Title: Understanding the molecular and cellular complexity of adult human cornea through single cell RNA-Seq analysis

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Purpose: Cornea is the transparent front part of the eye, which together with the lens focuses the light onto retina for visual processing. Corneal blindness is the second main cause of blindness worldwide accounting for 23 million patients. Clearly, there is an unmet need for the design of new smart biomaterials/stem cell therapies to create corneal structures that are indistinguishable from the native tissue. This requires a detailed understanding of corneal structure and function. This study has utilized the most recent advances in single cell RNA sequencing to reveal the intricacies of human adult corneal structure at the cellular and molecular level.

Methods: Human adult cornea was excised from four deceased donor eyes (51, 75, 83 and 86 years old) and dissociated to single cells. Approximately 10,000 cells were captured using the 10 X Chromium Single Cell 3' Library & Gel Bead Kit Genomics (version 3). Single cell libraries were sequenced to 50 k reads per cell on an Illumina NovaSeq 6000. The 10x Genomics software CellRanger was used to process the raw sequencing data. A quality control step was applied to remove technical noise.

Results: 21,343 cells were obtained from the four adult corneas after data integration. 21 cell clusters representing corneal epithelium, stroma and endothelium, conjuctival epithelium and endothelium, immune and red blood cells as well as limbal fibroblasts and melanocytes were identified. Three populations representing limbal progenitors (comprising 8.2% of the total), quiescent and mitotic limbal stem cells (comprising 4.2 and 3.9% of the total) were identified. Differential gene expression analysis between these three clusters combined with gene expression heat maps of all 21 clusters revealed: (i) expression of *S100A2, KRT14 and KRT15* specifically in the limbal, quiescent and mitotic LSC clusters; (ii) expression of *SLC6A6* in quiescent LSC and limbal fibroblasts clusters and (iii) *NRTK2* in the mitotic LSCs and limbal stroma and fibroblasts.

Conclusion: This study indicates the usefulness of single cell sequencing analyses for identification of stem, progenitor and differentiated cells in the adult cornea and specific extracellular matrix components, which can be utilised to enhance their *ex vivo* expansion for therapeutic applications.