Tissue- and Microstructural-level Deformation of Aortic Tissue under Viscoelastic/Viscoplastic Loading

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ABSTRACT

Mechanical function of tissues in health and disease is regulated through interactions of phenomena spanning across multiple length scales. The multiscale nature of tissue biomechanics should be incorporated into modeling approaches if a full description of soft tissue biomechanics is sought [1-4]. While gross changes in geometry are sufficient for quantifying tissue-level deformations, quantification of microstructural deformations is more challenging [5-6]. There have been some advances over the past decade [7], but there remains many issues regarding how best to characterize changes in microstructure. In this study, we examined the relation between deformation of bovine aortic tissue at the tissue- and microstructural scales, during uniaxial stretch and after loads are removed. Specifically, circumferential aortic samples were subjected to small and large stretches. Some specimens were chemically fixed during stretch, and others released to undergo free retraction before chemical fixation. Specimens were measured macroscopically before/after loading, sectioned and stained histologically, and analyzed to examine microstructure. Image-based analysis of histology images have been effective in quantifying microstructural changes in soft tissues [8-14] and served as guidance to assess tissue microstructure. At the tissue scale, it is observed that sample's elongation is accompanied with width/thickness shrinkage in an isochoric manner. Microstructural investigations reveal straightening of (undulated) fibrillar network when tissue is stretched circumferentially; with variations different at inner and outer layers of wall thickness. Once recovered, samples exhibited larger permanent deformation toward outer layer, which possesses sparse elastic lamina. This study provides a microstructural basis for observations of local permanent stretch in artery tissues, and paves the way for further development of multiscale models of cardiovascular biomechanics.

METHODS

Aortas of 291±23 mm length were obtained from Angus male cattle (average age of 20-24 months) at a local abattoir. Proximally, each aorta was cut near the heart and, distally, above the abdominal bifurcation, and then transferred to the laboratory immersed in cold phosphate buffered saline. The tissue was carefully cleaned of large remnants of fat and attached connective tissues. Areas of the aorta containing vascular branch points and abnormalities were discarded. Circumferential specimens of 30mm$\times$10mm size were excised from the aorta, with

thicknesses naturally varying in 3-8mm range. The specimens were preserved in phosphate buffer (PBS) solution with protease-inhibitor additives (per 1 Liter of PBS: 1mM of EDTA-Disodium salt, 1mM of EDTA-Tetrasodium salt, 5mM of Benzamadine, 10mM of NEM, 1mM of PMSF) until being used.

A custom apparatus was constructed to maintain specimens under controlled deformation over time and in solution. The apparatus consists of a frame and two movable clamps which can be secured at different distances apart on the stand, Fig. 1. Clamps were tightened over the tissue while relaxed. While one clamp was fixed, the second clamp was moved along the frame and secured at a known stretch. Some specimens were chemically fixed while held at a fixed stretch by immersing the clamps and tissue in fixative for 24 hours. For specimens to be tested after recovery from stretch, the specimen was released from the jig so it could retract for 45 minutes and then fixed for 24 hours.

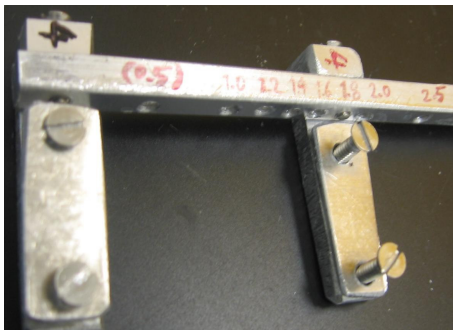

Fig. 1- Custom apparatus used to apply and hold the deformation of aortic specimens.

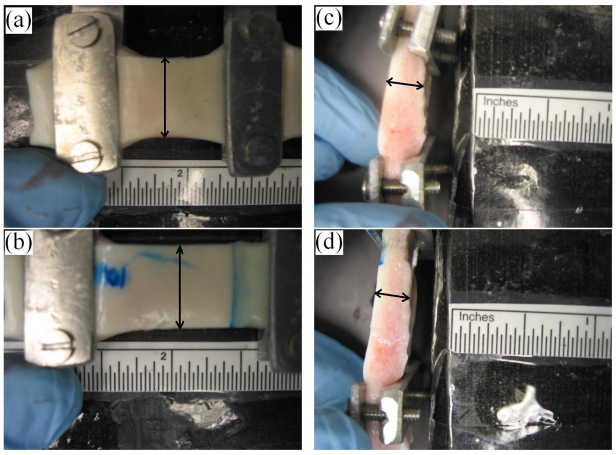

Fig. 2- Images acquired to analyze changes in specimen dimensions during tissue deformation. A ruler was included in the images to calibrate length measurements, carried out by the software ImageJ. Figure shows the width of the specimen before loading and after deformation recovery, (a) and (b), respectively; and the thickness of the specimen before loading and after deformation recovery, (c) and (d), respectively.

To quantify the tissue-scale changes in the specimens, images were acquired of specimens at each of the stages of unstretched, stretched, and after recovery (Fig 2). These images were analyzed using ImageJ software *(NIH, Bethesda, MD)* to obtain width and thickness measurements of each specimen.

For tissue fixation, we used a modified fixative solution consisting of 10% bleach and 15% formaldehyde in deionized water to better fix the elastin and collagen [15,16]. After fixing the microstructure, specimens went through paraffin-embedding tissue processing stages and sectioned and stained histologically. Histologic sections were made transverse to the layers to better describe the fibrillar orientation, and stained using a protocol which was a modification of Masson's trichrome with Verhoeff's hematoxylin [17,18]. In this protocol, the elastin is stained dark blue, the collagen is stained light blue, and proteoglycans are stained red. In a separate study, we sought to quantify the microstructural conformations seen in the histology images using different image-based techniques [11].

RESULTS

Different specimens at the unstretched state, under different levels of stretch, and after recovery from deformation were examined for microstructural measurements. Given the drastic change in the density of the elastin network

between inner and outer layers of the wall thickness, we separated our analyses into inner and outer layers of the aortic wall thickness. Figure 3 shows histology of an unstretched specimen (a), of specimens under 1.4 and 2.0 stretches (b) and (c), respectively, and of specimens after recovering from 1.4 and 2.0 stretches (d) and (e), respectively. Figure 4 shows images from outer regions of the wall, corresponding to the conditions shown in Figure 3.

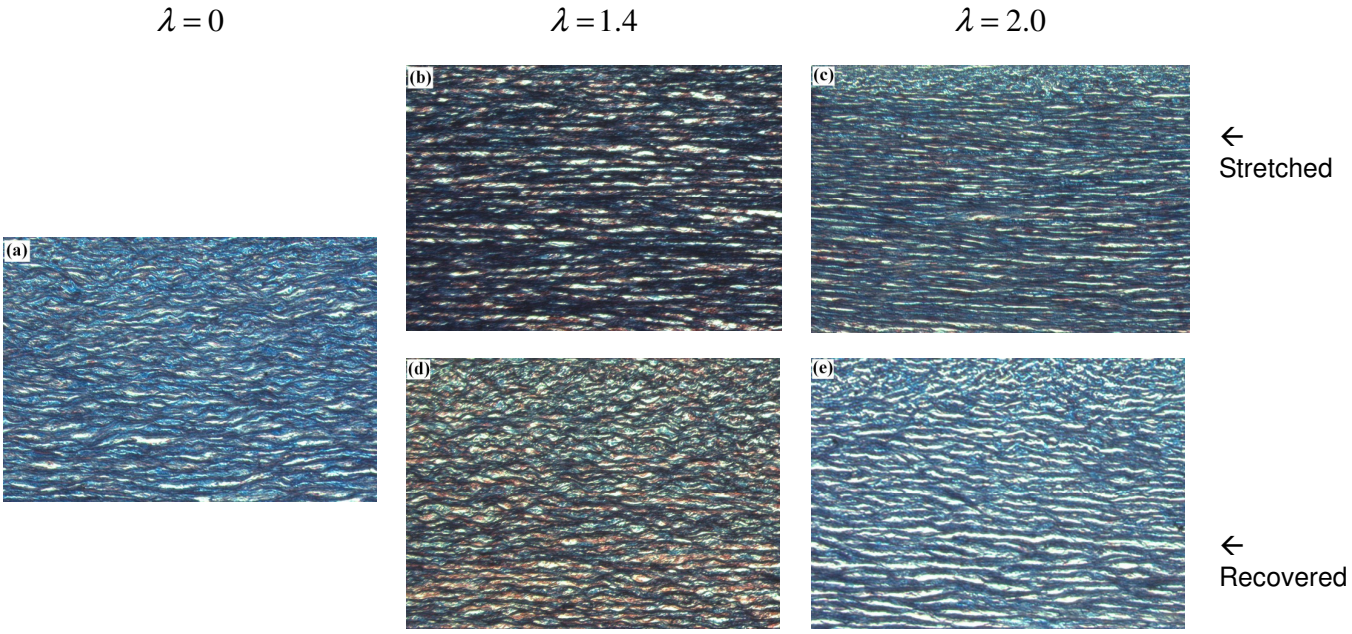

Figure 3- Histological sections of 'INNER' layer of aortic tissue. (a) Unstretched, (b) Stretched to 1.4 stretch, (c) Recovered from 1.4 stretch, (d) Stretched to 2.0 stretch, (e) Recovered from 2.0 stretch

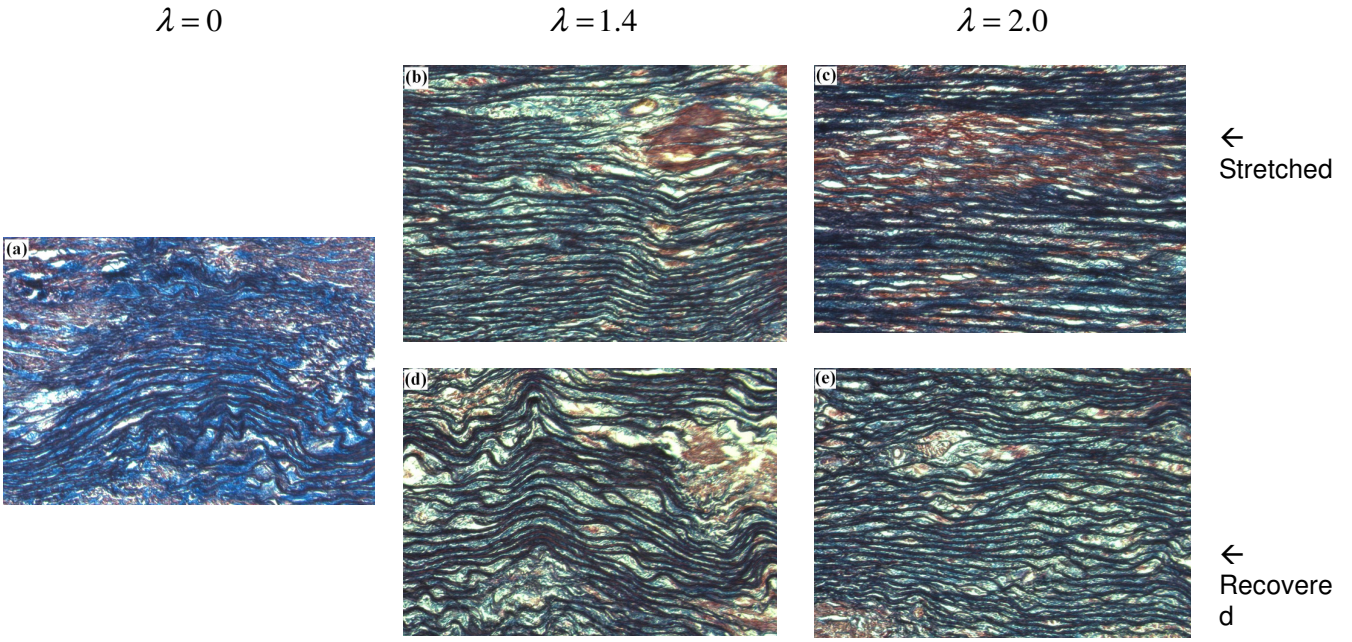

Figure 4- Histological sections of 'OUTER' layer of aortic tissue. (a) Unstretched, (b) Stretched to 1.4 stretch, (c) Recovered from 1.4 stretch, (d) Stretched to 2.0 stretch, (e) Recovered from 2.0 stretch

DISCUSSION

The main goal of this study was to probe the deformation of aortic specimens at the microstructural scale. The current understanding of governing mechanisms underlying microstructural alterations is limited, leaving many issues to be addressed before a comprehensive understanding of microstructural deformation can be obtained. We sought to examine the histological images obtained from different aortic specimens —in unstretched state, under stretch, and after recovering from stretch— to improve the knowledge base in this area of research.

Given our interest in the deformation of fibrillar elastin, we studied the inner and outer regions of the wall thickness separately. Distinctions between the histological observations of inner (intima, inner media) and outer (outer media, possible remnants of adventitia) regions of aortic wall thickness may be attributed to drastic increase in elastin's density and organization toward inner regions. Our observations suggest that the elastin network in the inner region is much more organized compared with that of the outer region, which undergoes deformations on the order of tissue-scale stretch.

Studying the microstructural alterations in specimens under increasing stretch shows that both in the inner and the outer media, stretching the tissue specimens has a direct effect on microstructure by straightening the fibrillar structure. Less intuitive observations were made for the microstructure of recovered tissue samples after being released from stretch. For samples recovered from 1.4 stretch, it was found that large portion of microstructural deformation is recovered, but does not fully recover to the initial conformation of unstretched tissue samples. Furthermore, when recovered from 2.0 stretch, even lower level of microstructural restoration occurs; hypothetically due to break down and dislocation of the extracellular matrix to which elatin network is anchored. These observations suggest that a certain amount of permanent deformation occurs during tissue loading. Such irrecoverable deformations have been observed in other tissues, but the mechanisms for restoration or remodeling of the tissue remain unknown.

We believe that the outcome of this study paves the way to achieve a better understanding of microstructural deformation. It should be noted that throughout the procedures we undertook in this study to prepare circumferential rectangular specimens from the originally cylindrical-shape aorta, the residual stress within the aortic wall is released [19,20], which, hypothetically, accompanies reconfiguration of a tissue's microstructure. As a result, we argue that the histological images obtained in this study might not accurately

represent those in intact aorta, but remain informative on how the fibrillar networks in the tissue can stretch and shrink as a tissue deforms.

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