Engineered Basement Membranes for Regeneration of the Corneal Endothelium

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Statement of Purpose: The cornea is responsible for 2/3 of the refractive power of the eye and optical clarity is critical for normal vision. In the developed world, the primary cause of corneal opacity is failure of the corneal endothelium (CE), a monolayer of cells on the posterior surface of the cornea that regulates transport into and out of the stroma. Loss of endothelial cells leads to impaired vision and the need for corneal transplantation. Current transplantation options to restore CE function include a full cornea transplant and lamellar techniques such as Descemet's Stripping Endothelial Keratoplasty (DSEK), where only a thin layer of stroma, Descemet's membrane and the CE from a donor cornea are transplanted. These surgeries are successful in restoring corneal clarity; however donor corneas are limited worldwide and rejection remains a serious issue, especially in pediatric populations. Corneal endothelial cells (CECs) are non-proliferative in vivo with minimal proliferation in vitro, making expansion of these cells for therapeutic application difficult. We hypothesized that culturing CECs on a substrate that mimics the biomechanics and protein composition of the native Descemet's membrane would enhance proliferation and maintain phenotype of CECs in vitro. Once expanded, we can then use the CECs to bioengineer a corneal endothelium suitable for transplantation into the eye using a DSEK-like procedure.

Methods: A screen was performed by creating polydimethylsiloxane (PDMS) substrates with tunable elastic modulus from 5 kPa to 1.7 MPa and protein coatings of laminin, fibronectin, collagen type I and collagen type IV (Col4) for a total of 36 substrate conditions.[1] Bovine corneal endothelial cells (CECs) were screened by seeding and culturing to form a monolayer and quantifying morphology. The optimal substrate was then used to expand CECs in large numbers using image analysis, immunofluorescent staining and qRT-PCR to assess maintenance of CEC phenotype relative to positive and negative controls. Cell density at confluence was calculated for each passage using phase contrast images up to passage 10. CECs on each substrate at passages 1, 5 and 8 were also fixed and stained for the nucleus (DAPI), tight junction protein ZO-1 (monoclonal antibody) and F-actin (phalloidin). The images of ZO-1 were used to quantify the morphology of the cells using the cell area and hexagon shape factor (HSF). **C**orneal endothelium were then formed by seeding CECs on engineered basement membranes. Briefly, collagen I gels were formed at 37°C,[2] and then a polymerized layer of fluorescently labeled collagen IV was transferred onto the gel surface using a surface initiated assembly process.[3] Transfer of the fluorescent COL4 sheet was confirmed using confocal laser scanning microscopy at each stage of fabrication. In vitro expanded bovine CECs were seeded onto the engineered basement membranes and cultured for 7 days to form continuous CE monolayers that were subsequently fixed and stained for the nucleus, ZO-1, collagen IV, and F-actin.

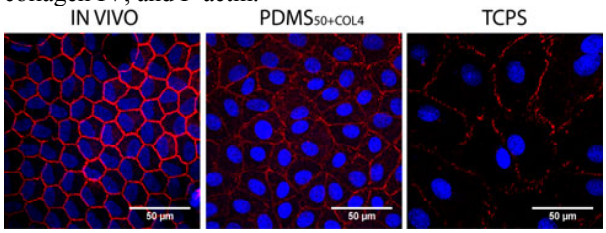

Figure 1. Representative images of CECs in vivo, on $PDMS_{50+COL4}$ and on tissue culture polystyrene (TCPS). CECs cultured on $PDMS_{50+COL4}$ that mimic the native Descemet's membrane maintain a phenotype closer to in vivo cells than those cultured on TCPS. Nucleus (blue) and ZO-1 (red).

Results: CECs were found to maintain phenotype including a polygonal shape, robust ZO-1 staining at the cell-cell border and a thin, cortical F-actin cytoskeletal on PDMS with an elastic modulus of 50 kPa and a COL4 coating ($PDMS_{50+COL4}$). CECs on the $PDMS_{50+COL4}$ could be expanded >3000-fold compared to <140-fold on polystyrene controls. At all passages, cells cultured on $PDMS_{50+COL4}$ had a smaller cell area and higher cell density. qRT-PCR indicated that the $PDMS_{50+COL4}$ prevented CECs from expressing fibroblastic genes such as collagen type III while maintaining expression of CEC genes such as the sodium bicarbonate transporter. Engineered CE was successfully formed on the engineered basement membranes using the in vitro expanded CECs. Confocal microscopy confirmed that fluorescently labeled COL4 was completely transferred onto the compressed collagen I gels and remained up to 7 days in culture. Bovine CECs formed a confluent monolayer on the engineered basement membranes and exhibited their characteristic hexagonal shape, cortical F-actin cytoskeleton and ZO-1 at the borders.

Conclusions: We have demonstrated that a biomimetic substrate that recapitulates the mechanical stiffness and COL4 composition of Descemet's membrane is able to enhance the in vitro expansion of CECs. These expanded CECs can be cultured on an engineered basement membrane that closely resembles the portion of the cornea transplanted during a DSEK procedure. These results, suggest that it is feasible to expand CECs in vitro and bioengineer a DSEK mimic that could be used for corneal repair instead of transplantation. Future studies include testing the ability of the engineered CE to pump fluid in a modified Ussing Chamber and evaluating whether human CECs can be expanded with the same system to create clinical relevant, engineered human CEs.

References:

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