**Nano-Structure and Mechanical changes in Sclera following Proteoglycan Depletion**

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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 proteoglycans (PGs). There is substantial evidence that profound biomechanical changes occur in the sclera with conditions such as myopia, which is characterised by scleral weakening. Alongside biomechanical changes, a reduction of collagen fibril diameter and content of PGs have been reported in myopic eyes. However, to date few studies have determined how PG content in the sclera affects its mechanical properties and nano-structure. In this study, in vitro degradation of PGs in the porcine sclera was conducted in order to determine how this affects nano-scale changes in its structure and mechanical properties.

Materials & Methods: Porcine scleras (n = 6 eyes) were treated with α-amylase solutions (2mg/ml α-amylase in PBS and in ultra clean distilled water) and control buffer 100% ultra clean distilled water and 100% PBS. The nanotopography and elastic modulus were measured before and after treatment with atomic force microscopy (AFM) using the Peakforce QNM method in both ambient conditions and in fluid. Sulphated glycosaminoglycans (sGAG) (a major component of PGs) content was analysed with the dimethylmethylene blue (DMMB) assay before and after treatment.

Results and Discussion: DMMB assays indicated that sGAG content was reduced after α-amylase treatment (18.1%). Collagen fibrils diameter significantly reduced (p<0.0001) in both groups incubated with amylase solutions and unchanged in other two groups. Collagen fibrils D-periodicity remain unchanged in all groups after incubation. Collagen fibrils Gap zone depth increased significantly (p<0.001) in both groups after incubation with amylase and decreased after 100%PBS incubation (p<0.0001). The elastic modulus decreased (p<0.05) in both groups after incubation with amylase solutions and significantly increased after incubation with 100 % PBS buffer (p<0.001). These data demonstrate that collagen fibril mechanical properties are substantially altered by salt concentration and that proteoglycans plays an important role in determining the nano- stiffness and structure of sclera. Although α-amylase only partially depletes PGs in the sclera, the amylase treatment had a significant effect on the mechanical properties and collagen structure after PG depletion.