Acta Biomaterialia 9 (2013) 4653–4660

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

On the biomechanical role of glycosaminoglycans in the aortic heart valve leaflet

Chad E. Eckert [a], Rong Fan [b], Brandon Mikulis [a], Mathew Barron [a], Christopher A. Carruthers [a], Vincent M. Friebe [c], Naren R. Vyavahare [c], Michael S. Sacks [b,*]

[a] Department of Bioengineering, Swanson School of Engineering, University of Pittsburgh, Pittsburgh, PA, USA
[b] Department of Biomedical Engineering, Institute for Computational Engineering and Sciences, University of Texas at Austin, Austin, TX, USA
[c] Cardiovascular Implant Research Laboratory, Department of Bioengineering, Clemson University, Clemson, SC, USA

ARTICLE INFO

Article history:
Received 12 May 2012
Received in revised form 9 September 2012
Accepted 25 September 2012
Available online 2 October 2012

Keywords:
Glycosaminoglycan
Heart valve
Mechanical properties
Mechanical test

ABSTRACT

While the role of collagen and elastin fibrous components in heart valve valvular biomechanics has been extensively investigated, the biomechanical role of the glycosaminoglycan (GAG) gelatinous-like material phase remains unclear. In the present study, we investigated the biomechanical role of GAGs in porcine aortic valve (AV) leaflets under tension utilizing enzymatic removal. Tissue specimens were removed from the belly region of porcine AVs and subsequently treated with either an enzyme solution for GAG removal or a control (buffer with no enzyme) solution. A dual stress level test methodology was used to determine the effects at low and high (physiological) stress levels. In addition, planar biaxial tests were conducted both on-axis (i.e. aligned to the circumferential and radial axes) and at 45° off-axis to induce maximum shear, to explore the effects of augmented fiber rotations on the fiber–fiber interactions. Changes in hysteresis were used as the primary metric of GAG functional assessment. A simulation of the low-force experimental setup was also conducted to clarify the internal stress system and provide viscoelastic model parameters for this loading range. Results indicated that under planar tension the removal of GAGs had no measureable affect extensional mechanical properties (either on- or 45° off-axis), including peak stretch, hysteresis and creep. Interestingly, in the low-force range, hysteresis was markedly reduced, from 35.96 ± 2.65% in control group to 25.00 ± 1.64% ($p < 0.001$) as a result of GAG removal. Collectively, these results suggest that GAGs do not play a direct role in modulating the time-dependent tensile properties of valvular tissues. Rather, they appear to be strongly connected with fiber–fiber and fiber–matrix interactions at low force levels. Thus, we speculate that GAGs may be important in providing a damping mechanism to reduce leaflet flutter when the leaflet is not under high tensile stress.

1. Introduction

Despite a phenomenally wide range of physiological functions and variation in specific behaviors, soft connective (non-muscular) tissues typically share the same principle constituents: collagen, elastin and glycosaminoglycans (GAGs). The unique properties of each tissue are a result of its distinctive structure, which is determined by the constituents present and their physical arrangements. Types I and III collagen form undulating fibers that provide support under tension, whereas elastin, an aggregate fiber from microfibrils, provides the ability to undergo large extensions and provides elastic properties. In contrast to these fibrous components, GAGs are composed of long unbranched polysaccharides usually attached to a protein core to form proteoglycans, high negative charge of which makes GAGs hydrophilic.

The native aortic valve (AV), a collagenous thin tissue structure, is a remarkable example of the highly developed structure and function [1]. When open, the AV allows for unobstructed flow of blood out of the ventricle during systole; with flow deceleration, it closes via Bernoulli effects, and the leaflet provides complete closure to prevent valve regurgitation. This is all achieved through the unique and complex structure of the AV tissue, which consists of three layers (the fibrosa, spongiosa and ventricularis), constructed principally of collagen, elastin and GAGs [2–7]. The ventricularis layer faces the left ventricle and is composed primarily of collagen and elastin [2,4–9]. On the opposite face of the valve is the fibrosa layer. This layer faces the aorta and is composed predominantly of a dense network of Type I collagen fibers, which exhibit macroscopic crimp and are aligned primarily in the circumferential direction [2,4]. The highly hydrophilic GAGs largely make up the central spongiosa, resulting in a highly hydrated layer [10–12]. Interestingly, all heart

* Corresponding author. Postal address: W. A. "Tex" Moncrief, Jr. Simulation-Based Engineering Science Chair, Institute for Computational Engineering and Sciences (ICES), The University of Texas at Austin, 201 East 24th Street, ACES 5.438, 1 University Station, C0200, Austin, TX 78712-0027, USA. Tel.: +1 512 232 7773.
E-mail address: msacks@ices.utexas.edu (M.S. Sacks).

1742-7061/$ - see front matter © 2012 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2012.09.031

valves have similar microanatomical arrangements, in that the GAG-rich layer (spongiosa) is always in te center of the leaflet [13,14].

At the tissue level, all three layers within the AV tissue work in unison to produce the net leaflet mechanical behavior [13]. In diastole, the AV leaflets are loaded as a result of diastolic pressure loading, and are virtually inextensible once fully straightened under full tensile load [2,13,15]. During systole, the valve opens and the collagen fibers recoil. The elastin fibers within the ventricularis, which are radially aligned, act to assist in the recovery of radial distention [4–6,16] after full forward flow. In conjunction with aortic root distention [14], this allows for complete unobstructed blood flow. At the end of systole, the blood flow starts to decelerate and, with very little reverse flow, the valve closes due to Bernoulli's principle and the developing vortices in the sinuses behind the leaflets [13,17]. Once again, the collagen fibers are fully straightened, rendering the leaflet inextensible and providing a sufficient coaptation area (amount of leaflet overlap) to prevent valve prolapse [18].

Since GAGs are the primary component of the central spongiosa layer, it has been speculated that this layer serves to reduce the shearing resulting from the differential movement of the fibrosa and ventricularis during leaflet deformations [2,8,10,19–21]. Moreover, the AV biaxial tensile biomechanical response comes primarily from the collagenous fibrosa layer [22]. From these and other studies, we have hypothesized that, under high tensile loading, biomechanical behavior is dominated by collagen and elastin. This is all the more important as changes in behavior with various tissue pathologies that alter tissue composition (e.g. myxomatous valve disease) suggest that substantial changes may occur in the tissue behavior. GAGs that occur in tendon, a dense collagenous tissue similar in composition and structure to the fibrosa layer, have been shown to manifest themselves mechanically in the low-stress regions, where the constituent collagen fibers remain mostly in the undulating state [23–25]. Mechanistically, GAGs are hypothesized to contribute substantially to the time-dependent behavior of the bulk tissue at lower physiological force levels through mechanical interactions with the partially undulating collagen fibers achieved by direct bonding. This has been used to explain why the relative amount of stress relaxation is reduced at higher tissue stress levels, wherein the bulk tissue response is dominated by the fully loaded and straightened collagen fibers [26]. While some insight into collagen–GAG interactions has been achieved in the mitral valve chordae [27], it is not known whether such mechanisms occur in valvular tissues.

How the GAGs contribute to heart valve tissue behavior, especially as they relate to the observed valvular tissue quasi-elastic at high tensile loading [28–30], also remains unclear. Such insights are important not only for our understanding of native tissue function, but also to lay the basis of novel approaches to chemically modify biologically derived tissues for heart valve biomaterials. The present study was thus undertaken to clarify and quantify the mechanical contribution of GAGs to AV leaflet tensile behavior. We developed a dual stress-level experimental design to elucidate both a low-stress (strip–biaxial configuration) and a high-stress (planar biaxial) tensile mode under both quasi-static and creep configurations. This novel two-tier measurement method was utilized due to the substantial differences in force levels required to encompass the physiological ranges of extensional deformation known to occur in the native valve in vivo [13]. Finally, we simulated the low-stress experimental configuration to gain insight into the internal stress distribution in this deformation mode to better interpret the experimental results. To elucidate the biomechanical role of GAGS, we preformed these tests on pre- and post-GAG removal specimens using an enzymatic technique to remove the GAGs from the leaflet tissue.

2. Methods

2.1. Tissue harvest

Fresh porcine aortic valves from adult animals of both sexes were acquired at the local abattoir (Thoma's Meat Market, Saxonburg, PA). Valves were excised intact, along with the aortic root, on-site by cutting the aorta distal to the aortic sinuses and then cutting the left ventricle outflow. The valves were then transported back to laboratory in ice-chilled phosphate-buffered saline (PBS). Valves were rinsed again with ice-cold PBS, then the individual leaflets were cut away from the aortic root with no differentiation between the non-, right and left coronary leaflets. Valve leaflets were then divided into three groups: native, enzyme treated and control.

Leaflets in the native group were stored covered in ice-cold PBS and underwent mechanical testing within 24 h of harvesting. The leaflets from each valve in the enzyme-treated group were processed using a standard protocol [31], modified to utilize higher concentrations of enzymes for shorter periods of time in order to minimize total processing time. The specimens were placed in a conical tube with 2.4 ml of enzyme degradation solution per leaflet, containing 30 U ml^{-1} hyaluronidase and 0.6 U ml^{-1} chondroitinase (Sigma Aldrich #H3631 and #C3667) in 100 mM ammonium acetate buffer solution (AABS), with the pH adjusted to 7.0. The tubes were shaken continuously for 2 h at 37 °C. The enzyme degradation solution was then removed and the leaflets were washed with ice-cold PBS three times, for 5 min each time. The enzyme-treated specimens were then stored covered in ice-cold PBS and underwent mechanical testing within 48 h of harvesting. The leaflets from the each valve in the non-treated control group were placed in a conical tube with 2.4 ml per leaflet of AABS, with the pH adjusted to 7.0. The tubes were continuously shaken for 2 h at 37 °C. The buffer solution was then removed and the leaflets were washed with ice-cold PBS three times, for 5 min each time. The control specimens were then stored covered in ice-cold PBS and underwent mechanical testing within 48 h of harvesting.

2.2. GAG content quantification

Quantification of GAG content was performed by hexosamine analysis using previously published methods [31,32]. In brief, leaflets were lyophilized, weighed and then hydrolyzed in 2 ml of 2 M HCl at 95 °C for 20 h. The solution was then evaporated under nitrogen gas and the hydrolysates dissolved in 2 ml of 1 M NaCl. This was followed by the addition of 2 ml of 3% acetylacetone in 1.25 M sodium carbonate. Next, 4 ml of absolute ethanol and 2 ml of Ehrlich's reagent (0.18 M *p*-diemethylaminobenzaldahyde in 50% ethanol) were added. Incubation at room temperature for 45 min yielded a color product indicative of hexosamine quantities, which was read for absorbance at 540 nm with a set of D (+) glucosamine (0–200 μg) controls. All values were normalized to their respective dry tissue weights and corrected for the fact that the hexosamine assay accounts for ~90 μg/10 mg of non-GAG related hexosamines, as shown previously [31,33].

2.3. Planar biaxial mechanical behavior

Specimens (native: group 1; control: group 2; enzyme treated: group 3; $n = 8$ for each group) were cut from the central belly region of the leaflet into an approximately 10 mm × 10 mm square (Fig. 1a), with the thickness measurement. A fourth group of native-tissue-only specimens (group 5, $n = 5$) was similarly prepared but, with their edges aligned at a 45° angle to the circumferential axis, as in Ref. [34], induced a state of high in-plane shear [35]. This