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Transmural variation in elastin fiber orientation distribution in the arterial wall

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ABSTRACT

The complex three-dimensional elastin network is a major load-bearing extracellular matrix (ECM) component of an artery. Despite the reported anisotropic behavior of arterial elastin network, it is usually treated as an isotropic material in constitutive models. Our recent multiphoton microscopy study reported a relatively uniform elastin fiber orientation distribution in porcine thoracic aorta when imaging from the intima side (Chow et al., 2014). However it is questionable whether the fiber orientation distribution obtained from a small depth is representative of the elastin network structure in the arterial wall, especially when developing structure-based constitutive models. To date, the structural basis for the anisotropic mechanical behavior of elastin is still not fully understood. In this study, we examined the transmural variation in elastin fiber orientation distribution in porcine thoracic aorta and its association with elastin anisotropy. Using multi-photon microscopy, we observed that the elastin fibers orientation changes from a relatively uniform distribution in regions close to the luminal surface to a more circumferential distribution in regions that dominate the media, then to a longitudinal distribution in regions close to the outer media. Planar biaxial tensile test was performed to characterize the anisotropic behavior of elastin network. A new structure-based constitutive model of elastin network was developed to incorporate the transmural variation in fiber orientation distribution. The new model well captures the anisotropic mechanical behavior of elastin network under both equi- and nonequi-biaxial loading and showed improvements in both fitting and predicting capabilities when compared to a model that only considers the fiber orientation distribution from the intima side. We submit that the transmural variation in fiber orientation distribution is important in characterizing the anisotropic mechanical behavior of elastin network and should be considered in constitutive modeling of an artery.

1. Introduction

Elastin is one of the major extracellular matrix (ECM) components that imparts elastic property to an artery in order to accommodate cyclic physiological deformation. In elastic arteries such as aorta, elastin fibers in the medial layer form concentric layers of elastic lamellae. Each elastic lamella alternates with a layer of smooth muscle cells and collagen fibers forming a lamellar unit, which is considered as a functional unit of the arterial wall (Wolinsky and Glagov, 1967). It is believed that elastin dominants the passive behavior of arteries at low strains whereas collagen are progressively recruited at higher strains (Roach and Burton, 1957). Using multi-photon microscopy, Chow et al. (2014) quantified the sequential engagement of elastin and collagen fibers in response to arterial deformation.

Blood vessels are generally considered to be anisotropic (Zhou and Fung, 1997). Various forms of strain energy function have been developed, however the contribution of elastin to arterial wall mechanics is usually assumed to take isotropic forms, and the anisotropic response of arterial tissue usually comes from preferred collagen fiber distribution (Wuyts et al., 1995; Holzapfel et al., 2000; Fung and Liu, 1989; Zulliger et al., 2004; Zeinali-Davarani et al., 2013). Several recent studies attempt to account for the anisotropic mechanical properties of elastin. Rezakhaniha and Stergiopulos (2008) considered a model with one family of axially oriented fibers embedded in an isotropic matrix to model elastin as a transversely isotropic material. Zou and Zhang (2009) developed a statistical mechanics-based hyperelastic anisotropic constitutive model to study purified elastin network. Kao et al. (2011) used an orthotropic representation that consists of two orthogonal families oriented in the axial and circumferential directions distributed in a matrix. Rezakhaniha et al. (2011) assumed a model with elastin fibers oriented in the circumferential direction and a better model predictability was reported. Wang et al. (2016) considered the unique

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contribution of elastin, medial collagen, and adventitial collagen fibers in a structure-based constitutive model of the arterial wall that incorporates fiber orientation and engagement of each ECM components (Wang et al., 2016).

Natural biological tissues and tissue-derived soft biomaterial often exhibit pronounced mechanical anisotropy due to preferred fibrous orientation (Sacks, 2000). Ligaments and tendon have anisotropic behavior with a preferred direction that can be represented by transverse isotropy (Hoffmeister et al., 1996; Ono et al., 1990; Weiss et al., 1996). Fiber structure revealed from small angle light scattering technique was found to be consistent with the direction and the degree of mechanical anisotropy of canine diaphragmatic central tendon (Chuong et al., 1991). Canine myocardium exhibits anisotropic behavior in the along-fiber and cross-fiber directions (Demer and Yin, 1983). Elastin network has been observed to have anisotropic mechanical behavior (Sherebrin et al., 1983; Zou and Zhang, 2009; Lillie et al., 2010). The structural basis for the anisotropic mechanical behavior of elastin, however, is still not clear. In this study, we focus on the transmural variation in elastin fiber orientation distributions and the association with the anisotropic behavior of elastin network. Multiphoton microscopy was used to image purified medial elastin network from both sides as well as sections from various depths to capture the transmural variation in elastin fiber distribution. Equi- and nonequi-biaxial tensile tests were performed on purified elastin network to characterize its mechanical behavior. Finally a structure-based constitutive model was developed to incorporate the measured elastin fiber distributions from the inner, middle, and outer media for the mechanical behavior of elastin network.

2. Material and methods

2.1. Sample preparation

Descending thoracic aortas were harvested from pigs of 12–24 month of age at a local abattoir and transported to laboratory on ice. After removing of adhesive tissue and fat, samples of approximately 20 × 20 mm square were cut with one edge parallel to the longitudinal direction and the other edge parallel to the circumferential direction of the artery. Purified elastin was obtained using a cyanogen bromide (CNBr) treatment to remove cells, collagen and other ECM components (Zou and Zhang, 2009). Aorta samples were kept in 50 mg/ml CNBr in 70% formic acid at room temperature for 19 h, then at 60 °C for 1 h with gentle stirring, followed by boiling for 5 min to inactivate CNBr. Samples were rinsed in DI water and 1 × phosphate buffered saline (PBS) several times and placed in PBS for mechanical testing and imaging.

2.2. Mechanical testing

Equi- and nonequi-biaxial tensile tests were performed using a biaxial tensile testing device to characterize the anisotropic mechanical properties of elastin network following protocols described previously (Zou and Zhang, 2009). A total of six samples were tested. Briefly, sandpaper tabs were glued to the edge of the samples, and nylon sutures were looped through the sandpaper tabs and then connected to linear carriages. Tension control experiments were performed through a custom LABVIEW program. There were two load cells, one in each axis, to monitor the load applied to the tissue sample in both loading directions. The position of the four carbon marker dots glued to samples was monitored using a CCD camera to measure tissue deformation. A preload of 5 N/m was applied to the samples in order to straighten the sutures. Samples were subjected to eight cycles of equi-biaxial tension of 40 N/m for preconditioning. Following preconditioning, eight cycles of biaxial tension with f_l:f_c = 100:100, 100:75, and 75:100 N/m were applied to achieve repeatable mechanical response, where f_l and f_c refers to tension in the longitudinal and circumferential directions, respectively. Here tension is calculated as force divided by the side length of the sample where the force is applied. Measurements from the last cycle were used for data analysis. Cauchy stresses were calculated by assuming plane stress and incompressibility as:

$$\sigma_1 = \frac{F_1\lambda_1}{tL_{20}},\ \sigma_2 = \frac{F_2\lambda_2}{tL_{10}} \tag{1}$$

where σ_i is Cauchy stress, F_i is the applied load, L_{i0} is the initial side length, and t is the initial thickness of the tissue. λ_i is stretch, which was calculated as $\frac{L_i}{L_{i0}}$, where L_i is the deformed side length. Subscripts i = 1, 2 correspond to the longitudinal and circumferential directions, respectively.

2.3. Multiphoton microscopy

A multiphoton microscopy system (Carl Zeiss LSM 710 NLO) with a 810 nm femtosecond IR pulse laser excitation was used to generate two-photon excited fluorescence (2PEF) from elastin (525/45 nm). Laser power at the sample was set to 25 mW to minimize thermal effects. The laser scanning system is coupled to an upright microscope with a 20 × water immersion objective lens. Each sample was imaged with a field view of 425 × 425 μm at five locations to obtain average structural properties of the samples. A total of six samples were imaged. Samples were placed with longitudinal direction of the tissue aligned vertically. Thus, fibers oriented at 0° and ± 90° are in the circumferential (C) and longitudinal (L) directions, respectively. Samples were imaged from both sides to assess the inner and outer medial elastin, which was referred to as inner media and outer media throughout the study. Regions between the inner and outer media were referred to as middle media. A custom-built device was used that allows the elastin samples to be imaged while under biaxial strain (Chow et al., 2014). The samples were imaged from the intimal side when subjected to up to 30% equi-biaxial strain at 10% increments. To examine elastin fiber reorientation under nonequi-biaxial deformation, samples were imaged at strains of 0%C-0%L, 30%C-30%L, 15%C-30%L, 30%C-15%L, where the strains represent grip-to-grip engineering strain. The elastin samples were then frozen, and from 400 μm beneath intima surface, three ~ 100 μm thick sections were cut in parallel to the intima surface using a microtome (MICROM cryostat HM 525). The sections were collected on slides for imaging. Samples were imaged to a depth of about 40 μm with scans every 2 μm. Maximum intensity projections of the Z-stacks were produced for further analysis. Sections of about 100 μm in thickness were also prepared to image the circumferential cross-sectional structure of the elastin network.

2.4. Imaging analysis

Two dimensional fast Fourier transform (2D-FFT) analysis using the Directionality plug-in (developed by Jean-Yves Tinevez) in FIJI (http://Fiji.sc/Fiji, Ashburn,VA) was performed on 2PEF images to determine fiber orientation distribution, following developer's instructions. The fiber orientation in the spatial frequency space was determined and a normalized histogram was generated to represent the amount of fibers at angles from − 90° to 90° at 2° increment (Chow et al., 2014). Each sample was imaged at five locations that cover about 1 cm^2 area. Average fiber orientation distributions from six samples, or 30 locations, were obtained and used for constitutive modeling.

The fiber orientation ratio of circumferentially to longitudinally oriented fibers, defined as the number of circumferential fibers (oriented between 0° ± 20°) divided by the number of longitudinal fibers (oriented between 90° ± 20°), was calculated to compare the degree of fiber alignment.

2.5. Constitutive modeling

To account for transmural variation in elastin fiber orientation