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MICROMECHANICAL MODEL OF MECHANOSENSITIVE COLLAGEN TISSUES

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INTRODUCTION

The mechanical behavior of soft collagenous tissues is largely influenced by the reinforcing collagen fiber microstructure. The anisotropic collagen microstructure can remodel in response to changes in mechanical loading, which can dramatically alter the mechanical properties of the tissues and the mechanical environment of the resident cells. It is important to study the remodeling mechanisms of collagen tissues to understand the pathophysiology of various connective tissue diseases. We hypothesize that the collagen structure actively changes in response to mechanical stimuli through concurrent processes of collagen deposition and degradation and that the rates of these processes are altered by collagen mechanochemistry, mechanosensitive collagen production, and cellular contraction. In prior studies, we developed micromechanical models of collagen tissues to investigate the role of collagen mechanochemistry and mechanosensitive collagen production in remodeling the collagen fiber structure and tissue growth.[1,2] We found that stress inhibition of enzymatic degradation and stimulation of collagen production can explain many phenomena, including remodeling the anisotropic collagen structure along the directions of the maximum principal stress and the development of stress homeostasis.

The goal of this study is to investigate the effect of mechanical loading on the active behavior of the cells. Our approach uses a model 3D microtissue system, self-assembled on a magnetically actuated two-pillar system (μTUG), to investigate these cell-collagen interactions and effects of mechanical loading.[4] The micropillar support allows for measurement of the active cellular contraction, while the magnetic tweezer allows for mechanical testing of the microtissue under a controlled stress rate. Digital image analysis is applied to measure the local two-dimensional (2D) strain field.

To analyze the mechanical measurements for mechanical properties of the collagen structure and active behavior of the cells, we developed a micromechanical model for the mechanical behavior of the microtissue. The micromechanical model includes the elastic behavior of the anisotropic collagen structure and the anisotropic active behavior of the cells. To describe mechanosensitive cellular contraction, we assume concurrent polymerization/depolymerization of actin filaments, where the polymerization rate increases with the fiber stress.[3]

In this paper, we will briefly summarize the model and describe an initial model validation by comparing to μTUG experiments measuring the stress-strain behavior of the microtissue to load-unload tests.

METHODS

Mechanical characterization: To validate our model we considered previous studies of Liu et al. [4] of microtissues composed of bovine pulmonary artery smooth muscle cells (SMCs) in model collagen/fibrinogen matrix. In this prior study, microwell array devices (μTUGs) were fabricated from poly(dimethylsiloxane) (PDMS) via replica molding. One of the two micropillars in each well was affixed with a 100μm nickel sphere for actuation by magnetic tweezers. Microtissues were seeded in the μTUG arrays from SMC-collagen/ fibrinogen mixtures at a density of ~300 cells per microwell. Over the course of 24-48 hours microtissues self-assembled on the pillars. During tissue formation, the cells contracted and compacted the extracellular matrix (ECM) causing the pillars to deflect. The deflection of the pillars was measured via phase-contrast imaging [4] and used to calculate the baseline active stress $\sigma_0 = 5.1$ kPa.

Uniaxial quasi-static load-unload tests were performed on individual microtissues by actuating the magnetic pillars via magnetic tweezers. The tissues were stretched using a magnetic force of 25-35 μN over 120 s and then unloaded to nominally zero stress at the same rate. The passive mechanical behavior of the ECM was directly measured with the same stretching protocol on tissues treated with Triton X-100 for 10 min, to lyse the cells.[4]

Micromechanical constitutive model of microtissue: We conceptualize the microtissue as an anisotropic network of wavy collagen fibrils arranged in an isotropic matrix that represents the

nonfibrous extracellular matrix materials and passive mechanical behavior of the cells. The wavy collagen fibers are modeled as planar sinusoidal elastica, with initial crimp angle $\Theta(\mathbf{X})$, subjected to a horizontal force, which causes the fibers to unbend and stretch axially. The axial stretch, $\lambda_f(\mathbf{X})$, and deformed crimp angle $\theta(\mathbf{X})$ can be determined as described by Comninou and Yannas [5]. The strain energy density of the fiber is given by,[1]

$$U_f = \int_0^1 \underbrace{\left[\frac{E\beta D^2}{2}\left(\theta(\bar{X}_1) - \Theta(\bar{X}_1)\right)^2\right]}_{U_{bend}} + \underbrace{\left[\frac{E}{2}\left(\lambda_f(\bar{X}_1) - 1\right)^2\right]}_{U_{axial}} d\bar{X}_1 \ , \quad (1)$$

Where E is the fiber axial modulus, β is the bending stiffness of the fiber cross-section, and D is the collagen diameter. The contractile cells exert a force on the collagen fibrils in their environment. The active stress of an actin filament is described by a Hill-Type law given in [2],

$$\sigma_{sf}(\theta) = \sigma_{\max} f(\lambda_\theta) g(\dot{\lambda}_\theta), \quad (2)$$

where $\sigma_{\max}$ is the isometric contraction of an actin filament and $f(\lambda_\theta)$ and $g(\dot{\lambda}_\theta)$ describe the strain sensitivity and rate sensitivity of the contractile stress. We assume the cellular component of the tissue can be described by an anisotropic network of stress fibers that undergo concurrent polymerization and depolymerization in response to stress. Furthermore, we assume that the rate of polymerization increases with the fiber stress. This process is incorporated into the model as a kinetic equation for the fiber volume fraction. [3]

$$\frac{\mathrm{d}\phi_{sf}(\theta)}{\mathrm{d}t} = \left(k_0^f + k_1^f \sigma_{sf}(\theta, \lambda_\theta\ , \dot{\lambda_\theta})\right)\phi_{am} - k_d \phi_{sf}(\theta) \quad (3)$$

Finally, we assume that active stress of the cells and passive stress of the extracellular matrix add to give the tissue stress response,

$$\sigma = \frac{1}{\pi}\phi_{sf}\int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \sigma_{sf}(\theta)\,\rho_{sf}(\theta)\mathrm{a}\otimes\mathrm{a}\,\mathrm{d}\theta + \frac{1}{\pi}\phi_{cf}\int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \frac{\partial U_f}{\partial\lambda_\theta}\frac{1}{\lambda_\theta}\rho_{cf}(\theta)\mathrm{F}\,\mathrm{a}\otimes\mathrm{F}\,\mathrm{a}\,\mathrm{d}\theta + \phi_m(\mu\mathrm{B} - p\mathrm{I}), \quad (4)$$

where ϕ_{sf}, ϕ_{cf}, and ϕ_m are volume fractions, ρ_{sf} and ρ_{cf} are angular distributions of fibers, $\mathbf{a}$ is the deformed orientation of the fibers, σ_{sf} is the active stress generated by the stress fiber. We applied the model to simulate the uniaxial tensile tests of the microtissues (Fig. 1C-D).

RESULTS

We fit the scaled isometric contraction $\phi_{sf}\sigma_{\max}$ =0.2 kPa to the baseline stress of the microtissue and use the parameters of $f(\lambda_\theta)$ and $g(\dot{\lambda}_\theta)$ determined in a prior study.[3] We used $\beta = 0.02$ and initial crimp angle $\Theta_0 = 29°$ determined in a prior study [1] and fit the axial modulus E = 1.2 MPa (Fig.1C). These results are used to validate the model response in Fig. 1D. This preliminary result shows that the model can recapitulate the loading curve for the untreated tissue. The model accurately predicted the modulus at small strain but overestimated the peak stress. In contrast, the model produced a poor fit for the Triton treated cells. Experiments showed that the Triton treated tissues also exhibited a small baseline stress σ_0 = 1.6 kPa caused by tissue compaction, which we neglected. The small baseline stress may have straightened the collagen fibers, such that subsequent stretch produced a linear stress-strain response. Fig. 1E shows the model prediction of the reorientation of stress fibers towards the loading direction.

DISCUSSION

This study proposed a model for the investigation of the interaction of mechanosensitive stress fiber evolution and the non-linear properties of the ECM in 3D microtissues. The current model demonstrates how physically derived parameters can describe the mechanical response of the microtissues. The model has some limitations. First, the model neglected the effects of tissue compaction and the viscoelastic behavior of the collagen structure. Consequently, the model cannot reproduce the hysteresis of the unloading response. The viscoelastic response of the collagen needs to be coupled with the contractile dynamics of the cells to investigate the shielding by the cells of the viscoelastic effects.

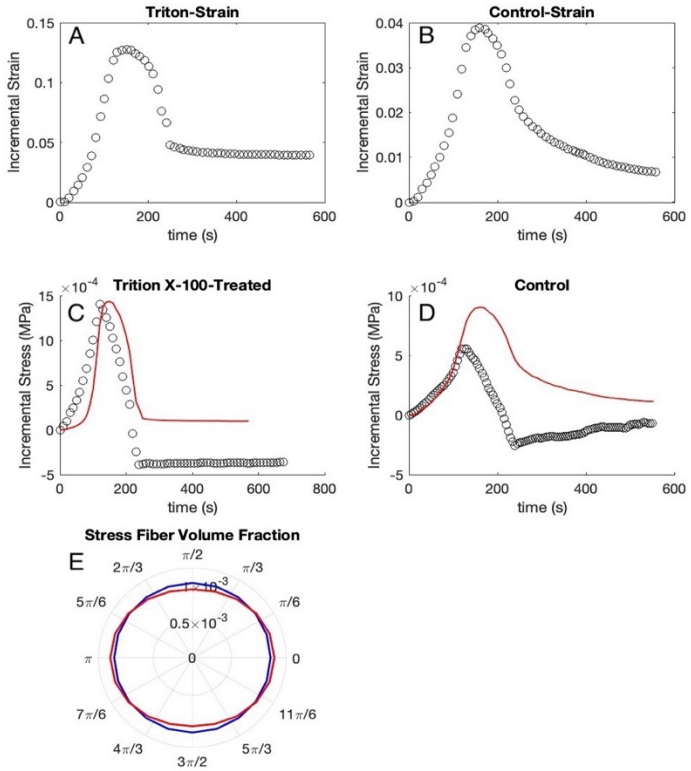

Figure 1: (A-B) Measured strain of Triton-treated and untreated tissue. (C) Model fit of the stress response of Triton-treated tissue. (D) Model prediction of the stress response of the untreated tissue. (E) Loading caused the initially isotropic stress fibers (blue) to align in the loading direction (red). Data in A-D from [4].

We also assumed an initial isotropic distribution of the collagen and stress fibers. However, confocal imaging of the matured tissues before loading revealed that the collagen and stress fibers exhibited a dominant axial orientation. To account for tissue maturation, we will incorporate the anisotropic collagen and actin fiber structure and the cell-mediated tissue compaction of the matured tissue.

In future studies, we apply a viscoelastic extension of the model to investigate the effect of strain rate on cell response. Furthermore, we will extend the model to include a fiber-level description of collagen remodeling and the effects of strain inhibition of collagen degradation and strain stimulation of collagen production.

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REFERENCES

[1] Tonge, T. K. et al., *Biophysical journal,* 109(12):2689-2700, 2015.
[2] Jia, Z. et al., *J Mech Behav Biomed Mater*, 98:96-107, 2019.
[3] Obbink-Huizer C et al., *Biomech Model Mechanobiol*, 13(1):227-236, 2014
[4] Liu A.S. et al., *Sci Rep*, 6:33919, 2016
[5] Comninou M et al., *J Biomech*, 9:427-433, 1976.6