Use of human umbilical cord mesenchymal stem cells to treat corneal opacity acquired through injury and infection

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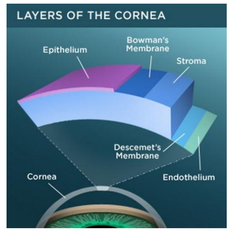
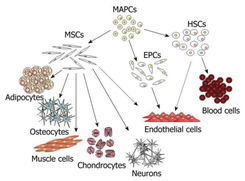
Introduction

Maintenance of corneal transparency is imperative for vision, as more than two-thirds of the eye's optical refractive power can be attributed to the cornea [1]. An important tissue within the cornea is the corneal stroma, which accounts for 90% of corneal thickness and which is composed of interwoven collagen fibrils, proteoglycans, and sparsely distributed keratocytes. The lattice arrangement and spacing of the collagen fibrils in the stroma allows for transparency, permitting light to refract through the stroma correctly. Disruption of the collagen fibril architecture from conditions such as corneal injury, bacterial infection, and complications from surgical interventions such as LASIK and PRK, can lead to a loss in vision acuity. While previous research has focused on developing an understanding of the role of collagen V in collagen fibril architecture regulation, this project aims to translate this learning into insight on how to efficaciously aid in corneal repair.



Because prior findings have shown that human umbilical cord mesenchymal stem cells (hUMSCs) have been able to rescue the thin, cloudy corneas of lumican-null [2] and collagen V-null mice with little to no immune rejection or inflammation, this therapeutic treatment was tested for its ability to recover corneal transparency in both corneal injury and bacterial keratitis infection.



Human UMSCs, like other mesenchymal stem cells, are multipotent stromal cells that have the ability to differentiate into osteocytes, adipocytes, and chondrocytes. These cells are also well-documented for their immunomodulatory effects and the immunosuppressive microenvironment that they can generate by secreting cytokines.

Hypothesis: Human umbilical cord mesenchymal stem cells will improve acquired corneal opacity in both the corneal injury and bacterial keratitis models.

Methods

Keratectomy
Mice were anesthetized by intraperitoneal (I.P.) administration of ketamine (95 mg/kg) and xylazine (20 mg/kg) and subsequently operated upon to remove a 2 mm in diameter section of corneal epithelium and the anterior corneal stroma.

Stem Cell Transplantation
hUMSCs were either transplanted onto the eye through the use of a fibrin gel. hUMSCs were added to a dilute thrombin solution, and then the thrombin with stem cells, or the control thrombin without stem cells was mixed with fibrin and applied directly to the eye to form a gel.

Bacterial Keratitis Infection
Corneas for the bacterial keratitis model were infected with *Pseudomonas aeruginosa* (strain PA01) by making three small scrapes into the anterior cornea with a 27 gauge needle and then applying 5 µL drops of bacteria at a concentration of ~ 2 x 10^9 CFU/mL.

Analysis of corneal stromal thickness and haze
Studies of corneal thickness and haze were performed by *in vivo* confocal microscopy (Heidelberg Retinal Tomograph, HRTII). Mice were given a single drop of GenTeal Gel on the eye before being placed next to the objective. 3D images were reconstructed using Axiovision imaging software and assessed for pixel intensity using ImageJ software. Student's t-test was used to determine statistical significance.

Analysis of corneal polarization by SHG imaging
Corneas were subject to nuclear staining using Syto 59 (1:1000). Confocal images were obtained using Second Harmonic Generation (SHG) to reveal stromal architecture.

Immunohistochemistry
Paraffin and frozen sections of whole eyes were produced and stained with antibodies that identified important cellular structures including collagen, lumican and CD45.

Corneal Injury and Scarification

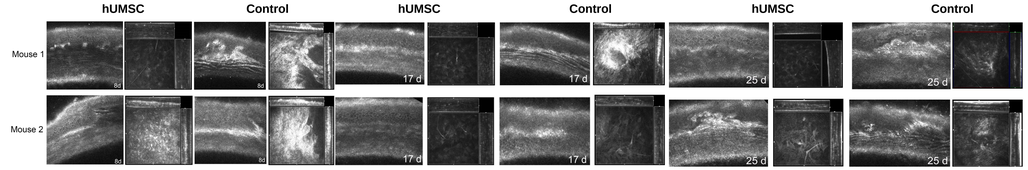


Figure 1. Treatment of corneal wounds with hUMSCs. A 2 mm central keratectomy wound was generated in C57BL/6 mice. hUMSCs were transplanted immediately following injury. Eyes were examined at 8 d, 17 d and 25 d post injury. hUMSC transplantation improved corneal transparency.

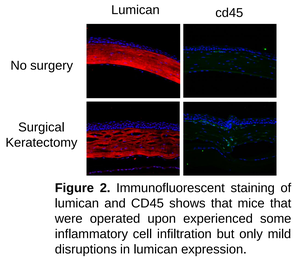


Figure 2. Immunofluorescent staining of lumican and CD45 shows that mice that were operated upon experienced some inflammatory cell infiltration but only mild disruptions in lumican expression.

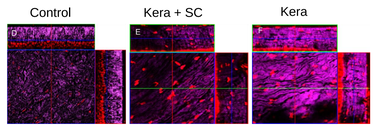


Figure 3. Disorganized collagen fibril structure in Col5a1-null cornea stroma. Back-scattering SHG images show disorganized lamellae in keratectomy and fibrin gel treated cornea **(F)** and control **(D)** compared to the more transparent and flattened lamellae in keratectomy and hUMSC treated cornea **(E)**. Keratocytes (red) visible due to Syto59 staining of the cornea.

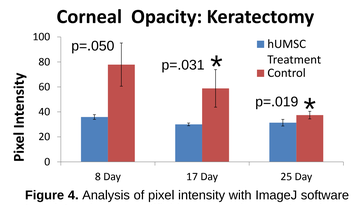


Figure 4. Analysis of pixel intensity with ImageJ software to determine quantifiable corneal opacity shows significant differences in treated and controlled subjects at the 17- and 25-day time points.

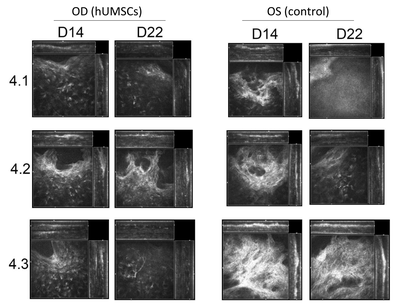


Figure 5. Treatment of corneal scarification with hUMSCs. A 2 mm central keratectomy was created and allowed to scarify for two weeks before any further experimentation. After 2 weeks, hUMSCs were transplanted, resulting in preliminary data indicative of improved corneal transparency.

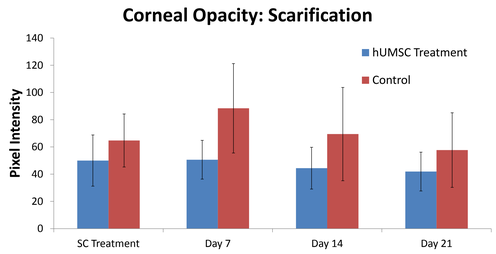


Figure 6. Quantification analysis of pixel intensity with ImageJ software to determine quantifiable corneal opacity shows no significant differences in treated and controlled subjects at the any time points after stem cells are applied to scarified corneas.

Bacterial Keratitis Infection

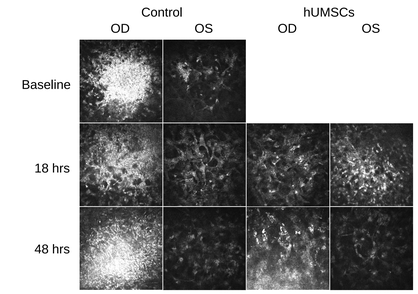


Figure 7. Representative images show that bacterial infection causes extensive opacification in the eye at baseline before any treatment. When treated with hUMSCs, opacification appears to be reduced as compared to controls. High variance in sample intensities, though, renders the observed differences not statistically significant.

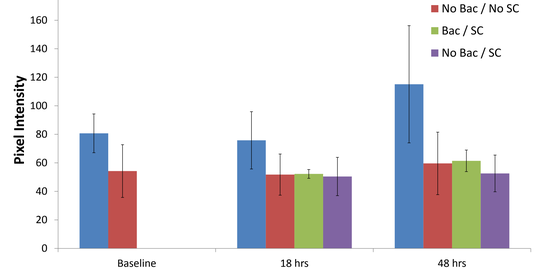


Figure 8. Corneas are infected with pseudomonas aeruginosa to simulate bacterial keratitis infection. After 24 hrs, a baseline of corneal opacity is taken with the Heidelberg Retinal Tomograph and stem cells are applied to select eyes. Analysis of pixel intensity with ImageJ software is then done to determine quantifiable corneal opacity at different time points in the two days following stem cell treatment. The data show no significant differences in treated and controlled subjects at the any time points after stem cells are applied to infected corneas.

Conclusion

1. Corneal opacity was successfully reduced in the acquired injury keratectomy mouse model with the use of human umbilical cord mesenchymal stem cells, implicating the efficacy of using hUMSCs in treating acquired corneal disease.
2. SHG Imaging showcases that injured corneas not treated with hUMSCs had prominent lamellae disorganization, whereas the injured corneas treated with hUMSCs had better collagen fiber organization.
3. hUMSCs did not contribute in a statistically significant way to recovering corneal transparency in the scarification and bacterial keratitis models of acquired corneal disease. Because, on manual inspection, marked improvement seemed to exist with the treatment of hUMSCs in both models, there is the possibility that this therapeutic treatment may indeed be efficacious. As evidenced by high variance among a limited sample size, further experimentation is needed to produce conclusive results.

References

[1] Busquets-Garcia A, Maldonado R, Ozaita. *New insights into the molecular pathophysiology of fragile X* 1] I. Fatt, *Physiology of the eye: An introduction to the vegetative functions*. London: Butterworths, 1978, pp. 1–42.

[2] Liu H, Zhang J, Liu C-Y, et al. Cell Therapy of Congenital Corneal Diseases with Umbilical Mesenchymal Stem Cells: Lumican Null Mice. Connon CJ, ed. *PLoS ONE*. 2010;5(5):e10707. doi:10.1371/journal.pone.0010707.

Future Work

- Further experimentation to expand the sample size of scarification and bacterial keratitis treatment models in order to render more conclusive results
- Assess CD45 and other immune markers upon stem cell treatment in injury and bacterial keratitis conditions to examine the immunomodulatory response caused by hUMSCs
- Transmission electron microscopy to assess collagen fibril structure
- Harvest infected corneas in the bacterial keratitis model at different time points and plate on *Pseudomonas aeruginosa*-specific plates to test for infection specificity
- Experimentation related to long-term chronic inflammation after antibiotic treatment
- Determine the therapeutic efficacy of hUMSCs in treating age-related corneal opacity