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The role of elastin and collagen in the softening behavior of the human thoracic aortic media

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ABSTRACT

In a previous study we were able to accurately fit experimental data on arterial tissues at supra-physiological loads using a material model that accounts for softening/damage only in the portion of the model associated with the collagen fibers (Weisbecker et al., 2012). Naturally, this result leads to the hypothesis that the softening behavior is related only to the collagen fibers, and not to the matrix material. In this study we test this hypothesis by conducting uniaxial extension tests on elastase and collagenase treated tissues and on untreated control specimens from the media of human thoracic aortas. We relate structural changes in the tissue after enzyme treatment to changes in the corresponding mechanical behavior. Collagenase treated tissue does not exhibit any softening behavior under quasi-static cyclic loading, a result supporting our hypothesis. Conversely, elastase treated tissue exhibits continuous softening under the same loading conditions, indicating that the integrity of the tissue is destroyed upon removal of the elastin. Finally, we fit isotropic and anisotropic constitutive models to the mechanical response of the collagenase treated arterial tissue, while our anisotropic model better approximates the response of collagenase treated arterial tissues, we show that an isotropic matrix model is sufficient to accurately reproduce the mechanical response of untreated control specimens, consistent with current practice in the literature.

1. Introduction

In a recent study we showed that softening in aortic tissues due to supra-physiological loads can be modeled by accounting only for the softening in the strain energy associated with the collagen fibers, and not for the matrix material (Weisbecker et al., 2012). This phenomenological result leads naturally to the hypothesis that the softening behavior is associated only with the collagen fibers. To investigate this hypothesis, we perform experiments on both collagenase treated tissues and elastase treated tissues, as well as untreated control tissues, to gain more insight into the softening mechanisms associated with the collagen fibers.

Roach and Burton (1957) conducted the first study which isolated the mechanically relevant constituents of arteries, namely elastin and collagen, and investigated the resulting mechanical responses. These authors showed that elastin is responsible for the initially compliant behavior at lower strains and that collagen is responsible for a stiffening behavior at higher strains. Experiments conducted by Dobrin et al. (1984) showed that the radius of the vessels increases after elastase treatment, indicating that elastin keeps the arterial wall in its original shape. Dobrin and Canfield (1984) stated that it is necessary to select enzyme activity and digestion time such that collagen is only partially digested, otherwise inflation tests are not possible due to leaking or rupture of the specimens.

Determining the mechanical properties of elastin, the main component of elastic fibers, has recently attracted increased attention. Gundiah et al. (2007) performed planar biaxial extension tests that show an isotropic stress–stretch behavior which they initially modeled using the neo-Hookean material model. However, the authors report that elastic fibers of porcine arteries are oriented axially in the intima and the adventitia, and circumferentially in the media. The authors believe that this finding motivates an anisotropic model for the non-collagenous matrix. As a result of their structural findings, they propose an invariant-based orthotropic strain-energy function (Gundiah et al., 2009).

Lillie et al. (2010) performed mechanical tests on purified elastic fiber network tissue obtained from the thoracic aorta of pigs. In uniaxial tests this tissue is stiffer in the circumferential direction compared to axial. The authors then compared four constitutive models to predict the pressure–circumferential

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stretch behavior. They conclude that models incorporating both anisotropy and nonlinearity best predict the mechanical behavior of the elastic fiber network.

Similarly, Zou and Zhang (2009) investigated the mechanical properties of the elastic fiber network under biaxial tensile loading for the bovine thoracic aorta. Their experiments also show an anisotropic mechanical response with the circumferential direction being stiffer than the axial direction. The authors propose an eight-chain statistical mechanics based microstructural approach to model the mechanical behavior of the elastic fiber network. The same authors also investigated the time-dependent behavior using biaxial stress relaxation and creep experiments (Zou and Zhang, 2011). Their experimental results reveal that the creep response is negligible.

In this study we perform uniaxial extension tests to study the mechanical behavior of the media of the human thoracic aorta under relatively large loads. While uniaxial extension tests do not mimic the physiological loading condition, meaningful results can be obtained when the resulting data are carefully fitted (Holzapfel, 2006). Planar biaxial extension tests better represent the physiological loading conditions. However, they are difficult to perform within the supra-physiological loading domain, and they may lead to poor estimates of the stress, as shown both experimentally (Waldman and Lee, 2002) and numerically (Sun et al., 2005). Pressure inflation tests mimic physiological conditions well, however, the size of our samples did not allow such tests. Furthermore problems may arise due to leakage through the small side branches, especially at supra-physiological pressures.

In this study we compare the mechanical behavior of elastase and collagenase treated media from human thoracic aorta to untreated control specimens. We seek to understand the role of elastin and collagen in the softening behavior observed in untreated specimens at physiological and supra-physiological loads. Furthermore, both the isotropic neo-Hookean model and an anisotropic model are used to mathematically describe the mechanical response of the elastic fiber network. We discuss the implications of our model fits for elastin on modeling the untreated aortic tissue. We also qualitatively describe anisotropy observed in the continuous softening of elastase treated specimens.

2. Materials and methods

We conduct uniaxial extension tests on circumferentially and axially aligned specimens of the media of 17 human thoracic aortas (66 ± 8 years, mean $\pm$ SD), under stretch magnitudes within the physiological and supra-physiological range.

We obtain the specimens from patients undergoing autopsy up to 24 h after death unrelated to cardiovascular disease and we stored them in physiological solution. When possible, we test tissue specimens within 48 h after excision. Otherwise, we immediately freeze the specimens and test them within 24 h of unfreezing. The Ethics Committee of the Medical University of Graz approved the use of autopsy material from human subjects.

We base our protocol for enzyme treatment on the work of both Dobrin and Canfield (1984) and Kratzberg et al. (2009). We perform the elastase treatment for 2 h in a 100 U/ml physiological solution for a complete digestion of elastin and the collagenase treatment for 16 h in a 4000 U/ml or 20 h in a 5000 U/ml physiological solution for partial or complete digestion of collagen, respectively, all at 37 °C.

For the collagenase treated tissue, we test seven specimens which contain negligible amounts of residual collagen (Donors 1–7) and five specimens with partially digested collagen (Donors 8–12). For the elastase treated tissue, we test a total of five specimens (Donors 13–17). If the tissue specimens are sufficiently large, we also test untreated control specimens from each donor for comparison.

In order to verify enzymatic digestion of the intended tissue constituent, we examine histological images using both Elastica van Gieson and Picrosirius red stains. If collagen is still visible in a histological image, we determine the area fraction of collagen in the cross-section using image analysis tools in Matlab (The MathWorks, Inc., Massachusetts, United States). We include specimens with visible collagen residues of less than 5% area fraction in our analysis, but they are marked with an asterisk $*$ to distinguish them from those specimens without visible collagen residues. Additionally, we have five specimens with larger residues in the range of 20–40% collagen area fraction. We compare the latter qualitatively to the corresponding data from control and enzyme-treated specimens.

In order to evaluate the ultrastructure of the tissue by transmission electron microscopy (TEM) we fix the aortic specimens in 2.5% glutaraldehyde in cacodylate buffer (pH 7.3) for 4 h at room temperature, followed by washing steps in cacodylate buffer solution. Subsequently, we postfix all specimens in 1% osmium tetroxide (OsO_4) solution for 2 h, wash in cacodylate buffer, and dehydrate in increasing concentrations of ethanol at room temperature, passed through propyleneoxide and embedded in Agar 100 resin. We then collect ultrathin sections cut with a ultramicrotome (Drukker International, Cuijk, The Netherlands) on mesh copper grids, stain using uranyl acetate and lead citrate, and examine the specimens with a Philips 100 electron microscope (Philips Electronics N.V., Eindhoven, The Netherlands).

The protocol for mechanical testing is similar to that reported previously in Weisbecker et al. (2012). Briefly, we cut dog bone specimens with a gage width of 4 mm from the aortic tissue in both circumferential and axial directions. Next, we remove the intima and adventitia using tweezers. We assume that the mechanical influence of internal and external elastic lamina, if present, can be neglected. To calculate the thickness we average four measurements taken using a videoextensometer (ME 46–350, Messphysik, Fürstenfeld, Austria).

We conduct the mechanical tests using a uniaxial testing machine (μ-strain ME 30–1, Messphysik, Fürstenfeld, Austria) with the tissue in a bath of physiological solution at 37 °C. The videoextensometer records the stretch in the gauge region of the specimen by tracking two black markers glued onto the specimen. We determine the unloaded length when the specimen is clamped and immersed in the bath at zero force. Labrosse et al. (2009) report, e.g., that the mean circumferential and axial stretches are 1.31 and 1.11, respectively, for the human thoracic aortic wall. Our force driven protocol prescribes the first Piola–Kirchhoff stress (engineering stress) to be 10, 25, 50, 100 kPa for the elastase treated specimens as well as the axially aligned collagenase treated specimens. Since they can bear more load, we load the circumferentially aligned collagenase treated specimens as well as the control specimens to 50, 100, 300, 500 kPa. In these tests we complete three cycles for each load step. For four additional circumferentially aligned, elastase treated specimens we apply cyclic loading to the first load step (10 kPa) to monitor the evolution of continuous softening in the specimen until rupture. In all cases the testing speed equals 5 mm/min to ensure quasi-static behavior of the tissue.

2.1. Data analysis

In all our subsequent analyses we convert uniaxial force-displacement data to Cauchy stress–stretch data for constitutive model fitting. We evaluate the Cauchy stress σ from the experimental data as

$$\sigma = \frac{f}{WT}\lambda, \tag{1}$$

where f is the current force, W and T are initial width and thickness of the specimen, and λ is the applied uniaxial stretch (Holzapfel et al., 2004; Weisbecker et al., 2012).

In all that follows we use convex strain-energy functions, say Ψ, defined per unit volume in the reference configuration, to model nonlinear materials undergoing finite strains. The Cauchy stress tensor $\boldsymbol{\sigma}$ follows from the strain-energy function as $\boldsymbol{\sigma} = 2J^{-1}\mathbf{F}(\partial\Psi/\partial\mathbf{C})\mathbf{F}^{\mathrm{T}}$, where $\mathbf{C} = \mathbf{F}^{\mathrm{T}}\mathbf{F}$ is the right Cauchy Green tensor, $\mathbf{F}$ is the deformation gradient tensor and $J > 0$ is the Jacobian, the determinant of the deformation gradient (Holzapfel, 2000). Note that we assume an incompressible behavior, and hence we have $J = 1$.

We determine the constitutive parameters using a nonlinear-least squares algorithm implemented in the MATLAB. The goodness-of-fit is reported using the square of the Pearson's correlation coefficient R^2.

2.1.1. Collagenase treated specimens

The macroscopic mechanical response of elastin in arterial tissues is commonly modeled using an incompressible neo-Hookean material, see e.g., Holzapfel et al. (2000) and Watton et al. (2009). Correspondingly, we fit the experimental data from the collagenase treated specimens using the strain-energy function

$$\Psi_{\mathrm{nH}} = \frac{\mu_{\mathrm{nH}}}{2}(I_1 - 3), \tag{2}$$

where $\mu_{\mathrm{nH}} > 0$ is the initial shear modulus and $I_1 = \mathrm{tr}\,\mathbf{C}$.

Since we observe an anisotropic mechanical response for all tissue specimens, and both Clark and Glagov (1985) and Gundiah et al. (2007) show that elastic fibers are arranged circumferentially in medial tissue, we propose a transversally isotropic strain-energy function Ψ_{ti} of the form

$$\Psi_{\mathrm{ti}} = \frac{\mu_{\mathrm{ti}}}{2}(I_1 - 3) + c_{\mathrm{ti}}(I_{4\mathrm{m}} - 1)^2, \tag{3}$$

where $\mu_{\mathrm{ti}} > 0$ and $c_{\mathrm{ti}} > 0$ are material parameters, and $I_{4\mathrm{m}} = \mathbf{M}_{\mathrm{m}} \cdot \mathbf{C}\mathbf{M}_{\mathrm{m}}$ is the square of the stretch in the direction $\mathbf{M}_{\mathrm{m}}$, describing the orientation of the elastic fibers in the reference configuration.