© Journal for Modeling in Ophthalmology 2018; 2:14-17
ESB-ITA 2017: Extended abstract

Nanostructural and mechanical changes in the sclera following proteoglycan depletion

Zhuola[1], Steve Barrett[2], Yalda Ashraf Kharaz[3], Eithne Comerford[3], Riaz Akhtar[1]

[1]Department of Mechanical Materials and Aerospace Engineering, University of Liverpool, Liverpool, UK; [2]Department of Physics, University of Liverpool, Liverpool, UK; [3]Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK

Abstract

The mechanical properties of ocular tissues, such as the sclera, have a major impact on healthy eye function, and are governed by the properties and composition of the microstructural components. For example, biomechanical degradation associated with myopia occurs alongside a reduction of proteoglycans (PGs). In this study, the role of PG degradation in the nanomechanical properties of the porcine sclera is explored. *In-vitro* enzymatic degradation of PGs was conducted with α-amylase and chondroitinase ABC enzymes. Collagen fibril morphology and nanomechanical stiffness were measured with atomic force microscopy (AFM). The elastic modulus of the tissue was reduced in all enzyme-treated samples relative to controls. In addition, collagen fibril organization was disrupted by PG depletion. Our data demonstrate that PGs play an important role in determining not only the mechanical properties at these length scales, but also collagen fibril arrangement.

Keywords: collagen structure, proteoglycan depletion, scleral mechanical properties

1. Introduction

The sclera is the dense outer coating of the eye, which provides the structural framework that defines the shape of the eye. It is mainly composed of collagen, elastin, and interfibrillar PGs. There is substantial evidence that profound bio-

Correspondence: Zhoula, Department of Mechanical Materials and Aerospace Engineering, University of Liverpool, Brownlow Hill, Liverpool L69 3GH, UK.
E-mail: zhuola@liverpool.ac.uk

mechanical changes occur in the sclera with conditions such as myopia, which is characterized by scleral weakening. Alongside biomechanical changes, a reduction of collagen fibril diameter and PG content have been reported in myopic eyes. However, few studies to date have determined how PG content in the sclera affects its mechanical properties and nanostructure. In this study, *in-vitro* degradation of PGs in the porcine sclera was conducted in order to determine how this affects nanoscale changes in its structure and mechanical properties.

2. Materials and methods

Porcine eyes were obtained from a local abbatoir (n = 5). The sclerae were dissected and cryosectioned for AFM testing following the methods and experimental procedure described previously.[1] The cryosectioned samples were treated with the following solutions: 2mg/ml α-amylase in phosphate buffered saline (PBS), 2mg/ml α-amylase in ultra-clean distilled water, chondroitinase ABC buffer, 100% ultra clean distilled water (control group), and 100% PBS (control group). The nanotopography and elastic modulus were measured before and after one-hour treatment at the same locations with AFM using the Peakforce QNM method.[1] All tests were conducted in liquid.

As shown in Figure 1, chondroitinase ABC depletes two types of major sulphated PGs in the sclera:[1] chondroitin sulfate PGss and dermatan sulfate PGs.[2] Sulphated glycosaminoglycan (sGAG, a major component of PGs) content was analyzed with the dimethylmethylene blue (DMMB) assay before and after α-amylase treatment.

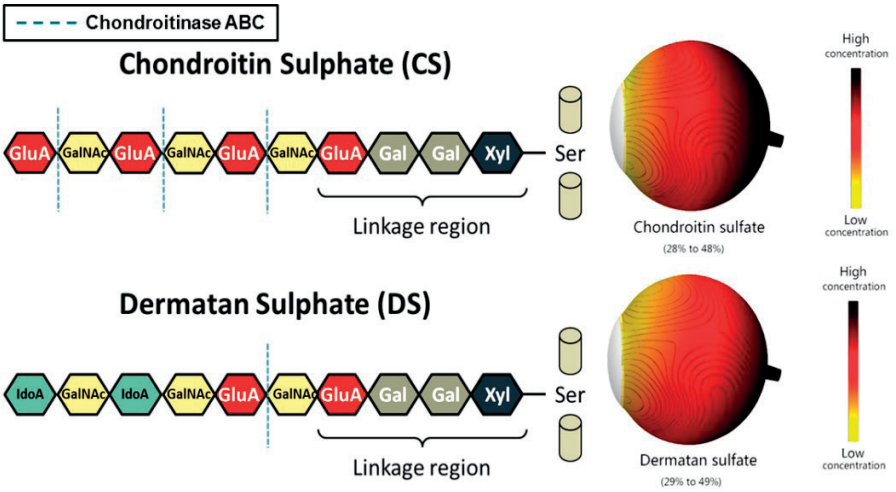

Fig. 1. PG depletion by using chondroitinase ABC.

Although α-amylase is one of the major enzymes found in tear fluid, few studies have shown which PGs in the eye are affected by α-amylase.

Nanomechanical properties were determined off-line by using NanoScope Analysis 1.7 (Bruker Nano Inc.; Nano Surfaces Division, Santa Barbara, CA, USA). AFM images from the height channel (topography images) were analyzed using Image SXM 1.99 (Steve Barrett, Image J; http://www.liv.ac.uk/~sdb/ImageSXM/) and Matlab 2013a (The MathWorks; Natick, MA, USA) for measuring scleral collagen fibril structure and distribution.

3. Results

DMMB assays indicated that sGAG content was reduced after α-amylase treatment in all three regions (8.9% to 22.6%). Collagen fibril diameter was significantly reduced in all groups incubated with enzyme solutions, and remained unchanged in control groups. Collagen fibril D-periodicity remained unchanged in all groups after incubation. The gap zone depth increased significantly in all groups after incubation with enzymes, decreasing after 100% PBS incubation. As shown in Figure 2, the elastic modulus decreased in all groups after incubation with enzyme solutions and significantly increased after incubation with 100% PBS buffer.

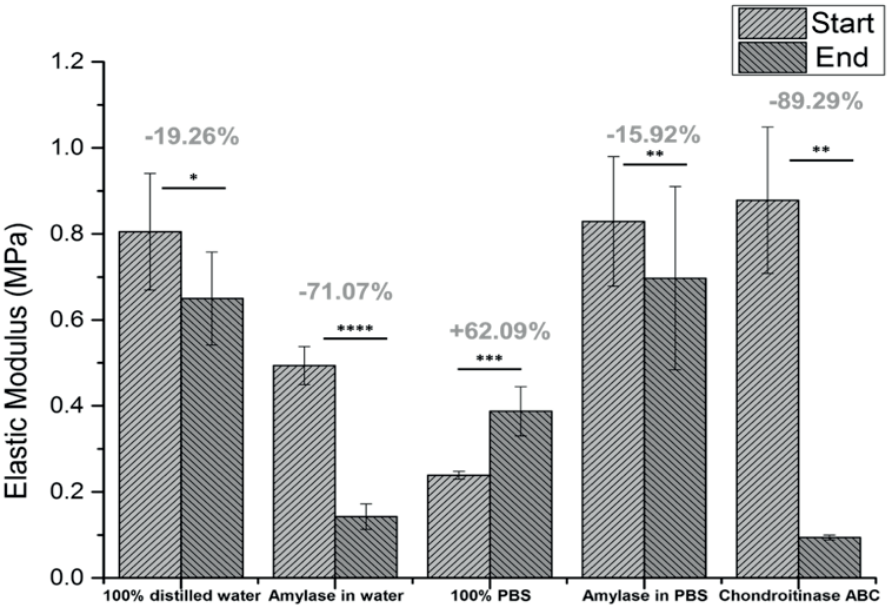

Fig. 2. Results of mechanical properties measured with AFM Peakforce QNM.

4. Conclusion

These data demonstrate that collagen fibril mechanical properties are substantially altered by salt concentration and that PGs play an important role in determining the nanostiffness and structure of the sclera. Collagen D-periodicity remained unchanged in all groups after treatment, indicating that PG depletion does not degrade collagen fibrils. Elastic properties were reduced in samples incubated with amylase solutions, decreasing even further in those incubated with chondroitinase solution. This result indicated that PG degradation will cause a reduction in the elastic modulus of collagen fibrils.

References

1. Papi M, Paoletti P, Geraghty B, Akhtar R. Nanoscale characterization of the biomechanical properties of collagen fibrils in the sclera. Appl Phys Lett. 2014;104(10):103703.
2. Murienne BJ, Chen ML, Quigley HA, Nguyen TD. The contribution of glycosaminoglycans to the mechanical behaviour of the posterior human sclera. J R Soc Interface. 2016;13(119):20160367.
3. Trier K, Olsen EB, Ammitzbøll T. Regional glycosaminoglycans composition of the human sclera. Acta Ophthalmol. 1990;68(3):304-306.