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ON INTRINSIC STRESS FIBER CONTRACTILE FORCES IN SEMILUNAR HEART VALVE INTERSTITIAL CELLS USING A CONTINUUM MIXTURE MODEL

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Abstract

Heart valve interstitial cells (VICs) play a critical role in the maintenance and pathophysiology of heart valve tissues. Normally quiescent in the adult, VICs can become activated in periods of growth and disease. When activated, VICs exhibit increased levels of cytokines and extracellular matrix (ECM) synthesis, and upregulated expression and strong contraction of α -smooth muscle actin (α -SMA) fibers. However, it remains unknown how expression and contraction of the α -SMA fibers, which vary among different VIC types, contribute to the overall VIC mechanical responses, including the nucleus and cytoskeleton contributions. In the present study, we developed a novel solid-mixture model for VIC biomechanical behavior that incorporated 1) the underlying cytoskeletal network, 2) the oriented α -SMA stress fibers with passive elastic and active contractile responses, 3) a finite deformable elastic nucleus. We implemented the model in a full 3D finite element simulation of a VIC based on known geometry. Moreover, we examined the respective mechanical responses of aortic and pulmonary VICs (AVICs and PVICs, respectively), which are known to have different levels of α -SMA expression levels and contractile behaviors. To calibrate the model, we simulated the combined mechanical responses of VICs in both micropipette aspiration (MA) and atomic force microscopy (AFM) experiments. These two states were chosen as the VICs were under significantly different mechanical loading conditions and activation states, with the α -SMA fibers inactivated in the MA studies while fully activated in the AFM studies. We also used the AFM to study the mechanical property of the nucleus. Our model predicted that the substantial differences found in stiffening of the AVIC compared to the PVICs was due to a 9 to 16 times stronger *intrinsic* AVIC α -SMA stress fiber

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Author Contributions

YS: Co-developed the research approach, developed the computational tools, re-analyzed the MA and AFM data, and co-wrote the paper. RMB: provided additional experimental data and assisted in cell physiological interpretations. MSS designed the overall research program, closely guided the theoretical formulation and computational implementation, co-wrote the paper, and provided funding.

contractile force. Model validation was done by simulating a traction force microscopy experiment to estimate the forces the VICs exert on the underlying substrate, and found good agreement with reported traction force microscopy results. Further, estimated nuclear stiffness for both AVICs and PVICs were similar and comparable to the literature, and were both unaffected by VIC activation level. These results suggest substantial functional differences between AVICs and PVICs at the subcellular level. Moreover, this first VIC computational biomechanical model is but a first step in developing a comprehensive, integrated view of the VIC pathophysiology and interactions with the valve ECM micro-environment based on simulation technologies.

Keywords

Valve interstitial cell; finite element method; active contraction; alpha-SMA stress fibers; heart valves; micropipette aspiration; atomic force microscopy

2. Introduction

Heart valves are specialized cardiac structures that ensure unidirectional blood flow [1, 2]. Heart valves undergo approximately 40 million cycles per year, with a total of at least 3 billion times over an average lifetime [3]. According to the American Heart Association, valvular heart disease resulted in 21,824 deaths directly and was a contributing factor in 45,062 deaths in the US in 2008 [4]. Thus, it is essential to understand the heart valve pathophysiology and to develop means to mitigate its effects using intervention approaches that range from pharmaceuticals to surgical interventions. While surgical interventions remain the gold standard, new therapeutic approaches that are based on an understanding of valve pathophysiology could potentially allow for novel approaches aimed at minimizing the onset and progression of valve disease.

Valvular interstitial cells (VICs) are located throughout heart valve leaflet tissues of all four heart valves. Studies of normal and pathological heart valves have demonstrated that VICs play critical roles in the maintenance of heart valve tissues and the onset and progression of heart valve disease [1, 3]. Heart valve leaflets experience dynamic and complex mechanical stress states over the cardiac cycle, including surface shear stress due to blood flow, flexure (opening and closing), and high in-plane tension (closed valve). The resulting forces are translated to the VICs through the complex micromechanical interactions with the extra cellular matrix (ECM) such as collagen stretch with reorientation and fiber compaction. VICs thus interact with the ECM in highly stress-dependent manner in a valve tissue [2, 3, 5]. Recently, the stiffness of mitral heart valve VICs (MVICs) within leaflet tissues under controlled biaxial loading were estimated using finite element simulations [6]. This study found that while the MVIC effective stiffness was quite similar within each of the four leaflet layers, MVICs experienced significantly different mechanical deformations resulting from differing layer microstructures. We speculated that this may induce the MVICs to respond in a layer-specific manner despite their similar phenotypic state, as evidenced by similar stiffnesses.

Such speculations are built on the knowledge that VICs respond to their biomechanical cues by upregulating ECM protein synthesis and the development of a highly contractile

cytoskeleton via α -smooth muscle actin (α -SMA) fibers. Activated VICs continuously maintain valvular ECM by synthesizing ECM and remodel collagen and other ECM components using matrix degrading enzymes [2, 7], all to maintain heart valve tissue homeostasis. After completion of remodeling and repair, the activated VICs are thought to revert back an inactivated state [3, 8] or are removed by apoptosis [9]. However, excessive and persisting environmental changes cause the improper regulations of VICs, and a clinically significant valve pathology may result [2]. The VICs in a diseased state cause the continuous force generation and excessive ECM production, resulting in pathological fibrosis, scarring, and fibrocontractile disease [3, 10]. Thus, it is important to investigate where the contractile activities of the differentiated VICs are generated.

In recent years, the mechanical properties of VICs have been investigated using various experimental techniques, such as micropipette aspiration (MA) [11–13], collagen-gel contraction [14], and atomic force microscopy (AFM) [14]. In these studies, VICs were isolated from the different heart valve leaflet tissues and suspended in an aqueous solution (MA), seeded on a 2D flat substrates (AFM), or seeded on collagen gels. The studies of the VICs and myofibroblasts in general suggest that the expression of α -SMA into the stress fibers upregulates the contraction and stiffness [15–20], with the disruption of α -SMA incorporation abolishes the contractile activity [15, 21, 22]. The properties of VICs have been also studied within the 3D hydrogels with varying material properties [23, 24] or under mechanical loading conditions [25]. These studies indicate that cell-matrix interactions such as cell adhesion, stiffness of the matrix, and mechanical cues due to different loading conditions influence the activation states of the VICs.

To integrate these various and complex behaviors, we need to develop a computational model of VICs that is capable of capturing a wide range of activation states and the effects of the surrounding microenvironment. Currently, linear elastic models have been widely used to interpret the experimental data of the MA and AFM experiments, such as Theret's model for MA [26] and Hertz model for AFM [27, 28]. While these models can capture some of the important trends in the stiffness values of the VICs [11, 14], more advanced models are necessary to study the complex physiology of the internal cell mechanics. For example, the stiffness values of the VICs measured by AFM are about 10 to 100 times larger than the ones measured by MA. It is likely that during MA experiments, VICs are suspended in a liquid and thus are in an inactivated state. In contrast, the during AFM experiments, VICs were seeded on a collagen substrate, and were thus in an activated state, generating a strong contractile stresses.

The complex behaviors of VICs cannot be readily derived with such simple approaches. Recently, advanced large-deformation cell models have been developed and applied to MA and AFM studies [29–31]. It should be noted that these models considered the cell as an isotropic hyper(visco)elastic material, and did not treat the stress fibers as a distinct mechanical phase. In VICs, stress fibers exhibit directionality [10] and are the main source of stiffness and force development [32]. In related studies, Na et al. [33, 34] used a constrained mixture model of the cell with fibers as distinct phases were used. However, the work has been limited to a local response of a cell under AFM experiment and did not provide insight to a cell mechanical response as a whole. Weafer et al. developed a bio-

chemo-mechanical model of a contractile osteoblasts under compression to analyze the cytoskeleton and nucleus stiffness and contraction strength [35]. However, in order to capture the mechanical responses of VICs with different expression and contraction levels of α -SMA fibers, we need to develop a comprehensive mechanical model of the VIC compatible with experimental data under different mechanical loading conditions and activation states.

Much of current heart valve disease research aims at characterizing the mechanisms that activate the VIC contractility and the mechanical interactions with the surrounding ECM [1]. Herein, we are interested in how the α -SMA fibers, under various activation and expression levels, affect the overall effective mechanical properties of the VICs. Towards addressing these issues, we developed a continuum mechanics model to better understand VIC micromechanics and mechanobiology. Several important aspects were considered, including: 1) the contributions of the underlying cytoskeletal network, 2) passive elastic response of the stress fibers, 3) the active contraction of the stress fibers, and 4) the nucleus. Model parameters were calibrated to and validated against extant VIC MA and AFM experimental data [11, 14]. We note too that this is the first attempt to comprehensively model VIC internal mechanics.

3. Methods

3.1 Overview

We aim to develop a VIC biosolid mixture model to simulate the mechanical responses of VICs in different activation states, expression levels of α -SMA fibers, and different mechanical loading conditions. In order to handle the material and geometric nonlinearity, we utilize the finite element method, with model parameters calibrated simulating two sets of extant experimental data: micropipette aspiration (MA) [11] and atomic force microscopy (AFM) [14]. We first simulated the mechanical response of the VICs during MA experiments to determine their mechanical properties in the *inactivated* state. The MA experimental data included the measurements from all four types of the VICs: aortic VIC, mitral VIC, pulmonary VIC, and tricuspid VIC, (AVIC, MVIC, PVIC, and TPVIC, respectively) where they each exhibited the different expression levels of the α -SMA fibers [11]. Then, we simulated the mechanical response of VICs during the AFM experiments to determine their mechanical properties in the *activated* states. The AFM experimental data include the measurements of the AVICs and PVICs, with different expression and contraction levels of α -SMA fibers [14].

3.2 Experimental procedures

Methods for both studies have been previously presented [11, 14]. Briefly, for the VIC MA studies were obtained from ovine hearts, with cells from each valve were divided into two groups for mechanical testing and protein quantification. Cells were trypsinized, pelleted, and resuspended in media prior to testing. Pressures were applied to the surface of a VIC through the micropipette via a custom-built water reservoir with an in-line pressure transducer having a resolution of 1 Pa. While pressure was applied, digital images of the cell aspiration were recorded. Cells were aspirated and the applied pressure and aspirated length

recorded. The effective stiffness, E , of the cell was determined with a half-space model (so-called Theret's model), referred to as the punch model, which assumes that the cell is an isotropic, elastic, incompressible, half-space material [36]. This model assumes the VIC to be a homogeneous material while ignoring discontinuities (organelles, nucleus, etc.) and viscous effects from the cytosol. Hence, as an elastic model, it was chosen to demonstrate the intrinsic stiffness of cell populations and was not intended to fully characterize the mechanical behavior of the cells. In this study, we used the aspiration length vs applied pressure relationship to calibrate the cytoskeleton (μ^{cyto}) and stress fiber shear (μ^{sf}) moduli, (see Sec 3.6.1).

For the AFM studies, porcine AVICs and PVICs were isolated from the same animal. A monolayer of seeded VICs were prepared for each cell type. Collagen was then placed on the cover slip surface and left overnight before being rinsed with PBS. VICs were trypsinized, plated on the cover slips, and allowed to attach for 3 hours. VIC height maps were made by AFM using tapping mode. For each VIC, approximately 70 indentions were made over the surface of each cell using a rectilinear grid sampling pattern. These measurements were carried out on a total of 7 cells from each cell type (AVIC and PVIC), for a total of 14 cells measured. The apparent stiffness of the cell, E , was calculated based on the Hertzian model of the AFM probe modeled as a conical tip with a probe opening angle of 35 degrees. In this study, we used the indentation depth vs force relationships at each point to calibrate the stress fiber contraction strength and nucleus shear modulus, which will be explained in Sec 3.6.2.

3.3 Mathematical Formulations

Overall, we considered the VIC cytoplasm to be a solid mixture of isotropic cytoskeleton and oriented α -SMA fibers which generate both passive elastic stress against stretch and active contractile stress in the direction of the fibers (Figure 1). The nucleus was considered as a distinct structural component. Also, we assume that the VICs are under quiescent conditions during the course of simulations, where no dynamic structural changes occur within the VIC that affects the cell response.

3.3.1 Governing Equations—We idealized a cell as an open bounded domain $\Omega_0 \subset \mathbb{R}^3$ with boundary Ω_0 as the undeformed reference configuration. Let $\mathbf{X}$ indicates the position vector of a particle in Ω_0 . Then the position of the particle in the current configuration Ω_t at time $t \geq 0$ is given by

$$\mathbf{x}(\mathbf{x}_0, t) = \varphi(\mathbf{x}_0, t) = \mathbf{x}_0 + \mathbf{u}(\mathbf{x}_0, t), \quad (1)$$

where $\varphi(\bullet, t): \Omega_0 \rightarrow \Omega_t$ is an orientation-preserving, continuously differentiable one-to-one mapping for all times $t \geq 0$, and $\mathbf{u} = \mathbf{x} - \mathbf{x}_0$ denotes the displacement of the particle. The deformation gradient tensor $\mathbf{F}$ and its determinant J are defined as

$$\mathbf{F} = \frac{\partial \varphi}{\partial \mathbf{x}_0} = \nabla_0 \varphi, \quad (2)$$

$$J = \det \mathbf{F}$$

where the operator ∇_0 denotes the gradient with respect to the reference configuration. The right Cauchy-Green deformation tensor $\mathbf{C}$ and the Green-Lagrangian strain tensor $\mathbf{E}$ are defined as

$$\mathbf{C} = \mathbf{F}^T \mathbf{F}$$

$$\mathbf{E} = \frac{1}{2}(\mathbf{C} - \mathbf{I}), \quad (3)$$

where $\mathbf{I}$ is the second-order identity tensor. In the absence of body and inertial forces, the conservation of linear momentum equation is

$$\nabla \bullet \mathbf{T} = 0 \text{ in } \Omega_t \times (0, T), \quad (4)$$

where $\mathbf{T}$ is the Cauchy stress tensor, and $\nabla \bullet$ denotes the divergence operator with respect to the current configuration.

3.3.2 Cytoskeleton and fiber phases—The VIC cytoplasm is considered as a solid mixture of two phases: 1) the underlying (basal) cytoskeleton, and 2) the α -SMA stress fibers. The cytoskeleton phase and stress fiber phase are expressed by superscript *cyto* and *sf*, respectively. Note that the “cytoskeleton” phase actually includes every mechanical structures of the VIC except stress fibers and nucleus, such as cell membrane and other organelles. We assume that the cytoskeleton and fiber phases move together and do not mechanically interact with each other. Thus, their displacements are equal, and their stress are additive, i.e.

$$\mathbf{u} = \mathbf{u}^{\text{cyto}} = \mathbf{u}^{\text{sf}}. \quad (5)$$

$$\mathbf{T} = \mathbf{T}^{\text{cyto}} + \mathbf{T}^{\text{sf}}$$

The constitutive equations for $\mathbf{T}^{\text{cyto}}$ and $\mathbf{T}^{\text{sf}}$ will be described below.

3.4 Constitutive model of underlying cytoskeleton

We assume that the cytoskeleton can be modeled as an isotropic hyperelastic material using a nearly-incompressible neo-Hookean model, where the strain energy density function is defined as

$$\Psi^{\text{cyto}} = \frac{\mu^{\text{cyto}}}{2}(\bar{I}_1 - 3) + \frac{1}{2}K(\ln J)^2. \quad (6)$$

Here, μ^{cyto} and K are the shear and bulk modulus of the cytoskeleton, respectively, and $\bar{I}_1 = J^{-2/3} \text{tr}(\mathbf{C})$ is the first invariant of the deviatoric part of the right Cauchy-Green tensor. In

order to enforce the incompressibility constraint, we set $K/\mu^{\text{cyto}}=10,000$, at which the change in volume ratio is numerically confirmed to be less than 1%.

3.5 Constitutive model of stress in stress fiber phase

The stress within the stress fiber phase is composed of two components: the passive elastic stress against stretch and active contractile stress. Here, we will introduce the continuum representation of distributed fiber orientations and derive the constitutive model of the stress in fiber phase.

3.5.1 Orientation of the α -SMA fibers—The direction of each fiber in its initial state is represented by a unit vector $\mathbf{m}_0$. We can characterize the vector $\mathbf{m}_0$ using two angles $\Theta \in [0, \pi]$ and $\Phi \in [0, 2\pi]$

$$\mathbf{m}_0(\Theta, \Phi) = \sin\Theta \cos\Phi \mathbf{e}_1 + \sin\Theta \sin\Phi \mathbf{e}_2 + \cos\Theta \mathbf{e}_3, \quad (7)$$

where $\mathbf{e}_1$, $\mathbf{e}_2$, and $\mathbf{e}_3$ represent the unit normal basis vector for the 3D Cartesian coordinates. Next, we assume the existence of an orientation density function $\Gamma_0(\mathbf{m}_0)$ that describes the distribution of the fibers in reference configuration Ω_0 . The orientation density function is defined so that $\Gamma_0[\mathbf{m}_0(\Theta, \Phi)] \sin\Theta d\Theta d\Phi$ represents the normalized number of fibers (no. of fibers/total no. of fibers) within the range $[(\Theta, \Theta+d\Theta), (\Phi, \Phi+d\Phi)]$ and $\Gamma_0(\mathbf{m}_0)$ satisfies the symmetry and normalization requirements

$$\Gamma_0(\mathbf{m}_0) = \Gamma_0(-\mathbf{m}_0)$$

$$\int_0^{2\pi} \int_0^\pi \Gamma_0[\mathbf{m}_0(\Theta, \Phi)] \sin\Theta d\Theta d\Phi = 1. \quad (8)$$

3.5.2 Stress in the α -SMA fiber phase—In order to model the stress in the α -SMA fiber phase, we first define the fiber stretch λ along initial direction $\mathbf{m}_0$ as

$$\lambda = \sqrt{I_4} = \sqrt{\mathbf{m}_0 \cdot \mathbf{C} \mathbf{m}_0}. \quad (9)$$

We can also represent the direction of the fibers initially oriented along $\mathbf{m}_0$ in their current state by $\mathbf{m}$,

$$\mathbf{m} = \lambda^{-1} \mathbf{F} \cdot \mathbf{m}_0. \quad (10)$$

The Cauchy stress in the stress fiber phase $\mathbf{T}^{\text{sf}}$, is given by integrating the contributions from every direction:

$$\mathbf{T}^{\text{sf}} = \int_0^{2\pi} \int_0^\pi \Gamma_t(\mathbf{m}) \left[H(I_4 - 1) \mathbf{T}^p(\mu^{\text{sf}}, \mathbf{C}, \mathbf{m}) + \mathbf{T}^a(f, \mathbf{C}, \mathbf{m}) \right] \sin\theta d\theta d\phi \quad (11)$$

$$\Gamma_t(\mathbf{m}) = \Gamma_0[\mathbf{m}_0(\mathbf{m}, t)]$$

where $\mathbf{T}^p$ and $\mathbf{T}^a$ are the Cauchy stresses due to passive stretch and active contraction, respectively, of the fibers currently oriented along $\mathbf{m}$, and $\Gamma_t(\mathbf{m})$ represents the current orientation distribution of the fibers with respect to the current direction $\mathbf{m}$. A Heaviside step function H was introduced to enforce that the passive stress arises only from fiber stretch. We introduce two constants, μ^{sf} and f , which describes the intrinsic shear modulus of the stress fibers and the strength of the active contraction, respectively.

3.5.3 Passive fiber stretch—We model the passive component of individual α -SMA fibers initially oriented along $\mathbf{m}_0$ as a transversely isotropic material with the axis of symmetry $\mathbf{m}_0$, whose stress arises only from the axial stretch. Thus, we define the strain energy density function $\psi_p^{sf} = \psi_p^{sf}(I_4)$, that captures the mechanical response of the α -SMA fibers

$$\psi_p^{sf}(I_4) = \frac{\mu^{sf} \bar{\phi}^{sf}}{2} (I_4 - 1)^2 \quad (12)$$

where μ^{sf} is the shear modulus of the α -SMA fibers, and $\bar{\phi}^{sf}$ is the effective expression levels of the α -SMA fibers, which is assumed uniform within the VIC. Here, we assumed that $\bar{\phi}^{sf}$ is proportional to the α -SMA protein levels within each VICs, which were experimentally measured previously [11]. We used the “effective” expression because only the actual protein levels of the α -SMA fibers are currently known. Thus, $\bar{\phi}^{sf}$ was normalized by the maximum value ($\bar{\phi}^{sf}$ of AVICs is one in Table 1). Note that as we considered the stress fibers to be a homogenized phase, the number, length, and thickness of the stress fibers were incorporated into the parameter $\bar{\phi}^{sf}$. Also, no interactions between fiber and solid phases were considered.

The Cauchy stress $\mathbf{T}^p$ can be obtained by

$$\mathbf{T}^p(\mu^{sf}, \mathbf{C}, \mathbf{m}) = 2 \frac{I_4}{J} \frac{\partial \psi_p^{sf}}{\partial I_4} \mathbf{m} \otimes \mathbf{m}, \quad (13)$$

where $\otimes$ denotes the tensor product.

3.5.4 Active fiber contraction—The contraction of the stress fibers within a cell has been modeled using the tension-velocity relationship [37–40], which states that the contraction strength decreases as the fiber shortening rate increases. Here, we assume that the AFM indentation speed is slow enough so that the fiber shortening rate does not influence the contraction strength. Thus, fibers exert maximum contraction along their current direction $\mathbf{m}$ with contraction strength f . Also, we hypothesized that the higher the α -SMA protein levels within the VIC, the more contractile the VIC becomes due to the presence of more contractile units. Thus, we assume that the active contraction strength is proportional to the VIC protein level $\bar{\phi}^{sf}$. We then derived the active stress $\mathbf{T}^a$ of the stress fiber

$$\mathbf{T}^a(f, \mathbf{C}, \mathbf{m}) = f \bullet \bar{\phi}^{\text{sf}} \frac{I_4}{J} \mathbf{m} \otimes \mathbf{m}. \quad (14)$$

Note that f is the intrinsic contraction strength per unit protein level.

3.5.5 The nucleus—The cell nucleus has been reported to be 2 to 10 times stiffer than the rest of the cell, and is typically modeled as a neo-Hookean material [40–43]. We thus model the nucleus as a nearly incompressible neo-Hookean material whose strain energy density function has the same form as (6), with the shear modulus μ^{nuc} . We further assume that there is no slip between the nucleus and cytoplasm, as it is completely bonded to cytoskeleton.

3.6 Simulation procedures

We determined four parameters: cytoskeletal shear modulus μ^{cyto} , fiber shear modulus μ^{sf} , contraction strength of the α -SMA stress fibers f , and shear modulus of a nucleus μ^{nuc} as follows. In order to avoid the complexity of determining these four parameters simultaneously, we used the following step-by-step process (Figure 2). First, we used the micropipette aspiration experimental data to estimate shear moduli of cytoskeleton and the passive α -SMA stress fibers (μ^{cyto} and μ^{sf} , respectively), assuming that the VICs were in inactivated state and nucleus did not contribute to the mechanical response. We then used these parameters in the AFM simulations and determined the contraction strength of the α -SMA stress fibers f and shear modulus of a nucleus μ^{nuc} . During the AFM parameter estimation process, the contraction strength was determined first using the indentation data at peripheral regions, where no nucleus deformation occurs. Then, shear modulus of the nucleus was calibrated using the indentation data at the central regions.

3.6.1 Micropipette aspiration simulations—Using the relationship between the applied pressure (P) and aspiration length (L) of the different types of VICs with different levels of α -SMA expressions, we calibrated the values of the cytoskeletal shear modulus (μ^{cyto}) and fiber shear modulus (μ^{sf}) (Figure 2, Calibration Step 1). The experimental data was obtained from the previous micropipette aspiration study by Merryman et al. [11] (Table 1). The initial shape of a VIC was assumed to be a sphere with radius 8 μm , and the pipette radius set to 4 μm to mimic the experimental setup. In order to represent the realistic geometry of a micropipette inner corner and for improved numerical stability, we chose a circular shape with the radius of $0.2R_p$, where R_p is the inner micropipette radius. This “smoothing” of the micropipette corner has been done for other micropipette aspiration finite element simulation studies, such as [29]. A contact-traction was applied on the boundary between the VIC and the micropipette using a penalty method for a sliding without any friction. We first applied ~60 Pa “tare” pressure, then the pressure was increased to ~260 Pa to mimic the experimental procedure. The aspiration length, L , was measured as the z -displacement at the tip with respect to “tared” state (Figure 3). The VICs were assumed in an inactivated state ($f=0$), and nucleus was ignored since it does not contribute to the mechanical responses of a cell during micropipette aspiration. Thus, the differences in the mechanical responses of different types of VICs arose from the different expression levels of their α -SMA fibers $\bar{\phi}^{\text{sf}}$, which was proportional to the experimentally measured α -SMA protein levels (Table 1). We used the initially isotropic fiber orientation

distribution ($\Gamma(\Theta, \Phi) = 1/4\pi$) since no fiber directionality was observed. Thus, we simulated the VICs with different $\bar{\phi}^{\text{sf}}$ values to match the experimental results with the applied pressure vs. aspiration length relationship using the nonlinear regression technique.

The total Cauchy stress used in the MA study is thus given by

$$\mathbf{T} = 2 \frac{1}{J} \mathbf{F} \frac{\partial}{\partial \mathbf{C}} \left[\frac{\mu^{\text{cyto}}}{2} (\bar{I}_1 - 3) + \frac{1}{2} K (\ln J)^2 \right] \mathbf{F}^T + \frac{1}{4\pi} \int_0^{2\pi} \int_0^\pi \left[H(I_4 - 1) 2 \frac{I_4}{J} \frac{\partial}{\partial I_4} \left(\frac{\mu^{\text{sf}} \bar{\phi}^{\text{sf}}}{2} (I_4 - 1)^2 \right) \mathbf{m} \otimes \mathbf{m} \right] \sin \theta d\theta d\phi. \quad (15)$$

We thus calibrated two parameters, μ^{cyto} and μ^{sf} , by minimizing the least square error between the simulated aspiration lengths and the experimental aspiration lengths, given the applied pressure (P) and normalized α -SMA expression ($\bar{\phi}^{\text{sf}}$) values (Table 1).

3.6.2 Atomic force microscopy simulations—Using the relationship between the indentation depth and reaction force during the AFM, we calibrated the contraction strength of the α -SMA stress fibers (f) and shear modulus of a nucleus (μ^{nuc}) at each indentation location for the AVICs and PVICs, as previously reported by Merryman et al. [14]. The AFM simulation domains are composed of three subdomains: the indenter, VIC cytoplasm, and nucleus. The indenter was considered as a rigid body with a prescribed displacement. The VIC cytoplasm and nucleus are considered as distinct mechanical structures with different mechanical properties. A sliding frictionless contact boundary condition was applied between the VIC and the indenter, with no slip between the cytoplasm and nucleus on the interface boundary and any contributions of the cell membrane was ignored. The bottom of the VIC was attached to the plate with no displacement resulting from perfect adhesion (i.e. perfectly bonded). We used the cytoskeleton and stress fiber shear moduli (μ^{cyto} and μ^{sf} , respectively) obtained by the previous MA calibrations, and incorporated the α -SMA expression levels data (Table 1).

The simulations were carried out in the simplified VIC geometry whose dimensions were taken from the VIC height and stiffness, as well as the nucleus dimensions taken from new microscopy data (Figure 4). We idealized the shape of the VIC as a half-ellipsoid (flat side facing downward) and the shape of a nucleus as a full-ellipsoid. The simulation of the AFM experiment consisted of two steps: 1) the VIC starts from the undeformed configuration and evolves to a fully contracted state, 2) the indentation was then applied. The calibration processes were also two-step. We first calibrated the contraction strength f of each VIC using the indentation data at the cell peripheral region, where no nucleus deformation occurs (Figure 2, Calibration Step 2). Then, we calibrated the nucleus stiffness μ^{nuc} using the calibrated contraction strength and indentation data at the central region, where the nucleus deformation occurs (Figure 2, Calibration Step 3).

It has been observed that the VICs seeded on a flat collagen substrate exhibit a flattened morphology, with stress fibers orient largely parallel to the bottom surface (Figure 5b). Thus, we used the (locally) cylindrically symmetric assumptions for the initial orientation of the α -SMA stress fibers (Figure 5a, left), assuming that the VICs do not exhibit any polarity in this study. For the cylindrically symmetric orientation, $\Gamma = 1/2\pi$ was used, where the orientation density function is only defined on the local 2D surface that is perpendicular to

the symmetry axis. The symmetry axis of the fibers was determined as a height-weighted average of the surface normals on top and bottom of the VIC. Thus, the fibers generally orient along the tangential direction of the top and bottom surfaces, depending on how close to the top or bottom. To probe the sensitivity of the VIC mechanical response to the fiber orientation, we also used the spherically symmetric initial fiber orientation assumptions (Figure 5a, right). For the spherically symmetric orientation, $\Gamma=1/4\pi$ was used uniformly in the VIC.

The total Cauchy stress used in the AFM study is thus given by

$$\begin{aligned} \mathbf{T} = & 2 \frac{1}{J} \mathbf{F} \frac{\partial}{\partial \mathbf{C}} \left[\frac{\mu^{\text{cyto}}}{2} (\bar{I}_1 - 3) + \frac{1}{2} K (\ln J)^2 \right] \mathbf{F}^T \\ & + \int_0^{2\pi} \int_0^\pi \left[\Gamma_t(\mathbf{m}) H(I_4 - 1) 2 \frac{I_4}{J} \frac{\partial}{\partial I_4} \left(\frac{\mu^{\text{sf}} \bar{\phi}^{\text{sf}}}{2} (I_4 - 1)^2 \right) \mathbf{m} \otimes \mathbf{m} \right] \sin \theta d\theta d\phi \\ & + \int_0^{2\pi} \int_0^\pi \left[\Gamma_t(\mathbf{m}) f \bullet \bar{\phi}^{\text{sf}} \frac{I_4}{J} \mathbf{m} \otimes \mathbf{m} \right] \sin \theta d\theta d\phi \end{aligned} \quad (16)$$

within the cytoplasm, and

$$\mathbf{T} = 2 \frac{1}{J} \mathbf{F} \frac{\partial}{\partial \mathbf{C}} \left[\frac{\mu^{\text{nuc}}}{2} (\bar{I}_1 - 3) + \frac{1}{2} K (\ln J)^2 \right] \mathbf{F}^T \quad (17)$$

within the nucleus. We thus calibrated two parameters, f and μ^{nuc} , by minimizing the least square error between the simulated and experimental indentation depth vs force curves (Figure 9) at each indentation location.

3.7 Numerical Methods

The system of partial differential equations presented earlier was solved by the open source finite element package FEBio [44]. The fiber passive and active stresses were implemented as a plugin extension to FEBio. The simulations were quasi-static, where the applied pressure P for MA and indentation depth d for AFM were varied over time, and the static solutions were calculated at each time step. The convergence of the algorithm has been numerically observed against the finest-mesh (“overkill”) solutions for both MA and AFM simulations. About 50,000 ~ 10,000 linear tetrahedron (TET4) elements were used for the typical computations, where we observed the sufficiently accurate results.

3.8 Statistical analysis

The parameter estimation was carried out using non-linear least square minimization technique, which was implemented by a custom Python script. For the MA simulations, values of μ^{cyto} and μ^{sf} were calibrated using the aspiration length stiffness and the (normalized) α -SMA expressions of the VICs from experimental data. For the AFM simulations, values of f and μ^{nuc} were calibrated using the indentation depth and force experimental data at different locations of the AVICs and PVICs. Thus, the means and standard errors of these values for each VIC type were calculated. Also, the statistical

significance of the difference in mean values of two sample populations was analyzed using the Student's t-test, with p-value < 0.05.

4. Results

4.1 Micropipette Aspiration Simulations

The μ^{cyto} and μ^{sf} values were calibrated by minimizing the L_2 norm of the residuals between the simulated and experimental aspiration lengths. The μ^{cyto} and μ^{sf} values obtained were 5.0 Pa and 390.0 Pa, respectively, where the L_2 error reaches the minimum (Figure 6). The coefficient of determination (r^2 value) for this calibration is 0.87. Using the calibrated values, we simulated the MA of different types of VICs with different applied pressures and compare the results to the experimental data (Figure 7). The simulation predicts the applied pressure vs aspiration length relationship within a reasonable range from the experimental data. As the expression levels of α -SMA increases, the stiffer the VIC becomes, which decreases the aspiration length for a given pressure.

4.2 Atomic Force Microscopy Simulations

4.2.1 Deformation due to AFM indentation—We first computed the deformation purely due to the AFM tip indentation as the difference in the displacement before and after the indentation, excluding the displacement due to contraction (Figure 8). The deformation due to AFM indentation was indeed localized around the indentation region. The indentation at the central region significantly deforms the nucleus while the indentation at the peripheral regions does not. In fact, the indentation depth vs force curves are significantly influenced by the underlying nucleus for both experimental data (Figure 9). Using the FEM simulation with calibrated parameters (which will be explained later), we successfully reproduced the indentation depth vs force curve in both the central and peripheral locations (Figure 9).

4.2.2 Active contraction strength—We first calibrated the active fiber contraction strength f of the AVICs and PVICs. Under the cylindrical symmetric fiber orientation assumption, the calibrated active contraction strength were 35.27 ± 3.42 kPa ($n=22$) and 3.86 ± 0.61 kPa ($n=21$) for AVICs and PVICs, respectively with the p-value of 0.01 (Figure 10 (a)). Under the spherical symmetric fiber orientation assumption, the calibrated active contraction strength the α -SMA fibers were 29.64 ± 3.58 kPa ($n=22$) and 1.74 ± 0.42 kPa ($n=21$) for AVICs and PVICs, respectively with the p-value of <0.01 (Figure 10 (b)). Generally, the cylindrically symmetric orientation yielded larger active contraction strength values than spherically symmetric orientation. We speculated that this is due to that fact that under the cylindrical symmetry orientations the fibers can only resist against the AFM indentation under certain directions while under the spherical symmetry orientations, the fibers can resist against any deformation. Nevertheless, these two different fiber orientations yielded the same trend: the AVICs contracts significantly stronger than PVICs, as the p-values represents. Thus, while we will require quantified 3D stress fiber geometry in future simulations, the current results suggest that our results are at least qualitatively correct. The ratios of the active contraction strength from the PVICs to AVICs are about 5 to 1 and 6 to 1 for cylindrically symmetric and spherically symmetric assumptions.

4.2.3 Nucleus shear modulus—We next estimated the nucleus shear modulus μ^{cyto} of the AVICs and PVICs using the AFM indentation data from the central regions. Under the cylindrical symmetric fiber orientation assumption, the calibrated nucleus shear moduli were 15.64 ± 2.46 kPa (n=9) and 16.06 ± 2.19 kPa (n=10) for AVICs and PVICs, respectively, with the p-value of 0.90 (Figure 10 (c)). Under the spherical symmetric fiber orientation assumption, the calibrated nucleus shear moduli were 14.91 ± 2.32 kPa (n=9) and 16.13 ± 2.30 kPa (n=10) for AVICs and PVICs, respectively, with the p-value of 0.73 (Figure 10 (d)). It is clear that the fiber orientation, at least for spherical or cylindrical symmetry assumptions, does not affect the measurement of the nucleus shear moduli for different types of VICs. Also, the p-values indicate that for both assumptions, there are no statistically significant differences in the nucleus shear moduli between AVICs and PVICs even though these VICs exhibit significant difference in their active contraction strengths.

5. Discussion

5.1 Primary findings

In the present study, we investigated how major functional aspects of VIC α -SMA fibers, such as their orientation, expression levels, and active contraction strength, contributed to the VIC effective mechanical properties. A solid mixture constitutive model was developed that captured VIC mechanical responses in various activation states and under different loading conditions. We used the finite element method to implement the model and simulated the mechanical responses of VICs during MA and AFM experiments. During MA simulation, the VICs were assumed to be in an inactivated state and thus exhibited no contraction. On the other hand, during AFM simulation, the VICs were in an activated state, exhibiting maximum contraction. Thus, by combining the experimental data from these two studies we were able to determine the mechanical properties of the α -SMA fibers, both passive and actively contracted. In addition, we were able to determine the mechanical properties of the underlying cytoskeleton and nucleus. Thus, a novelty of this study is the use of the unified model of a VIC that integrated the data from various experiments to develop a more unified picture of VIC function.

It is interesting to note that the correlation between the expression levels of the α -SMA fibers and stiffness of the VICs were initially estimated using linear elastic models [11, 14]. However, in these studies, the models were inadequate to separate the mechanical response of the VICs due to α -SMA fiber activations, basal cytoskeleton, nucleus, and cell geometry. The stiffness values measured by AFM is typically 100 times larger than the ones measured by MA. Using our new model, we attributed the discrepancy in the stiffness values to the difference in the activation state of the VICs. This study is consistent with our result that the AVICs exhibit 9 or 16 times stronger contraction strength than PVICs, depending on the fiber initial orientation assumptions. Moreover, gel contraction experimental studies indicated that when seeded on a collagen gel, AVICs contracted the surrounding gel significantly more and faster than the PVICs [14].

We also included the nucleus in our model for the AFM analysis. In related studies using the localized nature of the AFM measurements, it has been shown that AFM can be used to measure the stiffness of the underlying actin bundles [45]. Moreover, a plate compression

experimental technique with finite element simulations have been used to analyze the mechanical property of the nucleus [41]. Thus, as long as the AFM indentation depth is large enough, local variations in the indentation depth vs force relationship can be used to derive the mechanical property of the underlying nucleus, as we observed in our simulations (Figure 8). To our knowledge, this is the first study to calibrate the effective modulus of the nucleus within a cell from the AFM indentation data. The derived linearized stiffness of the nucleus in this study was around 45 kPa, which is relatively large compared to the previously reported values of 1 to 10 kPa for different cell types and experimental techniques [41, 46, 47]. The AFM study of the same VICs used the Hertz model and yielded the stiffness of the VICs within 10 kPa to 140 kPa range [14]. Assuming that this result effectively homogenized the cytoplasm and nucleus, our value of 45 kPa is within a reasonable range.

In the AFM analysis, we assumed that the initial fiber orientations are either locally cylindrically or spherically symmetric (Figure 5). The visual observation of the contractile fibroblasts and myofibroblasts indicates that the α -SMA fibers usually run laterally, with visually strong preferred directions (Figure 5(b)). Thus, we assigned the cylindrically symmetric fiber distributions, using the spherically symmetric fiber distributions to explore the model sensitivity to α -SMA fiber orientation. We also assumed that the VICs were non-polarized, based on the observation that during the gel contraction the VICs contracted the gel uniformly [14]. Interestingly, regardless of the exact fiber orientation distribution used, the estimated contraction strengths reveal similar trends: That the AVICs exhibits significantly stronger contraction than PVICs. Also, the determined shear modulus of the nucleus was not influenced by the fiber orientation assumptions.

In this study, we assumed that the VICs are in a steady-state condition, where no dynamic structural changes occur within the VIC during the course of measurements and simulations. Observations of VICs during MA experiment suggested that the VICs were in inert condition, where no stress fiber development occur. Also, during the AFM experiment, the stress fibers were fully developed before the indentation, and each indentation only lasts less than a second. Thus, it is unlikely that any biomechanical or biochemical changes occur during each indentation. Thus, we argue that our assumption is valid at least for simulating the VIC mechanical responses during MA and AFM experiments.

To better understand how VIC interact with the underlying substrate, we calculated the 2D traction field on the bottom of the cell (Figure 11b). For a spherically contracting VICs, the traction is uniformly zero on the bottom due to symmetry. For cylindrically contracting VICs, the magnitude of the traction is maximum on the cell edge. This roughly corresponds to the gel-contraction experimental results, where the VICs seeded on the collagen gel contracted uniformly, exerting traction force on the gel towards the center of the cell [14]. Similar behavior was observed by circular contractile human tendon fibroblasts by traction force microscopy experiments, where the cells pull the substrates inwards and exhibit maximum traction strengths on the edge (Figure 11a) [48]. For a cylindrically contracting VICs, the maximum traction force calculated by our simulations were: ~1 kPa for the PVICs and ~10 kPa for the AVICs, where the previous traction force microscopy studies reported the maximum traction forces of various cells as: 0.2 kPa to 2.0 kPa [20, 48–51]. Thus, the

cylindrically symmetric fiber orientation assumption captures the realistic VIC traction behaviors, although there are inevitable differences in the magnitude of traction resulting differing cell type

In order to verify the consistency of our model with previous small strain measures, we simulated the AFM on an AVIC with very small indentation depth with different contraction strength (Figure 12). Using the indentation depth vs reaction force relationship from the simulation, we calculated the Hertz stiffness of the VIC:

$$E_{Hertz} = \frac{F(1 - \nu^2)}{\pi \bullet \phi(\delta)}, \quad (18)$$

where F is the indentation force, δ is the indentation depth, ν is the Poisson's ratio, and ϕ is the function that depends on the shape of the tip. The resulting relationship between the contraction strength and the Hertz stiffness clearly shows that increase in contraction strength stiffen the VIC as expected (Figure 12b). Obviously, the increase in the contraction strength corresponds to more resistance against the deformation that stretches the individual fibers. Hence the "apparent" stiffness increases. In fact, the correlation between the stiffness measured by oscillatory magnetic cytometry and pre-stress, which is generated by the contraction of actin fibers, was observed in human smooth muscle cells [52]. The y-intercept in Figure 12b was around 400 Pa, which corresponds to the "pure" Hertz stiffness of the VIC, which combines the stiffness of the cytoskeleton phase, α -SMA fiber phase, and underlying nucleus without any contractile activity. Because the Hertz and Theret models use the same linear elasticity and infinitesimal deformation assumptions, this "pure" Hertz stiffness should correspond to the Theret stiffness measured in MA experimental study. Thus, we simulated the AFM indentation of a noncontractile VICs ($f=0$) with different expression levels of α -SMA. We then compared the Hertz stiffness values with Theret stiffness values from the MA experimental data from [11]. The results are shown in Table 2. These two types of stiffness values correspond well. Thus, we conclude that our model is consistent in two different simulation setups and can be used to simulate VICs under different mechanical loading conditions and activation states.

5.2 Limitations

Although our model successfully predicted the key responses of the VICs, it also has some limitations. First, we assumed that the initial α -SMA fiber orientations are cylindrically or spherically symmetric, and the fibers are uniformly expressed throughout the cytoplasm (i.e. α -SMA protein level is spatially uniform). However, currently we do not have quantitative data of the orientation and spatial density of the α -SMA fibers. We are planning to visualize the 3D geometry of α -SMA fibers and integrate the results to our VIC model. Secondly, we homogenized the intracellular structures, excluding the stress fibers and nucleus, into a single isotropic "cytoskeleton" phase. It is possible that these intracellular structures exhibit some anisotropic response or do not share the same displacement as the stress fiber phase. However, the analysis in Figure 12(b) indicates that the stress fibers are the main contributor of the VIC mechanical responses to the external stimuli (excluding the effect of nucleus). Thus, we argue that even if the cytoskeleton phase exhibits some anisotropy, its effect would be minimal. Thirdly, we only considered two cases of contraction: None (MA) or maximum

contraction (AFM). The time-dependent process of fiber contraction, remodeling, and re-orientation in response to biochemical or biomechanical signals should also be modeled as information on these phenomena become available for VICs. Largely unique to VICs, they undergo very large and highly dynamic deformations within the native tissues over the cardiac cycle [1, 53]. We also know VICs can behave as viscoelastic materials [12], yet have relatively little knowledge how these relate to in-vivo function. Finally, we modeled the cell adhesion during the AFM simulations using a zero-displacement boundary condition. It is known that the extracellular matrix rigidity influences the activation states of contractile myofibroblasts through the development of focal adhesion complexes. While sufficient for stable states as studied herein, it may be important to consider the adhesion processes in a complete VIC model.

5.3 Summary and conclusions

In conclusion, we have developed a novel solid mixture model of a VIC that consists of the different mechanical constituents that captured modulating mechanical responses of the VICs resulting from different activation states and loading conditions. Using the simulations, we revealed that α -SMA fiber expression and contraction was the major modulator of the overall mechanical response of the VIC. Also, we found that the activation states do not influence the mechanical behavior of a nucleus. We also showed that our model can be used to simulate the stiffening of the cell due to contractile activities and to analyze the traction that the VICs exert on the substrates. Our model elucidated that in inactivated state, the α -SMA fiber stiffness dominates over the underlying cytoskeleton stiffness, and that the significant increase in the stiffness of the VICs in activated states from inactivated states is due to the contraction of the α -SMA fibers. Interestingly we determined that AVIC α -SMA fiber stiffness is intrinsically higher than in PVICs. Thus, in order to understand the VIC mechanics more accurately, we need to understand the intrinsic stiffness and contractile force development of the stress fibers. We hope that our model can provide more holistic view of the physiology of the VICs. Also, while we took a continuum approach, this will allow us to easily incorporate it within a larger multiscale model of the entire valve. Then, our model can be used to study the mechanics of diseased heart valve tissues and the variations in the mechanical properties of the different heart valves.

Acknowledgements

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Nomenclature

μ^{cyto}	shear modulus of the cytoskeleton phase
K	bulk modulus of the cytoskeleton phase
ϕ^{sf}	expression level of the α -SMA stress fiber
μ^{sf}	shear modulus of the α -SMA stress fibers
f	contraction strength

μ^{nuc} shear modulus of the nucleus

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Research highlights

- We developed a biomechanical model of a contracting valve interstitial cell (VIC).
- Mechanical responses of VICs were simulated under different activation states.
- Aortic VICs exhibit significantly stronger active contraction than pulmonary VICs.
- Nucleus stiffness is unaffected by the activation states.

Figure 1.

VIC mechanical model. The nucleus is considered as an incompressible neo-Hookean material while the cytoplasm is considered as a solid mixture of the isotropic basal cytoskeleton and the actively contracting, oriented stress fibers. The stress fibers resist elastically to the stretch and also generate active contractile stress in the direction of the fibers.

1. Micropipette Aspiration

μ^{sf}	μ^{cyto}
f	μ^{nuc}

2. Atomic Force Microscopy on Peripheral Region

μ^{sf}	μ^{cyto}
f	μ^{nuc}

3. Atomic Force Microscopy on Central Region

μ^{sf}	μ^{cyto}
f	μ^{nuc}

Figure 2.

The step-by-step calibration process. 1. The cytoskeletal shear modulus (μ^{cyto}) and α -SMA fiber shear modulus (μ^{sf}) were calibrated using the micropipette aspiration data for different types of VICs with different expression levels of α -SMA fibers. 2. Using the AFM indentation data on the cell peripheral regions, the fiber active contraction strength (f) were calibrated for AVICs and PVICs. 3. Using the AFM indentation data on the cell central region, the shear modulus of nucleus (μ^{nuc}) were calibrated for AVICs and PVICs. The parameters in red represents the ones that are being calibrated in the step, the parameters in

gray represents the ones that are assumed not contribute to the mechanical response of the VICs (hence ignored) in the step, and the parameters in black represents the ones that are already calibrated from the previous steps and integrated into the model.

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Figure 3.

Micropipette aspiration simulation geometries. The negative pressure P was applied on the boundary of the VIC in the micropipette while zero-pressure was prescribed on the boundary outside. The slip with zero friction contact boundary condition was prescribed between the VIC surface and micropipette wall. The applied pressures were, 0 Pa (initial configuration), ~ 60 Pa (tared configuration), and ~ 260 Pa (aspirated configuration) for A, B, and C, respectively. The aspiration length was determined as the difference in the z -displacement of the cell front tip between the aspirated and tared configurations, represented by an arrow in the figure.

Figure 4.

Atomic force microscopy simulation setup with finite element mesh. We used a simplified geometry of the VIC and its nucleus, whose dimensions were taken from the experimental data. **(a)** The volume ratio of the nucleus was taken from the microscopy image of a VIC within the ECM. A scale bar represents 1 μm. **(b)** The dimension of the VICs were taken from the height map generated during the AFM experiments. The color represents the height at the point in μm and the scale bar represents 10 μm. The mesh was refined around the indentation region, with typically 10,000~50,000 linear tetrahedron elements. Note that this is a cross-section of the geometry, but we simulated the whole cell.

Figure 5.

Two types of initial fiber orientations considered. **(a)** Left: initially cylindrical symmetric fiber orientation. At each point, the fiber orient uniformly on the plane whose normal is parallel to the normal vector of the top surface (represented by a blue arrow). Thus, the fibers orient along the tangential directions of the top surfaces. Right: initially spherically symmetric fiber orientation. Note that the figure is not drawn in scale. The actual VICs exhibit a very flat morphology. **(b)** Typical VIC geometry and fiber orientation seeded on a flat collagen gel. A nucleus (blue) was stained by DAPI while F-actin (green) was stained by phalloidin-FITC. The actin fibers primarily orient parallel to the surface.

Figure 6.

The error surface generated by plotting the L_2 norm of the residuals between the simulated and experimental aspiration lengths for different μ^{sf} and μ^{cyto} values. A clear global minimum occurs at $\mu^{sf} = 390$ Pa and $\mu^{cyto} = 5.0$ Pa, represented by a star.

Figure 7.

Comparison of the applied pressure to the aspiration length to the experimental data. Data points shows the experiments data from Table 1, where the error bar represents the standard error. The VICs become stiffer as the expression levels of α -SMA stress fibers increases, causing the aspiration length to decrease for the same applied pressure.

Figure 8.

The deformation contour due to for the central (left) and peripheral (right) indentations. Note that the deformation of the VIC is localized around the indentation region, yet the indentation at the central region indeed deforms the nucleus while the indentation at the peripheral region does not. Thus, the AFM indentation with sufficient depth can be used to analyze the nucleus mechanical properties.

Figure 9.

Typical indentation depth vs force curve from the AFM experiment (dots) and simulation (lines) for the indentation on a cell central region and peripheral region. It is clear that the reaction force from the AFM indentation is significantly larger in the central region than the peripheral region, and the FEM simulation accurately capture the indentation depth vs force relationship in both regions.

Figure 10.

Top: Contraction strength of the α -SMA fibers in the AVICs and PVICs under spherically symmetric fiber orientation (a) and cylindrically symmetric fiber orientations (b). About **9:1** ratio in the contraction strength was observed from AVICs to PVICs with cylindrical symmetry while **16:1** ratio was observed in spherical symmetry. Although the calibrated values of the contraction strength are different for different fiber orientation assumptions, the general trend is the same: the AVICs exhibit significantly stronger contraction than PVICs. **Bottom:** Shear moduli of the AVICs and PVICs under spherically symmetric fiber orientation (c) and cylindrically symmetric fiber orientation (d). The shear moduli of the VICs irrelevant to the fiber orientation assumptions or the fiber contraction activities.

Figure 11.

(a) Traction field of a circular human tendon fibroblast obtained by the traction force microscopy method [48]. (b) Traction field of a contractile AVIC obtained by the simulation for cylindrical fiber orientation (**left**) and spherical fiber orientation (**right**). The color represents the magnitude of the traction while the arrows represent the directions. The spherically contracting VIC does not exert any traction on the substrate due to symmetry while the cylindrically contracting VIC pulls the substrates inward with maximum traction around the cell edge. The simulation of a cylindrically contracting VICs predicted very similar traction force pattern to the traction force microscopy experiments. The maximum traction force by cylindrically contracting VICs calculated are: ~1 kPa for the PVICs and ~10 kPa for the AVICs, where the typical traction force microscopy studies reported the maximum traction forces of various cells as: 0.2 kPa to 2.0 kPa [20, 48–51].

Figure 12.

Simulation of an infinitesimal AFM indentation. **(a)** The geometry before (top) and after (bottom) the indentation. **(b)** The relationship between the contraction strength and Hertz stiffness calculated from the simulated indentation depth vs reaction force relationship. Our simulations suggest that the more contractile cells exhibit higher stiffness values. Similar observations have been reported by previous studies: where the contractile cells exhibit higher stiffness values than non-contractile cells [14, 52]. Also, at no contraction ($f=0$ kPa), the Hertz stiffness converges to 400 Pa, which is consistent to Theret stiffness value measured by the MA study [11].

Table 1

MA experimental data, [11]

VIC type	Aspiration length (μm)	Tare pressure (Pa)	Applied pressure (Pa)	Normalized α -SMA protein levels
Symbol	L	P^0	P^1	φ^{st}
AVIC	2.10 ± 0.15	58	267 ± 3	1.00 ± 0.0036
MVIC	1.80 ± 0.21	62	260 ± 2	0.99 ± 0.0043
PVIC	2.55 ± 0.25	60	261 ± 3	0.80 ± 0.0038
TVIC	3.10 ± 0.25	63	274 ± 4	0.59 ± 0.0079

Table 2

Comparison of the Hertz stiffness derived from the AFM simulation and Theret stiffness measured by an MA experiments [11].

VIC type	Hertz stiffness (Pa)	Theret stiffness (Pa)
MVIC	515	499
AVIC	395	479
PVIC	340	276
TVIC	279	285