Comparison of Biomechanical Properties and Microstructure of Trabeculae Carneae, Papillary Muscles, and Myocardium in the Human Heart

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Trabeculae carneae account for a significant portion of human ventricular mass, despite being considered embryologic remnants. Recent studies have found trabeculae hypertrophy and fibrosis in hypertrophied left ventricles with various pathological conditions. The objective of this study was to investigate the passive mechanical properties and microstructural characteristics of trabeculae carneae and papillary muscles compared to the myocardium in human hearts. Uniaxial tensile tests were performed on samples of trabeculae carneae and myocardium strips, while biaxial tensile tests were performed on samples of papillary muscles and myocardium sheets. The experimental data were fitted with a Fung-type strain energy function and material coefficients were determined. The secant moduli at given diastolic stress and strain levels were determined and compared among the tissues. Following the mechanical testing, histology examinations were performed to investigate the microstructural characteristics of the tissues. Our results demonstrated that the trabeculae carneae were significantly stiffer (Secant modulus $SM_2 = 80.06 \pm 10.04$ KPa) and had higher collagen content ($16.10 \pm 3.80\%$) than the myocardium ($SM_2 = 55.14 \pm 20.49$ KPa, collagen content = $10.06 \pm 4.15\%$) in the left ventricle. The results of this study improve our understanding of the contribution of trabeculae carneae to left ventricular compliance and will be useful for building accurate computational models of the human heart. [DOI: 10.1115/1.4041966]

Keywords: trabeculae carneae, papillary muscles, myocardium, mechanical properties, heart failure with preserved ejection fraction, collagen, myocyte, impaired ventricular compliance

1 Introduction

Reduced left ventricular (LV) compliance is the hallmark of heart failure with preserved ejection fraction (HFpEF), which is the leading cause of all heart failure hospitalizations [1–3]. Hypertrophy and stiffening of the ventricular wall causes impaired ventricular relaxation during LV filling and leads to diastolic dysfunction [4]. LV hypertrophy is also associated with thickening and fibrosis of the trabeculae carneae [5,6]. Our recent study suggested that trabeculae carneae had a noticeable effect on the overall LV stiffness, and severing free-running trabeculae carneae can help increase the compliance of the LV in HFpEF patients [7]. Therefore, it is of clinical interest to better understand the mechanical and microstructural properties of trabeculae carneae.

Trabeculae carneae are irregular muscular bundles lining the interior of both ventricles (Fig. 1). Trabeculae carneae are among the first features to appear in the embryonic cardiac tube [8,9]. During the development of the heart, some trabeculae carneae condense to form the myocardium, papillary muscles, chordae tendineae, and septum [9,10]. The remaining trabeculae carneae along with papillary muscles account for a significant proportion of ventricular mass (12%–17%) in normal adult hearts and are correlated with ventricular end-diastolic volume [11]. Studies have shown that the size and mass of trabeculae carneae can be used as indicators of some cardiac disorders such as heart failure

and left ventricular noncompaction cardiomyopathy [12,13]. Recently, Gati et al. showed that highly trained athletes displayed a higher prevalence of increased LV trabeculation [14] and interestingly, reversible LV trabeculation occurred in women in response to LV preload during pregnancy [15].

There have been extensive studies on the mechanical properties of the myocardium in animal (canine, bovine, porcine, and ovine) hearts [16–21]. Trabeculae carneae and papillary muscles are often used in mechanical tests as a surrogate of the myocardium

Fig. 1 Photograph of a human heart opened by a frontal incision illustrating the trabeculae carneae and papillary muscles

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Table 1 Patient information of all investigated specimens

Patient no.	Age (yr)	Gender	Weight (kg)	Heart weight (g)	Cause of death	Heart-related disease
1	67	M	113	625	CR	HTN
2	42	M	90	493	CVA	HTN
3	47	M	101	526	CH	AFib
4	55	M	108	782	CVA	CVA, HTN
5	47	M	100	549	CR	A, HTN
6	59	M	108	676	CR	CVA, HTN
7	64	F	127	465	Unk	CAD, HTN
8	35	M	136	568	SA	None
9	70	F	61	423	CR	CAD, HTN
10	48	F	110	753	CR	CAD, HTN
11	52	F	100	840	CR	HTN
12	63	F	86	510	CVA	HTN
Mean $\pm$ SD	54.1 $\pm$ 10.8	—	103.3 $\pm$ 19.3	600.8 $\pm$ 134.4	—	—

CAD—coronary artery disease; CVA—cerebrovascular accident; HTN—hypertension; SA—seizure attack; CR—cardiac related; A—angina; CH—cholelithiasis; AFib—atrial fibrillation; and Unk—Unknown.

Fig. 2 (a) Trabeculae carneae isolated from human right and left ventricles, (b) specimen mounted using four rakes for biaxial testing, and (c) specimen mounted using two rakes for uniaxial testing

to understand its mechanical behavior [22,23]. For example, papillary muscles from rat and rabbit hearts have been used extensively in mechanical testing to understand the mechanical properties of the myocardium [24,25]. The active contraction of trabeculae has been studied in both animal hearts [26,27] and human hearts [28,29] to understand the myocardial wall properties and ventricular function. However, the similarities and differences among trabeculae, papillary muscles and myocardium have not been carefully compared. Reports of trabeculae microstructure in the literature are limited to a few animal species (rats, mice, and rabbits) [30,31]. Recently, Sommer et al. studied the mechanical properties and microstructure of human myocardium in both ventricles [32]. Still, little is known about the mechanical properties and microstructure of trabeculae carneae and papillary muscles in human hearts. This information is needed for better understanding of ventricular function and developing accurate computational models of the ventricles.

Therefore, the aim of this study was to quantify the passive mechanical properties and microstructural characteristics of human trabeculae carneae and papillary muscles compared to the myocardium in both ventricles.

2 Materials and Methods

2.1 Materials. Twelve human hearts from seven male and five female donors (average age 54.1 ± 10.8 -yr-old) were collected within 24 hours of death from the South Texas Blood and Tissue Center with permission. All except two donors had history of hypertension with cardiac related cause of death (Table 1). Consent was obtained from donor families and local Institutional Review Board's approval was exempted as all tissues and patient information were de-identified. Trabeculae carneae, papillary muscles, and sections of myocardial free wall were isolated from

both ventricles and specimens were dissected from these tissues (Fig. 2(a)). All specimens were kept in a 4 °C phosphate-buffered saline solution and tested immediately after collecting. The use of human tissues was approved by the Institutional Biosafety Committee of The University of Texas at San Antonio.

2.2 Mechanical Testing

2.2.1 Biaxial Tensile Test. Planar biaxial tensile testing was conducted using a CellScale BioTester (Waterloo, ON, Canada) with 1.5 ± 0.003 N load-cell [33,34]. Specimens of square slices were cut from papillary muscles and mid ventricular region of myocardial free wall where the muscle structure is more uniform [32]. The square slices had average dimensions of 10.28 ± 1.31 mm $\times$ 10.46 ± 2.16 mm and thickness of 1.15 ± 0.63 mm ($n = 77$) and were sliced such that one side was aligned with the mean fiber direction (F) and the other side with the cross-fiber (CF) direction [32]. The slices were mounted along the four sides using four rakes that are attached to actuators to control the displacement and pull the slices in the mean fiber and cross-fiber directions [33,34]. Each rake had five attachment points to achieve uniform strain field in the specimen (Fig. 2(b)) [35]. The actual average thickness was determined by weighing the specimens [36]. The initial cross-sectional areas were computed as effective side lengths (distance between attachment points of two opposite sides) multiplied by average sample thickness [36]. Specimens were immersed in a bath of phosphate-buffered saline at 37 °C throughout testing to maintain moisture and relax the myocytes [37].

The specimens were stretched and released slowly (at a stretch rate of ~ 6 mm/min) for five complete loading–unloading cycles for preconditioning and then one loading cycle was performed for data acquisition [38]. Stretch levels of up to 40% were applied

to fully cover the physiological in vivo range [39]. Our preliminary study showed that after five preconditioning cycles, the stress–strain curves became repetitive. To capture the anisotropic behavior of the material, three different loading schemes with different loading ratios in the fiber and cross-fiber directions (1F:1CF, 1F:0.5CF, 0.5F:1CF) were applied to samples [32].

A pilot study was done to compare the strain measurements using the displacements from the central region of the specimen (where the local deformation is more uniform) and using rake displacement [35]. For 18 specimens from three hearts, waterproof ink-marks were placed at the center of the sample for optical in-plane deformation tracking and measurement while the displacement between the attachment points was also recorded. Our results showed that there was no significant difference in the calculated strains using these two approaches. So displacement between attachment points was used in strain analysis in all samples.

The displacement and tensile forces were recorded for all loading schemes and used in data analysis. The dimensions after preconditioning were determined using incompressibility assumption in the analysis.

2.2.2 Uniaxial Tensile Test. Uniaxial tensile tests were performed on the free-running trabeculae carneae isolated from right and left ventricles (Figs. 2(a) and 2(c)). Free-running trabeculae carneae are connected either to the ventricle wall, papillary muscles or to the network of trabeculae carneae at extremities but free in the middle [40]. For comparison, uniaxial testing was also performed on the myocardium strips dissected from the middle layer of the left ventricular free wall. Specimens were loaded along the fiber direction using the same BioTester, with displacement control in one axis. The length of specimens were 12.41 ± 1.63 mm with a width of 2.06 ± 1.18 mm and thickness of 1.11 ± 0.75 mm ($n = 74$). Similar to the biaxial tensile test, specimens were preloaded five cycles for preconditioning followed by one measuring cycle with up to 40% stretch level.

2.3 Stress and Stain Analysis. The Cauchy stresses and Green strains were calculated from the force and displacement data and initial dimensions with incompressibility assumption and neglecting the shear in biaxial testing [17,41]. Using displacement data from the actuators, the Green strains were computed by

$$E_i = (\lambda_i^2 - 1)/2 \quad i = x, y, z \quad (1)$$

where λ_i is the stretch ratio. With incompressibility assumption, $\lambda_x \lambda_y \lambda_z = 1$, the deformed cross-sectional area A_i is equal to the initial cross-sectional area A_{0i} divided by the stretch ratio in the direction, $A_i = A_{0i}/\lambda_i$. So, the Cauchy stresses were calculated by

$$\sigma_i = \frac{F_i \lambda_i}{A_{0i}} \quad i = x \text{ for uniaxial; } i = x, y \text{ for biaxial} \quad (2)$$

where F is the load measured by the load-cell [16].

As all tested tissues demonstrated nonlinear stress–strain behavior, for direct comparison of the stiffness of these tissues, the secant modulus (SM) was calculated from equi-biaxial and uniaxial tensile tests at two different conditions: (a) at a stress of 5 KPa which represents the physiological diastolic stress (SM₁) and (b) at a stretch ratio of 15% which represents the physiological diastolic stretch (SM₂) [39,42,43]. For comparison of the degree of anisotropy, the tangent modulus (TM) ratio (TM in the mean fiber direction/TM in the cross-fiber direction) at 15% equi-biaxial stretch ratio was calculated [17].

2.4 Constitutive Equation. The cardiac tissue is known to be hyperelastic, anisotropic, and nearly incompressible [32,44,45]. The Fung exponential strain energy function which has been widely used in describing the myocardial tissue and vascular tissue [46] was used to describe the constitutive equations of the

trabeculae, papillary muscle, and myocardium. The two-dimensional (2D) Fung stress energy function is given by

$$W = \frac{1}{2} c (e^Q - 1), \quad Q = b_1 E_x^2 + b_2 E_y^2 + 2b_4 E_x E_y \quad (3)$$

where c , b_1 , b_2 , and b_4 are the four material constants and $b_2 = b_4 = 0$ for uniaxial testing. Accordingly, with incompressibility assumption, the Cauchy stress components can be obtained as [47]

$$\sigma_x = \lambda_x^2 (b_1 E_x + b_4 E_y) c e^Q \quad (4a)$$

$$\sigma_y = \lambda_y^2 (b_2 E_y + b_4 E_x) c e^Q \quad (4b)$$

The material constants were determined by fitting these equations with experimental data. For stability of the material under loading, all parameters must be positive and the strain energy function needs to be convex. The Fung strain energy is locally convex when the eigenvalues of the matrix $\begin{pmatrix} b_1 & b_4 \\ b_4 & b_2 \end{pmatrix}$ are positive [48].

Fitted material properties for each individual sample as well as group averaged material properties were obtained. The goodness of fit was evaluated by R^2 value and root-mean-square error (RMSE) percent based on the Levenberg–Marquardt nonlinear regression algorithm using in-house MATLAB (V8.3.0.532) program.

2.5 Histology. Histological examinations were performed on the myocardium, papillary muscles, and trabeculae carneae samples taken from right and left ventricles of four hearts (number 9, 10, 11, and 12). After mechanical testing, tissues were fixed in 10% formalin overnight and then moved to 70% alcohol. Later, samples were processed and embedded in paraffin. Sections of 5 μ m thickness in fiber direction and in cross-fiber direction were cut for each sample and processed for Hematoxylin and Eosin (H&E) staining and Picrosirius red staining.

2.5.1 Characterization of Myocytes Geometry. Sections in the cross-fiber direction stained with (H&E) were examined and photographed using an OLYMPUS CX41 microscope at 40 $\times$ magnification. The images were analyzed using ImageJ 1.47v (National Institute of Health, Bethesda, MD). Ten random regions from each slide were selected and ten myocytes were measured in each region. For each myocyte, the cross-sectional area, the maximum and minimum diameter and the aspect ratio (maximum diameter/minimum diameter) were measured. Only myocytes with central nuclei were measured, as described in our previous report [49]. The nuclei number (per mm²) and inter-myocyte white space were also determined. The inter-myocyte white space was measured as the percentage of area that was neither cell nor nucleus within each image.

2.5.2 Collagen Content. For the collagen density measurement, trabeculae, papillary muscles and the myocardial wall specimens were sectioned in the fiber direction and stained with Picrosirius red. Ten random regions from each slide were selected and photographed at 20 $\times$ magnification. The collagen content was measured using a semi-automated image analysis protocol in ImageJ and its concentration was determined as the percentage of the area occupied by collagen divided by the total image area excluding the empty inter-cellular spaces [49].

2.6 Statistical Analyzes. All statistical analyses were carried out using spss statistical software (IBM). All measured values are presented as mean $\pm$ standard deviation. Multiple group analysis was performed using One-way analysis of variance followed by a post-hoc Tukey honestly significant difference test with a p -value less than 0.05 indicating statistical significance.

Fig. 3 Comparison of average Cauchy stress versus stretch ratio curves: (a) Left ventricle myocardium (LVMY) and papillary muscles (LVPM) under equi-biaxial stretch in fiber (F) and cross-fiber (CF) directions ($n = 9$), (b) right ventricle myocardium (RVMY) and papillary muscles (RVPM) under equi-biaxial stretch in fiber (F) and cross-fiber (CF) directions ($n = 9$), (c) left ventricle trabeculae carneae (LVT) and right ventricle trabeculae carneae (RVT) under uniaxial stretch ($n = 9$), and (d) left ventricle trabeculae carneae (LVT, $n = 12$) and myocardium (LVMY-uniaxial, $n = 5$) under uniaxial stretch and equi-biaxial stretch (LVMY F, $n = 9$)

3 Results

The twelve human hearts used in this study had an average weight of 600.8 ± 134.4 g, and the average LV free wall thickness was 17.16 ± 3.74 mm, 16.35 ± 3.91 mm, and 12.21 ± 2.97 mm at the base, midventricle, and apex, respectively. A total of 151 specimens were dissected including 36 LV trabeculae carneae (LVT), 50 LV myocardial samples (LVMY), 15 LV papillary muscles (LVPM), 18 RV trabeculae carneae (RVT), 18 RV myocardial samples (RVMY), and 14 RV papillary muscles (RVPM).

3.1 Uniaxial and Biaxial Stress–Strain Curves. The average stress–stretch ratio curves for trabeculae, myocardium and papillary muscles are compared in Fig. 3 for the range up to a stretch level of 15% which covers the in vivo deformation of human hearts [39,42]. In comparison, LVPM were stiffer (higher slope) than the LVMY in both fiber and cross-fiber directions under equi-biaxial stretch (Fig. 3(a)), but there were little differences between RVPM and RVMY (Fig. 3(b)). The RVT had similar behavior as LVT with a slightly higher slope at the higher strain range (Fig. 3(c)). Generally, tissues show stiffer behavior under biaxial loading than under the uniaxial loading [16]. Hence, the trabeculae carneae response was compared to the uniaxial response of the myocardium. LVT were stiffer (steeper slope) than LVMY under uniaxial stretch (Fig. 3(d)).

3.2 Constitutive Model. The Fung strain energy function was able to capture both the uniaxial and biaxial behaviors of all tested tissues (trabeculae, papillary muscle, and myocardial wall) very well with an average R^2 value of 0.97 (Fig. 4). The average material coefficients are presented in Table 2.

Fig. 4 Representative stress–stretch ratio curves fitted with Fung strain energy function for (a) left ventricle trabeculae (LVT) under uniaxial stretch and (b) left ventricle myocardium (LVMY) under equi-biaxial stretch

Table 2 Fung strain energy coefficients for the six anatomical regions of the human heart: left ventricle myocardium (LVMY), left ventricle papillary muscles (LVPM), left ventricle trabeculae carneae (LVT), right ventricle myocardium (RVMY), right ventricle papillary muscles (RVPM), and right ventricle trabeculae carneae (RVT). Values are in Mean $\pm$ SD, $n = 8$.

Human	LVMY	LVPM	LVT	RVMY	RVPM	RVT
C (KPa)	5.74 ± 2.73	7.60 ± 1.20	2.25 ± 0.57	5.00 ± 0.56	5.65 ± 0.99	0.66 ± 0.28
b_1	7.99 ± 2.20	8.75 ± 5.19	36.4 ± 13.88	10.15 ± 1.50	13.53 ± 0.46	19.96 ± 10.64
b_2	3.92 ± 1.49	6.18 ± 3.80	—	7.99 ± 3.03	5.53 ± 2.52	—
b_4	2.35 ± 1.22	1.551 ± 0.49	—	1.03 ± 0.15	0.88 ± 0.20	—
RMSE%	10.18 ± 3.61	7.56 ± 2.53	9.54 ± 3.96	7.85 ± 3.07	17.58 ± 12.66	11.74 ± 1.84
R^2	0.98 ± 0.01	0.98 ± 0.01	0.98 ± 0.01	0.98 ± 0.01	0.97 ± 0.01	0.98 ± 0.01

Fig. 5 Comparison of Secant modulus SM_1 at 5 KPa for (a) left ventricle myocardium (LVMY) and papillary muscles (LVPM), right ventricle myocardium (RVMY), and papillary muscles (RVPM) under equi-biaxial stretch ($n = 9$) and (b) left ventricle trabeculae carneae (LVT, $n = 12$) and myocardium strip (LVMY-Uniaxial, $n = 5$), right ventricle trabeculae carneae (RVT, $n = 9$), under uniaxial stretch. Values are Mean $\pm$ SD. No significant difference is seen.

3.3 Comparison of Secant Modulus of Trabeculae Carneae, Papillary Muscle, and Myocardium. Direct comparisons of the secant moduli at a diastolic stress level of 5 KPa (SM_1) and at a diastolic stretch ratio of 15% (SM_2) are illustrated in Figs. 5 and 6, respectively. There was no significant difference in SM_1 ($p = 0.33$) between the LVMY and LVPM under diastolic stress condition. The SM_2 of LVPM was significantly higher than the LVMY ($p < 0.01$), and the LVT showed significantly higher SM_2 than the LVMY-Uniaxial ($p < 0.01$). The stiffness of the RVPM and the RVMY were not significantly different ($p = 0.35$ for SM_1 and $p = 0.36$ for SM_2). At end-diastolic stretch level, SM_2 of the RVMY and the RVT were significantly higher than the LVMY ($p < 0.05$) and the LVT ($p < 0.01$), respectively. There was no statistical difference between SM_2 of RVPM and LVPM ($p = 0.89$). The SM_1 of RVMY, RVPM, LVMY, and LVPM were not significantly different ($p = 0.9$).

The fiber to cross-fiber TM ratio (TM in mean fiber direction/TM in cross-fiber direction) was calculated as an index of

Fig. 6 Comparison of Secant modulus SM_2 at 15% stretch ratio for (a) left ventricle myocardium (LVMY) and papillary muscles (LVPM), right ventricle myocardium (RVMY) and papillary muscles (RVPM) under equi-biaxial stretch ($n = 9$). (**) and (*) indicate ($p < 0.01$) and ($p < 0.05$) versus LVMY, respectively, and (b) left ventricle trabeculae carneae (LVT, $n = 12$) and myocardium strip (LVMY-Uniaxial, $n = 5$), right ventricle trabeculae carneae (RVT, $n = 9$), under uniaxial stretch. (**) indicates ($p < 0.01$) versus LVT. Values are Mean $\pm$ SD.

anisotropy from equi-biaxial stretch tests of myocardium and papillary muscles at 15% stretch ratio (Fig. 7). The TM ratio of the LVMY and LVPM were significantly smaller than those of the RVMY and RVPM, respectively ($p < 0.01$). The anisotropic behavior of the myocardium and papillary muscles were not significantly different in each ventricle ($p = 0.14$).

3.4 Myocardial Morphology. H&E Staining rendered myocytes pink and nuclei dark blue (Fig. 8). We observed no significant difference in the number of nuclei in all tested tissues (Fig. 9(a)), but a decrease in the inter-myocyte space in LVPM compared to other tissues ($p < 0.05$) (Fig. 9(b)). Moreover, the myocyte cross-sectional area was found to be the smallest in the LVT and the largest in the LVPM ($p < 0.05$) (Fig. 10(a)). Comparing the minor diameter and aspect ratio of the myocyte cross section from different tissues of both ventricles showed that the LVT myocyte had the smallest diameter and highest aspect ratio while the LVPM had the largest diameter (Figs. 10(b) and 10(c)).

Fig. 7 Comparison of the equi-biaxial tangent modulus (TM) ratio (TM in fiber direction/TM in cross-fiber direction) for left ventricle myocardium (LVMY) and papillary muscles (LVPM), right ventricle myocardium (RVMY) and papillary muscles (RVPM) ($n = 9$). (**) and (##) indicate ($p < 0.01$) versus RVPM and RVMY, respectively. Values are Mean $\pm$ SD.

Our results showed no significant difference in the myocyte cross-sectional area and diameter between human left and right ventricles.

Collagen in the tissues was stained red with Picrosirius red stain (Fig. 8). The collagen content was found to be significantly higher in the LVT and RVT than in the LVMY ($p < 0.05$), but no significant difference with RVMY was found ($p = 0.22$) (Fig. 11).

4 Discussion

In this study, we tested and compared the mechanical properties and microstructural characteristics of human trabeculae carneae, papillary muscles, and myocardial wall. Our results demonstrated that LV trabeculae were significantly stiffer than the LV

Fig. 9 Comparison of (a) nuclei number, and (b) inter-myocyte space (% area) in human left ventricle myocardium (LVMY), papillary muscles (LVPM), trabeculae carneae (LVT), and right ventricle myocardium (RVMY), papillary muscles (RVPM), trabeculae carneae (RVT) ($n = 4$). (**) and (*) indicate ($p < 0.01$) and ($p < 0.05$) versus LVPM, respectively. Values are mean $\pm$ SD.

Fig. 8 Hematoxylin and Eosin staining of (a) left ventricle myocardium (LVMY), (b) left ventricle papillary muscles (LVPM), (c) left ventricle trabeculae carneae (LVT) of a human heart at 40 $\times$ magnification and Picrosirius red staining of (d) left ventricle myocardium (LVMY), (e) left ventricle papillary muscles (LVPM), and (f) left ventricle trabeculae carneae (LVT) of a human heart at 20 $\times$ magnification

Fig. 10 Comparison of the myocyte geometry indices (a) myocyte cross-sectional area, (b) myocyte diameter, and (c) myocyte aspect ratio in human left ventricle myocardium (LVMY), papillary muscles (LVPM), trabeculae carneae (LVT), and right ventricle myocardium (RVMY), papillary muscles (RVPM), trabeculae carneae (RVT) ($n = 4$). (*) indicates ($p < 0.05$) versus LVT. Values are mean $\pm$ SD.

myocardium under physiological uniaxial stretch and had higher collagen content than the myocardial wall. This indicates that trabeculae carneae are an important component of the LV and their role may need to be considered in analysis of LV function.

Fig. 11 Comparison of collagen content (% area) in human left ventricle myocardium (LVMY), papillary muscles (LVPM), trabeculae carneae (LVT), and right ventricle myocardium (RVMY), papillary muscles (RVPM), trabeculae carneae (RVT) ($n = 4$). (*) indicates ($p < 0.05$) versus LVMY. Values are mean $\pm$ SD.

In this study, we tested tissues from human hearts collected within 24 h of death. Fresh human cadaver hearts have been used in many studies for characterization of the biomechanical properties of myocardium [32,41], chordae tendineae [50], heart valves [51], and arteries [52], and no biomechanical differences between the explanted and postmortem tissues were reported. The tissues used in these studies were collected post-mortem from patients with a history of cardiovascular disease or a cardiac-related cause of death. Our results showed that Cauchy stress in the fiber and cross-fiber directions of LVMY at 15% equi-biaxial stretch were 21.00 ± 8.49 KPa and 16.29 ± 6.11 KPa, respectively. These values are very close to the values (18.9 KPa and 10.50 KPa, respectively) Sommer et al. obtained for human LVMY at the same loading condition [32]. Moreover, our results showed good agreement with the myocardial stiffness measured from biopsies of the LV wall in patients who underwent heart transplantation or coronary artery bypass grafting surgery [53,54]. In these studies, the maximum myocardium strip stress at 20% extension was estimated to be between 5 KPa and 29 KPa which is close to the average value of 21.77 ± 7.27 KPa that we obtained from uniaxial extension of myocardial strips at 20% stretch. The small difference may be due to differences in age, weight, collagen content, and hypertrophy of the heart [50,55]. In our current study, the average thickness of the LV free wall was more than 15 mm, which indicates hypertrophy [56,57]. Differences in specimen preparation and test protocols as well as different methods of choosing reference dimension and thickness of the specimens could also affect the stress and strain data. Furthermore, these studies demonstrated a stiffer human myocardium compared to the canine [16,17], bovine [18], and ovine [19] myocardium found in the literature, suggesting the necessity of testing human tissues to develop accurate constitutive models, material parameters, and computational simulation for reliable assessment of heart disease and planning surgical treatments [58,59].

We demonstrated that in the human heart, all the RV tissues were slightly stiffer than the corresponding LV tissues. The RVMY showed stiffer behavior than the LVMY under physiological conditions, which is consistent with previous reports [17,32]. The RVMY also showed a significantly greater degree of anisotropy than the LVMY. A possible reason is the different functional role, geometry, and cell biology of the RV [60]. In the RV, the direction of the maximum systolic deformation almost coincides with the mean fiber direction of the RVMY [17]. Therefore, the RVMY needs to be stiffer along the fiber direction to support large stresses. However, in the LV, there is no close relation between the mean fiber direction and the maximum deformation direction [61].

Histological assessments revealed no significant difference in the density of nuclei between trabeculae, papillary muscles and the myocardial wall of both ventricles. In humans with advanced age, LV wall thickening is associated with increased cardiomyocyte cross-sectional area, focal collagen deposition, and myocyte nuclei number, as well as decreased cardiac myocyte number [62–64]. The LVPM showed the highest nuclei density and cell size with the smallest intermyocyte space. Drawing analogy with aging, this can be interpreted as a compensatory mechanism of the LVPM to maintain valve function. The highly oval cross-sectional shape of the myocytes of all tested tissues indicates the development of hypertrophy in the tested hearts [65]. It should be noted that the hearts used in this study were from donors with a history of hypertension and cardiovascular disease, and an average LV wall thickness more than 15 mm. Therefore, we can expect the development of hypertrophy in these hearts [57,66].

The total amount, alignment, and configuration of collagen are important determinants of the passive mechanical properties of the myocardium [67]. Our histological analyses revealed that the collagen concentration of trabeculae carneae was higher than the myocardium and papillary muscles. Increased collagen may stiffen the trabeculae and weaken its contractility, which then limits the LV's ability to relax during diastolic filling [34,67]. This

indicates the potential contribution of trabeculae carneae to the LV's overall stiffness and diastolic function but may be of little contribution to the systolic function. In previous studies, the collagen concentration of the myocardium from the RV was found to be greater than that of the LV [68]. In this study, we also found that the collagen content of the RVT and RVPM were slightly more than the LVT and LVPM, respectively. This can also explain the stiffer behavior of the right ventricular tissues. It has been reported that the collagen concentration in human myocardium can be up to 20%–35% [31,68,69]. Our examination showed a smaller collagen concentration of 10%–15% in the myocardium. The difference could be explained by the effect of age on collagen concentration, as collagen content increases with age [69]. This increase is more significant in the subendocardial and subepicardial regions [69]. The higher mechanical stiffness of trabeculae in association with higher collagen content may manifest at the microstructure and play a regulatory role in the cells of the trabeculae carneae. Further studies are needed to measure the change in mechanical properties and microstructure of trabeculae in pathological conditions to validate these relationships.

There were some limitations in this study. The number of samples was relatively small. We were not able to compare the passive stiffness and microstructural characteristics of the hypertrophied hearts to healthy controls due to limitations of the tissue resource. Also, the medical history of donors was not fully available due to confidentiality requirement. These factors may have influenced the results. In addition, only uniaxial tests were conducted on trabeculae carneae due to their thin strip shape while only biaxial tests were conducted on papillary muscles based on their thick diameter. Both uniaxial and biaxial tensile tests were performed on the myocardium to facilitate the comparison to trabeculae carneae and papillary muscles, as well as comparison to results in the literature. It also demonstrated how the same tissue behaves differently under uniaxial and biaxial loading conditions. Strain uniformity in biaxial/uniaxial testing is sensitive to the grip attachment. We used five attachment points per side of the specimen to achieve uniform strain field in the specimen and minimize stress concentration and localized deformation [35]. Though shear deformation may occur in 2D testing, it can be minimized by using very thin slices and aligning fiber direction with the loading direction [41]. Thus, we used very thin square slices which were arranged so that one side was aligned with the mean fiber direction and the other side with the cross-fiber direction to minimize the in-plane shear stress during the biaxial testing [41]. Also, SM_1 and SM_2 criteria were defined based on the in vivo diastolic stress and stretch of myocardium [39,42,43]. The working stress–strain level for trabeculae carneae and papillary muscles were not available in the literature, thus we assumed they have a similar working stress–strain level as myocardium. We also compared stress–strain curves which do not depend on the working level. In addition, we did not study the contribution of elastin in myocardial stiffness [70]. Changes in the ratio of collagen and elastin can alter the diastolic stiffness of the myocardium [71]. Further studies are needed to explore the contribution of elastin in trabeculae and myocardial stiffness especially in the low strain range. Even though the 2D model and data cannot directly predict three-dimensional (3D) behavior [72], these material constants can be used for 3D computational modeling of human hearts with certain assumptions (such as transversally isotropic perpendicular to the fiber direction) and calibrations (with patient-specific end-diastolic pressure–volume relationships) [73].

Although limitations exist, to the best of our knowledge, this is the first study on the mechanical and microstructural properties of human trabeculae carneae and papillary muscles compared to the myocardium of both ventricles. The presented results demonstrate the possible contribution of trabeculae to the reduced ventricular compliance in HFP EF patients. The Fung strain energy material constants will be useful for building accurate computational

models of human hearts to accurately simulate cardiac function and develop new surgical treatments.

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