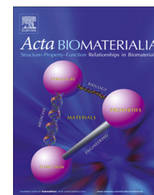
Acta Biomaterialia 12 (2015) 195–206

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat



The effects of glycosaminoglycan degradation on the mechanical behavior of the posterior porcine sclera

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ARTICLE INFO

Article history:
Received 30 May 2014
Received in revised form 28 August 2014
Accepted 23 October 2014
Available online 4 November 2014

Keywords:
Sclera
Glaucoma
Myopia
Chondroitinase ABC
Inflation

ABSTRACT

Pathological changes in *scleral* glycosaminoglycan (GAG) content and in scleral mechanical properties have been observed in eyes with glaucoma and myopia. The purpose of this study is to investigate the effect of GAG removal on the scleral mechanical properties to better understand the impact of GAG content variations in the pathophysiology of glaucoma and myopia. We measured how the removal of sulphated GAG (s-GAG) affected the hydration, thickness and mechanical properties of the posterior sclera in enucleated eyes of 6–9 month-old pigs. Measurements were made in 4 regions centered on the optic nerve head (O_{NH}) and evaluated under 3 conditions: no treatment (control), after treatment in buffer solution alone, and after treatment in buffer containing chondroitinase ABC (ChABC) to remove s-GAGs. The specimens were mechanically tested by pressure-controlled inflation with full-field deformation mapping using digital image correlation (DIC). The mechanical outcomes described the tissue tensile and viscoelastic behavior. Treatment with buffer alone increased the hydration of the posterior sclera compared to controls, while s-GAG removal caused a further increase in hydration compared to buffer-treated scleras. Buffer-treatment significantly changed the scleral mechanical behavior compared to the control condition, in a manner *consistent* with an increase in hydration. Specifically, buffer-treatment led to an increase in low-pressure stiffness, hysteresis, and creep rate, and a decrease in high-pressure stiffness. ChABC-treatment on buffer-treated scleras had opposite mechanical effects than buffer-treatment on controls, leading to a decrease in low-pressure stiffness, hysteresis, and creep rate, and an increase in high-pressure stiffness and transition strain. Furthermore, s-GAG digestion dramatically reduced the differences in the mechanical behavior among the 4 quadrants surrounding the ONH as well as the differences between the circumferential and meridional responses compared to the buffer-treated condition. These findings demonstrate a significant effect of s-GAGs on both the stiffness and time-dependent behavior of the sclera. Alterations in s-GAG content may contribute to the altered creep and stiffness of the sclera of myopic and glaucoma eyes.

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1. Introduction

The sclera, the white outer shell of the eye, is responsible for maintaining the eye shape in the presence of intraocular pressure and protecting the internal ocular structures from external forces. It is the main load-bearing tissue of the eye and studies have suggested that alterations to its mechanical properties, specifically at the posterior pole, contribute to the development of glaucoma and myopia [1]. Glaucoma is the second leading cause of blindness worldwide [2] and glaucoma damage is considered to be dependent on the scleral mechanical properties [3–5]. Myopia is a common refractive error that is ubiquitous in certain populations [6] and the associated abnormal axial elongation of the globe involves dramatic changes in the scleral mechanical properties [7].

Changes in the extracellular matrix (ECM) structure of the eye wall have been reported with both glaucoma and myopia. These include variations in the GAG content. GAGs are polysaccharide chains of various lengths, the majority of which bind to core proteins to form proteoglycans (PGs). The human posterior sclera is particularly rich in chondroitin sulfate GAG side chains [8], from the abundant presence of aggrecan [9], and in dermatan sulfate chains [8]. An abnormal accumulation of GAGs, including chondroitin sulfates, was reported in some portions of the anterior

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http://dx.doi.org/10.1016/j.actbio.2014.10.033
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segment [10] and in the ONH [11] of glaucomatous human eyes. Similarly, the rat ONH [12] and monkey lamina cribrosa [13] showed a higher chondroitin sulfate content when subjected to IOP elevation. A decrease in GAG content was found in the posterior sclera of human myopic eyes [14] and tree-shrews with form-deprivation myopia [15]. A decrease in GAG synthesis was also reported in the posterior sclera of form-deprived myopic tree shrew [16] and monkey eyes [17].

Changes in the tensile and viscoelastic properties of the sclera also occur in glaucoma and myopia. The posterior sclera of glaucoma eyes showed an increase in stiffness [18–20] and a decrease in creep rate [20]. Myopic posterior scleras, in which there is a decrease in GAG content, had the opposite biomechanical changes in some parameters, with an increase in tissue extensibility [14,21,22] and creep rate [22,23] and no change in stiffness [21,23].

GAGs play an important role in the structure and mechanical behavior of collagenous ECM tissues. GAGs fill the space between the collagen fibrils and the collagen–elastin network in the ECM. Although the core protein of some PGs is found at regular intervals on collagen fibrils [24–26], it seems that PG interactions with collagen not only occur through binding of the core protein [27], but also through electrostatic interaction [28] of the GAGs themselves as shown *in vitro* [28–31]. The majority of GAGs attract water and are highly polar, due to their negatively charged carboxyl and sulphated groups. Their fixed charge density also creates attraction/repulsion forces among themselves and with collagen fibrils. As a result, the GAG content helps to determine the tissue hydration [32] by determining the number of polar sites for water binding and the space for free water uptake [33], as well as the tissue swelling pressure [34–36], which is highly dependent on hydration [37]. Hydration is believed to regulate the spacing between collagen fibrils in the ECM [38,39]. GAGs have been shown to regulate collagen fibrillogenesis *in vitro* under certain conditions, specifically affecting the rate of fibril formation [40–42] and fibril diameter [43,44].

The mechanical functions of GAGs have been studied in various tissues, with tissue-specific and sometimes conflicting results, by comparing their mechanical behavior before and after GAG removal. In cartilage, GAG removal produced a faster creep rate [45]. This led the authors of the study to speculate that GAGs may have a role in the damping function of cartilage by providing friction and viscous resistance to interfibrillar sliding. In aortic heart valve leaflets, where native creep is negligible, no change in the creep behavior was observed after GAG removal [46]. GAG removal does not change the hysteresis observed during cyclic loading in ligaments [47] nor at the high stress range in heart valve leaflets [46]. In contrast, it decreased the hysteresis in aortic valve leaflets in the low stress range [46]. A faster relaxation was observed after GAG removal in the aorta [48], but no changes in relaxation were observed in ligaments [49]. The role of GAGs in the tissue stiffness is still controversial. No change in the uniaxial loading stiffness was found after GAG removal in cartilage [45] and tendons [50]. However, an increase in stiffness occurred in mesenteric arteries [51] and lungs [52]. In the aorta, the small-strain stiffness was higher and the high-strain stiffness lower after GAG removal, and similar changes were measured for incubation in buffer with or without a GAG-targeting enzyme compared to controls [48]. Similarly, ligaments showed a higher small-strain stiffness after GAG depletion [53], whereas no change in stiffness was measured in another study [47].

None of the studies comparing the biochemical and biomechanical characteristics of normal and pathological sclera have addressed the mechanical function of GAGs. In this study, we investigated the effect of s-GAG removal on the mechanical behavior of the posterior sclera to infer the mechanical relevance of pathological changes in scleral s-GAG content.

2. Methods

This section describes the methods used to evaluate the mechanical behavior and structural characteristics of the porcine posterior sclera.

2.1. Experimental design and glycosaminoglycan removal

All mechanical and structural outcomes were measured in 4 quadrants of the posterior scleral cup surrounding the ONH (superior–temporal (ST), superior–nasal (SN), inferior–temporal (IT), inferior–nasal (IN)). Different specimens were used for either hydration measurement (n = 18) or s-GAG quantification (n = 18) and they were divided into 3 experimental groups. In the first group, the specimens were processed immediately after preparation (control) (n = 6). In the second group, they were incubated for 18 h at 37 °C in a modified Trizma buffer at pH 8.0 (buffer-treated) (n = 6). In the third group, they were incubated for 18 h in Trizma buffer containing ChABC (C2905, Sigma–Aldrich, St. Louis MO) at 2 units/ml (enzyme-treated) (n = 6).

Other specimens used for both mechanical testing and thickness measurement (n = 12) were divided into 2 groups only. In one group, the specimens were mechanically tested after dissection and again after incubation in buffer alone for 18 h (protocol 1: control/buffer-treated) (n = 5). In another group, the specimens were soaked in buffer alone for 18 h after dissection, mechanically tested, soaked in enzyme solution for 18 h, and tested again (protocol 2: buffer-treated/enzyme-treated) (n = 7). Thickness data for 3 additional control/buffer-treated specimens and 2 additional buffer-treated/enzyme-treated specimens that were not inflation tested were added for statistical analysis. After incubation, the samples were always rinsed for 30 min in fresh Trizma buffer without enzyme. Table 1 summarizes the number of specimens used in each experiment.

The control specimens represented a baseline hydrated state in that the interior surface of the specimens was exposed to Dulbecco's Phosphate Buffered Saline (DPBS) during the inflation test and the specimens were tested in a humidity chamber at greater than 90% humidity. Comparing controls to specimens soaked in buffer alone allowed the effect of an increase in hydration on the scleral mechanical properties to be measured. The comparison between the control (baseline hydrated) and buffer-treated (swollen) states allowed the effect of hydration to be distinguished from that of s-GAG degradation when the specimens were treated with buffer containing ChABC.

2.2. Specimen preparation

Eyes from 6–9 month-old pigs (Animal Technologies Inc., Tyler TX) were received on wet ice 24 h after enucleation and used upon arrival. The skin, extraocular fat and muscles were first carefully removed from all eyes using fine dissection scissors to obtain a clean scleral surface. The eyes used for either mechanical testing and thickness measurement or s-GAG quantification were mounted on a custom-made acrylic holder, using a cyanoacrylate-based glue (Permabond 910, Electron Microscopy Sciences, Hatfield PA). The specimens were positioned such that the posterior scleral cup protruded through the holder opening centered about the ONH (Fig. 1(a)). After the glue polymerized, a cut was made through the cornea using a scalpel and the intraocular structures including the retina and choroid were removed with fine curved dissecting forceps, leaving only the scleral shell. The remaining anterior sclera was then scored through its thickness and glued to the back side of the holder. The eyes used for hydration measurement were prepared using an 8 mm biopsy punch to

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