Hyaluronan Supports Corneal Limbal Stem Cells

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Introduction

The region between the cornea and the conjunctiva is known as the corneal limbus. Substantial studies have shown that a population of stem cells, namely limbal epithelial stem cells (LESCs) exist in the basal layer of the limbal epithelium [1]. Previous work by our lab has shown that hyaluronan (HA) is a major component of the LESC extracellular matrix, providing a specialized environment [1]. In this specialized niche, HA has been shown to be abundantly present in the limbal area and is important for maintaining the LESC phenotype *in vivo* [1]. This phenotype includes characteristics such as high proliferative capacity, increased roundness, and positive staining for putative stem cell markers [2]. The aim of this study was to investigate whether HA can support LESCs *ex vivo*.

Methods

Single limbal stem cell (LESC) suspensions were performed with human corneas, from donors aged 29-65 years (less than 72 hours post-mortem) through sequential dispase and trypsin digestion. These isolated LESCs were then seeded on ColIV (collagen IV), PLL (poly-L-lysine), or PLL/HA (PLL/hyaluronan) coated petri dishes. This was done both in the presence or absence of 3T3 feeder cells. For data analysis, the Qu-Path program was used, to analyze both circularity and cell area at each passage. Cell adhesion and proliferation was determined through image analysis and proliferation rate was determined by the change in the number of cells over time. A colony formation assay (CFA) was performed at a seeding density of 1000 cells/cm² and the expression of LESC markers through immunofluorescent staining was analyzed at passage 3.

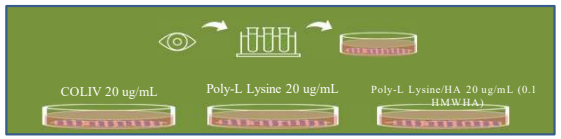

Figure 1: Schematic of the differently coated dishes used to maintain LESCs.

Results

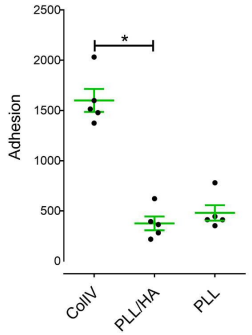

Figure 2: LESCs were seeded onto differently coated dishes (ColIV, PLL/HA, PLL) respectively, imaged, and analyzed using QuPath bio-image analysis software. Cells were quantified and the mean average was recorded.

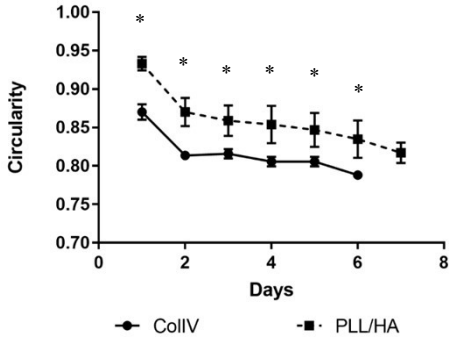

Figure 3: Cells were maintained on differently coated dishes and the cell circularity was determined after image analysis of cells adhered to the coated dishes (ColIV, PLL/HA) at Passage 0 for the ColIV and PLL/HA coats with QuPath. Increased cell circularity has previously been correlated with the LESC phenotype [3].

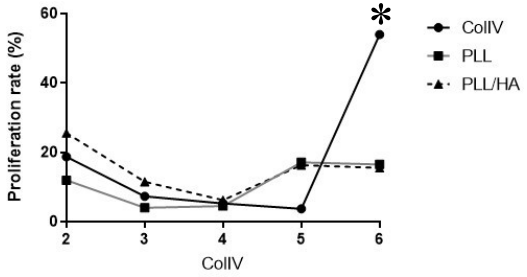

Figure 4: Proliferation rate of LESCs cultured on ColIV, PLL/HA or PLL coated dishes was determined through image analysis using the change in cell number from days 2 through 6.

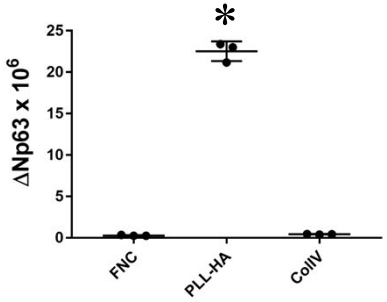

Figure 5: LESCs were cultured on differently coated dishes and subjected to qPCR analysis for DNp63, a putative stem cell marker. Cells cultured on PLL/HA have higher expression levels of DNp63 when compared to cells maintained on ColIV and fibrinectin (FNC).

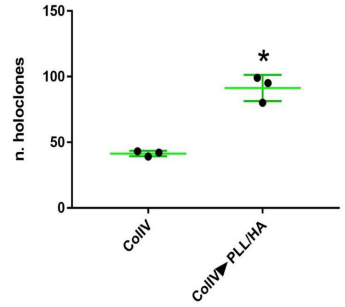

Figure 6: A colony formation assay was performed by seeding LESCs onto ColIV and PLL/HA coated dishes at a density of 1000 cells/cm². The nof holoclones greater than or equal to 500 cells were counted.

Conclusion

This study investigated the role of HA in supporting LESCs *ex vivo*. Our preliminary findings show that there is a positive correlation between cells cultured onto PLL/HA and the LESC phenotype. The LESCs cultured onto PLL/HA exhibited increased cell roundness at passage 2 and passage 3, indicating that PLL/HA maintains cell roundness to a higher extent than ColIV. LESCs cultured on PLL/HA also presented increased CFA capabilities and increased number of cells expressing the LESC marker, DNp63. LESCs are quiescent cells with high proliferative capabilities. Initially, PLL/HA had a higher proliferation rate than LESCs maintained on ColIV and PLL, however, over time LESCs maintained on ColIV coated dishes suffer a drastic increased in proliferation which culminates in significantly higher proliferation when compared to cells maintained on PLL/HA coated dishes. Overall, this research suggests HA can be used to support the expansion of LESCs for *ex vivo* prior to LESC transplantation.

References

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