Quantification of Structural Compliance of Aged Human and Porcine Aortic Root Tissues

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Abstract

The structural compliance of the aortic root has a significant implication for valve procedures such as transcatheter aortic valve implantation and valve-sparing aortic root replacement. However, a detailed quantification of human aortic roots structural compliance, particularly in different regions has been incomplete. In this study, the structural properties of human aortic roots (81 ± 8.74 years, n=10) were characterized and compared with those of porcine ones (6–9 months, n=10) using a vessel pressure-inflation test. The test involves tracking three-dimensional deformation of the markers affixed on the different surface regions of the aortic roots, including the three sinuses: the non-coronary sinus (NCS), the left coronary sinus (LCS) and the right coronary sinus (RCS), and at three regions along the longitudinal direction of each sinus: the upper sinus (US), the middle sinus (MS) and the lower sinus (LS), and the ascending aorta (AA) region above the NCS. We found that tissue stiffness at a physiological pressure range was similar among the three human sinuses. The regional structural stiffness of human aorta was observed. In the circumferential direction, the LS regions were the stiffest in the LCS and RCS sinuses, while NCS had relatively uniform stiffness. In the longitudinal direction, the human AA regions were more compliant than all sinuses. There was a significant difference in tissue stiffness between human and porcine aortic tissues, suggesting that the mechanical properties of porcine may not be analogous to aged human ones.

Keywords

Heart valve mechanics; Aortic root compliance; Inflation test; Surface deformation; Direct linear transformation

INTRODUCTION

Aortic root is defined as the portion of the left ventricular outflow tract which supports the leaflets of the aortic valve, delineated by the sinotubular ridge superiorly and bases of the valve leaflets inferiorly [1]. It comprises the sinuses, the aortic valve leaflets, the commissures, and the interleaflet triangles. The sinuses are expanded portions of the aortic root which are confined proximally by the attachments of the valve leaflets and distally by
the sinotubular junction. Common forms of aortic root diseases include aortic stenosis and aortic aneurysms. Surgical and transcatheter approaches have been used to treat these diseases with the purpose of restoring the normal function of the aortic root. Hence, the knowledge of both normal anatomy and physiological function of the human aortic root is critical to the clinical success of these operations [2]. Moreover, since aortic root diseases primarily affect patients with advanced age, there is a dire need for a complete understanding of biomechanical responses of aged human aortic tissues. However, the knowledge of structural compliance and regional variation of aged human aortic root tissue remains very limited.

The extensibility of the aortic root in the circumferential (CIRC) and longitudinal (LONG) directions was studied by several research groups [2–5] using pressure inflation tests with radiopaque markers or sonomicrometric crystals using either in vitro or in vivo animal models. The results were, however, limited to the overall dimensional changes of the whole aortic root, not specific to each of the sinuses. Studies using either planar biaxial tests [6–11] or uniaxial tests [12, 13] quantified the aortic root material properties at a specific location. Since the aortic root is heterogeneous, the overall aortic root structural compliance may not be accurately derived using such localized material testing data.

Much of our knowledge on human aortic root properties is derived from these studies of porcine and ovine aortic roots, partly due to the limited access of human tissues. With the advent of transcatheter valve intervention, new devices and approaches are being extensively tested using porcine and ovine animal models. Indeed, in recent years, there have been approximately 40 journal articles published on animal trials of various transcatheter valve interventions, with about 500 porcine, ovine, bovine animals used [14–24]. The number could be much higher considering those unpublished animal experiments. Although significant experience has been gained through animal and human clinical trials, disparity between the results of animal trials and those of human trials often exists. It should be noted that these animals were often young and healthy at the time of implantation. For instance, juvenile swine with weight of 35–46 kg [25] and 45–55 kg [26] was used to evaluate transcatheter aortic valve (TAV) device and delivery system. Wendt et al. [27] recently reported feasibility and safety of the transapical ACURATE TA™ (Symetis SA, Ecublens, Switzerland) TAV implantation using healthy porcine (57 – 71 kg) and ovine (36 – 52 kg) models. Although doubts always exist regarding the validity of animal models and their applicability to human ones, there is clearly a lack of scientific evidence and engineering quantification of the discrepancy between animal and human trials. Therefore, the current assumption taken for TAV pre-clinical trials - porcine and ovine animal models are similar to those of aged humans – is still prevailing.

In this study, human aortic roots were characterized and compared to young porcine ones. Structural compliance of both human and porcine aortic roots was analyzed at different regions, including the three sinuses: the non-coronary sinus (NCS), the left coronary sinus (LCS) and the right coronary sinus (RCS), and at three regions along the LONG direction of each sinus: the upper sinus (US), the middle sinus (MS) and the lower sinus (LS) near the aortic annulus, and the ascending aorta (AA) above the NCS.
MATERIALS AND METHODS

Tissue Preparation

Ten human cadaver hearts (age of 81.00 ± 8.74 years and weight of 563.50 ± 218.35g) without any valvular heart disease were obtained from the National Disease Research Interchange (NDRI, Philadelphia, PA). The use of human tissues was approved by the Institutional Review Board at the University of Connecticut. The characteristics of human specimens were listed in Table 1. All human hearts were fresh frozen within a post-mortem recovery interval (15.32 ± 6.51 hours) and remained frozen until delivery on the next day. All hearts were stored in −80°C freezer prior to testing. Ten fresh frozen porcine hearts with ascending aortas (6 – 9 months old and weight of 600.09 ± 75.46 g) were obtained from the Animal Technologies, Inc. (Tyler, TX) and stored in −80°C freezer. Prior to testing, each heart was submerged in a 37°C water bath until totally defrosted. Both human and porcine hearts were frozen and thawed using the same procedure. The aortic root including ascending aorta was carefully separated from the left ventricle and surrounding tissues. All adherent connective and fatty tissues on the surface of the aortic root were carefully removed from the specimens. The coronary arteries were occluded with sutures to prevent leakage during pressurization of the aortic root.

Experiment Setup

Each aortic root was cannulated at the AA using a plastic fitting, mounted vertically to a laboratory stand via a plastic ring clamp, and submerged in 0.9% saline solution. Fig. 1 shows the setup of the inflation test system. The plastic fitting was connected to a pressure transducer (model BLPR2, World Precision Instruments, Sarasota, FL) and a 100 mL surgical syringe through plastic barb fittings and flexible tubing. The syringe filled with saline solution was used to pressurize the aortic root. A hand-held pressure gauge with a range up to 300 mmHg was connected to the tubing for calibration of the pressure transducer. The pressure transducer was calibrated up to 200 mmHg against the pressure gauge with error less than 1 mmHg. After 10 cycles of preconditioning, the aortic root was inflated from 0 to 200 mmHg by smoothly injecting saline solution to the root. An inflation pressure of 200 mmHg was chosen because the aortic root may bear high loads during procedures such as TAV implantation. Two CCD cameras (model XC-ST50, Sony Corporation of America, Park Ridge, NJ), positioned approximately 30 degree apart from each other, were utilized to capture the motion of the markers on the specimen at 30 frames per second. An in-house LabVIEW (National Instruments, Austin TX) program was developed to simultaneously acquire videos from the two cameras and pressure data from the pressure transducer.

Surface Strain Computation

The marker layout on the aortic root is shown in Fig. 2a. The markers were used to calculate the mechanical responses of each sinus and at three regions along the LONG direction—US, MS and LS, as well as AA. To minimize the effect of coronary arteries on the strain measurement of RCS and LCS, the markers were affixed 3–5 mm away from coronary arterial ostia. The marker pattern on the specimen was arranged so that each region consists...
of 7 markers (Fig. 2b). Three dimensional (3D) spatial coordinates of the markers were reconstructed from the 2D images using the direct linear transformation method [28–30]. The 3D coordinates of all markers were captured at all frames to calculate the displacement gradients and the regional in-plane Green strain.

To compute the in-plane Green strain within each region, shell-based 2D isoparametric finite element shape functions [31] were used to fit the surface geometry of each region. Following the surface fitting methods reported by Sacks et al. 2002 [32], the local surface of aortic sinus, in the region delimited by the 7 markers, was approximated by a seven-node \(C^0\)-continuity quadratic Lagrangian element. The surface fitting of the initial state (Pressure = 0 mmHg) yields the reference configuration of the region. To determine the strain field at each frame, the displacements of each marker were computed as the difference between the reference and the deformed spatial marker positions. The displacement component was fitted separately using the same shape functions. This fitting was completed for each region at each frame. The in-plane components of Green strain (\(\varepsilon_{ij}\)) were determined by the fitted continuous displacement functions (\(u_i\)), as follows:

\[
\varepsilon_{ij} = \frac{1}{2} \left( \frac{\partial u_j}{\partial x_i} + \frac{\partial u_i}{\partial x_j} + \frac{\partial u_{i\alpha}}{\partial x_i} \frac{\partial u_{\alpha j}}{\partial x_j} \right),
\]

where \(x_i\) and \(x_j\) indicate differentiation with respect to the in-surface coordinate components, \(\alpha\) is the repeating index. The respected stretches \(\lambda_{11}\) and \(\lambda_{22}\) in the CIRC and LONG directions were calculated for each region by

\[
\lambda_{11} = \sqrt{u \cdot Cu} \quad \lambda_{22} = \sqrt{v \cdot Cv},
\]

where \(u\) and \(v\) are unit vectors along the CIRC and LONG directions, respectively, \(C = 2E + I\) denotes the right Cauchy-Green tensor, where \(I\) is the identity tensor.

**Data analysis**

To estimate the structural stiffness of aortic root tissues, the pressure–strain elastic modulus \(E_p\) is calculated [33] by

\[
E_p = \frac{P_{sys} - P_{dia}}{(\lambda_{sys} - \lambda_{dia})/\lambda_{dia}},
\]

where \(sys\) and \(dia\) denote the systolic and diastolic phases, respectively. We chose \(P_{dia} = 10.67\) kPa (80 mmHg) and \(P_{sys} = 16.00\) kPa (120 mmHg). \(\lambda_{sys}\) and \(\lambda_{dia}\) are corresponding stretches in either CIRC or LONG direction. The pressure-strain elastic modulus was used to compare the structure stiffness between different regions.

To further analyze the aortic compliance, the extensibility of the samples was calculated and compared via the areal strain as follows

\[
e = \lambda_{11p} \lambda_{22p} - 1,
\]
where $\lambda_{11p}$ and $\lambda_{22p}$ are the CIRC and LONG stretch values, respectively, at three pressure levels of $p = 10$, 15, and 25 kPa. A pressure of 25 kPa was chosen to represent a hypertensive condition. Patients' characterization including age, gender, hypertension (HTN) or normotensive (NTN) were related to the biomechanical parameters to determine any correlation.

**Statistical Analysis**

Statistical analyses were evaluated using SigmaPlot (V11.0, Systat Software Inc., San Jose, CA). Both one-way and two-way Repeated Measures ANOVA tests were used to compare the difference among the three sinuses and the regions along the LONG direction. The Tukey pairwise multiple comparison procedures were performed to identify which group is different, with $p < 0.05$ was considered a statistically significant difference and $p < 0.001$ indicates highly statistical significance. The Student’s t-test was used to determine significant differences between the parameters between human and porcine tissues.

**RESULTS**

**Pressure-Green strain responses**

The mean pressure-Green strain responses of human and porcine sinus tissues were shown in Figs. 3 and 4. The human tissues were distinctively stiffer than porcine tissues. Specifically, the human tissue stiffened rapidly at a low pressure range of 5 – 10 kPa, whereas porcine tissue responses behaved linearly up to 15 – 20 kPa. At the pressure of 27 kPa, the peak strains were similar in both LONG and CIRC directions for the human aorta, while for the porcine aorta the CIRC strains were higher than the LONG strains (Table 2).

Similar to the sinuses, the structural responses of human AA tissues were much stiffer than those of porcine tissues, as shown in Fig. 5.

There are no significant differences in the pressure-strain modulus among the three human sinuses, see Fig. 6a (i.e., the averaged responses of LS, MS and US of each sinus). However, various regional differences were observed, see Fig. 6c–d. In the CIRC direction, the LS regions were the stiffest in the LCS ($p = 0.023$) and RCS ($p = 0.048$) sinuses, while NCS had relatively uniform stiffness. In the LONG direction, there were no significant difference of stiffness among the three LS, MS and US regions. However, the AA regions were significantly more compliant than the LS regions of LCS ($p = 0.035$), RCS ($p = 0.028$), NCS ($p = 0.016$) and the MS regions of RCS ($p = 0.008$) and NCS ($p = 0.004$).

Significant differences between porcine sinuses were observed: NCS was stiffer than RCS ($p < 0.001$) in both CIRC and LONG directions and LCS ($p = 0.042$) in the CIRC direction. LCS was stiffer than RCS in the CIRC ($p = 0.027$) and LONG ($p < 0.001$) directions, as shown in Fig. 6b. For the regional variation, in the CIRC direction, LS was stiffer than US ($p = 0.001$) and MS ($p = 0.006$) of LCS, US ($p < 0.001$) of RCS, US ($p < 0.001$), MS ($p = 0.018$) of NCS and AA ($p < 0.001$). The MS regions were stiffer than US of RCS ($p = 0.033$) and AA ($p = 0.016$). No regional differences were observed in LONG direction of porcine specimens.
Areal strain comparison

No regional differences in areal strain were observed between sinuses and regions in human LCS and RCS specimens (Fig. 7). For human NCS, the US and MS regions were significantly more extensible than LS regions (US vs. LS: p < 0.001 at all pressure levels; MS vs. LS: p <0.05 at all pressure level). For porcine specimens, on the other hand, the LS regions were more extensible than other regions, particularly compared to US and MS regions in LCS (LS vs. US: p < 0.05; LS vs. MS: p <0.05) and NCS (LS vs. US: p < 0.05; LS vs. MS: p <0.05). For the RCS specimens, however, LS regions were similar to other regions at all pressure levels. The porcine AA specimens were more extensible than all sinus regions, p < 0.05. Note that for human specimens, no differences in areal strain were observed between the sinuses and AA specimens or between the pressure levels. A significant difference was observed in all pairwise comparison of areal strain between human and porcine aortic tissues.

Correlation between age and compliance

A correlation trend was observed between age and human aortic compliance. Fig. 8 shows the aortic tissue areal strain significantly decreased as age increases in LCS and NCS. While all regions in NCS specimens negatively correlated strongly with age, only the MS and LS of LCS decreased as age increases. The pressure-strain moduli correlated very weakly with age and were only significant for the LS (p = 0.045 for CIRC direction) and MS (p = 0.041 for LONG direction) of LCS specimens.

DISCUSSION

In this study, the structural properties of the aged human and porcine aortic tissues were investigated using a 3D marker tracking technique. This tracking technique allowed quantification and comparison of the structural properties of three regions along the LONG direction of the three sinuses, and AA. We found that tissue stiffness at the physiological pressure range was similar among the human three sinuses. The AA region were found to be more compliant than the sinuses, which is consistent with other studies. Martin et al. [10] found that AA tissues were more compliant than LCS and RCS tissues in the LONG direction. Azadani et al. [6] found that fresh human AA tissues were significantly more compliant than the sinus tissues in both the CIRC and LONG directions. Similarly, Gundiah et al. [11] also determined that their porcine sinus tissues were stiffer than AA tissues in both directions.

The regional structural stiffness of human aorta was observed. The CIRC LS regions were the stiffest in the LCS and RCS sinuses, while NCS had relatively uniform stiffness. The observation that human LS was stiffer than MS and US may be explained by the fact that calcification of human aortic roots is mainly localized at the region adjacent to the aortic annulus. The samples were selected from an advanced aged patient group. Light calcium deposition was observed in the lumen layer of the root, at the annular-sinus regions, commissure regions and sinotubular junction in most of patient samples as shown in Fig. 9, which could contribute to the high stiffness of the LS region. However, it did not affect the valve function, i.e. the valves were closed properly during testing. Another factor that might
have caused a high stiffness in structural tissue properties is hypertension. Hypertension is a significant risk factor for many diseases and is well known to induce changes in the mechanical properties of the cardiovascular [34] and other arteries [35]. A total of six out of ten patients had a record of systematic hypertension. We observed that these three out of six patients exhibited a higher physiological stiffness than the group mean in the LS region. Our observation suggests a trend towards an increase in physiological stiffness with either or both calcification and hypertension. In addition, a negative correlation between age and human tissue compliance was observed. The microstructural components such as elastin and collagen play an important role in tissue compliance. Studies have shown that the decrease in elastin functionality [36] and the increase in collagen fiber recruitments with aging [37, 38] might greatly impact the mechanical properties of aortic tissues, resulting in higher stiffness and lower areal strain. A future study on the changes of the microstructure in aging aorta in relation to changes in the structural properties as a result of the hemodynamic alternation is needed to elucidate an increase in stiffness of aged human aortic tissues.

Statistical significant differences in stiffness and areal strain between human and porcine aortic root tissues were found in this study, which is similar to a previous study [10] comparing the planar biaxial mechanical properties of human and porcine aortic tissues. One reason is that human specimens used in this study were obtained from older patients with mean age of 81 ± 8.74 years, which could result in an overall higher stiffness of human aortic tissues. As shown in Fig. 7, for human samples, there was no significant increase in the areal strain when pressure increased from 10 to 25 kPa. However, for porcine specimens, there was a significant increase in the areal strain as pressure increases. These differences indicate that porcine aortic tissues might not be analogous to human. As this phenomenon has been previously reported in aortic tissues [10] and other cardiovascular tissue studies [39], it is important to know the discrepancy between the animal and human models to avoid erroneous assumption when one attempts to apply animal data to human study, particularly in the pre-clinical animal study of prosthetic devices. Computational modeling has also been employed as a predictive tool to study the biomechanics of the aortic root and to better understand the complex interactions between prostheses and host tissues. To date, due to the lack of experimental data, many computational studies assumed homogeneity [40] of aortic root and of different locations on the root. The aortic root has been characterized as either linear elastic (isotropic or anisotropic) [41–45] and nonlinear hyperelastic [46–49]. The current experimental data provide a detailed quantification of regional aortic responses which could be used to derive tissue material responses using inverse finite element analysis [50] as well as to validate the simulation results.

Limitations

Several assumptions and simplifications were made in the present study. Both human and porcine tissues were frozen prior to testing. Therefore, the structural properties obtained might be different from those of fresh tissues. Due to the limited sample size, we were not able to identify any correlation between structural properties and age, gender and disease states such as aortic hypertension. Because our samples were collected from a subset of individuals with no-valvular disease in the medical record, we could not correlate aortic pathologies (e.g. aortic wall/root dilation and wall degenerative changes) with structural
properties. However, we observed light calcium deposition in most of our samples. As calcification might be one of the factors that might contribute to the regional high stiffness of the roots, future work may include histological and microstructural analysis to better understand the underlying pathologies responsible for such responses. The graphite markers on the LCS and RCS might not be affixed in the central area of the sinus due to the presence of the coronary arteries. In addition, the structural properties of the AA were measured approximately 5 – 10 mm above the NCS. We also assumed that the material properties of this region were not varied along the CIRC direction. The anterior and posterior regions of AA tissues were reported to be similar in the studies by Gundiah et al. and Azadani et al. [25, 26].

CONCLUSIONS

In this study, the regional structural compliance of aged human and porcine aortic tissues was quantified. The experimental results of ten aged human hearts revealed no significant difference in stiffness among the sinuses in both directions. The regional structural stiffness of human aortas in CIRC direction varied with the LS being the stiffest in left and right sinuses. The NCS regions were uniform in stiffness in both CIRC and LONG directions. The asymmetric structural properties were observed among porcine aortic sinuses, with the NCS being the stiffest in the CIRC direction and the RCS being the least stiff in both directions. Stiffness in porcine lower regions was higher than middle, upper sinuses and ascending aortas in the CIRC direction. Aged human aortic tissues were significantly stiffer than 6–9 months old porcine aortic tissues, indicating that the mechanical properties of porcine may not be analogous to aged human ones. These observations suggest that clinical interpretations of animal trials on prosthetic devices should be determined with caution.

Acknowledgments

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REFERENCES


Figure 1.
Schematic of the primary components in the inflation test system, including a specimen chamber, an inflation test system consisting of a pressure transducer, pressure gauge, syringe and two digital cameras.
Figure 2.

a) The marker layout on the surface of non-coronary sinus (NCS) demarcating the Upper Sinus (US), Middle Sinus (MS) and Lower Sinus (LS) regions and the ascending aorta (AA). Note the AA was tested on the region superior to NCS only; b) the numbering of the markers on each sinus region.
Figure 3.
Figure 4.
Figure 5.
The pressure-Green strain curves (mean and standard error) in the circumferential (CIRC) and longitudinal (LONG) directions of human (HH) and porcine (PH) ascending aortic tissues.
Figure 6.
Variation of the pressure-strain modulus in the circumferential (CIRC) and longitudinal (LONG) directions among the three sinuses of (a) human and (b) porcine tissues and among the regions of the sinuses in the (c) CIRC and (d) LONG directions of the human and porcine aortic tissues. * p < 0.05 compared to LS; † p < 0.05 compared to MS; § p < 0.05 compared to AA. All human aortic tissues were significantly stiffer than the corresponding porcine tissues.
Figure 7.
Variation in (a) circumferential (CIRC) and (b) longitudinal (LONG) pressure-strain modulus of the human and porcine aortic tissues. * p < 0.05 compared to LS; § p < 0.05 compared to AA. Difference between stress levels within sinus and regions are all significant for porcine, but not human tissues. All human aortic tissues were significantly stiffer than the corresponding porcine tissues.
Figure 8.
Correlations between age and areal strain for (a) LCS and (b) NCS specimens. Table shows the correlation coefficient, R, and p-values. Solid lines are the linear fits to the datasets.
Figure 9.
Representative images of the Left-Coronary Sinus (LCS), Right-Coronary Sinus (RCS) and Non-Coronary Sinus (NCS) of a human aortic root (Specimen 5). Asterisks indicate locations of calcium deposition in the lumen layer of the aortic wall. [1mm sub-division for all images]
### Table 1

Patients' clinical information

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Table 2

Maximum strains at a maximum stress of 27 kPa for both human and porcine aortas.

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