CHARACTERIZATION OF HEALTHY AND DISEASED HUMAN ASCENDING AORTA TISSUE

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Abstract: Little information is available on the structure or mechanical properties of the human ascending aorta (AA). Most studies to date have assumed homogeneous tissue mechanical properties. The objective of this study was to investigate the local variation in AA tissue structure and mechanical properties. Healthy and pathologic tissue samples of human AA were obtained at autopsy and surgical pathology. Each aortic ring was sectioned into quadrants; anterior, posterior, medial (inner curvature) and lateral (outer curvature). Samples from each quadrant were processed for histological analysis and biaxial tensile testing. The results from this study indicate that regional differences are present in both healthy and diseased human AA tissue. Overall, the medial quadrant contained significantly more elastin and mechanically, it was the thickest, least stiff and most likely to fail in comparison to the other quadrants. The assumption of homogeneity in AA tissue properties may not be a valid one.

Introduction

The ascending aorta (AA), the primary artery of the circulatory system; serves as the conduit for blood exiting through the left ventricle outflow tract (LVOT). As a result, the biomechanics within the AA characterize the pressure and flow for the entire vascular system. It is uniquely constructed to withstand the large fluid and tissue stresses occurring in the LVOT.

Changes in the AA dynamics that arise with age and/or disease can lead to cardiovascular complications and death. The importance of proper functioning of the AA is underscored by the high morbidity and mortality rates associated with diseases of the AA. The major pathologies of the AA include dissections, aneurysms and atherosclerosis. All of these vascular diseases involve the deterioration of the structural components of the blood vessel wall, referred to as medial degeneration.

The aorta is a major determiner of vascular compliance, contributing to 60-70% of total systemic compliance [1]. In the aortic wall, it is the structured medial layer consisting of elastic plates, interspersed collagen and smooth muscle cells that is responsible for the mechanical properties of the AA. The passive biomechanical response of vascular tissue is complex; it depends on the amount of the structural proteins such as collagen and elastin, their interaction and morphological arrangement in the arterial wall.

Although much has been learned about the pathology of aortic disease, past studies have assumed homogeneous and isotropic AA tissue properties. To date almost nothing is known about the heterogeneity of aortic disease. However, it has been hypothesized that pathologies which affect the LVOT result in focal AA tissue remodeling. The work conducted in this study investigated this hypothesis; the objective was to identify whether regional differences exist in the local properties of both healthy and pathologic human AA tissue (tricuspid and bicuspid tissue patients) and to compare the different tissue types. Specifically,

1. To identify if regional mechanical properties vary using biaxial tensile tests;
2. To define the variation in the content of elastin, collagen and SMCs from histological sections.

The information obtained was used to compare the structure and composition of the tissue with the observed mechanical response.

Materials and Methods

All tissue specimens were obtained under informed consent following the guidelines of the Tri-Council Policy Statement. Pathologic AA tissue was acquired from patients scheduled for cardiac surgery at the Montreal Heart Institute (MHI), requiring replacement of the AA (due to valvular disease, annulo-aortic ecstasia, atherosclerosis, aneurysms or dissections). Healthy AA tissue was obtained from autopsy from both the MHI and the University Health Network (UHN) Toronto General Hospital. All of the collected tissues were given an anonymous identification number.

Tensile Tests: A total of 11 surgical samples and 4 autopsy samples for mechanical testing were collected from the MHI and the UHN Toronto General Hospital. All of these samples consisted of a tubular section of the AA, Figure 1. The tissue was obtained from 9 men and 6 women with ages ranging from 30 to 74 years old.

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Figure 1: Gross photograph of a 42 year old male healthy human AA segment.

The intact specimen was grossed by taking digital pictures of the specimen, measuring the proximal and distal diameter of the aortic ring and noting the presence of, if any, remarkable features (such as atherosclerotic lesions). The tissue was then placed in the saline solution in a closed container and refrigerated at 4°C.

The tissue sample was placed on a surgical table, oriented such that the proximal end was located below the distal end. In this position, the quadrants are identified as the front quadrant as the anterior, the back quadrant as the posterior, the quadrant on right (the smallest section) as the medial and the quadrant to the left (the largest section) as the lateral. Using dissection scissors, representative tissue samples of the medial (inner curvature), anterior, lateral (outer curvature) and posterior quadrants were taken. The tissue was cut into square 1.5 cm by 1.5 cm samples with the edges aligned with the circumferential and axial directions of the aorta. The dimensions of the samples were taken and recorded using an electronic digital caliper (± 0.01 mm). Three measurements were taken of each of the circumferential and axial lengths and the thickness of the sample and averaged.

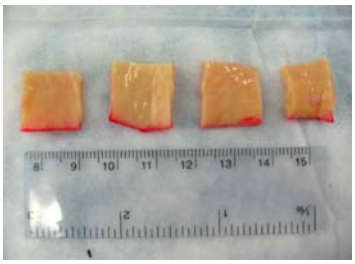

Figure 2: Sample pieces taken from each quadrant of a human aortic ring. Red India ink was used to stain the proximal circumferential edge of each sample.

The AA specimens were subjected to tensile testing with a constant strain rate using the EnduraTEC *elf*® 3200 biaxial tensile tester system supplied with WinTest® software (Minnesota, USA). The tissue was attached to the tester with 3-0 (0.2 mm diameter) silk sutures with pledgets used for reinforcement and floated in a saline bath at room temperature.

The tensile test was equi-biaxial with a constant strain rate of 0.1 mm/sec. The tissue was subjected to 13 loading and unloading cycles with a displacement of 5 mm. The first 10 cycles were used to precondition the tissue. The final three cycles were considered the experimental runs; only the data following preconditioning is reproducible. A final stretch resulting in a 12 mm displacement was then applied to bring the tissue to failure. The test, consisting of 13 cycles and a final stretch, lasted 20 minutes. For the duration, data was acquired at a rate of 4 Hz to collect continuous load cell measurements of force and transducer measurements of displacement.

Engineering stress-strain curves were generated to investigate the circumferential mechanical properties of healthy and pathologic AA tissue, Figure 3.

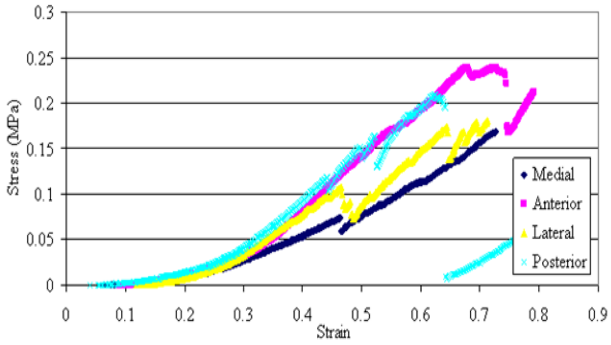

Figure 3: Example of stress-strain curves in the circumferential direction obtained from the final stretch for tissue. Each quadrant response of an aortic ring of a 58 year old male is plotted. The same graph was also plotted for the axial direction. The effect of tissue tearing can be seen in the discontinuity of the curves.

Histological Analysis: An additional piece of tissue (0.5 x 1.5 cm) adjacent to each sample used for the mechanical tests was also taken for histological processing. The tissue was placed in labelled histology cassettes in a closed container filled with 4% neutral buffered formalin solution to fix the tissue.

In addition, microscope slides of 18 past surgical samples and 3 autopsy samples were obtained from the UHN General Hospital MHI; resulting in a total of 37 specimens used in the histological analysis. The additional AA samples were obtained from 18 men and 4 women, with ages ranging from 32 to 83 years old.

The tissue samples were processed by the histology department at the MHI. Slides were prepared from the fixed tissue in formalin. The tissue was embedded in paraffin wax so that thin sections (approximately 3µm) could be cut for mounting onto microscope slides. The specimens were then stained with the Movat Pentachrome stain so that the different components of the medial layer of the tissue could be distinguished.

For each sample, 4 Movat pentachrome stained histology slides (one from each quadrant) were obtained. The slides were examined with a Leitz Diaplan upright microscope through a 40x objective. Digitized images were obtained with a Leica DC 300 digital camera affixed onto the microscope. A semi-quantization of the tissue composition was obtained using a program written in MATLAB that counted the

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pixels by colour and divided this number by the total pixels present in the digital image. For each patient, areas of known content were used to set the colour threshold for each component. The components of interest were elastin (black), collagen (yellow) and SMCs (red), Figure 4. The remainder of the components: mucopolysaccharides, interstitial fluids and fat (void spaces) were grouped as "other". Replicate readings were taken for statistical purposes.

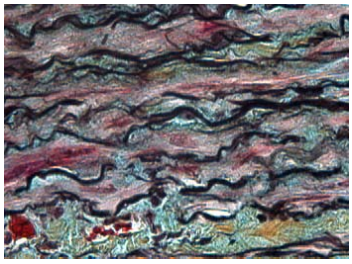

Figure 4: Movat pentachrome stain was used to distinguish elastin (black), collagen (yellow) and smooth muscle cells (red). This picture is an example of one of nine taken for a given histological slide; this one is from the anterior quadrant for the region closest to the adventitia.

Stastistical Analyses: Statistical analyses were carried out using GraphPad Prism version 4.01 (GraphPad Software, San Diego, California). All statistics are presented as mean values ± standard deviation (SD). Student *t*-tests and both one way and two way analysis of variance (ANOVA) were used. Bonferroni's multiple comparisons post tests were used to identify which groups were significantly different with P values <0.05 considered statistically significant. The spread of the data sets was illustrated with Box and Whisker plots by displaying the upper-quartile, inter-quartile and lower-quartile range (25%, 50% and 75%, respectively).

Results

For the tensile tests, 11 surgical samples of pathologic tissue (from surgery) and 4 samples of healthy tissue (from autopsy) were used. The pathologic samples were sub-grouped by aortic valve type as either bicuspid (BAV) or tricuspid (TAV). The aim was to investigate whether the aortic valve type was a factor in the circumferential mechanical response of the tissue, summarized in Table 1.

Table 1: Patient characteristics for biomechanical analysis. Pathologic tissue was grouped according to aortic valve type. BAV: bicuspid aortic valve and TAV: tricuspid aortic valve.

	Tissue Type		
	Healthy	**Pathologic**	
Patient Traits	TAV (*n*=4)	BAV (*n*=6)	TAV (*n*=5)
Mean Age	58 ± 20	57 ± 10	58 ± 9
M/F Gender	1/3	5/1	3/2

Tissue Thickness: The mean values for the tissue thickness of each quadrant for all of the samples were compared to investigate the general population tissue thickness characteristics. Significant differences (P=0.018, one way ANOVA, Bonferroni post test) were detected in the quadrant wall thicknesses between the medial vs. the lateral and posterior quadrants, Figure 5. The medial quadrant contained the thickest tissue.

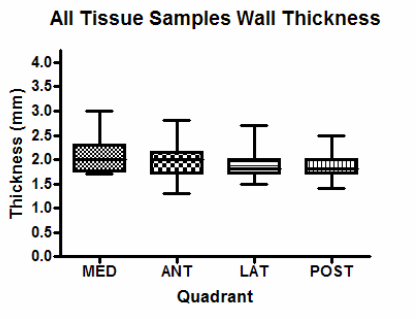

Figure 5: AA wall thickness comparison between the quadrants for all tissue. The medial quadrant is significantly thicker than the lateral and posterior quadrants. MED: medial, ANT: anterior, LAT: lateral and POST: posterior quadrants.

Similar trends were noted with the inclusion of tissue type in the analysis. Significant regional differences in tissue thickness were detected in healthy tissue between the medial and lateral quadrants and for pathologic tricuspid tissue between the medial and posterior quadrants. However, pathologic bicuspid tissue did not exhibit any local variation in thickness. Furthermore, pathologic bicuspid tissue had the thinnest tissue overall with a P value <0.0001 (two way ANOVA).

Mechanical Properties: For the statistical analyses, elastic moduli were fit to the final stretch stress-strain curves (for each quadrant of tissue) in both the low and high stress regions. The low stress elastic moduli (Ee) is associated with the elastin content of the tissue and the strain hardening region elastic moduli (Eec) is related to both the elastin and collagen content of the tissue. These two regions were selected in the loading

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curve; similar to the technique used by Vorp in the investigation of aortic aneurysms [2].

General statistics were performed to elucidate any trends from the general population (all groups lumped). Column statistics and one way ANOVA were performed to investigate the directional variation of the elastic moduli for the quadrants of the tissue. The mean values along with the standard deviation amongst the tissue are provided, Table 2. There were no statistical differences in either moduli for the axial or circumferential direction when the entire patient population was included.

Table 2: General population mean elastic moduli ± SD for both the circumferential and axial direction.

	Axial	**Circumferential**
E_e (MPa)	0.10 ± 0.05	0.07 ± 0.04
E_{ec} (MPa)	0.61 ± 0.41	0.49 ± 0.30

With the inclusion of tissue type and circumferential position in the analyses, statistical differences in the circumferential low stress slope (Ee) were observed, Figure 6. The differences were detected for both the circumferential position and tissue type with respective P values of 0.0319 and 0.0047 (two way ANOVA). Although the Bonferroni tests could not detect where the differences occurred, it was observed that the pathologic tissue from tricuspid patients had, on average, the lowest slope values. In addition, all three groups demonstrated similar circumferential variation, with the medial and anterior quadrants having the smallest slope values.

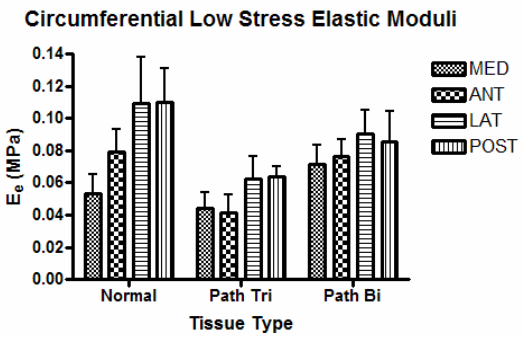

Figure 6: Circumferential low stress slope (Ee) comparison in healthy, pathologic tissue from bicuspid and tricuspid aortic valve patient tissue. The moduli varied for different circumferential positions and tissue type.

Though not statistically significant, a similar quadrant variation was also noted in the circumferential high stress moduli. Comparisons between the groups for the moduli in the axial direction did not show any significant differences.

To expose any general trends, the stiffest tissue quadrant and the occurrence and location of failure was also noted for each of the clinical samples tested. It was observed that in healthy tissue, the anterior quadrant failed every time. In addition, it was observed that regardless of the tissue type, failure generally occurred most often in the medial quadrant.

Histological Analysis: The grouping of clinical samples was the same as the biomechanical analysis. The patient characteristics are provided along with the standard deviation, Table 3.

Table 3: Patient characteristics for histological analysis. Pathologic tissue was grouped according to aortic valve type. BAV: bicuspid aortic valve and TAV: tricuspid aortic valve.

	Tissue Type		
	Healthy	**Pathologic**	
Patient Traits	TAV (*n*=7)	BAV (*n*=15)	TAV (*n*=15)
Age	61 ± 17	62 ± 16	59 ± 8
M/F Gender	3/4	14/1	11/4

General statistics were performed to elucidate any trends from the general population (all groups lumped). There were no significant differences in the tissue content (elastin, collagen, SMCs and other) detected between the quadrants. Radially however, for all types of tissue, a significantly higher SMCs content was detected in the region closest to the adventitia with a P value of 0.011 (one way ANOVA, Bonferroni post tests), Figure 7.

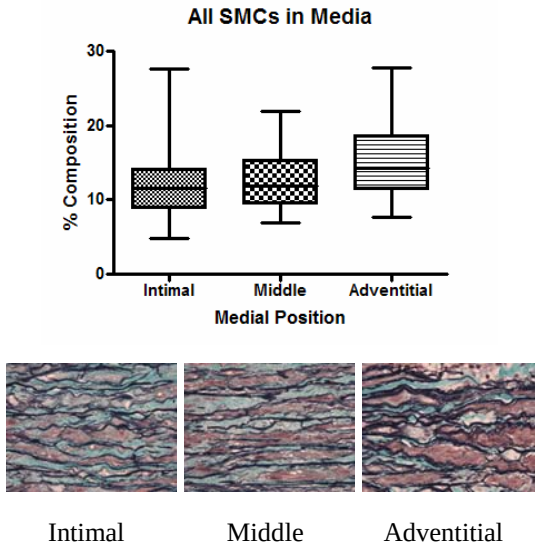

Figure 7: Medial SMCs (red) distribution for all tissue types. As can be seen in both the graph and histology images, the region closest to the adventitia contains on average more SMCs. In this case, thickening of the elastic plates was also observed in this region.

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In the analyses that grouped tissue by type, significant differences in the "other" content were detected. In healthy tissue, there was significantly less of the other components in the region closest to the adventitia (P=0.0177, one way ANOVA, Bonferroni's post test). The pathologic tissue demonstrated an opposite trend; the region closest to the adventitia had a higher content of the other components; each group had a P value <0.001. A loss of these components may be attributed to medial compaction. Thus these findings suggest that the medial compaction occurring with disease is concentrated in the region closest to the intima.

Significant differences were found between tissue types in the elastin content of the tissue as well, in both the radial and circumferential directions (P=0.0334 and P=0.0208 respectively, two way ANOVA), Figure 8. It was noted that the greatest difference was observed between healthy and pathologic tricuspid tissue, with the latter containing a higher percentage of elastin overall. Although it was not significant, it can also be seen that healthy and pathologic tricuspid tissue exhibit regional variation, unlike pathologic bicuspid tissue. The medial quadrant in both healthy and pathologic tricuspid tissue appears to have a higher percentage in elastin content.

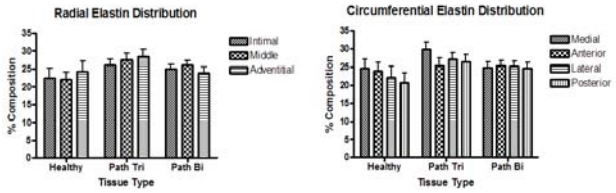

Figure 8: Radial and circumferential elastin distribution for all tissue types. A significant difference was detected in the comparison of tissue types.

Discussion

The key finding in this study was that regional differences do exist in human AA tissue, supporting the hypothesis of this study.

Local Variation: The results from this study have shown that the medial and anterior quadrants are generally thicker than the lateral and posterior quadrants. Comparisons betweens tissue types revealed significant differences between the thicknesses of the quadrants were detected for healthy tissue and for pathologic tricuspid tissue, but no significant variation was found for the pathologic bicuspid tissue.

Mechanically, the medial quadrant was generally less stiff circumferentially regardless of the tissue type in both the low and high stress regions. Overall, the mechanical variation between quadrants was most apparent in healthy tissue and least obvious in pathologic bicuspid tissue. This circumferential variation may reflect the circumferential thickness variation in the tissue as thickness inversely dictates the amount of stress a material experiences. Nicosia et al. also reported circumferential variation. They compared tissue from the anterior and posterior quadrants of porcine aortic root tissue using biaxial tensile tests [3]. Overall, they found tissue from the anterior region to be biaxially more extensible than that from the posterior region. However, it is important to note that the porcine aortic valve morphology is different, with the valve consisting of two large cusps and one small cusp; most likely contributing to different hemodynamic profiles than that in humans.

Histologically, although local variation throughout the media was seen, differences in tissue content between the quadrants were not detected. Local variations were observed between the quadrants only when the analyses were patient specific (no lumping of tissue samples). This suggests a large variation in tissue content between different samples.

The overall trends from the analyses have indicated that although the medial quadrant was less stiff and thicker, this tissue was also most likely to fail. This finding suggests that the medial quadrant has a tissue composition that leads to it being a weaker material than that of the other quadrants. Although not significant, in healthy and pathologic tricuspid tissue, the medial quadrant appeared to have a higher percentage of elastin content than the others. The structure and organization of the components in the medial quadrant may also be attributed to this finding. During the histological analysis, focal alterations were noted in the elastic plates, such as the breaking of the elastic lamellae. In some instances, the plates appeared to be thicker, most likely due to the fusion of adjacent plates occurring with the loss of SMCs.

Healthy, BAV and TAV Comparisons: The average composition of tissue was compared for the different tissue types: healthy, pathologic tricuspid, pathologic bicuspid. Although no significant differences were found, it appeared that healthy tissue contained significantly smaller percent composition of elastin, collagen and SMCs than pathologic tissue (Table 5.3). This contradicts past work that has cited that the pathologic media contains less of these components. This finding suggests the compaction of the medial layer (involved with medial degeneration) in pathologic tissue.

In the comparison of between tissue types, local differences occurred in both healthy and pathologic AA tissue, with the quadrant variation least evident in pathologic bicuspid tissue. Therefore, circumferential variation in tissue properties may be aortic valve dependent, that tissue remodeling is related to the hemodynamics in the AA. Since BAVs can have any leaflet orientation; it seems likely that classifying all pathologic bicuspid tissue together would not demonstrate a consistent local variation in tissue properties.

The results from the biomechanical analyses in the present study have found that AA tissue from BAV patients is significantly stiffer than tissue from TAV patients in the low stress region. This trend can be

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attributed to the fact that the average wall thickness of pathologic bicuspid tissue was thinner than the other types of tissue. Comparisons between the groups of tissue in the high stress moduli (associated with collagen content) in both directions revealed that the slopes were generally comparable as no significant differences were detected.

The histology results from comparisons of healthy and pathologic tissue based on valve type detected a significant difference in the elastin content. Although the post tests did not identify where the difference occurred; it was noted that pathologic bicuspid tissue contained less elastin than pathologic tricuspid tissue. Also, the pathologic bicuspid tissue had a higher percentage of collagen content than pathologic tricuspid tissue. This finding suggests that different forms of medial degeneration between pathologic tricuspid and pathologic bicuspid tissue may be present.

Work done by Bechtel et al. has indicated that pathologic AAs with a BAV have less severe aortic wall abnormalities than those with TAV despite a similar degree of aortic dilatation [4]. This includes less elastic plate fragmentation, medial necrosis, atherosclerosis and fibrosis (grading according to histological standard criteria [5]). Their results suggest that the pathology associated with BAV cannot be detected by light microscopy criteria and that there may be different mechanisms involved in AA dilatation with BAV. Their work, however, was qualitative; they did not quantify the amount of structural components present. In this study, however, a semi-quantitative approach was used and has been successful in identifying the differences between the types of tissue.

Parai et al. have found significant differences in elastin content between AAs in patients with BAV and TAV using morphometry [6]. They have concluded that pathologic bicuspid tissue contains less elastin than pathologic tricuspid tissue, consistent with the results from this work. Past work by Nistri et al. had found that the aorta in BAV patients is stiffer and less distensible than in TAV patients, which agrees with the results that pathologic bicuspid tissue contains more collagen [7]. In this study, pathologic tricuspid tissue had the smallest slope values in the low stress region.

Conclusions

In summary, this work has reported the mechanical and structural properties of human AA tissue obtained from autopsy and excised surgical tissue. This thesis investigated the retrospective tissue histology and *in vitro* passive tissue mechanics. This is the first study to show that local differences in tissue properties exist in both healthy and pathologic human AA.

Circumferential variability was observed in the tissue mechanics. Overall, the medial quadrant was the thickest, least stiff and most likely to fail. Histologically it was observed that this quadrant appeared to contain more elastin. Statistical differences were also evident when the pathological tissue samples were classified according to the aortic valve type (BAV/TAV). Comparisons between the patient groups revealed that healthy and pathologic tricuspid tissue exhibit similar variations in tissue content, thickness and mechanical response between the quadrants, whereas in pathologic bicuspid tissue, the differences were less pronounced or non existent. This suggests that the valve type influences the tissue remodeling in the AA, most likely due to hemodynamic differences. Although variations in tissue content were not evident amongst the four quadrants, significant radial variations were detected in the histological analysis.

The results from this study indicate that regional differences are present in both healthy and diseased human AA tissue, indicating that the assumption of homogeneity in tissue properties may not be a valid one.

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