Use of human umbilical cord mesenchymal stem cells to treat congenital and acquired corneal opacity

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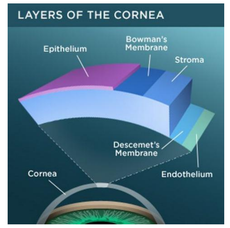
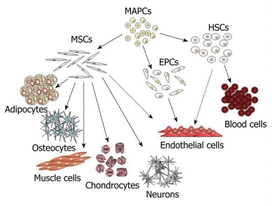
Introduction

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 keratocyte cells. 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 plana, corneal stromal congenital dystrophy, corneal injury, 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 (UMSCs) have been able to rescue the thin, cloudy corneas of lumican-null mice with little to no immune rejection or inflammation [2], this therapeutic treatment was tested for its ability to recover corneal transparency in both the congenital model deficient in collagen isoform Col5a1 and the acquired-condition model brought about by corneal injury. 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. In the scope of this project, the ability of UMSCs to differentiate into keratocytes may compensate for the lack of collagen V in the congenital model and the irregular arrangement of collagen fibrils in the acquired model to reduce corneal opacity.





Hypothesis: Human umbilical cord mesenchymal stem cells will improve acquired corneal opacity and will compensate for the loss of Col5a1 in the congenital model.

Methods

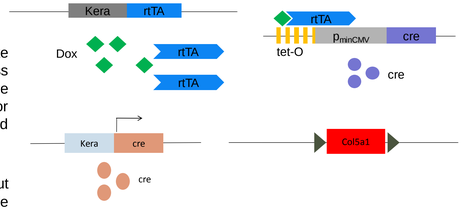
Congenital Mouse Models

Tri-transgenic *Kera-rtTA/tet-O-Cre/Col5a1*$^{f/f}$

This mouse model is tissue specific to cells like keratocytes in the corneal stroma which express keratocan. Upon induction of doxycycline, the rtTA/dox complex can bind to the tet-O operator driving the expression of cre recombinase and leading to the excision of *Col5a1*.

Uninducible bi-transgenic *Kera-Cre/Col5a1*$^{f/f}$

This mouse model is also tissue specific, but unlike the previous model, is not inducible through the use of doxycycline.



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 section of 2mm in diameter of corneal epithelium and partial layer of the anterior corneal stroma.

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.

Stem Cell Transplantation

hUMSCs were either transplanted into the eye by intrastromal injection or through the use of a fibrin gel.

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.

Whole mount corneal staining

Corneas were stained with phalloidin to identify F-actin and counterstained with DAPI (1:2000) and viewed on a Zeiss microscope.

Immunohistochemistry

Paraffin block and cryo-frozen sections of whole eyes were produced and stained with antibodies that identified important cellular structures including lumican and CD45.

Results

Congenital

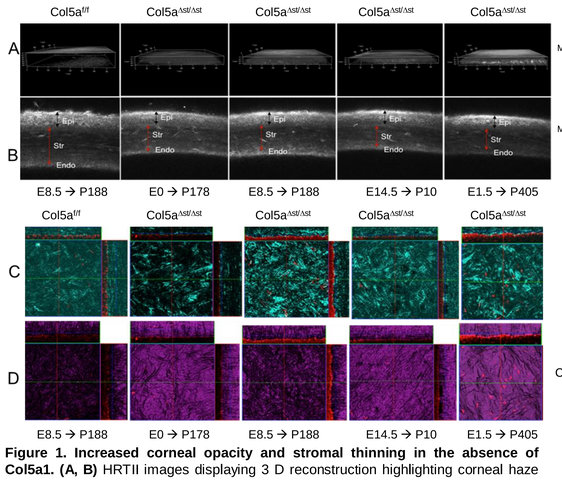


Figure 1. Increased corneal opacity and stromal thinning in the absence of Col5a1. (A, B) HRTII images displaying 3 D reconstruction highlighting corneal haze and cross-section images displaying stromal thinning. **(C, D)** SHG images showing the forward and backward scattering of light. Loss of Col5a1 shows increased fibril bundles and lamellar disorganization

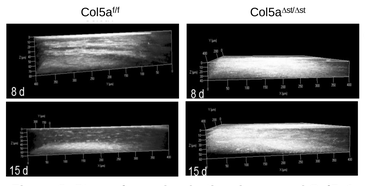


Figure 3. Stromal scarring in the absence of Col5a1 after injury. HRT images showing stromal haze 8 d and 15 d after keratectomy. Loss of Col5a1 fails to resolve stromal haze and scarring.

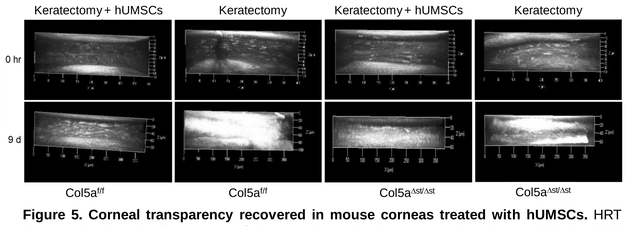


Figure 5. Corneal transparency recovered in mouse corneas treated with hUMSCs. HRT images showing significant stromal haze 9 days after keratectomy in mice at P30. hUMSC transplantation was able to improve corneal transparency in mice with Col5a1 but did so to a lesser degree in Col5a1 deficient corneas.

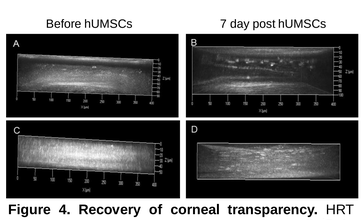


Figure 4. Recovery of corneal transparency. HRT images showing recovery of transparency in Col5a1-null corneas 7 days post-hUMSC transplantation. **(A-B)** Mouse 1 **(C-D)** Mouse 2. Mice were 70 days old.

Acquired

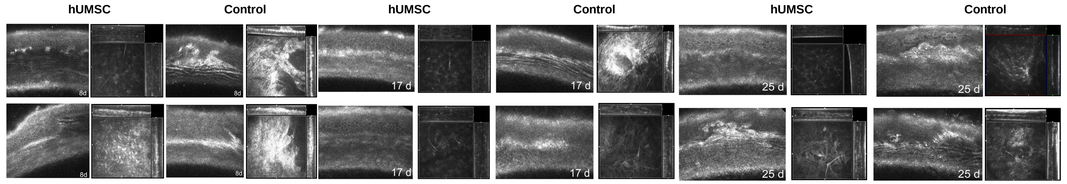


Figure 7. Treatment of corneal wounds with hUMSCs. A 2 mm central keratectomy wound was generated in C57BL/6 mice. hUMSCs were transplanted immediately following injury. Eyes were examined at 8 d, 17 d and 25 d post injury. hUMSC transplantation improved corneal transparency.

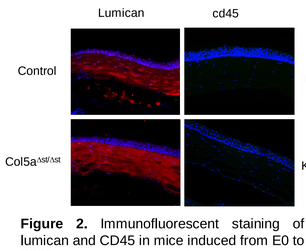


Figure 2. Immunofluorescent staining of lumican and CD45 in mice induced from E0 to P360. Loss of collagen V did not result in inflammatory cell infiltration or changes in lumican expression.

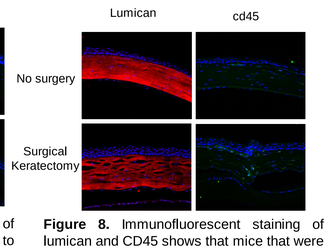


Figure 8. Immunofluorescent staining of lumican and CD45 shows that mice that were operated upon experienced some inflammatory cell infiltration but only mild disruptions in lumican expression.

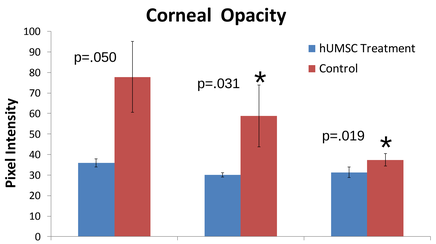


Figure 9. Analysis of pixel intensity with ImageJ software to determine quantifiable corneal opacity shows significant differences in treated and controlled subjects at the 17- and 25-day time points.

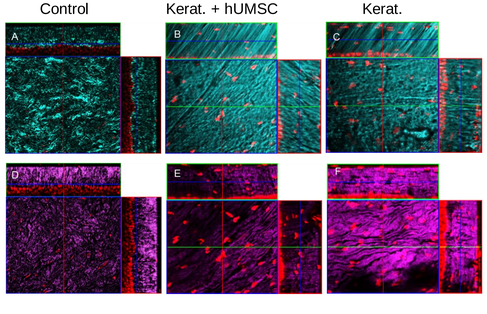


Figure 6. Disorganized collagen fibril structure in Col5a1-null cornea stroma. Forward-scattering SHG images (cyan) show enlarged fibrils in keratectomy and hUMSCs treated cornea **(B)** and keratectomy and fibrin gel treated cornea **(C)** compared to control **(A)**. Back-scattering SHG images (magenta) 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.

Conclusion

1. Corneal opacity was successfully reduced in the congenital and acquired mouse models with the use of human umbilical cord mesenchymal stem cells, implicating the efficacy of using hUMSCs in treating acquired and congenital corneal disease.
2. hUMSCs were successful in recovering some corneal transparency in both normal and Col5a1-null injured corneas.
3. SHG analysis showed the differences in collagen fibril and lamellae organization between corneas treated and not treated with hUMSCs; the more opaque corneas, that were injured and not treated with hUMSCs, had prominent lamellae disorganization, whereas the injured corneas treated with hUMSCs had better collagen fiber organization.

Future Work

- Immunofluorescent staining for mouse Col5a1 to ensure knockout
- Immunofluorescent staining for human-specific Col5a1 to affirm that Col5a1 is produced by hUMSCs
- Determine the therapeutic efficacy of hUMSCs in treating age-related corneal opacity in Col5a1-deficient mice.
- Assess the impact of scarification upon hUMSC efficacy
- Assess CD45 and lumican upon stem cell treatment to examine immunomodulatory response caused by hUMSCs
- Transmission electron microscopy to assess collagen fibril structure

References

[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.