QUANTITATIVE ANALYSIS OF CORNEAL COLLAGEN ARRANGEMENT AFTER IN VIVO PHOTO-CHEMICAL SURGERY USING SECOND HARMONIC IMAGING MICROSCOPY

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Second Harmonic Generation (SHG) microscopy has been shown to be a useful imaging tool to visualize collagen-based tissues such as the corneal stroma. The natural arrangement of the collagen fibers within the cornea can be modified under different factors, such as aging, pathology, external damage or surgery [1-3]. In particular, keratoconus is a non-reversible corneal pathology leading to blindness that deserves special attention. Compared to a normal tissue, a keratoconic cornea presents a non-organized structure [1]. To stop keratoconus progression collagen cross-linking (CXL) is the usual surgical treatment. This is a photochemical procedure based on the application of riboflavin-dextran (used as a photosensitizer) after corneal epithelium removal, followed by UVA irradiation. CXL generates intra- and inter-fibrillar covalent bonds leading to an increase in corneal stiffness. Despite its success, the changes produced in collagen distribution are still a topic of discussion. Moreover, fundamental studies on how CXL modifies the corneal stroma architecture are scarce in the literature. Here we propose to use SHG microscopy to analyze and quantify changes induced by CXL treatment in two animal models under similar experimental conditions.

A research multiphoton microscope was used to record SHG images of the corneal stroma of healthy rabbits and adult chickens [4]. The CXL treatment was performed in the left eye of both animal models (the fellow eye was used as control). SHG imaging was carried out 4 weeks after the CXL standard treatment, once the animals were sacrificed, eye globes enucleated and corneas excised. The structure tensor [5] was used to quantify the organization of the collagen fibers.

SHG images of untreated control corneas show different collagen arrangement for both animal models. Bundles are clearly outlined and differences in lamellar organization, thickness and spacing are easily observed. Results show that the effects of CXL depend on the initial arrangement of the corneal collagen. Whereas the treatment increases the order in corneas with a low level of initial organization, corneas presenting a fairly regular pattern are hardly affected. SHG microscopy is a useful technique to accurate and objectively determine the changes in stromal distribution after CXL. Future clinical implementations might help not only in surgical monitoring but also in early diagnoses of corneal pathologies.

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