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Computational investigation of in situ chondrocyte deformation and actin cytoskeleton remodelling under physiological loading

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ABSTRACT

Previous experimental studies have determined local strain fields for both healthy and degenerate cartilage tissue during mechanical loading. However, the biomechanical response of chondrocytes in situ, in particular the response of the actin cytoskeleton to physiological loading conditions, is poorly understood. In the current study a three-dimensional (3-D) representative volume element (RVE) for cartilage tissue is created, comprising a chondrocyte surrounded by a pericellular matrix and embedded in an extracellular matrix. A 3-D active modelling framework incorporating actin cytoskeleton remodelling and contractility is implemented to predict the biomechanical behaviour of chondrocytes. Physiological and abnormal strain fields, based on the experimental study of Wong and Sah (J. Orthop. Res. 2010; 28: 1554–1561), are applied to the RVE. Simulations demonstrate that the presence of a focal defect significantly affects cellular deformation, increases the stress experienced by the nucleus, and alters the distribution of the actin cytoskeleton. It is demonstrated that during dynamic loading cyclic tension reduction in the cytoplasm causes continuous dissociation of the actin cytoskeleton. In contrast, during static loading significant changes in cytoplasm tension are not predicted and hence the rate of dissociation of the actin cytoskeleton is reduced. It is demonstrated that chondrocyte behaviour is affected by the stiffness of the pericellular matrix, and also by the anisotropy of the extracellular matrix. The findings of the current study are of particular importance in understanding the biomechanics underlying experimental observations such as actin cytoskeleton dissociation during the dynamic loading of chondrocytes.

1. Introduction

Numerous in vitro studies have demonstrated that the actin cytoskeleton plays a role in the biomechanical response of chondrocytes to mechanical stimuli [1–4]. In addition, abnormal mechanical loads, which are believed to contribute to the development of osteoarthritis, can also affect the actin cytoskeleton [5]. Continuous high levels of hydrostatic pressure have been shown to alter the chondrocyte actin cytoskeleton such that it is similar to the actin cytoskeleton found in osteoarthritic chondrocytes [6]. However, the response of the actin cytoskeleton in chondrocytes in situ during physiological and abnormal mechanical loading is poorly understood.

Previous studies have experimentally determined local strains during cartilage on cartilage articulation [7,8]. In addition, Wong et al. [9] have shown that the shear strain in degenerate cartilage is markedly increased near the surface due to both increased friction and a reduction in the mechanical properties of the tissue. A focal defect (FD) is characterized as a partial or full thickness defect in a localized area of articular cartilage tissue, and is typically associated with acute injury or trauma [10,11]. Significantly, FDs have been shown to dramatically alter cartilage deformation during combined loading [12,13]. However, these experimentally determined strains, for both healthy and degenerate cartilage, have not previously been used to investigate the biomechanical response of chondrocytes. Understanding the biomechanical response of chondrocytes to abnormal strains is particularly important given the negative effect of high strain impact loads on chondrocyte activity [14].

Finite element modelling has previously been used to investigate the stress state and deformation in chondrocytes during mechanical loading. Chondrocytes have commonly modelled been as biphasic and isotropic in multi-scale models when simulating their response to deformation [15–18]. In addition, viscoelastic models have been employed to simulate chondrocyte behaviour during single cell micropipette aspiration [19,20] and shear experiments [21]. However, these models of chondrocytes have considered the cell as a passive, homogeneous material. A recent computational–experimental study by Dowling et al. [22] has demonstrated that an active modelling framework, based on the bio-chemo-mechanics of the actin cytoskeleton [23], must be used

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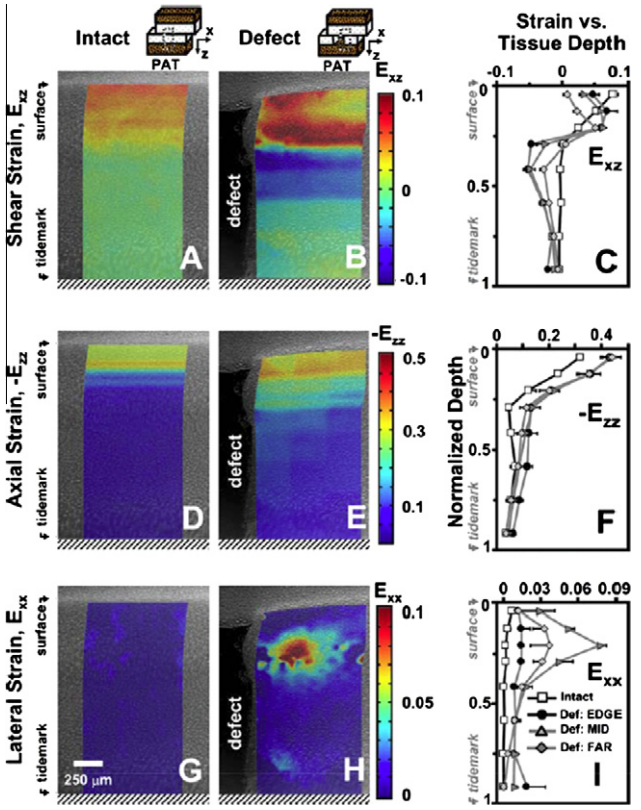

Fig. 1. Experimentally measured shear (A and B) and axial (D and E) and lateral (G and H) strain maps of patellar cartilage, either intact (A, D, and G) or containing a focal defect (B, E, and H), after articulation against trochlear cartilage. Local shear (C) and axial (F) and lateral (I) strain versus normalized tissue depth for patella cartilage, intact or with a focal defect (as a function of lateral distance from the defect edge (EDGE, MID, FAR)). Figure reproduced from Wong and Sah [11] with permission from Wiley Periodicals Inc., copyright © 2010 Orthopaedic Research Society.

to elucidate the mechanisms underlying the behaviour of in vitro chondrocytes during shear deformation. Dowling et al. [22] also demonstrated that a passive material model captures chondrocyte behaviour only if the actin cytoskeleton is disrupted using cytochalasin D (further details of this study can be found in Section 2.2).

The objective of the current work is to investigate the role of active remodelling of the actin cytoskeleton in the response of chondrocytes and the surrounding extracellular matrix (ECM) to physiological loading conditions. Specifically, an active bio-chemo-mechanical model based on remodelling and the contractility of the actin cytoskeleton [23] is implemented in order to simulate the biomechanical behaviour of chondrocytes. Physiological strain fields are chosen based on the experimental study of Wong and Sah [13] (Fig. 1). Abnormal strains [13] due to the presence of a FD are also simulated. Additionally, simulations are performed which investigate the effect of the pericellular matrix (PCM) and cartilage anisotropy on chondrocyte biomechanics and actin cytoskeleton remodelling. It is demonstrated that the distribution and remodelling of the actin cytoskeleton plays an important role in in situ chondrocytes during abnormal loading.

2. Methods

2.1. Active cell model formulation

In the present study the active framework for stress fibre remodelling and contractility proposed by Deshpande et al. [23] is used to model the actin cytoskeleton of chondrocytes. This framework offers a phenomenological description of the biochemistry and mechanics of stress fibre formation, remodelling and contractility. The actin cytoskeleton consists of an assembly of stress fibres, comprised of actin filaments and the motor protein myosin. The formation of the actin cytoskeleton is initiated by a biochemical or mechanical perturbation that triggers a signalling cascade within the cell. In response to an activation signal several intracellular pathways (such as Rac, Rho, Ca^{2+} and Cdc42) may stimulate the polymerization of actin filaments and phosphorylation of myosin II. For example, the signal can result in a large influx of calcium ions (Ca^{2+}) into the cell cytosol, leading to the formation of long actin filaments and the assembly of myosin into bipolar filaments, forming contractile stress fibres [24,25]. In the current study an activation signal is initially applied in order to determine the steady-state actin cytoskeleton distribution in the cell prior to the application of experimentally determined strain fields. The contractility of stress fibres occurs due to the cross-bridge cycling of actin–myosin pairs. In chondrocytes highly aligned bands of stress fibres are not observed; instead, the actin cytoskeleton has a more smeared appearance. However, as detailed in Section 2.2 below, the contribution of the actin cytoskeleton to chondrocyte biomechanics is highly important and is accurately modelled by a 3-D implementation of the active modelling framework.

Full details of the 3-D active stress fibre framework can be found in Deshpande et al. [26] and Ronan et al. [27]. In summary, a kinetic equation describes the formation and dissociation of the actin cytoskeleton:

$$\frac{\mathrm{d}\eta}{\mathrm{d}t} = [1-\eta]\frac{C\bar{k}_\mathrm{f}}{\theta} - \left[1-\frac{T}{T_\mathrm{o}}\right]\frac{\eta\bar{k}_\mathrm{b}}{\theta} \tag{1}$$

where η is the non-dimensional stress fibre activation level. The dimensionless constants $\bar{k}_\mathrm{f}$ and $\bar{k}_\mathrm{b}$ govern the rates of actin cytoskeleton formation and dissociation, respectively. An activation signal that decays exponentially over time $C = e^{(-t/\theta)}$, where θ is a decay constant and t is the time since the most recent signal) triggers formation of the actin cytoskeleton (Fig. 2A(i)), as specified by the first term on the right-hand side of Eq. (1). While actin cytoskeleton formation is driven by the activation signal, the steady-state actin cytoskeleton distribution is determined largely by the ability of the cell and the surrounding PCM to support stress fibre tension. When the tension (T) in a stress fibre is lower than the isometric tension (T_o), fibre dissociation occurs (Fig. 2A(ii)), as specified in the second term on the right-hand side of Eq. (1). The isometric tension is proportional to the activation level of the stress fibre $T_\mathrm{o} = \eta T_\mathrm{max}$) with T_max being the maximum isometric tension in a fully activated stress fibre ($\eta = 1$). Finally, the contractility strain rate relationship of the actin cytoskeleton (Fig. 2A(iii)) due to cross-bridge cycling between actin and myosin is described by a Hill-type equation:

$$\frac{T}{T_\mathrm{o}} = 1 + \frac{\bar{k}_\mathrm{v}}{\eta}\left(\frac{\dot{\varepsilon}}{\dot{\varepsilon}_0}\right) - \frac{\eta}{\bar{k}_\mathrm{v}} \leqslant \frac{\dot{\varepsilon}}{\dot{\varepsilon}_0} \leqslant 0 \tag{2}$$

where $\dot{\varepsilon}$ is the fibre contraction/extension strain rate, and the model parameters $\bar{k}_\mathrm{v}$ and $\dot{\varepsilon}_0$ control the slope of the Hill-type curve. Stress fibres yield at the isometric tension T_o when $\dot{\varepsilon} > 0$, and exert no tension when $\dot{\varepsilon}/\dot{\varepsilon}_0 < -\eta/\bar{k}_\mathrm{v}$.

The active formulation is implemented in a 3-D framework [27], and the model is implemented in the finite element software Abaqus 6.10 (Simulia, Providence, RI) via a user-defined material subroutine (UMAT) [23]. At every integration point the theoretical framework is solved in 240 evenly spaced directions in 3-D space, providing a full prediction of the distribution of the inhomogeneous anisotropic contractile actin cytoskeleton [27]. This active framework is placed in parallel with a neo-Hookean hyperelastic formulation, which represents the passive non-contractile material (E_cyto) surrounding the actin cytoskeleton. The total stress at any