00482 Quantitation of Collagen Architecture in the Post-operative Conjunctiva by Multiphoton Imaging

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Aims: Increase in collagen production in the post-operative conjunctiva contributes to scarring and surgical failure. Although collagen content can be measured by common molecular techniques, alterations in collagen architecture cannot be as easily quantitated. In this study, we quantitated progressive changes in collagen characteristics using quantitative multiphoton microscopy pioneered by HistoIndex Pte Ltd.

Methodology: The mouse model of glaucoma filtration surgery was performed on 57BL/6 mice with approval by the Institutional Animal Care and Use Committee. Each mouse was subjected to experimental GFS in one eye while the unoperated contralateral eye was used for baseline assessment. The time points for eye enucleation were days 2, 7, 14 and 21 post-surgery. The eyes were fixed and cryosectioned before being scanned and analysed using image analysis algorithms by HistoIndex.

Result: The operated conjunctiva in this mouse model is unique in that the operated tissue is enlarged from accumulation of aqueous humor when the filtering function remains effective. The progressive and significant decline in tissue area from day 2 to 14 therefore suggests reduction of functional filtration with time. Collagen fiber density was found to be significantly reduced on days 2 and 7, but then increased significantly on days 14 and 21 post-surgery, suggesting scarring has occurred by day 14.

Collagen reticulation was significantly increased from baseline from day 2 to 7 but returned to normal tissue levels on day 14, suggesting alteration of collagen structure in the operated tissue. Collagen length and thickness were both significantly reduced in the operated conjunctivas from day 7 to 21, suggesting that these collagen attributes deviate from normal in the scarred tissue.

Conclusion: These data indicate that collagen characteristics are altered in the process of post-operative wound healing/ scarring in the conjunctiva. Increase in understanding of these architectural changes will facilitate the evaluation of anti-fibrotic therapeutics.