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Ultrastructural and nanomechanical changes of the cornea following enzymatic degradation

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Abstract

Understanding of the ultrastructure and nanomechanical behavior of the cornea is important for a number of ocular disorders. In this study, atomic force microscopy (AFM) was used to determine nanoscale changes in the porcine cornea following enzymatic degradation. Different concentrations of amylase were used to degrade the cornea. A reduction in elastic modulus at the nanoscale, along with disrupted collagen morphology, was observed following enzymatic treatment. This study highlights the interplay between mechanical properties and collagen organization in the healthy cornea.

Keywords: amylase, corneal biomechanics, keratoconus

1. Introduction

There is a need to explore and develop a better understanding of corneal biomechanics at the nanolevel. Collagen fibrils are the main load-bearing components of the cornea, which are embedded in extracellular matrix, that is mainly formed of proteoglycans.[1] The structural organization and mechanical properties of collagen fibrils and proteoglycans play an important role in exhibiting normal corneal

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curvature.[2] With certain diseases, such as keratoconus, these properties are compromised.[3] This study aims to use *in-vitro* enzymatic degradation to understand how the ultrastructural and nanomechanical changes in collagen fibril networks and extracellular matrix may affect corneal properties.

2. Materials and methods

Sixteen porcine corneas obtained from a local abattoir. They were grouped into a control group (8 corneas) and amylase-treated group (8 corneas). Corneal samples were chosen from the apex after desquamating the epithelial layer. A previous study has suggested that tissue samples for AFM should be 5 µm.[4] Samples from the anterior one-third of the stroma were chosen for this study. For the control group, each cornea was cryosectioned to produce six slices (5 µm thick): three of these slices were washed with distilled water (DW), and the other three slices were washed with phosphate buffer saline solution (PBS). For the amylase test group, each cornea was cryosectioned to produce 24 slices (5 µm thick). The samples from each cornea were then sub-divided into two subgroups: 16 slices were treated with amylase dissolved in DW and the other 16 slices were treated with amylase dissolved in PBS. The slices of each subgroup underwent enzymatic degradation (n = 2 slices/subgroup) at the following concentrations: 0.2, 0.4, 0.8, 1, 1.2, 2 mg/ml amylase. Each slice was incubated for 40 min.

Stromal samples were tested by using Bruker Multimode-8 AFM (Bruker; Santa Barbara, CA, USA); a schematic of the AFM is shown in Figure 1. The AFM method and instrument used have been described previously by Papi *et al.*[5] The elastic modulus and topographical properties were determined by using Peak Force Quantitative

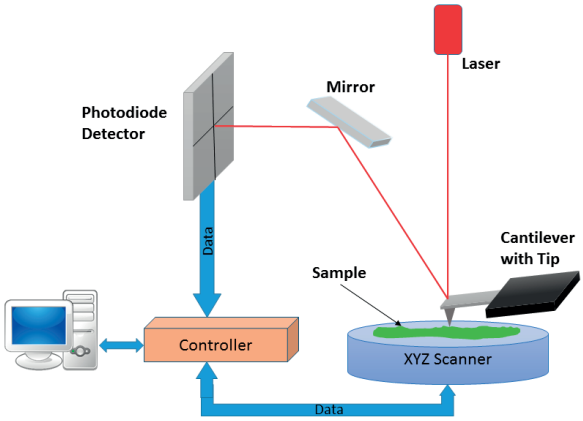

Fig. 1. Schematic of AFM.[5]

Nanomechanical Mapping (PF-QNM) mode in air. This AFM has been well-described in the literature.[6] The Derjaguin-Muller-Toporov (DMT) model was used to calculate elastic modulus. This model uses the loading force plus the adhesive force between the tip and the surface of the sample.[7] Aluminum-coated silicon probes (Bruker RTESPA-300) were used for all measurements. The AFM was calibrated to measure the tip radius and the spring constant of the cantilever by using a Vishay Photostress PS1 Polymer (Vishay; Wendell, NC, USA) reference sample with an elastic modulus of 2.7 GPa. The Poisson's ratio of the corneas was assumed to be 0.5. AFM images were analyzed and processed by using Bruker NanoScope Analysis Software, version 1.70. The temperature and humidity of the testing room were recorded as approximately 22.4 C° and 41%, respectively. All chemicals and reagents were obtained from Sigma-Aldrich (Dorset, UK).

3. Results and discussion

3.1. Topography

Topographical images revealed distinct collagen fibrils (type I) in the stroma when the samples were washed with either DW/PBS or treated by amylase (Fig. 2). The collagen fibrils had diameters ranging from 32–50 nm. The axial D-periodicity of collagen fibrils ranged from 64–70 nm.

Collagen fibril diameter was slightly higher in samples washed with DW as compared to PBS-washed. Deterioration in the collagen fibrils in samples treated with amylase was seen in some points (arrow in the zoomed image in Fig. 2). In addition, there was clear deterioration in the extracellular matrix.

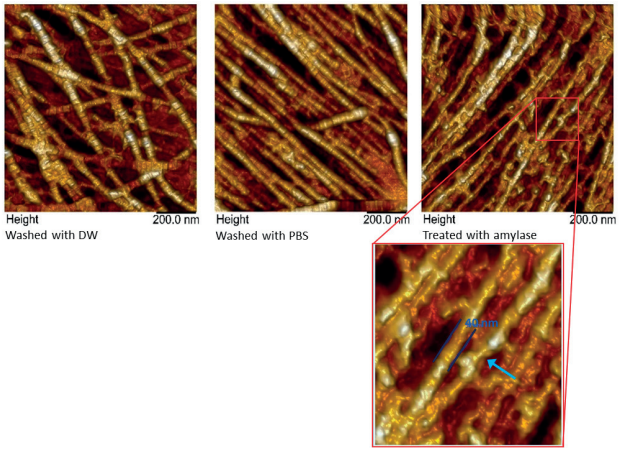

Fig. 2. Topographical images of corneal samples showing collagen fibrils.

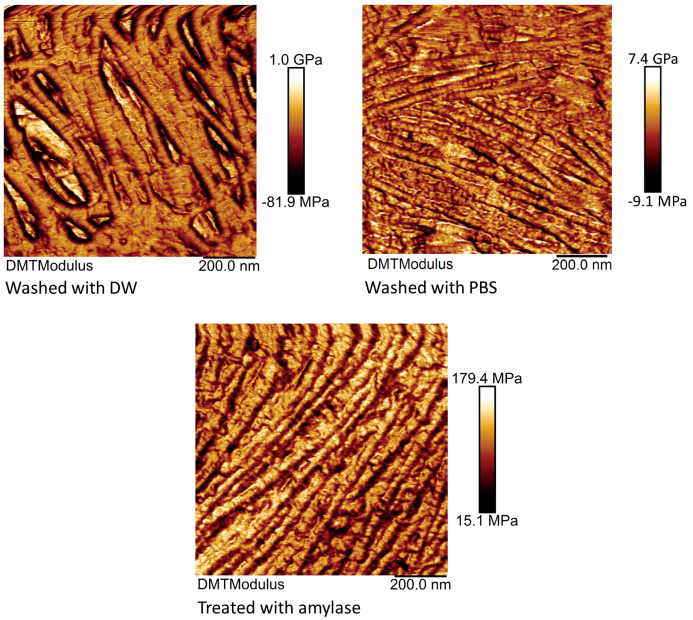

Fig. 3. Elastic modulus images of corneal samples

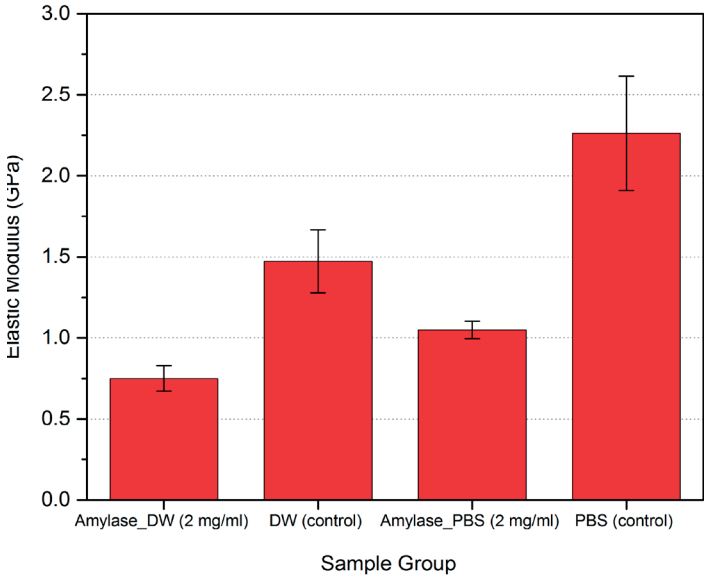

Fig. 4. Mean elastic modulus values of control group and amylase dissolved in DW (2 mg/ml) treated group.

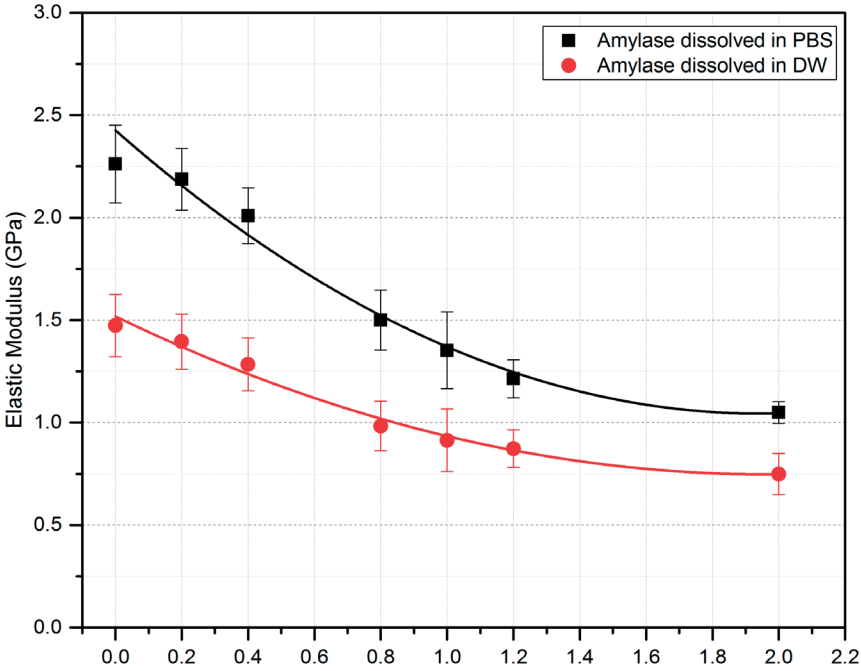

Fig. 5. Mean values of elastic modulus of corneal samples treated with varying concentrations of amylase.

3.2. Mechanical properties

Elastic modulus images of corneal samples in different solutions are shown in Figure 3. The elastic modulus was reduced by 37% for the samples washed by DW as compared to samples washed by PBS. The greatest reduction was seen following amylase (2 mg/ml) treatment. There was a 68% reduction in the elastic modulus as compared to samples washed by PBS, and a 49% reduction as compared with samples washed by DW (Fig. 4).

It was noticed that elastic modulus values decreased as the amylase concentration increased. The mean values of elastic modulus of corneal samples treated with varying amylase concentrations are shown in Figure 5. In both curves, the reduction seems to reach a steady state of proteoglycan digestion at higher amylase concentrations.

4. Conclusion

Amylasetreatment dramatically altered the collagen fibril structure and appeared to affect the surrounding proteoglycans, leading to deterioration of the mechanical

properties of the cornea. This enzymatic degradation method may serve as a model for understanding keratoconus.

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