Circumferential variations in passive and active mechanical properties of healthy and aneurysmal ascending aorta

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Abstract — **Healthy and pathologic tissues of human ascending aorta (AA) were obtained from autopsy and surgical pathology. Each aortic ring was classified as a healthy (non-dilated AA) or diseased (dilated AA) group. Within the diseased group, the samples were further sub-classified as tricuspid aortic valve (TAV), fused tricuspid aortic valve (fTAV) and congenital bicuspid aortic valve (BAV). Each aortic ring was sectioned into quadrants; anterior, posterior, inner curvature and outer curvature. Samples from each quadrant were processed for passive equi-biaxial tensile testing. Phenylephrine was used to contract vascular smooth muscle cells (VSMCs) for active equi-biaxial tensile testing. We observed local variations in stiffness in healthy, TAV, fTAV and BAV tissues. fTAV tissues were significantly stiffer than diseased TAV and BAV tissues and a different local remodeling between diseased fTAV and other diseased tissues exists. Also local variations of anisotropic properties in all groups exist and fTAV tissues are significantly more anisotropic than healthy tissues suggesting a change in microstructure. There was a significant increase in stiffness for tissues under active testing compared to tissues under passive testing. Moreover, we observed an increase of anisotropy for tissues under active testing.**

Keywords — **Aneurysm, Biaxial, Mechanical, Passive, Active.**

I. Introduction

The complex hemodynamics of the left ventricle outflow tract (LVOT) combine with the complex geometry of the ascending aorta (AA) produce a non-uniform shear stress distribution on the AA wall [1]. Endothelial cells are known to be sensitive to shear stress and can lead vascular remodeling. We hypothesize that a non-uniform morphological adaptation occurs in the AA tissue leading to the change of the local mechanical properties. Furthermore, this local remodeling should be dependant on the function on the aortic valve. It is known that AA's wall is composed of passive (elastin & collagen) and active elements (vascular smooth muscles cells). No data currently exists about active mechanical properties assessment under biaxial testing and very little is available on the passive properties of human AA tissue. We therefore evaluated both the passive and active mechanical properties of human AA order to fully characterize the mechanical differences between aneurysmal and healthy ascending aortas.

II. Materials and methods

A. Tissue collection

All human tissues have been collected under ethical committee approval. Ascending aorta (AA) tissues were classified either as healthy (non-dilated AA) or the diseased (dilated AA). Healthy human AAs were collected from autopsy cases at the Maisonneuve-Rosemont Hospital. Diseased human AAs were collected from AAs replacement surgery at the Montreal Heart Institute. Within the diseased group, the aortic tissues were further sub classified with respect to their aortic valve type: tricuspid aortic valve (TAV), fused tricuspid aortic valve (fTAV) or congenital bicuspid aortic valve (BAV). Overall, 30 healthy AA from cadavers and 24 diseased AA from patients have been tested under passive mechanical testing. Active testing have been performed on 3 pig AAs and 2 human AA with a fTAV. All pig tissues were collected from sacrificed healthy pig. AA of these pigs was classified as pig controls (PC). See table 1 for details.

Table 1 Cadavers, patients, and pig controls AA assessed for passive and active mechanical properties

Mechanical testing	Tissue Type		Number of aortic rings	Age (years ±std)
Active	Pig Control (PC)		3	0.3
	fTAV		2	49.0 ± 2.8
Passive	Healthy		30	53.3 ± 15.3
	Diseased	TAV	7	71.9 ± 4.1
		fTAV	14	61.6 ± 10.3
		BAV	3	49.7 ± 9.3

J. Vander Sloten, P. Verdonck, M. Nyssen, J. Haueisen (Eds.): ECIFMBE 2008, IFMBE Proceedings 22, pp. 1899–1902, 2008
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B. Tissue Preparation

Each aortic ring was opened along the axial direction. Four squared samples of 15.0 X 15.0 mm are carefully sectioned from each quadrant of the aortic ring: inner curvature (ic), anterior (ant), outer curvature (oc), and posterior (post), Figure 1. The circumferential side of each sample was carefully identified in order to distinguish the axial direction from the circumferential direction for further testing purposes. The thickness of all samples was measured at 5 points on tissue surface using a digital contact system equipped with constant force transducer (Litematic VL-50A, Mitutoyo USA).

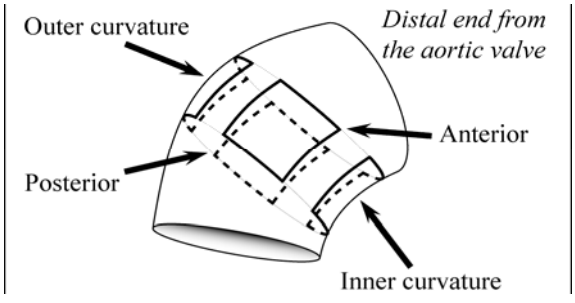

Fig. 1 Quadrants identification

All sample were attached to the grip of the tensile tester using sutures. Two sutures were placed on each side of the sample using silk surgical thread 4-0 with PTFE pledgets (Ethicon inc., USA) to prevent tissue from tearing at the suture points.

C. Passive Mechanical Testing

Mechanical testing was performed using a biaxial tensile tester, EnduraTEC Electro Force 3200 (Bose, Minnesota, USA). During tensile testing, tissue samples were immersed in a bath of Krebs-Ringer buffered solution (Sigma-Aldrich) maintained at 37 ^{0}C. The samples were put in solution for 15 minutes prior to testing in order to reach a zero-stress state. Then a tension of 0.1N was applied from both axes for 10 minutes. Afterward, a standard equi-biaxial test was performed. It consisted of 13 cycles: 10 preconditioning cycles to 30% strain, three experimental runs to 30% strain. The percent strain was based on the average relaxed excised dimensions measure between sutures. All tissue materials were tested at a strain rate of 0.1 mm/s.

D. Active Mechanical testing

At the end of each passive mechanical testing, the samples were kept in place under zero tension for a 15 minutes period in order to reach the zero-stress state. Then a tension of 0.1N was applied for 10 minutes. Phenylephrine at a concentration of 10^{-5}M was added to solution. Once maximum contraction of VSMCs was reached, biaxial testing was performed according to passive mechanical testing protocol in order to compare with the previous results obtained under the passive mechanical testing protocol.

E. Data Analysis

MATLAB programs developed in-house were used to convert force-displacement curves into stress-strain curves and then obtain the incremental moduli of elasticity. As the stress-strain response of biological tissue is non-linear, it was necessary to define the strain values from which incremental moduli of elasticity (stiffness) would be obtained. From the stress-strain curves the incremental moduli of elasticity were obtained at a low (7.5%) and a high strain value (25%), Figure 2.

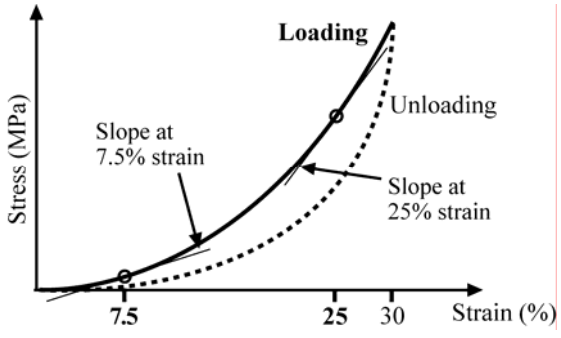

Fig. 2 Typical stress-strain curve

Since all tensile testing was performed using a biaxial tensile tester, it was possible to compute the degree of anisotropy of each tissue. We used an anisotropic index (AI) to compute the difference between the circumferential stiffness and the axial stiffness divided by the average of both stiffness values [equation 1].

$$AI = \frac{2(E_{circ} - E_{axial})}{E_{circ} + E_{axial}} \quad (1)$$

The contribution of VSMCs on stiffness was illustrated using a stiffness index (SI). This index is defined as the difference between stiffness obtained under passive and active testing divided by the average of both stiffness value [equation 2].

$$SI = \frac{2(E_{active} - E_{passive})}{E_{active} + E_{passive}} \quad (2)$$

III. Results and discussion

We found significant circumferential variations in stiffness for healthy and fTAV tissues under passive biaxial testing, Figure 3 (P-value < 0.05, One-way ANOVA, Turkey post test). Healthy tissues showed the outer curvature to be the stiffest. Indeed, the outer curvature is exposed to blood flow ejected from the aortic valve. Thus higher shear and pressure load is suggested to lead to an increase in stiffness in order to withstand the loads. On the other hand, the fTAV tissue group showed the posterior quadrant to be the stiffest. In fact, all aortic ring associated with a fTAV had the left and right coronary leaflets fused together. This configuration conducts the main blood flow through the non-coronary leaflet which is oriented oc-post hence the stiffer quadrants.

The averaged stiffness of each group showed fTAV tissues to be significantly stiffer than all groups (Figure 3; under the brace; P-value < 0.05, One-way ANOVA, Turkey post test). This finding can not be only explain by the effect of age on stiffness of aortic tissues as suggested by Okamoto *et al.* [2]. Indeed, it has been shown that aortic stiffness increase with age [3]. However, in our study, patients with a fTAV are younger than patients with a TAV. Therefore a different mechanism than ageing is responsible for this stiffness increase. Overall, our data suggest a strong dependency of the valve pathology on aneurysmal tissue stiffness.

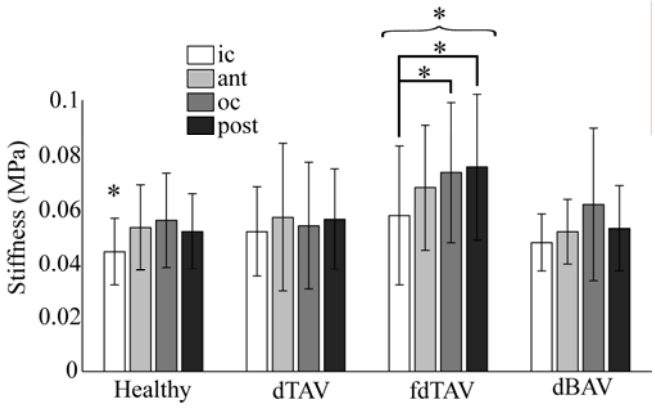

Fig. 3 Local stiffness at 25% strain under passive testing

Although we found local variations in the anisotropic properties within each group, only the fTAV tissues showed significant variations under passive mechanical testing (figure 4; P-value < 0.05; One-way ANOVA, Turkey post test). Positive and significantly different from zero index values indicate that tissues had anisotropic properties (represented by a cross; P-value < 0.05, hypothesis test). There is no existing data about local anisotropic properties assessment of aortic tissues. No significant variations between quadrants except for the anterior and posterior quadrants for fTAV tissues. However we found that all quadrants in the healthy group were anisotropic with the inner curvature possessing the largest difference in directional properties. The anisotropic property of aortic tissues have been showed in previous studies and are explained by the circumferentially oriented collagen network [4][5][6]. The averaged anisotropic index showed that fTAV tissue was significantly more anisotropic than the other groups (Figure 4; under the brace; P-value < 0.05, One-way ANOVA, Turkey post test). Moreover, these differences make fTAV tissues more anisotropic than the other groups. This suggests a change in the microstructure of the aortic wall; either a weakening of the elastin layers or an increase in collagen content oriented circumferentially.

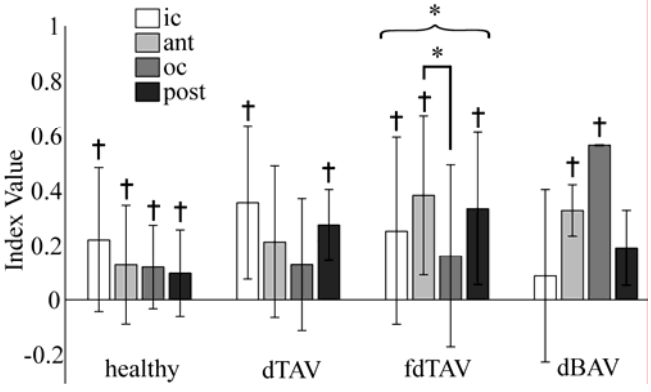

Fig. 4 Local anisotropic index at 25% strain under passive testing

Active testing showed that VSMCs have a non-negligible effect on aortic tissue mechanical properties. Pig and diseased human tissue testing showed a positive stiffness index (SI) meaning an increase of stiffness under active testing in comparison to passive testing, Figure 5 and 6. Owing to the small number of aortic tissue samples tested, we could not find any significant differences in the inner and outer curvatures or at different strains. However, in both figures, SI have a tendency to be the highest at low strain value. Previous studies have suggested that the effect of VSMC is relatively dependent of strain [7]. VSMCs may increase tissues stiffness at low strain but as tissues stretch, actin and myosin filaments don't overlap anymore and then active mechanical stiffness decrease toward passive mechanical stiffness value, that is a zero SI. Moreover, we observed a tendency that the inner curvature had a larger SI than the outer curvature suggesting either a higher VSMCs content or more active VSMCs in this quadrant.

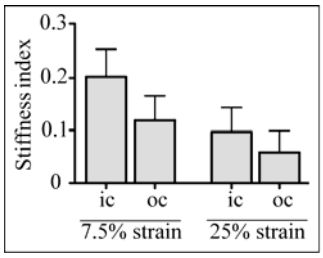

Fig. 5 SI for pig tissues

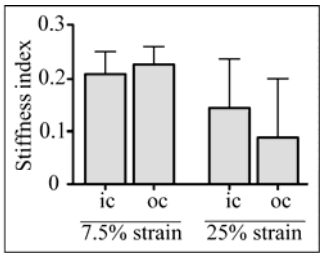

Fig. 6 SI for human tissues

Figure 7 shows the effect of VSMCs on the anisotropic properties of pig tissues. Here again, because of the number of aortic tissue tested, we did not find any significant differences. However, we observed an increase of anisotropy for all quadrants with increasing strain. This increase is positively related to the circumferential orientation of VSMCs. We did expect a larger variation between passive and active testing but the axial response had a larger response than expected. It has been suggest that extracellular matrix transmits VSMCs contraction not only in the circumferential direction but also in the axial direction [8]. We could not observe any variations or trends related to diseased human tissue because of the small number of patients (data not shown).

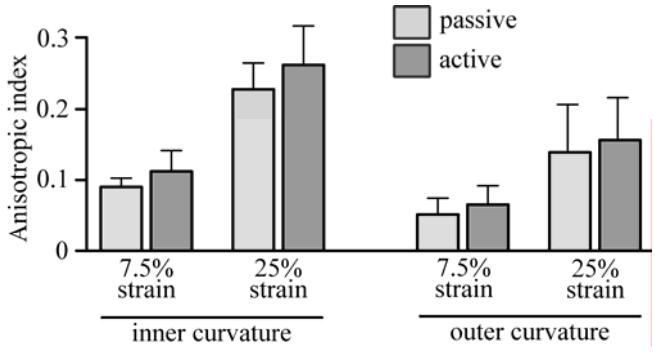

Fig. 7 Anisotropic index for pig tissues

IV. Conclusion

We have observed local variations in stiffness in healthy, TAV, fTAV and BAV tissues. Local variations within fTAV and healthy group showed a different local remodeling which is likely related to the aortic valve morphology. Overall, fTAV aortic tissue was significantly stiffer than diseased TAV and BAV tissue. Therefore stiffness is strongly dependant on valve morphology. Also local variations of anisotropic properties exist in all groups and fTAV tissues are significantly more anisotropic than healthy tissue suggesting a change in the microstructure. There is a significant increase in stiffness for pig and diseased human tissue when the VSMCs are activated as illustrated by the stiffness index. VSMCs appear to have a greater effect on the stiffness at low strain value than at high strain value. More data needs to be obtained to verify if a significant increase in anisotropy occurs under active testing and if active testing effects the local variations of anisotropic properties.

Acknowledgment

This work was funded in part by FQRNT, CIHR, CFI and Williwam Dawson Fund from McGill University.

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