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Shape of chondrocytes within articular cartilage affects the solid but not the fluid microenvironment under unconfined compression

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ABSTRACT

Metabolic activity of the chondrocytes in articular cartilage is strongly related to their zone-specific shape and the composition and mechanical properties of their surrounding extracellular matrix (ECM). However the mechanisms by which cell shape influences the response of the ECM microenvironment to mechanical loading is yet to be elucidated. This relationship was studied using a biphasic multiscale finite element model of different shaped chondrocytes in the superficial and deep zones of the ECM during unconfined stress relaxation. For chondrocytes in the superficial zone, increasing the cell's initial aspect ratio (length/height) increased the deformation and solid stresses of the chondrocyte and pericellular matrix (PCM) during the loading phase; for chondrocytes in the deep zone the effect of the cell shape on the solid microenvironment was time and variable dependent. However, for superficial and deep zone chondrocytes the cell shape did not affect the fluid pressure and fluid shear stress. These results suggest that mechanotransduction of chondrocytes in articular cartilage may be regulated through the solid phase rather than the fluid phase, and that high stresses and deformations in the solid microenvironment in the superficial zone may be essential for the zone-specific biosynthetic activity of the chondrocyte. The biphasic multiscale computational analysis suggests that maintaining the cell shape is critical for regulating the microenvironment and metabolic activity of the chondrocyte in tissue engineering constructs.

Statement of significance

We investigated the effect of chondrocyte shape on the cellular microenvironment using a biphasic multiscale finite element analysis. Our study showed that cell shapes affects the solid but not the fluid microenvironment of the chondrocyte, and that maintaining the cell shape is critical for regulating the microenvironment and metabolic activity of the chondrocyte in native cartilage and tissue engineering constructs. As far as we know, this is the first study on the mechanotransduction mechanisms by which cell shape influences the response of the microenvironment to mechanical loading. This study is important for understanding cell mechanobiology, not only for regulation of cell phenotype in tissue engineered constructs but, as important, for understanding changes in normal chondrocyte function after post-traumatic injury and in the initiation and progression of osteoarthritis.

1. Introduction

Articular cartilage is a low-friction, load-bearing material covering the surfaces of diarthrodial joints. Mature cartilage has a zone-dependent inhomogeneous composition: collagen fibers are oriented parallel to the cartilage surface in the superficial zone (SZ), become more randomly oriented in the middle zone (MZ), and align perpendicular to the subchondral bone in the deep zone (DZ) [1,2]. Chondrocytes are the only cell type in articular cartilage and are responsible for the homeostasis of the extracellular matrix (ECM), including the synthesis of type II collagen, proteoglycan and other proteins [3]. Chondrocytes also exhibit zonal shape dependency: flattened or disc-shaped cells aligned with the articular surface in the SZ, spherical shaped and randomly spaced in the MZ, and somewhat elongated cells arranged in columns aligned perpendicular to the subchondral bone in the DZ [4]. Mechanical stimuli modulate the metabolic response of the chondrocytes[5–9], which is influenced by the local profile of interstitial fluid flow and matrix deformation [10–12] and thus their location within the different zones [13].

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Chondrocyte mechanotransduction, the mechanism by which chondrocytes convert mechanical signals into biochemical responses [14], involves the integration and transduction of multiple biophysical signals, such as solid stresses and strains, fluid pressurization, fluid shear stress, and osmotic pressure [15]. Articular cartilage responds to mechanical compression by initiating intracellular calcium transients during and immediately following tissue compression [16]. Calcium is a second messenger directly involved in many cellular processes, including matrix synthesis, cytoskeletal remodeling, cell hyperpolarization and cell death [17]. The zone-specific metabolic activity and gene expression of chondrocytes is strongly related to chondrocyte shape [18], yet the mechanisms by which chondrocyte shape and function are related to the mechanical response of their ECM microenvironment when subjected to external joint loading is yet to be elucidated.

In an attempt to repair a cartilage defect to its native zonal organization, stratified tissue engineering techniques use zonal chondrocyte subpopulations to generate cartilage constructs with zonal features and biomechanical properties [19–22]. Yet, to date, a relatively small number of *in vitro* studies have used this technique, and current *in vivo* treatment strategies do not reflect native zonal differences [23]. Understanding the relationship between cell shape and their microenvironment is important for understanding cell mechanobiology, not only for regulation of cell phenotype in tissue engineered constructs but, as important, for understanding changes in normal chondrocyte function after post-traumatic injury and in the initiation and progression of osteoarthritis.

Because of the difficultly using experimental studies to quantify chondrocyte biomechanical behavior within its ECM, computational models were developed to investigate the biomechanical microenvironment of the chondrocyte [24]. Most computational studies modeled the chondrocytes as spherical in all zones, ignoring the true zonal morphologies [25]. The aim of this study was to investigate how the different shape of chondrocytes in the SZ and DZ would affect the mechanical response of the chondrocytes and their pericellular and extracellular microenvironments when the articular cartilage was loaded in unconfined stress relaxation. The chondrocytes, pericellular matrix (PCM), and ECM were modeled using an inhomogeneous finite-deformation biphasic multiscale finite element analysis. The solid deformation, stress and strain, and the fluid pressure of the chondrocytes and PCM in the SZ and DZ were investigated.

2. Methods

2.1. Finite deformation biphasic theory

The current study used the hyperelastic biphasic theory proposed by Holmes and Mow [26]. The governing equations are

$$\nabla \cdot (\sigma^{e} - pI) = 0 \tag{1}$$

$$\nabla \cdot (v^{s} - k\nabla p) = 0 \tag{2}$$

where p is the fluid pressure, I the identity tensor, $v^{s} = \frac{du}{dt}$ the solid phase velocity, k the permeability, and σ^{e} the effective stress of the solid matrix, defined as

$$\sigma^{e} = \frac{1}{J} F \cdot \frac{\partial \Psi^{s}}{\partial \varepsilon} \cdot F^{T} \tag{3}$$

where J is the volume ratio, $J = \det(F)$, F the Jacobian determinant of the deformation gradient, and $\varepsilon = \frac{1}{2}\left(\nabla u + (\nabla u)^{T} + \nabla u (\nabla u)^{T}\right)$ the Green-Lagrangian strain tensor [27]. The strain energy density function is defined by [26]

$$\Psi^{s} = \alpha_0 \frac{e^{\alpha_1 (I_1 - 3) + \alpha_2 (I_2 - 3)}}{I_3^{\beta}} \tag{4}$$

where I_1, I_2, and I_3 are the invariants of the right Cauchy-Green deformation tensor, $C = F^{T}F$, α_0, α_1, and α_2 positive material parameters, and the dimensionless nonlinear stiffening coefficient $\beta = \alpha_1 + 2\alpha_2$. The parameters α_0, α_1, and α_2 are related to β, aggregate modulus, H_A, and Poisson's ratio, ν, by

$$\alpha_0 = \frac{H_A}{\beta}, \quad \alpha_1 = \frac{1 - 3\nu}{1 - \nu}\beta, \quad \alpha_2 = \frac{\nu\beta}{1 - \nu} \tag{5}$$

The deformation-dependent permeability [28] is defined by

$$k = k_0 J^{m} \tag{6}$$

where k_0 is the initial permeability in the undeformed state, and m a material parameter.The fluid shear stress [29] is estimated by

$$\tau = \omega_{\infty} \sqrt{\mu_f / k} \tag{7}$$

where ω_{∞} is the fluid velocity, and μ_f the viscosity of the fluid (1 mPa s for water).

2.2. Biphasic multiscale finite element simulations

The hyperelastic biphasic theory was implemented in COMSOL Multiphysics (Burlington, MA). Solid mechanics in the Structural Mechanics Module and Darcy's Law in the Earth Science Module were used [30,31]. User-defined function in COMSOL was used to input the strain energy density function (Eq. (4)) [32].

In the biphasic multiscale approach, an axisymmetric macroscale model (Fig. 1, left side) representing articular cartilage in unconfined compression was first analyzed to determine the solid displacement and fluid pressure at each node of the finite element model. The width and thickness of the tissue were 2.5 mm and 1.5 mm, respectively. Quad meshes with a size of 10 × 10 μm were used (~size of chondrocytes). A displacement of 0.075 mm (5% axial compression) was linearly applied in 100 s to the top surface of the articular cartilage and thereafter held as constant. The top and bottom boundaries of the cartilage were assumed frictionless, with the top boundary free to slide in the radial direction while a roller boundary condition was applied to the bottom boundary. A free draining boundary condition was applied to the peripheral (vertical) boundary of the macroscale model. The model was solved to 3000 s at which time an equilibrium condition was achieved. Thereafter the solid displacement and fluid pressure at each chondrocyte location (i.e. 20 × 40 μm rectangle) were extracted (Fig. 1, middle).

Two chondrocytes, one in the superficial zone and one in the deep zone, were studied in detail; their radial and axial (r, z) coordinates (in mm) were (0, 1.44) and (0, 0.15), respectively. Since gradients of solid displacement and fluid pressure around a cell at the axisymmetric axis of the macroscale model were less than 0.1% [25], average solid displacement and fluid pressure at the boundaries of the 20 × 40 μm rectangle were used as boundary conditions for the microscale model (Fig. 1, right side). In the microscale model, the chondrocyte and PCM were modeled as ellipses, with the PCM having a uniform thickness of 2.5 μm and the chondrocyte filling the entire area within the PCM. The initial aspect ratio of the chondrocyte ϕ_0 was defined as the ratio of the major axis to the minor axis. The major axis of the SZ chondrocyte was in the radial direction, while that of the DZ chondrocyte was in the axial direction. The length of major axis for both chondrocytes was fixed as 15 μm. Five aspect ratios, from 1 to 5, were studied for each chondrocyte.

The zone-dependent aggregate modulus for articular cartilage was obtained from published measurements [33–35] (Fig. 2). The

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