. Electrical resistance measurements of conditioned medium differentiated CEC-like cells,, control HUVECs, and immortalised CECs, over a 160-hour period (A). FITC-dextran dye-leak measurements of conditioned medium differentiated CEC-like cells, control HUVECs, and immortalised CECs (B). Asterisks indicate statistical significance ($p \leq 0.05$).

Following the infliction of a wound, differentiated CEC-like cells showed recovery by 28 hours post injury, demonstrating their regenerative properties.

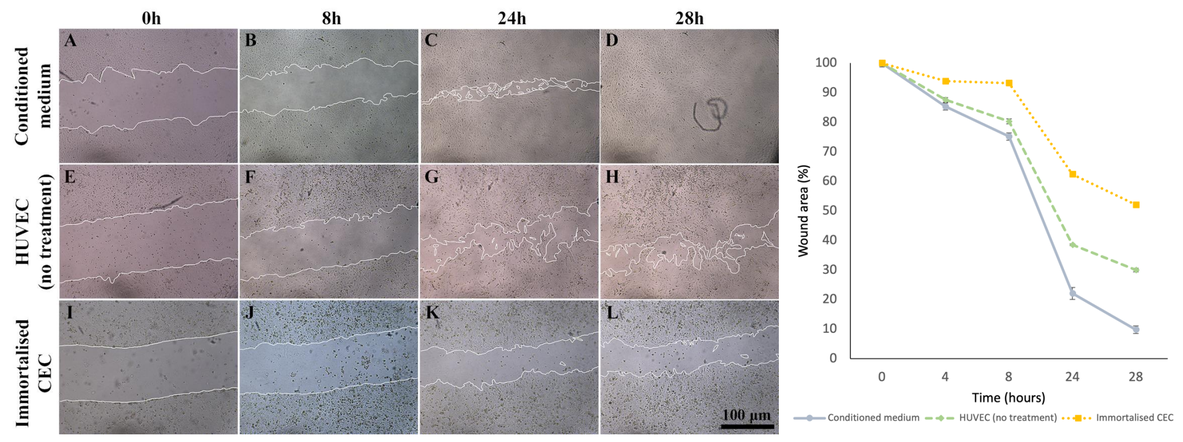



Figure 7. Scratch assay of conditioned medium differentiated CEC-like cells, control HUVECs, and immortalised CECs, over a 28-hour period (A). Graph showing wound area at different timepoints of recovery (B).

## Conclusions

- It has been shown that CEC-like cells display a polygonal morphology, and express CEC markers, in particular ZO1 is present along cell membranes.
- Functional tests show that CEC-like cells possess barrier properties, evidenced by the formation of tight junctions between cells which resist the flow of current and reduced solute leakage across the membrane.
- Next steps of this research include further functional testing using an Ussing chamber, and an in vivo (animal) trial.
- Overall, this research holds potential to be developed into a cell replacement therapy for the treatment of corneal endothelial damage, which will help address the global shortage of donor corneal tissue.

References
1. Meeney A, Mudhar HS. Histopathological reporting of corneal pathology by a biomedical scientist: the Sheffield Experience. Eye. 2013 Feb;27(2):272-6.
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