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The role of the cytoskeleton in the viscoelastic properties of human articular chondrocytes

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Abstract

Biomechanical factors are believed to play an important role in regulating the metabolic activity of chondrocytes in articular cartilage. Previous studies suggest that cytoskeletal proteins such as actin, vimentin, and tubulin influence cellular mechanical properties, and may therefore influence the mechanical interactions between the chondrocyte and the surrounding tissue matrix. In this study, we investigated the role of specific cytoskeletal components on the mechanical properties of individual chondrocytes isolated from normal or osteoarthritic hip articular cartilage. Chondrocytes were exposed to a range of concentrations of chemical agents that disrupt the primary cytoskeletal elements (cytochalasin D for F-actin microfilaments, acrylamide for vimentin intermediate filaments, and colchicine for microtubules). Chondrocyte mechanical properties were determined using the micropipette aspiration technique coupled with a viscoelastic solid model of the cell. Chondrocyte stiffness (elastic modulus) was significantly increased with osteoarthritis. With increasing cytochalasin D treatment, chondrocyte stiffness decreased by up to 90% and apparent viscosity decreased by up to 80%. The effect of cytochalasin D was greater on normal chondrocytes than those isolated from osteoarthritic cartilage. Treatment with acrylamide also decreased the moduli and viscosity, but only at the highest concentration tested. No consistent changes in cell mechanical properties were observed with colchicine treatment. These findings suggest that microfilaments and possibly intermediate filaments provide the viscoelastic properties of the chondrocyte, and changes in the structure and properties of these cytoskeletal elements may reflect changes in the chondrocyte with osteoarthritis.

Keywords: Cell mechanics; Osteoarthritis; Mechanical properties; Actin; Tubulin; Vimentin; Micropipette aspiration; Biomechanics

Introduction

Osteoarthritis is a painful and debilitating disease of the synovial joints, affecting nearly 40 million people in the United States [42]. An important hallmark of this disease is the progressive degeneration of the articular cartilage, indicating that the normal balance of anabolic and catabolic activities of the chondrocytes has been severely disrupted [41]. Chondrocyte metabolic activity is regulated by the local microenvironment, consisting of soluble mediators (e.g., cytokines), extracellular matrix composition, and biophysical factors such as mechanical stress that are engendered by normal physiologic loading of the joint. Under abnormal loading conditions, however, mechanical factors are believed to play a significant role in the initiation and progression of joint disease [18,21]. The specific sequence of events through which chondrocytes convert mechanical signals to an intracellular biochemical response is not fully understood (reviewed in [13]), and a better understanding of these mechanisms may provide new insight into the role of biomechanical factors in the etiopathogenesis of osteoarthritis.

The cytoskeleton plays an important role in the physical interactions between the chondrocyte and its extracellular matrix [2,10,34,38] and may therefore be involved in the process of mechanical signal transduction in articular cartilage. Within articular cartilage, the chondrocyte cytoskeleton comprises a dynamic three-dimensional network consisting of principally of the proteins actin, vimentin, and tubulin, that are organized into microfilaments, intermediate filaments, and microtubules, respectively [2,6,33]. The cytoskeleton is also believed to contribute to the biomechanical properties of the chondrocyte, which also influence the interactions between the cell and its pericellular and extracellular

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matrices [12]. In previous studies, we have shown that the mechanical behavior of the chondrocyte is that of a viscoelastic solid [26,48], and it has been assumed that the chondrocyte cytoskeleton is responsible for this "solid-like" elastic response due to its distribution and structure within the cell [10]. However, the sources of viscoelasticity in the cell are not fully understood and may be attributed to flow-dependent (fluid–solid interactions and fluid viscosity) [28,46] as well as flow-independent mechanisms (viscoelasticity of the cytoskeleton) [5,8,20,43,45,47].

The goal of this study was to test the hypothesis that the cytoskeleton is responsible for the viscoelastic properties of the chondrocyte, and that these cellular properties are altered with osteoarthritis. The micropipette aspiration test was used to determine the effect of various chemical agents that disrupt specific cytoskeletal elements (cytochalasin D for microfilaments, acrylamide for intermediate filaments, and colchicine for microtubules) on the viscoelastic properties of isolated human chondrocytes from non-osteoarthritic (macroscopically and histologically normal) and osteoarthritic cartilage. Indirect immunofluorescence and confocal microscopy were used to visualize the distribution of the cytoskeleton in control and treated cells to confirm the action of the different disrupting agents as well as to examine the possibility of non-specific action of these agents on other cytoskeletal proteins.

Materials and methods

Articular cartilage was obtained as surgical waste tissue from human femoral heads at the time of joint replacement surgery ($N = 16$). For each joint, cartilage was removed from sections of the femoral head that appeared macroscopically similar. Cartilage from the same region was also examined by histology. A section of the cartilage and bone from this region was fixed in formalin, paraffin embedded, sliced perpendicular to the surface, and stained with toluidine blue or hematoxylin and eosin (H&E) (Fig. 1). The femoral head and histology sections were then graded on a scale of 1–4 (1 being normal) in four categories: the gross appearance of the femoral head, surface fibrillation, chondrocyte cloning, and proteoglycan staining. If the average of these values was less than or equal to 1.5, the cartilage was classified as non-osteoarthritic ($N = 8$, ages 20–69 yrs). Cartilage with a mean grade of greater than 1.5 was classified as osteoarthritic ($N = 8$, ages 44–79 yrs). The average grades for non-osteoarthritic and osteoarthritic cartilage were 1.47 ± 0.09 and 3.09 ± 0.83, respectively.

Chondrocytes were enzymatically isolated with 1% pronase (90 min, 37 °C) and then with 0.4% collagenase as described previously (180 min, 37 °C) [31,35]. The cells were then suspended in 1.2% alginate beads and maintained at 37 °C and 5% CO_2 in culture medium, containing Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (Gibco, Grand Island, NY) with 10% fetal bovine serum (Hyclone, Logan, UT) and 1% penicillin/streptomycin solution (Gibco). All experiments were performed between 24 and 48 h after cell isolation.

Prior to testing the chondrocytes, alginate beads were incubated for 3 h in control, low, medium and high concentrations of chemical agents that disrupt the three principal cytoskeletal elements (cytochalasin D for microfilaments [44], acrylamide for intermediate filaments [7], and colchicine for microtubules [17]). These concentrations were based on studies reported in the literature [1,3,4,7,44,49,50], as well as our own previous work [16], showing a range of structural and biological influences on the cytoskeletal proteins that we targeted in this study (Table 1).

Table 1
Concentrations of cytoskeletal disrupting agents and number of patients (N) and cells (n) for each experimental condition

Treatment	Concentration	Non-OA	OA
Cytochalasin D			
Control	0	$N = 5$, $n = 46$	$N = 5$, $n = 46$
Low	0.2 μM	$N = 5$, $n = 46$	$N = 5$, $n = 44$
Medium	2 μM	$N = 5$, $n = 45$	$N = 5$, $n = 48$
High	20 μM	$N = 5$, $n = 48$	$N = 5$, $n = 47$
Acrylamide			
Control	0	$N = 5$, $n = 48$	$N = 5$, $n = 47$
Low	0.4 mM	$N = 5$, $n = 48$	$N = 5$, $n = 45$
Medium	4 mM	$N = 5$, $n = 46$	$N = 5$, $n = 45$
High	40 mM	$N = 5$, $n = 48$	$N = 5$, $n = 47$
Colchicine			
Control	0	$N = 4$, $n = 38$	$N = 5$, $n = 47$
Low	0.2 μM	$N = 4$, $n = 38$	$N = 5$, $n = 46$
Medium	2 μM	$N = 4$, $n = 39$	$N = 5$, $n = 47$
High	20 μM	$N = 4$, $n = 36$	$N = 5$, $n = 47$

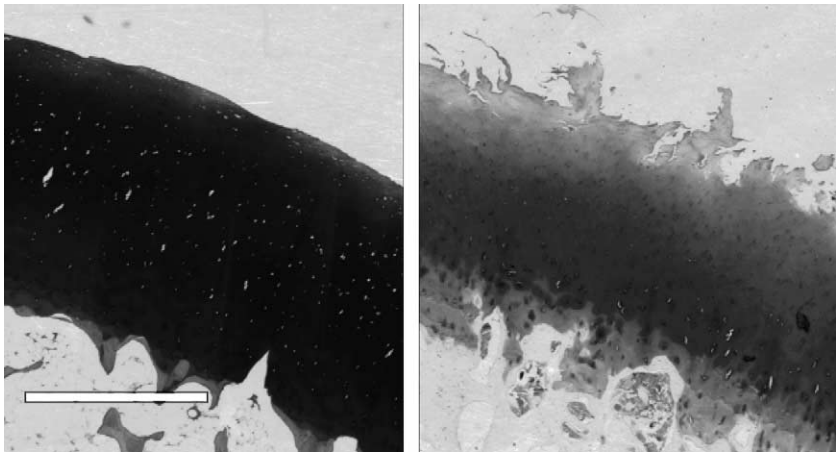

Fig. 1. Histologic sections of non-osteoarthritic (left) and osteoarthritic (right) cartilage labeled with toluidine blue. Osteoarthritic specimens were characterized by fibrillation, loss of toluidine blue labeling, and the presence of chondrocyte clonal clusters. Scale bar = 1 mm.

For each experiment, five alginate beads were then dissolved in 300 μl of 50 mM sodium chloride and 55 mM sodium citrate solution, 10 μl of 0.1% (5 units) hyaluronate lyase (Sigma) and the corresponding concentration of each disrupting agent for approximately 30 min. This solution was then centrifuged and the supernatant removed. The cells were then resuspended in 1 ml of testing solution, consisting of Hank's Balanced Salt Solution, 100 μl of 1% bovine albumin, 0.001% hyaluronate lyase, 2.5×10^{-2} μM ethidium homodimer-1 (Molecular Probes, Eugene, OR), and the specified concentration of the cytoskeletal disrupting agent.

Micropipette aspiration was used to determine the viscoelastic properties of the chondrocytes, as described previously [48]. Briefly, the solution and cells were placed in a chamber that allows for the entry of a micropipette from the side [20]. Micropipettes were made by drawing glass capillary tubes (A-M Systems, Inc., Carlsborg, WA) with a pipette puller (David Kopf Instruments, Tujunga, CA) and fracturing them on a microforge to an inner diameter of approximately 6 μm. The micropipettes and bottom coverslip of the microscope chamber were coated with Sigmacote (Sigma) to prevent cell adhesion. The micropipette diameter was measured using an end-on view through a light microscope.

Pressures were applied to the surface of a chondrocyte through the micropipette with a custom-built adjustable water reservoir and measured with an in-line pressure transducer having a resolution of 1 Pa (Model no. DP15-28, Validyne Engineering Corp., Northridge, CA), as described previously [48]. During the application of pressure, video images of cell aspiration into the micropipette were recorded on a S-VHS video cassette recorder at 60 fields/s with a CCD camera (COHU, San Diego, CA) through a bright-field microscope (Diaphot 300, Nikon Inc., Melville, NY), using a 60× oil immersion objective (numerical aperture = 1.40) (Nikon) and a 10× wide field eyepiece (Edmund Scientific Co., Barrington, NJ). The applied pressures and time were displayed on a video monitor using a digital multiplexer (Vista Electronics, Ramona, CA) and recorded to videotape. The length of cell aspiration was measured with a video caliper system (resolution ±0.2 μm). The horizontal and vertical diameters of each cell were measured prior to testing, and cell diameter was reported as the average of these two measurements.

For creep testing of individual chondrocytes, a tare pressure (0.01 kPa) was first applied to the chondrocyte and allowed to equilibrate for 60s. A step increase in pressure ranging from 0.03 to 0.68 kPa was then applied and the aspiration length of the cell was measured for 300 s. Because cell stiffness varied considerably among the treatment groups, the pressure magnitude was prescribed for each treatment group separately in order to maintain a similar length of aspiration among the experiments. Some cells were observed to respond actively to micropipette aspiration such that after an equilibrium deformation had been reached (generally after 120 s), the aspiration length then began to either increase or decrease. A small number of cells showed active motile behavior during micropipette aspiration. This behavior was characterized by a deviation from the typical monotonic creep curve that is characteristic of viscoelastic solid-like materials during aspiration. The specific criterion used to define this behavior was: (1) a decrease (rather than increase) of the aspiration length in two consecutive data points; (2) an increase or decrease of aspiration length for two consecutive data points following equilibrium; or (3) a correlation coefficient R^2 of less that 0.75 for the non-linear regression. Cells that exhibited active behavior prior to reaching an equilibrium deformation were excluded from the analysis. Following each test, epifluorescence microscopy was used to ensure that that the tested cell had remained viable. The testing solution contained the membrane-impermeant dye ethidium homodimer (Molecular Probes), which is excluded from viable cells. Cells that showed positive nuclear labeling with this dye following micropipette aspiration were excluded from the analysis.

The viscoelastic properties of the chondrocytes were calculated based on an analytical solution of the micropipette aspiration experiment [43]. This model assumes that the cell is a homogeneous linear viscoelastic three-parameter solid half-space, and provides a closed-form solution for the length of aspiration, L, in terms of the time, t, the applied pressure, Δp, the inner micropipette radius, a, the wall parameter, Φ (dependent on the ratio of the thickness to the radius of the micropipette, approximately equal to 2.1). The viscoelastic parameters, k_1, k_2, and μ and the relaxation time constant τ, were calculated by fitting the length of aspiration to the following equations using a non-linear regression analysis (Kaleidagraph, Synergy Software, Reading, PA):

$$L = \frac{a\Delta p\Phi(\eta)}{\pi k_1}\left[1 - \frac{k_2}{k_1+k_2}\exp\left(-\frac{k_1k_2}{(k_1+k_2)\mu}t\right)\right]$$

$$\tau = \frac{(k_1+k_2)\mu}{k_1k_2}$$

The mean and standard deviation of all parameters (k_1, k_2, k_1+k_2, τ and μ) was determined for each treatment condition. Statistical analysis was performed using a multivariate analysis of variance with Newman–Keuls post hoc test, and all results are reported as the mean ± one standard deviation.

Fluorescence immunolabeling was used to visualize the structure of the cytoskeleton under the control and treated conditions. One day after isolation, while still in alginate beads, chondrocytes were exposed to the high concentration of each cytoskeletal disrupting agent (Table 1) for 3 h using the same protocol that was described earlier for micropipette aspiration. The beads were then dissolved for 30 min in the sodium chloride/sodium citrate solution (60 μl per bead) containing 10 μl of 0.1% hyaluronate lyase per 300 μl sodium chloride/sodium citrate, and the appropriate concentration of each cytoskeletal disrupting agent. The cells were centrifuged and the supernatant removed. Culture medium (including the appropriate cytoskeletal disrupting agent) was added to the cells, and this solution was transferred to a Lab-Tek II chamber slide (Nalge Nunc Intl, Naperville, IL) previously coated with 0.1% poly-L-lysine. The slides were stored in an incubator at 37 °C and 5% CO_2 for 1 h. In this manner, cells were maintained in a spherical configuration, as when tested with the micropipette technique. The cells were washed once with PBS, fixed with 50%/50% methanol/ethanol (5 min, −20 °C), and washed three times for 10 min each.

Primary antibodies for actin (JLA20, a mouse IgM anti-chicken) (Jim Jung-Ching Lin, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), vimentin (AMF-17b, a mouse IgG anti-embryonic myofibril chick) (Alice Fulton, Developmental Studies Hybridoma Bank), and β-tubulin (E7, a mouse IgG anti-E. coli) (Michael Klymkowsky, Developmental Studies Hybridoma Bank) were used to visualize microfilaments, intermediate filaments, and microtubules, respectively. The fixed cells were preincubated in PBS with 1% BSA for 30 min, exposed to the primary antibody for 1 h at 0.1 ml/ml PBS, washed three times for 10 min each in PBS, and then exposed to the secondary antibody (Alexa 488 goat anti-mouse IgG, Molecular Probes) for 30 min at 0.04 mg/ml in PBS. Cells were then washed three times for 10 min each with PBS. The slides were allowed to dry and then coated with a Prolong Antifade Kit solution (Molecular Probes) and covered with a coverslip. After drying overnight, the cells were visualized using a confocal laser scanning microscope (LSM 510, Zeiss Inc., Thornwood, NY) with a 63× water immersion objective (Zeiss). An optical slice of 2 μm was used for imaging.

Results

In response to a step pressure, chondrocytes exhibit viscoelastic solid creep behavior characterized initially by a jump in displacement followed by a monotonically decreasing rate of deformation that generally reached an equilibrium displacement within 120 s (Fig. 2a–c). Disruption of the chondrocyte cytoskeleton generally resulted in a dose-dependent alteration in cellular mechanical properties (Figs. 3 and 4). At the highest concentrations of cytochalasin D treatment, some cells exhibited fluid-like behavior [8], flowing completely into the micropipette under a small applied pressure (Fig. 2d–f). For non-osteoarthritic hip chondrocytes, the moduli decreased significantly, by up to 90% from the control value, with increasing concentration of cytochalasin D

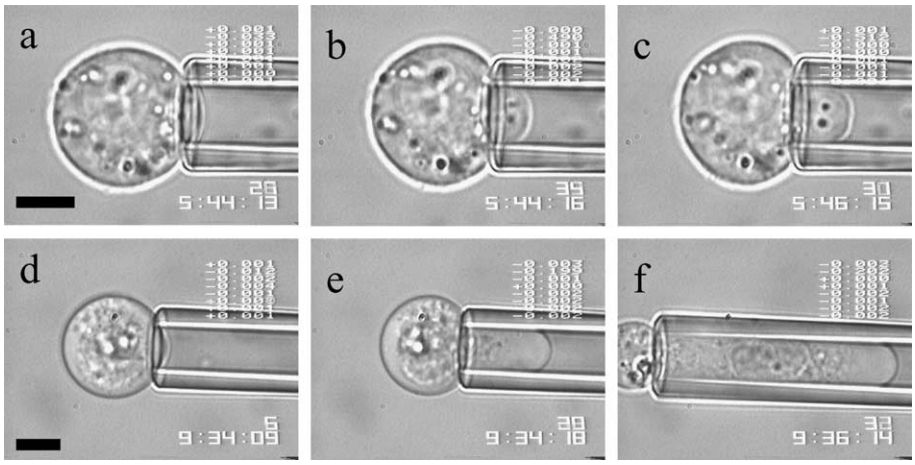

Fig. 2. Micropipette aspiration of chondrocytes under control conditions (a–c) or following treatment with a high concentration (20 μM) of cytochalasin D (d–f). Images are shown prior to aspiration (a,d), 4 s after application of the test pressure (b,e), and 120 s after application of the test pressure (c,f). Disruption of F-actin significantly increased the aspiration length and the relaxation time constant under similar magnitudes of applied pressure. In some cases, disruption of the F-actin network at high concentrations of cytochalasin D caused the cell to behave as a fluid-like material rather than a solid.

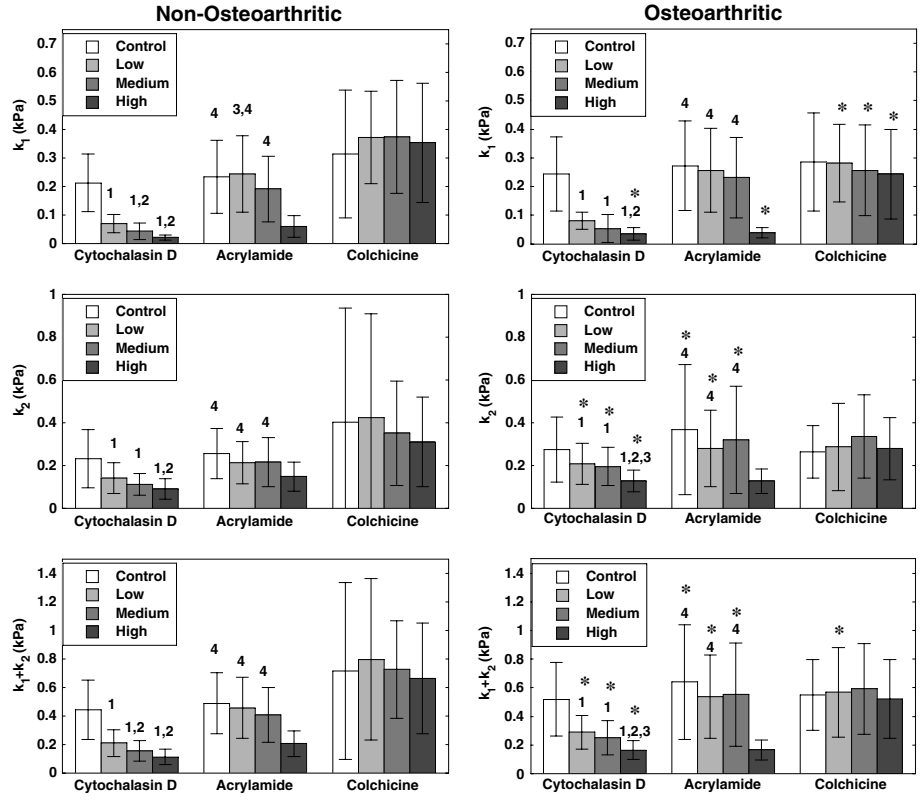

Fig. 3. Elastic moduli of non-osteoarthritic (left) and osteoarthritic (right) chondrocytes exposed to various cytoskeletal-disrupting agents. (1) $p < 0.05$ as compared to control. (2) $p < 0.05$ as compared to low concentration. (3) $p < 0.05$ as compared to medium concentration. (4) $p < 0.05$ as compared to high concentration. $^{*}p < 0.05$ as compared to non-osteoarthritic cells as the same concentration.

concentration ($p < 0.0001$) (Fig. 3). Cytochalasin D also significantly increased the time constant τ and decreased the viscosity μ. (Fig. 3). Only the highest concentration of acrylamide showed a significant effect on cell mechanical properties ($p < 0.01$). Colchicine had no significant effect on any of the properties at any concentration ($p > 0.3$). Cell diameter was unaffected by any of the treatments ($p > 0.1$).

Similar trends were observed in the viscoelastic properties of chondrocytes isolated from osteoarthritic

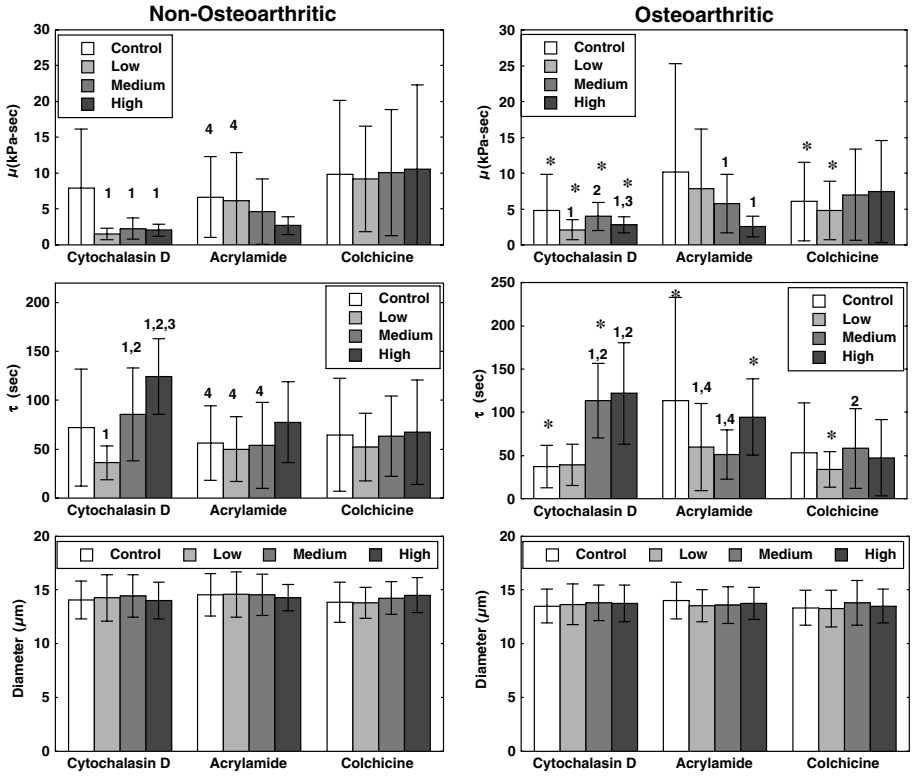

Fig. 4. Apparent viscosity, time constant, and cell diameter of non-osteoarthritic (left) and osteoarthritic (right) chondrocytes exposed to cytoskeletal disrupting agents. (1) $p < 0.05$ as compared to control. (2) $p < 0.05$ as compared to low concentration. (3) $p < 0.05$ as compared to medium concentration. (4) $p < 0.05$ as compared to high concentration. $^{*}p < 0.05$ as compared to non-osteoarthritic cells at the same concentration.

hips. With exposure to cytochalasin D, the moduli at all three concentrations were significantly less than the control (up to 90% less) ($p < 0.02$) (Fig. 4). The time constant, τ, significantly increased from the control and low concentrations with the addition of the medium and high concentrations of cytochalasin D ($p < 0.0001$). While the viscosity, μ, showed some significant differences, there was no clear pattern to this response (Fig. 4). The high concentration of acrylamide significantly decreased all moduli parameters, by up to an 85% ($p < 0.001$). In this group, μ was significantly less than the control with the medium and high concentrations ($p < 0.05$), while τ did not exhibit a dose-response effect. There were no significant difference in the parameters with the addition of colchicine ($p > 0.05$), except for an increase in τ from the low to medium concentration ($p = 0.04$). The diameter did not significantly change with any of the treatments ($p > 0.1$).

By analysis of variance, it was observed that cytochalasin D affected k_1 similarly for non-osteoarthritic and osteoarthritic chondrocytes, but had a greater effect on k_2, $k_1 + k_2$, and μ in non-osteoarthritic cells than in osteoarthritic cells. The time constant, τ, however, was affected by cytochalasin D to a greater extent in osteoarthritic cells than non-osteoarthritic cells. With acrylamide treatment, osteoarthritic cells were more affected than the normal cells at the high concentration for all parameters except τ.

Fluorescence immunolabeling showed significant affects of the various cytoskeletal disrupting agents on the distribution of the three cytoskeletal proteins. Due to the qualitative nature of this analysis, the results are shown only for the high concentration of each disrupting agent. Under control conditions, no apparent differences were observed in the cytoskeletal distribution between the non-osteoarthritic and osteoarthritic cells.

Control cells exhibited a bright solid ring of F-actin around the periphery (cortex) of the cell (Fig. 5a). At the high concentration of cytochalasin D, the staining was more punctate, and the cortical localization of the microfilaments was longer apparent (Fig. 5b). With the addition of 40 mM acrylamide, the F-actin distribution was again more punctate (Fig. 5c). Chondrocyte treated with colchicine showed variable F-actin distribution, in some cells exhibiting cortical localization and in others more punctate labeling (Fig. 5d).

In control cells, vimentin was distributed as networks throughout the cytosol, extending from the nucleus to the cell membrane (Fig. 5e). With the high concentration of acrylamide, the networks were less apparent and sometimes were not present (Fig. 5g). There was little or

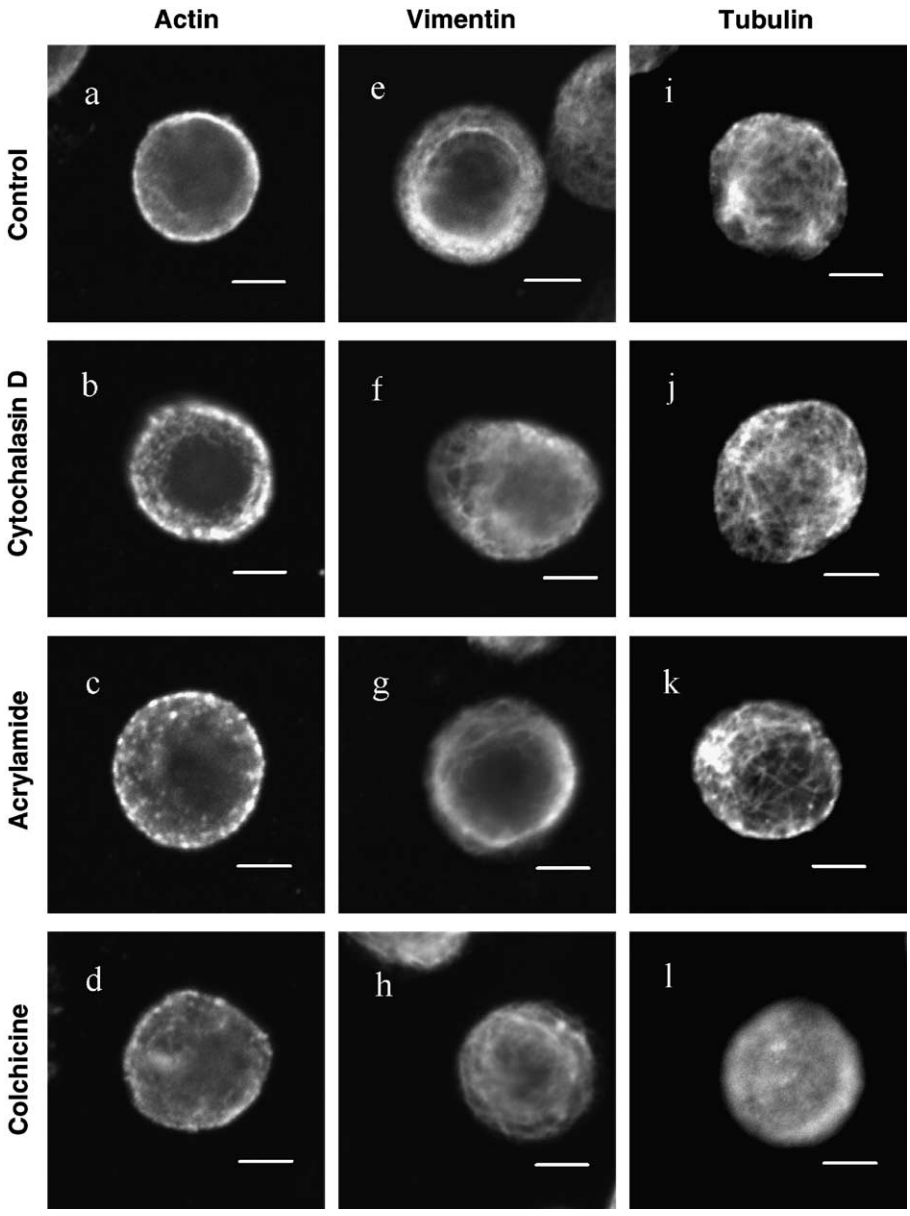

Fig. 5. Fluorescent labeling of F-actin (a–d), vimentin (e–h), and tubulin (i–l) exposed to control (a,e,i), 20 μM cytochalasin D (b,f,j), 40 mM acrylamide (c,g,k), or 20 μM colchicine (d,h,l). Scale bar = 5 μm.

no change in the distribution of vimentin with cytochalasin D or colchicine treatment (Fig. 5f and h).

Tubulin was distributed completely throughout the cytosol, with clear filamentous structure that extended from a single site (the centrosome) (Fig. 5i). With colchicine treatment, network structure was disrupted and tubulin was distributed throughout the cell (Fig. 5l). Treatment with cytochalasin D or acrylamide did not affect the distribution of tubulin (Fig. 5j and k).

Discussion

The findings of this study suggest that specific elements of the chondrocyte cytoskeleton may be responsible for determining the mechanical properties of the chondrocyte. The relatively strong influence of cytochalasin D and acrylamide suggests that microfilaments and intermediate filaments play the dominant role in governing the solid-like viscoelastic behavior of these cells. In contrast, disruption of the microtubules with colchicine had no consistent effect on chondrocyte mechanical properties. One explanation for the differential biomechanical role of these various cytoskeletal components may be the innate differences in their molecular properties and localization within the chondrocytes [24]. Actin is distributed around the cortex and generally forms stiff networks that can fluidize at high strains [25]. It has also been shown recently that chondrocytes express a contractile actin isoform, alpha-smooth muscle

actin [27] that may provide for active cell contraction. In contrast, intermediate filament networks of vimentin stiffen at high strains but have a lower shear modulus than actin at low strains and are located throughout the cytosol [24]. In non-proliferating cells, microtubules are believed to be in a state of relatively rapid turnover termed ''dynamic instability'', constantly polymerizing and depolymerizing [39], and therefore may not contribute significantly to the mechanical properties of the chondrocyte.

These findings are generally consistent with those observed in other cell types, although some specific differences appear to exist in the potential biomechanical role of different cytoskeletal proteins. Specific to chondrocytes, cytochalasin D has been shown to alter the relationship between the deformation of the cartilage extracellular matrix and that of the chondrocyte nucleus [10], suggesting that F-actin was responsible, in part, for transmitting mechanical deformation across the cell membrane. In other studies, however, it has been suggested that cytochalasin D may increase the deformability of the nucleus [37], suggesting that the observed effects may reflect changes in the properties of the cytosol as well as those of the nucleus. Although the chondrocyte nucleus is significantly stiffer than the cytoplasm [14], the ratio of cell to nucleus volume is large ($\sim$10:1) [9], suggesting that the influence of the nucleus on the apparent properties is relatively low.

In other studies, hypo-osmotic stress of chondrocytes has been shown to induce a transient dispersion and reorganization of the cortical F-actin of chondrocytes, leading to a decrease in k_1, k_2, and μ [11]. In endothelial cells, cytochalasin B decreased k_1, μ, and τ [43], while in hepatocytes, cytochalasin D decreased k_1, k_2, and μ [50]. Finally, studies of intervertebral disc cells show that cell of the nucleus pulposus, which exhibit greater actin organization and content in comparison to cells of the anulus fibrosus or transition zone, also possess significantly greater stiffness and apparent viscosity [15]. Taken together, these different studies consistently indicate that F-actin contributes significantly to the moduli of different cell types, including chondrocytes.

Our findings showed little or no effect of colchicine on any of the measured properties, suggesting that microtubules do not contribute to the mechanical properties of the chondrocyte. In contrast to our findings, however, previous studies have shown that high concentrations of colchicine resulted in a decrease of k_2 and μ in neutrophils [4], a decrease of k_1 and μ in endothelial cells [43], and an increase in k_1 in hepatocytes [50]. These differences in the response to colchicine may depend on a number of factors, particularly those relating to the state of cell division. In the present study, chondrocytes were not allowed to divide and were tested within 48 h of isolation. Cells that are grown in monolayer and allowed to divide may therefore be tested in various portions of the cell cycle that exhibit a greater organization of intracellular tubulin. In support of this hypothesis, we have previously observed that chondrocytes in monolayer assume a rounded shape when treated with colchicine [16], while no morphologic changes were induced by colchicine in the present study.

The increased stiffness (moduli) of osteoarthritic chondrocytes relative to non-osteoarthritic chondrocytes is generally consistent with those reported previously [48]. Although little difference in cytoskeletal distribution was seen in this study between non-osteoarthritic and osteoarthritic cells, previous studies with electron and confocal microscopy showed cytoskeletal alterations that were associated with cartilage degeneration. Previous studies have described multiple cell types in normal and diseased cartilage, showing that ''clonal cells'' found in fibrillated cartilage had varying amounts of cytoskeletal actin and vimentin, as opposed to the non-clonal (i.e., ''normal'') cells, which always displayed relatively constant amounts of these cytoskeletal proteins [29]. It is important to note that osteoarthritic cartilage is generally lacking a surface, or superficial zone, suggesting that the population of cells studied in this case includes a greater proportion of middle or deep zone cells as compared to non-osteoarthritic cartilage. In recent studies, we have shown little or no zonal variations in the mechanical properties of porcine chondrocytes from surface zone cartilage as compared to middle/deep zone tissue (Guilak, unpublished results), suggest that the observed differences between osteoarthritic and non-osteoarthritic chondrocytes are not simply due to alterations in the ratio of surface versus middle/deep cells studied [48].

Cytoskeletal alterations with osteoarthritis may also be related to changes in the distribution or quantity of cell surface integrins, which may be increased in osteoarthritic cartilage [36]. In one study, chondrocytes in embryonic day 14 chick sterna were exposed to blocking antibodies to β1, α2, and α3 integrins [19]. In response, the usual F-actin bundles around the cortex of the chondrocyte were instead organized in condensed foci, and disruption of the microfilament structure with dihydrocytochalasin B was associated with reduced integrin expression. This finding provides further indirect support for the hypothesis that the increased moduli of osteoarthritic chondrocytes reflect increased F-actin organization and associated integrin expression.

The intracellular distribution of different cytoskeletal proteins observed in this study was generally consistent with that observed in situ in normal articular cartilage [2,6,33,34]. In cartilage explants and in chondrocytes seeded in 3% agarose, actin is localized predominantly at the cortex of the chondrocyte, with spikes radiating centrally to the nucleus [6,33,34]. This distribution is similar to the pattern of immunolabeling that we observed in this study. In situ, intermediate filaments have

been shown to form networks at the cortex and surrounding the nucleus, with filaments extending radially between these networks [6]. In agarose, there was a similar trend with more intense networks between the nucleus and periphery [34]. Interestingly, previous studies have reported conflicting results, in that one showed no vimentin staining [6] and another showed the most intense vimentin staining in the surface zone of cartilage explants [33]. In the present study, however, the vimentin was more generally distributed throughout the cytosol and near the nucleus in defined filaments. With respect to microtubules, the distribution of tubulin reported in situ is generally consistent with the findings of the present study. In explants of articular cartilage and chondrocytes seeded in agarose, microtubules were observed to radiated from a centrosome near the nucleus [6] and existed as networks distributed throughout the cytosol [33].

Despite the similarities noted in cytoskeletal distribution between our findings and those previously reported for normal cartilage in situ, it is important to note that cell isolation may influence the architecture and arrangement of cytoskeletal proteins [6,22]. For this reason, it is not possible to conclude our findings of altered cellular properties with osteoarthritis directly reflect in situ differences in the structure of chondrocyte cytoskeleton. For example, the hyaluronan cell membrane receptor CD44 has been found to be associated with microfilaments [32]. Without hyaluronan in the extracellular environment, as would occur with enzymatic isolation, CD44 is internalized and is no longer phosphorylated or associated with microfilaments [23, 35,40], potentially leading to reorganization of F-actin. Integrins may also play a role in determining cytoskeleton organization. Loeser et al. [36] used a non-human primate model of naturally occurring osteoarthritis to examine integrin expression in culture and in cartilage. When these cells were treated with cytochalasin B, levels of $\alpha 5$ and $\beta 1$ integrins were reduced, suggesting a direct connection with the integrins and microfilaments. The impact of an extracellular matrix environment is also indicated in the study of chondrocytes in agarose [34]. In these experiments, most cells did not exhibit an organized cytoskeletal structure and showed diffuse staining throughout the cell for the first two days; furthermore, an organized cytoskeleton was only observed after six days in culture.

In summary, our findings indicate that microfilaments and intermediate filaments are predominantly responsible for determining the viscoelastic properties of the chondrocyte. The cytoskeleton may therefore serve an important biomechanical function in governing cell–matrix interactions in articular cartilage, and may also play a role in regulating the response of the chondrocyte to mechanical stimuli. Therefore, changes in mechanical properties of chondrocytes that occur with hip osteoarthritis may reflect changes in the structure and composition of these cytoskeletal proteins [30]. At this point, it is not possible to determine whether these changes are causally related to osteoarthritis, or simply phenomena that occur secondary to the disease process. A further understanding of the biomechanical interactions between the chondrocyte and the extracellular matrix in both normal and diseased conditions may provide new insight into the etiopathogenesis of osteoarthritis.

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