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Second Harmonic Imaging of Corneas After Collagen Cross-Linking

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Footnotes

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Abstract

Purpose: : To investigate microscopic changes in the morphology of the corneal stroma after collagen cross-linking (CXL) treatment in bovine and porcine eyes using second harmonic generation (SHG) multiphoton imaging.

Methods: : A two-photon (non-linear) microscope was used to register SHG tomographic (3D) images of the corneal stroma. The microscope used a Ti:Sapphire femtosecond laser as excitation source and a photon-counting unit as detection device. A motorized stage allowed optical sectioning across the entire cornea. The instrument is computer-controlled with custom-developed software for image acquisition and processing. Enucleated bovine and porcine eyes were analyzed after CXL treatment and

without treatment as control. The CXL treatment was performed as follows: the corneal epithelium was removed, 0.125% riboflavin solution was instilled and the eye illuminated with UV-light (365 nm, 2.038 mW, 12-mm diameter beam) for 30 minutes. For SHG imaging, the eyes were placed up-side-down on a glass bottom dish filled with saline solution. Control and CXL treated eyes were measured under the same experimental conditions. Tomographic (XZ or YZ) SHG images (lateral and axial resolution: 0.5 microns/pixel and 1 micron/pixel respectively) were recorded as a function of time after treatment. Moreover SHG XY-plane images at different depths (10 microns apart) through the cornea were also acquired.

Results: : The SHG images showed a reduction of ~1/3 in corneal thickness immediately after treatment, which partially recovers with time. SHG signal decreased with depth in both the CXL and control (untreated) corneas. The maximum SHG signal was found close to the Bowman's layer and its value and location were independent of the corneal thinning resulting from the CXL treatment. The characteristic arrangement of the collagen fibers throughout the entire stroma is revealed in the SHG images. In the control corneas, the sets of lamellae are densely packed with singular undulations. This spatial pattern was changed in CXL treated corneas, where some abnormal structures, such as thicker collagen bundles appear in some localized areas. These differences were only observed in the first third of the outer stroma.

Conclusions: : SHG microscopy imaging was used to characterize the structural changes in the corneal stroma after CXL treatment. Significant changes in the corneal morphology after CXL treatment were revealed: a reduction in thickness and modifications of the local organization of the collagen bundles.

Keywords: cornea: stroma and keratocytes • microscopy: light/fluorescence/immunohistochemistry • imaging/image analysis: non-clinical