2E2-2

High-frequency Quantitative Ultrasound-based Assessment of Microstructural Change in Myopic Guinea Pig Sclera

高周波超音波を用いた近視モデルモルモット強膜の構造評価

Kazuyo Ito[1†], Jonathan Mamou[2], Kazuki Tamura[3], Cameron Hoerig[2], Sally A. McFadden[4], and Quan V. Hoang[1,5,6] ([1]Singapore Eye Research Institute, Singapore National Eye Centre, Duke-NUS Medical School; [2]Riverside Research; [3]Hamamatsu Univ. School of Medicine; [4]The University of Newcastle, Australia; [5]National Univ. of Singapore; [6]Columbia Univ. Irving Medical Center)

1. Background

Myopia, also known as near-sightedness is one of the common eye disorders. While myopia is not often a significant cause for concern when mild, eyes with high myopia (HM, defined as more than -6.0 diopters (D) of near sightedness) can progress to pathologic myopia, with up to 70% of patients threatened with blindness or visual impairment.

As myopia progresses, posterior eye elongation is caused by changes in the microstructural properties of the sclera including decreased collagen and fibril diameters. High-frequency quantitative ultrasound (QUS) allows non-invasive measures of biomechanical parameters associated with changes in tissue microstructure. This study investigated microstructural changes occurring in the posterior sclera (PS) in myopic guinea pig (GP) eyes by means of QUS with a 20-µm spatial resolution.

2. Materials and methods

2.1. Animals

Form-deprivation myopia (FDM) was induced in young GPs by diffusers worn over the right eye from 6 days of age for 1, 2 or 3 weeks (n = 5, 9 and 4 animals respectively). Untreated paired left eyes were used as controls. On the last day of treatment, cycloplegic spherical equivalent refractive error (SERE) and axial length (AxL) were measured. Within minutes after the eye enucleation, the eye was flash-frozen and stored at -80C until thawed immediately prior to ultrasound measurement. All procedures were approved under Australian animal ethics legislative requirements and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Procedure

Fig. 1 illustrates the configuration of the ex-vivo ultrasound measurement. The eye was immersed in PBS with posterior pole facing up to minimize ultrasound attenuation. To mimic *in-vivo* conditions, the eye was suspended with partial thickness corneal sutures to orient the eye with near zero-tension. Three-dimensional radio-frequency (RF) data was acquired with an 80-MHz single element transducer. The transducer had a focal length of 2.2 mm, an F-number of 2, and a -6-dB bandwidth extending from 41 to 109 MHz.

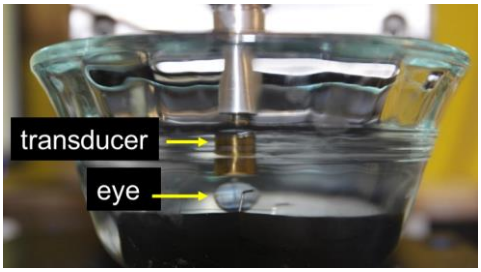


Fig. 1 Configuration of the measurement.

2.3. QUS analysis and post-processing

Two independent approaches were used[1]: an envelope-statistic related QUS estimates with Homodyned-K (HK) model (k)[2], and backscatter spectral quantification with a spherical Gaussian scattering model. Three parameters were generated being the effective scatterer size (ESS), effective acoustic concentration (EAC)[3] and k. ESS and EAC can be related to collagen fibril diameter and scattering power respectively. k represents the ratio of coherent to the diffused signal and is associated with the orientation of the collagen fibrils. Prior to the QUS parameter computation, the sclera was automatically segmented in three dimensions to crop out the optic nerve head and any defocused tissue. Two-dimensional QUS parameter map in bird's eye view was then reconstructed as shown in **Fig 2**. This 2D QUS map was divided into quadrants; inferior (INF), superior (SUP), nasal (NAS), and temporal (TMP). Finally, inter-ocular differences (IOD) were calculated within each animal and the average IOD and standard deviation was calculated for each quadrant. Linear regression analysis were performed to evaluate correlations between QUS estimates and biometric measures. To examine the differences

between the groups, statistical tests were also performed (Kruskal-Wallis test for the examination among groups, two-sided Wilcoxon rank sum test for the examination between two groups).

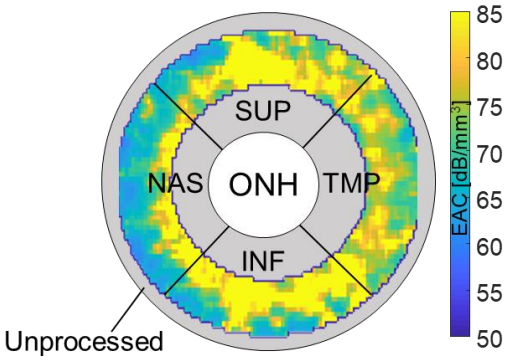


Fig. 2 Color-coded two dimensional map of effective acoustic concentration (EAC). The gray colored area was unprocessed because of defocus or non-scleral area.

3. Results and Discussion

3.1. Correlation with the biometric measures

Within the nasal (NAS) region, which contains the central axis and posterior pole, SERE was positively correlated with the PS ESS ($R^2 = 0.45$, $p = 0.04$), and negatively correlated with EAC ($R^2 = -0.58$, $p = 0.006$). Hence this result suggested that QUS parameters relate to structural changes secondary to eye elongation.

3.2. Regional differences with FDM duration

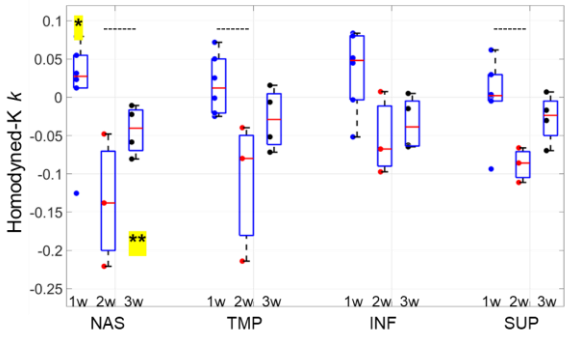
Fig. 3 illustrates the regional differences with different FDM durations. Changes in HK *k* varied with durations of induced myopia (**Fig. 3**(a)). ESS tended to decrease with the FDM duration and was significantly lower in the INF region after three weeks of FDM (**Fig. 3** (b)). To note, ESS in myopic eye and control eyes were significantly different after three weeks of FDM in all quadrants.

Several studies have reported that in myopia, there is decreased collagen fibril diameters and greater fiber bundle dissociation, which likely relates to a greater variation in fiber orientation and diameters[4]. The fibril diameter reduction increases with myopia progression[4] consistent with our findings that the ESS decreased with longer durations of myopia inducement, implicating the development of smaller collagen fibril diameters over time.

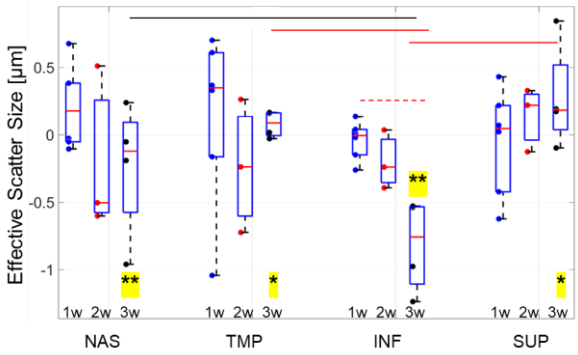
4. Conclusion

QUS has the potential to quantitatively characterize microstructural changes that occur in the posterior sclera during myopia development and progression.

Contacts: Kazuyo Ito (ito.kazuyo@seri.com.sg)
Quan V. Hoang (donny.hoang@singhealth.com.sg)



(a)



(b)

Fig. 3 The regional differences with varying durations of induced myopia. Inter-ocular differences (RE-LE) with four quadrants are shown with either 1, 2 or 3 weeks of induced myopia for: (a) Homodyned-K (HK) *k* parameter, and (b) Effective scatter size (ESS). Black horizontal bars indicate a difference between the two groups at a p value of 0.05 and red bars indicate a difference at $p < 0.01$. Solid horizontal bars represent regional differences, and dashed lines represent differences between the treatment duration. Asterisks within a given boxplot indicates a significant difference between myopic and control eyes for that given time point.

Acknowledgments

This work was supported in part by the National Institutes of Health (EB028084 (JM) and EY023595 (QVH); G1401348 (SAM)), Hunter Medical Research Institute (G1400967 (SAM)), and the Singapore National Medical Research Council (CSIRG/MOH-000531/2021 (QVH)).

References

1. J. Mamou *et al.*: Ultrason. Med. Biol. **37** (2011) 345-357.
2. F. Destrempes *et al.*: SIAM J. Imaging Sci. **6** (2013) 1499-1530.
3. M.L. Oelze et al.: J. Acoust. Soc. Am. **112** (2002) 1202-1211.
4. N.A. Mcbrien *et al.*: Invest. Ophthalmol. Vis. Sci. **42** (2001).