Published in final edited form as:

Acta Biomater. 2019 February ; 85: 241–252. doi:10.1016/j.actbio.2018.12.029.

Comparative mechanical, morphological, and microstructural characterization of porcine mitral and tricuspid leaflets and chordae tendineae

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Abstract

Background: Healthy function of tricuspid valve (TV) structures is essential to avoid tricuspid regurgitation (TR) and may significantly improve disease prognosis. Mitral valve (MV) structures have been extensively studied, but little is known about the TV and right-sided heart diseases. Therefore, clinical decisions and finite element (FE) simulations often rely heavily on MV data for TV applications, despite fundamentally different mechanical and physiological environments.

Method/Results: To bridge this gap, we performed a rigorous mechanical, morphological, and microstructural characterization of the MV and TV leaflets and chordae in a porcine model. Planar biaxial testing, uniaxial testing, second harmonic generation imaging and Verhoeff Van Gieson staining were performed. Morphological parameters, tissue moduli, extensibility, and anisotropy were quantified and compared. No major differences in leaflet mechanics or structure were found between TV and MV; chordal mechanics, morphology, and structure were found to compensate for anatomical and physiological loading differences between the valves. No differences in chordal mechanics were observed by insertion point within a leaflet; the septal tricuspid leaflet (STL) and posterior mitral leaflet (PML) did not have distinguishable strut chords, and the STL had the shortest chords. Within a valve, chords from septally-located leaflets were more extensible. MV chords were stiffer.

Conclusions: This study presents the first rigorous comparative mechanical and structural dataset of MV and TV structures. Valve type and anatomical location may be stronger predictors of chordal mechanics. Chords from septally-located leaflets differ from each other and from their

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⁶CONFLICTS OF INTEREST

Dr. Wei Sun is a co-founder and serves as the Chief Scientific Advisor of Dura Biotech. He has received compensation and owns equity in the company.

intravalvular counterparts; they merit special consideration in surgical and computational applications.

Graphical Abstract

Keywords

tricuspid valve mechanics; tricuspid regurgitation; chordae tendineae; biaxial testing; uniaxial testing

1. INTRODUCTION

The tricuspid valve (TV) apparatus (TA) is a complex structure, composed of the valve leaflets, tricuspid annulus, the papillary muscles (PM), and the chordae tendineae of the right ventricle, which originate from the papillary muscles and attach on the ventricular side of the leaflets. The chords maintain proper coaptation during systole by preventing the leaflets from billowing into the right atrium (RA) and ensuring unidirectional flow of blood through the right ventricle (RV) [1]. Thus, proper TV coaptation is critical for maintaining a healthy function of the right side of the heart may have a significant impact on the clinical condition and prognosis of the patient [2].

Impairment of any of the TV components (TV leaflets, PM, and tricuspid chordae tendineae) may lead to tricuspid regurgitation (TR), which is the most common TV disease in North America [3]. Moderate to severe TR is associated with a poor prognosis, just a 63.9% one-year survival rate [4]. The most common form of TR, accounting for greater than 80% of cases, is referred to as “functional” TR, and occurs due to dilatation of the annulus as a result of increased pulmonary or RV pressure and is often secondary to right heart valve disease [2, 5]. There are a number of challenges associated with TR repair, such as a lack of a reliable method to quantify TR severity or assess the true RV function. Currently, the clinical decision is crucial [2]; in the case of TR surgical intervention is complicated due to the geometrical limitations of the TV chordae, especially in the case of abnormally short and thick chordae, as in the case of congenital TV aberrations [6]. Additionally, differences in the left and right ventricular hemodynamics contribute to the differences in the physiological and mechanical environment of the ventricles and may account for the disparity of the efficacy of valve repair outcomes between the MV and TV [7].

It has been shown that chordal tension in the mitral valve (MV) is directly correlated to the leaflet malcoaptation, which results in mitral regurgitation (MR) [8, 9]. While the valvular and the chordal mechanics in the left side of the heart have been extensively studied [10, 11], less is known about the right side [12, 13]. The mechanical properties of the chordae tendineae of the mitral and the tricuspid valves in particular have not been thoroughly

investigated and compared, and most of what is known about these valvular structures is based on MV investigations. Few studies utilized human and animal models to study TV chordal function, but comparative studies are almost nonexistent [7, 14, 15].

Finite element (FE) models have also been extensively used to gain a deeper understanding of the left heart mechanics [16–18], however FE modeling of various TV diseases remains stalled by the lack of material properties of these tissues in the literature. As a result, FE simulations on the right side of the heart rely heavily on available left heart data, and assume similar mechanical properties for the TV leaflets and chords. A 2010 study by Stevanella et al. created a preliminary, although limited, model of the TV [3], wherein valve geometry was based on experimental observations of human and porcine tissue, mechanical properties of TV leaflets were obtained from MV studies, and the mechanical properties of chords were approximated by interpolating the mechanical results presented by Lim [7]. Additionally, recent studies have shown the mitral and tricuspid valve leaflets to also differ in their mechanical properties [19], making modeling efforts based on left heart mechanics possibly insufficient for the investigation of TV diseases.

In order to bridge the gap in the knowledge of right heart mechanics, the current study aims to characterize the mechanical properties of TV leaflets and chordae tendineae and compare them to their MV counterparts. An improved understanding of the biomechanics of the leaflets and chordae regarding morphology, geometry, elasticity, strength, and microstructure has a twofold benefit: firstly, it will be essential for the development and refinement of TV leaflets and chordal repair and replacement techniques; secondly, it will serve as a baseline to inform computational models of the right heart about the relevant physiological characteristics of the leaflets and chordae tendineae to be used in simulations of the TV mechanics. Thus, this study focuses on the morphological, structural, and mechanical characterization and computational modeling of TV leaflets and chords of porcine tissue, as well as on the comparison to those of the MV leaflets and chords from the same subjects.

2. METHODS

2.1. Sample Procurement and specifications

Porcine hearts were obtained fresh from the local abattoir (Holifield Farms, Covington, GA), ranging between 1 and 5 years of age. The MV and TV leaflets and chords were cryopreserved in 90%/10% RPMI-1640/DMSO solution upon explantation and stored at –80°C until testing could be performed [20, 21].

The leaflets, anterior mitral (AML, n = 10), posterior mitral (PML, n = 15), anterior tricuspid (ATL, n = 9), posterior tricuspid (PTL, n = 12), and septal tricuspid (STL, n = 16), were tested biaxially. Chordae specimens were classified by leaflet type and insertion point on the leaflet and tested uniaxially (Figure 1). Chords with an insertion point along the free margin of the leaflet were identified as marginal (M), while the rest with attachment points away from the margins were identified as basal (B) [14, 16, 22]. Literature review also points to the presence of two thicker basal chords near the center of the AML valve leaflets, called struts (S) which are absent in the PML, which role is considered pivotal for the preservation of the left ventricle geometry and systolic function [22–24]. The strut chords

are distinguished as the two largest and thickest chords originating from the papillary muscles and inserting into the ventricular surface of the anterior leaflet between the 4 and 5 o'clock positions and between 7 and 8 o'clock positions [14, 22–24]. The PML is not associated with strut chordal structures and confirmed by our observations, and similarly we did not observe two identifiable strut chords with the STL, as shown in Figure 1.

2.2. Biaxial Testing

Planar biaxial tensile testing was conducted according to the methods described by Sacks and Sun [25]. Briefly, the leaflet was mounted onto the testing device such that the X_1 axis corresponded with the circumferential direction and X_2 with the radial. Thickness was measured and averaged in three distinct locations throughout the testing region using a Mitutoyo 7301 rotating thickness gauge (Aurora, IL, ± 0.01 mm resolution).

A square testing region was delimited by 16 suture hooks, 4 per side. Four graphite markers delineating a $2\text{ mm} \times 2\text{ mm}$ square region were fixed with cyanoacrylate adhesive in the center of the testing region for strain tracking. The uniformity of the strain field was ensured by selecting a small target region located away from the hook attachments and re-zeroing the marker locations after each load-unload protocol. The sample was mounted onto a custom-made testing machine in a trampoline-like fashion, submerged in a 0.9% saline solution maintained at 37°C for the duration of the test, and subjected to a load-controlled testing protocol. The ratio of the normal Lagrangian stress components P_{11} : P_{22} was predefined with shear terms $P_{12} = P_{21} = 0$. Samples were subjected to a minimum of 30 equibiaxial preconditioning cycles at maximum loads chosen to not exceed the physiological operating range of the tissue such as to minimize hysteretic effects, followed by 7 testing protocols, with P_{11} : $P_{22} = 1:1, 0.75:1, 0.5:1, 0.3:1, 1:0.75, 1:0.5, 1:0.3$. The maximum loading range (300–400g) chosen for the biaxial testing resulted in equibiaxial stresses in the range of 100–200 kPa which corresponds with physiological loading conditions [26, 27]. Repeatable loading curves achieved after preconditioning indicate that the chosen maximum loads did not induce mechanical damage in the specimens.

2.3. Uniaxial Testing

Morphological parameters such as chordal width and length were determined from images of the whole leaflet with attached chords and analyzed using ImageJ. The length of the chord was defined from the insertion point at the papillary muscle to the insertion point at the leaflet. Prior to leaflet testing, the chords were explanted and prepared for uniaxial failure testing. Graphite markers were fixed along the sample for strain tracking. The axial force was measured by means of a 500 lbf load cell (TestResources SM-500–294). Samples were clamped with sand paper into the machine grips and subjected to 10 preconditioning cycles before loading at a rate of 3 mm/min until failure. Samples were kept hydrated with saline solution throughout testing.

2.4. Histological Staining

Representative histological chordae specimens were extracted, fixed in 10% formalin for at least 24 hours, processed, and embedded in paraffin. Samples were sectioned into $6\text{ }\mu\text{m}$ -thick slices along the length of the chordae, and subjected to Verhoeff Van-Gieson (VVG)

staining. Analysis of tissue microstructure was performed with the Zeiss Axio Scope. Images were taken at 20x magnification level and processed with the Zeiss Zen imaging software. The VVG slides show both collagen (pink) and elastic fibers (black).

2.5. Second Harmonic Generation Imaging

Representative leaflet specimens were also subjected to second harmonic generation (SHG) imaging to visualize local collagen fiber architecture in the belly region of the leaflet, as for biaxial tensile testing. Leaflets were imaged on a Zeiss 710 NLO inverted confocal microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY, USA) equipped with a mode-locked Ti:Sapphire Chameleon Ultra laser (Coherent Inc., Santa Clara, CA) in combination with non-descanned detection (NDD). The laser excitation was set to 800 nm and backward SHG signal was filtered from 380–430 nm. Samples were kept hydrated with saline solution during imaging to prevent drying artifacts and covered with #1.5 coverslips. SHG was collected from the atrial side of the leaflets using a Plan-Apochromat 63x oil immersion objective for correlation of local collagen fiber architecture to the local biaxially-derived mechanical properties. Image resolution was set to $0.44 \times 0.44 \mu\text{m}^2$ per pixel at 8-bit pixel depth.

2.6. Constitutive Modeling

The average biaxial mechanical response for each leaflet type was fit in the least squares sense to the Fung-type model [28], as the valve leaflet materials are assumed to be homogeneous and incompressible, and exhibited nonlinear, hyperelastic, and anisotropic responses [19]. Additionally, the viscoelastic effect of the heart valves is ignored [29], and tissues are assumed to exhibit strain-rate insensitive behavior [30, 31]. Leaflet mechanical responses were fit according to the strain energy function below, where c and A_i are material constants and E_{ij} are the components of the Green strain tensor:

$$W = \frac{c}{2}(e^Q - 1) \quad (\text{Eq. 1})$$

$$Q = A_1 E_{11}^2 + A_2 E_{22}^2 + 2A_3 E_{11} E_{22} + A_4 E_{12}^2 + 2A_5 E_{11} E_{12} + 2A_6 E_{22} E_{12}$$

Although the applied loads are perpendicular to the tissue edges and aligned with the material axes thus predefining zero shear terms, the inclusion of E_{12} terms accounts for minimal shear contributions given rise due to experimental nature of the procedure. The elastic portion of the chordal mechanical responses was averaged by leaflet type and insertion point and fit according to the Ogden strain energy function [32], where μ and a are material constants, and λ_j represent the principal stretches:

$$W = \frac{2\mu}{a}(\lambda_1^a + \lambda_2^a + \lambda_3^a - 3) \quad (\text{Eq. 2})$$

2.7. Data Analysis

All results are presented as mean $\pm$ standard error of the mean (SEM). Tissue thickness was obtained from each individual group. All biaxial plots show Green Strain, calculated as $E = \frac{1}{2}(\mathbf{F}^T \mathbf{F} - \mathbf{I})$, and Second Piola-Kirchoff Stress, calculated as $\mathbf{S} = \mathbf{F}^{-1} \frac{\mathbf{f}}{hL}$, where $\mathbf{F}$ is the deformation gradient, $\mathbf{f}$ is the current force value in the circumferential and longitudinal directions, h and L are the initial unloaded thickness and length, respectively. From the biaxial tensile testing data, stiffness was quantified in both radial and circumferential direction by means of the tangent modulus (TM) in the low and high linear regions of the equibiaxial response curve, along with the extensibility (EXT) (Figure 2). The data points within each region were fitted in the least-square sense by means of a custom MATLAB code (MathWorks, Natick, MA). TM was calculated from the slope of the fitted line, and extensibility was defined as the intersection of the fitted line from the high linear region with the x-axis. The degree of anisotropy (DA) was quantified as the ratio of the maximum strains reached in the equibiaxial response. For the anisotropy calculations the chosen level of stress ensured full engagement of the collagen fibers (100 kPa for ATL, PTL, STL, and AML and 65 kPa for the PML). For uniaxially tested chords, lower (LTM) and upper (UTM) tangent moduli, as well as EXT were calculated in a similar manner and compared among leaflets as well as by chord type.

All results were tested for normality of distribution using the Shapiro-Wilk and Kolmogorov-Smirnov tests and then subjected to the one-way analysis of variance test. Normally distributed results were compared by use of the unpaired student's t-test, while the Dunn's test was utilized for non-parametric results. p-values less than 0.01 were considered significant, and p-values less than 0.001 were considered highly significant.

3. RESULTS

3.1. Atrioventricular leaflets

All mechanical and morphological parameters for the leaflets are summarized in Table 1.

3.1.1. Leaflet Morphology—The MV, on average, had thicker leaflets (1.14 ± 0.04 mm and 1.30 ± 0.03 mm for the AML and PML, respectively) than the TV (ATL: 0.81 ± 0.05 mm, PTL: 0.73 ± 0.02 mm, STL: 0.89 ± 0.01 mm), although this finding was not statistically significant. The PML was found to be the thickest leaflet.

3.1.2. Leaflet Mechanics—Figure 3 depicts the average equibiaxial response for the TV (A) and MV (B) leaflets. All leaflets exhibited the nonlinear, anisotropic response characteristic of collagenous tissues, with the circumferential direction being significantly stiffer and less extensible than the radial direction.

No significant differences were observed within the circumferential or radial UTM values among any of the leaflets (Figure 4). In the circumferential LTM, no differences were observed within the TV leaflets, however the PML was significantly more compliant than the STL and AML (307 ± 54 kPa vs. 2383 ± 495 kPa and 1237 ± 428 kPa, respectively), but

not significantly against the ATL and PTL (3710 ± 1495 kPa and 4652 ± 1319 kPa, respectively). No significant differences were observed in radial LTM values.

Circumferentially, the STL was found to be significantly less extensible than both the AML and PML (0.01 vs. 0.11 and 0.05, respectively), but on par with ATL and PTL (0.02) (Figure 5). No significant differences were found in radial EXT or DA values.

3.1.3. Leaflet Microstructure—Appendix Figure A1 shows representative SHG images for each of the atrioventricular leaflets. No visible differences were observed in collagen fiber crimp, dispersion, or orientation. Fibers show a general alignment in the circumferential direction, agreeing with the observed mechanical anisotropy (Figures 3–5). Additionally, the lack of observable structural differences in the collagen fibers agrees with the similarity of UTM values (Figure 4).

3.2. Atrioventricular chords

All mechanical and morphological parameters for the chords are summarized in Table 2.

3.2.1. Chordal Morphology—Leaflets were found to have a similar chordal layout, with a dispersion of basal and marginal chords. The ATL, PTL, and AML were also found to have two thicker identifiable strut chords, while the PML and STL did not. Figure 6A shows the thickness of the strut, basal, and marginal chords for each leaflet group. While no significant morphological differences were observed between chords within a chordal type, the strut chords were consistently found to be significantly thicker than the corresponding marginal chords within the same leaflet. Aside from the AML chords, no significant differences were found between basal and marginal chords.

Additionally, significant differences in chordal length were found. The strut chords were found to be significantly longer than the basal and marginal chords within a leaflet (Figure 6B). Between leaflets, additional differences were observed. The AML strut chords were found to be significantly longer than their ATL and PTL counterparts (29 ± 3 mm, 20 ± 2 mm, 16 ± 1 mm, respectively). In the basal chords, no differences were observed among MV leaflets, although the STL was found to have significantly shorter basal chords (11.0 ± 0.2 mm) than all of the other leaflets (ATL: 13.7 ± 0.4 mm, PTL: 12.0 ± 0.3 mm, AML: 16 ± 2 mm, PML: 14.8 ± 0.8 mm). The PML was found to have significantly shorter marginal chords than both the AML and ATL (8.3 ± 0.9 mm vs. 11.5 ± 0.7 mm and 12.3 ± 0.6 mm, respectively), but statistically on par with the marginal STL chords (10 ± 3 mm). The marginal STL chords were also found to be significantly shorter than those of the ATL.

3.2.2. Chordal Mechanics—Figure 7 shows the average uniaxial response for the chords for each leaflet and each chord type. Figure 8 shows the chordal UTM (A), LTM (B), and EXT (C) divided by leaflet and chord type. Aside from LTM in the STL chords, no inter-leaflet differences were found between chordal groups. Strut chords were not found to differ in modulus or extensibility between any leaflet groups. Additionally, no significant differences were found between the ATL and PTL or between the AML and PML leaflets.

The STL was found to have significantly more extensible basal and marginal chords (0.11 ± 0.01) than the ATL (0.02 ± 0.01 and 0.02 ± 0.01 , respectively) and PTL (0.06 ± 0.01 and 0.03 ± 0.01 , respectively). With respect to LTM, the STL had significantly more compliant basal chords (2.9 ± 0.2 MPa) than both the ATL and PTL (4.7 ± 0.6 MPa and 5.3 ± 0.6 MPa), although with respect to UTM marginal chords were only significantly more compliant against ATL. Additionally, STL chords were also more compliant than both AML and PML chords. The STL chords were similarly extensible as compared to the AML (0.09 ± 0.02 and 0.05 ± 0.01 , respectively), although PML basal chords were significantly less extensible (0.04 ± 0.01) than their STL counterparts.

3.2.3. Chordal Microstructure—The representative VVG images (Appendix Figure A2) highlight the relationship between the chordal microstructure and chordal mechanics. VVG stain was utilized to visualize the architecture of the elastic fibers in the chordae tendinae in order to relate matrix organization to the exhibited mechanical properties of the chordae tendinae. Comparing the MV chords, the PML had similar crimp but more interfibrillar gaps than the AML, possibly contributing to its similar compliance but lower extensibility. Additionally, while the PTL and STL had more interfibrillar gaps than the ATL, the decreased crimp and presence of elastin in the PTL as compared to the STL contributed to its higher LTM and lower extensibility.

3.3. Constitutive model fitting

Table 3 shows the Fung-type and Ogden model parameters as obtained for the leaflets and chords, respectively. The strain response within a group was averaged for each stress level among all tested specimens within this group. The average mechanical response within a group was then fitted to obtain “average” constitutive parameters representative of the mechanical response of the group. The models were able to accurately fit the mechanical response, with $R^2 > 0.9$ for every fit.

4. DISCUSSION

4.1. Summary

The mechanical and structural properties of the left side of the heart have been extensively characterized in the literature [10, 11, 14, 16, 19, 33–35]. In stark contrast, the right side of the heart and its structures have been largely understudied, especially in the context of the atrioventricular leaflets and chords [7, 36]. Moreover, side-by-side comparisons of the left and right leaflet mechanics and structure are very scarce [19, 34], and similarly those of the chordae tendinae are nonexistent to the authors’ current knowledge. In an attempt to bridge this gap, facilitate clinical decisions, and inform computational simulations, our study presents a novel and thorough parallel characterization of the elastic, macro- and microstructural properties of the leaflets and chords of both the MV and TV in a porcine model. While the leaflet mechanics and structure were found to be relatively similar across leaflets and valve types, marked differences in the mechanical behavior of the chordae tendinae were observed, which correlated to their anatomical locations (oriented in the septal vs. non-septal position) around the ventricle, as well as valve type.

4.2. Comparison to the literature

It is well-known that the mechanical environment determines to a large extent the function of biological structures. During the cardiac cycle the mitral and tricuspid valves are subjected to a dynamic environment that significantly differs between the left and right side of the heart [15] and comprises displacement of the annulus, motion of the ventricle and contraction of the papillary muscles [10]. However, no consensus has been reached in the literature when it comes to defining the parameters that determine leaflet and chordal mechanics, with several studies attributing chordal insertion point as a predictor of mechanics [10, 16, 35, 37], while others attributing mechanical differences to valve type [15]. The results of this study, however, allude to a more complex physiological explanation: while the different loading conditions between the left and the right side of the heart may contribute to intervalvular differences in structural stiffness, anatomic location around the ventricle (septal vs. non-septal location) may play an important role in extensibility as well as intravalvular mechanical properties.

4.3. Mechanics and structure of the atrioventricular leaflets

At high stresses, no differences between the different leaflet types were found in either circumferential or radial tensile modulus, a finding corroborated by the lack of differences in collagen fiber architecture as found by SHG imaging. All five groups exhibited very similar collagen fiber architecture, with fiber orientation aligned with the circumferential direction (Appendix Figure 1). Collagen alignment with the circumferential direction renders a much stiffer mechanical response in the circumferential direction along the collagen fibers as compared to the compliant response when the leaflet is loaded in the radial direction or against the collagen fibers, which gives rise to the well-characterized leaflet anisotropy and higher circumferential tensile stresses in all groups [38, 39].

The only differences in leaflet mechanics were observed along the circumferential direction at low stresses, which correspond to the initial uncrimping of the highly kinked collagen fibers and govern tissue extensibility. No significant differences in either LTM or circumferential EXT were found between TV leaflets, however, the STL was stiffer and significantly less extensible in the circumferential direction than the AML (Figure 5A). Differences in the STL mechanical properties in the low stress regime could be due to the inherent geometrical characteristics and orientation of the septal tricuspid leaflet, which is the smallest of the three cusps of the TV, and is attached from the inferoseptal commissure on the posterior wall and angled across the membranous septum to the antero-septal commissure [36]. Ensuring proper coaptation would necessitate larger deformation and bending of the smaller septal cusp in order to achieve proper alignment with the non-septal leaflets.

It is known that there are large physiologic differences in the lower peak transvalvular pressure between the two ventricles (120 mmHg in MV and 30 mmHg in the TV [12]); however, very few differences in mechanical and structural properties of the TV and MV leaflets were found. These findings therefore allude to the necessity of structural and mechanical differences in the chordae tendineae between the two valves in order to ensure

their proper function under considerably different conditions and may suggest a chordal compensatory mechanism.

4.4. Mechanics and structure of the atrioventricular chords

4.4.1. Chordal morphology—For all leaflets, strut chords were found to be thicker than the marginal chords (Figure 6), agreeing with reported data [14, 33, 40]. Also, similar to previous findings, there were statistically significant differences in the thicknesses of the basal and the marginal chords of the mitral leaflets [33]. Interestingly, we observed no differences in the thicknesses of the marginal and basal chords of the tricuspid leaflets. Additionally, there were no differences in the chordal thickness among the different leaflets of TV and MV.

4.4.2. Mechanics between chordal types—Intra-leaflet chordal comparisons on porcine models [16, 19, 33] report larger extensibilities and lower modulus of basal chordae as compared with marginal chordae of the mitral valve [16], and even though not statistically significant, our findings confirm these trends. Interestingly, aside from the STL chords, which in accordance with the findings of Kunzelman et al exhibited statistically stiffer response for the marginal chords as compared to the basal chords, no intra leaflet differences were observed for the mechanics of the different chordal types, indicating that intra-leaflet geometric variations do not necessarily translate to differentiated mechanics. On the contrary, distinct inter-leaflet mechanical differences were observed, which correlated with both valve type as well as the anatomical location of the leaflet.

4.4.3. Mechanics within a valve—UTM, LTM, and EXT were not found to significantly differ between the two mitral leaflets. On the contrary, the STL chords were found to be significantly more compliant and more extensible than the non-septal TV chords (Figure 8). The non-septal chords were not found to significantly differ from each other in upper modulus or extensibility. This can be explained in view of the anatomic location of the septal TV leaflet, which is oriented almost parallel to the septum, as well as its relatively shorter chords. In order to ensure valve closure and proper leaflet coaptation, the STL undergoes a large angular displacement that needs to be accommodated by its compliant and highly extensible chords.

4.4.4. Mechanics between valves and anatomic location—The MV chords were on average stiffer than the TV chords, but the AML and PML chords were not found to significantly differ from each other. Interestingly, in regard to anatomical location, the non-septal leaflets of both the MV and TV were found to have comparable moduli and extensibilities. However, the septally-located AML chords were significantly stiffer in the post- transitional region than the septally-located STL chords, despite having similar extensibilities. The geometrically similar locations of the AML and STL are suggestive of similar mechanical function and correlate well with their comparable extensibilities. The differences in their corresponding stiffness values can be attributed to their different physiological loading conditions, which underlies the significance of location. Taken together, these findings suggest the presence of a compensatory chordal mechanism which

ensures proper coaptation of leaflets of similar mechanical characteristics, despite their markedly different physiological loading conditions.

4.5. Limitations

It was not always feasible to test every chord from every leaflet due to large variability in branching and attachments points, so the dominating branch was selected for mechanical testing. Additionally, true failure criteria (ultimate tensile stress and strain) were not reported in the present study, as boundary effects were unavoidable due to the small length of the chords. However, this is universally true throughout the literature. A further limitation is the qualitative nature of the histological analysis. A more rigorous collagen and elastin content analysis is recommended as a future effort to characterize the observed differences in material properties.

5. CONCLUSIONS

The present study thoroughly investigated the comparative mechanical and structural properties of the atrioventricular leaflets and chordae tendineae in a porcine model. Despite the lack of major mechanical and structural differences between MV and TV leaflets, their respective chords were found to compensate for their different physiological loading environments. The mechanical properties of the chordae tendineae were not found to differ by chordal insertion point, but to instead vary by valve type and anatomical location around the ventricle. To that end, the chordae of the septally-located leaflets in each valve were found to be more extensible than their non-septal counterparts. Within the septally-located leaflets, the mitral chords were found to be stiffer than the tricuspid, potentially due to their higher mechanical loading environment. The findings from this study could facilitate TV and chordal repair by providing baseline comparative MV and TV chordal mechanics. Additionally, this study provided a complete dataset of fitting parameters of the constituents of the mitral and tricuspid apparatuses for computational modeling applications.

ACKNOWLEDGMENTS

Special thanks to Juliana Rios, Mynah Holloway, Sydney Platt, Rachel Mann, and Kathleen Szabo for their experimental and data analysis support. Thanks also to Aqua Asberry for assistance with histological staining. This research is funded in part by NIH HL127570 and NIH HL104080 grants. Additionally, Fatiesa Sulejmani is supported by the Georgia Institute of Technology-Emory University-Peking University Global Biomedical Engineering Research and Education Fellowship.

Appendix Figure A1.: Representative leaflet SHG images at 63x magnification of the ATL (A), PTL (B), STL (C), AML (D), and PML (E).

Circumferential direction is aligned with horizontal, radial direction is aligned with vertical. Collagen is shown in red. Scale bar represents 50 μm .

Appendix Figure A2.: Representative chordal VVG images at 20x magnification of the ATL (A), PTL (B), STL (C), AML (D), and PML (E).

Collagen is shown in red, elastin and nuclei in black. Scale bar represents 100 μm .

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A better understanding of the tricuspid valve (TV) and its associated structures is important for making advancements towards the repair of tricuspid regurgitation. Mitral valve structures have been extensively studied, but little is known about the TV and right-sided heart diseases. Clinical decisions and computational simulations often rely heavily on MV data for TV applications, despite fundamentally different environments. We therefore performed a rigorous mechanical, morphological, and microstructural characterization of atrioventricular leaflets and chordae tendineae in a porcine model.

Finding that valve type and anatomical location may be strong predictors of chordal mechanics, chords from septally-located leaflets differ from each other and from their intravalvular counterparts; they merit special consideration in surgical and computational applications.

Figure 1.

Representative images of porcine ATL (A), PTL (B), STL (C), AML (D), PML (E) leaflets post-explantation. Strut chords are labeled with S, marginal chords with M, and basal chords with B. Red arrows represent the circumferential direction, blue arrows represent the radial direction.

Figure 2. Sample equibiaxial (A) and uniaxial (B) mechanical responses specifying determination of mechanical parameters. UTM - upper tangent modulus, LTM - lower tangent modulus, EXT - extensibility. CIRC, C - circumferential, RAD, R - radial.

Figure 3.

Average equibiaxial response for the TV leaflets (A) and MV leaflets (B). Error bars represent SEM.

Figure 4. Leaflet equibiaxially-derived tangent moduli grouped by leaflets and loading direction. Panels A and B delineate the UTM along the circumferential and radial directions, respectively, and panels C and D represent the LTM in the circumferential and radial directions, respectively. Error bars represent SEM. Bars indicate $p < 0.01$.

Figure 5. Leaflet equibiaxially-derived extensibility in the circumferential (A) and radial (B) directions, and degree of anisotropy (C). Error bars represent SEM. Bars indicate $p < 0.01$.

Figure 6.

Chordal thickness (A) and length (B) for each leaflet grouped by chordal insertion point (Strut, Basal, Marginal). Error bars represent SEM. Bars indicate $p < 0.01$, ** show high statistical significance ($p < 0.001$). Horizontal bars represent strut chords, solid bars represent basal chords, and diagonal bars represent marginal chords.

Figure 7.

Chordal average uniaxial mechanical response for ATL (A), PTL (B), STL (C), AML (D), PML (E) grouped by chordal insertion point. Strut chords are shown with triangles, basal chords with squares, and marginal chords with circles.

Figure 8.

Chordal UTM, LTM, and EXT for each leaflet grouped by chordal insertion point (Strut, Basal, Marginal). Error bars represent SEM. Bars indicate p < 0.01, ** show high statistical significance (p < 0.001). Vertical bars represent strut chords, solid bars represent basal chords, and diagonal bars represent marginal chords.

Table 1.

Morphological and equibiaxially-derived mechanical parameters for the leaflets. Results are presented as mean $\pm$ SEM.

Parameter	TV			MV	
	ATL	PTL	STL	AML	PML
<i>Thickness (mm)</i>	0.81 $\pm$ 0.05	0.73 $\pm$ 0.02	0.89 $\pm$ 0.01	1.14 $\pm$ 0.04	1.30 $\pm$ 0.03
<i>UTM_C (kPa)</i>	50245 $\pm$ 34367	351956 $\pm$ 9274	24092 $\pm$ 4137	27128 $\pm$ 11157	14550 $\pm$ 6543
<i>UTM_R (kPa)</i>	665 $\pm$ 195	3598 $\pm$ 1311	2275 $\pm$ 573	2481 $\pm$ 450	1686 $\pm$ 436
<i>LTM_C (kPa)</i>	3710 $\pm$ 1495	4652 $\pm$ 1319	2383 $\pm$ 495	1237 $\pm$ 428	307 $\pm$ 54
<i>LTM_R (kPa)</i>	60 $\pm$ 16	281 $\pm$ 89	113 $\pm$ 41	77 $\pm$ 29	46 $\pm$ 10
<i>EXT_C</i>	0.02 $\pm$ 0.01	0.02 $\pm$ 0.00	0.01 $\pm$ 0.00	0.11 $\pm$ 0.02	0.05 $\pm$ 0.01
<i>EXT_R</i>	0.24 $\pm$ 0.03	0.19 $\pm$ 0.03	0.24 $\pm$ 0.01	0.32 $\pm$ 0.02	0.16 $\pm$ 0.01
<i>DA</i>	0.09 $\pm$ 0.02	0.15 $\pm$ 0.02	0.06 $\pm$ 0.00	0.15 $\pm$ 0.02	0.22 $\pm$ 0.01

Table 2.

Morphological and uniaxially-derived mechanical parameters for the chordae grouped by insertion point and leaflet type. Results are presented as mean $\pm$ SEM.

Valve	Leaflet	Chord	Thickness (mm)	Length (mm)	UTM (kPa)	LTM (kPa)	EXT
TV	ATL	S	1.36 $\pm$ 0.07	20 $\pm$ 2	116 $\pm$ 17	8 $\pm$ 1	0.03 $\pm$ 0.01
		B	0.99 $\pm$ 0.05	13.7 $\pm$ 0.4	91 $\pm$ 10	4.7 $\pm$ 0.6	0.02 $\pm$ 0.01
		M	0.99 $\pm$ 0.07	12.3 $\pm$ 0.6	136 $\pm$ 20	5 $\pm$ 1	0.02 $\pm$ 0.01
	PTL	S	1.23 $\pm$ 0.07	16 $\pm$ 1	117 $\pm$ 19	7 $\pm$ 1	0.05 $\pm$ 0.01
		B	0.99 $\pm$ 0.03	12.0 $\pm$ 0.3	96 $\pm$ 10	5.3 $\pm$ 0.6	0.06 $\pm$ 0.01
		M	0.86 $\pm$ 0.03	10.3 $\pm$ 0.5	96 $\pm$ 10	8 $\pm$ 2	0.03 $\pm$ 0.01
	STL	B	0.96 $\pm$ 0.02	11.0 $\pm$ 0.2	71 $\pm$ 5	2.9 $\pm$ 0.2	0.11 $\pm$ 0.01
		M	0.89 $\pm$ 0.04	9.6 $\pm$ 0.5	53 $\pm$ 9	3.2 $\pm$ 0.5	0.11 $\pm$ 0.01
		S	1.8 $\pm$ 0.1	29 $\pm$ 3	189 $\pm$ 57	3 $\pm$ 1	0.07 $\pm$ 0.03
MV	AML	B	1.16 $\pm$ 0.05	16 $\pm$ 2	178 $\pm$ 22	5 $\pm$ 1	0.09 $\pm$ 0.02
		M	0.85 $\pm$ 0.05	11.5 $\pm$ 0.7	166 $\pm$ 24	10 $\pm$ 2	0.05 $\pm$ 0.01
	PML	B	0.97 $\pm$ 0.05	14.8 $\pm$ 0.8	154 $\pm$ 14	11 $\pm$ 2	0.04 $\pm$ 0.01
		M	0.71 $\pm$ 0.05	8.3 $\pm$ 0.9	160 $\pm$ 20	10 $\pm$ 2	0.05 $\pm$ 0.01

Table 3.

Average constitutive modeling parameters for the and Ogden (A) and Fung-type (B) models.

(A)		TV						MV					
Parameter	ATL			PTL			STL		AML			PML	
	S	B	M	S	B	M	B	M	S	B	M	B	M
μ (MPa)	0.15	0.18	0.49	0.12	0.05	0.34	0.07	0.02	0.02	0.05	0.07	0.09	49.34
α	86.49	57.75	35.37	42.57	44.85	26.89	27.02	32.71	65.27	38.95	32.42	68.21	0.41
(B)		TV						MV					
Parameter	ATL			PTL			STL		AML			PML	
C	3.00			2.80			14.37		30.94			2.80	
A ₁	100.11			280.51			119.51		5.72			280.51	
A ₂	17.26			20.22			10.66		2.73			20.22	
A ₃	8.93			12.35			17.22		3.37			12.35	
A ₄	8.67			100.00			10.00		3.48			100.00	
A ₅	3.57			-32.53			3.31		5.49			-32.53	
A ₆	0.28			2.10			0.45		-1.52			2.10	