Characterizing the Composition of the Corneal Limbal Epithelial Stem Cell Niche

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Background

Limbal epithelial stem cells (LESCs) are essential for the maintenance of the corneal epithelium and are required to regenerate the corneal epithelium post injury. The loss of LESCs or their niche can lead to limbal stem cell deficiency (LSCD). LSCD causes corneal erosions and conjunctivalization of the cornea, which leads to ocular pain and vision loss. Hyaluronan (HA) is a constituent of the LSCN that is necessary to maintain LESCs in their stem cell state.

We sought to characterize the HA-specific LSCN by identifying HA bound proteins and proteoglycans (PGs) within the limbal region of human and porcine corneas **(Fig 1)**. We created a protocol for PG extraction withing the limbal regions and corneas in order to analyze the extracts through Western Blot and agarose gel electrophoresis **(Fig 2)**.

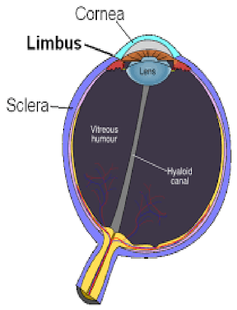


Figure 1.
Anatomy of an eye distinguishing the limbus and cornea

Methodology

Tissue Digestion
- 4 samples (Human Limbus, Human Cornea, Pig Limbus, and Pig Cornea) were subjected to tissue digestion in a solution of 4M guanidinium chloride, 0.05M sodium acetate, pH 6 containing 2% Triton X-100.
- Scissors used to break down tissues followed by low intensity sonication. Tissues were vortexed, centrifuged once they were homogenous, and the supernatant was collected.

Buffer Exchange
- A 10mL chromatography column was packed with Sephadex G-50 resin.
- The column was equilibrated with 7M Urea.
- Samples were added in 3mL increments and washed through with Urea (flowthrough was collected).

Fast Flow Anion Exchange
- A 50mL chromatography column was packed with 8mL of Q-Sepharose resin.
- Column was equilibrated with a low salt buffer.
- Sample was added in 15mL increments and the flow through was discarded.
- High salt buffer was used to wash out the PGs and GAGs (flow through collected).

Desalting
- Samples were put in snakeskin dialysis bags and placed into a 1L beaker filled with DI water.
- Water was changed twice a day and this process was repeated 3 days, allowing the samples to desalt.
- Samples were then concentrated through lyophilization.

PG Analysis
- Samples were quantified using a BCA kit.
- Western Blot was done using an approximated 20-50 ug of protein.
- Agarose gel electrophoresis also performed.

Samples	Wet Weight
Human Cornea	80 mg
Human Limbus	90 mg
Pig Cornea	830 mg
Pig Limbus	958 mg

Table 1. Wet Weights
4 sample tissues were obtained, and the mass was recorded.

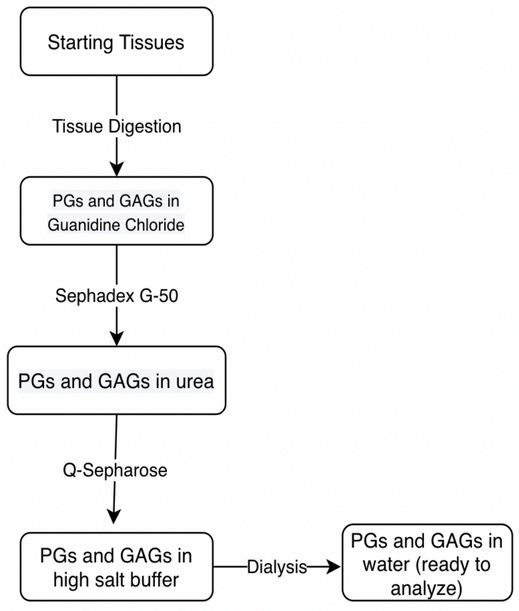
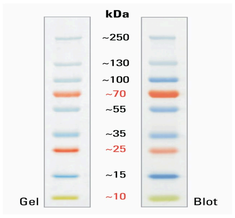


Figure 2. Extraction Protocol
A detailed protocol was developed in order to properly extract PGs and GAGs and analyze them

Results

After several Western Blot gels were run along with immunohistochemistry, Inter-Alpha Inhibitor (IaI) was identified in our samples **(Fig. 3)**. This molecule forms specific HA matrices with anti-inflammatory properties.



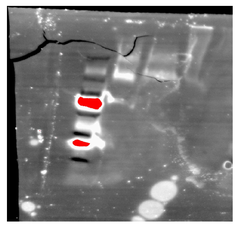


Figure 3.
The Western Blot gel identifies the the IaI at around 250 KD. Lanes 1-4 are Human Cornea, Human Limbus, Pig Cornea, Pig Limbus respectively.

Conclusion

With the identification of the IaI **(Fig. 4)**, future studies will work on characterizing the role of this HA/IaI and HA/TSG-6/IaI matrix within the corneal limbus. Characterizing the role of the LSCN is vital for establishing novel mechanisms for treating LSCD.

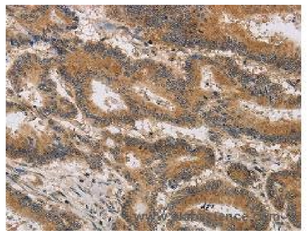


Figure 4.
Imaging of anti-ITIH antibodies

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