Non-invasive assessment of corneal crosslinking changes using polarization sensitive optical coherence tomography

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Abstract

Collagen crosslinking (CXL) has shown promising results in the prevention of the progression of keratoconus and corneal ectasia. However, techniques for *in vivo* and *in situ* assessment of the treatment are limited. In this study, *ex vivo* porcine eyes were treated with a chemical CXL agent (glutaraldehyde), during which polarization sensitive optical coherence tomography (PS-OCT) recordings were acquired simultaneously to assess the sensitivity of the technique to assess changes in the cornea. The results obtained in this study suggest that PS-OCT may be a suitable technique to measure CXL changes *in situ* and to assess the local changes in the treated region of the cornea.

Keywords: cross-linking, OCT, polarization sensitive, biomechanics

1. Introduction

Collagen crosslinking (CXL) procedures are used to change the biomechanical properties of the cornea by increasing its rigidity and to stop the progression of ectasia. This is achieve thru mechanisms of crosslinking by forming intra- and inter- fibrillar bonds between collagen fibrils. Due to the clinically promising results obtained with the CXL procedure [1], there is an increased interest in developing non-invasive techniques capable of assessing and imaging *in situ* changes in the cornea associated with the CXL treatment. A few studies have shown techniques that are able to discriminate those changes. Raud et al. [2, 3] used a chemical cross-linking agent (glutaraldehyde) and showed that nonlinear optical microscopy with second harmonic generation and two photon-excited autofluorescence can be used to characterize the biomechanical changes in the collagen properties. With similar imaging techniques but a different CXL treatment (UVA+riboflavin), Bueno et al. [4] also characterized the collagen changes. Brillouin optical microscopy [5] techniques have also shown the ability to discriminate the effect of cross-linking.

Doors et al. [6] used a commercial anterior segment OCT to visualize the changes in human patients after crosslinking. However, the stromal demarcation line, which marks the end of the treatment zone, was only visible in the intensity OCT images at 1 month after surgery. Seiler and Hafezi [7] reported the demarcation line is detectable in slit lamp biomicroscope examination as early 2 weeks after treatment.

Polarization sensitive OCT (PS-OCT) can measure the polarization state of the light reflected from the cornea, which is one of the ocular tissues that changes the polarization of the light. Thus, PS-OCT could potentially evaluate stromal changes of the cornea after crosslinking, as the change on the collagen fibre structure (orientation and organization) may induce a change in the birefringence information. PS-OCT technology has already been used for other applications of the anterior segment of the eye, such as tissue discrimination [8], visual enhancement of the trabecular meshwork [9] and keratoconus detection [10]. Pircher et al. [11] provide an in-depth review on a variety of different applications of PS-OCT to image the human eye. However, to date no study has shown the applicability of this technology to characterize the changes in the cornea after chemical CXL.

In this study we aim to assess the use of PS-OCT technology to image and discriminate the changes produced by the CXL on the porcine cornea *ex vivo*. A well-documented drug (glutaraldehyde) is used to stimulate crosslinking [12], in a set of freshly enucleated porcine eyes. We were particularly interested in observing the changes in the polarization information, while also imaging the cornea with standard OCT intensity images to allow later comparison. We expected that the changes produced by glutaraldehyde CXL may have an influence on the organization/orientation of the collagen fibres and therefore on the birefringence obtained from PS-OCT. We aimed to identify whether PS-OCT imaging shows significant differences when comparing baseline measurements with measurements taken during and after the crosslinking treatment.

2. Method

Four porcine eyes were equally divided into two groups, a control group (n=2) and a CXL group (n=2). The whole eyes were fully immersed in a container with the convex cornea facing upwards. The control group was immersed in phosphate buffered saline solution (PBS) to avoid dehydration, while the CXL group was immersed in PBS with 4% glutaraldehyde to stimulate chemical CXL. PS-OCT data of the cornea were acquired with a custom-built PS-OCT prototype [13], just after immersion (baseline), and every 10 minutes for a total period of 30-minutes (total soaking time). The PS-OCT system is based on a swept-source OCT (center wavelength = 1.3 µm, bandwidth = 110 nm), which provides an axial resolution of 9.2 µm in air.

For this study the phase retardation was obtained, and this measurement is the accumulated phase retardation in depth as the light passes through birefringent tissues. As previously reported [14], the phase retardation measurements can be drastically affected by the signal to noise ratio (SNR), which can decrease the reliability of the technique to quantify changes. This systematic error is especially significant at low SNR, which is typical in corneal measurement. In this study, a Monte-Carlo-Based (MCB) phase retardation estimator [15] was used to

reduce the systematic error in the measurement and to relax the harsh SNR requirements for accurate phase retardation measurement in the cornea. Additionally, data points with a SNR below 10dB were removed from further analysis.

The PS-OCT instrument acquires volumetric scans at the center of the cornea (4 x 4 mm^2; 512 A-scans and 128 B-scans). For each B-scan, we have two components; the intensity and phase retardation. Using the intensity image, the front (epithelial) and back (endothelial) surfaces of the cornea were automatically segmented. These surface shapes were used as a reference to extract an *en face* maps of MCB phase retardation at different corneal depths. During the experiment, the instrument and sample (immersed eyes) were not moved, to avoid potential artefacts in the results.

3. Results and discussion

The mean *en face* corneal profiles in depth for both the intensity and MCB phase retardation are shown in Figure 1. Representative data are presented at the four elapsed measurement times (0 min, 10 min, 20 min and 30 min) while the samples were immersed in the control PBS (left-column, A & C) and glutaraldehyde CXL (right-column, B & D) solutions. The top row presents the intensity values while the bottom row presents the MCB phase retardation values. Each plot shows the depth profile on the x-axis from the epithelium side (left) to the endothelium side (right). A line at about 90 µm depth indicates the transition zone between the epithelial and stromal layers.

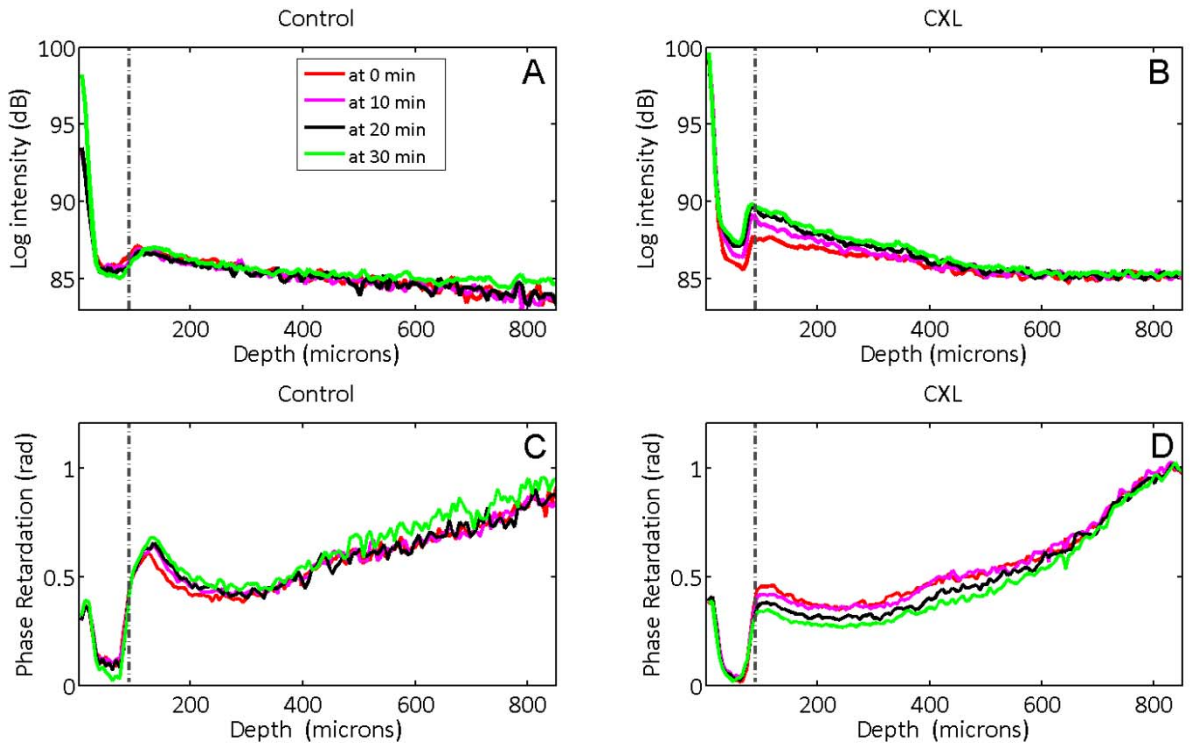

Figure 1. Mean values in depth for the intensity (A,B) and phase retardation (C,D) values. The plot represent two representative samples, a control (A,C) and a CXL (B,D). Corneal epithelium (outer surface) is on the left and endothelium (inner surface) on the right side of the plots.

From the intensity plots, it is clear that while the control PBS sample shows no intensity change, the glutaraldehyde CXL treated sample shows an increase in the intensity component. This opacity change in the CXL sample was also observed in the corneal histological slices as a yellowing, and it is consistent with the effect of the chemical agent. Regarding the phase retardation values, no change was observed in the control samples while the CXL samples present a decrease in the phase retardation in the stroma, with the effect most noticeable close to the epithelium (left side of the plot).

Focusing on the CXL samples, we looked in detail at the different layers of the cornea and made some observations of interest. In the stromal region (central thickest layer of the cornea) the intensity presents clear changes up to 400 μm depth, while the phase retardation shows the greatest changes in the deeper layers up to 600 μm. Thus, there seems to be a disparity between the effects of the CXL on these two measurements. The epithelium region (outer-most layer of the cornea) presents an increased intensity. However, the phase retardation in this region did not change. Thus, the epithelium does not seem to be affected by CXL treatment, and this is potentially due to the different physiological structure of this layer, which does not have a collagen matrix. Hence, CXL may not affect the polarization properties of the epithelial layer.

The intensity change (increase) induced by the CXL procedure can potentially create an artefact in the phase retardation results, since the SNR is linked to the intensity and as mentioned before, it also affects the phase retardation quantification [14]. However, taking into account the threshold on the SNR and the use of the MCB phase retardation estimator, it is unlikely that the CXL effect observed in this study is an artefact for two reasons: (i) the magnitudes of changes observed in this study are higher than the potential artefact that the intensity/SNR could generate, as reported in [15], and (ii) the intensity change did not produce a phase retardation change at the epithelium layer, which is not likely to have polarization properties changes during the CXL procedure, due to its different tissue properties. Thus, we believe that the CXL effect can be assessed with PS-OCT.

To further assess the CXL effect on samples, Figure 2 shows the intensity versus the phase retardation in depth, where the depth information has been colour coded. For the CXL samples, it is easy to appreciate the change in the phase retardation and intensity, which is especially significant in the region closest to the epithelium (i.e. blue colours on the Figure 2).

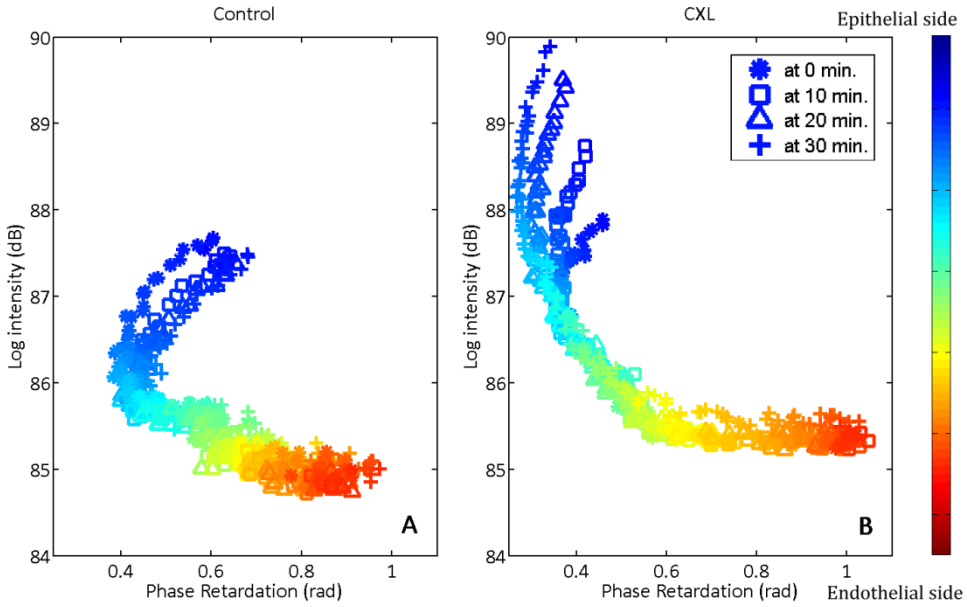

Figure 2. Phase retardation versus intensity across the stomal region. The colour codes the depth information. (A) Control and (B) CXL samples.

4. Conclusion

We have demonstrated the potential of PS-OCT to detect the changes in the cornea after chemical CXL produced by soaking the sample in glutaraldehyde. The PS-OCT phase retardation showed a significant change at the outer section of the corneal stroma (epithelium side) in the intensity and phase retardation values. However, the intensity may not be the best (or unique) indicator to discriminate CXL changes after the treatment. Unlike previous studies, where the intensity change was observed weeks after the procedure [6, 7], an immediate increase of the intensity was recorded in this study. However, the different CXL procedure used in the studies (glutaraldehyde vs. UVA+riboflavin) might be responsible for the different outcomes.

Despite the resolution of the PS-OCT system being lower that the micro-collagen structure, the phase retardation seems to provide an overall measure of the effect of the CXL. Based on the preliminary results on a keratoconic cornea [13] and the results presented in this study, PS-OCT may be a promising technique to measure collagen changes in the cornea.

In summary, PS-OCT seems to be a useful *in situ* imaging technique to monitor the change in the cornea associated with CXL. Further work with a larger sample size is needed to better understand the changes in phase retardation of the cornea under the influence of CXL.

References

[1]. G. Wollensak, "Crosslinking treatment of progressive keratoconus: new hope," *Current Opinion in Ophthalmology* 17(4), 356 (2006)

[2]. C. B. Raub, A. J. Putnam, B. J. Tromberg and S. C. George, "Predicting bulk mechanical properties of cellularized collagen gels using multiphoton microscopy," *Acta Biomaterialia* 6(12), 4657-4665 (2010)

[3]. C. B. Raub, V. Suresh, T. Krasieva, J. Lyubovitsky, J. D. Mih, A. J. Putnam, B. J. Tromberg and S. C. George, "Noninvasive assessment of collagen gel microstructure and mechanics using multiphoton microscopy," *Biophysical Journal* 92(6), 2212-2222 (2007)

[4]. J. M. Bueno, E. J. Gualda, A. Giakoumaki, P. Perez-Merino, S. Marcos and P. Artal, "Multiphoton microscopy of ex vivo corneas after collagen cross-linking," *Investigative Ophthalmology & Visual Science* 52(8), 5325-5331 (2011)

[5]. G. Scarcelli, R. Pineda and S. H. Yun, "Brillouin optical microscopy for corneal biomechanics," *Investigative Ophthalmology & Visual Science* 53(1), 185-190 (2011)

[6]. M. Doors, N. G. Tahzib, F. A. Eggink, T. T. J. M. Berendschot, C. A. B. Webers and R. M. M. A. Nuijts, "Use of anterior segment optical coherence tomography to study corneal changes after collagen cross-linking," *American Journal of Ophthalmology* 148(6), 844-851. e842 (2009)

[7]. T. Seiler and F. Hafezi, "Corneal cross-linking-induced stromal demarcation line," *Cornea* 25(9), 1057-1059 (2006)

[8]. A. Miyazawa, M. Yamanari, S. Makita, M. Miura, K. Kawana, K. Iwaya, H. Goto and Y. Yasuno, "Tissue discrimination in anterior eye using three optical parameters obtained by polarization sensitive optical coherence tomography," *Optics Express* 17(20), 17426-17440 (2009)

[9]. Y. Yasuno, M. Yamanari, K. Kawana, M. Miura, S. Fukuda, S. Makita, S. Sakai and T. Oshika, "Visibility of trabecular meshwork by standard and polarization-sensitive optical coherence tomography," *Journal of Biomedical Optics* 15(6), 061705 (2010)

[10]. E. Götzinger, M. Pircher, I. Dejaco-Ruhswurm, S. Kaminski, C. Skorpik and C. K. Hitzenberger, "Imaging of birefringent properties of keratoconus corneas by polarization-sensitive optical coherence tomography," *Investigative Ophthalmology & Visual Science* 48(8), 3551-3558 (2007)

[11]. M. Pircher, C. K. Hitzenberger and U. Schmidt-Erfurth, "Polarization sensitive optical coherence tomography in the human eye," *Progress in Retinal and Eye Research* 30(6), 431-451 (2011)

[12]. G. Wollensak and E. Spoerl, "Collagen crosslinking of human and porcine sclera," *Journal of Cataract & Refractive Surgery* 30(3), 689-695 (2004)

[13]. Y. Lim, M. Yamanari, S. Fukuda, Y. Kaji, T. Kiuchi, M. Miura, T. Oshika and Y. Yasuno, "Birefringence measurement of cornea and anterior segment by office-based polarization-sensitive optical coherence tomography," *Biomedical optics express* 2(8), 2392-2402 (2011)

[14]. S. Makita, M. Yamanari and Y. Yasuno, "Generalized Jones matrix optical coherence tomography: performance and local birefringence imaging," *Optics express* 18(2), 854-876 (2010)

[15]. L. Duan, S. Makita, M. Yamanari, Y. Lim and Y. Yasuno, "Monte-Carlo-based phase retardation estimator for polarization sensitive optical coherence tomography," *Optics Express* 19(17), 16330-16345 (2011)