Modeling of cardiac muscle thin films: Pre-stretch, passive and active behavior

Citation

Published Version
doi:10.1016/j.jbiomech.2011.11.024

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:11949243

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Open Access Policy Articles, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#OAP

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
Modeling of Cardiac Muscle Thin Films: Pre-stretch, Passive and Active Behavior

Jongmin Shim¹, Anna Grosberg², Janna C. Nawroth³, Kevin Kit Parker², and Katia Bertoldi¹

¹School of Engineering and Applied Science, Harvard University, Cambridge, MA
²Disease Biophysics Group, Wyss Institute for Biologically Inspired Engineering, School of Engineering and Applied Science, Harvard University, Cambridge, MA
³Division of Biology, California Institute of Technology, Pasadena, CA

March 18, 2014
Abstract

Recent progress in tissue engineering has made it possible to build contractile bio-hybrid materials that undergo conformational changes by growing a layer of cardiac muscle on elastic polymeric membranes. Further development of such muscular thin films for building actuators and powering devices requires exploring several design parameters, which include the alignment of the cardiac myocytes, the thickness and Young’s modulus of elastomeric film. Moreover, while the pre-stretched deformation of unconstrained thin films during myogenesis and tissue development has to be taken into account, very little research has been reported on this issue. To help exploring these design parameters, we propose a 3-D phenomenological constitutive model which accounts both for the pre-stretch induced deformation and the active and the passive behavior of the cardiomyocytes. The proposed 3-D constitutive model is implemented within a finite element framework, and can be used to improve the current design of bio-hybrid thin films and help developing bio-hybrid constructs capable of complex conformational changes.
1 Introduction

The field of tissue engineering is rapidly moving toward rebuilding living tissues and organs through the development of stem-cell-derived cells and in vitro manufacturing of extracellular matrix proteins (Place et al., 2009). Organs and tissues adopt complex 3-D structures and dynamics, so the capability to model the conformation of engineered tissues is essential for their design. Contractile bio-hybrid materials have been built by growing a monolayer of spatially aligned cardiac myocytes on synthetic elastomeric thin films (Feinberg et al., 2007). When the bio-hybrid film is released into solution and electrically stimulated, the myocytes contract, forcing the construct into adopting a 3-D conformation (Feinberg et al., 2007; Alford et al., 2010). The development of such muscular thin films (MTFs, i.e., a rectangular construct actuated by aligned cardiomyocytes) for building actuators and powering devices requires exploring several design parameters which include the arrangement of the cardiac myocytes and the thickness and Young’s modulus of the elastomeric film.

Finite element (FE) analysis provides an efficient way to explore those design parameters, and it will allow researchers to create contractile constructs with non-trivial 3-D geometries (e.g., flexible pumps). Such modeling capability could play an important role in designing constructs that would finish the building process, or self-assemble in vivo. Additionally, it could greatly aid in the development of in vitro assays to test pharmaceutical agents for efficacy and toxicity as well as evaluation of stem cell-derived tissues.

Along with the advances in tissue engineering, FE methodology has been used to investigate the behavior of anisotropic bio-materials (Spencer, 1984; Weiss et al., 1996) and skeletal muscle (Blemker et al., 2005; Böl and Reese, 2008; Calvo et al., 2010). Cardiac muscle is, like skeletal muscle, characterized by highly organized striated myofibrils; however, it also exhibits spontaneous activity as well as mechanical, chemical and electrical cell-to-cell coupling (functional syncytium) leading to through-conduction of impulses and, subsequently, a highly synchronous response. Recently, a few researchers (Sainte-Marie et al., 2006; Sermesant et al., 2006; Chapelle et al., 2010) proposed physiology-based models for cardiomyocytes, which considers the mechanism of the involuntary contractile behavior of cardiac muscle due to the action potential under calcium flux and captures the characteristic energy dissipative process of the cardiomyocyte active contribution. However, those physiology-based models are mathematically complex and the identification of the model parameters is very challenging. To overcome this difficulties, Böl et al. (2009) proposed a phenomenological model which
neglects the energy dissipation of the active contribution, leading to a formulation where parameter identification is much easier. In this article, authors put emphasis on the simplicity of the model, taking a phenomenological approach while capturing all the main features of the cardiac muscle cell behavior.

A successful constitutive model of cardiac muscle should capture the active behavior including both systole and diastole and the passive behavior inducing pre-stretch deformation of the muscle cells. Currently there is no phenomenological model capable of capturing all those features. Although Böll et al. [2009] successfully modeled the active behavior of cardiac muscle cells on thin films using 1-D truss-type elements, their model is not able to predict the deformation observed in unconstraint MTFs.

Here, we propose a 3-D phenomenological constitutive model which accounts for both the thin film deformation due to pre-stretch during the cell maturation and the active and passive behavior of the cardiac muscle cells. The proposed constitutive model is implemented within a FE framework, and used to reproduce the experimental results of diastolic and systolic conformations in a MTF. Particularly, in order to accommodate the variation in mechanical properties observed in cultured cardiac tissue, a range of values is identified for both the induced pre-stretch and the isometric twitch stress. This paper is organized as follows: Section 2 details the experimental data analysis and the corresponding results. In Section 3, the constitutive model is presented for both the elastomeric silicone layer and the cardiac muscle layer. Section 4 demonstrates the model parameter identification results based on literature and experiments with straight cell alignment along the longitudinal direction of MTF. As a validation of the proposed model, Section 5 shows comparison between the numerical and experimental results for a construct with tilted cell alignment. In addition, parametric studies are also presented to investigate the influence of the thin film thickness, isometric twitch stress, and pre-stretch deformation on MTF conformational changes.

In the following, we will refer to “active” behavior when describing magnitude and time course of stresses and MTF (i.e., a rectangular construct actuated by aligned cardiomyocytes) deformation resulting from cardiomyocytes contraction. These strains and stresses are increasing during the systolic phase until peak systole is reached, and are decreasing during diastole until a fully relaxed state is attained. However, even at this fully relaxed state, MTF curvature might be present due to the pre-stress of the cells, and resulting pre-stretch of the substrate, introduced during tissue maturation. These pre-stresses and strains that are present even at the fully relaxed state will be refereed to as “passive” behavior.
2 Experiments

This section focuses on the data analysis procedure used to obtain the MTF curvature from recorded data movies; the detailed experimental protocols to fabricate MTFs (see Fig. 1A for the controlled intracellular alignment of sarcomeres and Fig. 1B for its schematic) are provided in Section S1 of the supporting material.

2.1 Data Analysis

Two different cell alignments were investigated. In the first case, the cells were aligned along the longitudinal axis of the thin film (for short, straight cell alignment, Fig. 1C (top)), and in the second case, the cells were aligned at an angle of approximately 30° relative to the longitudinal axis of the thin film (diagonal cell alignment, Fig. 1C (bottom)). While the former sets of experiments were designed to identify the material parameters of the proposed 3-D constitutive model, the latter sets were used to validate the proposed model.

We assume that the deformed shape of the film is described by the arc of a circle, so that the curvature of the film, $K$, can be calculated by measuring the projection length $x$ and following the simple relation:

$$ x = \begin{cases} R \sin \left( \frac{L_0}{R} \right), & \text{if } x > \frac{L_0}{\pi} \\ R, & \text{if } x \leq \frac{L_0}{\pi} \end{cases} $$

where $R = 1/K$ is the radius of curvature, and $L_0$ is the longitudinal length of MTF. The geometric relation of $x$, $L_0$ and $R$ is shown in Fig. 1D.

After the experiments were completed the data movies were filtered to remove noise, made binary using image processing software (ImageJ, NIH), and processed using MatLab (Mathworks, Natick, MA) to calculate the curvature of the film. For the case of straight cell alignment, the MTF curvature is obtained from the measured projection length, by directly applying Eq. (1). However, with diagonal cell alignment case, due to the non-trivial conformation of the bio-hybrid film, a more elaborated procedure was developed. A parameter, $\alpha$, is defined as the angle between the film’s free and fixed edges (see Fig. 1C (bottom)). We define the base of the film as the line marking the border between the portion of the film lying on the glass and the free section (black dashed line in Fig. 1C). The $x$-projection length is defined as the distance between the base of the film and the free edge of the film (green solid line in Fig. 1C). Then, the average of temporal MTF curvature (i.e., the prevailing curvature of the MTF) was obtained.
by minimizing the standard deviation of the temporal curvature collection calculated along the bottom edge of the film. In order to have consistent comparison between the FE model and the experiment results, the movies produced by the simulations were also analyzed using the same software as the experimental data movies.

2.2 Experimental Results

We tested the contractile response of MTFs with three different polydimethylsiloxane (PDMS, Sylgard 184-Dow Corning, Midland, MI) thickness (i.e., 14.5\(\mu\)m, 18.0\(\mu\)m, 23.0\(\mu\)m) for the constructs with straight cell alignment and single PDMS thickness (i.e., 14.5\(\mu\)m) for the constructs with tilted cell alignment case. Test results for the straight cell alignment case are presented in Fig. 2A-D; Fig. 2A for the a snapshots from the recorded movie and Figs. 2B-D for the time-curvature plots. Similarly, Figs. 2E and 2F summarize test results from diagonal cell alignment case. A non-negligible variation of the time-curvature plots under identical experimental conditions can be easily observed. This is due to the inherent variability of biological samples, and it is accommodated by identifying a range of values for two material constants entering into the proposed 3-D constitutive, as described in Section 4.

3 Constitutive Modeling of Bio-hybrid Films

While the detailed development of the proposed constitutive model is provided in Section S2 of the supporting material, the key equations are highlighted in this section.

Let \(\mathbf{F} = \frac{\partial \mathbf{x}}{\partial \mathbf{X}}\) be the deformation gradient mapping a material point from the reference position \(\mathbf{X}\) to its current position \(\mathbf{x}\), and \(J = \det \mathbf{F}\) be its determinant. The behavior of nearly incompressible materials is effectively described by splitting the deformation locally into volumetric (denoted by superscript \(v\)) and isochoric (denoted by superscript \(i\)) components as

\[
\mathbf{F} = \mathbf{F}^v \cdot \mathbf{F}^i, \quad \text{where} \quad \mathbf{F}^v = J^{1/3} \mathbf{1}, \quad \mathbf{F}^i = J^{-1/3} \mathbf{F}. \tag{2}
\]

3.1 Elastomeric Substrate

The elastomeric substrate is fabricated using PDMS, and its behavior is well captured using a neo-Hookean model. Based on the kinematic assumption
shown in Eq. (2) with $C_i = F^i T \cdot F^i$ and $\bar{I}_1 = \text{tr} C_i$, a decoupled form of the strain energy density (Gurtin et al., 2010) is given by

$$
\psi = \psi^v (J) + \psi^i (\bar{I}_1) = \frac{\tilde{\kappa}}{2} (J - 1)^2 + \frac{\tilde{E}}{6} (\bar{I}_1 - 3),
$$

where $\tilde{\kappa}$ and $\tilde{E}$ denote the bulk modulus and the initial elastic modulus of the elastomer, respectively. The Cauchy stress $T$ is found by differentiating $\psi$ with respect to $C$, yielding

$$
T = \tilde{\kappa}(J - 1)1 + \frac{\tilde{E}}{3J} \text{dev}(B^i).
$$

where $B^i = F^i T \cdot F^i$ and “dev” stands for deviatoric part of 2nd order tensors.

### 3.2 Cardiac Muscle Cells

The characteristic energy dissipative process of the cardiomyocyte active contribution can be captured by recently developed physiology-based models, which consider mechanism of the involuntary contractile behavior of cardiac muscle due to the action potential under calcium flux (Sainte-Marie et al., 2006; Sermesant et al., 2006; Chapelle et al., 2010). However, this approach is mathematically complex, and model parameter identification is challenging. In this article, instead, we take a simple, but effective phenomenological approach, neglecting the energy dissipative process. While recently Böl et al. [2009] developed a phenomenological model of cardiac muscle cells, their model neglects the important effect of the pre-stretch that the muscle cells develop as they mature. This paper presents a 3-D phenomenological model that captures the two major features of cardiac muscles: the passive behavior inducing pre-stretched deformation and their active behavior including systole and diastole.

#### 3.2.1 Kinematics including pre-stretch

Even when resting, muscle cells in vivo experience a state of stress due to their pre-stretched conditions. This can be clearly observed from the experimental data reported in Figs. 2B-D and F, showing a non-negligible MTF curvature during diastole. In order to account for such pre-stretched conditions, previous constitutive models introduced an ad hoc strain-shift in the stress-strain curve (Blemker et al., 2005; Böl et al., 2009; Calvo et al., 2010) leading to a non-unique material response that depends on the level...
of pre-stretch of muscle cells. Moreover, such approach does not capture the non-negligible MTF curvature observed during diastole. Thus, in this paper, a different approach is adopted for pre-stretch modeling; inspired by the multiplicative decomposition introduced by Kroner [1960] and Lee [1969], the isochoric deformation gradient $F^i$ in Eq. (2) is decomposed into load-induced, $F^{iL}$, and pre-stretched, $F^{iS}$, contributions

$$F^i = F^{iL} \cdot F^{iS}. \quad (5)$$

Here, for the sake of simplicity, the pre-stretch is assumed to be fully developed during cell differentiation and maturation prior to the experiment, and not to be affected by the cell active response. If we also assume that the pre-stretch deformation is incompressible and the cells deform affinely, the pre-stretched contribution to the deformation gradient $F^{iS}$ is given by

$$F^{iS} = QAQ^T, \quad (6)$$

with

$$\Lambda = \hat{\lambda}_S \mathbf{e}_1 \otimes \mathbf{e}_1 + \left(\hat{\lambda}_S\right)^{-\frac{1}{2}} (\mathbf{e}_2 \otimes \mathbf{e}_2 + \mathbf{e}_3 \otimes \mathbf{e}_3), \quad (7)$$

$$Q = \cos \hat{\theta} (\mathbf{e}_1 \otimes \mathbf{e}_1 + \mathbf{e}_2 \otimes \mathbf{e}_2) + \sin \hat{\theta} (-\mathbf{e}_1 \otimes \mathbf{e}_2 + \mathbf{e}_2 \otimes \mathbf{e}_1) + \mathbf{e}_3 \otimes \mathbf{e}_3, \quad (8)$$

where $\hat{\lambda}_S$ is the pre-stretch induced into the cardiac myocytes lying in the $x_1$-$x_2$ plane during maturation (i.e., myogenesis and tissue development) and $\hat{\theta}$ is the angle identifying the cell alignment in the undeformed configuration (see Fig. 1B). The diastole curvatures of the experimental data reported in Figs. 2B-D and F clearly show a deviation in the pre-stretched response of the cells as a result of the diversity in cell conditions. Therefore, we expect the parameter $\hat{\lambda}_S$ not to be uniquely determined, but to be influenced by experimental factors such as small variations in temperature/humidity, local density variations, local intracellular architecture, and cardiac origin of the cells (left or right ventricle) of the cells.

### 3.2.2 Constitutive equations for passive and active behavior

When an isotropic material is reinforced by a family of fibers with direction $a_0 = \cos \theta \mathbf{e}_1 + \sin \theta \mathbf{e}_2$ in the reference configuration (Fig. 1B), the isochoric part of the strain energy can be expressed as a function of not only the invariants of $C$, but also additional invariants depending on $a_0$. To describe both passive (representing resting status) and active (including systole and
diastole) behavior of cardiomyocytes, we specify a decoupled form of free energy as

\[ \psi = \psi^v(J) + \psi^i_{\text{iso}}(\bar{I}_1) + \psi^p_{\text{ani}}(\bar{I}_4, q), \]  

(9)

where \( \bar{I}_1 = a_0 \cdot C^i \cdot a_0 \) and \( q \) is the activation level of cardiac muscle cells. In the proposed model, while \( \psi^v \) and \( \psi^i_{\text{iso}} \) reflect the volumetric and isotropic contributions of the intercellular part, \( \psi^p_{\text{ani}} \) and \( \psi^a_{\text{ani}} \) represent the passive and the active contributions of anisotropic effect of the myofibril, respectively. Their detailed forms can be found in the Section S2 of the supporting material. By differentiating \( \psi \) with respect to \( C \), the resulting Cauchy stress for the cardiac muscle cells is obtained as

\[ T = T^v + T^i_{\text{iso}} + T^p_{\text{ani}} + T^a_{\text{ani}}, \]  

(10)

where

\[ T^v = \hat{\kappa} (J - 1) \mathbf{1}, \]  

(11)

\[ T^i_{\text{iso}} = \frac{\hat{E}_c}{3J} \text{dev}(\mathbf{B}^i), \]  

(12)

\[ T^p_{\text{ani}} = \frac{\hat{P}_q}{\hat{\lambda}} \left[ e^{\hat{\alpha} (\hat{\lambda} - 1)} - 1 \right] \text{dev}(\bar{a} \otimes \bar{a}), \]  

(13)

\[ T^a_{\text{ani}} = \begin{cases} \frac{\hat{P}_q}{\hat{T}} \left[ 1 - \left( \frac{\hat{\lambda} - \hat{\lambda}_o}{1 - \hat{\lambda}_o} \right)^2 \right] \text{dev}(\bar{a} \otimes \bar{a}), & \text{if } 1 < \hat{\lambda} < (2\hat{\lambda}_o - 1), \\ 0, & \text{otherwise.} \end{cases} \]  

(14)

Here, \( \hat{\lambda} = \sqrt{\bar{I}_4}, \bar{a} = \mathbf{F}^i \cdot a_0 / \hat{\lambda}, \)

\[ q(t) = \left( \frac{t}{\hat{T}} \right)^2 \exp \left[ 1 - \left( \frac{t}{\hat{T}} \right)^2 \right], \]  

(15)

and the physical meaning of all the model parameters (i.e., \( \hat{\kappa}, \hat{E}_c, \hat{E}_p, \hat{\alpha}, \hat{P}, \hat{\lambda}_o, \hat{T} \)) is summarized in Table 1.

### 3.2.3 Model Implementation

The constitutive model outlined in this section has been implemented into the commercial finite element code ABAQUS/Explicit, using a user-defined subroutine VUMAT, and Table 2 summarizes the model and its implementation. Each FE analysis consists of two steps: the first step simulates the
development of pre-stretch into the non-activated cells; in the second step, the active response of MTFs is simulated under fully pre-stretched conditions.

4 Model Parameter Identification

In this section, we present the procedure followed to identify the parameters entering in the constitutive model. Additional details are provided in S3 of the supporting material.

While the material parameters for the elastomeric substrate ($\tilde{E} = 1.5 \text{MPa}, \tilde{\kappa} = 25 \text{MPa}$) are provided by the manufacturers, the parameters needed for the cardiac muscle cells are obtained both from literature and from contraction assays performed with neonatal rat ventricular myocytes micropatterned on PDMS thin films.

4.1 Parameters Identified from Literature

The paper by Weiwad et al. [2000] provides in a single article nearly all the required test results to identify the material parameters entering into the proposed material model. Moreover, all their data are in a good agreement with similar test results for the heart muscle of adult rats (i.e., Granzier and Irving [1995] and Palmer et al. [1996] for passive behavior and Nishimura et al. [2000] and Palmer et al. [1996] for active behavior). Thus, the test results presented in Weiwad et al. [2000] are used to identify five material-specific parameters entering into the proposed constitutive model.

From their tensile tests of the non-activated cells (shown in Fig. 3A), we determine three model parameters, i.e. $\hat{E}_c = 21 \text{kPa}, \hat{E}_p = 2.3 \text{kPa}$, and $\alpha = 5.5$ by curve-fitting the test results with Eq. (12) and Eq. (13) specialized to uniaxial loading conditions. The experimental results obtained for the isometric twitch stress (Fig. 3B) guide us to propose a quadratic form of active stress profile shown in Eq. (14), and they determine $\hat{\lambda}_o = 1.24$. Finally, for the sake of simplicity, we assume that the cardiac muscle is nearly incompressible and its volumetric behavior is determined by the passive response, resulting in a bulk modulus of $\hat{\kappa} = 380 \text{kPa}$.
4.2 Parameters Identified from MTF Tests with Straight Cell Alignment

Experiments with straight cell alignment (Section 2) are used to identify the remaining three model parameters \((\hat{P}, \hat{\lambda}_S, \hat{T})\). While \(\hat{P}\) and \(\hat{\lambda}_S\) are experimental-conditions-specific, \(\hat{T}\) denotes the characteristic time scale of contractile behavior under the stimulating electric pulse. First, \(\hat{T} = 0.21\, \text{sec}\) is identified by curve-fitting a typical profile of the active amplitude history shown in Fig. 3C with the proposed function for \(q(t)\).

Secondly, values for both \(\hat{\lambda}_S\) and \(\hat{P}\) are determined using experimental data presented in Figs. 2B-D. As in the experiments, MTFs with PDMS substrate of thickness \(\hat{t} = 14.5, 18.0, \) or \(23.0\, \mu \text{m}\) and cardiac myocytes thickness \(\hat{t} = 4\, \mu \text{m}\) are modeled and simulated. Since the experiments show a significant variability in the material response for each curvature history shown in Figs. 2B-D, both \(\hat{\lambda}_S\) and \(\hat{P}\) are determined to achieve the best fit which leaving all the other model parameters unchanged. Thus, a range of values is identified for \(\hat{\lambda}_S\) and \(\hat{P}\) whose distributions are shown in Figs. 3D and E, respectively. More specifically, we obtain (a) \(\hat{\lambda}_S \in [1.11, 1.16]\) and \(\hat{P} \in [2.8, 7.3]\, \text{kPa}\) for \(\hat{t} = 14.5\, \mu \text{m}\), (b) \(\hat{\lambda}_S \in [1.12, 1.14]\) and \(\hat{P} \in [9.2, 21.6]\, \text{kPa}\) for \(\hat{t} = 18.0\, \mu \text{m}\), and (c) \(\hat{\lambda}_S \in [1.16, 1.18]\) and \(\hat{P} \in [6.9, 8.5]\, \text{kPa}\) for \(\hat{t} = 23.0\, \mu \text{m}\). While for the considered tests rather small variation in the pre-stretch of 1.11 \(\sim\) 1.18 is observed, we find a quite large variation in the isometric twitch stress ranging from 2.7 to 21.6\, \text{kPa}. Figures 3F-H clearly show that the model correctly capture the pre-stretch curvature induced during cell maturation and the the active response period.

5 Numerical Simulation Results

In this section, we first validate the proposed model by simulating MTFs with diagonal cell alignments, and then present a series of parametric studies to investigate the effect of PDMS thickness, isometric twitch stress, and pre-stretch of cells.

5.1 Model Validation for MTFs with Diagonal Cell Alignment

In order to validate the proposed model, the identified model parameters listed in Table 1 are used to simulate the behavior of MTF with diagonal cell alignment. To incorporate the variation of cell conditions observed in
MTFs with straight cell alignment, we perform simulations with two extreme sets of parameters: one with $\lambda_S = 1.11$ and $P = 2.8kPa$ (weakest cell conditions), and the other with $\lambda_S = 1.18$ and $P = 21.6kPa$ (strongest cell conditions). As shown in Figs. 4A-B, the FE simulations qualitatively capture the experimentally observed behavior of the MTFs with diagonal cell alignment (Fig. 2E). In addition, Fig. 4C clearly show that all the experimental time-curvature data are bounded by the simulation results obtained using extreme cell conditions.

5.2 Parametric Studies

Various formulations (Atkinson, 1995; Alford et al., 2010) have been proposed to evaluate stress within multi-layer thin films because the direct measurement of the stress within the cells is experimentally much more challenging than the curvature measurement. Yet, most of these are essentially extensions or modifications of Stoney’s approximate plate analysis (Stoney, 1909). Using the proposed 3-D constitutive model, FEM simulations can provide more accurate predictions for stress and strain distribution. Here, we explore the effect of important parameters within MTF (e.g., the PDMS thickness, the isometric twitch stress of cells, and the pre-stretch of cells) on the stress history. While the details of the simulations are summarized in Section S4 of the supporting material, this section focuses on the effect of those parameters on the stress history within the cells.

Figure 5A shows the effect of PDMS thickness on the average of maximum principal stress within the cardiac cells, denoted by $\bar{S}$. In order to obtain $\bar{S}$, we collect the time history of the maximum principal stress within all the cardiac cell elements, and then calculated the spatial average. It is interesting to observe that the results of the FEM simulations show a negligible effect of the PDMS thickness on the cells stress. To better quantify this behavior, we decompose $\bar{S}$ into the contribution of passive ($\bar{S}_p$) and active response ($\bar{S}_a$),

$$\bar{S} = \bar{S}_p + \bar{S}_a,$$

and plot their maximum values ($\max(\bar{S}_p)$, $\max(\bar{S}_a)$) separately in Fig. 5B, confirming no effect of the PDMS thickness on the cells stress.

In the experiments, we observed that the active response of cells largely changes from test to test. In the proposed model, such variation in the cell response can be studied by exploring the value of the isometric twitch stress, $\hat{P}$. Simulations results reported in Figs. 5C-D show that the active stress...
within the cells linearly increases as the isometric twitch stress increases, as formulated in Eq. (2).

Finally, it is well known that the pre-stretch within the cells is largely affected by the substrate and the mechanical/chemical stimulus during maturation [Wang et al., 2002; Griffin et al., 2004; Engler et al., 2008]. We investigate systematically the effect of pre-stretch ($\lambda_S$), and the results are reported in Figs. 5E-F. Figure 5F shows that a linear model, $\bar{S}_{\text{linear}} = (\dot{E}_c + \dot{E}_p)\ln\lambda_S$, poorly predicts the stress distribution within the cells (denoted by $\bar{S}_{\text{linear}}$). It is interesting to observe that the case with no pre-stretch (i.e., $\lambda_S = 1.00$) results in no stress within the cells. The simulations also recover the exponential form of stretch-stress relation for the passive fiber response and the quadratic relation for the active fiber response. From the quadratic relation between the pre-stretch and the active stress contribution, we observe an optimal pre-stretch value at which a maximum level of active response/stress (i.e. maximum active mobility) for the structures is achieved.

6 Discussion and Conclusions

6.1 Discussion

In this paper, we report experimental results obtained from two different cell alignments of MTFs (straight and diagonal cell alignments), where the curvature of the films are calculated from the recorded movies. Using the simple procedure based on the measured projection length, we quantified the curvature of the straight and tilted cell alignment MTFs, suggesting that the procedure can be used for non-trivial shapes of MTFs such as swimmers. In order to reproduce and predict the observed experimental behavior of MTFs, we propose a 3-D phenomenological constitutive model that captures the pre-stretch induced deformation as well as the active and the passive behavior of the cardiomyocytes. Inspired by the multiplicative decomposition introduced by Kroner [1960] and Lee [1969], the isochoric deformation gradient is decomposed into load-induced and pre-stretched contributions so that the effect of the pre-stretch can be independently investigated. With the purpose of introducing a simple model parameter identification procedure, we propose a phenomenological model in which a simple elastic model is adopted to describe the cardiomyocyte active contribution and all the different aspects of constitutive behaviors are decoupled. All the parameters entering into the proposed model are identified from literature and a reference test set (i.e., MTFs with straight cell alignment). Particularly, the
variation in mechanical properties observed in cultured cardiac tissue experiments with MTFs are accounted for by identifying a range of values the induced pre-stretch ($\hat{\lambda}_S$) and the isometric twitch stress ($\hat{P}$). By considering those two parameters as experiment/cell-dependent variables, we confirms that the FE simulation quantitatively and qualitatively captures the behavior of the MTFs with diagonal cell alignment. However, the proposed model does have the following modeling limitations.

Scalar internal variables have been often introduced into the free energy in order to describe the rich behavior of plasticity (Brown et al., 1989) as an energy dissipating mechanism. However, the scalar internal variable, $q$ in Eq. (9), introduced in this study represents the active cell response energy which is supplied from the external system or generated within the cell. Thus, the introduction of the activation level $q$ leads to a formulation that does not satisfy the free energy imbalance conditions. In order to resolve this thermodynamical inconsistency, several researchers have proposed physiology-based constitutive models which introduce a chemical potential into the constitutive model, inducing the mechanical motions of the muscle (Sainte-Marie et al., 2006; Sermesant et al., 2006; Chapelle et al., 2010). However, the introduction of the corresponding chemical potential leads to mathematically complex constitutive models which require challenging model parameter identification procedure; for example, Sermesant et al. [2006] used a sophisticated optimization procedure based on the magnetic resonance imaging of hearts. Thus, we take a simple, but effective phenomenological approach (Blemker et al., 2005; Böl et al., 2009), where the active behavior is assumed to be elastic and its model parameters can be easily identified. Our distinctive contribution compared to the previous phenomenological models is that the proposed phenomenological model accounts for the thin film deformation due to pre-stretch during the cell maturation as well as the active and passive behavior of the cardiac muscle cells.

Most theories of metal plasticity (Brown et al., 1989), approximately incompressible elastic materials (Anand, 1996), and fiber-reinforced composite (Spencer, 1984) have been based on a separable form of free energy (which is so-called separability hypothesis (Gurtin et al., 2010)) considering separately the contributions of plastic, volumetric and anisotropic deformation. In these fields, the decoupling approach has been considered as an effective way to incorporate various aspect of the complex material behavior. Inspired by this hypothesis, we decouple all the aspects of the muscle as shown in Eq. (9) although it is not based on experimental evidence. By taking this approach, the procedure for identifying model parameters has
been substantially simplified; most model parameters can be identified from literature, and only a few parameters need to be identified from reference tests on the cardiomyocytes of interest.

The proposed model has been numerically implemented for two/three dimensional solid (brick) elements, which are ideal for bulky structures having similar dimensions in all two/three directions. Thus, simulations of the shell-type structures require high computational cost since the smallest dimension (i.e., thickness) controls the critical mesh size for explicit integration technique. Future work would include the implementation of formulation for shell elements. Lastly, the current simulations neglect the contribution of the fluid surrounding the MTF, which is present for all the cardiomyocyte experiments. To account for the effect of the surrounding fluid, the proposed constitutive model may be used within a suitable fluid-structure interaction scheme.

6.2 Conclusions

The 3-D phenomenological constitutive model presented is capable of capturing the MTF deformation due to pre-stretch during the cell maturation as well as the active and passive behavior of the cardiac muscle cells. While a range of values for two model parameters (induced pre-stretch $\lambda$ and isometric twitch stress $\tilde{P}$) are identified to account for the variation in mechanical properties observed in cultured cardiac tissue experiments, all other model parameters are identified from literature. The proposed model is implemented within a FE framework and used to reproduce the experimental results of diastolic and systolic conformations in a muscular thin film. With the identified model parameters, the FE simulations showed an excellent agreement with experiment for the straight cell alinement. The proposed model qualitatively captured the behavior of MTFs with diagonal cell alignment, and all the experimental time-curvature plots were bounded by the simulation results obtained from the two extreme cell conditions.

The proposed constitutive model can be immediately extended to the analysis of constructs with non-trivial 3-D initial geometries. This will greatly aid in the engineering of soft muscle-powered robots. Furthermore, the model has the potential to take into account fluid-structure interactions, which will have application in creating swimming constructs, actuators, micro-fluidics, and modeling passive film behavior.
Acknowledgements

This work has been supported by the Harvard Materials Research Science and Engineering Center under NSF award number DMR-0820484 (KB), DMR-0213805 (KKP), and NIH grant 1 R01 HL079126 (KKP). We are grateful to the Center of Nanoscale Systems at Harvard University for the use of their cleanroom facilities, and to Alexander P. Nesmith for developing the brick patterned stamps.
References


Figure 1: (A) Top: Intracellular alignment of sarcomeres having nuclei (blue), actin (green), alpha-actinin (red). Bottom: a bright-field image of the same tissue showing the overall alignment of cells in the tissue. The length of both scale bars represent $20\mu m$. (B) Schematic of the muscular thin film (MTF). Here, $a_0$ and $\hat{\theta}$ represent the cell alignment vector and the corresponding angle, respectively. (C) Schematic showing the projection of the MTF with straight cell alignment (top) and with diagonal cell alignment (bottom). The $x -$projection is indicated in green, and $\alpha$ in blue. (D) Schematic of MTF illustrating the geometric relation of $x$, $L_0$ and $R$ in Eq. [1].
Figure 2: Experimental results from MTFs with straight cell alignment (A-D) and with diagonal cell alignment (E-F). (A) Snapshot of contractile behavior of MTFs with straight cell alignment (PDMS thickness of 14.5µm) taken at $t = 1.215\text{sec}$. (B-D) Time-curvature plots of MTFs with straight cell alignment for three different PDMS thicknesses, i.e. (B) $\tilde{t}_{PDMS} = 14.5\text{µm}$, (C) $\tilde{t}_{PDMS} = 18.0\text{µm}$, and (D) $\tilde{t}_{PDMS} = 23.0\text{µm}$. (E) Snapshots of contractile behavior MTF with diagonal cell alignment (PDMS thickness of 14.5µm). The green line marks the border of the portion of the film lying on glass. The blue line denotes the film when it is completely flat on the glass. The length of the red segment corresponds to $x$ in Eq. [1], while the sum of the lengths of the purple segment and red segment is $L_0$ in Eq. [1]. (F) Time-curvature plots of MTFs with diagonal cell alignment (PDMS thicknesses of $\tilde{t}_{PDMS} = 14.5\text{µm}$).
Figure 3: Model parameter identification from Weiward et al. (2000) (A-B) and from experiments with straight cell alignment (C-H). (A) Tensile behavior of non-activated cells. Blue circle markers denote experimental data, and the red line corresponds to curvefit results with Eq. (S25). (B) Isometric twitch stress as a function of calcium concentration. Markers denote experimental data, and the lines correspond to curvefit results with Eq. (S25). (C) A typical profile of the active amplitude history from tests performed on MTFs with straight cell alignment. Markers denote experimental data, and the line correspond to curvefit results with \( q(t) \) in Eq. (15). (D-E) Range of values identified for \( \lambda \) and \( P \) (F-H) Time-curvature plots of MTFs with straight cell alignment from FE simulations. Note that one-to-one correspondence between experimental (Figs. 2B-D) and numerical (Figs. 3F-H) curves.
Figure 4: Contractile behavior of MTF with diagonal cell alignment (PDMS thickness of 14.5µm) from FE simulations. (A) Snapshots of FE simulation with strongest cell conditions (i.e., pre-stretch of $\lambda_S = 1.18$ and isometric twitch stress of $P = 21.6kPa$), (B) Snapshots of FE simulation with weakest cell conditions (i.e., pre-stretch of $\lambda_S = 1.11$ and isometric twitch stress of $P = 2.8kPa$). (C) Comparison of experimental and numerical time-curvature plots. The green shaded region corresponds to the area bounded by simulations with the two extreme cell conditions considered in this study.
Figure 5: The left column reports the the averaged history of maximum principal stress within cardiac cells ($\bar{S}$) from FE analysis while the right column accounts for the stress contribution of pre-stretch and active response ($\text{max}(S_p)$ and $\text{max}(S_a)$). (A-B) effect of PDMS thickness, (C-D) Effect of isometric twitch stress of cells, (E-F) effect of cell pre-stretch.
Table 1: Summary of model parameters and their physical meaning.

<table>
<thead>
<tr>
<th>Material</th>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMS</td>
<td>$\tilde{E}$</td>
<td>$1.5 MPa$</td>
<td>Initial elastic modulus</td>
</tr>
<tr>
<td></td>
<td>$\tilde{\kappa}$</td>
<td>$25 MPa$</td>
<td>Initial bulk modulus</td>
</tr>
<tr>
<td>Cardiac Muscle Cell</td>
<td>pre-stretched</td>
<td>$\hat{\theta} = 0^\circ, 30^\circ$</td>
<td>Angle defining the initial alignment of the cells within the $x_1 - x_2$ plane</td>
</tr>
<tr>
<td></td>
<td>$\hat{\lambda}_S$</td>
<td>$[1.11, 1.18]$</td>
<td>Pre-stretch developed in the cardiomyocytes during cell maturation</td>
</tr>
<tr>
<td></td>
<td>volumetric</td>
<td>$\hat{\kappa} = 380 kPa$</td>
<td>Initial bulk modulus</td>
</tr>
<tr>
<td></td>
<td>isotropic</td>
<td>$\hat{E}_c = 2.3 kPa$</td>
<td>Initial elastic modulus of intercellular part</td>
</tr>
<tr>
<td></td>
<td>anisotropic</td>
<td>$\hat{E}_p = 21 kPa$</td>
<td>Initial elastic modulus of fiber</td>
</tr>
<tr>
<td></td>
<td>passive</td>
<td>$\hat{\alpha} = 5.5$</td>
<td>Constant related to the slope of stress-strain relation of passive fiber</td>
</tr>
<tr>
<td></td>
<td>active</td>
<td>$\hat{P} \in [2.8, 21.6] kPa$</td>
<td>Isometric twitch stress</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\hat{\lambda}_a = 1.24$</td>
<td>Optimal stretch for the maximum active stress</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\hat{T} = 0.21 sec$</td>
<td>Characteristic time scale for contraction</td>
</tr>
</tbody>
</table>
Table 2: Summary of the model described in Section 3. Note that $g(\tau)$ is an arbitrary time history describing the cell pre-stretch development.

<table>
<thead>
<tr>
<th>Given at time $\tau$</th>
<th>$F^{ABQ}(\tau)$: deformation gradient given by ABAQUS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kinematics</strong></td>
<td>$J(\tau) = \det F^{ABQ}(\tau)$</td>
</tr>
<tr>
<td></td>
<td>$F^{\nu}(\tau) = J(\tau)1$</td>
</tr>
<tr>
<td></td>
<td>$F^{iL}(\tau) = J(\tau)^{-1/3}F^{ABQ}$</td>
</tr>
<tr>
<td></td>
<td>$\Lambda = \hat{\lambda}_S e_1 \otimes e_1 + \left(\hat{\lambda}_S\right)^{-\frac{1}{2}}(e_2 \otimes e_2 + e_3 \otimes e_3)$</td>
</tr>
<tr>
<td></td>
<td>$Q = \cos \hat{\theta} (e_1 \otimes e_1 + e_2 \otimes e_2) + \sin \hat{\theta} (-e_1 \otimes e_2 + e_2 \otimes e_1) + e_3 \otimes e_3$</td>
</tr>
<tr>
<td></td>
<td>$F^{iS}(\tau) = g(\tau)Q \cdot \Lambda \cdot Q^T$</td>
</tr>
<tr>
<td></td>
<td>$F(\tau) = F^{\nu}(\tau) \cdot F^{iL}(\tau) \cdot F^{iS}(\tau)$</td>
</tr>
<tr>
<td><strong>Constitutive equation</strong></td>
<td>$a_0(\tau) = \cos \hat{\theta} e_1 + \sin \hat{\theta} e_2$</td>
</tr>
<tr>
<td></td>
<td>$C^i(\tau) = F^{iT}(\tau) \cdot F^{iT}(\tau)$</td>
</tr>
<tr>
<td></td>
<td>$\hat{\lambda} = \sqrt{a_0 \cdot C^i \cdot a_0}$</td>
</tr>
<tr>
<td></td>
<td>$B^i(\tau) = F^i(\tau) \cdot F^{iT}(\tau)$</td>
</tr>
<tr>
<td></td>
<td>$\bar{a}(\tau) = F^i(\tau) \cdot a_0 / \hat{\lambda}$</td>
</tr>
<tr>
<td></td>
<td>$q(\tau) = \left(\frac{\tau}{T}\right)^2 \exp \left[1 - \left(\frac{\tau}{T}\right)^2\right]$</td>
</tr>
<tr>
<td></td>
<td>$T^u(\tau) = \dot{\hat{\lambda}}(J(\tau) - 1)$</td>
</tr>
<tr>
<td></td>
<td>$T^i_{iso}(\tau) = \frac{E_i}{3J(\tau)} \text{dev} (B^i(\tau))$</td>
</tr>
<tr>
<td></td>
<td>$T^p_{ani}(\tau) = \frac{E_p(\hat{\lambda}(\tau) - 1)}{a(\tau)J(\tau)} \text{dev} (\bar{a}(\tau) \otimes \bar{a}(\tau))$</td>
</tr>
<tr>
<td></td>
<td>$0,$ if $1 &lt; \hat{\lambda}(\tau) &lt; (2\hat{\lambda}_o - 1)$</td>
</tr>
<tr>
<td></td>
<td>otherwise</td>
</tr>
<tr>
<td></td>
<td>$T(\tau) = T^u(\tau) + T^i_{iso}(\tau) + T^p_{ani}(\tau) + T^i_{ani}(\tau)$</td>
</tr>
</tbody>
</table>

24