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 visual acuity. While previous research has focused on understanding the role of collagen V in corneal fibril architecture regulation, this project aims to translate this learning into insight on how efficaciously aid in corneal repair.

Because prior findings have shown that human umbilical cord mesenchymal stem cells (hUMSCs) have been able to reduce the thin, cloudy corneas of kera-nectin-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 models.

Methods

Keratitis

Mice were anesthetized by intraperitoneal (i.p.) administration of ketamine (50 mg/kg) and xylazine (50 mg/kg) and subsequently operated upon to remove a 2-mm diameter section of corneal epithelial and/or anterior stromal cornea.

Stem Cell Transplantation

hUMSCs were transplanted subconjunctivally into the eye through a 2-mm incision. hUMSCs were added to a subconjunctival solution, and then the incision with stem cells, or the control incision without stem cells was closed with suture and applied directly to the eye to form a gel.

Bacterial Keratitis

Corneas were harvested from C57BL/6J mice infected with bacterial cultures and prepared for confocal and/or SHG imaging.

Analysis of corneal stromal thickness and haze

Studies of corneal thickness and haze were performed by in vivo corneal microscopy (Hidex Retina Topograph, HRTII). Mice were anesthetized with ketamine (80 mg/kg) and xylazine (4 mg/kg) and the corneal stroma was assessed after wound healing is assessed after pain intensity using imaging software. Subjects were used to determine statistical differences.

Analysis of corneal polarization by SHG imaging

Corneas were collected from donor mice using 8% sodium azide (1:100). Corneal images were obtained using Second Harmonic Generation (SHG) microscopy on an upright epi-fluorescence microscope.

Immunohistochemistry

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

Immunofluorescence

Results

Figure 1. Treatment of corneal wounds with hUMSCs. A 2 mm central keratolytic wound was generated in C57BL/6J mice. hUMSCs were transplanted immediately following injury. Eyes were examined at 0, 1, 7, and 25 days post-injury. hUMSC transplantation improved corneal transparency.

Figure 2. Intracameral injection of SHG and CD45 shows that mice that were opened experienced some inflammatory cell infiltration but only mild disruption of stromal expression.

Figure 3. Disaggregated collagen fibril structure in Cornea-null (CNg) mouse corneas. Back-scattering SHG images show disaggregated collagen in tectonic fibrils get treated (CNg) and control (CNg) in the mouse. The tectonic fibrils in the control mouse is visible due to bulk staining of the cornea.

Figure 4. Analysis of total intensity with ImageJ software to determine quantitative corneal opacity shows no significant differences in treated and control mice at the any time points after stem cells are applied to the injured cornea.

Figure 5. Quantitation analysis of pixel intensity with ImageJ software to determine quantitative corneal opacity shows no significant differences in treated and control mice at any time points after stem cells are applied to the injured cornea.

Figure 6. Corneas are infected with pseudomonas aeruginosa to simulate bacterial keratitis infection. After 24 hrs, a sample of corneal opacity is taken with the Hidex retinal topography and stem cells are applied to the eye. Analysis of stem positivity with ImageJ software is then done to determine the changes in corneal opacity.

Figure 7. Representative images show that bacterial infection causes severe 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.

Conclusions

The stem cell treatment was successfully reduced the acquired injury keratolytic mouse model with the use of human umbilical cord mesenchymal stem cells, implying the efficacy of using hUMSCs in treating acquired injury keratolytic mouse model.

Bacterial Keratitis Infection

Corneal Opacity: Keratocystomy

Corneal Opacity: Scarring

References


Future Work

Further experimentation to expand the sample size of scarring and bacterial keratitis treatment models in order to render more conclusive results.

Assess CDS and other immune pathways upon stem cell treatment and injury and bacterial keratitis conditions to examine the immunomodulatory responses caused by hUMSCs.

Transplantation electron microscopy to assess collagen fibril structure.

Immunohistochemistry in bacterial keratitis at different time points and plate on Pseudomonas aeruginosa specific plates to test for infection specificity.

Experimentation related to long-term chronic infection and antibiotic treatment.

Determine the therapeutic efficacy of hUMSCs in treating age-related corneal opacity.

Conclusion

The stem cell treatment was successfully reduced the acquired injury keratolytic mouse model with the use of human umbilical cord mesenchymal stem cells, implying the efficacy of using hUMSCs in treating acquired injury keratolytic mouse model.