



# An investigation of how specimen dimensions affect biaxial mechanical characterizations with CellScale BioTester and constitutive modeling of porcine tricuspid valve leaflets

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## ABSTRACT

Biaxial mechanical characterizations are the accepted approach to determine the mechanical response of many biological soft tissues. Although several computational and experimental studies have examined how experimental factors (e.g., clamped vs. suture mounting) affect the acquired tissue mechanical behavior, little is known about the role of specimen dimensions in data acquisition and the subsequent modeling. In this study, we combined our established mechanical characterization framework with an iterative size-reduction protocol to test the hypothesis that specimen dimensions affect the observed mechanical behavior of biaxial characterizations. Our findings indicated that there were non-significant differences in the peak equibiaxial stretches of tricuspid valve leaflets across four specimen dimensions ranging from  $4.5 \times 4.5$  mm to  $9 \times 9$  mm. Further analyses revealed that there were significant differences in the low-tensile modulus of the circumferential tissue direction. These differences resulted in significantly different constitutive model parameters for the Tong–Fung model between different specimen dimensions of the posterior and septal leaflets. Overall, our findings demonstrate that specimen dimensions play an important role in experimental characterizations, but not necessarily in constitutive modeling of soft tissue mechanical behavior during biaxial testing with the commercial CellScale BioTester.

## 1. Introduction

Finite element simulations of heart valve function require detailed descriptions of how the tissues respond to applied loading (Sacks et al., 2019). These mechanical behaviors are often derived from experimental characterizations, including uniaxial and biaxial tensile tests (Sacks, 2000). Although these methods have been widely accepted as the standard approaches to determine the mechanical response of soft tissues, inconsistencies in published protocols may confuse comparisons and the use of the data in *in silico* simulations.

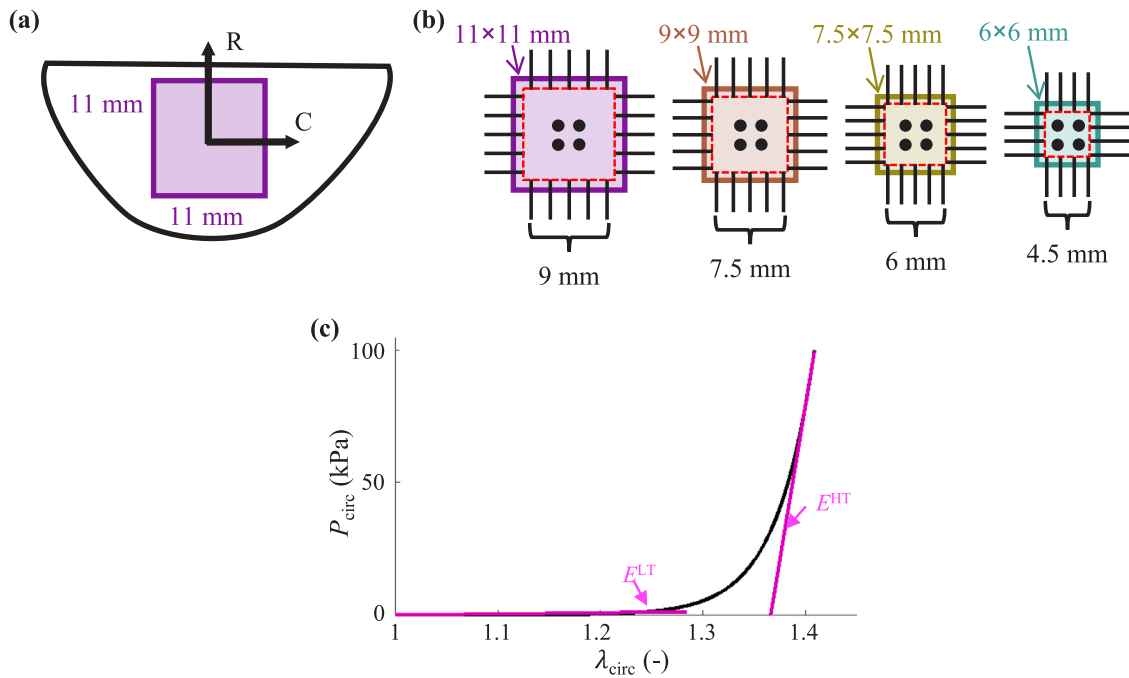
Inspired by the seminal work of Nielsen et al. (1991), early studies focused on refining the experimental protocols using finite element

models that emulated the testing apparatus. For example, Sun et al. (2005) explored how different attachment methods (e.g., clamp or sutures) or specimen shapes (square vs. cruciform) influenced the homogeneity of the predicted tissue strains and stresses. Eilaghi et al. (2009) later expanded this work by exploring how the distribution of attachment points altered the experimental results. The authors noted that non-equibiaxial protocols were particularly influenced by the attachment properties. Jacobs et al. (2013) then used finite element analysis to determine a correction factor that could account for the stress shielding and other boundary loading effects. This approach and the resulting metrics were later re-examined and expanded

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**Fig. 1.** (a) Central region of the tricuspid valve leaflet was prepared and (b) mounted to the commercial biaxial tester for an iterative approach to determine the effect of specimen sizes on the observed mechanical properties. (Red dashed lines denote the effective tissue region of each specimen size.) (c) Determination of the low-tensile modulus  $E^{LT}$  and the high-tensile modulus  $E^{HT}$  for a representative tissue direction. ( $P_{circ}$  and  $\lambda_{circ}$  denote the first Piola–Kirchhoff stress and tissue stretch in the circumferential direction, respectively.).

by Nolan and McGarry (2016). Recent computational investigations have attempted to compare the numerical predictions with experimental findings (Avanzini and Battini, 2016) or to investigate emerging commercial testing platforms (Fehervary et al., 2016).

On the other hand, benchtop studies have also advanced our knowledge on how experimental factors influence biaxial tensile characterizations of heart valve leaflets. For example, Jett et al. (2018) showed that atrioventricular heart valve leaflets become more compliant in the radial direction with increasing tissue temperature. Later, Salinas et al. (2019) further demonstrated that testing media osmolality was also crucial to acquiring accurate mechanical behaviors with non-physiological deionized water, resulting in reduced tissue extensibility. Other studies by Duginski et al. (2020) and Salinas et al. (2020) explored how freezer storage may alter the observed mechanical behavior and found that temporary freezer storage between  $-14^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$  did not significantly alter the mechanical behavior of heart valve leaflets. Recently there has also been an interest in understanding the appropriate choice of the stress-free reference configuration (Laurence et al., 2022) and the refined strain fields via dense speckle patterning (Sugerman et al., 2023) or polarized light techniques (Dover et al., 2022). Collectively, these experimental studies provide the guidance for the development of frameworks and protocols to mechanically characterize the heart valve leaflets.

An area that still needs to be explored, particularly in the field of experimental tissue biomechanics, is how specimen dimensions influence the observed biomechanical behavior. We hypothesize that the specimen dimensions influence the observed mechanical behaviors as there is ample evidence that the insertion properties alter the homogeneity of the quantified tissue stress and strain fields. This would also lead to erroneous comparisons between studies with different specimen dimensions, as well as inaccurate estimates of the constitutive parameters and their use in subsequent *in silico* predictions.

Therefore, the aim of this work is to test this hypothesis and to quantify how specimen dimensions affect the mechanical behavior, mechanical properties and derived constitutive model parameters of tricuspid heart valve leaflets—our *tissue application of choice*. We achieve this goal by using our established biaxial mechanical characterization technique with an iterative reduction in specimen dimensions.

The resulting mechanical behavior is analyzed to determine typical biomechanical properties (e.g., low- and high-tensile moduli). Furthermore, we fit the experimental data to three typical strain–energy functions to understand whether the differences in specimen dimensions alter the constitutive modeling that is typically used in numerical simulations/investigations of heart valve function.

## 2. Methods

### 2.1. Tissue acquisition and preparation

Adult porcine hearts ( $n = 8$ , 80–140 kg, 1–1.5 years of age) were obtained from a USDA-approved abattoir (Country Home Meats Company, Edmond, OK) and shipped to our laboratory. The hearts were cleaned to remove excess blood and the whole organ was immediately stored in a freezer at  $-14^{\circ}\text{C}$ . The frozen hearts were thawed in a water bath at room temperature within a week, and the three TV leaflets were dissected using our established technique (Jett et al., 2018; Ross et al., 2019b). All three dissected TV leaflets ( $n = 8$  per leaflet), i.e., anterior leaflet (TVAL), posterior leaflet (TVPL), and septal leaflet (TVSL), were then temporarily stored in the freezer prior to the experimental characterization within 1–2 days. This temporary frozen storage is supported by our previous investigation which found that short-term frozen storage of atrioventricular valve leaflets had negligible effects on the characterized mechanical properties (Duginski et al., 2020).

In preparation for biaxial mechanical testing, the excised leaflet was thawed in phosphate-buffered saline (PBS). Then a  $13 \times 13$  mm specimen was cut from the central region of each TV leaflet (Fig. 1(a)), and the upper right corner of the specimen was marked with a surgical pen for orientation. The average thickness of the tissue was determined from three separate measurements across the specimen with a digital caliper (Westward 1AAU4, 0.01 mm resolution). The tissue was next mounted on a BioTester (CellScale, Canada) using BioRakes to delineate an effective test region of  $9 \times 9$  mm (Fig. 1(b)). Then four glass beads (300–500  $\mu\text{m}$ ) were glued in a square configuration within the central one-third area of the effective test region (i.e., central  $3 \times 3$  mm).

for subsequent calculations of tissue deformations/strains using digital image correlation (DIC). Once the glass beads were attached to the tissue surface, the specimen was immersed in a 37 °C PBS bath (Jett et al., 2020).

## 2.2. Biaxial mechanical characterization

For biaxial tests, the target peak force  $f^{\text{target}}$  for the TV leaflet specimen was determined using the approximate maximum physiological stress  $P^{\text{target}}$  of 115 kPa according to previous literature (Jett et al., 2018; Khoiy and Amini, 2016), the initial effective edge length  $L_0$  of the tissue, and the undeformed thickness  $t_0$ :  $f^{\text{target}} = P^{\text{target}} L_0 t_0$ . The leaflet was first subjected to 10 cycles of force-controlled equibiaxial preconditioning to ensure repeatable mechanical behaviors. The post-preconditioned configuration was recorded for later analyses. Next, the tissue was returned to its initial mounted configuration and the functionality of the BioTester was used to determine the biaxial displacements associated with the varying ratios  $f_{\text{circ}}^{\text{max}}:f_{\text{rad}}^{\text{max}} = 1:1, 1:0.667, 1:0.333, 0.667:1, 0.333:1$ . The specimen was then allowed to rest at the mounted configuration for 3 min. Finally, the tissue underwent biaxial mechanical characterization that included three loading/unloading cycles for each of the above-mentioned force ratios (i.e., protocols) at a loading rate of 1.31 N/min to emulate the quasi-static loading condition.

During the biaxial test, images were taken at 1280 × 960 resolution using the charge-couple device (CCD) camera and the load cell forces and time displacements at 15 Hz. Data from the last loading cycle was used in the stress/strain analysis and constitutive modeling discussed in Section 2.4.

## 2.3. Iterative approach to determine the effect of specimen dimensions

In order to investigate the effect of the specimen sizes on the characterized mechanical behavior, a sequential test procedure was carried out (Fig. 1(b)). Immediately after mechanical characterization of the 9 × 9 mm effective test region, the specimen removed from the BioTester and the BioRake holes were used as guidelines to procure a smaller specimen of 9 × 9 mm. The 9 × 9 mm specimen was then mounted to the BioTester with the second effective test region of 7.5 × 7.5 mm to repeat the mechanical characterization procedure described in Section 2.2. This iterative process was repeated for the 7.5 × 7.5 mm and 6 × 6 mm specimen sizes, resulting in the characterization of a total of four effective test regions (9 × 9 mm, 7.5 × 7.5 mm, 6 × 6 mm, and 4.5 × 4.5 mm) referred to in this study. Where possible, the original glass beads from the first specimen size were retained throughout the iterative procedure. However, it was sometimes necessary to swap out the glass beads for the smallest dimensions to keep the position of the fiducial markers within the central one-third of the specimen and allow for a homogeneous strain calculation.

## 2.4. Calculations of tissue stretches, stresses, and other derived mechanics metrics

The time-dependent fiducial marker positions were determined using the DIC functionality of the CellScale LabJoy software. The two-dimensional in-plane deformation gradient was calculated using a four-node bilinear finite element and the marker displacements (Sacks, 2000), i.e.,

$$[\mathbf{F}] = [\mathbf{F}(\mathbf{X}, t)] = [\mathbf{I}] + \begin{bmatrix} \sum_{I=1}^4 B_{XI} u_I(t) & \sum_{I=1}^4 B_{YI} u_I(t) \\ \sum_{I=1}^4 B_{XI} v_I(t) & \sum_{I=1}^4 B_{YI} v_I(t) \end{bmatrix}. \quad (1)$$

Here,  $\mathbf{I}$  is the second-order identity tensor,  $B_{XI}$  and  $B_{YI}$  are the shape function derivatives of marker  $I$  and  $u_I(t)$  and  $v_I(t)$  are the time-varying

$X$ - and  $Y$ -displacements of the  $I^{\text{th}}$  marker with respect to the stress-free mounted configuration. The tissue stretches  $\lambda_{\text{circ}}$  and  $\lambda_{\text{rad}}$  were calculated as the square roots of the principal values of the right-Cauchy tensor  $\mathbf{C} = \mathbf{F}^T \mathbf{F}$  (Holzapfel, 2000), and the preconditioning stretches  $\lambda_{\text{circ}}^{\text{PC}}$  and  $\lambda_{\text{rad}}^{\text{PC}}$  were determined after the preconditioning step using the configuration recorded in Section 2.3.

**Remark 1.** Note that the BioRake time-based testing scheme minimizes shear deformations of the tissue in contrast to existing suture-based methods in the literature. Therefore, we have assumed negligible shear deformations in the calculated deformation gradient, Eq. (1), which is consistent with the study of Narang et al. (2021) that demonstrated minimal *in vivo* shear deformations in the central region of the heart valve leaflets.

Next, the first Piola–Kirchhoff stresses  $P_{\text{circ}}$  and  $P_{\text{rad}}$  were determined as

$$[\mathbf{P}] = \text{diag}[P_{\text{circ}}, P_{\text{rad}}] = \frac{1}{I_0 L_0} \text{diag}[f_{\text{circ}}, f_{\text{rad}}], \quad (2)$$

where  $f_{\text{circ}}$  and  $f_{\text{rad}}$  are the forces measured in the circumferential and radial directions of the tissue, respectively.

The resulting stress–stretch curves were further analyzed to determine relevant engineering mechanics metrics, including the low-tensile moduli  $E_{\text{circ}}^{\text{LT}}$  and  $E_{\text{rad}}^{\text{LT}}$  using the experimental data < 10 kPa, the high-tensile moduli  $E_{\text{circ}}^{\text{HT}}$  and  $E_{\text{rad}}^{\text{HT}}$  using the last 5 data points of the loading curve (Fig. 1(c)), and the anisotropy index  $AI$  (May-Newman and Yin, 1995; Pham et al., 2017; Wells et al., 2012), calculated as the ratio of radial stretch to circumferential stretch under equibiaxial loading, i.e.,  $\lambda_{\text{rad}}/\lambda_{\text{circ}}$ .

## 2.5. Constitutive modeling

Furthermore, we examined the influence of the specimen dimensions on the determined constitutive model parameters for each leaflet ( $n = 8$ ). Instead of performing a model fit to the average experimental data (e.g., Khoiy et al. 2018), we wanted to test the null hypothesis that specimen dimensions do not change specimen-specific constitutive model parameters. To test this, data from the equibiaxial loading protocol ( $P_{\text{circ}}:P_{\text{rad}} = 1:1$ ) for all four effective test sizes were fit with three widely used constitutive models of heart valves and artery tissues. Note that only the equibiaxial configuration was considered to avoid complications when fitting with multiple protocols and to isolate the effect of specimen dimensions on the optimal model parameters.

The first constitutive model is the Lee–Sacks model typically used for the heart valve leaflets (Lee et al., 2014), i.e.,

$$\Psi_{\text{LS}} = C_{10}(I_1 - 3) + \frac{c_1}{2} \{ \delta \exp[c_2(I_1 - 3)^2] + (1 - \delta) \exp[c_3(I_4 - 1)^2] - 1 \}, \quad (3)$$

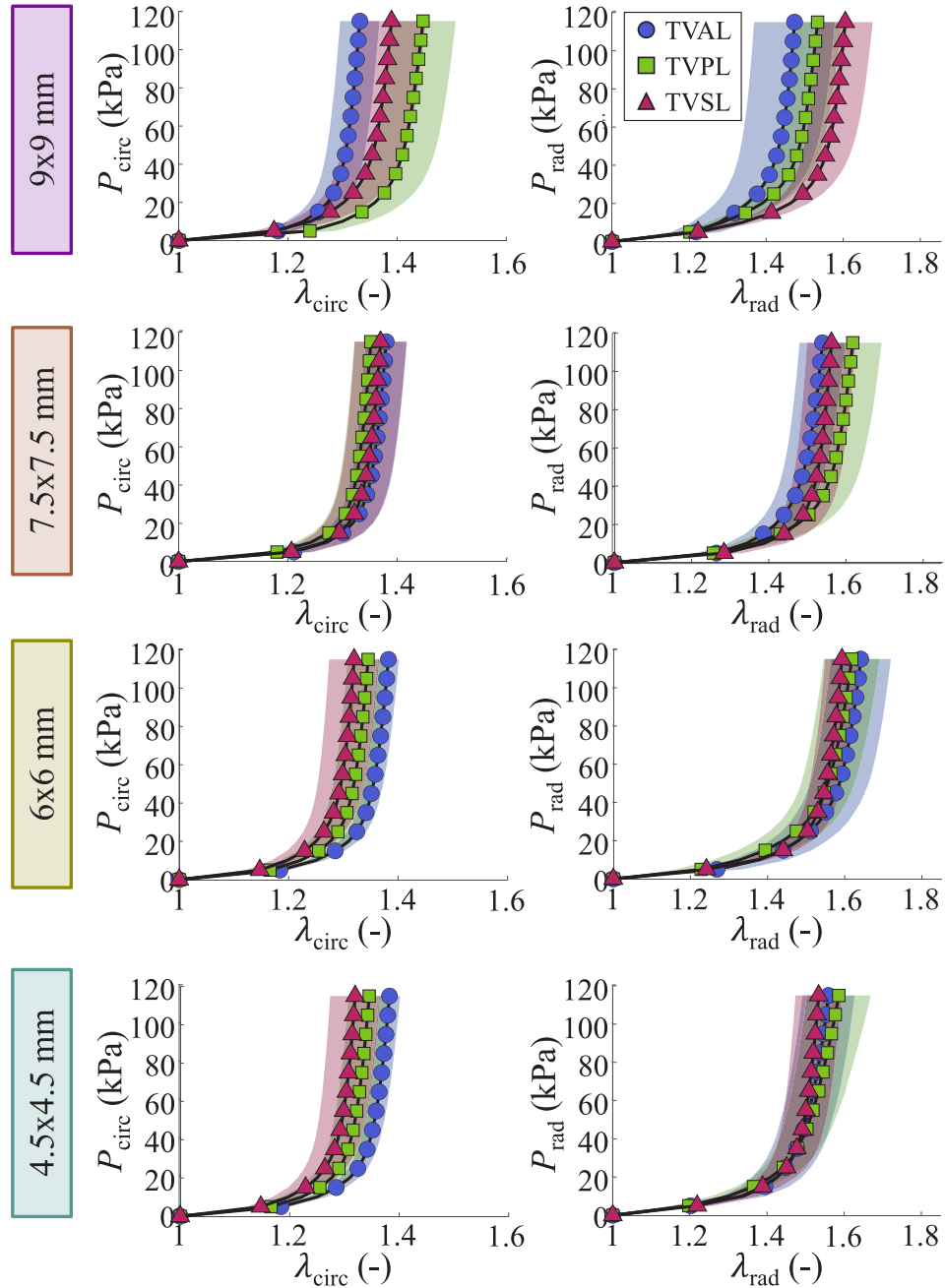
in which  $\Psi_{\text{LS}}$  is the strain–energy function,  $C_{10}$ ,  $c_0$ ,  $c_1$ ,  $c_2$  and  $c_3$  are the model parameters to be determined,  $I_1 = \text{tr} \mathbf{C}$  is the first invariant of  $\mathbf{C}$ ,  $I_4 = \mathbf{N} \cdot (\mathbf{C} \mathbf{N})$  is the fourth pseudo-invariant of  $\mathbf{C}$ ,  $[\mathbf{N}] = [\cos \theta, \sin \theta, 0]^T$  denotes the matrix form of the unit vector  $\mathbf{N}$ , which describes the collagen fiber orientation with the angle  $\theta$ , and  $\delta \in [0, 1]$  describes the material anisotropy.

The second model considered is the phenomenological constitutive model for soft biological tissues proposed by Tong and Fung (1976), i.e.,

$$\Psi_{\text{TF}} = C_{10}(I_1 - 3) + \frac{c}{2} [\exp(a_1 \hat{E}_{\text{circ}}^2 + a_2 \hat{E}_{\text{rad}}^2 + a_3 \hat{E}_{\text{circ}} \hat{E}_{\text{rad}}) - 1], \quad (4)$$

where  $C_{10}$ ,  $c$ ,  $a_1$ ,  $a_2$ , and  $a_3$  are the model parameters, and  $\hat{E}_{\text{circ}}$  and  $\hat{E}_{\text{rad}}$  are the circumferential and radial Green–Lagrange strains, respectively. Here, the Green–Lagrange strain tensor is computed by  $\mathbf{E} = \frac{1}{2}(\mathbf{C} - \mathbf{I})$ .

**Remark 2.** Although this model has certain shortcomings, e.g., ignoring shear strains, Narang et al. (2021) showed negligible *in vivo* shear deformations in the central belly region of the leaflet, which was the focus of this work.



**Fig. 2.** Mechanical behavior for equibiaxial tensions ( $P_{\text{circ}} : P_{\text{rad}} = 1 : 1$ ) of the three TV leaflets represented as mean (solid curves)  $\pm$  standard error of the mean (shaded areas).  $P_{(\cdot)}$  and  $\lambda_{(\cdot)}$  denote the first Piola–Kirchhoff stress and tissue stretch, respectively. TVAL: TV anterior leaflet, TVPL: TV posterior leaflet, and TVSL: TV septal leaflet.

The final constitutive model was proposed by Gasser et al. (2006), the Gasser–Ogden–Holzapfel (GOH) model, i.e.,

$$\Psi_{\text{GOH}} = C_{10}(I_1 - 3) + \frac{k_1}{2k_2} \left( \exp \left\{ k_2 [\kappa(I_1 - 3) + (1 - 3\kappa)(I_4 - 1)]^2 \right\} - 1 \right). \quad (5)$$

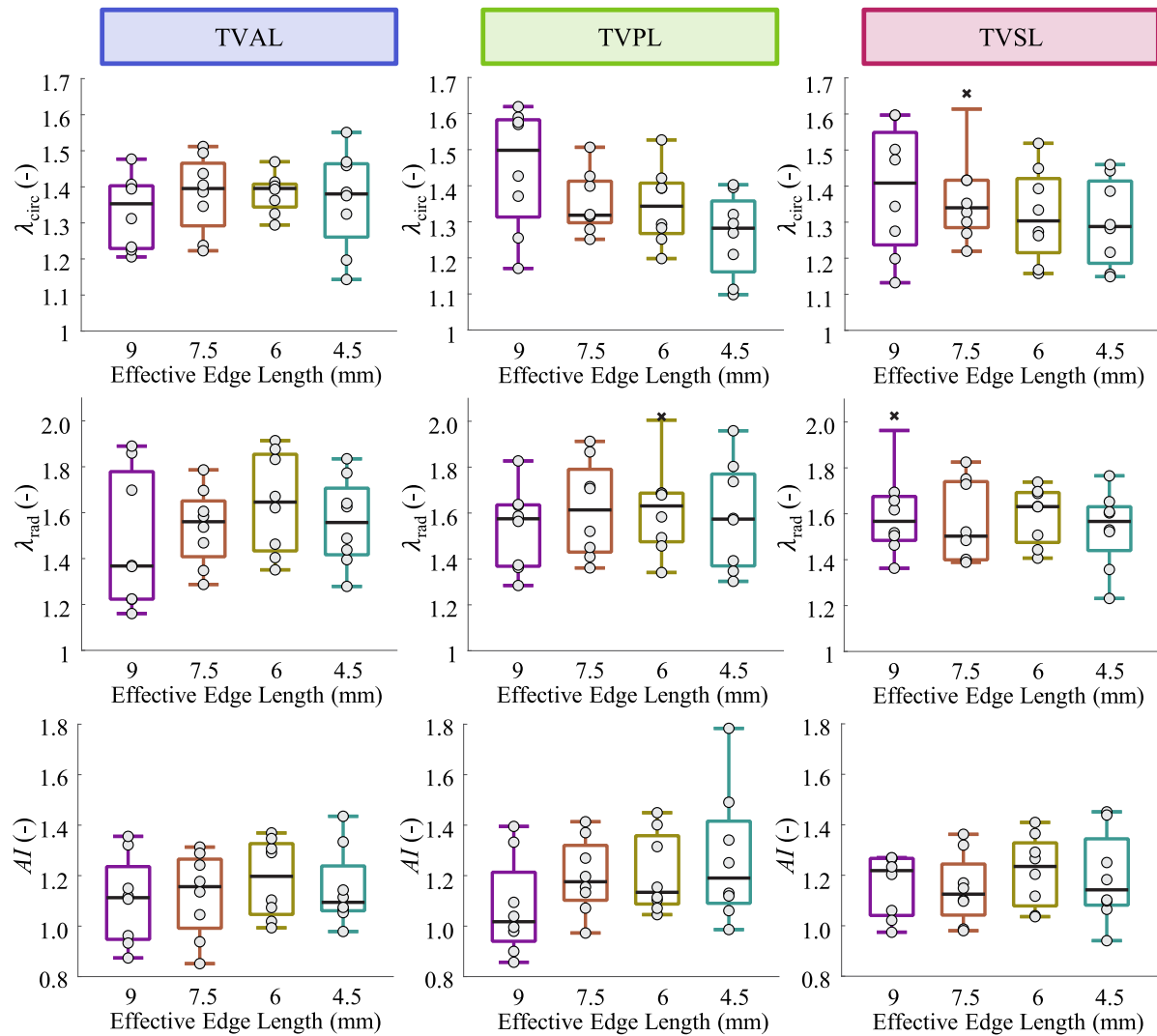
Herein,  $C_{10}$ ,  $k_1$ ,  $k_2$ , and  $\kappa$  are the model parameters, while the parameter  $\kappa \in [0, 1/3]$  describes the collagen fiber dispersion.

An in-house differential evolution optimization (DEO) algorithm was employed to estimate the material model parameters in Eqs. (3)–(5) by nonlinear least-squares regression (Yu et al., 2014). For all three constitutive models, it was assumed that  $C_{10} = 5$  kPa to reduce the number of parameters to be estimated and to ensure realistic and

consistent low-stress behavior. Briefly,  $n_{\text{pop}}$  uniformly distributed sets of material parameters were generated, and the residual  $r$  was computed for each parameter set  $j$ . Thus,

$$r_j = \frac{1}{n_{\text{data}}} \left[ \sum_{i=1}^{n_{\text{data}}} (S_{\text{circ}}^{\text{exp},i} - S_{\text{circ},j}^{\text{model},i})^2 + (S_{\text{rad}}^{\text{exp},i} - S_{\text{rad},j}^{\text{model},i})^2 \right]^{1/2}, \quad (6)$$

where  $n_{\text{data}}$  denotes the number of experimental data points,  $S_{\text{circ}}$  and  $S_{\text{rad}}$  are the circumferential and radial second Piola–Kirchhoff stresses, respectively, and the superscript denotes either the experimental data or the model predictions. The  $n_{\text{pop}}$  residuals and their Euclidian distances to the best parameter set were used with the two-step DEO algorithm to iteratively update the parameter sets until the residual



**Fig. 3.** (Top) Peak circumferential stretch, (middle) peak radial stretch and (bottom) anisotropy index (AI) for the four specimen sizes of all three TV leaflets. × denotes outliers,  $\lambda_{\text{circ}}$  denotes the stretch, TVAL: TV anterior leaflet, TVPL: TV posterior leaflet, and TVSL: TV septal leaflet.

threshold was reached ( $\max r_j < 5 \times 10^{-7}$ ), or the change in the residual was minimal ( $< 1 \times 10^{-7}$ ) for three consecutive iterations.

## 2.6. Statistical analyses

Visualization of the data in MATLAB using quantile–quantile (Q–Q) plots showed the data were not normally distributed (not shown here). Therefore, two-factor comparisons (size vs. leaflets) were performed using the non-parametric aligned rank transform in R version R-4.2.2 (Wobbrock et al., 2011). Comparisons were performed where  $p < 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Mechanical behavior with equibiaxial tension

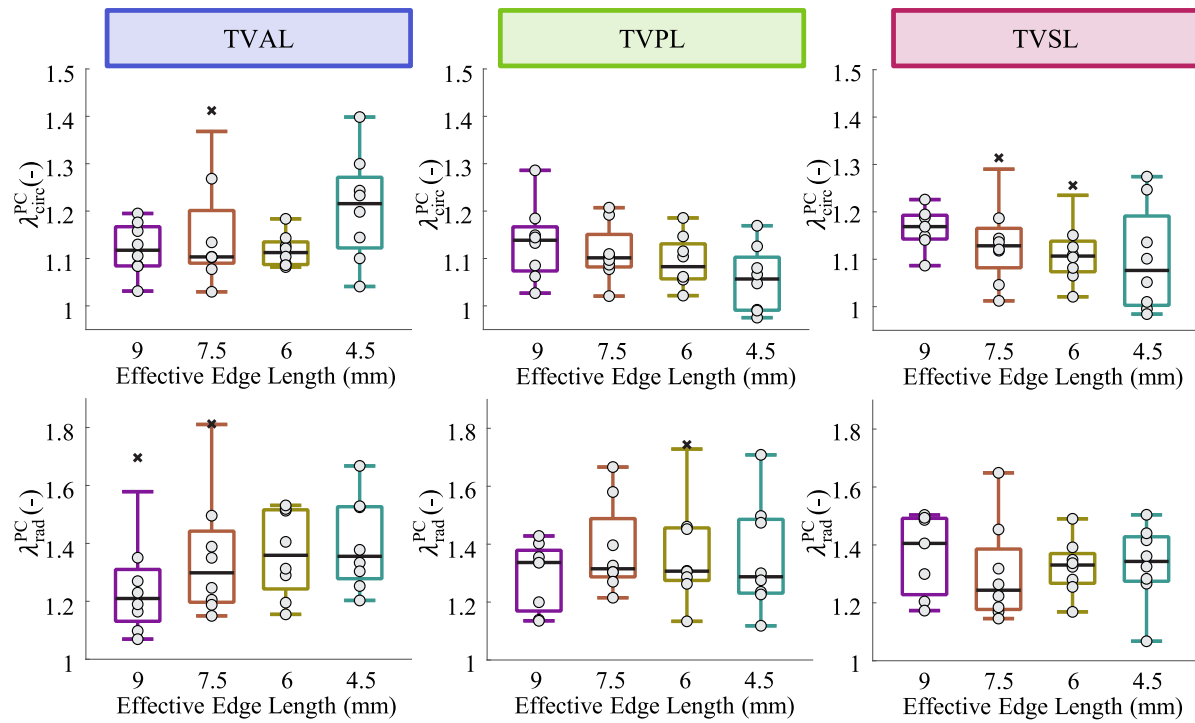
The TV leaflets showed nonlinear, anisotropic mechanical behavior under equibiaxial loading for all four specimen sizes, as shown in Fig. 2.

Detailed comparisons of the peak stretches are provided in Fig. 3. Results for other biaxial tension protocols are given in Appendix A.

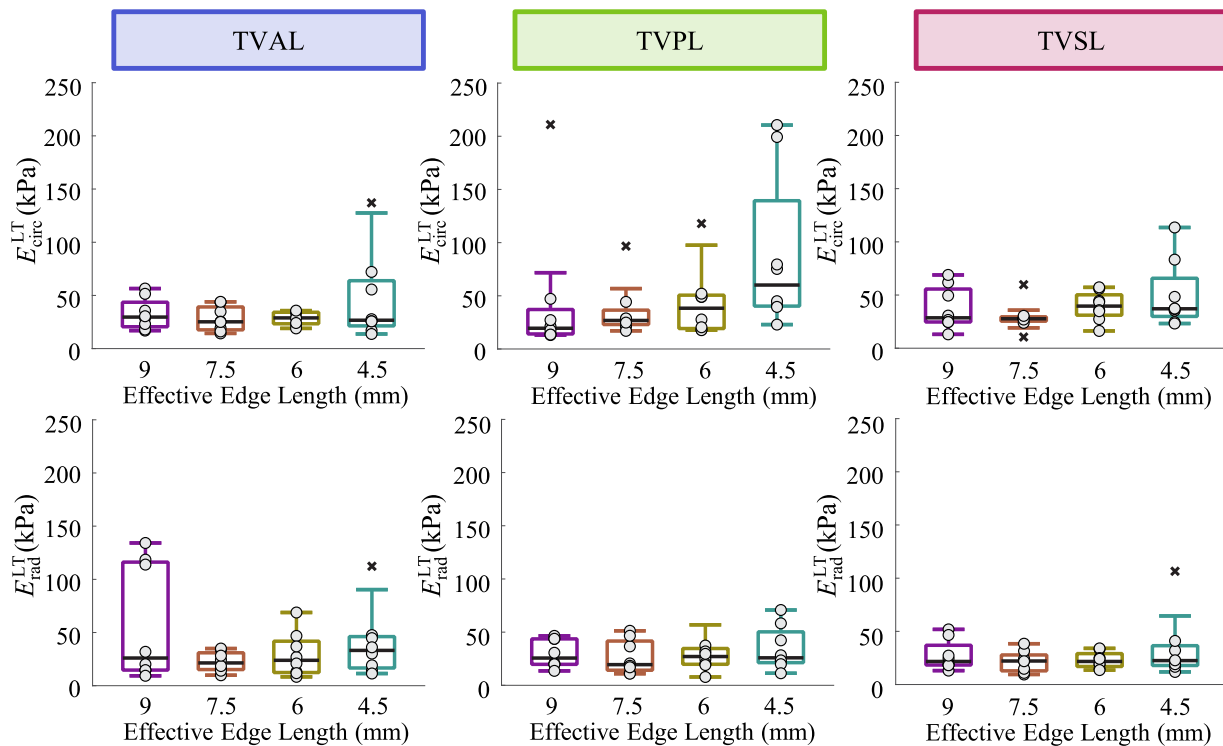
As expected, the circumferential directions of the leaflets were consistently stiffer than the radial directions due to collagen fiber reinforcement along the circumferential direction. For the circumferential direction, we observed stiffer stretches between the smallest ( $4.5 \times 4.5$  mm) and largest ( $9 \times 9$  mm) specimen dimensions for the TVPL (1.28 vs. 1.50) and TVSL (1.29 vs. 1.41). Specimen dimensions had minimal effects on the radial tissue stretches between the smallest and largest specimen dimensions of TVPL (1.57 vs. 1.58) and TVSL (1.57 vs. 1.57). Interestingly, the TVAL had an opposite trend for the circumferential stretches (1.38 vs. 1.35) and the differences in the radial stretches (1.56 vs. 1.37) were more apparent.

### 3.2. Preconditioning stretches

The quantified stretches after the preconditioning step ( $\lambda_{\text{circ}}^{\text{PC}}$  and  $\lambda_{\text{rad}}^{\text{PC}}$ ) are presented in Fig. 4. For both TVPL and TVSL in the circumferential (1.06–1.08 vs. 1.14–1.17) and radial (1.29–1.34 vs. 1.34–1.41)



**Fig. 4.** Preconditioning stretches for the four specimen sizes of all three TV leaflets in (top) circumferential and (bottom) radial directions, where  $\times$  denotes an outlier;  $\lambda_{\text{circ}}^{\text{PC}}$  denotes the quantified stretches after the preconditioning step. TVAL: TV anterior leaflet, TVPL: TV posterior leaflet, and TVSL: TV septal leaflet.



**Fig. 5.** Low-tensile moduli ( $E_{\text{circ}}^{\text{LT}}$  and  $E_{\text{rad}}^{\text{LT}}$ ) for the four specimen sizes of all three TV leaflets in (top) circumferential and (bottom) radial directions, where  $\times$  denotes an outlier. TVAL: TV anterior leaflet, TVPL: TV posterior leaflet, and TVSL: TV septal leaflet.

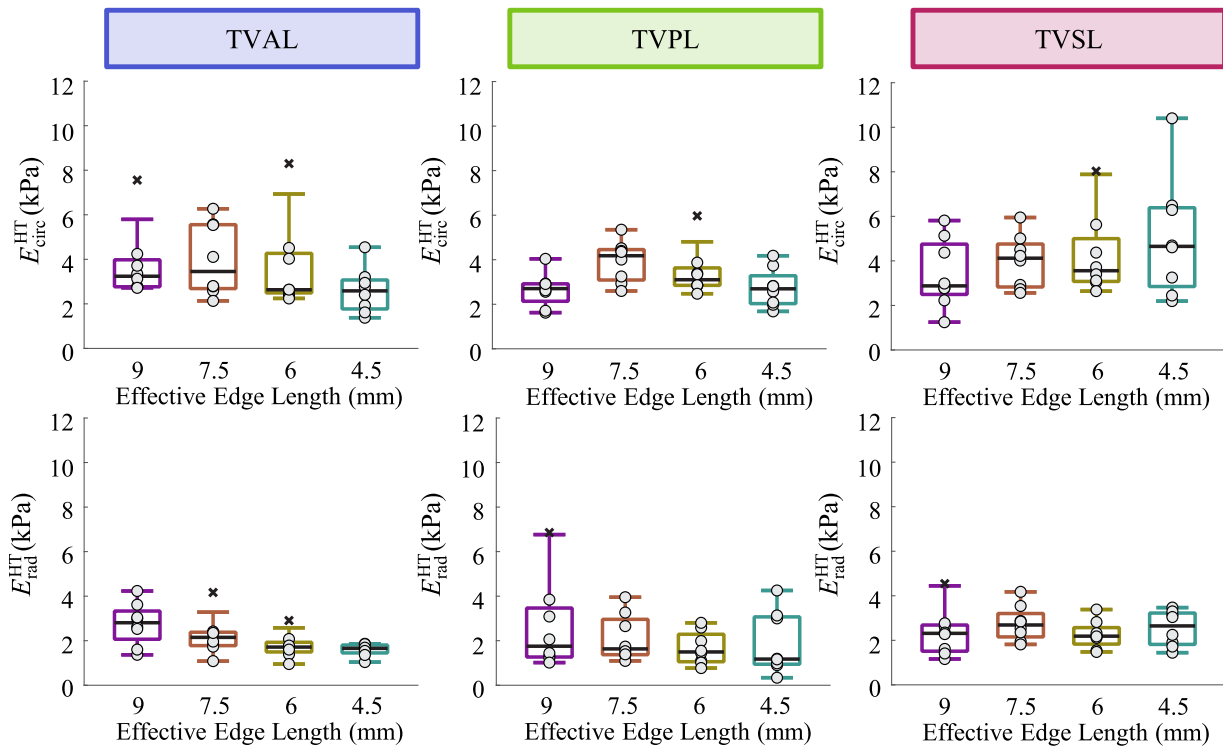


Fig. 6. High-tensile moduli ( $E_{\text{circ}}^{\text{HT}}$  and  $E_{\text{rad}}^{\text{HT}}$ ) for the four specimen sizes of all three TV leaflets in (top) circumferential and (bottom) radial directions, where  $\times$  denotes an outlier. TVAL: TV anterior leaflet, TVPL: TV posterior leaflet, and TVSL: TV septal leaflet.

Table 1

Estimated parameters for the Lee-Sacks model, i.e., Eq. (3), fitted to the equibiaxial mechanical response of all three tricuspid valve leaflets. The data presented include the median, first quartile (1st), and third quartile (3rd). AL: anterior leaflet, PL: posterior leaflet, and SL: septal leaflet.

| Parameter       | Leaflet | Size 1 |       |       | Size 2 |       |       | Size 3 |       |       | Size 4 |       |       | p-value |      |             |
|-----------------|---------|--------|-------|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|---------|------|-------------|
|                 |         | Median | 1st   | 3rd   | Median | 1st   | 3rd   | Median | 1st   | 3rd   | Median | 1st   | 3rd   | Leaflet | Size | Interaction |
| $c_0$ (kPa)     | AL      | 0.45   | 0.06  | 3.03  | 0.29   | 0.06  | 0.66  | 0.22   | 0.01  | 2.52  | 1.03   | 0.04  | 6.57  | 0.56    | 0.14 | 0.21        |
|                 | PL      | 0.35   | 0.09  | 4.15  | 0.23   | 0.03  | 0.66  | 1.32   | 0.11  | 5.66  | 3.45   | 0.73  | 4.09  |         |      |             |
|                 | SL      | 0.34   | 0.08  | 0.57  | 0.03   | 0.02  | 0.30  | 0.26   | 0.04  | 0.72  | 0.15   | 0.01  | 1.67  |         |      |             |
| $c_1$ (kPa)     | AL      | 2.82   | 1.00  | 6.28  | 1.86   | 1.12  | 2.79  | 1.58   | 1.00  | 2.36  | 1.44   | 1.07  | 2.30  | 0.06    | 0.22 | 0.17        |
|                 | PL      | 1.15   | 1.00  | 2.02  | 1.31   | 1.00  | 2.71  | 1.00   | 1.00  | 1.88  | 1.05   | 1.00  | 1.90  |         |      |             |
|                 | SL      | 1.20   | 1.00  | 2.13  | 2.52   | 1.98  | 3.19  | 1.29   | 1.15  | 1.83  | 1.61   | 1.00  | 2.57  |         |      |             |
| $c_2$ (-)       | AL      | 6.92   | 2.90  | 13.92 | 6.31   | 3.72  | 15.53 | 5.65   | 2.42  | 7.17  | 4.16   | 3.42  | 6.37  | 0.20    | 0.38 | 0.21        |
|                 | PL      | 3.29   | 2.62  | 11.73 | 5.02   | 3.34  | 9.25  | 2.55   | 2.20  | 6.25  | 2.50   | 1.57  | 6.42  |         |      |             |
|                 | SL      | 3.26   | 1.21  | 9.44  | 6.87   | 4.76  | 8.55  | 4.27   | 3.29  | 6.31  | 7.26   | 6.02  | 15.40 |         |      |             |
| $\delta$ (-)    | AL      | 0.00   | 0.00  | 0.06  | 0.00   | 0.00  | 0.28  | 0.00   | 0.00  | 0.49  | 0.00   | 0.00  | 0.41  | 0.89    | 0.73 | 0.83        |
|                 | PL      | 0.00   | 0.00  | 0.30  | 0.00   | 0.00  | 0.42  | 0.00   | 0.00  | 0.01  | 0.00   | 0.00  | 0.00  |         |      |             |
|                 | SL      | 0.00   | 0.00  | 0.61  | 0.00   | 0.00  | 0.49  | 0.00   | 0.00  | 0.00  | 0.00   | 0.00  | 0.26  |         |      |             |
| $\theta$ (deg.) | AL      | 44.14  | 41.01 | 46.27 | 41.33  | 20.18 | 45.40 | 39.71  | 19.50 | 41.71 | 43.10  | 19.73 | 43.99 | 0.78    | 0.95 | 0.86        |
|                 | PL      | 45.11  | 31.53 | 47.25 | 40.55  | 38.70 | 42.11 | 40.80  | 37.48 | 43.35 | 39.37  | 36.06 | 42.87 |         |      |             |
|                 | SL      | 40.75  | 29.56 | 42.38 | 42.08  | 25.82 | 44.17 | 40.11  | 38.89 | 42.34 | 42.35  | 36.89 | 43.17 |         |      |             |

directions, we consistently observed smaller preconditioning stretches at the smallest specimen size ( $4.5 \times 4.5$  mm) than the largest specimen size ( $9 \times 9$  mm). The TVAL, on the other hand, exhibited opposite trends with larger preconditioning stretches at the smallest specimen dimensions (1.22–1.36) compared to the largest specimen dimensions (1.12–1.21). Significant differences were only found between leaflets for  $\lambda_{\text{circ}}^{\text{PC}}$  ( $p = 0.03$ ).

### 3.3. Derived mechanics-related metrics

The quantified low-tensile moduli  $E_{\text{circ}}^{\text{LT}}$  and  $E_{\text{rad}}^{\text{LT}}$ , high-tensile moduli  $E_{\text{circ}}^{\text{HT}}$  and  $E_{\text{rad}}^{\text{HT}}$  and anisotropy indices  $AI$  are given in Figs. 5–6 and Fig. 3, respectively.

We find that the circumferential low-tensile moduli  $E_{\text{circ}}^{\text{LT}}$  increased with smaller specimen dimensions for the TVPL (60.10 kPa vs. 19.51 kPa) and TVSL (37.1 kPa vs. 28.6 kPa), where the radial low-tensile moduli  $E_{\text{rad}}^{\text{LT}}$  remained consistent. These differences were significant between specimen sizes ( $p = 0.01$ ). On the other hand, the circumferential and radial high-tensile moduli decreased with smaller specimen sizes for TVAL (1655.5–2583.6 kPa vs. 2806.9–3239.4 kPa) and TVPL (1176.2–2692.9 kPa vs. 1753.3–2702.6), but increased with smaller specimen sizes for the TVSL (2645.5–4650.5 kPa vs. 2312.1–2878.4 kPa). Significant differences were only found between leaflets ( $p < 0.05$ ) and not between specimen dimensions ( $p > 0.15$ ). Finally, the  $AI$  was found to be with non-significant differences at smaller specimen

**Table 2**

Estimated parameters for the Tong–Fung model, i.e., Eq. (4), fitted to the equibiaxial mechanical response of all three tricuspid valve leaflets. The data presented include the median, first quartile (1st), and third quartile (3rd). AL: anterior leaflet, PL: posterior leaflet, SL: septal leaflet, and \* denotes statistically significant pairwise comparison.

| Parameter   | Leaflet | Size 1 |      |       | Size 2 |      |       | Size 3 |      |       | Size 4 |      |       | p-value |       |             |
|-------------|---------|--------|------|-------|--------|------|-------|--------|------|-------|--------|------|-------|---------|-------|-------------|
|             |         | Median | 1st  | 3rd   | Median | 1st  | 3rd   | Median | 1st  | 3rd   | Median | 1st  | 3rd   | Leaflet | Size  | Interaction |
| $c$ (kPa)   | AL      | 0.29   | 0.08 | 0.80  | 0.21   | 0.02 | 0.64  | 0.28   | 0.02 | 1.94  | 0.73   | 0.14 | 2.01  | <0.01   | <0.01 | 0.16        |
|             | PL      | 0.32   | 0.15 | 1.22  | 0.16   | 0.02 | 0.49  | 0.91   | 0.15 | 2.87  | 0.86*  | 0.44 | 3.04  |         |       |             |
|             | SL      | 0.34   | 0.14 | 0.50  | 0.04*  | 0.02 | 0.14  | 0.17   | 0.05 | 0.25  | 0.14   | 0.02 | 0.58  |         |       |             |
| $a_1$ (–)   | AL      | 4.30   | 1.45 | 6.03  | 3.28   | 0.96 | 5.40  | 1.72   | 0.29 | 3.27  | 2.85   | 0.42 | 6.47  | 0.78    | 0.94  | 0.90        |
|             | PL      | 0.76   | 0.07 | 2.98  | 2.34   | 0.54 | 7.78  | 2.28   | 0.08 | 5.12  | 2.59   | 0.21 | 7.40  |         |       |             |
|             | SL      | 2.19   | 0.60 | 20.58 | 1.85   | 0.00 | 11.40 | 1.91   | 0.10 | 14.76 | 0.66   | 0.00 | 11.60 |         |       |             |
| $a_2$ (kPa) | AL      | 3.28   | 1.90 | 6.01  | 1.74   | 0.39 | 3.75  | 1.78   | 0.94 | 2.99  | 1.67   | 0.87 | 3.77  | 0.33    | 0.94  | 0.29        |
|             | PL      | 0.21   | 0.02 | 1.85  | 1.96   | 1.41 | 4.11  | 1.67   | 0.92 | 2.78  | 1.66   | 1.08 | 5.78  |         |       |             |
|             | SL      | 1.65   | 0.71 | 5.50  | 2.97   | 1.42 | 4.84  | 2.27   | 1.61 | 3.32  | 2.66   | 1.82 | 3.59  |         |       |             |
| $a_3$ (kPa) | AL      | 9.74   | 1.63 | 15.60 | 3.89   | 2.38 | 7.14  | 2.92   | 1.67 | 3.92  | 3.13   | 1.10 | 5.51  | 0.43    | 0.44  | 0.33        |
|             | PL      | 3.81   | 3.00 | 5.24  | 2.57   | 0.68 | 10.51 | 2.51   | 1.25 | 5.81  | 2.71   | 0.81 | 5.10  |         |       |             |
|             | SL      | 2.03   | 0.64 | 3.90  | 5.14   | 1.64 | 7.18  | 4.65   | 1.99 | 6.05  | 7.03   | 2.99 | 9.19  |         |       |             |

**Table 3**

Estimated parameters for the Gasser–Ogden–Holzapfel (GOH) model, i.e., Eq. (5), fitted to the equibiaxial mechanical response of all three tricuspid valve leaflets. The data presented include the median, first quartile (1st), and third quartile (3rd). AL: anterior leaflet, PL: posterior leaflet, and SL: septal leaflet.

| Parameter       | Leaflet | Size 1 |       |       | Size 2 |       |       | Size 3 |       |       | Size 4 |       |       | p-value |      |             |
|-----------------|---------|--------|-------|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|---------|------|-------------|
|                 |         | Median | 1st   | 3rd   | Median | 1st   | 3rd   | Median | 1st   | 3rd   | Median | 1st   | 3rd   | Leaflet | Size | Interaction |
| $k_1$ (kPa)     | AL      | 0.46   | 0.08  | 74.49 | 0.45   | 0.06  | 3.33  | 0.22   | 0.04  | 24.15 | 11.05  | 0.08  | 42.16 | 0.20    | 0.36 | 0.89        |
|                 | PL      | 0.40   | 0.09  | 17.69 | 0.86   | 0.03  | 9.72  | 8.18   | 0.12  | 18.39 | 15.58  | 1.66  | 49.86 |         |      |             |
|                 | SL      | 0.49   | 0.12  | 2.80  | 0.09   | 0.04  | 8.78  | 0.30   | 0.07  | 1.02  | 0.97   | 0.03  | 3.74  |         |      |             |
| $k_2$ (–)       | AL      | 14.29  | 2.87  | 37.92 | 6.33   | 4.86  | 8.69  | 5.31   | 2.73  | 9.42  | 5.76   | 3.42  | 10.49 | 0.24    | 0.34 | 0.58        |
|                 | PL      | 7.26   | 2.62  | 12.52 | 5.30   | 3.09  | 19.27 | 6.15   | 3.67  | 7.01  | 5.72   | 3.01  | 10.25 |         |      |             |
|                 | SL      | 4.74   | 3.26  | 7.50  | 6.87   | 5.08  | 13.29 | 5.40   | 3.84  | 9.53  | 7.55   | 6.11  | 11.36 |         |      |             |
| $\kappa$ (–)    | AL      | 0.00   | 0.00  | 0.25  | 0.00   | 0.00  | 0.14  | 0.00   | 0.00  | 0.31  | 0.00   | 0.00  | 0.30  | 0.31    | 0.81 | 0.83        |
|                 | PL      | 0.00   | 0.00  | 0.28  | 0.00   | 0.00  | 0.30  | 0.00   | 0.00  | 0.28  | 0.17   | 0.00  | 0.28  |         |      |             |
|                 | SL      | 0.00   | 0.00  | 0.19  | 0.00   | 0.00  | 0.16  | 0.00   | 0.00  | 0.00  | 0.00   | 0.00  | 0.25  |         |      |             |
| $\theta$ (deg.) | AL      | 42.95  | 39.28 | 47.59 | 41.24  | 26.77 | 45.40 | 39.44  | 19.16 | 44.21 | 42.86  | 19.72 | 43.68 | 0.69    | 0.29 | 0.74        |
|                 | PL      | 45.11  | 37.36 | 46.54 | 39.07  | 0.38  | 42.11 | 39.47  | 0.00  | 43.72 | 19.10  | 0.00  | 43.14 |         |      |             |
|                 | SL      | 40.59  | 29.88 | 42.78 | 42.59  | 39.13 | 44.36 | 40.11  | 38.89 | 42.34 | 41.22  | 35.03 | 42.38 |         |      |             |

**Table 4**

Existing investigations in the literature characterizing the mechanical properties of the tricuspid heart valve leaflets.

| PI            | Paper                           | Dissected dimensions | Mounted dimensions | Stress measure | Deformation measure |
|---------------|---------------------------------|----------------------|--------------------|----------------|---------------------|
| Chung-Hao Lee | Jett et al. (2018)              | 8 mm                 | 6.5 mm             | 1PK            | Stretch             |
|               | Laurence et al. (2019)          | 6 mm                 | 4 mm               | MT             | Stretch             |
|               | Ross et al. (2019a)             | 10 mm                | 7/5.5 mm           | MT             | Stretch             |
|               | Kramer et al. (2019)            | 4 mm                 | 3.5 mm             | 1PK            | Stretch             |
|               | Duginski et al. (2020)          | 10 mm                | 6.5–8.5 mm         | MT             | Stretch             |
|               | Hudson et al. (2020)            | ND                   | 10 mm              | MT             | Stretch             |
|               | Ross et al. (2021)              | 10 mm                | 7.5/6 mm           | MT             | Stretch             |
| Manuel Rausch | Meador et al. (2020a)           | 7 mm                 | ND                 | MT             | Stretch             |
|               | Meador et al. (2020b)           | 7 mm                 | ND                 | MT             | Stretch             |
| Rouzbeh Amini | Khoi and Amini (2016)           | 11 mm                | 7.6 mm             | MT             | Stretch             |
|               | Salinas et al. (2022)           | 11 mm                | 7.6 mm             | 1PK            | Green Strain        |
|               | Salinas et al. (2019)           | 11 mm                | 7.6 mm             | 1PK            | Green Strain        |
|               | Clarín et al. (2023)            | 11 mm                | 7.6 mm             | 1PK            | Green Strain        |
|               | Salinas et al. (2020)           | 11 mm                | 7.6 mm             | 1PK            | Green Strain        |
| Wei Sun       | Pokutta-Paskaleva et al. (2019) | ND                   | ND                 | 2PK            | Green Strain        |
|               | Pham et al. (2017)              | ND                   | 10 mm              | 2PK            | Green Strain        |

(ND: Not Defined, 1PK: First Piola–Kirchhoff, MT: Membrane Tension, 2PK: Second Piola–Kirchhoff).

dimensions for TVAL (1.09 vs. 1.12) and TVSL (1.14 vs. 1.22) but increased for TVPL (1.19 vs. 1.02).

### 3.4. Constitutive modeling

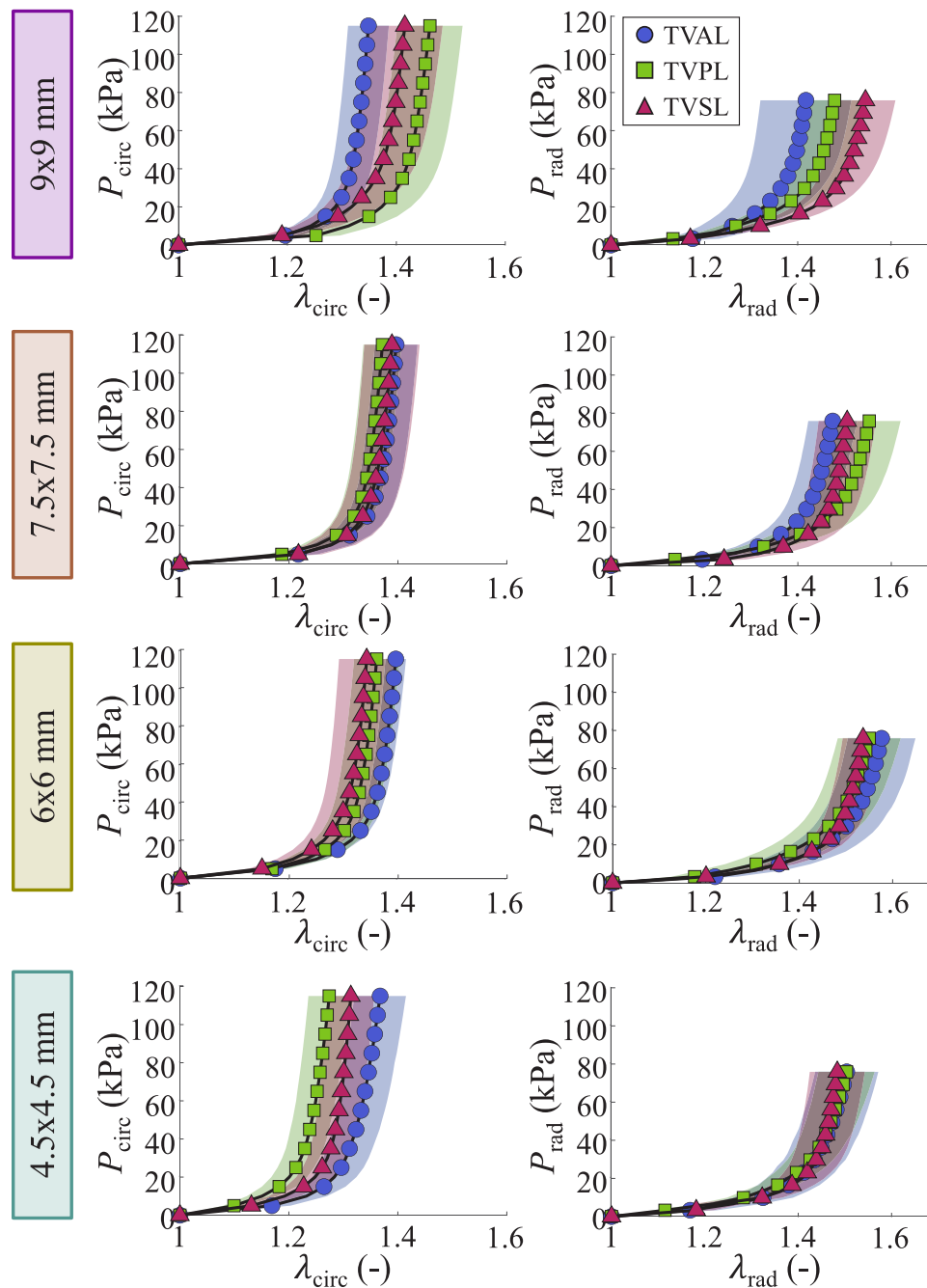
The parameters for the Lee–Sacks constitutive model, i.e., Eq. (3), the Tong–Fung constitutive model, i.e., Eq. (4), and the GOH constitutive model, i.e., Eq. (5) are summarized in Tables 1–3. Interestingly, there was only one statistically significant difference for the Tong–Fung constitutive model parameter ( $c$ ) between leaflets ( $p < 0.01$ ) and specimen sizes ( $p < 0.01$ ). When considering the interactions between

these effects, the only significant comparison was between TVSL Size #2 ( $c = 0.04$  kPa) and the TVPL Size #4 ( $c = 0.86$  kPa). Therefore, the specimen dimensions do not have a significant effect on the derived constitutive model parameters for a given TV leaflet.

## 4. Discussion

### 4.1. Overall findings

In this study, we performed biaxial tensile tests on TV leaflets to understand how specimen dimensions affect mechanical behavior,

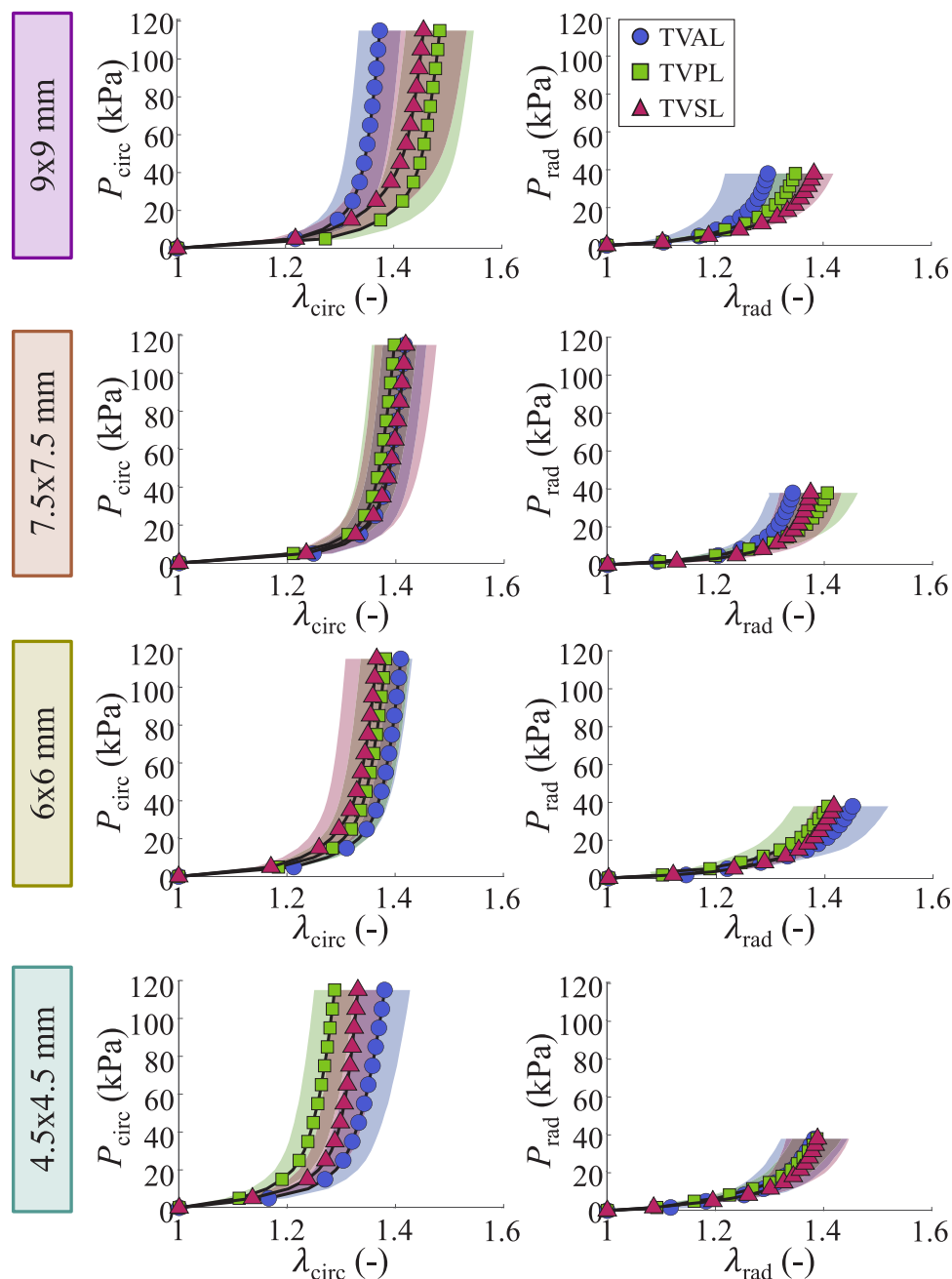


**Fig. A1.** Mechanical behavior for biaxial tensions ( $P_{\text{circ}}:P_{\text{rad}} = 1:0.667$ ) of the three TV leaflets represented as mean (solid curves)  $\pm$  standard error of the mean.  $P_{(\cdot)}$  and  $\lambda_{(\cdot)}$  denote the first Piola–Kirchhoff stress and the stretch, respectively. TVAL: TV anterior leaflet, TVPL: TV posterior leaflet, and TVSL: TV septal leaflet.

mechanical properties, and estimated constitutive model parameters. Our results demonstrated that reducing specimen size resulted in a slightly stiffer circumferential behavior and a slightly more compliant radial behavior. However, none of these differences were considered significant from the standpoint of statistical analysis. Further comparison of the derived mechanical properties illustrated a slight increase in low-tensile stiffness, a decrease in high-tensile stiffness, and an increase in  $AI$  with decreasing specimen dimensions, with the exception of TVSL. We detected one significant difference in the Tong–Fung constitutive model stiffness  $c$  between two specimen sizes of the TVPL and TVSL.

#### 4.2. Comparisons with existing literature

Although, to our knowledge, this is the first *experimental* study for exploring how specimen dimensions affect biaxial mechanical characterization and constitutive modeling, some have attempted to study this feature *numerically*. Most notably, [Fehervary et al. \(2016\)](#) used finite element simulations of planar biaxial testing to understand how certain mechanical testing factors affected the homogeneity of the observed strain field within the fiducial markers and the accuracy of the constitutive model behavior. Through their *correction factor metric*, they observed that the predicted stresses increased (i.e., stiffer



**Fig. A2.** Mechanical behavior for biaxial tensions ( $P_{\text{circ}} : P_{\text{rad}} = 1 : 0.333$ ) of the three TV leaflets represented as mean (solid curves)  $\pm$  standard error of the mean.  $P_{(\cdot)}$  and  $\lambda_{(\cdot)}$  denote the first Piola–Kirchhoff stress and the stretch, respectively. TVAL: TV anterior leaflet, TVPL: TV posterior leaflet, and TVSL: TV septal leaflet.

tissue) as the fiducial markers got closer to the tine insertions. The experimental findings in this work generally support this observation, with the exception of the TVSL, which was slightly more deformable with smaller sample dimensions. Another study by Eilaghi et al. (2009) underlined the importance of the insertion point distribution along the effective test region. In this study, we did not attempt to test this factor experimentally; however, we used different sets of tines for each specimen edge length. Therefore, our data presented here may be useful for further investigations along this direction.

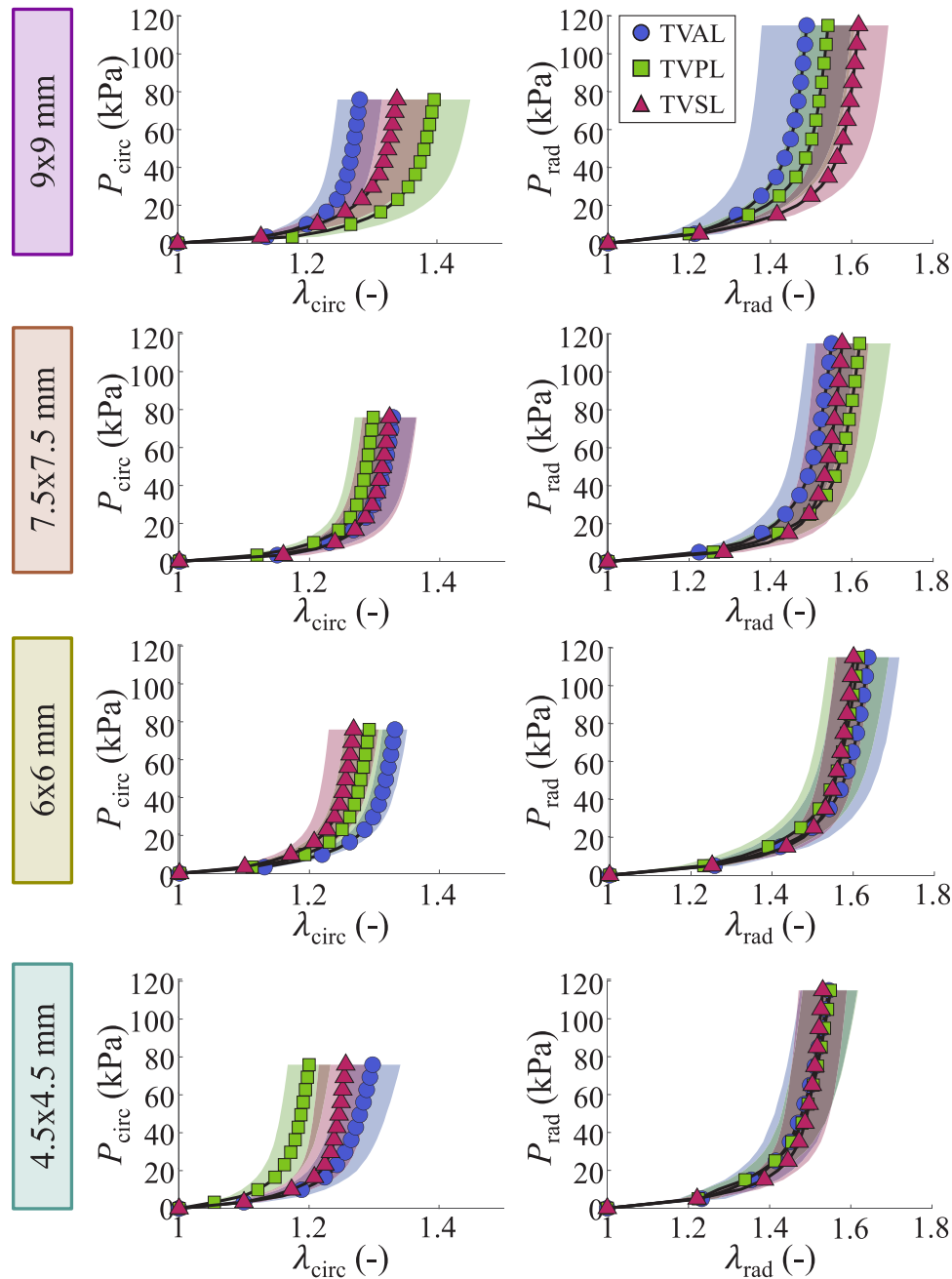
Recent advances in polarized spatial frequency domain imaging have enabled the simultaneous acquisition of collagen fiber architectural properties and tissue strains (Dover et al., 2022). In their work, Dover et al. (2022) assessed how the size of the region of interest affects strain homogeneity and analyses of tissue mechanical properties. They found that regions farther away from the boundary insertion

points exhibited stiffer circumferential behavior and more compliant radial behavior (i.e., the strains are only homogeneous within the central 80% of the specimen dimension).

Our results from iterative biaxial mechanical characterization reflect this result and underscores the importance of specimen dimensions and defining an appropriate region of interest.

#### 4.3. Implications of our findings

Our results have significant implications for existing mechanical studies using iterative experimental protocols. For example, our group used iterative enzyme-based approaches to characterize how glycosaminoglycans, collagen, and elastin contribute to the TV leaflet mechanical behavior (Ross et al., 2019a, 2021). Control tissues were



**Fig. A3.** Mechanical behavior for biaxial tensions ( $P_{\text{circ}} : P_{\text{rad}} = 0.667 : 1$ ) of the three TV leaflets represented as mean (solid curves)  $\pm$  standard error of the mean.  $P_{\text{c}}$  and  $\lambda_{\text{c}}$  denote the first Piola–Kirchhoff stress and the stretch, respectively. TVAL: TV anterior leaflet, TVPL: TV posterior leaflet, and TVSL: TV septal leaflet.

larger than enzyme-treated tissues in these studies to ensure that previous tine insertion holes did not affect the biomechanical performance. However, as our present study indicates, this reduction in specimen size results in higher circumferential stretches and lower radial stretches for the enzyme-treated specimens, which may result in misleading comparisons between the treated and control groups.

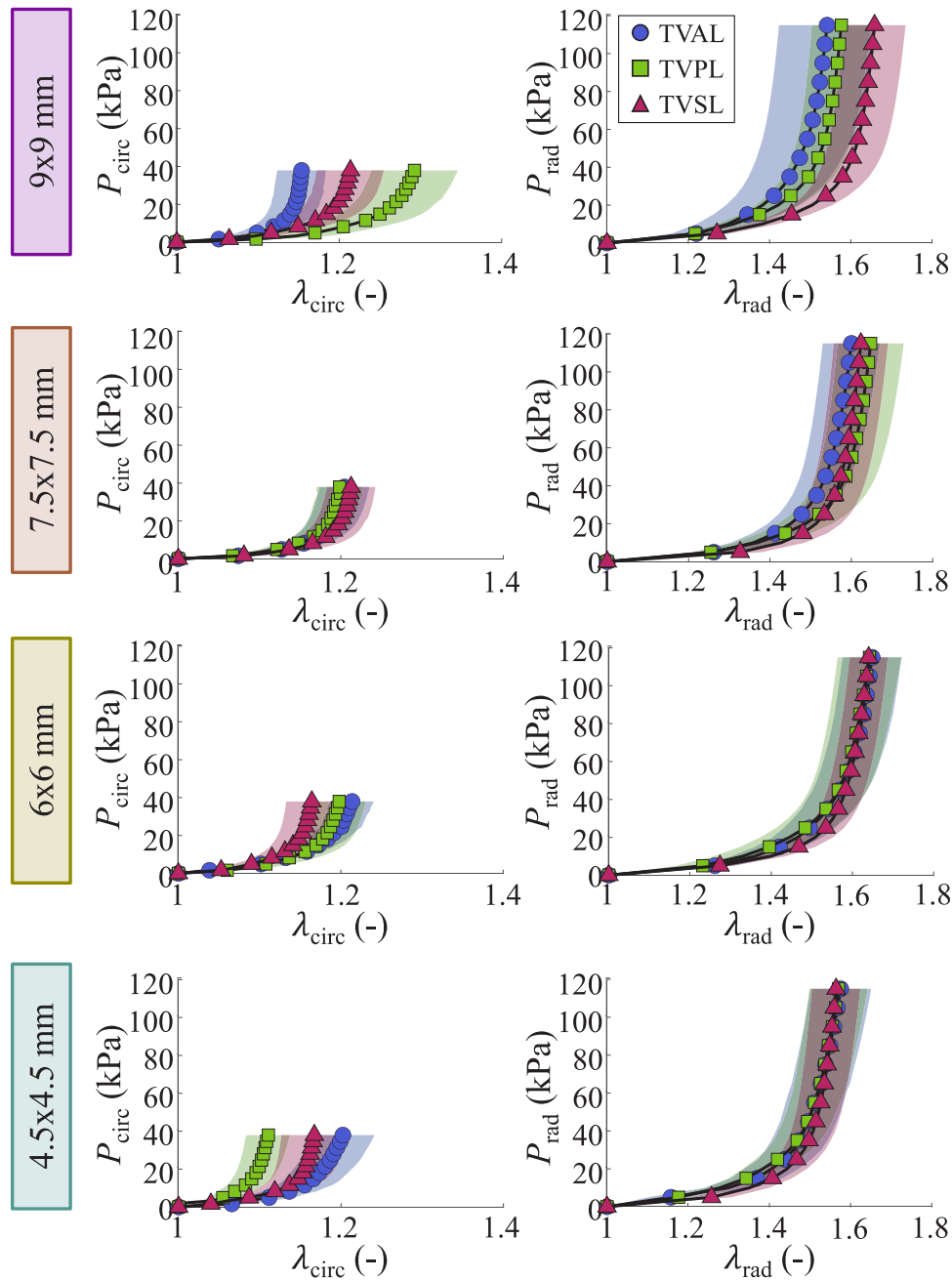
Beyond the scope of individual experimental investigations, our results also suggest that specimen size must be considered when comparing the mechanical behavior within the existing literature. Researchers typically do not control for specimen size in their studies (Table 4), which means direct comparisons can be problematic due to the specimen size effect presented herein. Additionally, studies can use different stress-free reference configurations such as the mounted specimen, the PPC specimen, or the floating specimen. Our finding that PC stretch depends on specimen dimensions will affect these comparisons and

further exacerbate known differences in mechanics between reference configurations (Laurence et al., 2022).

Finally, our results demonstrate that the use of different specimen dimensions in experimental characterizations does not have a significant effect on the estimated constitutive model parameters. The only significant difference was the Tong–Fung constitutive model parameter ( $c$ ) between different sizes of TVPL and TVSL. Therefore, we have shown that the considered experimental parameters of the commercial BioTester device do not significantly affect the estimated parameters of the constitutive model for use in *in silico* simulations.

#### 4.4. Study limitations and future extensions

There are several limitations in this study. First, the specimens were taken from the biaxial tester between tests on specimens of



**Fig. A4.** Mechanical behavior for biaxial tensions ( $P_{\text{circ}} : P_{\text{rad}} = 0.333 : 1$ ) of the three TV leaflets represented as mean (solid curves)  $\pm$  standard error of the mean.  $P_{(\cdot)}$  and  $\lambda_{(\cdot)}$  denote the first Piola–Kirchhoff stress and the stretch, respectively. TVAL: TV anterior leaflet, TVPL: TV posterior leaflet, and TVSL: TV septal leaflet.

different sizes. This likely resulted in inconsistent, stress-free mounted configurations that could affect our comparisons. An additional control group may have helped to understand these effects, but we expect only a minimal influence on our results since the tests were performed after preconditioning, at approximate *in vivo* forces and considering fiducial markers within the central 70% of the effective test region (see also the **Supplementary Material**). Interestingly, our unpublished preliminary data suggest that tissue swelling may play a more important role in the long-term mechanical characterization of the TV leaflets and will be explored in future investigations. Second, we used *rigid* BioRake tines to mount the tissues to the biaxial testing device that limits possible shear deformation of the tissue. A previous study by [Fehervary et al. \(2016\)](#) demonstrated that this mounting scheme is suitable for characterizing the *biaxial tensile* properties of soft tissues. Third, the number/distribution of BioRake tines and apron dimensions

were different for each specimen size in the present investigation. The computational study of [Fehervary et al. \(2016\)](#) showed that the apron dimensions do not have a significant influence on the experimental characterizations, whereas a uniform distribution of tines was critical to ensure strain homogeneity. On the other hand, [Eilaghi et al. \(2009\)](#) came up with conflicting findings, stating that the number of tines is critical to ensure homogeneous strains. The *in silico* investigations contained in the **Supplementary Material** to this article showed that the mounting configurations used in the associated experiments should not have a significant affect on the interpretation of our results. Finally, the constitutive modeling did not consider the average mechanical response for each specimen size. This was intentional to test whether the specimen dimensions affect specimen-specific model parameters. Due to the highly nonlinear nature of the constitutive models, the

median model parameters should not be used to represent the average size-specific behavior (Khoiy et al., 2018).

In addition, there are several potential extensions of this investigation. For example, the experimental protocol could be modified to account for a dense speckle pattern. This would allow the determination of a refined strain field that could be analyzed to understand how specimen dimensions alter strain heterogeneity within the test region. This was partially done in a recent study by Dover et al. (2022) in which the authors used polarized spatial frequency domain imaging with  $10 \times 10$  mm bovine pericardial specimens and determined that edge effects were minimal within the central  $8 \times 8$  mm, which was similar to the central 70% region found in our *in silico* studies shown in the **Supplementary Material**. A second extension of this work could focus on the large number of data from existing TV biomechanical studies (Table 4) to see if the observed (or missing) trends are confirmed for a larger number of samples and other specimen dimensions. We have attempted to capture the range of specimen dimensions in the literature, but the existing studies often use specimen dimensions within that range appropriate to their investigation and purpose.

#### 4.5. Conclusion

This study provided the first experimental investigation of how specimen dimensions affect the mechanical behavior, mechanical properties, and constitutive model parameters obtained from biaxial tensile characterizations. It was found that smaller specimen dimensions generally exhibited a stiffer circumferential behavior and a more compliant radial behavior, although the differences were not statistically significant. Furthermore, the derived mechanical properties were also affected by specimen dimensions, with the low-tensile modulus increasing, the high-tensile modulus decreasing, and the anisotropy index increasing as specimen dimensions became smaller. Finally, the equibiaxial tension data were fitted with three constitutive models used for soft biological tissues. Interestingly, for the Tong–Fung constitutive model, we found only one significant difference between two specimen sizes of TVPL and TVSL. This underscores that the choice of specimen dimensions in experimental characterizations does not necessarily play an important role in the development of computational models of heart valves that utilize the experimental mechanical data.

#### CRedit authorship contribution statement

**Devin W. Laurence:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Shuodao Wang:** Writing – review & editing, Conceptualization. **Rui Xiao:** Writing – review & editing. **Jin Qian:** Writing – review & editing. **Arshid Mir:** Writing – review & editing. **Harold M. Burkhart:** Writing – review & editing, Funding acquisition. **Gerhard A. Holzapfel:** Writing – review & editing. **Chung-Hao Lee:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

#### Declaration of competing interest

We hold no financial or personal relationships with other people or organizations that could inappropriately influence or bias our work. We hereby also declare that all authors were fully involved in the study and preparation of the manuscript and the material within has not been and will not be submitted for publication elsewhere.

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#### Appendix A. Results for the non-equibiaxial protocols

The mechanical behavior of all three TV leaflets under non-equibiaxial protocols (i.e.,  $f_{\text{circ}}^{\text{max}}:f_{\text{rad}}^{\text{max}} = 1:0.667, 1:0.333, 0.667:1, 0.333:1$ ) are presented in this Appendix, i.e., Figs. A1–A4.

#### Appendix B. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.jbiomech.2023.111829>.

#### References

- Avanzini, A., Battini, D., 2016. Integrated experimental and numerical comparison of different approaches for planar biaxial testing of a hyperelastic material. *Adv. Mater. Sci. Eng.* 2016, <http://dx.doi.org/10.1155/2016/6014129>.
- Clarín, J., Dang, D., Santos, L., Amini, R., 2023. Mechanical characterization of porcine tricuspid valve anterior leaflets over time: Applications to ex vivo studies. *ASME Open J. Eng.* 2, 021032. <http://dx.doi.org/10.1115/1.4062477>.
- Dover, C.M., Goth, W., Goodbrake, C., Tunnell, J.W., Sacks, M.S., 2022. Simultaneous wide-field planar strain–fiber orientation distribution measurement using polarized spatial domain imaging. *Ann. Biomed. Eng.* 50 (3), 253–277. <http://dx.doi.org/10.1007/s10439-021-02889-7>.
- Duginski, G.A., Ross, C.J., Laurence, D.W., Johns, C.H., Lee, C.-H., 2020. An investigation of the effect of freezing storage on the biaxial mechanical properties of excised porcine tricuspid valve anterior leaflets. *J. Mech. Behav. Biomed. Mater.* 101, 103438. <http://dx.doi.org/10.1016/j.jmbbm.2019.103438>.
- Eilaghi, A., Flanagan, J.G., Brodland, G.W., Ethier, C.R., 2009. Strain uniformity in biaxial specimens is highly sensitive to attachment details. *J. Biomech. Eng.* 131 (9), <http://dx.doi.org/10.1115/1.3148467>.
- Fehervary, H., Smoljić, M., Vander Sloten, J., Famaey, N., 2016. Planar biaxial testing of soft biological tissue using rakes: A critical analysis of protocol and fitting process. *J. Mech. Behav. Biomed. Mater.* 61, 135–151. <http://dx.doi.org/10.1016/j.jmbbm.2016.01.011>.
- Gasser, T.C., Ogden, R.W., Holzapfel, G.A., 2006. Hyperelastic modelling of arterial layers with distributed collagen fibre orientations. *J. R. Soc. Interface* 3 (6), 15–35. <http://dx.doi.org/10.1098/rsif.2005.0073>.
- Holzapfel, G.A., 2000. *Nonlinear Solid Mechanics. A Continuum Approach for Engineering*. John Wiley & Sons, Chichester.
- Hudson, L.T., Jett, S.V., Kramer, K.E., Laurence, D.W., Ross, C.J., Towner, R.A., Baumwart, R., Lim, K.M., Mir, A., Burkhart, H.M., et al., 2020. A pilot study on linking tissue mechanics with load-dependent collagen microstructures in porcine tricuspid valve leaflets. *Bioengineering* 7 (2), 60. <http://dx.doi.org/10.3390/bioengineering7020060>.
- Jacobs, N.T., Cortes, D.H., Vresilovic, E.J., Elliott, D.M., 2013. Biaxial tension of fibrous tissue: using finite element methods to address experimental challenges arising from boundary conditions and anisotropy. *J. Biomech. Eng.* 135 (2), <http://dx.doi.org/10.1115/1.4023503>.
- Jett, S.V., Hudson, L.T., Baumwart, R., Bohnstedt, B.N., Mir, A., et al., 2020. Integration of polarized spatial frequency domain imaging (pSFDI) with a biaxial mechanical testing system for quantification of load-dependent collagen architecture in soft collagenous tissues. *Acta Biomater.* 102, 149–168. <http://dx.doi.org/10.1016/j.actbio.2019.11.028>.
- Jett, S.V., Laurence, D.W., Kunkel, R.P., Babu, A.R., Kramer, K.E., et al., 2018. An investigation of the anisotropic mechanical properties and anatomical structure of porcine atrioventricular heart valves. *J. Mech. Behav. Biomed. Mater.* 87, 155–171. <http://dx.doi.org/10.1016/j.jmbbm.2018.07.024>.
- Khoiy, K.A., Amini, R., 2016. On the biaxial mechanical response of porcine tricuspid valve leaflets. *J. Biomech. Eng.* 138 (10), 104504. <http://dx.doi.org/10.1115/1.4034426>.

- Khoiy, K.A., Pant, A.D., Amini, R., 2018. Quantification of material constants for a phenomenological constitutive model of porcine tricuspid valve leaflets for simulation applications. *J. Biomech. Eng.* 140 (9), 094503. <http://dx.doi.org/10.1115/1.4040126>.
- Kramer, K.E., Ross, C.J., Laurence, D.W., Babu, A.R., Wu, Y., Towner, R.A., Mir, A., Burkhart, H.M., Holzapfel, G.A., Lee, C.-H., 2019. An investigation of layer-specific tissue biomechanics of porcine atrioventricular valve anterior leaflets. *Acta Biomater.* 96, 368–384. <http://dx.doi.org/10.1016/j.actbio.2019.06.049>.
- Laurence, D.W., Ross, C.J., Hsu, M.-C., Mir, A., Burkhart, H.M., Holzapfel, G.A., Lee, C.-H., 2022. Benchtop characterization of the tricuspid valve leaflet pre-strains. *Acta Biomater.* 152, 321–334. <http://dx.doi.org/10.1016/j.actbio.2022.08.046>.
- Laurence, D.W., Ross, C.J., Jett, S.V., Johns, C.H., Echols, A., Baumwart, R., Towner, R., Liao, J., Bajona, P., Wu, Y., Lee, C.-H., 2019. An investigation of regional variations in the biaxial mechanical properties and stress relaxation behaviors of porcine atrioventricular heart valve leaflets. *J. Biomech.* 83, 16–27. <http://dx.doi.org/10.1016/j.jbiomech.2018.11.015>.
- Lee, C.-H., Amini, R., Gorman, R.C., Gorman III, J.H., Sacks, M.S., 2014. An inverse modeling approach for stress estimation in mitral valve anterior leaflet valvuloplasty for in-vivo valvular biomaterial assessment. *J. Biomech.* 47 (9), 2055–2063. <http://dx.doi.org/10.1016/j.jbiomech.2013.10.058>.
- May-Newman, K., Yin, F.C., 1995. Biaxial mechanical behavior of excised porcine mitral valve leaflets. *Am. J. Physiol. Heart Circ. Physiol.* 269 (4), H1319–27. <http://dx.doi.org/10.1152/ajpheart.1995.269.4.H1319>.
- Meador, W.D., Mathur, M., Sugerman, G.P., Jazwiec, T., Malinowski, M., Bersi, M.R., Timek, T.A., Rausch, M.K., 2020a. A detailed mechanical and microstructural analysis of ovine tricuspid valve leaflets. *Acta Biomater.* 102, 100–113. <http://dx.doi.org/10.1016/j.actbio.2019.11.039>.
- Meador, W.D., Mathur, M., Sugerman, G.P., Malinowski, M., Jazwiec, T., Wang, X., Lacerda, C.M., Timek, T.A., Rausch, M.K., 2020b. The tricuspid valve also mal-adapts as shown in sheep with biventricular heart failure. *Elife* 9, e63855. <http://dx.doi.org/10.7554/eLife.63855>.
- Narang, H., Rego, B.V., Khalighi, A.H., Aly, A., Pouch, A.M., Gorman, R.C., Gorman III, J.H., Sacks, M.S., 2021. Pre-surgical prediction of ischemic mitral regurgitation recurrence using in vivo mitral valve leaflet strains. *Ann. Biomed. Eng.* 49, 3711–3723. <http://dx.doi.org/10.1007/s10439-021-02772-5>.
- Nielsen, P., Hunter, P., Smaill, B., 1991. Biaxial testing of membrane biomaterials: Testing equipment and procedures. *J. Biomech. Eng.* 113 (3), 295–300. <http://dx.doi.org/10.1115/1.2894887>.
- Nolan, D., McGarry, J., 2016. On the correct interpretation of measured force and calculation of material stress in biaxial tests. *J. Mech. Behav. Biomed. Mater.* 53, 187–199. <http://dx.doi.org/10.1016/j.jmbbm.2015.08.019>.
- Pham, T., Sulejmani, F., Shin, E., Wang, D., Sun, W., 2017. Quantification and comparison of the mechanical properties of four human cardiac valves. *Acta Biomater.* 54, 345–355. <http://dx.doi.org/10.1016/j.actbio.2017.03.026>.
- Pokutta-Paskaleva, A., Sulejmani, F., DelRocini, M., Sun, W., 2019. Comparative mechanical, morphological, and microstructural characterization of porcine mitral and tricuspid leaflets and chordae tendineae. *Acta Biomater.* 85, 241–252. <http://dx.doi.org/10.1016/j.actbio.2018.12.029>.
- Ross, C.J., Laurence, D.W., Echols, A.L., Babu, A.R., Gu, T., Duginski, G.A., Johns, C.H., Mullins, B.T., Casey, K.M., Laurence, K.A., et al., 2021. Effects of enzyme-based removal of collagen and elastin constituents on the biaxial mechanical responses of porcine atrioventricular heart valve anterior leaflets. *Acta Biomater.* 135, 425–440. <http://dx.doi.org/10.1016/j.actbio.2021.08.043>.
- Ross, C.J., Laurence, D.W., Richardson, J., Babu, A.R., Evans, L.E., Beyer, E.G., Childers, R.C., Wu, Y., Towner, R.A., Fung, K.-M., et al., 2019a. An investigation of the glycosaminoglycan contribution to biaxial mechanical behaviours of porcine atrioventricular heart valve leaflets. *J. R. Soc. Interface* 16 (156), 20190069. <http://dx.doi.org/10.1098/rsif.2019.0069>.
- Ross, C., Laurence, D., Wu, Y., Lee, C.-H., 2019b. Biaxial mechanical characterizations of atrioventricular heart valves. *J. Vis. Exp.* (146), e59170. <http://dx.doi.org/10.3791/59170>.
- Sacks, M.S., 2000. Biaxial mechanical evaluation of planar biological materials. *J. Elasticity Phys. Sci. Solids* 61, 199–246. <http://dx.doi.org/10.1023/A:1010917028671>.
- Sacks, M.S., Drach, A., Lee, C.-H., Khalighi, A.H., Rego, B.V., Zhang, W., Ayoub, S., Yoganathan, A.P., Gorman, R.C., Gorman III, J.H., 2019. On the simulation of mitral valve function in health, disease, and treatment. *J. Biomech. Eng.* 141 (7), 070804. <http://dx.doi.org/10.1115/1.4043552>.
- Salinas, S.D., Clark, M.M., Amini, R., 2019. Mechanical response changes in porcine tricuspid valve anterior leaflet under osmotic-induced swelling. *Bioengineering* 6 (3), 70. <http://dx.doi.org/10.3390/bioengineering6030070>.
- Salinas, S.D., Clark, M.M., Amini, R., 2020. The effects of –80 °C short-term storage on the mechanical response of tricuspid valve leaflets. *J. Biomech.* 98, 109462. <http://dx.doi.org/10.1016/j.jbiomech.2019.109462>.
- Salinas, S.D., Farra, Y.M., Amini Khoiy, K., Houston, J., Lee, C.-H., Bellini, C., Amini, R., 2022. The role of elastin on the mechanical properties of the anterior leaflet in porcine tricuspid valves. *PLOS One* 17 (5), e0267131. <http://dx.doi.org/10.1371/journal.pone.0267131>.
- Sugerman, G., Yang, J., Rausch, M., 2023. A speckling technique for DIC on ultra-soft, highly hydrated materials. *Exp. Mech.* 63, 585–590. <http://dx.doi.org/10.1007/s11340-023-00938-x>.
- Sun, W., Sacks, M.S., Scott, M.J., 2005. Effects of boundary conditions on the estimation of the planar biaxial mechanical properties of soft tissues. *J. Biomech. Eng.* 127 (4), 709–715. <http://dx.doi.org/10.1115/1.1933931>.
- Tong, P., Fung, Y.-C., 1976. The stress-strain relationship for the skin. *J. Biomech.* 9 (10), 649–657. [http://dx.doi.org/10.1016/0021-9290\(76\)90107-X](http://dx.doi.org/10.1016/0021-9290(76)90107-X).
- Wells, S.M., Pierlot, C.M., Moeller, A.D., 2012. Physiological remodeling of the mitral valve during pregnancy. *Am. J. Physiol.-Heart Circ. Physiol.* 303 (7), H878–H892. <http://dx.doi.org/10.1152/ajpheart.00845.2011>.
- Wobbrock, J.O., Findlater, L., Gergle, D., Higgins, J.J., 2011. The aligned rank transform for nonparametric factorial analyses using only anova procedures. In: *Proceedings of the SIGCHI Conference on Human Factors in Computing Systems*. pp. 143–146. <http://dx.doi.org/10.1145/1978942.1978963>.
- Yu, W.-J., Shen, M., Chen, W.-N., Zhan, Z.-H., Gong, Y.-J., Lin, Y., Liu, O., Zhang, J., 2014. Differential evolution with two-level parameter adaptation. *IEEE Trans. Cybern.* 44 (7), 1080–1099. <http://dx.doi.org/10.1109/TCYB.2013.2279211>.