On the effect of prestrain and residual stress in thin biological membranes

Manuel K. Rausch, Ellen Kuhl

Article info

Article history:
Received 3 November 2012
Received in revised form
25 February 2013
Accepted 15 April 2013
Available online 3 May 2013

Keywords:
Prestrain
Residual stress
Parameter identification
Finite element method
Mitral leaflet

Abstract

Understanding the difference between ex vivo and in vivo measurements is critical to interpret the load carrying mechanisms of living biological systems. For the past four decades, the ex vivo stiffness of thin biological membranes has been characterized using uniaxial and biaxial tests with remarkably consistent stiffness parameters, even across different species. Recently, the in vivo stiffness was characterized using combined imaging techniques and inverse finite element analyses. Surprisingly, ex vivo and in vivo stiffness values differed by up to three orders of magnitude. Here, for the first time, we explain this tremendous discrepancy using the concept of prestrain. We illustrate the mathematical modeling of prestrain in nonlinear continuum mechanics through the multiplicative decomposition of the total elastic deformation into prestrain-induced and load-induced parts. Using in vivo measured membrane kinematics and associated pressure recordings, we perform an inverse finite element analysis for different prestrain levels and show that the resulting membrane stiffness may indeed differ by four orders of magnitude depending on the prestrain level. Our study motivates the hypothesis that prestrain is important to position thin biological membranes in vivo into their optimal operating range, right at the transition point of the stiffening regime. Understanding the effect of prestrain has direct clinical implications in regenerative medicine, medical device design, and tissue engineering of replacement constructs for thin biological membranes.

1. Introduction

Since Fung's classical opening angle experiment in arteries more than two decades ago (Fung and Liu, 1989; Fung, 1991), we all know, at least theoretically, that biological tissues display residual stresses, stresses that are relieved when biological substrates are isolated from their native environment. In fact, engineers have utilized the concept of residual stresses in prestressed concrete to structurally strengthen high-rise buildings and long-span bridges since the early 1950s (Timoshenko, 1953). Because of their elegant shape and aesthetic appearance, prestrained thin membrane structures have become an architecturally innovative design element in large outdoor roofs and pavilions (Ramm and Mehlhorn, 1991). While residual stresses in engineering structures may result from well-defined fabrication processes such as tensile elements, plastic deformation, or heat treatment, residual stresses in biological structures typically originate from...
development, growth, or remodeling, phenomena which, themselves, are only poorly understood (Ambrosi et al., 2011; Rausch et al., 2012). It is therefore not surprising that we often forget about the existence of residual stresses when analyzing living systems. Since we only know so little about them, why can we not simply ignore the effects of residual stresses in biological systems?

Prestrained thin architectural membranes find their natural equivalent in the mitral valve leaflet, a thin biological membrane supported by a stiff reinforcing ring, the mitral annulus, and by tension cables, the chordae tendineae, see Fig. 1. The mitral leaflet has become an object of intense study throughout the past four decades (Sacks and Yoganathan, 2007), tested first uniaxially (Clark, 1973; Ghista and Rao, 1973; Kunzelman and Cochran, 1992) then biaxially (Grande-Allen et al., 2005; May-Newman and Yin, 1995), harvested from animals (Kunzelman and Cochran, 1992; May-Newman and Yin, 1995) and from humans (Clark, 1973; Ghista and Rao, 1973; Grande-Allen et al., 2005), characterized initially ex vivo (Grande-Allen et al., 2005; Kunzelman and Cochran, 1992; May-Newman and Yin, 1995) and now in vivo (Krishnamurthy et al., 2008; Itoh et al., 2009). While it would be overly optimistic to expect ex vivo and in vivo studies to yield identical mechanical characteristics, it is surprising that the reported stiffness values differ by up to three orders of magnitude (Krishnamurthy et al., 2008). When comparing the reported ex vivo and in vivo data more closely, we observe three major inconsistencies, one in kinematics, one in equilibrium, and one in the constitutive response.

The kinematic controversy manifests itself in significantly larger strains measured ex vivo than in vivo. Ex vivo, in a left heart simulator designed to reproduce the leaflet’s natural environment, explanted mitral leaflets displayed stretches in the order of 1.2–1.3 when subjected to physiological conditions (He et al., 2005; Jimenez et al., 2007). In vivo, in controlled animal experiments in sheep, both sonomicrometry (Sacks et al., 2006) and video fluoroscopy (Rothe et al., 2011; Rausch et al., 2011) revealed stretches in the order of 1.05–1.1, less than half of the ex vivo values. If membrane stretches were less than 1.1 in vivo, the true load carrying capacity of collagen associated with the uncrimping of collagen fibrils in stretch regimes beyond 1.2 (Grande-Allen et al., 2005) would not be activated at all under physiological conditions.

The equilibrium controversy manifests itself in larger stresses estimated in vivo than measured ex vivo. Ex vivo, measured failure stresses of healthy human mitral leaflets were in the order of 900 kPa (Grande-Allen et al., 2005). In vivo, computationally predicted stresses were as large as 3000 kPa in a forward finite element analysis based on in vivo data (Krishnamurthy et al., 2009). If membrane stresses were in the order of megapascales in vivo, more than three times larger than the failure stress, leaflets would be in serious danger of rupturing under physiological conditions.

The constitutive controversy manifests itself in significantly larger stiffness values identified in vivo than measured ex vivo. Ex vivo, measured pre- and post-transition moduli, the tangent stiffnesses before and after the onset of stress locking in collagenous tissues, were consistently reported in the orders of 10 kPa and 1 MPa in all previous studies (Clark, 1973; Ghista and Rao, 1973; Kunzelman and Cochran, 1992; May-Newman and Yin, 1995; Grande-Allen et al., 2005). In vivo, the mitral leaflet stiffness identified from in vivo data using linear inverse finite element analyses was in the order of 10–100 MPa (Krishnamurthy et al., 2008, 2009). Nonlinear constitutive models displayed a similar mismatch with significantly larger material parameter values when fitted to in vivo data (Rausch et al., in press) than when compared to ex vivo data (Prot et al., 2007). In a finite element simulation with the ex vivo fitted parameters, the computational analysis overestimated structural deformations by a factor two (Prot et al., 2007) indicating that the assumed stiffness was too low. If the membrane stiffness was up to three orders of magnitude larger in vivo than ex vivo, what are the mechanisms responsible for this tremendous stiffness increase in vivo?

Here we demonstrate that the concept of residual stress, or rather the concept of prestrain, is capable of explaining all three inconsistencies, in strain, in stress, and in stiffness, between ex vivo and in vivo data. A recent study revealed that mitral leaflets in vivo are indeed exposed to significant residual strains which can be released upon leaflet explantation.
Leaflets contracted by 17% in area when excising the heart from the body and by a total of 43% when further excising the leaflet from the heart. The ease of measuring kinematic changes suggests to characterize the difference between in vivo and ex vivo phenomena in terms of strains rather than stresses, and use a kinematic approach towards modeling residual stresses (Hoger, 1985). In particular, we propose to adopt the concept of fictitious configurations (Hoger, 1997; Johnson and Hoger, 1995), in which we decompose the total elastic deformation gradient multiplicatively into prestrain-induced and load-induced parts, see Fig. 2. In this approach, we parameterize the stored energy as a function of the total elastic strain (Alastrué et al., 2009). The degree of prestrain defines the remaining stored energy upon removal of all in vivo loads and naturally introduces the notion of residual stress (Buganza Tepole et al., 2011; Menzel, 2007). In cylindrical structures, residual stresses have been visualized using the classical opening angle experiment both in arteries (Fung and Liu, 1989; Fung, 1991) and in the heart (Omens and Fung, 1990). First mathematical models for residual stresses in cylindrical structures are now on their way (Cardamone et al., 2009; Chen and Eberth, 2012; Taber and Humphrey, 2001), and algorithmic protocols have been developed to efficiently incorporate prestrain in finite element simulations (Balzani et al., 2006; Famaey et al., 2013). In thin films, prestrain has been thoroughly characterized analytically, numerically, and experimentally using nano- and micro-indentation (Begley and Macking, 2004; Zamir and Taber, 2004). In thin biological membranes, prestrain has been recognized to play a critical role in tissue engineered artificial heart valves (Mol et al., 2005). Prestrain has recently been characterized experimentally ex vivo (Amini et al., 2012) and has been identified as an important mechanism in mitral valve mechanics (Rausch et al., in press). However, to date, the effects of prestrain and residual stress in thin biological membranes have never been quantified systematically in vivo. This is the goal of the present paper.

The remainder of this paper is organized as follows. In Section 2, we summarize the general kinematics of prestrain and specify this concept to model prestrain in thin membranes. In Section 3, we illustrate the general constitutive equations for prestrained systems and specify the free energy functions for prestrained isotropic circular thin films and prestrained transversely isotropic mitral leaflets. In Section 4, we discuss the computational modeling of prestrain and specify the benchmark problem of circular thin films and the clinical problem of mitral leaflets. In Section 5, we summarize the results of both problems with a particular focus on their sensitivity with respect to the prestrain level. In Section 6, we discuss our results and compare them to existing studies in the literature, before we conclude by reiterating the role of prestrain and residual stress in thin biological membranes in Section 7.

2. Kinematics of Prestrain

To characterize the kinematics of prestrain, we adopt the formulation of finite strain kinematics based on the deformation map \( \varphi \), which maps material points from the in vivo unloaded configuration \( B_0 \) to the in vivo loaded configuration \( B_t \). Its spatial gradient, the deformation gradient

\[
F = \nabla \varphi : \ TB_0 \rightarrow TB_t
\]

maps elements from tangent space of the in vivo unloaded configuration \( TB_0 \) to the tangent space of the in vivo loaded configuration \( TB_t \). Throughout this paper, we assume that the in vivo unloaded configuration \( B_0 \) is neither stress- nor strain-free. We interpret prestrain as the strain required to bring the membrane from the ex vivo unloaded configuration \( B_e \) to the in vivo unloaded configuration \( B_0 \) and denote the associated tangent map with \( F^p : TB_e \rightarrow TB_0 \). While it is difficult to measure \( F^p \) directly, we can experimentally measure the inverse prestrain \( F^{p\, -1} : TB_0 \rightarrow TB_e \) as the kinematic change upon tissue explantation (Amini et al., 2012). After characterizing the deformation gradient \( F \) and the prestrain \( F^p \), we can

---

**Fig. 2.** Kinematics of finite deformation with prestrain. The elastic tensor \( F^e = F - F^p \) is multiplicatively decomposed into a prestrain-induced part \( F^p \) and a load-induced part \( F = \nabla \varphi \), where the latter is the gradient of the in vivo deformation map \( \varphi \) from the in vivo unloaded configuration \( B_0 \) to the in vivo loaded configuration \( B_t \). While it is difficult to explicitly quantify the prestrain \( F^p \), we can easily measure the inverse prestrain \( F^{p\, -1} \) as the membrane shrinkage upon tissue explantation.
determine the second order elastic tensor
\[ F^* = F : F^0 : \mathcal{T}_B \to \mathcal{T}_B, \]
which we multiplicatively decompose into volumetric and isochoric parts,
\[ F^* = F_{\text{vol}}^* \cdot F^e \quad \text{with} \quad F_{\text{vol}}^* = (J^*)^{1/3} I \quad \text{and} \quad F^e = (J^*)^{-1/3} F^e. \]
This decomposition implies that \( J^* = \det(F^e) = 1 \) and thus \( F_{\text{vol}}^* = F^e = \det(F^e) \geq 0 \). We can then determine the elastic right Cauchy-Green deformation tensor \( \mathbf{C}^e \) and its relation to the total right Cauchy-Green deformation tensor \( \mathbf{C} \) and to its isochoric part \( \mathbf{C}^e \),
\[ \mathbf{C}^e = \mathbf{F}_v^e \cdot \mathbf{F}^e = \mathbf{F}_v^e \cdot \mathbf{C} \cdot \mathbf{F}^e = (J^*)^{2/3} \mathbf{C}^e \quad \text{with} \quad \mathbf{C} = \mathbf{F} \cdot \mathbf{F}^e \quad \text{and} \quad \mathbf{C}^e = \mathbf{F}_v^e \cdot \mathbf{F}_v^e. \]
We account for the characteristic tissue microstructure with a single family of collagen fibers modeled through the structural tensor \( N = n_0 \otimes n_0 \), where \( n_0 \) with \( \|n_0\| = 1 \) denotes the unit vector in the ex vivo unloaded configuration \( \mathcal{B}_e \). Accordingly, we introduce the following three elastic invariants:
\[ J^* = \det(F^e) \quad \text{and} \quad T^e_1 = \mathbf{C}^e : I \quad \text{and} \quad T^e_4 = \mathbf{C}^e : \mathbf{N}, \]
where the Jacobian \( J^* = \det(F^e) \) characterizes the incompressible response, the first isochoric invariant \( T^e_1 = \mathbf{C}^e : I \) characterizes the isotropic response, and the fourth isochoric invariant \( T^e_4 = \mathbf{C}^e : \mathbf{N} \) characterizes anisotropic response.

**Remark 1** (Kinematics of prestrained thin membranes). The simplest approach to model prestrain in thin membranes, which we adopt here, is to assume that the membrane prestrain is transversely isotropic with respect to the membrane normal \( m_0 \),
\[ F^0 = \lambda^p [I - m_0 \otimes m_0] + [m_0 \otimes m_0]/\lambda^p. \]
This allows us to parameterize prestrain in terms of a single scalar-valued variable, the in-plane prestretch \( \lambda^p \). The first term, \( \lambda^p [I - m_0 \otimes m_0] \), is associated with the in-plane prestretch \( \lambda^p \) and with an area change \( \lambda^p \), while the second term, \( [m_0 \otimes m_0]/\lambda^p \), is associated with the thickness contraction \( 1/\lambda^p \). This implies that our prestrain is incompressible, i.e., \( J^* = \det(F^0) = 1 \). Because of its simple rank-one update structure, we can explicitly invert the membrane prestrain,
\[ F^{0\text{-}1} = [I - m_0 \otimes m_0]/\lambda^p + \lambda^p m_0 \otimes m_0, \]
using the Sherman-Morrison formula. We can visualize and measure the inverse prestretch \( 1/\lambda^p \) as the membrane contraction upon tissue explantation, see Fig. 2.

**3. Constitutive equations for prestrained systems**

To model the constitutive response of prestrained systems, we adopt an incompressible, transversely isotropic, hyperelastic free energy function, characterized through a volumetric part \( U \) and an isochoric part \( \psi \), both parameterized exclusively in terms of the elastic invariants \( J^*, T^e_1, \text{and} \ T^e_4 \),
\[ \psi = U(J^*) + \varphi(T^e_1, T^e_4). \]  
where the volumetric and isochoric parts take the following explicit representations:
\[ S^e_{\text{vol}} = 2 \frac{\partial U}{\partial C} = J^* \hat{p} (C^e)^{-1} \]
\[ S^e_{\text{iso}} = 2 \frac{\partial \varphi}{\partial C} = (J^*)^{-2/3} \hat{p} : \mathbf{S}^e. \]
The volumetric stress \( S^e_{\text{vol}} \) depends primarily on the derivative \( \hat{p} := \partial U/\partial C^e \). The isochoric stress \( S^e_{\text{iso}} \) depends on the second order tensor \( \mathbf{S}^e \),
\[ \mathbf{S}^e = 2 \frac{\partial \varphi}{\partial C^e} = 2 \hat{\varphi}^e \mathbf{I} + 2 \hat{\varphi}^e \mathbf{N}, \]
whose weighting factors \( \hat{\varphi}_1^e = \partial \varphi^e / \partial C^e \) we will determine later when we specify the particular form of the isochoric free energy function \( \varphi \). In Eq. (8.2), \( P^p = I - \frac{1}{2} (C^0)^{-1} \otimes \mathbf{C}^e \) denotes the isochoric projection tensor in terms of the fourth order identity tensor \( I = \frac{1}{2} (\mathbf{C}^0 \otimes \mathbf{I}) + \mathbf{I} \otimes \mathbf{I} \), with the understanding that \( [\mathbf{C}^0 \otimes \mathbf{I}]_{ijkl} = [\mathbf{I}]_{ij} [\mathbf{C}^0]_{kl} \) and \( [\mathbf{I} \otimes \mathbf{C}^0]_{ijkl} = [\mathbf{I}]_{il} [\mathbf{C}^0]_{jk} \). To efficiently solve the nonlinear boundary value problem, we linearize the Piola-Kirchhoff stress \( \mathbf{S} \) with respect to the right Cauchy-Green deformation tensor \( \mathbf{C} \) to obtain the fourth order tangent moduli
\[ \mathbf{C} = 4 \frac{\partial^2 \psi}{\partial \mathbf{C} \otimes \partial \mathbf{C}} = [\mathbf{C}^p \otimes \mathbf{P}^p] : \mathbf{C}^e : [\mathbf{P}^p \otimes \mathbf{C}^p] \quad \text{with} \quad \mathbf{C}^e = 2 \frac{\partial \mathbf{S}^e}{\partial \mathbf{C}^e} = C^e_{\text{vol}} + C^e_{\text{iso}}. \]
which we can again decompose into volumetric and isochoric parts,

\[
\begin{align*}
\mathcal{C}_{vol} & = 2 \frac{\partial \mathcal{E}_{vol}}{\partial \mathcal{C}} = f'(\hat{\kappa}) + f''(\hat{\kappa}) (\mathcal{C})^{-1} \otimes (\mathcal{C})^{-1} - 2f''(\hat{\kappa}) \mathcal{I}_{vol}, \\
\mathcal{C}_{iso} & = 2 \frac{\partial \mathcal{E}_{iso}}{\partial \mathcal{C}} = (f')^{-4/3} \mathfrak{p}^e : \mathcal{T}^e = \mathfrak{p}^{et} + \frac{2}{3} [(f')^{-2/3}] \mathfrak{S}^e : \mathcal{C} \mathfrak{p}^e - (\mathfrak{S}^e \otimes (\mathcal{C})^{-1})^{sym}].
\end{align*}
\]

(11)

The volumetric part depends primarily on the second derivative \( \hat{\kappa} : = \partial^2 \mathcal{E} / \partial f'^2 \). The isochoric part depends on the fourth order tensor

\[
\mathcal{T}^e = 2 \frac{\partial \mathcal{E}^e}{\partial \mathcal{C}} = 4[\mathfrak{p}_{11} \mathcal{I} \otimes \mathcal{I} + 2 \mathfrak{p}_{14} (\mathcal{I} \otimes \mathcal{N}) + \mathfrak{p}_{44} \mathcal{N} \otimes \mathcal{N}],
\]

(12)

whose weighting factors \( \mathfrak{p}_{ij} = \partial \mathcal{E}^e / \partial f'^2 \mathfrak{p}_{ij} \) we will specify later. In Eq. (11.2), \( \mathcal{T}^e = \mathcal{I}_{vol} - \frac{1}{2} \mathcal{C}^{-1} \otimes \mathcal{C}^{-1} \) and \( \mathcal{I}_{vol} = \frac{1}{2} [(\mathcal{C})^{-1} \otimes (\mathcal{C})^{-1} + (\mathcal{C})^{-1} \otimes (\mathcal{C})^{-1}] \) are two additional fourth order tensors related to the isochoric projection. The proposed approach is generally applicable to incompressible, transversely isotropic materials. In Sections 3.1 and 3.2, we specify the isochoric free energy function \( \mathcal{E}^e \) for isotropic and transversely isotropic materials and determine its derivatives \( \mathfrak{p}_{ij} \) and \( \mathfrak{p}_{ij}^e \) to specify the isochoric stress \( \mathfrak{S}^e \) and the isochoric tangent moduli \( \mathcal{T}^e \).

### 3.1. Prestrained circular thin films

To specify the isochoric free energy \( \mathcal{E}^e \) of Eq. (6) for the benchmark problem of thin circular films, we select a simple isotropic Neo-Hookean material model parameterized exclusively in terms of the first invariant \( \mathcal{T}_1 \) weighted by the shear modulus \( c_0 \).

\[
\mathcal{E}^e = c_0 (\mathcal{T}_1 - 3].
\]

(13)

From the first derivative of this isochoric free energy function with respect to the first and fourth invariants,

\[
\mathfrak{p}_{11} = c_0 \quad \text{and} \quad \mathfrak{p}_{14}^e = 0, \tag{14}
\]

we conclude that, for this particular model, the isochoric stress \( \mathfrak{S}^e \) of Eq. (9) depends only on the shear modulus \( c_0 \). Accordingly, the second derivatives of the isochoric free energy function with respect to the first and fourth invariants are all zero,

\[
\mathfrak{p}_{11}^e = 0 \quad \text{and} \quad \mathfrak{p}_{14}^e = 0 \quad \text{and} \quad \mathfrak{p}_{44}^e = 0, \tag{15}
\]

and the isochoric tangent moduli \( \mathcal{T}^e \) of Eq. (12) vanish identically.

### 3.2. Prestrained mitral leaflets

To specify the isochoric free energy \( \mathcal{E}^e \) of Eq. (6) for the clinical problem of mitral leaflets, we select a well-calibrated constitutive model for mitral valve tissue (May-Newman and Yin, 1998; Prot et al., 2007). Transversely isotropic in nature, the mitral leaflet is characterized through a single representative fiber family. Its isochoric free energy is based on an exponential function in terms of the first and fourth invariants \( \mathcal{T}_1 \) and \( \mathcal{T}_4 \) weighted by three material parameters \( c_0, c_1, \) and \( c_2, \)

\[
\mathcal{E}^e = c_0 [\exp(c_1 (\mathcal{T}_1 - 3)^2 + c_2 (\mathcal{T}_4 - 1)^2) - 1].
\]

(16)

The free energy function is polyconvex in the case of incompressibility provided the fibers are only subjected to tension, i.e., \( \mathcal{T}_4 \geq 1 \) (Prot et al., 2007). To determine the isochoric stress \( \mathfrak{S}^e \) in Eq. (9), we evaluate the first derivatives of the isochoric free energy function with respect to the first and fourth invariants \( \mathfrak{p}_{ij} = \partial \mathcal{E}^e / \partial f'^2 \mathfrak{p}_{ij} \),

\[
\begin{align*}
\mathfrak{p}_{11} & = 2c_0c_1 (\mathcal{T}_1 - 3) \exp(c_1 (\mathcal{T}_1 - 3)^2 + c_2 (\mathcal{T}_4 - 1)^2) \\
\mathfrak{p}_{14}^e & = 2c_0c_2 (\mathcal{T}_4 - 1) \exp(c_1 (\mathcal{T}_1 - 3)^2 + c_2 (\mathcal{T}_4 - 1)^2). \tag{17}
\end{align*}
\]

To determine the isochoric tangent moduli \( \mathcal{T}^e \) in Eq. (12), we evaluate the second derivatives of the isochoric free energy function with respect to the first and fourth invariants \( \mathfrak{p}_{ij}^e = \partial^2 \mathcal{E}^e / \partial f'^2 \mathfrak{p}_{ij} \),

\[
\begin{align*}
\mathfrak{p}_{11} & = 2c_0c_1 [1 + 2c_1 (\mathcal{T}_1 - 3)^2] \exp(c_1 (\mathcal{T}_1 - 3)^2 + c_2 (\mathcal{T}_4 - 1)^2) \\
\mathfrak{p}_{14} & = 4c_0c_1c_2 (\mathcal{T}_1 - 3) (\mathcal{T}_4 - 1) \exp(c_1 (\mathcal{T}_1 - 3)^2 + c_2 (\mathcal{T}_4 - 1)^2) \\
\mathfrak{p}_{44} & = 2c_0c_2 [1 + 2c_2 (\mathcal{T}_4 - 1)^2] \exp(c_1 (\mathcal{T}_1 - 3)^2 + c_2 (\mathcal{T}_4 - 1)^2). \tag{18}
\end{align*}
\]

Unlike most transversely isotropic models for non-living materials, this particular constitutive model introduces an inherent constitutive coupling between the isotropic and anisotropic response through the first and fourth invariants \( \mathcal{T}_1 \) and \( \mathcal{T}_4 \). This coupling manifests itself in non-vanishing mixed second derivatives, \( \mathfrak{p}_{14}^e \neq 0 \).
4. Computational modeling of prestrain

To simulate prestrain using a standard, commercially available finite element solver, we adopt a three-step prestrain protocol as illustrated in Fig. 2: First, we virtually create the ex vivo configuration $B_0$. Starting with a known in vivo unloaded configuration $B_0$, for a given Green-Lagrange prestrain $F^p = [λ^p - 1]/2$, we shrink the geometry with the inverse membrane prestrain $F^{p^{-1}} = [1 - \lambda m_0 \otimes m_0] / \lambda^0 + \lambda^0 m_0 \otimes m_0$ with $\lambda^p = [2\lambda^p + 1]^{1/2}$. Second, we recreate the in vivo unloaded configuration $B_0$. Starting with the virtually created ex vivo configuration $B_0$, we apply the membrane prestrain $F^p = \lambda^p [1 - \lambda m_0 \otimes m_0] + \lambda^0 m_0 \otimes m_0 / \lambda^2$ and verify the prescribed prestrain level. Third, we create in vivo loaded configuration $B_1$. Starting with the in vivo unloaded configuration $B_0$, now prestrained, we apply the in vivo Dirichlet and Neumann boundary conditions along with the in vivo loading to solve for the deformation $\phi$ and the deformation gradient $F = V\phi$. We calculate the resulting stresses and tangent moduli using the elastic tensor $F = F : F$ as the composition of the mappings $F^p$ and $F$ from steps two and three. In what follows, we adopt this three-step protocol to explore the effect of prestrain in the benchmark problem of circular thin films and in the clinical problem of mitral leaflets. For both cases, we study different prestrain levels by systematically varying the in-plane Green-Lagrange prestrain $F^p = \frac{1}{2}[λ^p - 1]$ in increments of 10%.

4.1. Prestrained circular thin films

To illustrate the effect of prestrain on thin membranes, we simulate two easily reproducible, simple, generic benchmark problems. For the simulation, we use the commercially available, implicit finite element solver ABAQUS/Standard Version 6.9 (Abaqus, 2009). For easy reproducibility, we adopt a simple incompressible Neo-Hookean material model according to Section 3.1 with a shear modulus of $4000$ MPa. In particular, we utilize the framework UNISOHYPER_INV for user-defined anisotropic hyperelastic material models parameterized in an invariant formulation. We virtually generate prestrain in the film, we adopt the three-step prestrain protocol for prestrain levels of $F^p = [0\%, 10\%, 20\%, 30\%]$: first, starting with a circular disc of radius $1$ mm, we virtually create the unloaded configuration $B_0$, by shrinking the disc with the inverse membrane prestrain $F^{p^{-1}}$. Second, from this configuration $B_0$, we start the simulation and apply the membrane prestrain $F^p$ to recreate the configuration $B_0$. Third, we clamp the membrane at its outer edges and apply the in vivo-equivalent loading to solve for the deformation $\phi$ and the deformation gradient $F = V\phi$, which characterize the configuration $B_1$. For the first benchmark problem motivated by the mechanics of the mitral valve (Rausch et al., in press), we simulate the inflation of the thin film with a homogeneous pressure. For the second benchmark problem motivated by prestrain studies in the literature (Begley and Macking, 2004; Zamir and Taber, 2004), we simulate the indentation of the thin film with a frictionless spherical indenter of an indenter-to-film radius of 0.1. We gradually increase both the inflation pressure and the indentation force until the center deflection of the thin film reaches a deflection-to-radius ratio of 0.4.

4.2. Prestrained mitral leaflets

To explore the effect of prestrain on mitral valve mechanics, we implement the constitutive model described in Section 3.2 as a user subroutine into the commercially available, implicit finite element solver ABAQUS/Standard Version 6.9 (Abaqus, 2009). In particular, we utilize the framework UNISOHYPER_INV for user-defined anisotropic hyperelastic material models parameterized in an invariant formulation. We virtually create the ex vivo configuration $B_0$. Starting with the virtually created ex vivo configuration $B_0$, by shrinking the disc with the inverse membrane prestrain $F^{p^{-1}}$. Second, from this configuration $B_0$, we start the simulation and apply the membrane prestrain $F^p$ to recreate the configuration $B_0$. Third, we clamp the membrane at its outer edges and apply the in vivo-equivalent loading to solve for the deformation $\phi$ and the deformation gradient $F = V\phi$, which characterize the configuration $B_1$. For the first benchmark problem motivated by the mechanics of the mitral valve (Rausch et al., in press), we simulate the inflation of the thin film with a homogeneous pressure. For the second benchmark problem motivated by prestrain studies in the literature (Begley and Macking, 2004; Zamir and Taber, 2004), we simulate the indentation of the thin film with a frictionless spherical indenter of an indenter-to-film radius of 0.1. We gradually increase both the inflation pressure and the indentation force until the center deflection of the thin film reaches a deflection-to-radius ratio of 0.4.

Fig. 3. Computational model of the anterior mitral leaflet created from 23 discrete marker positions (left). The resulting finite element model consists of $1920 \ S3R$ linear triangular finite strain shell elements (middle). Discrete collagen fiber orientation maps were extracted from tissue histology (right).
100 MPa (Rausch et al., in press). From the experimentally measured marker coordinates acquired at 60 frames per second, we select eight consecutive time frames starting with the image just before leaflet separation, which we define as the in vivo unloaded reference configuration $B_0$. We then go backwards in time towards the image at end systole, which we define as the in vivo loaded configuration $B_1$ (Krishnamurthy et al., 2008). In this particular simulation interval, the mitral valve is closed, hemodynamic effects are negligible, and the possible effects of contracting smooth muscle cells are minimized (Rausch et al., in press). To account for the characteristic transversely isotropic microstructure of the mitral leaflet, we create discrete collagen fiber orientation maps from tissue histology (Rausch et al., in press) and confirm the results with collagen orientations reported in the literature (Cochran et al., 1991; Prot et al., 2009), see Fig. 3, right. We support the leaflet belly through chordae tendinae, which we model as incompressible Neo-Hookean tension-only rods inserting into the leaflet center (Prot et al., 2009). We assume a chordae stiffness of 20 MPa and a total cross-sectional area of 1 mm² for each branch. Throughout all eight time steps, we apply inhomogeneous Dirichlet boundary conditions to all boundary nodes using the experimentally measured boundary marker coordinates. At the same time, we pressurize the membrane from underneath with the experimentally measured transvalvular pressure, the pressure difference between the left ventricle and the left atrium acquired using catheter micromanometer pressure transducers (Krishnamurthy et al., 2008). To virtually generate prestrain in the mitral leaflet, we adopt the three-step prestrain protocol for prestrain levels of $E = \{0\%, 10\%, 20\%, \ldots, 100\%\}$: First, for each prestrain level, we virtually create the corresponding ex vivo leaflet geometry $B_e$ by shrinking the experimentally acquired in vivo leaflet coordinates with the inverse membrane prestrain $F^{p, -1}$. Second, from the ex vivo leaflet geometry, we start the simulation and apply the membrane prestrain $F^p$ to recreate the in vivo unloaded leaflet geometry $B_0$. Third, we apply the in vivo acquired inhomogeneous Dirichlet boundary conditions and the in vivo acquired transmembrane pressure to solve for the deformation $\phi$ and the deformation gradient $F = \nabla \phi$, which characterize the in vivo loaded leaflet geometry $B_t$. To identify the material parameters for the different prestrain levels, we perform an inverse finite element analysis. We apply a genetic algorithm using MATLAB to minimize the average nodal displacement error $e$ by systematically varying the material parameters $c_0$, $c_1$, and $c_2$. We start with an initial parameter set and perform a first generation of finite element simulations. After the simulation, for each parameter set, we calculate the error $e = \sum_{n=1}^{n_{\text{m}}(m)} \sum_{m=1}^{n_{\text{m}}} \| \varphi_{e, t} - \varphi_{s, t} \| / (n_{m} n_{t})$ as the distance between all $m = 1, \ldots, n_{m}$ experimentally measured inner leaflet markers $\varphi_{e, t}$ and all computationally simulated inner leaflet markers $\varphi_{s, t}$ summed over all $t = 0, \ldots, n_{t}$ time steps. For our particular case, the number of inner markers is $n_{m} = 9$ and the number of time steps is $n_{t} = 8$. Whenever the simulation does not converge, we assign an error value that is larger than previously encountered values for converged solutions. Based on the average nodal displacement error $e$, the genetic algorithm generates a new input parameter set through 20% mutation and 80% cross-over. The genetic algorithm iteratively minimizes the error until it reaches a user-defined convergence criterion. After finding a convergence parameter set, we repeat the optimization algorithm for varying population sizes and initial parameter sets to ensure that the converged solution represents a global minimum.

Remark 2 (Kinematic compatibility of prestrain). Here, for the sake of simplicity, we assume that the prestrain tensor $F^p$ is kinematically compatible, i.e., that it can be constructed as the gradient of a prestrain deformation field. In this sense, we conceptually adapt a well-accepted two-step protocol to model prestrain in arteries (Balzani et al., 2006; Famaey et al., 2013), in which an open arterial segment is first closed to calculate the prestrain tensor $F^p$, before the in vivo loads are applied to calculate the in vivo deformation gradient $F = \nabla \phi$. This successive application of prestrain and deformation simplifies the algorithmic formulation, in that the finite element algorithm can conveniently work with the elastic tensor $F^e = F \cdot F^p$ as the composition of both mappings. From this elastic tensor, we can easily calculate the stresses $S^e = 2 \partial \phi / \partial C^e$ and the elastic tangent moduli $C^e = 2\partial S^e / \partial C^e$ introduced in Eqs. (7) and (10) with reference to an assumed-to-be-known ex vivo unloaded configuration $B_0$. Alternatively, if the ex vivo configuration $B_0$ is unknown, we can work with the total stresses $S = F^p \cdot S^e \cdot F^p$ and with the total tangent moduli $C = \left[ F^p \otimes F^p \right] : C^e : \left[ F^p \otimes F^p \right]$ using pushed forward operations to map the stresses and tangent moduli to the known in vivo unloaded configuration $B_0$.

5. Results

5.1. Prestrained circular thin films

Fig. 4 illustrates the maximum principal elastic Green Lagrange strains and the corresponding elastic stretches for the benchmark problem of prestrained circular thin films. The first column displays the ex vivo unloaded configuration, which is mapped onto the in vivo unloaded configuration displayed in the second column through the membrane prestrain $F^p$. The third and fourth columns display the elastic strains in the prestrained membrane, either subject to inflation or to indentation, characterized through the deformation gradient $F = \nabla \phi$. The color-coded elastic strains are a result of the composition of both mappings, $F^e = F \cdot F^p$, influenced through both the prestrain $F^p$ and the deformation gradient $F$. Each row corresponds to a different prestrain level, increasing gradually from 0% to 30% in increments of 10%. In agreement with intuition, increasing the membrane prestrain increases the elastic strains to generate the same vertical center deflection of 0.4 mm upon membrane inflation and upon membrane indentation. In agreement with the literature, increasing the membrane prestrain induces a sharper indentation profile associated with a more localized deformation pattern (Zamir and Taber, 2004).
Fig. 4. Maximum principal elastic Green Lagrange strains and corresponding elastic stretches for different prestrain levels. From the ex vivo unloaded configuration in the first column to the in vivo unloaded configuration in the second column, the membrane is prestrained through a prestrain \( F^p \) of different levels. From the in vivo unloaded configuration to the in vivo loaded configurations the membrane is either subjected to inflation, third column or to indentation, fourth column, reflected through the deformation gradient \( F = \nabla \varphi \). The color-coded elastic strains are a result of the composition of both mappings, \( F^e = F / C \cdot F^p \). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

Fig. 5. Inflation pressure vs. vertical displacement (left), and indentation force vs. vertical displacement (right), for circular thin films at different prestrain levels. The membrane stiffness, the slope of the curves, increases markedly with increasing membrane prestrain.
Fig. 5 illustrates the effect of prestrain on the constitutive response of circular thin films. It displays the inflation pressure as a function of the central vertical displacement, left, and the indentation force as a function of the central vertical displacement, right. To highlight the continuous dependence of the constitutive behavior on the prestrain level, we color-coded the regions between the curves of the individual prestrain levels ranging from red at no prestrain to blue at the highest simulated prestrain. In agreement with the literature, for both benchmark problems, the inflation test and the indentation test, the membrane stiffness represented through the slope of the curves increases markedly with increasing prestrain (Begley and Macking, 2004; Zamir and Taber, 2004).

Table 1
Material parameter values for mitral leaflet tissue identified for different prestrain levels.

<table>
<thead>
<tr>
<th>Prestrain (%)</th>
<th>Prestretch (-)</th>
<th>$c_0$ (kPa)</th>
<th>$c_1$ (-)</th>
<th>$c_2$ (-)</th>
<th>Error (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.000</td>
<td>119,020.7</td>
<td>152.4</td>
<td>185.5</td>
<td>0.561</td>
</tr>
<tr>
<td>10</td>
<td>1.095</td>
<td>817.9</td>
<td>16.2</td>
<td>27.3</td>
<td>0.551</td>
</tr>
<tr>
<td>20</td>
<td>1.183</td>
<td>71.6</td>
<td>5.0</td>
<td>15.6</td>
<td>0.567</td>
</tr>
<tr>
<td>30</td>
<td>1.265</td>
<td>35.9</td>
<td>2.3</td>
<td>9.8</td>
<td>0.575</td>
</tr>
<tr>
<td>40</td>
<td>1.342</td>
<td>23.0</td>
<td>1.2</td>
<td>5.6</td>
<td>0.582</td>
</tr>
<tr>
<td>50</td>
<td>1.414</td>
<td>10.6</td>
<td>1.0</td>
<td>4.7</td>
<td>0.587</td>
</tr>
<tr>
<td>60</td>
<td>1.483</td>
<td>4.4</td>
<td>0.6</td>
<td>3.4</td>
<td>0.588</td>
</tr>
<tr>
<td>70</td>
<td>1.549</td>
<td>7.5</td>
<td>0.4</td>
<td>2.5</td>
<td>0.590</td>
</tr>
<tr>
<td>80</td>
<td>1.613</td>
<td>5.2</td>
<td>0.3</td>
<td>2.0</td>
<td>0.592</td>
</tr>
<tr>
<td>90</td>
<td>1.673</td>
<td>4.8</td>
<td>0.3</td>
<td>1.7</td>
<td>0.593</td>
</tr>
<tr>
<td>100</td>
<td>1.732</td>
<td>4.0</td>
<td>0.2</td>
<td>1.5</td>
<td>0.595</td>
</tr>
</tbody>
</table>

Fig. 6. Material parameter values for mitral leaflet tissue identified for different prestrain levels.
5.2. Prestrained mitral leaflets

The parameter identification for the mitral leaflet has converged successfully for all eleven prestrain levels. For each prestrain level, we have identified an optimal parameter set $c_0$, $c_1$, and $c_2$, minimizing the total error between the experimentally measured and the computationally simulated inner marker coordinates.

Table 1 summarizes the identified parameter sets for Green Lagrange prestrains $E^P = [\lambda^P - 1]/2$ varying from 0% to 100% corresponding to a membrane prestretch of $\lambda^P$ varying from 1.000 to 1.732. All associated error values are smaller than

![Table 1: Prestrained Parameter Sets](image_url)

### Table 1: Prestrained Parameter Sets

<table>
<thead>
<tr>
<th>Prestrain</th>
<th>Ex Vivo Unloaded</th>
<th>In Vivo Unloaded</th>
<th>In Vivo Loaded</th>
<th>Prestretch</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td></td>
<td></td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>10%</td>
<td></td>
<td></td>
<td>1.095</td>
<td></td>
</tr>
<tr>
<td>20%</td>
<td></td>
<td></td>
<td>1.183</td>
<td></td>
</tr>
<tr>
<td>30%</td>
<td></td>
<td></td>
<td>1.265</td>
<td></td>
</tr>
<tr>
<td>40%</td>
<td></td>
<td></td>
<td>1.342</td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td></td>
<td></td>
<td>1.414</td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td></td>
<td></td>
<td>1.483</td>
<td></td>
</tr>
<tr>
<td>70%</td>
<td></td>
<td></td>
<td>1.549</td>
<td></td>
</tr>
<tr>
<td>80%</td>
<td></td>
<td></td>
<td>1.613</td>
<td></td>
</tr>
<tr>
<td>90%</td>
<td></td>
<td></td>
<td>1.673</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td></td>
<td></td>
<td>1.732</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 7. Maximum principal elastic Green Lagrange strains and corresponding elastic stretches for different prestrain levels. From the ex vivo unloaded configuration $B_e$ to the in vivo unloaded configuration $B_0$, the membrane is prestrained through a prestrain $F^P$ of different levels. From the in vivo unloaded configuration $B_0$ to the loaded configuration $B_t$, the membrane is subjected to the experimentally measured load resulting in the deformation gradient $F = \nabla \varphi$. The color-coded elastic strains are a result of the composition of both mappings, $F = F \cdot F^P$. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)
1 mm, which lies within the range of the expected experimental measuring error. Variation between error values is minimal with approximately 6% difference between the maximal and minimal values. All three material parameters decrease monotonically with increasing levels of prestrain. While the isotropic and anisotropic parameters \( c_1 \) and \( c_2 \) decrease by

\[
\begin{align*}
\sigma_{11} \quad [\text{kPa}] & \quad \lambda_1 \\
\sigma_{22} \quad [\text{kPa}] & \quad \lambda_2
\end{align*}
\]

levels of prestrain.

**Fig. 8.** Homogeneous biaxial tension test for different prestrain levels. Cauchy stresses parallel to fiber direction \( \sigma_{11} \) vs. elastic stretch \( \lambda_1 \) (left), and Cauchy stresses perpendicular to fiber direction \( \sigma_{22} \) vs. elastic stretch \( \lambda_2 \) (right). To calculate the stress–stretch curves, we used the individual parameter sets for the different prestrain levels summarized in Table 1.

**Fig. 9.** Homogeneous biaxial tension test for different prestrain levels. Cauchy stresses parallel to fiber direction \( \sigma_{11} \) vs. in vivo stretch \( \lambda_1 = \lambda_p \) (left), and Cauchy stresses perpendicular to fiber direction \( \sigma_{22} \) vs. in vivo stretch \( \lambda_2 = \lambda_p \) (right). To calculate the stress–stretch curves, we used the individual parameter sets for the different prestrain levels summarized in Table 1.
approximately two orders of magnitude, the stiffness parameter $c_0$ decreases by four orders of magnitude. Parameter $c_2$ associated with the anisotropic invariant $I_2$ is consistently larger than parameter $c_1$ associated with the isotropic invariant $I_1$. The ratio between $c_2$ and $c_1$ increases with prestrain from a ratio of approximately 1.2 at 0% prestrain to a ratio of 7.5 at 100% prestrain.

Fig. 6 illustrates the prestrain dependence of the three model parameters. For illustration purposes, we have fitted an exponential function to the discrete parameter values. All three parameters, $c_0$, $c_1$, and $c_2$, display a sharp exponential decay. Their initial rapid decrease flattens out at higher prestrain levels. The graphs confirm that the parameter $c_2$ associated with the anisotropic invariant $I_2$ is consistently larger than the parameter $c_1$ associated with the isotropic invariant $I_1$, which displays a faster exponential decay.

Fig. 7 visualizes the three configurations of the thin membrane for prestrain levels varying from 0% to 100%. All leaflets are color-coded for the maximum principal elastic Green Lagrange strain and are drawn to scale. The first column contains the leaflets in the ex vivo unloaded configuration $B_0$, before we apply prestrain. Elastic Green Lagrange strains are zero across all the leaflets. With increasing levels of prestrain, the leaflet size in the first column decreases as we isotropically scale down the leaflet dimensions. The second column contains the leaflets in the in vivo unloaded configuration $B_0$, after we applied the corresponding prestrain level. Elastic Green Lagrange strains in the second column are homogeneous and present the individual prestrain levels. By construction, the elastic Green Lagrange strains of the in vivo unloaded configuration $B_0$ increase with the prestrain level. The third column contains the leaflets in the in vivo loaded configuration $B_0$ after we applied prestrain and the in vivo loading. Elastic Green Lagrange strains in the third column are inhomogeneous and increase nonlinearly with the prestrain level.

Fig. 8 illustrates the effect of the different parameter sets on the constitutive response of a thin biological membrane in a homogeneous biaxial tension test. It displays the Cauchy stresses parallel and perpendicular to the collagen fiber direction $\sigma_{11}$, left, and $\sigma_{22}$, right, as functions of the corresponding elastic stretches $\lambda_1^c$ and $\lambda_2^c$. To highlight the continuous dependence of the stress–stretch behavior on the prestrain level, we color-coded the regions between the curves of the individual prestrain levels. The curves demonstrate the typical nonlinear stress–elastic–stretch behavior characteristic for collagenous soft biological tissues. As we increase the prestrain level, the material parameters $c_0$, $c_1$, and $c_2$ decrease, and the overall response seems to soften. Consequently, the stress–stretch curves flatten with increased prestrain levels.

Fig. 9 further illustrates the effect of the different parameter sets on the constitutive response of a thin biological membrane in a homogeneous biaxial tension test. In contrast to Fig. 8, Fig. 9 displays the Cauchy stresses parallel and perpendicular to the collagen fiber direction $\sigma_{11}$, left, and $\sigma_{22}$, right, as functions of the corresponding total stretches $\lambda_1 = \lambda_1^c / \lambda_0^p$ and $\lambda_2 = \lambda_2^c / \lambda_0^p$. This implies that we have virtually removed the prestrain $\lambda_0^p$. Accordingly, the curves shift to the left, depending on the prestrain level. The stretch-axis intercepts at $\sigma = 0$ indicate the inverse prestrain level $1 / \lambda_0^p$ and the stress-axis intercepts at $\lambda = 1$ indicate the residual stress induced through prestrain. While the stress–elastic–stretch curves shown in Fig. 8 seem to flatten with increased prestrain level parameters, the stress–stretch curves in Fig. 9 demonstrate that these curves actually display a significant exponential stiffening at comparable stretch levels $\lambda$.

6. Discussion

Residual stresses, stresses that are relieved when a biological sub-system is isolated from its natural environment (Fung and Liu, 1989; Fung, 1991), are inherent to virtually all living systems (Cardamone et al., 2009; Taber and Humphrey, 2001); yet, we know very little about them. While residual stresses are usually difficult to measure, residual strains are relatively easy to access as the inverse kinematic change upon isolation from the living system (Amini et al., 2012). The concept of fictitious configurations (Hoger, 1997; Johnson and Hoger, 1995) provides an elegant theoretical framework to model prestrain and residual stress through the multiplicative decomposition of the total elastic deformation into prestrain-induced and load-induced parts, see Fig. 2. In the present study, we extracted the load-induced kinematics and pressure values from in vivo experiments in sheep (Rausch et al., 2011, in press), see Figs. 1 and 3. To explore the effect of prestrain, we systematically prescribed different prestrain levels, and studied their impact on the overall mechanical characteristics of the mitral leaflet, see Fig. 7, left column. Our central finding is that prestrain has a drastic effect on strain, stress, and stiffness. Because of the multiplicative nature of the kinematic model, a linear increase in prestrain is associated with a nonlinear increase in the overall strain, see Fig. 7, right column. Because of the characteristic exponential strain–stiffening behavior of collagenous biological tissues, a linear increase in prestrain is associated with an exponential decrease in the apparent material stiffness, see Table 1 and Fig. 6. For similar reasons, a linear increase in prestrain is associated with a nonlinear alterations in stress, see Figs. 8 and 9.

6.1. Comparison to previous studies

Our in vivo mitral leaflet kinematics agree nicely with previously reported strains in sheep from sonomicrometry (Sacks et al., 2006) and from video fluoroscopy (Bothe et al., 2011; Rausch et al., 2011). Circumferential and radial stretches of approximately 1.05 and 1.08 are in good qualitative agreement with values found for both techniques. However, our in vivo stretches are significantly lower than the ex vivo circumferential and radial stretches of 1.2 and 1.5 measured in porcine leaflets in a left heart simulator under physiological loading conditions (Grashow et al., 2006; He et al., 2005; Jimenez et al., 2007). This difference between in vivo and ex vivo kinematics has previously been noted (Rausch et al., 2011), but has never
been explained to date. In view of the present study, we attribute this difference to the prestrain $F^p$ between the ex vivo unloaded configuration $B_c$ and the in vivo unloaded configuration $B_0$, see Fig. 2. Under similar physiological loading conditions, the ex vivo experiments record the total elastic deformation $F = F^p + F^c$, i.e., the deformation between the ex vivo configuration $B_c$ and the in vivo loaded configuration $B_0$, while the in vivo experiments only record the in vivo deformation $F = V_0$, i.e., the deformation between the in vivo unloaded configuration $B_0$ and the in vivo loaded configuration $B_1$. This difference is visualized in Figs. 8 and 9, where we plot the stresses of a biaxial tension test against the ex vivo recordable total elastic stretches $\lambda$ and against the in vivo recordable load-induced stretches $\lambda = x^\lambda/\lambda^\lambda$. First experimental studies on mitral leaflets in the early 1970s were based on ex vivo uniaxial testing of explanted human leaflets in the circumferential direction. To account for the fundamentally different constitutive response before and after collagen stiffening, these early studies introduced two different tangent moduli and reported the pre- and post-translational leaflet stiffnesses to $E_{cc}^{\text{pre}} = 10$ kPa and $E_{cc}^{\text{post}} = 4833$ kPa averaged over four human leaflets (Ghista and Rao, 1973), and to $E_{cc}^{\text{pre}} = 11.3$ kPa and $E_{cc}^{\text{post}} = 2970$ kPa averaged over 25 human leaflets (Clark, 1973). In the early 1990s, similar ex vivo uniaxial experiments were performed on porcine mitral leaflets, but now in both circumferential and radial directions with $E_{rr}^{\text{pre}} = 44$ kPa, $E_{cc}^{\text{post}} = 5976$ kPa, $E_{rr}^{\text{pre}} = 16$ kPa, and $E_{rr}^{\text{post}} = 1557$ kPa (Kunzelman and Cochran, 1992). Stiffness ranges were similar to the human leaflet, however, this study revealed the characteristic leaflet anisotropy with a three times larger stiffness circumferentially than radially, i.e., in the direction of the principal collagen fiber orientation. A combined uniaxial and biaxial study of porcine mitral leaflets in the mid 1990s confirmed these observations with $E_{cc}^{\text{pre}} = 89.3$ kPa, $E_{cc}^{\text{post}} = 8960$ kPa, $E_{rr}^{\text{pre}} = 60.5$ kPa, and $E_{rr}^{\text{post}} = 2400$ kPa (May-Newman and Yin, 1995). Last, a systematic study on healthy and diseased human mitral leaflets about a decade ago reported the healthy post-translational moduli to $E_{cc}^{\text{post}} = 3550$ kPa and $E_{rr}^{\text{post}} = 2292$ kPa, and found these values to double in the diseased state (Grande-Allen et al., 2005). In summary, stiffness values of all studies lie consistently in the same range, even for different species, with a stiffening of approximately two orders of magnitude between the pre- and post-translational regimes.

A few years ago, a study based on marker videofluoroscopy and linear inverse finite element analysis reported the first in vivo mitral leaflet stiffness in the beating ovine heart (Krishnamurthy et al., 2008). Surprisingly, the study identified the circumferential and radial leaflet stiffnesses to $E_{cc} = 43,000$ kPa and $E_{rr} = 11,000$ kPa, stiffness values that were consistently one and three orders of magnitude larger than the previously reported pre- and post-translational stiffness values (Krishnamurthy et al., 2009). This tremendous stiffness difference between ex vivo and in vivo data stimulated vivid discussions and ongoing debate about the possible mechanisms of in vivo stiffening. A potential explanation is active muscle cell contraction, present in vivo but not ex vivo (Ioth et al., 2009; Skallerud et al., 2011). To quantify the impact of active contraction throughout the cardiac cycle, stiffness values were identified during isovolumetric contraction and isovolumetric relaxation with circumferential and radial stiffnesses increasing by 41.3% and 54.5% during the contractile phase (Krishnamurthy et al., 2009); yet, not enough to explain a difference of orders of magnitude. To increase active contraction, the mitral valve complex was subjected to vagal nerve stimulation to further increase the circumferential and radial stiffnesses by 63.6% and 80.0% (Ioth et al., 2009); again, not enough to explain a difference of orders of magnitude. The effect of prestrain, as discussed in this paper, could easily explain these controversies, however, only in combination with a nonlinear constitutive equation.

Up until the mid 1990s, it was quite common in the bioengineering community to model the constitutive behavior of biological membranes through a bi-linear model with two distinct stiffness values in the pre- and post-translational regimes. The first study to use an exponential Fung-type free energy function for biological membranes was proposed two decades ago (Humphrey et al., 1992). It was soon adapted for porcine mitral leaflets using the free energy function $\psi = c_0 [\exp(c_1 (f_{rr}^2 - 3))^2 + c_2 (f_{cc}^2 - 1)^2] - 1$ with the following three material parameter values $c_0 = 0.399$ kPa, $c_1 = 4.325$, $c_2 = 1446.5$ (May-Newman and Yin, 1998) calibrated with earlier ex vivo data (May-Newman and Yin, 1995). Here, we used a slight modification of this free energy function $\psi = c_0 [\exp(c_1 (f_{rr}^2 - 3)^2 + c_2 (f_{cc}^2 - 1)) - 1]$ as introduced in Eq. (16). When calibrated with the same ex vivo data (May-Newman and Yin, 1995), its parameters take the following values $c_0 = 0.0520$ kPa, $c_1 = 4.63$, and $c_2 = 22.6$ (Prot et al., 2007). When calibrated with in vivo data using marker videofluoroscopy and nonlinear inverse finite element analysis, we found its parameters to take the following values $c_0 = 119.020.7$ kPa, $c_1 = 152.4$, and $c_2 = 185.5$, see Table 1. Qualitatively, these in vivo parameters of the nonlinear model lie within the same range as the in vivo parameters of the linear model (Krishnamurthy et al., 2008). However, consistent with the ex vivo and in vivo parameters of the linear constitutive model, the ex vivo and in vivo parameters of the nonlinear constitutive model differ by orders of magnitude. This raises the question which parameters we should use in future simulations? Or, more provocingly, how useful are ex vivo determined material parameters when we try to make predictions of a living system?

In linear finite element simulations with the in vivo fitted parameters, the computational analysis overestimated the stresses by a factor three beyond the failure stress (Krishnamurthy et al., 2009). In nonlinear finite element simulations with the ex vivo fitted parameters, the computational analysis overestimated the structural deformations by a factor two (Prot et al., 2007). The nonlinear model we propose here is calibrated to in vivo data (Rausch et al., in press); its computational analysis inherently predicts the correct structural deformations for all prestrain levels. So the question is rather, what is the correct prestrain level?

Recent prestrain measurements indicate an area reduction of 43% upon leaflet explantation (Amini et al., 2012). Assuming the prestrain is isotropic in the leaflet plane $F^p = \lambda^p [I - m_0 \otimes m_0] + [m_0 \otimes m_0]/\lambda^{2p}$, this would correspond to a prestretch of $\lambda^p = 1/\sqrt{[1.00 - 0.43]} = 1.32$. For this prestretch level, we interpolate the following parameter values $c_0 = 26.7$ kPa, $c_1 = 1.5$, and $c_2 = 6.8$ from the data in Table 1, illustrated also in Fig. 6. Although not identical, these in vivo
parameter values with $\lambda^p = 1.32$ prestretch are much closer to the previously reported ex vivo parameter values of $c_0 = 0.052$ kPa, $c_1 = 4.63$, $c_2 = 22.6$ (Prot et al., 2007).

6.2. Limitations

We view this study as a first prototype analysis of prestrain and residual stress in thin biological membranes. As such, it provides valuable insight into the interplay between prestrain-induced and load-induced deformation. Despite these promising first results, some limitations are inherent to the proposed method. Some have already been addressed in detail in the previous publications, e.g., limitations related to the experimental data acquisition (Bothe et al., 2011; Kvitting et al., 2010), limitations related to data averaging over 57 animals (Rausch et al., 2011, 2012), limitations related to creating a smooth surface from 23 discrete points (Göktepe et al., 2010), limitations related to the inverse finite element analysis itself (Krishnamurthy et al., 2008), limitations related to additional parameters such as chordae stiffness and leaflet thickness (Rausch et al., in press), and limitations related to differences in species such as pig, sheep, and human (Rausch et al., 2011). Additional potential limitations specific to this particular study are limitations related to this specific constitutive model (May–Newman and Yin, 1998; Prot et al., 2007), limitations related to the assumption of a transversely isotropic prestrain (Amini et al., 2012), and limitations related to the compatibility of prestrain (Buganza Tepole et al., 2011; Famaey et al., 2013).

7. Conclusion

This study has, for the first time, systematically quantified the effects of prestrain and residual stress in thin biological membranes in vivo. Previous studies had revealed three unresolved discrepancies in kinematic, equilibrium, and constitutive properties derived from ex vivo and in vivo measurements: Ex vivo strains were larger by a factor two than in vivo strains; in vivo stresses were larger by a factor three than ex vivo failure stresses; and, most drastically, in vivo stiffnesses were up to three orders of magnitude larger than ex vivo stiffnesses. Here we have shown that all three discrepancies can be explained by the concept of prestrain. Since the degree of prestrain in thin biological membranes has not been fully characterized to date, we systematically explored the effect of different prestrain levels, first in an in vivo parameter identification, then in an ex vivo biaxial test. Our studies of the anterior mitral leaflet reveal that the reported area reduction of 43% upon leaflet explantation associated with a prestretch of 1.32 would indeed reduce the membrane stiffness from 119,020.7 kPa for the prestrain-free case to 26.7 kPa for the reported prestrain level, an apparent stiffness reduction of four orders of magnitude. While these numbers might be specific to the anterior mitral leaflet, we believe that other thin collagenous membranes such as the fetal membrane, liver capsule, renal capsule, ear drum, pericardium, peritoneum, pia mater, dura mater, and skin display conceptually similar characteristics. Our findings suggests that prestrain plays a critical role in the mechanics of thin biological membranes. Neglecting its effects might fundamentally change the underlying load carrying mechanisms and might result in significantly under- or over-estimated material and structural properties. As such, our findings have direct implications in medical device design, in tissue engineering, and in other fields of material sciences targeted at designing replacement materials which resemble the native characteristics of thin biological structures.

Acknowledgments

This study was supported by the Stanford University BioX Fellowship to Manuel Rausch, and by the National Science Foundation CAREER award CMMI 0952021, by the National Science Foundation INSPIRE Grant 1233054, and by the National Institutes of Health Grant U54 GM072970 to Ellen Kuhl.

References
