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In-Vitro Estimation of O_2 Concentrations during Corneal Cross-Linking (CXL) for Porcine Corneas and Collagen Type-I Gels

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Abstract

Purpose : The presence of molecular oxygen (O_2) during corneal cross-linking (CXL) plays a pivotal role in the biomechanical properties of the cornea. We previously measured the role of O_2 and its distribution across the stroma before and during CXL in porcine corneas using phosphorescent nanoparticle probes. The purpose of this study is to compare collagen type-I monomer gel solution with porcine measurements.

Methods : Porcine globes were obtained from the local slaughterhouse 4 hours post mortem and kept at 4°C until used. The epithelium was removed and the corneas were stained with 0.1% riboflavin and different phosphorescent O_2 nanoparticle probes for 1 hour to achieve maximum diffusion. Each globe was analysed at 37° C at 21% ambient O_2 with phosphorescent lifetime microscopy (PLIM) with a 5x/0.25 Fluar objective, excitation at 488 nm and emission collected at 750-810 nm. The cornea was imaged over 15 sec at a fixed stromal depth of 50µm. The same method was applied to 6 samples of collagen type-I with an average pachymetry of 50 µm. CXL was achieved through periodic 20-30 illumination cycles with UV-A LED light (7 mW/cm^2) whilst imaging. Photon distributions and phosphorescence decay curves were analysed and lifetime values and O_2 concentrations calculated.

Results : We optimised staining with various O_2 probes and measurement conditions for porcine corneas and collagen type-I, and performed proof-of-principle PLIM experiments before and after CXL. We observed efficient uniform in-depth staining allowing high-resolution O_2 maps to be generated and monitor dynamics during CXL. PLIM revealed a slight increase in O_2 concentrations post UV illumination suggesting a role of reactive oxygen species (ROS) during the photochemical CXL process.

Conclusions : The use of phosphorescent O_2 probes allows for efficient and minimally-invasive determination of O_2 concentrations prior to and during CXL. Results indicate that collagen type-I is a more efficient model for measurements of O_2 due to restrictions in hydration control when using ex-vivo tissue. 2D and 3D maps of O_2 concentrations during CXL will allow better understanding of the role of O_2. Future work will focus on the suitability of the O_2 PLIM method for in-vivo use along with increased imaging depth profiles beyond 100µm for porcine and collagen type-I samples.

This is an abstract that was submitted for the 2017 ARVO Annual Meeting, held in Baltimore, MD, May 7-11, 2017.