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INTERLAYER MICROMECHANICS OF THE AORTIC HEART VALVE LEAFLET

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Abstract

While the mechanical behaviors of the fibrosa and ventricularis layers of the aortic valve (AV) leaflet are understood, little information exists on their mechanical interactions mediated by the GAG-rich central spongiosa layer. Parametric simulations of the interlayer interactions of the AV leaflets in flexure utilized a tri-layered finite element (FE) model of circumferentially oriented tissue sections to investigate inter-layer sliding hypothesized to occur. Simulation results indicated that the leaflet tissue functions as a tightly bonded structure when the spongiosa effective modulus was at least 25% that of the fibrosa and ventricularis layers. Novel studies that directly measured transmural strain in flexure of AV leaflet tissue specimens validated these findings. Interestingly, a smooth transmural strain distribution indicated that the layers of the leaflet indeed act as a bonded unit, consistent with our previous observations (Stella and Sacks, 2007) of a large number of transverse collagen fibers interconnecting the fibrosa and ventricularis layers. Additionally, when the tri-layered FE model was refined to match the transmural deformations, a layer-specific bimodular material model (resulting in four total moduli) accurately matched the transmural strain and moment-curvature relations simultaneously. Collectively, these results provide evidence, contrary to previous assumptions, that the valve layers function as a bonded structure in the low-strain flexure deformation mode. Most likely, this results directly from the transverse collagen fibers that bind the layers together to disable physical sliding and maintain layer residual stresses. Further, the spongiosa may function as a general dampening layer while the AV leaflets deforms as a homogenous structure despite its heterogeneous architecture.

Key Terms

aortic valve; flexure; hyperelastic; interlayer; micromechanics; bimodular

1 - INTRODUCTION

Over 5 million Americans currently suffer from heart valve disease (Go et al., 2013), estimating a prevalence of 2.5% in the US population. The AV (Fig.1-a) must perform under a unique mechanically stressed environment for an estimated 3 to 4 billion cycles throughout an average lifetime. For example, all valvular tissues must exhibit low flexural stiffness

during valve opening coupled with high tensile stiffness during closure (Sacks and Yoganathan, 2007). This behavior is attainable through a unique hierarchical structure in which all elements act in unison to provide seamless transition over the cardiac cycle. Despite this level of functional efficiency, the onset and progression of valve disease ultimately requires repair or full replacement in many patients.

The AV leaflet is a heterogeneous structure composed of three distinct layers: the fibrosa, spongiosa, and ventricularis (Fig. 1-b) (Thubrikar, 1990, Sacks et al., 1998, Schoen and Levy, 1999, Yacoub and Cohn, 2004, Stephens et al., 2008, Wiltz et al., 2013). Each layer contains varying amounts of collagen, glycosaminoglycan (GAG), and elastin (Carruthers et al., 2012). The AV valve has multiple biomechanical properties crucial to enabling proper function (Thubrikar et al., 1977, Missirlis and Chong, 1978, Thubrikar et al., 1979, Sacks et al., 2009). It has been speculated that the layered configuration of the leaflet facilitates valves under physiological conditions (Sauren et al., 1980, Sacks et al., 2009). Yet, while the outer fibrous layers of the AV leaflet have been extensively investigated (Christie and Stephenson, 1989, Vesely and Noseworthy, 1992, Vesely, 1996, Stella and Sacks, 2007), little information exists on interlayer micromechanics.

To date, only two studies have investigated the mechanical behavior of individual leaflet layers using micro-dissection techniques. Vesely et al. observed the extensibility of intact tissue under uni-axial tension to be significantly different from the individual layer responses (Vesely and Noseworthy, 1992). In the second study, Stella et al. observed measurably different behavior under biaxial loading of the separated layers (Stella and Sacks, 2007). The intact tissue response was intermediate to the separated responses. Interestingly, It has been assumed previously that the spongiosa layer enables sliding between the fibrosa and ventricularis during opening and closing (Mohri et al., 1972, Vesely and Boughner, 1989, Song et al., 1990, Thubrikar, 1990, Talman and Boughner, 1995), yet little data exists to support this theory. Vesely and Boughner (1989) and Song et al. (1990) claim evidence of measured sliding between the layers; however, the loading conditions used were artificial and not representative of physiological flexural conditions. Thus, the degree of bonding that exists between the two layers and its affects on AV leaflet bending *in vivo* remains unclear. Moreover, based on these findings, it is apparent that the individual layers function quite differently in the intact configuration than separated and must be evaluated in the intact state.

Historically, the term “tissue engineering” is attributed to Y.C. Fung (Woo and Seguchi, 1989). The term underscored the importance of “the application of principles and methods of engineering and life sciences toward a *fundamental understanding of structure-function* relationships in normal and pathologic mammalian tissues and the development of biological substitutes to restore, maintain, or improve tissue function.” Thus, it is imperative that fundamental structure-function understanding guides the reproduction of native tissue if it is to emulate its native counterpart successfully. Clearly, the complex nature of valve biomechanical behavior and function (Sacks et al., 2009) cannot be duplicated with simple homogenous biomaterials. Consequently, to develop replacement valvular tissues we must more fully understand the fundamental micromechanics of the tissue in both healthy and diseased states (Butler, Goldstein et al. 2000).

In the present study, we conducted an integrated simulation-experimental investigation utilizing flexural deformations of intact AV leaflets as a means to probe interlayer interactions. In addition to being a natural choice to study interlayer micromechanics, flexure is a major deformation mode of the cardiac cycle (Iyengar et al., 2001, Sacks and Yoganathan, 2007) and has been extensively used for valve tissue mechanical studies (Gloeckner et al., 1998, Gloeckner et al., 1999, Engelmayr et al., 2005, Merryman et al., 2006, Mirnajafi et al., 2006). Parametric simulations of interlayer interactions were first conducted using a tri-layered leaflet tissue finite element (FE) model to simulate interlayer sliding hypothesized to occur. To validate these findings and further refine the model, experimental studies on porcine AV leaflet tissue were conducted to examine bi-directional flexural response, and relative interlayer movement using actual transmural strain responses.

2 - METHODS

2.1 Overview

The flexural deformation mode not only represents a major deformation mode of heart valve leaflets, but also allows direct examination of individual layer responses in tension and compression. It should be noted that AV leaflets experience complex bidirectional flexure *in vivo* (Thubrikar et al., 1980, Thubrikar et al., 1986). To simplify the problem, we focused on bending in the circumferential direction only, as it is the major curvature change in leaflets (Sugimoto and Sacks, 2013). A circumferentially oriented rectangular leaflet tissue strip configuration, located below the Nodulus of Arantius (Fig. 2-a), was used for simulation since the central belly region is structurally most representative of the leaflet (Billiar and Sacks, 2000). First, an initial model was used to conduct a parametric study of the deformation through the thickness of the leaflet at varying degrees of layer connectivity. The results of this model were verified with novel experimental flexure studies that quantified the transmural variations in transmural strain. Based on these results, a refined flexural model was then developed to simultaneously match the moment-change in curvature ($M-K$, where $\kappa = \kappa - \kappa_0$ and κ_0 the initial curvature) relationship and transmural deformation of the AV in both bending directions. The end result was a clearer picture of interlayer mechanical interactions of the low strain behavior that occurs in flexure.

2.2 Initial simulations of AV leaflet tissue flexure

A finite element (FE) model was developed to simulate AV leaflet tissue in flexure using the software package COMSOL Multiphysics v4.3 (Burlington, MA). A rectangular model geometry was used, set to 14 mm in length (circumferential direction) by 3 mm in width (radial) by 0.4 mm thick. The tissue layer geometry was derived on previous histological data (Carruthers et al., 2012), which showed that the fibrosa represented 45% of the volume, the spongiosa 30%, and ventricularis 25%. Boundary conditions simulating three-point bending were avoided to ensure no point-loading effects would occur in the center of tissue and end-loading conditions were used instead. The boundary conditions thus consisted of pins at both ends of the model tissue, and a horizontal load was applied at one pin in the X_1 direction, causing the model tissue to undergo transverse deflection (Fig. 2-b). The mesh consisted of 3612 8-node brick elements, and shape functions for pressure (linear) were set

to one order lower than displacement (quadratic) to avoid locking. The geometry was modeled as symmetric along the X_3 axis to decrease computing time (Fig. 2-b).

To establish both the material model and to obtain an initial set of material parameters, we began with previously published AV flexure data for inactivated tissues (Merryman et al., 2006, Sacks et al., 2009). The following Ogden model, assuming incompressibility, was chosen as it provides an additional level of flexibility compared to a Neo-Hookean model

$$W = \sum_{p=1}^{N=1} \frac{\mu_p}{\alpha_p} \left(\lambda_1^{\alpha_p} + \lambda_2^{\alpha_p} + \lambda_1^{-\alpha_p} \lambda_2^{-\alpha_p} - 3 \right) \quad (1)$$

where α is a constant, μ the shear modulus, and λ_1 and λ_2 are the principle stretches. When $\alpha = 2$ and $N=1$, Eq. 1 will simplify to the standard neo-Hookean form. The classic analytical solution of an incompressible isotropic beam under flexure (Rivlin, 1949) was used to establish an initial material model for a homogenized single layer beam from Eqn. 1. This was accomplished by matching the non-linear shape of the M - κ relationship of the leaflet bending data to the Ogden model analytical solution from a straight to circular beam (Fig. 3).

2.3 Interlayer bonding simulation

To simulate the effects of various levels of interlayer bonding, the following parametric simulation was performed. The fibrosa and ventricularis moduli were set to 45 kPa based on the prediction of the initial flexure simulation described above. Next, bending simulations were performed up to curvature changes of 0.1, 0.2 and 0.3 mm^{-1} with values for the spongiosa shear modulus μ_S varied. A value of 1 Pa represented relatively unbonded fibrosa and ventricularis layers; then μ_S was assigned increasing values ($\mu_S = 0.1$ –10 kPa) to simulate increasing levels of bonded states. Lastly, the spongiosa was assigned the same layer properties as the fibrosa and ventricularis to represent a perfectly bonded state. The deformation gradient tensor was derived from the reference (X_1, X_2) and deformed (x_1, x_2)

coordinates of the element nodes using $\mathbf{F}_{ij} = \frac{\partial x_i}{\partial X_j}$. The resulting deformation gradient tensor $\mathbf{F}$ was decomposed into stretch and rotation tensors, $\mathbf{U}$ and $\mathbf{R}$, respectively. The transmural

variation in total axial stretch, $\Lambda_1 = \sqrt{\mathbf{U}_{11}^2 + \mathbf{U}_{12}^2}$, with respect to the thickness of the tissue was used to determine the overall effects of bonding on transmural deformations.

Normalized thickness was defined with the ventricularis at the origin and the fibrosa at unity.

2.4 Transmural strain experimental validation studies

A custom device performed flexure tests on AV leaflets, described in detail in (Lam, 2004). Briefly, two optical systems simultaneously collect flexural rigidity and transmural deformation data. A macro imaging system tracks markers on one edge of the sample. From these markers the curvature was calculated as well as the applied load via a marker on the bending bar transverse linkage. On the opposite edge of the sample a micro imaging system employs micron-sized ink marks to determine transmural strain via post processing

techniques. A specially designed tank and tissue holder maintains placement and physiological conditions of the sample being tested.

Native porcine aortic roots were removed from hearts obtained from an abattoir within three hours of sacrifice. Leaflets were dissected from the root along their attachment points, and cut into a circumferentially oriented rectangular strip of tissue for testing using a fresh razor blade to ensure smooth flat surfaces in the reference state (Fig. 2-a). On average, specimens were 318 ± 46 microns thick, 13.7 ± 2.1 mm long, and 2.8 ± 0.2 mm wide (Lam, 2004). Five small red ink markers were created on the side of the tissue farthest from the free edge for curvature calculation using the macro imaging system (Fig. 2-c). Airbrushing the edge of the tissue opposite to the red macro markers using a Badger Sotar airbrush (Badger Air-Brush Co., Franklin Park, IL) and black India ink created microscopic markers for tracking with the micro-imaging system (Fig. 2-d). A stainless steel sleeve was glued to one end of the tissue using cyanoacrylate and then slipped onto a steel dowel to allow for free rotation and restricted translation of the tissue. The tank-tissue holder assembly was mounted to a bi-directional (X_1 axis movement) stage fitted with two precision linear actuators (model MM-4M-EX80, National Aperture Inc., Salem NH). A small amount of cyanoacrylate glue attached a second stainless steel sleeve to the loading end of the tissue; this sleeve then slipped over a vertically mounted 316V stainless steel bending bar of known stiffness. The stiffness of the bar determined the amount of force produced as a function of the bar's displacement, eliminating the necessity of load cells. Actuation of the bi-directional stage in the X_1 -direction moved the entire tank and sample, causing the transverse linkage to bend the bending bar. In this design, both positive and negative changes in curvature could be tracked, allowing a single-run experiment in both the with-curvature (WC) and against-curvature (AC) testing directions.

For the bending data, the following information was recorded for the duration of the experiment: coordinates of red macro markers, displacement of the bending bar, and the current curvature of the tissue. Analysis of the bending data was detailed by (Engelmayr et al., 2003) and summarized here. A custom-written Mathcad (PTC, Needham, MA) program computed curvature, change in curvature, and moment at the middle five markers for each time point of the experiment. The resulting marker positions were fit to a fourth-order polynomial so that the curvature of the tissue could be determined. The initial curvature, κ_0 , was recorded by the control system and subtracted from consequent curvature measurements to obtain the change in curvature $\kappa = \kappa - \kappa_0$. 18 specimens were tested to a curvature change up to 0.3 mm^{-1} based on recent *in vitro* measurements (Sugimoto and Sacks, 2013). For clarity, results are reported for a change in curvature of 0.2 mm^{-1} and the full transmural results are reported in Appendix II. The moment was determined by using the position of the central marker and the displacement of the bending bar (Eq. 2 and Fig. 2-b)

$$M = Py \quad (2)$$

where M is the applied moment, y is the deflection from the horizontal axis, and P is the axial force. The displacement of the bending bar was tracked in real-time since the applied axial force, P , was a function of the displacement. The deflection, y (Fig. 2-b) was computed using the spatial y coordinate of the central tissue marker and the y coordinate of the

horizontal axis drawn from post to post. The resulting data is reported as the averaged M/I vs. κ response of the 18 specimens to normalize with respect to specimen geometry.

To determine transmural deformation, captured images from the micro-imaging system that tracked the markers on the edge of the tissue were analyzed (Fig. 2-d). A telecentric lens was used to avoid loss of accuracy from taking measurements from surface bending, resulting in surfaces not orthogonal to the imaging system, on the strain measurements. From the macro-level images, LabVIEW software (National Instruments, Austin, TX) identified and numbered markers and stored their centroidal coordinates. For the micro images used to determine the transmural stretch, an image-based particle tracking strain-mapping method, detailed in the Appendix I, computed the local deformation gradient $\mathbf{F}$ over the imaged region. Briefly, the reference and deformed states determined the displacement field and were fitted to obtain $\mathbf{F}$. Rigid body deformation was removed using polar decomposition. The particle tracking method was shown to measure stretch as small as 1.001 with 0.05% net accuracy (Appendix I) (Lam, 2004). The location of the neutral axis was determined by plotting Λ_1 against the thickness of the tissue to determine the location where $\Lambda_1=1$.

2.5 Validation of experimental/simulation method

Validation studies were carried out with 1 mm thick silicone rubber sheets cut into rectangular strips of the same dimensions as the experimental studies described above, 14mm in length by 3 mm in width. The shear modulus of the strips was determined using tensile testing with a MTS Tytron 250 Mechanical Test System and in flexure using the same custom flex device. The estimated shear moduli values obtained from the three methods were compared to ensure accuracy of both the computational and experimental methods.

3 - RESULTS

3.1 Initial tissue material model

The resulting M - κ relation from the analytical solution was normalized to the maximum achieved moment at a curvature of 0.28 mm^{-1} , the maximum observed experimentally measured value. Although there was some variation with applied moment, we determined that a value for α of ~ 2.0 captured the shape of the M - κ (Fig. 3, $r^2=0.93$). It is important to note that the analytical solution utilized a single layer and simplified boundary conditions (Rivlin, 1949), and was thus only used to set the value of the α parameter. Based on these results, we returned to the isotropic incompressible hyperelastic neo-Hookean material model to simulate the AV tissue in flexure

$$W = \frac{\mu_L}{2} (I_1 - 3) - p(I_3 - 1) \quad (3)$$

where I_1 and I_3 are the first and third invariant of the left Cauchy-Green deformation tensor $\mathbf{C}=\mathbf{F}^T\mathbf{F}$, respectively, p is the Lagrange multiplier to enforce incompressibility, and L indicates the layer ($L=F, S$, or V). With this model a value of $\mu_L = 45 \text{ kPa}$ fit the data well (0.974) and was used for the fibrosa and ventricularis layers in the unimodular simulations.

3.2 Parametric interlayer bonding model study

As expected, the simulated transmural Λ_1 distribution demonstrated substantial sliding between the fibrosa and ventricularis layers at extremely low spongiosa layer moduli values (Fig. 4). As the spongiosa modulus more closely matched that of the outer layers (i.e. layers becoming more bonded), the transmural variation in Λ_1 became nearly linear. Both bending directions showed an expected shift in the neutral axis (NA) location towards the fibrosa layer since it was assigned a modulus stiffer than the ventricularis based on previous studies. From these basic simulations, we determined that in order for the spongiosa to exhibit measurable differences in transmural stretch from the fibrosa and ventricularis, μ_s must be less than 1 kPa. This estimated threshold is independent of imposed curvature as discussed in Appendix II.

3.3 Transmural strain experimental results

The M - κ response of AV tissue strips was observed to be slightly nonlinear in both bending directions (Fig. 5). It should be noted that our previous work assumed a linear M - κ relationship to simplify parameter estimation (Sacks et al., 2009). The current approach was more accurate since the moduli were estimated directly from the M - κ data (Fig. 5), including accounting for the observed small non-linearity. Interestingly, as in our three-point bending study (Sacks et al., 2009), no significant directional differences were found in the overall flexural response (Fig. 5)

However, we noted that the transmural strain measurements varied with bending direction. AV results revealed specimens flexed in the AC direction exhibited a maximum Λ_1 stretch that increased from 1.016 to 1.026, with a respective increase in the change of curvature from 0.1 to 0.3 mm^{-1} . The minimum Λ_1 stretches decreased, indicating an increase in strain, with increasing curvature with typical values progressing from 0.984 to 0.969. The average neutral plane location for all specimens showed a shift towards the fibrosa in the AC direction, but was not statistically significant (Table 2). The NA results indicated a shift towards the stiffer fibrosa layer.

3.4 Refined leaflet model

The motivation for refining the initial model was the observed differences between the WC and AC bending directions in the transmural Λ_1 distributions (Fig. 6). That is, the M - κ and transmural strain responses could not be simultaneously fit in both bending directions (Fig. 6c-d). This suggests an asymmetry in the tissue layer tensile-compressive responses. Thus, to capture the bidirectional M - κ and transmural response of the leaflet observed in flexure, a refined FE model was created using a bimodular material model. Further, since the parametric interlayer bonding study and experimental validation indicated the leaflet tissue is a functionally bonded unit, we developed the following refined model by absorbing the spongiosa equally into the two outer layers, resulting in a bilayer model. Geometry and initial curvature of the model was matched to the AV experimental specimens. The following bimodular incompressible neo-Hookean material model was implemented

$$W^{\pm} = \frac{\mu_{\pm}^{\pm}}{2} (I_1 - 3) - p(I_3 - 1) \quad (4)$$

which has four total moduli, $\mu_F^{\pm}$ and $\mu_V^{\pm}$, with the subscripts indicating the fibrosa and ventricularis layer and the superscripts indicating in tension (+) or compression (-), respectively.

As in the first model, we conducted simulations in both flexural directions and simultaneously matched to the experimental data. Selected nodal positions on the edge of the specimen, representing tissue marker positions used in experimental configuration, were used to compute and equivalent change in curvature for each applied moment. The moment was plotted with respect to curvature change taken from the point of maximum curvature at the center of the specimen. The experimental M- κ data was averaged and fit to the FE model. The fitting procedure was carried out in a two-step process. First, the interrelationship of the moduli for each bending direction was determined by matching the location of the NA to experimental data for both directions (Table 2). Secondly, the layer moduli values were determined by fitting the M- κ with the experimental data using a minimization of the mean squared error (MSE) (Table 3). The MSE was defined as

$\frac{1}{n}(\hat{y} - y)^2$, where n is number of experimental data points and $\hat{y}$ and y are the simulation and experiment derived M- κ relation, respectively. It was crucial to perform the fitting procedure in this way to ensure both the transmural deformation and the M- κ aligned with the experimental measurements. The magnitude values reported are within ± 0.5 kPa, and the computed Λ_1 through the thickness of the tissue is within the range experimental error (± 0.0141) of the experimental measurements (Fig. 6).

An aspect of our approach is accounting for distortion of a beam of rectangular cross section undergoing large strains, which is a well-known effect (Timoshenko, 1953). This distortion causes out-of-plane warping, and was observed experimentally in the AV tissue strips. In the present experimental setup, out-of-plane warping (in the X_3 direction) was estimated to affect the transmural stretch measurements by ~ 0.02 in the net axial stretch between the center of the specimen and the edge (Fig 7). Appendix II further investigates the effects of specimen geometry on the out-of-plane warping. Warping also affected the measured location of the neutral axis, making it appear almost unchanged with each bending direction when taken from the edge of the specimen (Figs. 7 b-c). This edge warping affected approximately 40% of the tissue volume (20% from each edge), leaving the interior 60% of the tissue largely unaffected. To account for this warping effect, we utilized the edge deformations to match the experimental data, as well as reported the predicted interior deformation responses that represent bulk tissue behavior as would occur *in vivo*.

The resulting simulations indicated that Λ_1 in the center of the specimen (representing the bulk of the tissue) demonstrated significant changes in NA location with bending direction (Fig. 8). In the WC bending direction, the NA shifted towards the ventricularis approximately 0.35 of the normalized thickness. Interestingly, this indicates the whole fibrosa is under compression and the tensile load is carried entirely by the ventricularis (Fig.

8). This shift in the NA towards the ventricularis was reflected in the estimated moduli (Table 3) indicating greater tensile stiffness in the ventricularis than compressive stiffness in the fibrosa (a ratio of approximately 2:1). In the AC bending direction the NA shifts to approximately 0.79 of the normalized thickness, indicating the fibrosa was much stiffer under tension than compression (Fig. 8). Again, this shift was reflected in the estimated moduli values, indicating greater tensile stiffness of the fibrosa compared with compressive stiffness of both the fibrosa and ventricularis (ratio of approximately 4:1).

3.5 Model validation

Silicone rubber test specimens demonstrated good agreement with tensile test shear moduli measurements. Specifically, the model estimated 6.454 ± 0.168 MPa, the experimental flex measurement was 6.658 ± 0.366 , and the tensile tests measuring 6.211 ± 0.225 MPa (Table 1).

4 - DISCUSSION

4.1 Overview

The present study investigated the interlayer micromechanics of the AV leaflet undergoing flexure using an integrated simulation/experimental approach. While the modeling effort was rather straightforward, we noted the need for examining both the macro-level flexural behavior ($M-\kappa$) and the micro-level transmural deformation, integrated into a 3D beam model, to accurately capture AV leaflet layer interactions. Our major findings were that 1) the AV leaflet layers function in flexure as a perfectly bonding unit, and 2) the spongiosa layer did not have measurably different mechanical properties compared to the fibrosa and ventricularis. This last point was primarily evidenced by the smooth transmural stretch distributions (Fig. 4). Thus, in the low strain environment, the spongiosa may be described mechanically as contiguous extension of the two outer layers.

4.2 Bimodular material model for leaflet tissue in flexure

It is important to emphasize here that a standard Neo-Hookean material, single layer model was only used in the first part of the study to set the basic model form and a range for the material model parameters. Interestingly in the refined model, a bimodular material model for the AV leaflet tissue in flexure was identified, similar to approaches used for other fibrous tissues and part of a larger sub-class of bimodular materials (Curnier et al., 1995, Ateshian, 2007). We also determined that both the fibrosa and ventricularis have greater stiffness in tension than in compression (a ratio of approximately 4:1, Table 3). The fibrosa was found to be consistently stiffer than the ventricularis, approximately 2:1 in both tension and compression (Table 3). The bimodular behavior was not entirely surprising based on the unique arrangement of ECM components throughout the three layers. Moreover, the transmural deformation of the AV leaflet tissue indicated that the NA shifted closer to the ventricularis in the WC direction at all curvature levels. This implied that when the ventricularis was under mainly tensile loading in the circumferential direction and the fibrosa was fully under compression, the ventricularis provided most of the stiffness (Fig. 8). When the valve flexed in the opposite direction (AC), the NA shifted closer to the fibrosa, leaving the entire ventricularis and one third of the fibrosa under compressive loading in the

circumferential direction, and the remaining two thirds of the fibrosa under tension, providing most of the stiffness (Fig. 8). Structurally, this behavior was likely due to the greater concentration of the comparatively stiff type I collagen within the fibrosa and the greater concentration of the more compliant elastin found in the ventricularis. Previous studies have shown that the elastin in the ventricularis forms a honeycomb network around the collagen fibers, allowing the fibers to stretch and return to their initial state (Scott and Vesely, 1995, Vesely, 1998). The collagen fibers in the fibrosa do not possess an extensive or organized elastin network and are bound together more tightly (Schoen and Levy, 1999). Additionally, proteoglycans intimately bind to collagen fibers and may provide significant reinforcement to the fibrosa in doing so, compared to the collagen-depleted ventricularis.

4.3 Implications for AV micromechanical function

Functionally, the leaflet's ability to present low flexural stiffness during valve opening (WC) and high stiffness during closure (AC) is only attainable through its distinctive hierarchical structure in which all elements act in unison to provide seamless transition from the diastole to systole cycle. The present study indicated that, contrary to previous hypotheses (Mohri et al., 1972, Vesely and Boughner, 1989, Song et al., 1990, Thubrikar, 1990, Talman and Boughner, 1995), the spongiosa did not allow the fibrosa and ventricularis layers to slide with respect to one another. If this did occur (i.e. when functioning as loosely bonded layers), each layer would have its own neutral axis (Fig. 4), greatly complicating intra-layer deformation patterns. Instead, the leaflets appear to function as a fully bonded unit. These findings are also consistent with our observations (Stella and Sacks, 2007) of the presence of a large number of collagen fiber interconnections between the fibrosa and ventricularis layers. While recent work by Tseng et al. (Tseng and Grande-Allen, 2012) shows intriguing results of elastin presence in the spongiosa layer, the nature of the fibrous interconnections between the layers at a gross level appears to be dominated by collagenous fibers. However, the exact composition and structure of these interconnecting fibers remains unknown. More importantly, the composition of these fibers has no effect on the findings of the current study, since what is important is that they exist and appear to bind the fibrosa and ventricularis layers.

The question remains: what is the mechanical role, if any, of the spongiosa layer? In a recent study we noted that viscoelastic behavior of heart valve tissues only manifested themselves in the low strain region (Eckert et al., 2013). Also, other studies have demonstrated a functionally elastic behavior for native valvular tissues in tension (Grashow et al., 2006a, Grashow et al., 2006b, Stella et al., 2007). Yet, it can be speculated that the centrally located spongiosa layer functions to reduce high frequency motions of the leaflet during the opening/closing phases. Such dampening-like behavior might be a mechanism to reduce hemodynamic energy loss during the opening and closing phases. Current information on this aspect of valve function is scant, and must be the subject of future investigations.

4.4 Limitations and the need for an integrated experimental/simulation approach

In actual heart valves, flexure occurs during the opening/closing phases and is bidirectional (Iyengar et al., 2001, Sugimoto and Sacks, 2013). Since it is not currently possible to experimentally investigate this behavior directly, we chose instead to approximate this

behavior with a strip model in circumferential bending (Sugimoto and Sacks, 2013). While not accounting for bending in both directions, this is also the primary fiber direction and any measured behavior should approximate the native tissue response *in vivo*. It should also be noted that Vesely et al. (1989) investigated the flexural behavior of native AV leaflets and found that the NA was close to the fibrosa surface under AC flexure, suggesting a very low compressive modulus compared to tensile in agreement with the present study. However, testing was performed under unrealistic loading conditions, making it difficult to correlate to *in vivo* function. In the present approach, an end-loading configuration was used, which should be more representative of the *in vivo responses* of the bulk leaflet tissue since it avoided any artificial contact points. We also note that the measured transmural deformation data must be matched with the computational model at the edge of the specimen and then reported from the central region in order to represent the bulk tissue behavior accurately.

4.5 Conclusions

In summary, the behavior of AV leaflet tissue as a composite beam was determined by examining the M - k relationship and the stretch along the edge of the tissue during bending. We conclude that while it has been previously speculated that valve layers slide with respect to one another during valve opening/closing, our evidence suggests that the leaflet layers function as a *single bonded unit*. Layers appear to be bonded by transverse collagen fibers that hold residual strains in place. Thus, despite a heterogeneous structure and differences in stress distribution throughout the layers, the AV leaflet deforms as a homogenous structure. Furthermore, the valve tissue in flexure requires a bimodular material model. Further studies are needed to investigate the leaflet micromechanics in activated cellular conditions that represent healthy and diseased physiological conditions.

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Nomenclature

AV	aortic valve
AC	Flexure direction directed <u>against</u> the natural curvature of the leaflet
ECM	extracellular matrix
FE	finite element
GAG	glycosaminoglycans
I	second moment of inertia
k	change in valve leaflet curvature during flexure testing
M	applied bending moment
PG	proteoglycan

μ	shear modulus
TE	tissue engineering
W	strain energy function
WC	Flexure direction directed <u>with</u> the natural curvature of the leaflet

Appendix I

Transmural Deformation Analysis

The images captured using the micro-imaging system (Fig. 2D) were analyzed by locating the markers that had been airbrushed onto the edge of the tissue. A custom program was written in LabVIEW (National Instruments, Austin, TX) to post-process the images taken by the micro-imaging system so that displacement fields could be determined. The markers were identified, numbered, and their areas and centroid coordinates were determined. This procedure was performed simultaneously for the reference image and for the deformed image. The software then displayed both altered images concurrently so that the user of the program could match markers between the reference image and the deformed image.

From the resulting images the coordinates of the reference markers were referred to as the (x_1, x_2) system and the deformed coordinates were referred to as the (X_1, X_2) system. The displacements, u and v , were calculated from the former using $u=X_1-x_1$ and $v=X_2-x_2$ respectively. These quantities were then fitted to the surface described by Eq. (A.1).

$$u=a_0+a_1X_1+a_2X_2+a_3X_1X_2+a_4X_1^2+a_5X_2^2 \quad v=b_0+b_1X_1+b_2X_2+b_3X_1X_2+b_4X_1^2+b_5X_2^2 \quad (\text{Eq. A.1})$$

The surface fit to the u and v coordinates achieved an r^2 value of approximately 0.9. A higher order fit could have been used resulting in a higher r^2 , this would produce a rough surface due to variations in marker location from thresholding. The lower order fit maintains a smooth surface, true to the nature of the sample tested, by not overfitting the curve to all variations in marker location. By evaluating Eq. (A.2), the deformation gradient was obtained.

$$\mathbf{F}=\mathbf{H}+\mathbf{I}=\begin{bmatrix} \frac{\partial u_1}{\partial X_1} & \frac{\partial u_1}{\partial X_2} \\ \frac{\partial u_2}{\partial X_1} & \frac{\partial u_2}{\partial X_2} \end{bmatrix}+\begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}=\begin{bmatrix} \lambda_1 & \kappa_1 \\ \kappa_2 & \lambda_2 \end{bmatrix} \quad (\text{Eq. A.2})$$

The $\mathbf{F}$ was then decomposed into its stretch and rotation tensors, $\mathbf{U}$ and $\mathbf{R}$, respectively Eq. (A.3). The polar decomposition of the deformation tensor removes rigid body motion effects into the rotation tensor, leaving only the stretch deformation information in the stretch tensor. The rigid body rotation information in $\mathbf{R}$ was calculated to determine the degree of rotation experienced by the tissue during flexure. Higher levels of rigid body rotation were determined to be coincident with measurements taken away from the center of the tissue.

$$\mathbf{F}=\mathbf{R}\mathbf{U} \quad \mathbf{U}^2=\mathbf{F}^T\mathbf{F} \quad \mathbf{R}=\mathbf{F}\mathbf{U}^{-1} \quad (\text{Eq. A.3})$$

The stretch tensor components U_{11} and U_{22} correspond to local tissue strains in the X_1 and X_2 directions, respectively. Thus, the location of the neutral axis was determined by plotting U_{11} against the thickness of the tissue. The depth of the tissue that coincided with the U_{11} value of unity was the corresponding location of the neutral axis. Rotation that occurred in the displacement field was characterized by determining the angle of rotation, α , incurred in the deformed system from the reference state.

Appendix II

Parametric Interlayer Bonding Study Supplement

The reported results for the parametric bonding study represented a curvature change of 0.2mm^{-1} solely for sake of clarity, as the same trends were observed at curvature changes of 0.1, 0.2 and 0.3mm^{-1} (Figure A1a-c). These choices of curvature change were taken from our *in-vitro* measurements (Sugimoto and Sacks, 2013). Not surprisingly, we noted that the presence of interlayer sliding (not magnitude) either simulated (Figure A1) or experimentally (Figure A2) was not a function of the level of bending (i.e. κ), but only of the ratio of the ventricularis and fibrosa:spongiosa moduli for the simulations. Thus, the estimated μ_s threshold is independent of imposed curvature. Greater bending simply created greater sliding.

Parametric Out-of-Plane Warping Study Supplement

To investigate the effects of leaflet geometry on the simulation findings of out-of-plane warping effects, a parametric simulation was performed varying the thickness of the leaflets as well as the curvature change. The specimen length and width remained constant for the simulations. Figure 7 demonstrates the significant change in net axial stretch between the center of the specimen and the edge. Therefore, this change in absolute axial stretch (Λ_1) was used as a metric of warping and plotted against the change in specimen thickness (Figure A3). Results found that increasing thickness of the specimens exaggerated the degree of warping. Furthermore, as expected, this warping effect increases with increasing curvature change.

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Figure 1.

(a) The aortic valve (b) A 1 mm×1 mm section from the central belly region illustrating the 3D the tri-layered leaflet structure.

Figure 2.

(a) A schematic showing the orientation of the tissue strip and leaflet, and the location of the transmurial strain measurement, (b) A free body diagram of tissue mounted in the testing device with a force, P , applied to its free end. The orientation of the X_1 and X_2 coordinate system specifies the X_1 axis coincident with the circumferential direction and the X_2 axis to reflect the thickness of the leaflet, (c) Markers used by the macro-imaging system shown on the edge of the tissue strip, (d) Transmurial strain images obtained using micro camera, showing the reference and deformed tissue states.

Figure 3.

Comparison of the M - κ data (Merryman et al., 2006) with the analytical solution of the one-term Ogden model using various α parameter values. Note that the experimental data was normalized to the maximum achieved moment at a κ of 0.28 mm^{-1} . A value for α of 2.0 approximately captured the shape of the M - κ .

Figure 4.

Transmural stretch Λ_1 simulation results plotted against the normalized leaflet thickness. For measurable differences to occur in transmural stretch between the fibrosa and ventricularis, simulations indicate the spongiosa must possess a shear modulus less than 1 kPa. The WC bending direction simulation results are shown for sake of clarity, although the same simulation results were observed for the AC bending direction. The curvature change reported is 0.2mm^{-1} since the reported results demonstrated independence of curvature change (Appendix II)

Figure 5.

The $M-\kappa$ response of a native porcine AV leaflet in the AC and WC directions that has been bent to a κ of 0.2 mm^{-1} in either direction. Simulations using a unimodular (dashed line) and bimodular material model (solid line) are superimposed over experimental data. The bimodular model represents the bidirectional bending behavior slightly better than the unimodular material.

Figure 6.

Experimental transmural Λ_1 results plotted against the normalized thickness for (a,c) WC and (b,d) AC samples at $\kappa=0.2 \text{ mm}^{-1}$, along with the corresponding unimodal (dashed line) and bimodal (solid line) simulation results. (a)-(b) represent strain matched in the WC direction, showing the resulting discrepancy in the AC fit using a unimodal model. Similarly, (c)-(d) represent strain matched in the AC direction, resulting in a poor WC fit using the unimodal model. Although the M- κ behavior is captured with unimodal model, the transmural deformation response can only be captured in both the WC and AC directions using the bimodal material model.

Figure 7.

(a) Transmurular Green-Lagrange strain distribution (E_{11}) from the simulated flexure model demonstrating a disruption at the edges due to standard warping that occurs during bending. (b) A significant shift (2% stretch) in axial deformation is observed by plotting Λ_1 vs. normalized thickness in both the WC and (c) AC bending directions. It is important to note the associated shift in the neutral axis location when observing deformation at the edge or in the unaffected central region of the tissue.

Figure 8.

A schematic summarizing the changes in NA location experienced by the bulk tissue for both bending directions. In the WC bending direction, the NA shift towards the ventricularis approximately 0.35 of the normalized thickness, demonstrating the whole fibrosa under compression (–) and the majority of the ventricularis under tension (+). In the AC bending direction the NA shifts to approximately 0.79 of the normalized thickness. These results indicate that both the ventricularis and fibrosa are much stiffer under tension (+) than compression.

Figure A1.

Transmural deformation results of parametric bonding simulation, λ_1 is plotted against the normalized leaflet thickness for a curvature change of 0.1, 0.2, and 0.3 mm^{-1} . A tri-layered rectangular strip represented the AV and shear moduli values ranging from 1.0 Pa to 45 kPa were assigned to the central spongiosa layer to emulate varying degrees of connectivity between the outside layers and identify the following relationship: $\mu_F = \mu_V : \mu_S$. Results indicate for all curvature levels that for measurable sliding to occur between the fibrosa and ventricularis, the spongiosa must possess a shear modulus less than 1 kPa.

Figure A2.

Experimental results obtained from transmural bending tests performed on native aortic valve tissue. The tissue was bent to three different changes of curvature, 0.1, 0.2, and 0.3 mm⁻¹. As curvature increased, the deformation increased as expected, yet no sliding is observed.

Figure A3.

The effect of leaflet thickness on the out-of-plane warping estimated by the simulation (Figure 7). The degree of warping, measured by the absolute change in net axial stretch (Δ_1), increases with increasing leaflet thickness. Additionally, this relationship is maintained and exaggerated with increasing curvature (0.1, 0.2 and 0.3 mm⁻¹).

Table 1

Silicone material validation study, showing E (MPa)

Specimen	Model	Flexure	Tensile
1	5.973	5.805	5.551
2	6.501	6.558	6.557
3	6.593	6.677	6.333
4	6.749	7.591	6.403
Average	6.454	6.658	6.211
SEM	0.168	0.366	0.225

Table 2

Predicted and measured neutral axis locations

κ (mm ⁻¹)	Against Curvature (AC)		With Curvature (WC)	
	Experiment	Simulation	Experiment	Simulation
0.1	0.5987 ± 0.0929	0.6209	0.5453 ± 0.1012	0.4935
0.2	0.5903 ± 0.1022	0.5994	0.5571 ± 0.1021	0.5495
0.3	0.6026 ± 0.0730	0.5835	0.5629 ± 0.0315	0.5670

Table 3

Normalized material parameters

n=18 MSE=	Estimated Moduli Values (kPa)		Relative Moduli Values		Layer moduli ratios
	μ^+	μ^-	μ^+	μ^-	μ^+/μ^-
Fibrosa	139.5	29.55	100%	21.2%	4.72:1
Ventricularis	65.00	16.74	46.6%	12.0%	3.88:1