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Investigation of Inhomogeneous and Anisotropic Material Behavior of Porcine Thoracic Aorta Using Nano-Indentation Tests

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Abstract

This study investigates the inhomogeneity and anisotropy of porcine descending thoracic aorta in three dimensions using a custom-made nano-indentation technique and a quasi-linear viscoelastic modeling approach. The indentation tests were conducted in axial, circumferential, and radial orientations with about 100 μm spatial resolution. The ratio of the elastic moduli obtained in different orientations was used to quantify the tissue local anisotropy. The distal sections were generally stiffer than the proximal ones in both axial and circumferential indentations. Four distinct layers were identified across the thickness with significantly different mechanical properties. The stiffness of the medial quadrant was significantly lower than all other quadrants in axial indentation. The anisotropic behavior of the tissue was more pronounced in the lateral quadrant of the distal sections. The results of this study can be used to better understand the mechanisms of aorta deformation and improve the spatial accuracy of computational models of aorta.

Graphical abstract

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Conflict of interest statement

There are no conflicts of interest of any kind in the work presented in the manuscript.

Keywords

Thoracic Aorta; Aorta Material Properties; Inhomogeneity; Anisotropy; Nano-indentation; Viscoelasticity

1. Introduction

Characterization of the biomechanical behavior of aortic wall is essential for quantifying the effect of disease on aortic elastic recoil, and also for developing computational models to predict injury and rupture due to pathology or trauma. The focus of previous studies on aorta mechanical behavior has been primarily on the abdominal aorta to address Abdominal Aortic Aneurysm (AAA) (Vorp, 2007) and on thoracic aorta to address Thoracic Aortic Aneurysm (TAA) and Traumatic Aortic Rupture (TAR) (Iliopoulos et al., 2009a; Shah et al., 2006). The finite element method has been the main tool in predicting aortic tissue stresses and strains in aneurysm or injury models (Raghavan et al., 2000; Richens et al., 2004) and while in these models the anatomical geometry of aortic wall is included to a large extent, the material is often simplified to be homogeneous and isotropic. Several studies have shown that the aortic wall is inhomogeneous in the longitudinal (Purslow, 1983; Sokolis et al., 2008; Zeinali-Davarani et al., 2015), circumferential (Choudhury et al., 2009; Iliopoulos et al., 2009b; Kim and Baek, 2011), and radial (Hemmasizadeh et al., 2015; Pena et al., 2015) directions. Also, the circumferential direction has been shown to be generally stiffer than the axial direction (Kim et al., 2012; Marra et al., 2006; Mohan and Melvin, 1983, 1982). These studies, however, are not conclusive because they used different methodologies, species, and spatial resolution. The goal of this study was to use a cohesive methodology to characterize the three-dimensional inhomogeneity as well as anisotropy of thoracic aorta from one set of samples and with high spatial resolution.

The studies that address the inhomogeneity of thoracic aorta mostly used uniaxial and biaxial tests to characterize the mechanical properties. Due to the nonlinear behavior of aorta, the response was divided in either two or three segments based on the range of stress and strain. Regarding the inhomogeneity in the longitudinal direction, there is an agreement that distal sections are stiffer than the proximal ones. This is explained based on the higher ratio of collagen to elastin content in the distal sections determined from X-ray diffraction technique (Roveri et al., 1979) or histology (Sokolis et al., 2008). Additionally, the change in the undulation of collagen fibers in adventitia, with more undulation in the proximal sections, has been associated with this change of stiffness (Zeinali-Davarani et al., 2015). In a study that addresses the inhomogeneity in the circumferential direction, the circumference of samples of human ascending thoracic aorta were divided into four quadrants, which were tested using a biaxial tensile apparatus (Choudhury et al., 2009). This study concluded that the elastic modulus depends on the location of the segment, even though no significant difference was found in the tissue histology. The medial (inner curvature) and lateral (outer curvature) quadrants exhibited the least stiff and the stiffest behavior respectively, in low and high stress states and in both test directions. Iliopoulos et al. (2009b) observed similar behavior based on uniaxial tensile tests on healthy and aneurysmal human ascending thoracic aorta in the longitudinal direction. The higher modulus in the lateral quadrant was

later explained based on the orientation of the extracellular matrix that was more aligned with the longitudinal direction compared to the medial quadrant (Sokolis et al., 2012a). Kim and Baek, (2011) investigated the variations of the biomechanical properties of porcine proximal DTA in the circumferential direction using inflation tests and showed that the posterior quadrant was significantly stiffer than the anterior quadrant.

A few studies have addressed the inhomogeneity of aorta through the wall thickness. In previous studies conducted in our laboratory, the inhomogeneous biomechanical properties of porcine Descending Thoracic Aorta (DTA) in the radial direction and in the medial quadrant were characterized using a nano-indentation technique and a viscoelastic modeling approach (Hemmasizadeh et al., 2015, 2012). Two distinct layers of equal thickness were determined with significantly different material and morphological properties, with an increasing trend in the instantaneous elastic modulus (stiffness), elastic lamella density, total protein, and elastin circumferential percentage from the inner to the outer layers. Sokolis et al. (2012b) and Sokolis (2015) characterized the inhomogeneous layer-specific material properties and residual deformation of human aneurysmal ascending thoracic aorta and their variations with direction and circumferential location using uniaxial tests and showed that the intima and adventitia were stiffer than the media. Pena et al. (2015) found similar results for porcine DTA using uniaxial and biaxial tests and showed that the intima and adventitia were more isotropic than the media in the proximal sections.

In this study, the method of nano-indentation tests introduced in Hemmasizadeh et al. (2012) was expanded to three orientations to characterize the inhomogeneity and anisotropy of porcine DTA in three dimensions, i.e., in the longitudinal, circumferential and radial directions. The spatial resolution is also significantly improved compared to our previously reported results.

2. Materials and Methods

2.1 Tissue Preparation and Testing

Seven fresh porcine aorta specimens were obtained from young pigs (about 6 months old) from a local slaughterhouse (Hatfield Quality Meats, Hatfield, PA). Fat and excessive tissues were removed and six transverse sections (rings), 8–10 mm in length, were excised from the specimens (Figure 1-a). The sections were divided into three groups according to their location along the aortic tree (z-direction) including Upper-DTA (above the 1st intercostal artery), Mid-DTA (between the 1st and the 2nd intercostal arteries), and Lower-DTA (between the 3rd and 4th intercostal arteries) (Hemmasizadeh et al., 2012). In order to investigate the effect of circumferential location (θ -direction), four quadrants, i.e., anterior (ANT), posterior (POST), medial (MED), and lateral (LAT) quadrants, were identified as indicated in Figure 1-b. The wall thickness was measured at the four quadrants for the three section groups. The average (and standard deviation) of the wall thickness was 1.55 (0.54) mm.

Using a custom-made nano-indentation test setup that is described in Hemmasizadeh et al., 2012, indentation tests were performed for material characterization. Briefly, samples were moved upward toward the conical indenter (62.5° semi-vertical angle) with a ramp and hold

displacement with 60 μm indentation depth, 25 ms ramp time and 20 s hold time (Figure 2). All the tests were conducted within eight hours post-mortem.

In order to characterize the material properties in three dimensions, first, axial indentations were conducted on about 10 points along the thickness (r -direction) at four quadrants (Figure 1-c). Next, 5–6 mm rectangular blocks were cut from each section at the medial and lateral quadrants, which were used for circumferential and radial indentations (Figure 1-d). Circumferential tests were also performed on about 10 points along the thickness that corresponded approximately to the axial indentation points. The radial tests were conducted on the lumen side ($r = 0$) and repeated at two points. The spatial resolution of indentation was about 100 μm and the first and last points were about 100 μm from the edge to minimize the boundary effects.

2.2 Formulation

A Quasi-Linear Viscoelastic (QLV) constitutive equation was used to describe the relationship between the indenter force history, $P(t)$, and the indentation depth, $h(t)$, for a conical indenter with the semi-vertical angle of β . The formulations used were:

$$P(t) = \int_0^t G(t - \tau) \frac{\partial P^e(h)}{\partial h} \frac{\partial h}{\partial \tau} d\tau \quad (1)$$

$$P^e = \frac{1}{\alpha} \frac{2E_0 \cot(\beta)}{\pi(1 - \nu^2)} h^2 \quad (2)$$

$$G(t) = G_\infty + \sum_{i=1}^4 G_i \exp(-\beta_i t) \quad (3)$$

in which $G(t)$ is the reduced relaxation function describing the time dependency of the response and $P^e(h)$ is the instantaneous elastic force function. E_0 is the instantaneous elastic (Young's) modulus, and ν is the Poisson's ratio which was assumed to be 0.49. α is the correction factor introduced in Hemmasizadeh et al. (2012) that accounts for the non-ideal geometry of the indenter, imperfections in contact area, and the nonlinearity of system compliance among other factors. For the indentation depth used in this study ($h = 60 \mu\text{m}$), $\alpha \approx 3$. G_i are the reduced relaxation amplitudes and β_i are the corresponding decay rates that were assumed to be constants and spanned the four orders of magnitude of time that were in the experiments ($\beta_{1..4} = 0.1, 1, 10, 100 \text{ s}^{-1}$). For statistical analysis, $G(t)$ and the elastic modulus $E(t) = E_0 G(t)$ were calculated and compared at specific times of relaxation corresponding to $t = 0.01, 0.1, 1, 10$, and ∞ .

The QLV material parameters (Equations 2 and 3) were determined by comparing the experimental data with the model and a least square optimization for G_i and E using an in-house MATLAB code. The relaxation data was resampled logarithmically to give

approximately equal weights to different time scales. The moduli measured from indentations conducted in the axial, circumferential, and radial orientations are represented by subscripts A , C , and R , respectively (Figure 1-c and d).

2.3 Statistical Analysis

The statistical analysis was performed, using JMP SAS, Version 11, Cary, NC. To determine whether the elastic moduli were significantly dependent on the orientation of indentation (A , C and R), as well as the three-dimensional locations, z (Upper-, Mid-, and Lower-DTA), θ (ANT, LAT, MED, and POST quadrants), and r (11 regions), a 4-factor multi-variate analysis of variance (MANOVA) followed by Tukey's HSD *post-hoc* test was conducted. Additional *post-hoc* contrast analysis was performed to evaluate whether adjacent regions in the radial direction can be grouped in significantly distinct layers. The effect of relaxation was studied as an additional factor with repeated measures. In order to eliminate the inter-specimen variability in comparing the data, all the analyses were paired. The results are presented as mean (standard deviation) and standard error of the mean is used for the error bar in the graphs. The threshold for the p -value to indicate statistical significance was considered at 0.05.

3. Results

The samples wall thickness results (average and standard deviation) are summarized Table 1 which shows that the values generally decreased from proximal to distal sections and were dependent on the quadrant. The thickness in Lower-DTA was significantly smaller than Upper- and Mid-DTA ($p < 0.0001$). The highest and lowest thicknesses were in the ANT and POST quadrants and the two were significantly different ($p = 0.020$).

Figure 2 shows representative force and displacement time history curves of a typical indentation test and provides a comparison between the experimental data with the corresponding QLV model. This comparison demonstrates that the force predicted by the viscoelastic model closely followed the experimental force data. The transient vibration observed in the force data (due to the sample natural frequencies) was relatively small and was ignored in the viscoelastic characterization.

3.1 Tissue Anisotropy

Comparison between E_C and E_A , when pooled for z , θ , and r directions, showed that they are significantly different (Figure 3-a) with $E_C > E_A$ at all relaxation times ($p < 0.001$). Specifically, on average, the circumferential direction was 13% and 25% stiffer than the axial direction for $E(0)$ and $E(\infty)$ respectively. This means that $G(t)$ and E_0 both showed anisotropy. The difference between the circumferential and longitudinal instantaneous moduli was further studied when the effects of z -, θ -, and r -directions were separated. When the effect of z was separated, the anisotropy was significant in Mid- and Lower-DTA ($p < 0.002$) with $E_C(0)/E_A(0) = 1.17$ in both sections. However, anisotropy was not significant in the Upper-DTA ($p = 0.300$) with $E_C(0)/E_A(0) = 1.02$ (Figure 3-b). When the effect of θ was separated, the anisotropy was significant at LAT quadrant ($p < 0.00002$) with $E_C(0)/E_A(0) = 1.25$ but not at MED quadrant ($p = 0.167$) with $E_C(0)/E_A(0) = 1.03$ (Figure 3-c). At the

lumen side ($r = 0$), the elastic relaxation function in the three directions were compared (Figure 3-d) and it was found that E_C and E_R were indistinguishable ($p = 0.461$) and E_A was significantly lower ($p < 0.0002$) at all times. Quantitative comparison between the instantaneous and steady state moduli showed that $E_C(0)/E_A(0) = 1.24$, $E_R(0)/E_A(0) = 1.29$, $E_C(\infty)/E_A(\infty) = 1.31$, and $E_R(\infty)/E_A(\infty) = 1.10$. As can be seen, anisotropy in this case was more pronounced in E_0 and less pronounced in $G(t)$. The effect of r -direction on the anisotropy was more pronounced in Layer I ($p < 0.001$) with $E_C(0)/E_A(0) = 1.29$.

3.2 Tissue inhomogeneity

To quantify the tissue inhomogeneity, the changes in E_A , E_C , and E_R based on the three-dimensional location were studied separately. The pooled values of E_0 and G_i for each dimension are summarized in Table 2 and are explained below. To determine the values in each row of this table, the other two dimensions were averaged. Table S1 in supplementary material provides more detailed data of the viscoelastic properties grouped by section, quadrant, and layer for axial, circumferential, and radial indentations.

3.2.1 Effect of r -direction—The position along the r -direction (thickness) was normalized, where $r = 0$ and 1 represented the innermost and outermost layers, respectively and categorized into 11 regions as $r_0 = 0..0.05$, $r_1 = 0.05..0.15$, ..., and $r_{10} = 0.95..1.0$. In order to clarify how E_A and E_C depend on r , the elastic relaxation functions were normalized based on the average E_A for each specimen. This normalization eliminated the inter-specimen variability and showed that 4 layers could be distinguished. The innermost layer (Layer I: r_0-r_1), with a relatively high stiffness, included the elastic lamina interna (Mills, 2007). The next two layers (Layer II: r_2-r_5 and Layer III: r_6-r_9), were named inner and outer media and had relatively low and high stiffness respectively. The last layer (Layer IV: r_{10}), corresponded to the adventitia and showed the lowest stiffness. The variations of the normalized E_A and E_C at $t = 0$, designated by $A(0)$ and $C(0)$, are depicted in Figure 4 overlaid on a representative section of porcine aorta. The aorta section was stained with a Verhoeff-van Gieson protocol that showed elastin fibers as dark brown and collagen fibers as light red. Contrast analysis results showed that Layer I was stiffer than Layer II at all times ($p = 0.034$ for E_A , $p = 0.005$ for E_C). Layer III was also stiffer than Layer II but this result was not significant for E_C ($p = 0.019$ for E_A , $p = 0.140$ for E_C). If $t = 0$ is excluded, then the difference between Layer III and Layer II for E_C becomes statistically significant ($p < 0.05$). Layer IV was significantly more compliant than all other Layers at all times ($p < 0.0001$).

The comparison between $G_A(t)$ showed that Layer I and Layer IV were significantly different from Layers II and III combined ($p = 0.003$ and $p = 0.009$ respectively). Layer I showed a significantly different relaxation behavior compared to Layer IV ($p = 0.0005$). In the case of $G_C(t)$, the difference was only significant between Layer I and Layers II and III combined ($p = 0.033$).

3.2.2 Effect of z -direction—There was generally an increase in the elastic moduli from proximal to distal aortic sections in all three indentation orientations (Table 2). The lower-DTA was 12%, 18%, and 26% stiffer than the Upper-DTA in axial, circumferential, and radial indentation, respectively. In terms of E_A , Upper-DTA was not statistically different

from Mid- and Lower-DTA ($p = 0.069$ and 0.058 , respectively); however Mid-DTA was statistically less stiff than Lower-DTA ($p = 0.0006$). When the effect of relaxation time was separated, Mid-DTA was significantly more compliant than Upper-DTA at $t = \infty$ ($p = 0.014$). In the case of E_C , Mid-DTA was not statistically different from Upper- and Lower-DTA ($p = 0.242$ and 0.067 , respectively) while Upper-DTA was statistically less stiff than Lower-DTA ($p = 0.002$). Lower-DTA was significantly stiffer than Mid- and Upper-DTA at $t = \infty$ ($p < 0.05$). In the radial indentation results, the increases in versus z were all statistically significant at all relaxation times (Upper- vs Mid-DTA: $p = 0.035$; Upper- vs Lower-DTA: $p < 0.0001$; Mid- vs Lower-DTA: $p = 0.012$).

The comparison between $G_A(t)$ showed that Upper-DTA was statistically different from Mid- and Lower-DTA ($p = 0.003$ and 0.021 , respectively); however Mid-DTA was not statistically different from Lower-DTA ($p = 0.725$). Specifically, Lower-DTA showed more relaxation compared to Upper-DTA ($p = 0.010$). In the case of $G_C(t)$, the difference was only significant between Mid- and Lower-DTA ($p = 0.012$).

3.2.3 Effect of θ -direction—The axial indentation results (Table 2) showed that was E_A significantly more compliant in the MED quadrant ($p < 0.005$) at all relaxation times. However, a more detailed analysis showed that this effect was only significant in the Mid- and Lower-DTA ($p < 0.007$) but not in Upper-DTA ($p = 0.117$). For the other two orientations, comparison between LAT and MED quadrants showed that they are statistically indistinguishable ($p = 0.453$ for E_C and $p = 0.381$ for E_R). More detailed analysis showed that for E_C , the MED quadrant in Upper-DTA was, in fact, significantly stiffer than LAT ($p = 0.0003$), e.g., for $E_C(0)$ the MED is 23% stiffer than LAT. In terms of viscoelasticity, the POST quadrant showed a significantly different relaxation in axial indentation than ANT and LAT quadrants ($p = 0.023$ and $p = 0.010$, respectively).

4. Discussion

The material behavior of porcine DTA was characterized using a nano-indentation technique and QLV modeling. Using this method, the inhomogeneity or spatial resolution of the material properties, compared with existing data that are primarily obtained from uniaxial and biaxial tests (e.g., Sokolis et al., 2008; Zeinali-Davarani et al., 2015), was substantially improved. Particularly, the material properties were characterized across the aorta thickness in 10 locations that to the best of our knowledge has not been done before. The spatial resolution was approximately $100 \mu\text{m}$ since the test area was a circle with $62 \mu\text{m}$ diameter and the distance between two test sites was $100 \mu\text{m}$.

In order to quantify the tissue local anisotropy, the indentation tests were conducted in two (or three at $r = 0$) perpendicular orientations on the same sample. The material elastic moduli that were obtained using Equation (2), i.e., E_A , E_C , and E_R , are based on the equivalent isotropic solution, which is shown to be valid for axisymmetric indenters and an anisotropic half-space (Vlassak et al., 2003). They are not, however, equivalent to the cylindrical anisotropic moduli in the longitudinal (E_z), circumferential (E_θ), and radial (E_r) directions. The tissue in the vicinity of the indenter undergoes a complex state of stress (Fischer-Cripps, 2007). However, since the compressive stress in the direction of indentation is the dominant

component, it is expected that E_A would be more affected by E_z , E_C by E_θ , and E_R by E_r . The reported E_i in this study are representatives of the moduli in the toe region (small deformation). One expects that for small deformations, the material behavior of aorta be similar in compression and tension.

The mean stress experienced by the tissue under indentation loading in this study is approximately 60 to 90 kPa. The corresponding obtained $E_A(0)$ is consistent with the range of 80–170 kPa reported by Sokolis et al. (2008) for axial elastic modulus of porcine DTA in the physiologic stress region (40–80 kPa). The results of the current study for $E_A(0)$ are on average 64% stiffer than the values reported by Hemmasizadeh et al. (2012) which can be explained by the tissue nonlinearity with respect to the depth of indentation. The indentation depth in the current study (60 μm) was 50% higher than theirs (40 μm).

The majority of previous studies have also reported that human and porcine DTA exhibit higher stiffness in the circumferential direction compared to the axial direction although the ratios vary considerably (e.g., Peña et al., 2015; Purslow, 1983; Shah et al., 2006). The effect of anisotropy was more pronounced in the distal sections which is in agreement with the results of Zeinali-Davarani et al. (2015) where a similar trend was observed. The degree of anisotropy was more pronounced in Layer I which is consistent with the trend observed by Peña et al., (2015) where the intima was more anisotropic than the media as going from proximal to distal sections.

There was an increasing trend observed in E from proximal to distal sections which is consistent with previous studies (Peña et al., 2015; Purslow, 1983; Sokolis et al., 2008; Zeinali-Davarani et al., 2015) where this change was associated primarily with the collagen content and their undulation. Zeinali-Davarani et al. (2015) found that in distal sections compared with proximal ones, the collagen fibers had a larger contribution to the mechanical properties compared to elastin and an earlier engagement in the load bearing process.

The fact that the medial quadrant was significantly more compliant than the other quadrants in axial indentations is in agreement the results of Choudhury et al., (2009) for human ascending aortic tissue (58 $\pm$ 11 yrs).

The aorta wall thickness was divided into more sub-layers compared to Hemmasizadeh et al. (2012), which was focused only the axial indentation at the medial side. In order to compare the findings of the current study with their results, the average values of $E_A(0)$ at the medial quadrant were found in the inner half ($r_1 - r_5$) and the outer half ($r_6 - r_9$). The outer half found to be 12% stiffer than the inner layer (113.66 (32.27) vs 101.58 (26.69) kPa), which was similar to what they found (15% increase). This increase in the stiffness of the media from the inner layers toward the outer ones has been shown to be strongly related to certain wall morphological quantities, i.e., the elastic lamellae density, the total protein content and the elastin circumferential percentage of fibers (Hemmasizadeh et al., 2015).

In our results for across the thickness, two radial locations, r_0 and r_9 , showed relatively higher stiffness, which can be associated with the elastic lamina interna and externa respectively (Oie et al., 2009). In the last radial location, r_{10} , that encompasses the adventitia, the stiffness was decreased significantly. Zeinali-Davarani et al. (2015) showed

that the collagen fibers in media, regardless of their location along the aorta, are recruited gradually from the beginning of loading, while in adventitia, they start to become load bearing with a delay. This delay can partly explain why in nano-indentation tests, where the deformations are small, the adventitia would yield a lower stiffness. The results reported by Peña (2015), however, show that the adventitia was generally stiffer than the media. It should be noted that the outer layer in their study was 0.67 ± 0.15 and 0.55 ± 0.03 mm for proximal and distal DTA, respectively, and therefore thicker than layer r_{10} , which suggests that some of the media, especially the elastic lamina externa may have been included in their outer layer.

The distributions of stresses in aorta can be highly affected by the residual stresses present in the tissue (Han and Fung, 1996). In the test method used here, the axial residual stress was clearly released but in the axial indentation tests, the circumferential residual stress was preserved. In case of circumferential and radial indentations, the circumferential residual stress was also released to expose the surfaces. It is therefore possible that the anisotropic ratio between E_C and E_A was underestimated. A recent study (Sokolis, 2015) has shown that the residual deformation of aorta depends on the radial and circumferential locations. Including the effect of residual stress in the material model was beyond the scope of this work and should be considered in the future.

Aging can significantly affect the aortic tissue anatomy and material properties. It has been shown that after age 60, the human aorta becomes stiffer and more anisotropic due to the changes in the tissue microstructure (Haskett et al., 2010). In this study, porcine aorta was used as a surrogate for young and healthy human aorta. For this reason, the sections of DTA contained mostly media and showed no arterial wall disease. The methodology presented here needs to be applied to human DTA tissue in the future to characterize the effects of aging, diseases such as atherosclerosis, and changes in the microstructural anatomy.

The results of this study deal with the material behavior of aorta in the initial linear (toe) region of its generally nonlinear mechanical response. However, it is expected that the trends that were observed in the linear region would continue in the nonlinear region although, perhaps, with different ratios. For example, our results showed that the medial quadrant in axial indentation was more compliant compared to the other quadrants. As the failure in aorta is primarily strain based (Mohan and Melvin, 1983), this result would predict that in axial loading, DTA failure should start from the medial side. This prediction agrees with the clinical observations in patients who experienced TAR (medial and inner: Bertrand et al., (2008)) and the experimental work that show axial loading of aorta as one of the primary mechanisms of rupture (Shah et al., 2006).

5. Conclusions

Using a custom-made nano-indentation technique, the inhomogeneous and anisotropic mechanical properties of porcine descending thoracic aorta were characterized with a quasilinear viscoelastic model. The comparison of $E(t)$ showed that the circumferential direction is stiffer than the axial direction and this difference was more pronounced in the distal sections, at the lateral quadrant, and in the inner radial layer. The elastic moduli

increased from proximal to distal sections for all three indentation orientations and the medial quadrant showed the most compliant behavior compared to other quadrants in axial indentations. Four distinct layers were distinguishable based on the variations of the elastic moduli across the aortic thickness that were matched with the anatomical features.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nomenclature

α	Indentation Correction factor
β	Indenter Semi-vertical angle
β_i	Viscoelastic decay rates
ν	Poisson's ratio
E_0	Instantaneous elastic modulus
$E(t)$	Elastic Relaxation function
G_i	Reduced Relaxation amplitudes
$G(t)$	Reduced relaxation function
$h(t)$	Indentation depth history
$P(t)$	Indentation force history
P^e	Instantaneous elastic force
r	Radial direction
t	Time
z	Longitudinal direction
θ	Circumferential direction

Subscript

A	Axial indentation
C	Circumferential indentation
R	Radial indentation

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Highlights

- Inhomogeneity and anisotropy of porcine aorta were studied by nano-indentation.
- The effect of anisotropy was more pronounced in distal sections.
- The distal sections were stiffer than the proximal ones.
- The medial quadrant showed the most compliant response in axial indentation.
- Four distinct layers were identified across the aortic thickness.

Figure 1.

a) Locations of excised sections (rings) along the descending thoracic aorta (DTA). b) Top view of a cylindrical section with the medial, anterior, lateral, and posterior quadrants marked. c) Schematic representation of the locations of axial indentation, d) Schematic diagram of the locations for radial and circumferential indentations on excised blocks at the medial and lateral quadrants.

Figure 2.

Force and displacement time history curves from a typical indentation test and comparison with the corresponding QLV model.

Figure 3.

Tissue anisotropy based on the material properties obtained from indentation tests at different orientations. a) comparison of pooled relaxation moduli from axial and circumferential indentation. Variation of $E(\theta) = E_0$ with b) z-direction and c) θ -direction. d) comparison of pooled relaxation moduli at $r = 0$.

Figure 4.

Variation of average normalized elastic moduli along the thickness of the aorta overlaid on a representative stained section of porcine DTA. The innermost layer (Layer I: r_0-r_1), with a relatively high stiffness, included the elastic lamina interna. The next two layers (Layer II: r_2-r_5 and Layer III: r_6-r_9), were named inner and outer media and had relatively low and high stiffness respectively. The last layer (Layer IV: r_{10}), corresponded to the adventitia and showed the lowest stiffness. Axial (left axis) and circumferential (right axis) indentation results are shown in yellow and cyan, respectively. Verhoeff-van Gieson protocol is used for staining which shows elastin fibers as dark brown and collagen fibers as light red.

Table 1

Aortic wall thickness grouped by quadrant and section.

θ – Quadrant	Thickness (mm)	z – Section	Thickness (mm)
ANT	1.72 (0.67)	Upper-DTA	1.87 (0.33)
LAT	1.51 (0.51)	Mid-DTA	1.82 (0.42)
MED	1.59 (0.45)	Lower-DTA	0.99 (0.29)
POST	1.33 (0.60)		

Table 2

Summary of instantaneous elastic modulus and relaxation amplitudes grouped by section, quadrant, and layer for a) Axial, b) Circumferential, and c) Radial indentations.

	$E_0(\text{kPa})$	G_1	G_2	G_3	G_4	G_∞
a) Axial Indentation						
<i>r</i> – Layer						
Layer I	123.73 (37.15)	0.07 (0.02)	0.10 (0.01)	0.06 (0.02)	0.46 (0.05)	0.30 (0.05)
Layer II	114.10 (37.51)	0.06 (0.02)	0.10 (0.02)	0.07 (0.02)	0.47 (0.04)	0.29 (0.05)
Layer III	123.99 (42.51)	0.06 (0.02)	0.10 (0.01)	0.06 (0.02)	0.47 (0.04)	0.30 (0.05)
Layer IV	94.76 (27.68)	0.06 (0.01)	0.11 (0.01)	0.06 (0.02)	0.49 (0.04)	0.28 (0.05)
<i>z</i> – Section						
Upper-DTA	118.44 (34.25)	0.07 (0.02)	0.10 (0.02)	0.06 (0.02)	0.46 (0.04)	0.31 (0.05)
Mid-DTA	112.11 (36.46)	0.06 (0.02)	0.10 (0.01)	0.07 (0.02)	0.47 (0.04)	0.30 (0.04)
Lower-DTA	132.49 (44.48)	0.07 (0.02)	0.10 (0.01)	0.06 (0.02)	0.47 (0.05)	0.29 (0.05)
θ – Quadrant						
ANT	123.26 (40.35)	0.06 (0.01)	0.10 (0.01)	0.06 (0.02)	0.47 (0.04)	0.30 (0.04)
LAT	126.04 (39.88)	0.06 (0.02)	0.10 (0.01)	0.07 (0.02)	0.47 (0.04)	0.30 (0.05)
MED	107.22 (29.87)	0.07 (0.02)	0.10 (0.02)	0.07 (0.02)	0.47 (0.04)	0.29 (0.05)
POST	125.46 (51.64)	0.06 (0.02)	0.10 (0.01)	0.07 (0.02)	0.48 (0.05)	0.29 (0.05)
b) Circumferential Indentation						
<i>r</i> – Layer						
Layer I	154.00 (56.6)	0.07 (0.03)	0.10 (0.02)	0.04 (0.02)	0.48 (0.05)	0.32 (0.04)
Layer II	125.49 (46.74)	0.06 (0.01)	0.10 (0.01)	0.05 (0.02)	0.46 (0.05)	0.32 (0.05)

	$E_0(\text{kPa})$	G_1	G_2	G_3	G_4	G_∞
Layer III	132.48 (43.42)	0.06 (0.01)	0.10 (0.01)	0.05 (0.03)	0.45 (0.04)	0.34 (0.05)
Layer IV	100.32 (29.13)	0.08 (0.02)	0.11 (0.02)	0.05 (0.02)	0.46 (0.04)	0.29 (0.06)
z – Section						
Upper-DTA	121.32 (41.66)	0.07 (0.02)	0.10 (0.02)	0.06 (0.04)	0.45 (0.05)	0.32 (0.06)
Mid-DTA	131.77 (43.86)	0.06 (0.01)	0.10 (0.01)	0.05 (0.02)	0.47 (0.05)	0.32 (0.04)
Lower-DTA	142.98 (54.27)	0.06 (0.01)	0.10 (0.01)	0.05 (0.02)	0.45 (0.03)	0.34 (0.04)
θ – Quadrant						
LAT	130.42 (45.39)	0.06 (0.02)	0.10 (0.02)	0.05 (0.02)	0.46 (0.05)	0.33 (0.05)
MED	134.05 (49.91)	0.07 (0.02)	0.10 (0.01)	0.05 (0.03)	0.46 (0.04)	0.33 (0.05)
c) Radial Indentation (lumen side)						
z – Section						
Upper-DTA	141.41 (36.74)	0.13 (0.03)	0.14 (0.03)	0.08 (0.02)	0.39 (0.05)	0.26 (0.04)
Mid-DTA	159.47 (39.07)	0.13 (0.02)	0.15 (0.02)	0.09 (0.01)	0.39 (0.03)	0.25 (0.03)
Lower-DTA	177.88 (42.05)	0.14 (0.02)	0.13 (0.02)	0.08 (0.02)	0.39 (0.04)	0.27 (0.04)
θ – Quadrant						
LAT	163.68 (45.18)	0.13 (0.02)	0.14 (0.02)	0.08 (0.02)	0.4 (0.05)	0.25 (0.03)
MED	155.50 (37.92)	0.14 (0.02)	0.14 (0.03)	0.08 (0.02)	0.39 (0.03)	0.26 (0.04)