Acta Biomaterialia 14 (2015) 133–145

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Structure-based constitutive model can accurately predict planar biaxial properties of aortic wall tissue

S. Polzer [a,*], T.C. Gasser [b], K. Novak [a], V. Man [a], M. Tichy [c], P. Skacel [a], J. Bursa [a]

[a] Institute of Solid Mechanics, Mechatronics and Biomechanics, Technicka 2896/2, 616 69, Brno University of Technology, Czech Republic
[b] Department of Solid Mechanics, The Royal Institute of Technology, Stockholm, Sweden
[c] 1st Department of Pathological Anatomy, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno, Czech Republic

ARTICLE INFO

Article history:
Received 16 April 2014
Received in revised form 13 October 2014
Accepted 20 November 2014
Available online 29 November 2014

Keywords:
Biaxial testing
Collagen structure
Thoracic aorta
Constitutive modeling

ABSTRACT

Structure-based constitutive models might help in exploring mechanisms by which arterial wall histology is linked to wall mechanics. This study aims to validate a recently proposed structure-based constitutive model. Specifically, the model's ability to predict mechanical biaxial response of porcine aortic tissue with predefined collagen structure was tested. Histological slices from porcine thoracic aorta wall ($n = 9$) were automatically processed to quantify the collagen fiber organization, and mechanical testing identified the non-linear properties of the wall samples ($n = 18$) over a wide range of biaxial stretches. Histological and mechanical experimental data were used to identify the model parameters of a recently proposed multi-scale constitutive description for arterial layers. The model predictive capability was tested with respect to interpolation and extrapolation. Collagen in the media was predominantly aligned in circumferential direction (planar von Mises distribution with concentration parameter $b_M = 1.03 \pm 0.23$), and its coherence decreased gradually from the luminal to the abluminal tissue layers (inner media, $b = 1.54 \pm 0.40$; outer media, $b = 0.72 \pm 0.20$). In contrast, the collagen in the adventitia was aligned almost isotropically ($b_A = 0.27 \pm 0.11$), and no features, such as families of coherent fibers, were identified. The applied constitutive model captured the aorta biaxial properties accurately (coefficient of determination $R^2 = 0.95 \pm 0.03$) over the entire range of biaxial deformations and with physically meaningful model parameters. Good predictive properties, well outside the parameter identification space, were observed ($R^2 = 0.92 \pm 0.04$). Multi-scale constitutive models equipped with realistic micro-histological data can predict macroscopic non-linear aorta wall properties. Collagen largely defines already low strain properties of media, which explains the origin of wall anisotropy seen at this strain level. The structure and mechanical properties of adventitia are well designed to protect the media from axial and circumferential overloads.

1. Introduction

The aorta is uniquely designed to cope with the large hemodynamic forces and tissue stresses that are created [1] and its proper biomechanical function is of great importance not only for its own structural and functional integrity, but also for the entire vascular system. Aorta physiology relies on a complex spatial distribution of organized cells and extracellular matrix (ECM), and pathological changes are associated with the micro-histological alterations [2,3]. Passive biomechanical properties of the aortic wall are determined mainly by elastin, collagen and proteoglycans (PG), i.e. the major structural proteins of the ECM [4,5]. Functional as well as structural modifications of these proteins contribute to a loss of aortic wall integrity and progressive dilatation or dissection of the vessel segment. Specifically, vascular pathologies such as aortic aneurysms are directly linked to the organization and synthesis of collagen [6–8].

The aorta macroscopic mechanical properties depend strongly on the amount of fibrillar collagen [9–11] as well as its spatial organization [12], such that there is a need to understand the organization of collagen structures at different length scales. Several microscopy techniques such as classical microscopy [16], polarized light microscopy (PLM) [13,14], multi-photon microscopy [56,57] or small-angle light scattering [15] together with various image-processing techniques (see Ref. [16] and references therein) have been suggested to study the collagen organization in the arterial wall.

Phenomenological and structurally motivated constitutive models have been suggested to capture the complex mechanical

* Corresponding author. Tel.: +420 777 236 456.
E-mail address: polzer@seznam.cz (S. Polzer).

http://dx.doi.org/10.1016/j.actbio.2014.11.043
1742-7061/

properties of arterial wall. Although phenomenological approaches [17–20] may reflect the biomechanical properties of the vascular wall, their material parameters lack physical interpretation, and these models are unreliable for predictions beyond the strain range used in parameter estimation. In contrast, histomechanical constitutive models [12,21–25,58], i.e. structurally motivated mathematical descriptions of the tissue biomechanical properties, are supposed to overcome this drawback. Most important, histomechanical constitutive models allocate macroscopic stress to different microstructural components, and are able to link the macroscopic loading to potential cellular responses. Consequently, histomechanical models play a key role in studying the principles of normal and pathological growth and remodeling. Histomechanical models can be considered a subclass of statistical multiscale descriptions, since they link macroscopic tissue deformation to the corresponding microscopic fiber deformation typically through affine deformation, i.e. local fiber and continuum deformations match. In addition to microstructural data, in vitro aortic tissue testing provides mechanical input information for model parameter identification, where biaxial testing [9,19,21,26,27] is specifically capable of closely reflecting the in vivo mechanical environment of vascular tissue.

While many histomechanical models for vascular tissue in various forms have been proposed and also implemented in a computational framework [22,28–30,37], their robust evaluation and rigorous validation remain quite limited. Recent ability to get detailed fiber orientation [16] and recruitment [31] data makes this approach all the more relevant, such that structural and mechanical model parameters can be separately identified by image analysis of the histological slices and mechanical experiments, respectively. However, to the best of the authors' knowledge, apart from very limited studies of the aneurysmatic aorta [28,32] and carotid artery [52], no constitutive model for the (normal) aorta has been validated using independently measured collagen fiber orientation distribution.

The present study investigated wall samples from the porcine thoracic aorta, where biaxial tensile testing was combined with automatic Fast Fourier Transform (FFT)-based collagen fiber orientation measurements [16] throughout the whole wall thickness. A recently proposed constitutive model for arterial layers [22], integrating mechanical and histological data, and its predictive capability were tested with respect to interpolation and extrapolation.

2. Methods

2.1. Quantitative histology

2.1.1. Cohort and specimen preparation

Wall samples (18×18 mm) were taken from the pig aorta ($n = 9$; age, 10 month; weight, 105–120 kg) supplied by the local slaughterhouse. All specimens were taken from the straight anterior part of thoracic aorta and then flattened by four pins on plywood without stretching them. In order to track the specimen alignment, a non-symmetric triangular piece was cut away from the bottom left corner of the wall sample. Specimens were fixed in 10% solution of formaldehyde for 24 h, embedded in paraffin, cut into 5-μm-thick tissue sections, deparaffinized and hydrated. The samples were stained in picro-sirius red for 15 min (Sigma–Aldrich, India), i.e. collagen fibers appeared red. Nuclei were stained with Weigert's hematoxylin for 10 min (Merck KGaA, Germany). Every 120 μm along the radial direction, a slice was taken and, depending on the sample wall thickness, 12–14 slices from each sample were prepared in total.

In order to verify that fixing wall samples with four pins on plywood gives similar results to a previously reported method [28], two additional wall specimens were squeezed between glass plates during tissue fixation. Flattening the originally tubular specimen induces bending strains that also alter fiber orientation. However, a simple kinematic analysis showed that the deformation induced by specimen flattening rotates a collagen fiber at the most by 1.7°. This rotation was neglected, and the data were not corrected for this effect.

2.2. Image analysis

Histological slices were analyzed with a standard biological Padim microscope (Drexx s.r.o., Czech Republic), and images (see Fig. 1) were taken with a Bresser microcam 5 MP, (Bresser GmbH, Germany). Images were analyzed using a previously reported method [16], described briefly below.

After removing edge artifacts with suitable weight functions, the classical FFT provided the amplitude spectrum:

$$|F(u,v)| = \left| \frac{1}{a} \sum_{m=0}^{a-1} \left[\frac{1}{a} \cdot \sum_{n=0}^{a-1} e^{\left(\frac{-2\cdot\pi\cdot i\cdot n\cdot v}{a}\right)} \cdot S(m,n) \right] \cdot e^{\left(\frac{-2\cdot\pi\cdot i\cdot m\cdot u}{a}\right)} \right| \tag{1}$$

where a denotes the dimensions of the image $S(m, n)$.

The parts of the spectrum not carrying any relevant information were neglected, and only frequencies of the FFT image that corresponded to a length range of 3–20 μm were analyzed further. The upper half of the image was split into wedges 2° wide (sectors), and pixel intensities in each of these sectors were summed up and normalized to yield the orientation density function $h(\alpha)$. Then the spectrum $h(\alpha)$ was powered by $w \geq 1$, which finally defined the orientation density function

$$s(\alpha) = h^w(\alpha) \text{ for } 0 < \alpha < 180^\circ \tag{2}$$

of the collagen fiber distribution. It is emphasized that the FFT automatically enforces symmetry of the distribution, i.e. $s(\alpha) = s(\alpha + \pi)$ holds. The powering value w was set through a calibration procedure (see Appendix B) for medial and adventitial tissues separately. This is necessary, since the appearance of collagen differs significantly among those tissues (see Fig. 1) and the FFT-based image-processing algorithms are known to be sensitive to the contrast of individual structures [16].

After calibration, each histological slice was captured in 80–250 pictures (magnification 100×; image size, 2592×1944 pixels) and processed with the algorithm described above. Naturally, the present authors tried to exclude artifacts such as torn, twisted or folded parts. Histological slices at the media–adventitia interface showed typically both medial and adventitial tissue. These were analyzed separately, and their contributions to the final orientation distribution weighted accordingly. In order to derive the information presented in this study, ~9000 images in total were captured and analyzed.

The collagen fiber distributions obtained were least-square fitted to a planar von Mises distribution function:

$$\rho(\alpha) = \frac{\exp\left[b\cos\left(\frac{\pi(\alpha-\varphi)}{180}\right)\right]}{\frac{1}{180\pi}\int_0^\pi \exp\left[b\cos(\beta)\right]\mathrm{d}\beta} \tag{3}$$

where the angle φ denotes the mean fiber orientation in degrees, while the concentration parameter b quantifies its anisotropy. Isotropic and unidirectional (Dirac Delta) fiber distributions are defined by $b = 0$ and $b \to \infty$, respectively.

2.3. Planar biaxial mechanical testing

2.3.1. Cohort and specimen preparation

Wall samples were taken from the pig aorta ($n = 18$; age, 10 month; weight, 105–120 kg) supplied from the local slaughter-