Planar biaxial testing of heart valve cusp replacement biomaterials: Experiments, theory and material constants

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ABSTRACT

Objectives: Aortic valve (AV) repair has become an attractive option to correct aortic insufficiency. Yet, cusp reconstruction with various cusp replacement materials has been associated with greater long-term repair failures, and it is still unknown how such materials mechanically compare with native leaflets. We used planar biaxial testing to characterize six clinically relevant cusp replacement materials, along with native porcine AV leaflets, to ascertain which materials would be best suited for valve repair.

Methods: We tested at least six samples of: 1) fresh autologous porcine pericardium (APP), 2) glutaraldehyde fixed porcine pericardium (GPP), 3) St Jude Medical pericardial patch (SJM), 4) CardioCel patch (CC), 5) PeriGuard (PG), 6) Supple PeriGuard (SPG) and 7) fresh porcine AV leaflets (PC). We introduced efficient displacement-controlled testing protocols and processing, as well as advanced convexity requirements on the strain energy functions used to describe the mechanical response of the materials under loading.

Results: The proposed experimental and data processing pipeline allowed for a robust in-plane characterization of all the materials tested, with constants determined for two Fung-like hyperelastic, anisotropic strain energy models.

Conclusions: Overall, CC and SPG (respectively PG) patches ranked as the closest mechanical equivalents to young (respectively aged) AV leaflets. Because the native leaflets as well as CC, PG and SPG patches exhibit significant anisotropic behaviors, it is suggested that the fiber and cross-fiber directions of these replacement biomaterials be matched with those of the host AV leaflets.

Statement of Significance

The long-term performance of cusp replacement materials would ideally be evaluated in large animal models for AV disease and cusp repair, and over several months or more. Given the unavailability and impracticality of such models, detailed information on stress-strain behavior, as studied herein, and investigations of durability and valve dynamics will be the best surrogates, as they have been for prosthetic valves. Overall, comparison with Fig. 3 suggests that CC and SPG (respectively PG) patches may be the closest mechanical equivalents to young (respectively aged) AV leaflets. Interestingly, the thicknesses of these materials are close to those reported for porcine and younger human AV leaflets, which may facilitate surgical implantation, by contrast to the thinner APP which has poor handling qualities. Because the native leaflets as well as CC, PG and SPG patches exhibit anisotropic behaviors, from a mechanistic perspective alone, it stands to reason that cardiac surgeons should seek to intraoperatively match the fiber and cross-fiber directions of these replacement biomaterials with those of the repaired AV leaflets.

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1. Introduction

Native human aortic valve (AV) leaflets (or cusps) exhibit large deformations under comparatively small forces, and then undergo proportionally smaller deformations under larger forces (Fig. 1a) [1–3]. This mechanical response to loading, typical of soft biological tissues in general, is dependent on the direction of the load,
Therefore, as new testing equipment made it possible to report, it has been shown that tears can occur as well as to promote leaflet calcification and durability, as elevated mechanical stresses have been reported. Recurrent aortic insufficiency [6,8], a finding which may result from the limitations of the existing biomaterials available for cusp repair. A material patch (patch repair) can also be used to improve restrictive raphe of a bicuspid AV, requires the placement of a biological patch. A patch can also be used to improve cusp coaptation in pediatric AV repair surgery, or to reconstruct a commissure when creating a bicuspid valve from a unicuspid AV, or a tricuspid valve from a bicuspid AV [6]. However, the use of a patch has been associated with an increased risk of long-term recurrent aortic insufficiency [6,8], a finding which may result from the limitations of the existing biomaterials available for cusp reconstruction. It is assumed here that the mechanical behavior of such materials may have a significant bearing on ease of implantation and durability, as elevated mechanical stresses have been reported to cause tears as well as to promote leaflet calcification [9,10]. Therefore, as new testing equipment made it possible to refine previous work by our group [11], the first objective of the present study was to identify the material properties of various commercially available cusp replacement biomaterials, and evaluate which one(s) may be the closest mechanical equivalent(s) to native AV leaflets.

As a tool for material characterization of rubber-like and biological membranes, planar biaxial testing has been used for decades, resulting from the understanding, grounded in continuum mechanics, that uniaxial testing is insufficient when one's goal is to characterize anisotropic materials [12,13]. Planar biaxial testing makes it possible to explore a wide range of loads, understood here as forces or displacements, applied at once in two orthogonal directions. The loads applied in both directions may the same (equibiaxial loading), may be kept in constant proportion between both directions (proportional loading), or independent from each other (general biaxial loading). The work from the pioneers of planar biaxial testing strongly supports that series of protocols including equibiaxial and proportional loadings should be used for best material characterization [12–15]. Unfortunately, most experimental set-ups used to date have been custom-designed, to the effect that a wide disparity in experimental conditions has been the norm (e.g. displacement vs. force controlled protocols; grip displacements vs. optical tracking for determination of strains; rigid grips vs. suture lines). With the advent of commercially available, integrated biaxial testing equipment specially designed for biological soft tissues, such as the Biotester (CellScale, Waterloo, Canada) used herein, it is timely to revisit the practice of planar biaxial testing [16]. This is the second objective of the present work.

First, we will go over the details of the materials and methods (both experimental and theoretical, with insights from continuum mechanics), and then will describe and discuss our findings in the contexts of aortic cusp repair in particular and biaxial testing in general. In an effort to share methods across laboratories, which is our third objective, the interested reader can send a request to M.R.L. for our specially created Matlab programs that can be used with the Biotester to set up the experimental protocols, expedite the sorting of relevant images before image processing for strain calculations and finally, determine material constants for two Fung-like material models.

2. Methods

2.1. Tissue preparation

Six potential cusp replacement biomaterials were included in the study: 1) fresh autologous porcine pericardium (APP), 2) glutaraldehyde fixed porcine pericardium (GPP), 3) St Jude Medical pericardial patch (SJM; St Jude Medical, St Paul, Minnesota, USA), 4) CardioCel patch (CC; Admedus, Minneapolis, Minnesota, USA), 5) PeriGuard (PC; Synovis, St Paul, Minnesota, USA) and 6) Supple PeriGuard (SPG; Synovis, St Paul, Minnesota, USA). APP and GPP were used as surrogates for the fresh or glutaraldehyde fixed autologous human pericardium samples that have been used in the operating room as cusp replacements [5,7,8]. The porcine tissues were harvested on hearts from adult pigs weighing approximately 105 kg obtained from a local abattoir within hours of slaughter, and stored in saline solution at 4 °C. The GPP samples were dipped in 0.6% glutaraldehyde for 20 min before testing [17].

We initially tested nine samples of each material. The samples were cut using parallel steel razor blades into 6.5 × 6.5 mm² squares whose sides were aligned with, or orthogonal to, the direction of fiber reinforcement, as determined by back-lighting and careful visual inspection. In addition, we tested the three AV leaflets in each of two porcine hearts. All the AV cusp samples (PC) were taken out of the belly region (Fig. 1b).
2.2. Equipment and experimental protocol

Each sample had its thickness evaluated from the average of three measurements in three different locations using an electronic thickness gauge (Model 700-118-20, Mitutoyo, Japan) [18], before it was mounted on the 5-N capacity biaxial testing equipment (Biotester, CellScale, Waterloo, Canada) using four tungsten rakes, each fitted with five 0.7 mm spaced tines. The samples were immersed in a temperature-controlled saline bath at 37°C for 10 min before testing, but were tested out of the bath to mitigate reflections impairs image tracking.

The Biotester comes with an integrated software interface, LabJoy, whose data collection module allows the user to set the parameters for the test phases (preloading, stretching, holding, recovering and resting) of load cycles gathered into a test sequence, as well as saline bath temperature and image acquisition frequency (here, 5 Hz). Given the repetitive nature of the machine set up work and to minimize human error, we wrote a special Matlab program (Matlab R2013a, The MathWorks, Natick, MA, USA) to interface with LabJoy and automatically set the machine according to the user input. Because the equipment worked best in displacement control, all the tests were implemented in this mode using ramp functions and a target strain rate of approximately 0.04 s⁻¹ (no significant effect is expected by changing this value by at least one order of magnitude [19,20]). However, as can be seen from Fig. 1a, a test run under equibiaxial force control has the potential to give a more representative description of the upward turns expected from the force-displacement curves. This observation led us to determine the maximum displacements to be used during equibiaxial preconditioning for every biomaterial such that the upward turns expected from the load-displacement curves could be seen in both axes. In so doing, we adjusted the equibiaxial displacement-controlled protocol to approximate a force-controlled one. After 10 preconditioning cycles, each sample was submitted to the same succession of nine experimental protocols as detailed in (Fig. 2, Table 1). The non-equibiaxial protocols were designed to maintain the maximum forces acting in both directions at a similar level.

2.3. Image and data processing for stress-strain plots

It is well known that soft tissue or biomaterial samples may be subjected to less strain than calculated from the grip displacements due to attachment site effects and potential tissue tearing [21]. This motivated the use of the image tracking module in LabJoy to determine the actual strain distribution within the sample. Given the large number of protocols (hence, images) to be processed, and that only the loading portion of the cycles was of interest (because the loading and unloading paths were relatively close to each other, as can be appreciated in Fig. 2), another Matlab program was written to expedite the sorting of only those images that needed processing. Then, we used LabJoy to manually create a 9 by 9 node grid in the central region of each sample and launch the image tracking algorithm. After visual inspection of the strain
results in this region for each protocol, we manually selected 4 nodes in a sub-region where the strains were most homogeneous [22], and saved their information to one file per protocol for ultimate analysis. The mathematical formulation of such 2-D strain energy functions should ideally be based on a whole range of experimental tests and theoretical considerations [23]. However, as a shortcut, one often uses popular strain energy functions, as done here. For example, Sacks proposed a seven-parameter Fung model in terms of the Green strain tensor components such that $\mathbf{w} = \frac{1}{2} \mathbf{Q}(Q - 1)$, where $Q = c_1 E_{11} + c_2 E_{22} + 2c_4 E_{12} + c_6 E_{33} + 2c_8 E_{13} + 2c_9 E_{23} + 2c_{10} E_{12} + 2c_{11} E_{23} + 2c_{12} E_{32}$ [25]. Alternatively, we will also consider four-parameter Guccione et al’s material model [26], in the form: $\mathbf{w} = \frac{1}{2} \mathbf{Q}(Q - 1)$, where $Q = c_1 E_{11} + c_2 E_{22} + \frac{1}{2} ( \lambda - 1)^2$ + $2c_4 E_{12}^2$, with $\lambda = (2E_{11} + 1)/(2E_{22} + 1)$. Details of the implementation are given for both Sacks’ and Guccione et al’s material models in Appendix 3. With Sacks’ material model, we want to match the following experimental and theoretical membrane tensions (noted $\mathbf{T}_{11,exp}$ and $\mathbf{T}_{11,thre}$ respectively):

$\mathbf{T}_{11,exp} = \lambda_1 f_1/(J_2D_2)$ and $\mathbf{T}_{11,thre} = c_1 \exp(Q) [c_2 E_{11} + c_4 E_{22} + c_6 E_{33}]$,

$\mathbf{T}_{22,exp} = \lambda_2 f_2/(J_2D_1)$ and $\mathbf{T}_{22,thre} = c_1 \exp(Q) [c_3 E_{22} + c_4 E_{11} + c_6 E_{33}]$, as well as

$\mathbf{T}_{12,exp} = -f_1 f_2/(J_2D_1)$ and $\mathbf{T}_{12,thre} = c_1 \exp(Q) [c_3 E_{12} + c_6 E_{11} + c_6 E_{22}]$.

One can ensure that the experimental curves for second P-K membrane tensions vs. Green strains are recovered by the model using nonlinear optimization of the material constants $c_i$, e.g. by minimizing the objective function $\| \mathbf{T}_{11,exp} - \mathbf{T}_{11,thre} \| + \| \mathbf{T}_{22,exp} - \mathbf{T}_{22,thre} \| + \| \mathbf{T}_{12,exp} - \mathbf{T}_{12,thre} \|$. Where each entry is a vector array of all the relevant data points, and $\| . \|$ represents the Euclidian norm. The data points may be collected from one or several protocols; herein, we included all protocols simultaneously, after averaging the results from several samples for each protocol. The data were analyzed using the post-preconditioning state as the reference state.

### 2.4. Determination of material constants

Assuming that a repeatable mechanical response is achieved after preconditioning (according to the pseudo-hyperelasticity concept introduced by Fung [23]), and focusing here on the loading part of it, a 3-D strain energy functions $W$ may be postulated, relating the second PK stress tensor to the Green strain tensor by $\mathbf{S} = \partial W/\partial \mathbf{E}$. Consistent with in-plane stress conditions, $W$ only depends on $E_{11}, E_{22}$, and possibly $E_{33}$, such that $W = W(E_{11}, E_{22}, E_{33})$ [24]. Because of the assumed material incompressibility, $E_{33}$ may be directly expressed in terms of $E_{11}, E_{22}$ and $E_{12}$. Therefore, a reduced strain energy function $W$ may be introduced, such that $W = W(E_{11}, E_{22}, E_{12}) = W(E_{11}, E_{22}, E_{12}, E_{33})$. However, it is not possible to identify a suitable form for $W$ just from planar biaxial testing [24]. Instead, a practical form for $W$ is postulated to depend only on $E_{11}, E_{22}, E_{12}$ and not on $E_{33}$, such that $\mathbf{S} = \begin{bmatrix} \partial W/\partial E_{11} & \partial W/\partial E_{12} & 0 \\ \partial W/\partial E_{21} & \partial W/\partial E_{22} & 0 \\ 0 & 0 & 0 \end{bmatrix}$.

Introducing a 2-D strain energy function $w = H \mathbf{W}$, the second PK membrane tensions can also be written as $\mathbf{T}_{11} = \partial w/\partial E_{11}$, $\mathbf{T}_{22} = \partial w/\partial E_{22}$, and, owing to the symmetry of $\mathbf{S}$, $\mathbf{T}_{12} = \partial w/\partial E_{12}$, as well as components $\mathbf{T}_{11}$, $\mathbf{T}_{22}$, and $\mathbf{T}_{12}$ that are independent of the thickness measurement.

### Table 1

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Maximum displacement</th>
<th>Cycles #</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-direction</td>
<td>Y-direction</td>
<td></td>
</tr>
<tr>
<td>Preconditioning</td>
<td>$U_{max,X}$</td>
<td>$U_{max,Y}$</td>
</tr>
<tr>
<td>1</td>
<td>$U_{max,X} + U_{adj,X}$</td>
<td>$U_{max,Y} + U_{adj,Y}$</td>
</tr>
<tr>
<td>2</td>
<td>$U_{max,X} + 3/4U_{adj,X}$</td>
<td>$U_{max,Y} + 3/4U_{adj,Y}$</td>
</tr>
<tr>
<td>3</td>
<td>$U_{max,X} + 1/2U_{adj,X}$</td>
<td>$U_{max,Y} + 1/2U_{adj,Y}$</td>
</tr>
<tr>
<td>4</td>
<td>$U_{max,X} + 1/4U_{adj,X}$</td>
<td>$U_{max,Y} + 1/4U_{adj,Y}$</td>
</tr>
</tbody>
</table>

$U_{max,X}, U_{max,Y}$: maximum displacements in the X- and Y-directions, respectively, determined by trial and error such that they result in the same force in both axes.

$U_{adj,X}$: adjustment in X-displacement so that the maximum force in the X-direction in Protocols 1–4 is close to the maximum force reached in Protocol 5.

$U_{adj,Y}$: adjustment in Y-displacement so that the maximum force in the Y-direction in Protocols 6–9 is close to the maximum force reached in Protocol 5.

### Appendix 1

The orthonormal transformation gradient tensor $\mathbf{F}$ is symmetric. For consistency with other groups, e.g. [3], we reported herein second P-K membrane tensions vs. Green strains, as such curves are unaffected by thickness measurements. As detailed in Appendix 2, the components of second P-K stress resultants (or membrane tension, in N/m²) tensor are $T_{11} = \lambda_1 f_1/(J_2L_2)$, $T_{22} = \lambda_2 f_2/(J_2L_1)$ and, owing to the symmetry of $\mathbf{S}$, $T_{12} = -f_2 f_1/(J_2L_2)$, $T_{21} = -f_1 f_2/(J_2L_1)$, where external forces $f_1$ and $f_2$ are applied in directions 1 and 2.
In identifying the material constants, attention should be paid to their producing a physically meaningful response [23]. Herein, this will be accomplished by tracing the predicted second P-K membrane tensions vs. Green strains for all experimental protocols, to visually verify whether or not they match the (physical) experimental data. Another concern related to the material constants pertains to the convexity of the strain energy function as it required for the stability and accuracy of the solution using computational methods such as finite element analysis in which Newton-Raphson’s method of solution is often used [27].

\[
\begin{bmatrix}
C_{11} & C_{12} & C_{14} \\
0 & -(C_{12} - C_{11}C_{22})/C_{11} & (C_{11}C_{24} - C_{12}C_{44})/C_{11} \\
0 & 0 & (C_{11}C_{24} + C_{22}C_{14} + C_{44}C_{12} - C_{11}C_{22}C_{44} - 2C_{12}C_{14}C_{24})/(C_{12} - C_{11}C_{22})
\end{bmatrix}
\]

Convexity of the strain energy function necessitates its Hessian to be positive definite [28]. The Hessian of the strain energy function is the fourth-order elasticity tensor (or tangent modulus) \( \mathbf{C} \) defined by \( \mathbf{C} = \nabla^2 \phi = \nabla^2 W/\nabla^2 \mathbf{E} \). For in-plane stress conditions, the components of \( \mathbf{C} \) may be written as

\[
\mathbf{C}_{20} = \begin{bmatrix}
C_{11} & C_{12} & C_{14} \\
C_{12} & C_{22} & C_{24} \\
C_{14} & C_{24} & C_{44}
\end{bmatrix},
\]

where \( C_{ij} = \partial^2 W/\partial \epsilon_{ij}, \) \( C_{12} = \partial^2 W/\partial \epsilon_{11} \partial \epsilon_{22}, \) \( C_{14} = \partial^2 W/\partial \epsilon_{11} \partial \epsilon_{12}, \) \( C_{22} = \partial^2 W/\partial \epsilon_{22} \partial \epsilon_{22}, \) \( C_{24} = \partial^2 W/\partial \epsilon_{22} \partial \epsilon_{12} \) and \( C_{44} = \partial^2 W/\partial \epsilon_{12} \partial \epsilon_{12} \). A symmetric and real (i.e. Hermitian) matrix such as \( \mathbf{C}_{20} \) will be positive definite if and only if all its eigenvalues are positive [29]. Since the spectral theorem guarantees all eigenvalues of a Hermitian matrix to be real, the positivity of eigenvalues can be checked using Descartes’ rule of alternating signs for the characteristic polynomial \( X^n - \sum \mathbf{C}_{ij} X^{n-j} \), which is

\[
X^3 - [C_{11} + C_{22} + C_{44}]X^2 + [C_{11}C_{22} + C_{11}C_{44} + C_{22}C_{44} - C_{12}^2 - C_{14}^2 \\
- C_{24}^2]X + C_{11}C_{24} + C_{22}C_{14} + C_{44}C_{12} - C_{11}C_{22}C_{44} - 2C_{12}C_{14}C_{24} = 0.
\]

Descartes’ rule of alternating signs states that if the terms of a single-variable polynomial with real coefficients are ordered by descending variable exponent (as above), then the number of positive roots of the polynomial is either equal to the number of sign differences between consecutive nonzero coefficients, or is less than it by an even number [29]. Here, for the characteristic polynomial to have three positive roots, there must be three sign differences between consecutive nonzero coefficients. Namely, one must have:

\[-C_{11} - C_{22} - C_{44} < 0,\]

\[-C_{12} + C_{14} + C_{24} - C_{11}C_{22} - C_{11}C_{44} - C_{22}C_{44} < 0,\]

\[-C_{11}C_{24} + C_{22}C_{14} + C_{44}C_{12} - C_{11}C_{22}C_{44} - 2C_{12}C_{14}C_{24} < 0.\]

Alternatively, a Hermitian matrix such as \( \mathbf{C}_{20} \) will be positive definite if and only if its leading principal minors are all positive [29]. The \( k \)-th leading principal minor of a matrix is the determinant of its upper-left \( k \) by \( k \) submatrix. According to Sylvester’s criterion, a matrix is positive definite if and only if all these determinants are positive [29]. Practically, the matrix is reduced to an upper triangular matrix by using elementary row operations, as in the first part of the Gaussian elimination method, taking care to preserve the sign of its determinant during pivoting process. Since the \( k \)-th leading principal minor of a triangular matrix is the product of its diagonal elements up to row \( k \), Sylvester’s criterion is equivalent to checking whether its diagonal elements are all positive [29]. An upper triangular matrix for \( \mathbf{C}_{20} \) is

\[
\begin{bmatrix}
C_{11} & C_{12} & C_{14} \\
0 & -(C_{12} - C_{11}C_{22})/C_{11} & (C_{11}C_{24} - C_{12}C_{44})/C_{11} \\
0 & 0 & (C_{11}C_{24} + C_{22}C_{14} + C_{44}C_{12} - C_{11}C_{22}C_{44} - 2C_{12}C_{14}C_{24})/(C_{12} - C_{11}C_{22})
\end{bmatrix}.
\]

For all diagonal components to be positive, one must have

\[-C_{11} < 0,\]

\[C_{12} - C_{11}C_{22} < 0,\]

\[C_{11}C_{24} + C_{22}C_{14} + C_{44}C_{12} - C_{11}C_{22}C_{44} - 2C_{12}C_{14}C_{24} < 0.\]

For robustness, we simultaneously implemented the two partially different sets of constraints obtained above.

In addition to using data from all averaged biaxial protocols simultaneously, we investigated the soundness of using only experimental data from the averaged equibiaxial protocol to characterize the different materials of interest.

2.5. Statistical analysis

Results are reported as mean with the standard deviation, unless reported otherwise, shown in parentheses. A dedicated Matlab program was written to implement the constrained nonlinear optimization (using the \textit{fmincon} function), determine the material constants with their 95% confidence intervals (CI) and Pearson correlation coefficients, allow the user to graphically verify the convexity of the strain energy function, and plot the predicted stress-strains curves on top of the experimental ones for comparison.

3. Results

3.1. Stress-strain plots

The average experimental data from all protocols for all materials are presented in Appendix 4. As a summary, Fig. 3 shows averaged equibiaxial protocols for all materials.

3.2. Thickness and material constants

Table 2 lists the thicknesses of all the materials tested and the number of samples retained for determination of the material constants. Indeed, due to some tearing near the tines, or due to image tracking issues, some samples had to be discarded. Table 2 also includes values of the Green strains in both the fiber and cross-fiber directions under a reference second P-K membrane tension of 60 N/m [30,3]. These values can be used to compare how stretchable the different materials are in either direction, and the ratio between both values, if different from unity, illustrates the anisotropic nature of the materials [30].
The material constants obtained from simultaneous use of all averaged experimental protocols for both Sacks’ and Guccione et al.’s model are listed with the half span of the 95% CI and Pearson correlation coefficients in Tables 3 and 4, respectively. All the constants listed satisfied the strain energy convexity constraints (see one example in Fig. 4). The Pearson correlation coefficients were on average 0.96 (0.05) for Sacks’ model, while they were 0.95 (0.04) for Guccione et al.’s model. They reflect the overall excellent

The values in parentheses report the standard deviation.

E_Fd@60 N/m: Green strain in the fiber direction, under 60 N/m 2nd P-K membrane tension.

E_Xd@60 N/m: Green strain in the cross-fiber direction, under 60 N/m 2nd P-K membrane tension.

The values in parentheses report half the span of the 95% confidence interval.

R2-FD: Pearson correlation coefficient between the experimental and predicted membrane tensions in the fiber direction.

R2-XD: Pearson correlation coefficient between the experimental and predicted membrane tensions in the cross-fiber direction.
match between experimental and predicted data that can be visually appreciated in Appendix 4. For APP and PC, the fit provided by Guccione et al.’s model was only fair, with Pearson correlation coefficients at 0.91 (0.04). For all materials except APP and PC, the membrane tension vs strain curves predicted by Sacks’ and Guccione et al.’s models were virtually indistinguishable, for any given protocol.

3.3. Using only the equibiaxial protocol to derive material constants

The material constants obtained only from the equibiaxial experimental protocols for both Sacks’ and Guccione et al.’s model are listed with the half span of the 95% CI in Tables 5 and 6. Again, all the constants listed satisfied the strain energy convexity constraints. The Pearson correlation coefficients were on average 0.91 (0.08) for Sacks’ model, while they were 0.94 (0.05) for Guccione et al.’s model. Compared to the case including all experimental

<table>
<thead>
<tr>
<th>Material</th>
<th>APP</th>
<th>GPP</th>
<th>SJM</th>
<th>CC</th>
<th>PG</th>
<th>SPG</th>
<th>PC</th>
</tr>
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<tbody>
<tr>
<td>$c_1$ [N/m]</td>
<td>0.90</td>
<td>2.06</td>
<td>4.81</td>
<td>3.04</td>
<td>6.97</td>
<td>8.69</td>
<td>0.90</td>
</tr>
<tr>
<td>(0.11)</td>
<td>(0.13)</td>
<td>(0.19)</td>
<td>(0.14)</td>
<td>(0.22)</td>
<td>(0.25)</td>
<td>(0.08)</td>
<td></td>
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<tr>
<td>$c_2$ [-]</td>
<td>43.77</td>
<td>14.73</td>
<td>348.57</td>
<td>54.03</td>
<td>432.30</td>
<td>86.02</td>
<td>74.38</td>
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<tr>
<td>(3.61)</td>
<td>(0.45)</td>
<td>(9.48)</td>
<td>(1.46)</td>
<td>(9.89)</td>
<td>(1.79)</td>
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<td>$c_3$ [-]</td>
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<td>10.99</td>
<td>160.83</td>
<td>27.24</td>
<td>78.90</td>
<td>14.80</td>
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<tr>
<td>(2.52)</td>
<td>(6.30)</td>
<td>(3.36)</td>
<td>(0.01)</td>
<td>(1.50)</td>
<td>(0.25)</td>
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<td>$c_4$ [-]</td>
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<td>(73.87)</td>
<td>(24.20)</td>
<td>(18.80)</td>
<td>(13.10)</td>
<td>(3.47)</td>
<td>(6.98)</td>
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<td>$R^2$-FD</td>
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</table>

The values in parentheses report half the span of the 95% confidence interval.

$R^2$-FD: Pearson correlation coefficient between the experimental and predicted membrane tensions in the fiber direction.

$R^2$- XD: Pearson correlation coefficient between the experimental and predicted membrane tensions in the cross-fiber direction.

The values in parentheses report half the span of the 95% confidence interval.

$R^2$-FD: Pearson correlation coefficient between the experimental and predicted membrane tensions in the fiber direction.

$R^2$- XD: Pearson correlation coefficient between the experimental and predicted membrane tensions in the cross-fiber direction.

---

**Table 4**

Material constants for Guccione et al.’s model, derived from all protocols.

<table>
<thead>
<tr>
<th>Material</th>
<th>APP</th>
<th>GPP</th>
<th>SJM</th>
<th>CC</th>
<th>PG</th>
<th>SPG</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_1$ [N/m]</td>
<td>0.90</td>
<td>2.06</td>
<td>4.81</td>
<td>3.04</td>
<td>6.97</td>
<td>8.69</td>
<td>0.90</td>
</tr>
<tr>
<td>(0.11)</td>
<td>(0.13)</td>
<td>(0.19)</td>
<td>(0.14)</td>
<td>(0.22)</td>
<td>(0.25)</td>
<td>(0.08)</td>
<td></td>
</tr>
<tr>
<td>$c_2$ [-]</td>
<td>43.77</td>
<td>14.73</td>
<td>348.57</td>
<td>54.03</td>
<td>432.30</td>
<td>86.02</td>
<td>74.38</td>
</tr>
<tr>
<td>(3.61)</td>
<td>(0.45)</td>
<td>(9.48)</td>
<td>(1.46)</td>
<td>(9.89)</td>
<td>(1.79)</td>
<td>(3.47)</td>
<td></td>
</tr>
<tr>
<td>$c_3$ [-]</td>
<td>53.34</td>
<td>10.99</td>
<td>160.83</td>
<td>27.24</td>
<td>78.90</td>
<td>14.80</td>
<td>24.64</td>
</tr>
<tr>
<td>(2.52)</td>
<td>(6.30)</td>
<td>(3.36)</td>
<td>(0.01)</td>
<td>(1.50)</td>
<td>(0.25)</td>
<td>(0.84)</td>
<td></td>
</tr>
<tr>
<td>$c_4$ [-]</td>
<td>0.00</td>
<td>0.13</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.72</td>
</tr>
<tr>
<td>(73.87)</td>
<td>(24.20)</td>
<td>(18.80)</td>
<td>(13.10)</td>
<td>(3.47)</td>
<td>(6.98)</td>
<td>(52.29)</td>
<td></td>
</tr>
<tr>
<td>$R^2$-FD</td>
<td>0.88</td>
<td>0.94</td>
<td>0.97</td>
<td>0.96</td>
<td>0.99</td>
<td>0.98</td>
<td>0.91</td>
</tr>
<tr>
<td>$R^2$- XD</td>
<td>0.87</td>
<td>0.92</td>
<td>0.99</td>
<td>0.99</td>
<td>0.98</td>
<td>0.96</td>
<td></td>
</tr>
</tbody>
</table>

The values in parentheses report half the span of the 95% confidence interval.

$R^2$-FD: Pearson correlation coefficient between the experimental and predicted membrane tensions in the fiber direction.

$R^2$- XD: Pearson correlation coefficient between the experimental and predicted membrane tensions in the cross-fiber direction.

Fig. 4. Plots of strain energy values vs. Green strains in the case of the CardioCel patch modeled with Sacks’ material constants listed in Table 3. These plots are designed to only verify the convexity of the strain energy function over a range of direct and shear strains around zero strain, therefore the actual values of the strain energy are not provided. All other materials and cases produced similar looking plots; therefore, only one was included.

**Table 5**

Material constants for Sack’s model, derived from the equibiaxial protocol.

<table>
<thead>
<tr>
<th>Material</th>
<th>APP</th>
<th>GPP</th>
<th>SJM</th>
<th>CC</th>
<th>PG</th>
<th>SPG</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_1$ [N/m]</td>
<td>0.06</td>
<td>1.14</td>
<td>5.97(0.26)</td>
<td>3.08</td>
<td>9.07</td>
<td>10.42</td>
<td>0.21</td>
</tr>
<tr>
<td>(0.00)</td>
<td>(0.07)</td>
<td>(0.07)</td>
<td>(0.07)</td>
<td>(0.28)</td>
<td>(0.21)</td>
<td>(0.01)</td>
<td></td>
</tr>
<tr>
<td>$c_2$ [-]</td>
<td>126.24</td>
<td>19.99</td>
<td>245.29</td>
<td>39.25</td>
<td>369.12</td>
<td>23.59</td>
<td>240.31</td>
</tr>
<tr>
<td>(438.63)</td>
<td>(84.53)</td>
<td>(149.31)</td>
<td>(5.67)</td>
<td>(21.67)</td>
<td>(9.13)</td>
<td>(4.46)</td>
<td></td>
</tr>
<tr>
<td>$c_3$ [-]</td>
<td>107.71</td>
<td>15.31</td>
<td>213.25</td>
<td>26.51</td>
<td>121.74</td>
<td>15.80</td>
<td>77.02</td>
</tr>
<tr>
<td>(248.62)</td>
<td>(56.36)</td>
<td>(57.76)</td>
<td>(4.88)</td>
<td>(2.65)</td>
<td>(0.54)</td>
<td>(1.51)</td>
<td></td>
</tr>
<tr>
<td>$c_4$ [-]</td>
<td>104.90</td>
<td>5.29</td>
<td>190.77</td>
<td>31.29</td>
<td>42.06</td>
<td>18.83</td>
<td>23.39</td>
</tr>
<tr>
<td>(330.20)</td>
<td>(68.95)</td>
<td>(90.93)</td>
<td>(5.35)</td>
<td>(5.46)</td>
<td>(1.90)</td>
<td>(1.09)</td>
<td></td>
</tr>
<tr>
<td>$c_5$ [-]</td>
<td>0.14</td>
<td>2.81</td>
<td>5.84</td>
<td>16.76</td>
<td>24.06</td>
<td>3.09</td>
<td>9.65</td>
</tr>
<tr>
<td>(160.16)</td>
<td>(438.01)</td>
<td>(129.23)</td>
<td>(86.73)</td>
<td>(18.01)</td>
<td>(33.50)</td>
<td>(297.87)</td>
<td></td>
</tr>
<tr>
<td>$R^2$-FD</td>
<td>0.82</td>
<td>0.94</td>
<td>0.97</td>
<td>0.96</td>
<td>0.99</td>
<td>0.98</td>
<td>0.94</td>
</tr>
<tr>
<td>$R^2$- XD</td>
<td>0.74</td>
<td>0.92</td>
<td>0.99</td>
<td>0.99</td>
<td>0.98</td>
<td>0.96</td>
<td></td>
</tr>
</tbody>
</table>

The values in parentheses report half the span of the 95% confidence interval.

$R^2$-FD: Pearson correlation coefficient between the experimental and predicted membrane tensions in the fiber direction.

$R^2$- XD: Pearson correlation coefficient between the experimental and predicted membrane tensions in the cross-fiber direction.
Table 6
Material constants for Guccione et al.'s model, derived from the equibiaxial protocol.

<table>
<thead>
<tr>
<th>Material</th>
<th>APP</th>
<th>GPP</th>
<th>SJM</th>
<th>CC</th>
<th>PG</th>
<th>SPG</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_1$ [N/m]</td>
<td>0.90 (0.28)</td>
<td>0.90 (0.07)</td>
<td>4.89 (0.21)</td>
<td>2.01 (0.05)</td>
<td>7.09 (0.05)</td>
<td>6.88 (0.22)</td>
<td>0.90 (0.18)</td>
</tr>
<tr>
<td>$c_2$ [-]</td>
<td>80.88 (10.98)</td>
<td>19.65 (0.56)</td>
<td>273.10 (8.56)</td>
<td>60.50 (7.68)</td>
<td>527.67 (8.78)</td>
<td>93.46 (1.23)</td>
<td>58.19 (6.05)</td>
</tr>
<tr>
<td>$c_3$ [-]</td>
<td>43.46 (5.05)</td>
<td>13.62 (0.35)</td>
<td>162.37 (3.67)</td>
<td>30.93 (1.44)</td>
<td>78.69 (0.18)</td>
<td>16.02 (2.07)</td>
<td>26.39 (2.07)</td>
</tr>
<tr>
<td>$c_4$ [-]</td>
<td>0.00 (208.91)</td>
<td>0.00 (93.89)</td>
<td>0.00 (19.37)</td>
<td>0.00 (6.00)</td>
<td>0.00 (3.06)</td>
<td>0.01 (4.84)</td>
<td>1.53 (58.37)</td>
</tr>
</tbody>
</table>

The values in parentheses represent the range of the 95% confidence interval.

R$^2$-FD: Pearson correlation coefficient between the experimental and predicted membrane tensions in the fiber direction.

R$^2$-XD: Pearson correlation coefficient between the experimental and predicted membrane tensions in the cross-fiber direction.

protocols, the correlation coefficients decreased on average by 0.05 and 0.01 for Sacks' and Guccione et al.'s models, respectively. In the worst fit cases, the correlation coefficients decreased by 0.22 and 0.24 with Sacks' model for APP and SJM, respectively, while they decreased by 0.04 and 0.03 with Guccione et al.'s model for APP and PC, respectively.

4. Discussion

4.1. General comments

The methods presented above made it possible to successfully and robustly characterize the planar mechanical behavior of all materials considered, from a minimum of six samples per material. To the best of the author's knowledge, this is the first time that complete biaxial experimental data are reported on cusp replacement materials. Our group's initial study [11] presented results from an ex vivo porcine model of AV repair with clinically relevant biomaterials, studied in a left-heart simulator. The experimental data were assembled with finite element simulations. These simulations relied on material constants that were determined, for lack of better equipment at the time, from rolling the biomaterials into cylinders, and pressurization tests. Planar biaxial testing is obviously better suited, and allows increased accuracy.

As expected from a planar biaxial testing setup without shear, shear deformations were less than 5% of the deformations in the fiber and cross-fiber directions in most cases, and the resulting stresses were negligible compared to direct components, as illustrated in the experimental curves reported in Appendix 4. Therefore, only the Pearson correlation coefficients relative to the fiber and cross-fiber membrane tensions were stated. In addition, a rationale for constraining the strain energy function to be convex near the origin has been introduced that complements and extends previous presentations [27,31,32]. Although only two phenomenological material models were used herein, the experimental results listed in Appendix 3 make it possible to identify the material constants of yet other material models, either phenomenological or structure based (e.g. [33,34]). Still, because all the materials were tested using the same equipment and strain rates, and because all the data were processed using the same software, the opportunity arises for meaningful comparisons between the materials.

4.2. Comparison of heart valve cusp replacement biomaterials with native AV leaflets

As can be seen from Fig. 3 and Table 2, the tested materials exhibited nonlinear tension vs. strain behaviors spanning different ranges of tensions and strains, and featuring various levels of anisotropy. That APP was observed to be more elastic than APP has already been reported by others [34], even though the findings from earlier studies have not always been consistent ([34] and references therein). Regardless, the information gathered from this study is aimed at determining the closest mechanical equivalent(s) to native AV leaflets among six other materials considered. Although other studies have reported on porcine and human AV leaflet properties [35–42], they used different experimental methods whose results cannot be quantitatively compared with ours. On the other hand, Martin and Sun [3] used multi-protocol, planar biaxial testing to measure the mechanical properties of fresh AV leaflets from porcine (used as representative of younger humans) as well as from older humans. Their findings for porcine leaflets are in good agreement with ours, with Green strains at a load of 60 N/m of about 0.033 and 0.148 in the fiber and cross-fiber directions, respectively. In older humans (mean age of 80.6), they measured Green strains at a load of 60 N/m of about 0.007 and 0.095 in the fiber and cross-fiber directions, respectively (in both instances, we obtained these numbers by digitizing and averaging their experimental curves for all three leaflets). Martin and Sun [3] presented compelling arguments to explain why they found smaller strains than those reported earlier from biaxial testing in [30,42], especially in the cross-fiber direction. Similarly to Martin and Sun's, our reference state was defined after preconditioning, as more physiologically relevant than the freely floating configuration (unavailable in our set up with rigid rakes as sample attachments) used in [30,42]. In addition, the porcine AV leaflets thickness measurements that we report in Table 2 (0.27 (0.09) mm) are a good match to those reported in [42] – 0.39 (0.01) mm – when measured from histology. The large value – 0.43 (0.02) mm – also reported in [42], from caliper measurements was likely an overestimate, as demonstrated in [18].

4.3. Using only the equibiaxial protocol to derive material constants

In spite of recommendations to use biaxial data from multiple protocols, many studies have only used equibiaxial experimental data to identify material constants, often for lack of general biaxial data. The Pearson correlation coefficients listed in Tables 3–6 provide detailed information on the quality of the fits obtained from both approaches. Guccione et al.'s model performed slightly less well when using only the equibiaxial protocol, but still, maintained good overall accuracy, and showed good consistency between both approaches, as illustrated with similar material constants and relatively narrow 95% CI. On the other hand, Sacks' model performed best only when all protocols were included, and exhibited degraded performance when only the equibiaxial protocol was used.

4.4. Clinical relevance

The long-term performance of cusp replacement materials would ideally be evaluated in large animal models for AV disease and cusp repair, and over several months or more. Given the unavailability and impracticality of such models, detailed information on stress-strain behavior, as studied herein, and investigations of durability and valve dynamics will be the best surrogates, as they have been for prosthetic valves [43]. As mentioned, we previously reported results from experiments in a left-heart simulator where the non-coronary cusp of porcine AV's were excised and replaced with various clinically relevant biomaterials [11]. While the hemodynamic performance of the repaired valves was little, if at all, affected by the choice of material and matched the original valves very well (in terms of left ventricular work, maximum geometric orifice area, valve opening and closing velocities), finite
element analysis revealed a large variation in stress values in the AV leaflets, depending on the replacement materials. This motivated the present follow-up study to determine the material properties of the replacement materials with increased accuracy.

Overall, comparison with Fig. 3 suggests that CC and SPG (respectively PG) patches may be the closest mechanical equivalents to young (respectively aged) AV leaflets. Interestingly, the thicknesses of these materials are close to those reported for porcine and younger human AV leaflets, which may facilitate surgical implantation, by contrast to the thinner APP which has poor handling qualities [11]. Because the native leaflets as well as CC, PG and SPG patches exhibit anisotropic behaviors, from a mechanistic perspective alone, it stands to reason that cardiac surgeons should seek to interopatively match the fiber and cross-fiber directions of these replacement biomaterials with those of the repaired AV leaflets. Practically, tweezers can be used to probe the stretching abilities of the replacement patch in various directions, and readily determine which orientation is the stiffest. This direction should be aligned with the circumferential direction of the AV leaflet.

4.5. Study limitations

Determination of the fiber direction in the tested materials was based on visual assessment, for lack of more accurate modalities such as described in [30,2,44]. This precluded precise alignment of the fiber direction with one specific axis of the testing equipment. However, the consistency of the measurements, as illustrated by the relatively small error bars in Appendix 4, suggests that alignment of the samples was not an issue. Another current limitation of the work is the incomplete information about the strength of the native leaflets [45] and of the replacement materials [46], especially in combination with sutures. This will be the focus of future work, to determine whether or not some biomaterials are more prone to tearing at the sutures than others. It is also important to note that this study only succeeded in identifying the materials whose loading stress-strain behavior most closely matched that of native human tissues; other considerations such as fatigue durability will need attention as well. Lastly, whether using equibiaxial only or general biaxial data to identify material abilities of the replacement patch in various directions, and readily determine which orientation is the stiffest. This direction should be aligned with the circumferential direction of the AV leaflet.

Appendix 1. Determination of the components of \( \mathbf{F} \)

As discussed in Section 2.3, stretch ratios \( \lambda_1, \lambda_2 \) as well as components \( F_{12} \) and \( F_{21} \) over a region of interest of the sample can be post-processed from the images obtained during biaxial testing by following the procedure outlined in [19]. Herein, the variables of interest are the displacements \( u \) and \( v \) along the coordinate axes, as well as positions \( X_1 \) and \( X_2 \) of the 4 corner nodes of a quad finite element in the reference configuration. In the corresponding square parent coordinate system, variable of interest \( \varphi_r(s) = (N_1, N_2, N_3, N_4) \), \( r = 1, 2, 3, 4 \), \( N_i \) are known in all relevant deformed configurations. The coordinates \( (r, s) \) of the corner nodes are as follows: Node 1 (1, 1), Node 2 (−1, 1), Node 3 (−1, −1) and Node 4 (1, −1). Obviously, \( \partial N_i / \partial r = 1/2(1 + r_1)(1 + s_1) \) and \( \partial N_i / \partial s = 1/2(1 + r_1)(1 + s_1) \), from which \( \varphi_r(s) = 1/4(1 + r_1 − r_1 − 1 + r_1 − 1 − r_1 \varphi_r(s') \). For displacement \( w \) (e.g. \( u \) or \( v \)), using the chain rule of differentiation and by inversion,

\[
\begin{align*}
\frac{\partial u}{\partial X_1} &= \frac{1}{\varphi_r(s)} - \frac{\partial s}{\partial X_1} \frac{\partial w}{\partial X_2}, \\
\frac{\partial u}{\partial X_2} &= \frac{\partial w}{\partial X_1}, \\
\frac{\partial v}{\partial X_1} &= \frac{\partial w}{\partial X_2}.
\end{align*}
\]

In the latter equation, all the expressions on the right-hand side can be evaluated at any \( (r, s) \) by substituting \( \partial \varphi_r(s)/\partial r \) and \( \partial \varphi_r(s)/\partial s \) with their expressions in terms of \( r, s \) and the respective nodal variables \( \varphi_r \). For instance, when \( \varphi_r = X_1 \) (respectively \( u \)), one needs the 4 values of \( X_1 \) (respectively \( u \)) at the 4 corner nodes. Finally, stretch ratios \( \lambda_1, \lambda_2 \) and components \( F_{12} \) and \( F_{21} \) can be obtained from the definition of \( \mathbf{F} \) whereby \( \mathbf{F} = \mathbf{u}/\mathbf{X} + \mathbf{L} \). In 2-D, this results in

\[
\begin{align*}
\lambda_1 &= F_{12} / F_{21}, \\
\lambda_2 &= F_{21} / F_{12}.
\end{align*}
\]

Appendix 2. Determination of the stress components

With only external forces \( f_1 \) and \( f_2 \) applied in directions 1 and 2, respectively, the only non-zero Cartesian components of the first Piola-Kirchhoff (P-K, or Lagrangian) stress tensor \( \mathbf{P} \) (applying current forces onto undeformed areas) are \( P_{11} = f_1/HL_2 \) and \( P_{22} = f_2/HL_1 \). Assuming that the components of \( \mathbf{P} \) do not vary significantly through the (undeformed) thickness of the sample, the components of first P-K stress resultant (or membrane tension, in N/m) tensor obtained by integration over the undeformed thickness are \( T_{11} = f_1/HL_2 \) and \( T_{22} = f_2/HL_1 \). Alternatively, introducing the second P-K stress tensor (without physical meaning) \( \mathbf{S} = \mathbf{P} \mathbf{F}^T \), the components of second P-K stress resultant (or membrane tension, in N/m) tensor obtained by integration over the undeformed thickness are \( T_{11} = f_1/HL_2 \) and \( T_{22} = f_2/HL_1 \) and, owing to the symmetry of \( \mathbf{S} \), \( T_{12} = T_{21} = -F_{12} f_1/HL_2 = T_{21} = -F_{12} f_2/HL_1 \). Alternatively yet, introducing the Cauchy stress tensor \( \mathbf{T} = 1/\mathbf{F} \mathbf{S} \mathbf{F}^T = 1/\mathbf{F} \mathbf{P} \mathbf{F}^T \) (applying current forces onto current areas: this is the true stress), with \( j = \det \mathbf{F} = 1 \) herein, the components of the Cauchy stress resultant (or membrane tension, in N/m) tensor could be obtained by integration over the deformed thickness are \( T_{11} = f_1/HL_2 \) and \( T_{22} = f_2/HL_1 \) and, owing to the symmetry of \( \mathbf{T} \), \( T_{12} = T_{21} = f_1 f_2/HL_2 \).

Appendix 3. Convexity constraints for two Fung-like materials

For simplicity, we will note \( W \) instead of \( w \), keeping in mind that in expressions with \( w \), scaling constant \( c_1 \) has units of a membrane tension (N/m), whereas it has units of stress (Pa) with
In both Fung-like models considered herein, \( W = c_1/2[\exp(Q) - 1] \) and we require \(-c_1 < 0\) so that \( W \geq 0 \) in general. The components of the 2-D elasticity tensor can be found using
\[
\frac{\partial^2 W}{\partial \varepsilon_{ij} \partial \varepsilon_{kl}} = \frac{2}{Q} \exp(Q)(\partial^2 Q/\partial \varepsilon_{ij} \partial \varepsilon_{kl}) + (\partial Q/\partial \varepsilon_{ij})(\partial Q/\partial \varepsilon_{kl}),
\]
where \( \alpha, \beta = 11, 22 \) or 12.

### 3.1. Sack’s model (7 constants)

With \( Q = c_2 E_{11}^2 + c_1 E_{22} + 2 c_4 E_{11} E_{22} + c_5 E_{12}^2 + 2 c_6 E_{11} E_{22} + 2 c_7 E_{22} E_{12} \),

\[
\begin{align*}
C_{11} & = \frac{c_1}{2} \exp(Q)[2 c_2 + (2 c_2 E_{11} + 2 c_4 E_{22} + 2 c_6 E_{12})^2], \\
C_{22} & = \frac{c_1}{2} \exp(Q)[2 c_3 + (2 c_3 E_{22} + 2 c_4 E_{11} + 2 c_7 E_{12})^2], \\
C_{44} & = \frac{c_1}{2} \exp(Q)[2 c_5 + (2 c_5 E_{12} + 2 c_6 E_{22} + 2 c_7 E_{22})^2], \\
C_{12} & = \frac{c_1}{2} \exp(Q)[2 c_4 + (2 c_2 E_{11} + 2 c_4 E_{22} + 2 c_6 E_{12})/(2 c_1 E_{22} + 2 c_4 E_{11} + 2 c_7 E_{12})], \\
C_{14} & = \frac{c_1}{2} \exp(Q)[2 c_6 + (2 c_2 E_{11} + 2 c_4 E_{22} + 2 c_6 E_{12})/(2 c_3 E_{12} + 2 c_4 E_{11} + 2 c_7 E_{22})], \quad \text{and} \\
C_{24} & = \frac{c_1}{2} \exp(Q)[2 c_7 + (2 c_3 E_{22} + 2 c_4 E_{11} + 2 c_7 E_{12})/(2 c_3 E_{12} + 2 c_4 E_{11} + 2 c_7 E_{22})].
\end{align*}
\]

At zero strain, i.e. for \( E_{11} = E_{22} = E_{12} = 0 \), \( \mathbb{E} [\mathbb{C}_{2D}] = \)

\[
\begin{bmatrix}
c_2 & c_4 & c_6 \\
c_4 & c_3 & c_7 \\
c_6 & c_7 & c_5
\end{bmatrix}
\]

According to Section 2.4, the constraints on the material constants for the strain energy function to be convex at least at the origin are:

\[-c_2 < 0, -c_4 - c_5 - c_6 < 0, c_2^2 - c_4 c_5 < 0, c_2^2 + c_4^2 + c_5^2 - c_2 c_3 - c_4 c_6 - c_5 c_7 < 0 \]

\[-c_4 c_6 c_7 - 2 c_4 c_6 c_7 < 0. \]

### 3.2. Guccione et al.’s model (4 constants)

With \( Q = c_2 E_{11}^2 + c_1 (E_{22}^2 + E_{33}^2) + 2 c_4 E_{12}^2 \), where \( E_{33} = (1/2)(((1/\Lambda) - 1), \text{and} \ \Lambda = (2 E_{11} + 1)(2 E_{22} + 1), \)

\[
\begin{align*}
C_{11} & = \frac{c_1}{2} \exp(Q) \left[ 2 c_2 + \frac{c_3}{(2 E_{11} + 1)^2} \left( \frac{6}{\Lambda^2} - \frac{4}{\Lambda} \right) \\
& - \left( 2 c_2 E_{11} - \frac{c_3}{\Lambda(2 E_{11} + 1)} \left( \frac{1}{\Lambda} - 1 \right) \right)^2 \right], \\
C_{22} & = \frac{c_1}{2} \exp(Q) \left[ 2 c_3 + \frac{c_3}{(2 E_{22} + 1)^2} \left( \frac{6}{\Lambda^2} - \frac{4}{\Lambda} \right) \\
& + \left( 2 c_2 E_{22} - \frac{c_3}{\Lambda(2 E_{22} + 1)} \left( \frac{1}{\Lambda} - 1 \right) \right)^2 \right], \\
C_{24} & = \frac{c_1}{2} \exp(Q)[2 c_4 + (2 c_4 E_{12})^2], \\
C_{12} & = \frac{c_1}{2} \exp(Q) \left[ c_2 \left( \frac{4}{\Lambda^2} - \frac{2}{\Lambda} \right) + \left( 2 c_2 E_{11} - \frac{c_3}{\Lambda(2 E_{11} + 1)} \left( \frac{1}{\Lambda} - 1 \right) \right) \\
& - \left( \frac{c_3}{\Lambda(2 E_{22} + 1)} \left( \frac{1}{\Lambda} - 1 \right) \right)^2 \right], \\
C_{24} & = \frac{c_1}{2} \exp(Q) \left( 2 c_3 E_{12} - \frac{c_3}{\Lambda(2 E_{22} + 1)} \left( \frac{1}{\Lambda} - 1 \right) \right) 2 c_4 E_{12}, \quad \text{and} \\
C_{14} & = \frac{c_1}{2} \exp(Q) \left( 2 c_2 E_{11} - \frac{c_3}{\Lambda(2 E_{11} + 1)} \left( \frac{1}{\Lambda} - 1 \right) \right) 2 c_4 E_{12}. \\
\end{align*}
\]

At zero strain, i.e. for \( E_{11} = E_{22} = E_{12} = 0 \), \( \mathbb{E} [\mathbb{C}_{2D}] = \)

\[
\begin{bmatrix}
c_2 + c_3 & 0 \\
c_3 & 2 c_3 \\
0 & 0 \\
c_4
\end{bmatrix}
\]

According to Section 2.4, the constraints on the material constants for the strain energy function to be convex at least at the origin are:

\[-c_2 - c_3 < 0, -c_2 - 3 c_3 - c_4 < 0, -c_2^2 - 2 c_3 c_1 < 0, c_1^2 - 2 c_2 c_3 - c_2 c_4 - 3 c_3 c_4 < 0 \]

and \(-c_2^2 - 2 c_2 c_3 c_4 < 0.\)
Appendix 4. Experimental and model-fit membrane tensions vs. strains for all materials

Experimental data and model fit with Sack’s model for APP.

Experimental data and model fit with Guccione et al.’s model for APP.
Experimental data and model fit with Sack's model for GPP.

Experimental data and model fit with Guccione et al.'s model for GPP.
Experimental data and model fit with Sack’s model for SJM.

Experimental data and model fit with Guccione et al.’s model for SJM.
Experimental data and model fit with Sack's model for CC.

Experimental data and model fit with Guccione et al.’s model for CC.
Experimental data and model fit with Sack’s model for PG.

Experimental data and model fit with Guccione et al.’s model for PG.
Experimental data and model fit with Sack's model for SPG.

Experimental data and model fit with Guccione et al.'s model for SPG.
Experimental data and model fit with Sack’s model for PC.

References
