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MODELING CHONDROCYTE DEATH CAUSED BY MICROSCALE STRAIN IN CARTILAGE

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INTRODUCTION

Osteoarthritis (OA) is a disease of the synovial joint, with degeneration and loss of articular cartilage as one hallmark change. Despite the multifactorial nature of OA, mechanical strains and stresses play a key role in the destructive evolution of the disease. Both overloading (e.g. trauma) and reduced loading (e.g. immobilization) of cartilage induce molecular and microstructural changes that lead to cell death, mechanical softening, fibrillation, and erosion. Crucially, there is currently no direct method to correlate spatially resolved intra-tissue mechanics with progression of OA in individual patients. This unmet need renders us unable to identify the most significant stimuli in the progression of OA, and thus unable to identify reliable targets for treatment or to design new therapies.

Here we implemented a model of chondrocyte death within our established constitutive model of cartilage [3,4,5], and attempted to reproduce the experimental results of Bonnevie et al. [2]. An interplay of isotropically distributed, densely packed and negatively charged proteoglycans and a highly anisotropic and heterogeneously oriented fiber network of collagens governs the intra-tissue mechanics of cartilage. Chondrocytes (the only cells in cartilage) synthesize the components of extra cellular matrix, i.e. the proteoglycans and collagen fibers. Thus irregular death of chondrocytes causes dysfunction in maintaining good cartilage health and eventually leads to the development and progression of OA. Recent experimental evidence indicates that frictional strain correlates with chondrocyte death [2]. We simulated two mixed-mode mechanical tests of cartilage, submerged in either synovial fluid or in phosphate-buffered saline (PBS), and predicted the fraction of dead chondrocytes based on a proposed relation using strain measures [2]. We directly compared our predicted fractions of dead chondrocytes to the corresponding experimental results to validate our constitutive model and modeling approach.

METHODS

Constitutive Model. We modeled articular cartilage as a biphasic (swelling) continuum $\phi = \phi^{\mathrm{S}} + \phi^{\mathrm{F}}$ consisting of a porous solid phase ϕ^{S} saturated with an interstitial fluid phase ϕ^{F}. We defined the Helmholtz free-energy function of the solid ψ^{S} as [3,5]

$$\psi^{\mathrm{S}} = \psi^{\mathrm{S}}_{\mathrm{OP}}(J_{\mathrm{S}}) + (1-\nu)\psi^{\mathrm{S}}_{\mathrm{IM}}(J_{\mathrm{S}}, I_1) + \nu\psi^{\mathrm{S}}_{\mathrm{FN}}(\mathbf{C}_{\mathrm{S}}, \mathbf{M})\,, \qquad (1)$$

where $\psi^{\mathrm{S}}_{\mathrm{OP}}$, $\psi^{\mathrm{S}}_{\mathrm{IM}}$, and $\psi^{\mathrm{S}}_{\mathrm{FN}}$ are the strain energies of the osmotic pressure, isotropic matrix, and network of collagen fibers, respectively, J_{S}, $\mathbf{C}_{\mathrm{S}}$, and $\mathbf{M}$ are the Jacobian, the right Cauchy-Green tensor, and the reference angular fiber orientation, respectively, and $I_1 = \mathrm{tr}(\mathbf{C}_{\mathrm{S}})$. We modeled the contribution of osmotic pressure as [5]

$$\psi^{\mathrm{S}}_{\mathrm{OP}} = R\theta c^{\mathrm{fc}}_{\mathrm{OS}} n^{\mathrm{F}}_{\mathrm{OS}} \left[\frac{2\bar{c}_{\mathrm{m}}}{c^{\mathrm{fc}}_{\mathrm{m}}} - \frac{\sqrt{4(\bar{c}_{\mathrm{m}})^2 + (c^{\mathrm{fc}}_{\mathrm{m}})^2}}{c^{\mathrm{fc}}_{\mathrm{m}}} + \sinh^{-1}\!\left(\frac{c^{\mathrm{fc}}_{\mathrm{m}}}{2\bar{c}_{\mathrm{m}}}\right) \right], \qquad (2)$$

where $R = 8.314$ MPa mm^3/Kmol, $\theta = 310.15$ K, $c^{\mathrm{fc}}_{\mathrm{OS}}$ is the initial concentration of fixed charges, $n^{\mathrm{F}}_{\mathrm{OS}}$ is the initial fluid volume fraction, $\bar{c}_{\mathrm{m}}$ is the ion concentration of the external solution, and $c^{\mathrm{fc}}_{\mathrm{m}}$ is the initial concentration of fixed charge. We modeled the contribution of proteoglycan as [3,5]

$$\psi^{\mathrm{S}}_{\mathrm{IM}} = \frac{1}{\rho^{\mathrm{S}}_{\mathrm{OS}}}\left[U(J_{\mathrm{S}}) + \frac{1}{2}\mu^{\mathrm{S}}(I_1 - 3) \right], \qquad (3)$$

where $\rho^{\mathrm{S}}_{\mathrm{OS}}$ and μ^{S} are the reference partial density and shear modulus, respectively. We modeled the contribution of the fiber network as [3,5]

$$\psi^{\mathrm{S}}_{\mathrm{FN}} = \frac{1}{\rho^{\mathrm{S}}_{\mathrm{OS}}} \int_{\Omega} \rho(\mathbf{M}) \frac{k_1}{2k_2} \left\{ e^{[k_2(I_4-1)]^2 - 1} \right\} \mathcal{H}(I_4 - 1)\, \mathrm{d}\Omega\,, \qquad (4)$$

where $\rho(\mathbf{M})$ is an orientation distribution function, $k_1$and k_2 are material parameters, $I_4 = \mathbf{M} \cdot \mathbf{C}_{\mathrm{S}}\mathbf{M}$, and $\mathcal{H}$ is a Heaviside function [3,4,5]. We modeled the corresponding permeability of cartilage as [3,5]

$$\mathbf{K}_{\mathrm{F}} = k_{\mathrm{os}} \left(\frac{n^{\mathrm{F}}}{1 - n^{\mathrm{S}}_{\mathrm{OS}}} \right)^{m} \frac{1}{4\pi} \int_{\Omega} \frac{\rho(\mathbf{M})}{I_4} \mathbf{m} \otimes \mathbf{m} \, \mathrm{d}\Omega \,, \qquad (5)$$

where k_{os} is the initial Darcy permeability, n^{F} is the current volume fraction of interstitial fluid, $n^{\mathrm{S}}_{\mathrm{OS}}$ is the reference volume fraction of solid, and $\mathbf{m}$ is the current angular fiber orientation.

Chondrocyte-Death Model. We modeled the fraction of dead chondrocytes $(1 - \hat{n}_{\mathrm{CH}})$, where $\hat{n}_{\mathrm{CH}}$ is the fraction of living chondrocytes) as, cf. [2],

$$(1 - \hat{n}_{\mathrm{CH}}) = A + B e^{\alpha\left(1.99 \cdot E_{\mathrm{yy}} + 4.96 \cdot 2 \cdot E_{\mathrm{xy}}\right)} \qquad (6)$$

where $\alpha = 6.68$, $A = -0.000246$, and $B = 0.000995$ are fitted parameters, and E_{yy} and E_{xy} are the absolute values of the compressive and shear Green-Lagrange strains in the sliding plane, respectively.

Cartilage Specimen with Compression and Sliding. We simulated the experiment performed by Bonnevie et al. [2] and predicted through-thickness patterns in the fraction of dead chondrocytes. We modeled a 2 mm × 2 mm specimen of articular cartilage (and using plane-strain conditions with a 0.1 mm cross-section) with a mesh of 400 linear hexahedral elements. Through the cartilage thickness we modeled the superficial (16%), middle (54%), and deep zones (30%). We compressed the cartilage specimen into a rigid glass surface using 15% bulk axial strain and slid the specimen back and forth on the glass (a reciprocating cycle) at a speed of 1 mm/s. To mimic the experiment, we fixed the subchondral bone in all degrees of freedom and applied (plane strain) symmetry boundary conditions at the cross-sectional surfaces. We also set the fluid flux normal to these surfaces to zero. We kept the remaining surfaces free to displace and set the corresponding fluid pressure to zero. We present the model properties for our analyses in Table 1 [3,4,5].

Table 1: Model parameters for cartilage.

Parameter		Unit
μ^{S}	0.5 (SZ); 1.0 (MZ); 1.8 (DZ)	MPa
k_1, k_2	0.43, 8	MPa, -
$n^{\mathrm{S}}_{\mathrm{OS}}$	0.16 (SZ); 0.54 (MZ); 0.30 (DZ)	-
ν	$0.43(z^*)2 - 0.60(z^*) + 0.85$	-
$J^{\mathrm{S}}_{\mathrm{cp}}$	$0.36 + 0.11(z^*)$	-
k_{OS}	$1 \times 10^{-3} - 0.9 \times 10^{-3}(z^*)$	$\mathrm{mm}^4/\mathrm{Ns}$
m	$3 + 5.0(z^*)$	-
$c^{\mathrm{fc}}_{\mathrm{OS}}$	0.5×10^{-6}	$\mathrm{mol/mm}^3$
$\bar{c}_{\mathrm{m}}$	0.15×10^{-6}	$\mathrm{mol/mm}^3$

$z^* \epsilon [0,1]$ is the normalized tissue thickness from subchondral bone to the articular surface. SZ–Superficial Zone, MZ–Middle Zone, DZ–Deep Zone.

We predicted through-thickness patterns in the fraction of dead chondrocytes during axial compression plus cyclic sliding in both synovial fluid ($\mu_{\mathrm{SF}} = 0.08$) and phosphate-buffered saline (PBS, $\mu_{\mathrm{PBS}} = 0.24$). We performed our simulations using FEBio (University of Utah, USA) and plotted our results using PostView.

RESULTS

We present our numerical predictions versus the corresponding experimental measurements in Fig. 1. Figure 1(a) shows a plot of the through-thickness predictions of the fraction of dead chondrocytes vs. the corresponding experiment from Bonnevie et al. [2] for mixed-mode loading (compression plus cyclic frictional sliding that generates shear) in both synovial fluid and PBS. Figure 1(b) illustrates the predicted pattern of the fraction of dead chondrocytes for mixed-mode loading in synovial fluid, while Fig. 1(c) illustrates the same for loading in PBS.

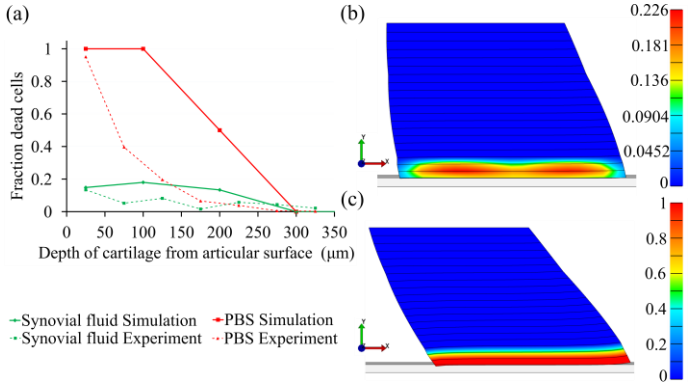

Figure 1: Numerical predictions of the fraction of dead chondrocytes during mixed-mode loading compare favorably to experimental measurements from Bonnevie et al. [2]: (a) plot of through-thickness predictions vs. experiments; and contour plots of the fraction of dead chondrocytes after compression and cyclic sliding in (b) synovial fluid and (c) phosphate-buffered saline.

DISCUSSION

Our simulations of neonatal bovine cartilage successfully predict patterns of the fraction of dead chondrocytes determined in the experiments of Bonnevie et al. [2]. Patterns of the fraction of dead chondrocytes correlate with local Green-Lagrange strains in cartilage and thus cell death is more severe during compression and cyclic sliding against glass within a PBS bath (Fig. 1(c)) than within a bath of synovial fluid (Fig. 1(b)). The superficial zone has a lower effective shear modulus than the middle and deep zones, and hence it is more deformed (compressed and sheared) and presents great cell death. In Fig. 1(a) all of the chondrocytes are dead within 100 μm of the cartilage surface under loading in PBS due to the relatively large friction coefficient and thus shear strain. Note that both the experimental and numerical analyses represent an extreme loading condition (and thus extreme chondrocyte death) as both the maximum compressive and shear strains are reduced *in vivo*, to ~10% and ~12% respectively [6]. Nevertheless, identical loading causes less cell death in synovial fluid versus PBS.

Limitations and outlook. The correspondence between predictions and experiments [2] could be improved. Perhaps refining the mesh using higher order elements could reduce the difference. In this study we only considered the fraction of dead chondrocytes during mechanical loading. However, these elevated loading conditions would likely also damage the other constituents, e.g. collagen and proteoglycan, and thus change the response of cartilage during cyclic sliding (not included in the current study). Our predictions of the fraction of dead chondrocytes form a foundation facilitating the establishment of a new class of computational tools that allow, for the first time, patient-specific modeling of OA progression considering the biomechanics and kinetics of cell and extracellular matrix turnover, both *in vitro* and *in vivo*.

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