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EVALUATION OF BIAXIAL MECHANICAL PROPERTIES OF MEDIAL LAMELLAE OF AORTIC WALL USING MULTISCALE MODELING

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Abstract. Arterial wall is one of the most complicated tissues in both structural and functional aspects and its pathological states are among leading causes of world mortality. Mechanical properties of the arterial tissue highly depend on wall constituents. Hence, evaluation of mechanical properties of wall components is necessary for establishing a mechanical model applicable in various physiological and pathological conditions such as remodeling, growth, and plaque formation. In this contribution, we proposed a new model for evaluation of mechanical properties of wall lamellae based on biaxial testing of the arterial wall to characterize wall mechanical behavior. We assumed composite structure of aortic media to be composed of two sets of concentric lamellae, namely sheets of elastin (layer I)and interstitial layers composed of mostly Smooth Muscle Cells, collagen bundles and fine elastin fibers (layer II). A pair of two adjacent layers is often called the structural unit of media. In this contribution, we carried out biaxial tensile tests as well as histological tests on human descending thoracic aorta samples. According to the experimental data, a new microstructural model was proposed. Because of the isotropic nature of elastin sheets, we assumed a Neo-Hookian strain energy density function for the layer I. On the other hand, for layer II a four-parameter exponential strain energy density function was allocated due to the anisotropy.

Considering experimental data and above functions, we applied optimization algorithms to find unknown material parameters for lamellae. The range of otained parameters was to be in good agreement with recent publications. Resulting lamellar model is capable of modeling different phases of arterial tissue behavior.

1 INTRODUCTION

Arterial wall is one of the most complicated tissues in both structural and functional aspects. Successful biomechanical investigations of the aortic wall go back to only recent decades due to such complications [1]. The importance of such studies becomes multifold considering that pathological conditions of cardiovascular system such as atherosclerosis and hypertension are among leading causes of death [2, 3].

Since mechanical properties of soft biological tissues highly depend on their microstructure, proposing a reliable mechanical model for these tissues including arterial wall depends on the level of microstructure integration attained in the constitutive model. The early proposed models assumed arterial wall as a single continuous medium and suggested different forms of Strain Energy Density Function (SEDF) to characterize its mechanical behavior. These models were useful in providing primary insights to mechanical features of arteries but more realistic models with focus on microstructure were vital to fully understand mechanisms invovled in the mechanical modeling of arteries. As a consequence, a new category of arterial tissue models called microstructural models were adopted.

Microstructural models of the arterial wall can be categorized into two groups. In the first class, wall is regarded to be composed of three main layers, i.e. intima, media and adventitia [4, 5]. In the second group, different strain energy density functions are proposed for main wall constituents such as Elastin and Collagen [6, 7].

It is well known that mechanical behavior of the arterial wall depends mainly on mechanical properties of the media and its composite like lamellar structure [8-10]. Anatomical organization of the wall media is reported previously [11-13]. O'connel et al provided 3D schematic of this structure using confocal and electron microscopy imaging in which elastin sheets, SMCs and collagen bundles and fine elastin fibers are distinguishable; Concentric elastin sheets are almost identical in terms of thickness and composition and SMCs and collagen fibers together with fine elastic mesh lay in between [13]. Hence, the arterial wall media is considered as two sets of consequent and concentric lamellae, namely sheets of elastin (layer I) and interstitial layers mostly composed of collagen bundles with diverse fine elastin fibers among them together with smooth muscle cells (layer II). A pair of layer I and layer II often is regarded as the structural unit of the media. Approximately 60 structural units form aortic media in thoracic aorta of an adult human [8, 11].

To the best of our knowledge, mechanical analysis of the lamellar structure and its contribution to the whole wall mechanics is not considered before; as a result we have proposed sets of biaxial tests on samples of human thoracic aortas accompanied by histological staining for geometical assessment of lamellae. Resulting data were fed to an optimization algorithm and the stress-strain curves of layers I and II were obtained and compared to that of the wall.

2 MATERIAL AND METHODS

In thoracic region of the descending aorta diameter changes are minimal and hence microstructural parameters, i.e. thickness and number of lamellae, do not vary notably. As a result, samples of human descending thoracic aorta were used. These tissues were provided from brain death patients after organ donation according to ethical committee instructions of Masih Daneshvari hospital, the main site of organ donation and transplant in Iran. Sections from these samples were prepared for biaxial tests and some adjacent blocks were extracted for histological staining.

2.1 SPECIMEN PREPARATION

Aortic samples were preserved in Phosphate Buffered Saline (PBS) immediately after harvest and transferred to our lab. The adventitial and loose connective tissues were carefully removed using a surgical scalpel (Fig. 1). The remaining media-intima was submerged in PBS and then refrigerated at 4^{o}C until the test time preferably within the same day. Prior to tests, samples were allowed to reach to room temperature.

Cylinders with a height of 11 mm were cut and 11mm ×11mm squares were extracted for biaxial tests. Simultaneously, rings of 2-3mm height were cut to be used in histological staining procedures.

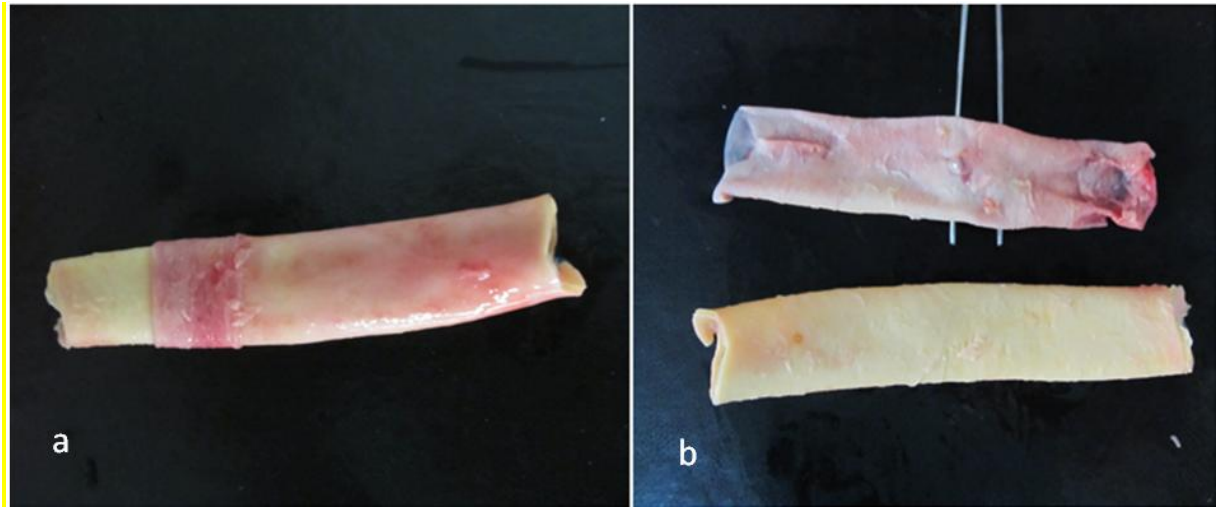

Figure 1: (a) Removal of adventitial tissue, and (b) resulting intima-media composite and loose adventitial layer

2.2 BIAXIAL TESTING

Biaxial testing of soft tissue samples is difficult yet necessary to fully capture mechanical features due to anisotropic nature. Some researches have used uniaxial test data to characterize mechanical properties [10]; Others utilized variety of biaxial tests including planar biaxial tests [14] or extension-inflation tests [5]. In the present study, we carried out planar biaxial tests using a custom-made biaxial test apparatus consisting of four stepping-

motors to stretch the samples and two load cells for recording loads in perpendicular test directions during extensions. We used hooks to mount samples to the test machine (Fig. 2a). To record displacements, 4 markers were used on the central part of the squared specimens and a CCD camera used to track marker coordinates during tests. Considering homogenous strain in each direction, the resulting force- displacement data were converted to Second Piola-Kirchhoff stress and Green Strain.

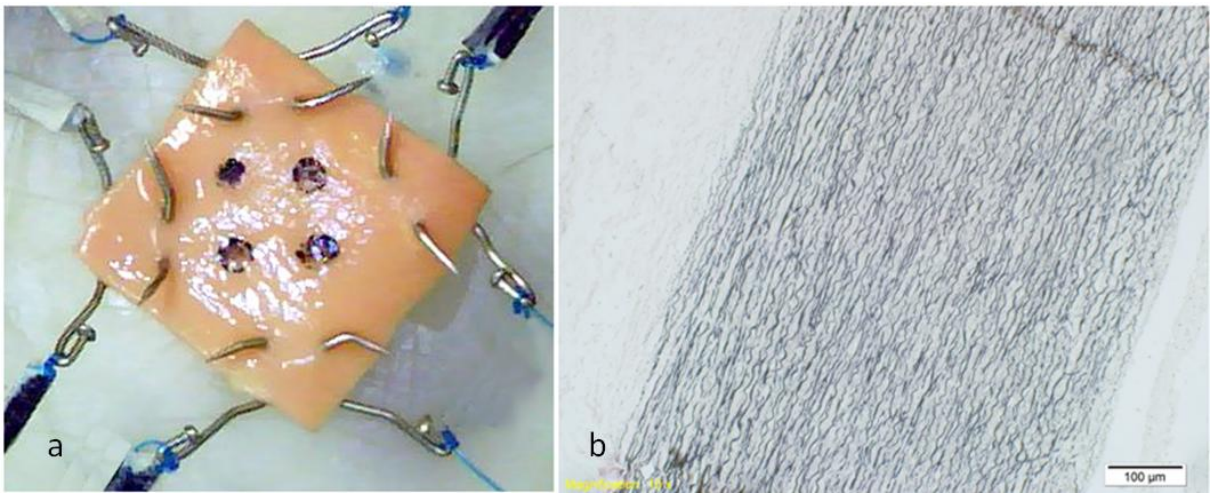

Figure 2: needed data collected from (a) biaxial test and (b) histological staining

2.3 HISTOLOGICAL STAINING

To fully setup our optimization, dimensions and volume fractions of lamellae are required. These features were extracted by histological staining. After fixation in formalin and paraffin-embedding, rings were cut into some micron height tubes using microtome and then put on slides for staining. We used Verhoff Van-Geison (VVG) stain to distinuish adjacent layers as elastin layers (Layer I) become colored (Fig. 2b). After staining, slides were photographed under microscope and the resultant images were processed by a MATLAB code to find the volume fractions. To do so, images were converted to black & white pictures and after removal of artifacts the percentage of the black (layer I) and white (layer II) zones were computed as the volume fractions. To minimize locatoin dependency, we measured volume fraction in six equally apart radial sites.

2.4 STRAIN ENERGY DENSITY FUNCTIONS (SEDF) AND OPTIMIZATION

Since Layer I is made of almost entirely elastin sheets, it can be assumed to be isotropic and its mechanical behavior is linear [15], hence simple forms of SEDFs such as Neo-Hookian may be adopted to describe its mechanical behavior (eq. 1) [16, 17]. On the other hand, for the interstitial layers a four-parameter exponential SEDF was allocated due to the anisotropic nature (eq. 2) [18, 19]. After assigning SEDFs to layers, Strain Energy for the whole wall can be written as eq. 3.

$$W_I = c_1(I_1 - 3) \tag{1}$$

$$W_{II} = c_2[\exp(a_1 E_{11}^2 + a_2 E_{22}^2 + 2a_3 E_{11} E_{22}) - 1] \quad (2)$$

$$W_{wall} = f_I W_I + f_{II} W_{II} \quad (3)$$

In which f_I and f_{II} describe volume fractions of layer I and layer II respectively, and W denotes strain enregy density functin for layer I (W_I), layer II (W_{II}) and the whole media (W_{wall}). Also, E_{ij} stands for green strain and c_i and a_i are unknown material parameters.

The equilibrium equations for the wall in terms of layer stresses can be written as follows:

$$\begin{cases} f_1 S_I^c + f_{II} S_{II}^c = S_{wall}^c \\ f_1 S_I^a + f_{II} S_{II}^a = S_{wall}^a \end{cases} \quad (4)$$

As a consequence of isotropic nature of Elastin sheets (layer I), corresponding stress terms in axial (S_I^a) and circumferential (S_I^c) directions are identical.

Experimental dispalcements devided by initial length of the marker pairs to give engineering strains and these data are then converted to stretch ratios. Finally Green strain is computed from stretches for each of test frames. Also collected force data was devided by initial sample area to give engineering stress and then converted to Second-Piola stress. The experimental data are plotted for both axial and circumferential directions in Fig. 3.

Experimental stresses can be seen in right side of eq. 4 as S_{wall}. Stress terms on the left side of the eq. 4 contain unknown material parameters. These parameters should be defined such that set of equilibrium equatoins remain valid for the range of experimental strains (E_{ij}).

A MATLAB code was developed to simultaneously consider equations in (4) and find optimum material parameters.

3 RESULTS

Table 1 indicates calculated material coefficients for typical human thoracic aorta and the volume fraction of each layer. The obtained material parameters are in good agreement with recent reports of mechanical properties [15].

Table 1: Compumted volume fractions and obtainded material parameters

f_I	f_{II}	c_1	c_2	a_1	a_2	a_3
0.377	0.623	65.7KPa	31.3KPa	1.889	2.286	0

Additionally, stress-strain response predicted by the model is plotted together with the Experimental data for both axial and circumferential directions in Figure 3. Good concurrency of model and experimental data indicates the ability of proposed lamellar analysis in describing the mechanical behavior of the aortic media.

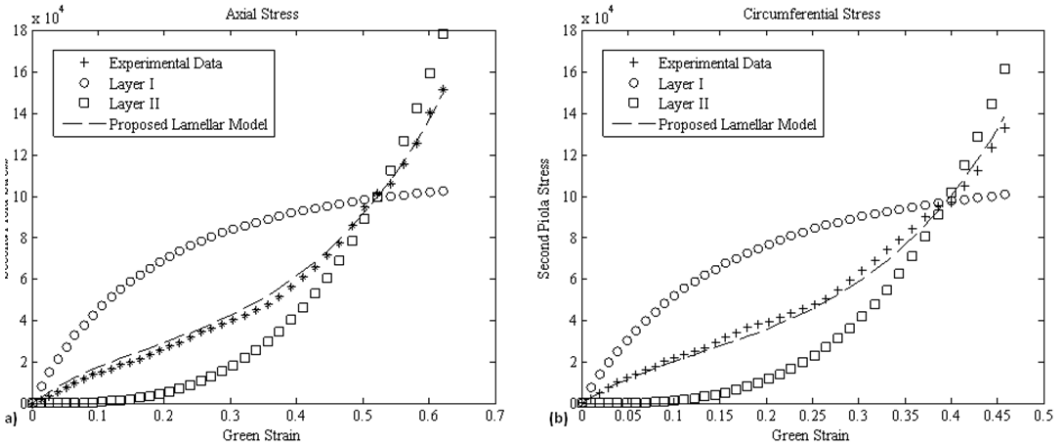

Figure 3: Contribution of Layer I & Layer II on the mechanical behavior of the wall in different Green strains: a) Axial direction b) Circumferential direction

4 DISCUSSION AND CONCLUSIONS

In this study, lamellar model of the arterial media is proposed and related material parameters are obtained. Samples of human thoracic aorta were examined biaxially and the resultant data were utilized to find material parameters. Other required data, i.e. volume fractions of layers, were computed from stained aortic rings and using image processing techniques.

Three distinct phases can be distinguished in the stress-strain relation of the arterial wall tissue [10, 20] (Fig. 3). In the first phase, elastin is the main contributor to the mechanical behaviorand thus the stress strain curve is nearly linear in this region. The second phase (named the "toe region" within the literature) includes engagement of some collagen fibers and can be considered as a transitional phase. In the last phase, most of stiffer collagen fibers are recruited and their behavior becomes dominant. An interesting fact that can be inferred from Fig. 3, is that the strain corresponding to the intersection point in circumferential direction is lower compared to axial direction. One can conclude that more collagen fibers are aligned in circumferential direction than in the axial direction. In recent studies, preferred direction of collagen fibers was inquired and the same dominancy in circumferential direction has been reported [21, 22]. The proposed microstructural model integrates isotropic role of elastin sheets and anisotropic behavior of interstitial layer mainly of collagen fibers simultaneously. Such model gives an appropriate estimate of the aortic media behavior compared to Fung model [18], since it incorporates a separate term (neo- Hookian function assigned to layer I) to follow the initial nearly linear part of the stress- strain curve and the exponential part to model the nonlinear behavior of the tissue within the third phase.

The lamellar model presented here is useful in establishing roles of micro constituents of aortic media on its macro behavior; moreover this model is capable of following arterial mechanical behavior in its functional phases. The intended model, can give some novel insights on the contributions of elastin and collagen to the whole mechanical behavior of the media. Further investigation is needed in terms of experiments and structural elements taken into account in the model. Mechanical properties of elastin sheets, collagen bundles, SMCs and also interaction of this components are not fully understood and need novel experimental

protocols. Exact mechanical properties of these components along with realistic geometry will lead to more accurate models and new aspects of arterial mechanics.

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