ADAPTIVE-OPTICS MULTIPHOTON MICROSCOPY OF PHOTOCURABLE POLYMER INTRASTROMAL IMPLANTS IN EX-VIVO CORNEAS

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Multiphoton (non-linear) microscopy has been established as an emerging tool for the analysis of non-stained ocular tissues. The stroma represents over 90% of the corneal thickness and its structural element is type-I collagen, with a natural non-centrosymmetric organization that generates a strong second harmonic generation (SHG) signal [1]. This allowed exploring the collagen distribution in both normal and pathological corneas using SHG microscopy [2,3]. Changes after corneal surgical treatments have also been visualized with this technique [4,5]. In this work, we investigate the ability of an adaptive-optics (AO) multiphoton microscope to follow corneal changes after a photo-polymerisable hydrophilic material (PEG/Irg) [6] was implanted in ex-vivo corneas.

We used a custom backscattered AO multiphoton microscope for SHG imaging of freshly enucleated porcine eyes [5]. For each cornea, a longitudinal lamellar pocket (~ 5-mm in width) was manually created after the epithelium was removed. A certain amount of PEG/Irg in liquid form was injected in each pocket. The cornea was then irradiated with UV light (390nm) to polymerize the PEG/Irg forming a rigid film. A series of eyes without treatment were used as control.

Both tomographic and regular XY SHG images were recorded from both control and treated corneas. In control corneas SHG signal decreased uniformly as deeper corneal layers were imaged. On the opposite, treated corneas exhibited an abrupt lost of SHG signal at the location of the implant. The use of AO-microscope permitted to detect SHG signal at deeper corneal layers, showing that the lamellar arrangement was kept intact.

Multiphoton microscopy was able to accurately visualize and localize intrastromal implants within the corneal tissue in a minimally invasive manner. Collagen patterns were not affected in the regions surrounded the implant. The absence of SHG signal at the implant location allows the assessment of its dimensions and locations within the corneal stroma.


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