THE ROLE OF THE CYTOSKELETON IN THE VISCOELASTIC PROPERTIES OF OSTEOARTHRITIC HUMAN CHONDROCYTES

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Introduction: The cytoskeleton plays an important role in the physical interactions between the chondrocyte and its extracellular matrix [1, 2] and may therefore be involved in the process of mechanical signal transduction in articular cartilage. The chondrocyte cytoskeleton consists of filamentous proteins such as actin, tubulin, and vimentin which form the microfilaments, microtubules, and intermediate filaments, respectively. Biomechanically, the chondrocyte behaves as a viscoelastic solid structure, and has been modeled using linear elastic and viscoelastic models [3, 4], as well as with biphasic and triphasic continuum models [5, 6]. However, the roles of the different cytoskeletal components in chondrocyte properties are not fully understood. The goal of this study was to test the hypothesis that the major cytoskeletal proteins play a significant role in the viscoelastic properties of the chondrocyte. The micropipette aspiration test was used to determine the effect of various cytoskeletal disrupting agents (cytochalasin D (CD) for microfilaments, acrylamide for intermediate filaments, and colchicine for microtubules) on the viscoelastic properties of isolated human chondrocytes.

Methods: Chondrocytes were isolated from osteoarthritic articular cartilage from human femoral heads retrieved at the time of joint replacement surgery (N=5). All specimens were harvested from sites exhibiting significant macroscopic and histologic characteristics of end-stage osteoarthritis. Chondrocytes were isolated by tissue digestion with 1% pronase and 0.4% collagenase and were stored in 1.2% alginate beads until testing. Alginate beads were incubated in different concentrations of various cytoskeletal disrupting agents for 3 hrs prior to testing (0 μM [control], 0.2 μM [low], 2 μM [medium], and 20 μM [high] of cytochalasin D or colchicine, or 0 μM [control], 0.4 mM [low], 4 mM [medium], and 40 mM [high] of acrylamide). The elastic and viscoelastic properties of single cells were measured using the micropipette aspiration test (n=26-29 at each concentration). A tare pressure (0.01 kPa) was applied to a chondrocyte through the micropipette (~ 5 μm diam.). A step increase in pressure ranging from 0.06-0.59 kPa was then applied and the aspiration length of the cell was measured for 300 s. The viscoelastic properties of the chondrocytes were calculated with a least squares fit of the length versus time plot using a standard three-parameter viscoelastic solid model which assumes the cell to be a homogeneous half-space [7]. If the cell showed any active response by increasing or decreasing in aspiration length after reaching a steady equilibrium, only the passive segment of the creep behavior was used for the curve fit. Statistical analysis was performed using a multivariate analysis of variance.

Results: Exposure of chondrocytes to various cytoskeletal disrupting agents generally resulted in a dose-dependent alteration in cellular mechanical properties. The equilibrium modulus (k_1) decreased significantly with increasing concentration of the different agents, down to 15% of control values with CD, 13% with acrylamide treatment, and to 63% of control values with colchicine treatment. A dose-dependent decrease was observed in the apparent viscosity (μ) with acrylamide treatment, and in k_2 and k_1+k_2 (the instantaneous modulus) with CD or acrylamide treatment. There was little effect of colchicine on these parameters. The relaxation time constant (τ)was increased with CD concentration, but was generally unaffected by acrylamide or colchicine.

Discussion: The findings of this study suggest that all three major cytoskeletal proteins play a role in the mechanical properties of the chondrocyte. In particular, the relatively strong influence of CD and acrylamide suggests that microfilaments and intermediate filaments play the dominant role in governing the "solid-like" viscoelastic behavior of chondrocytes. In fact, at the highest concentrations of these disruptors, the cells sometimes exhibited "fluid-like" behavior and completely flowed into the micropipette under a small applied pressure [8]. Disruption of the microtubules with colchicine, however, had a relatively minor influence and only affected the equilibrium modulus.

These findings are in general agreement with previous studies of the role of cytochalasins and colchicine on the viscoelastic properties of other cells. In intact cartilage, CD was shown to alter the relationship between deformation of the chondrocyte nucleus and that of the cartilage extracellular matrix [2]. In neutrophils, high concentrations of colchicine resulted in a decrease of k_2 and μ [9]. In endothelial cells, k_1 and μ decreased with the addition of 2 μM colchicine, and k_1, μ, and τ decreased with the addition of 2μM cytochalasin B [7]. Similarly, treatment with CD resulted in a decrease in k_1, k_2, and μ in hepatocytes, although colchicine resulted in an increase in k_1 [10].

One explanation for the differential biomechanical role of these various cytoskeletal components may be the intrinsic differences in their molecular properties [11]. Actin forms stiff networks that can fluidize at high strains, while intermediate filament networks of vimentin have a lower shear modulus than actin at low strain but stiffen at high strains. Microtubules polymerize and depolymerize rapidly, and therefore may not be able to provide a significant mechanical contribution. Furthermore, significant differences exist in the distribution of these various molecules in chondrocytes [1,12]. F-actin is predominantly found in the cortex of the chondrocyte with spikes which radiate centrally to the nucleus. Intermediate filaments form networks at the cortex and surrounding the nucleus, with filaments extending radially between these networks. Microtubules radiate from a centrosome near the nucleus. These molecular components may serve an important biomechanical function in governing cell-matrix interactions in articular cartilage, and therefore play a role in regulating the response of the chondrocyte to mechanical stimuli.

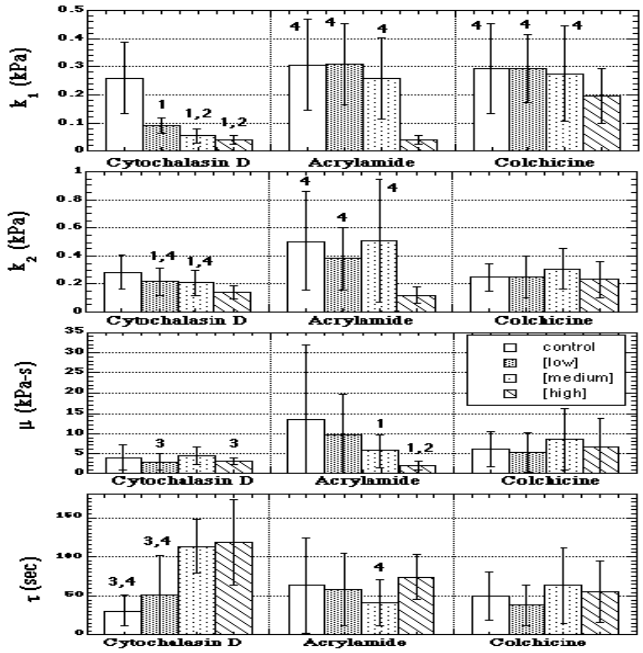

Figure 1. Viscoelastic properties of chondrocytes exposed to cytoskeletal disruptors **1** - $p<0.05$ vs. control, **2** - $p<0.05$ vs. [low], **3** - $p<0.05$ vs. [med], **4** - $p<0.05$ vs. [high]

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