The Effects of Osmotic Stress on the Viscoelastic and Physical Properties of Articular Chondrocytes

Farshid Guilak,* Geoffrey R. Erickson,* and H. Ping Ting-Beall†
*Orthopaedic Research Laboratories, Departments of Surgery, Biomedical Engineering and †Mechanical Engineering and Materials Science, Duke University Medical Center, Durham, North Carolina 27710 USA

ABSTRACT The metabolic activity of chondrocytes in articular cartilage is influenced by alterations in the osmotic environment of the tissue, which occur secondary to mechanical compression. The mechanism by which osmotic stress modulates cell physiology is not fully understood and may involve changes in the physical properties of the membrane or the cytoskeleton. The goal of this study was to determine the effect of the osmotic environment on the mechanical and physical properties of chondrocytes. In isoosmotic medium, chondrocytes exhibited a spherical shape with numerous membrane ruffles. Normalized cell volume was found to be linearly related to the reciprocal of the extracellular osmolality (Boyle van't Hoff relationship) with an osmotically active intracellular water fraction of 61%. In deionized water, chondrocytes swelled monotonically until lysis at a mean apparent membrane area 234 ± 49% of the initial area. Biomechanically, chondrocytes exhibited viscoelastic solid behavior. The instantaneous and equilibrium elastic moduli and the apparent viscosity of the cell were significantly decreased by hypoosmotic stress, but were unchanged by hyperosmotic stress. Changes in the viscoelastic properties were paralleled by the rapid dissociation and remodeling of cortical actin in response to hypoosmotic stress. These findings indicate that the physicochemical environment has a strong influence on the viscoelastic and physical properties of the chondrocyte, potentially through alterations in the actin cytoskeleton.

INTRODUCTION

Articular cartilage is the avascular connective tissue that lines the surfaces of the diarthrodial joints, providing a nearly frictionless bearing surface for transmitting and distributing mechanical loads between bones of the skeleton (Mow et al., 1992). This tissue is maintained in a state of constant turnover by a balance of the anabolic and catabolic activities of the chondrocytes, the single cell type that is present in articular cartilage. The metabolic activity of these cells is strongly influenced by environmental factors such as soluble mediators, matrix composition, and various biophysical factors related to the stress history of the tissue. For example, previous studies have shown that static (i.e., constant) mechanical compression leads to suppression of metabolic activity, whereas cyclic or intermittent compression at physiological frequencies increase the biosynthesis of matrix macromolecules (Guilak et al., 1997). However, the biophysical and biochemical events involved in the transduction of mechanical stress to a cellular response are not fully understood. A full understanding of these signaling mechanisms has been elusive because of the complex ultrastructure and physicochemical properties of the constituents of the extracellular matrix, as well as those of the cell.

Articular cartilage consists primarily of water dissolved with small electrolytes (Na^+, Cl^-, Ca^{2+}, etc.). The remaining solid matrix is composed of collagen (mainly type II) and the proteoglycan aggrecan, with smaller amounts of other proteoglycans, proteins, lipids, and cells (Heinegård and Oldberg, 1989). Collagen forms an anisotropic and inhomogeneous fibrous network that provides primarily the tensile and shear properties of the tissue (Kempson et al., 1973; Mow and Ratcliffe, 1997). Aggrecan is a large aggregating proteoglycan that contains keratan sulfate and chondroitin sulfate glycosaminoglycan chains, consisting of numerous carboxyl and sulfate groups (Hardingham et al., 1994). These groups become negatively charged when dissolved in the interstitial fluid, giving rise to intercharge repulsive forces such that a considerable swelling pressure exists within the tissue. This swelling pressure, which is resisted by the collagen network, will influence the hydration state of the tissue, as well as the mechanical response to applied load or deformation (Maroudas, 1979). An important characteristic of the biomechanical and physicochemical behavior of cartilage involves the transport of the interstitial water and ion phases (Lai et al., 1991; Mow et al., 1992). Mechanical compression of the extracellular matrix causes exudation of interstitial water, which leads to an increase in the apparent concentration of proteoglycans (i.e., increased fixed-charge density) and associated changes in interstitial ion concentrations (Maroudas, 1979). The increased fixed-charge density and ion concentrations within the tissue are believed to contribute to the changes in cell shape and volume that are observed with tissue compression (Guilak, 1995; Guilak et al., 1995; Wong et al., 1997). Furthermore, in early osteoarthritis, disruption of the collagen network leads to increased water content of the tissue (Guilak et al., 1994) and a corresponding decrease in the pericellular osmolality (Maroudas et al., 1985). These

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Address reprint requests to: Farshid Guilak, PhD, Orthopaedic Research Laboratories, 375 MSRB, Box 3093, Duke University Medical Center, Durham, North Carolina, 27710. Tel.: 919-684-2521; Fax: 919-681-8490; Email: guilak@duke.edu.

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changes in fixed-charge density correspond to changes of up to 150 mOsm in the interstitial osmolality of the tissue (Lai et al., 1991; Maroudas, 1981) and may have a significant effect on chondrocyte metabolic activity (Urban et al., 1993).

Most cells of the body are highly sensitive to their physicochemical environment and respond to osmotic stress by activating various mechanisms to restore their volume to its original state. These processes may involve coordinated organization of the cytoskeleton and activation of various transporters in the plasma membrane that rapidly mediate the mobilization of osmotically active solutes (Sarkadi and Parker, 1991). Such changes in intracellular ion concentrations, particularly those that function as second messengers (e.g., Ca^{2+}), may influence other cellular responses such as gene expression and cellular metabolism, as well as reorganization of the cytoskeleton. In this regard, the relationships among compression of the extracellular matrix and cell volume, membrane stretch, cytoskeletal structure, and cellular mechanical properties may play an important role in the process of mechanical signal transduction in chondrocytes (Borghetti et al., 1995; Erickson et al., 2001; Hall, 1995; Mobasheri et al., 1998; Urban et al., 1993).

The goals of this study were to quantify the physical and biomechanical properties of the chondrocyte and to examine the hypothesis that the physicochemical (osmotic) environment of the cell influences these properties. Isolated chondrocytes were exposed to anisoosmotic conditions, and various microscopy techniques were used to determine changes in cell morphology and apparent membrane area. Micropipette aspiration was used in conjunction with a theoretical viscoelastic model of the chondrocyte to determine the effects of osmotic stress on the viscoelastic properties of the cell. Fluorescent confocal microscopy was used to qualitatively examine alterations in F-actin distribution in response to osmotic changes.

MATERIALS AND METHODS

Cell culture and isolation

Chondrocytes were isolated from articular cartilage of the femoral condyle of skeletally mature pigs ($N = 4$ animals) immediately after sacrifice. Cells were isolated with sequential pronase and collagenase treatment, encapsulated in 1.2% (w/v) alginate beads at a density of 10^6 cells/ml, and cultured in Dulbecco modified eagle medium (DMEM, Gibco BRL Products, Life Technologies, Grand Island, NY) with 10% fetal bovine serum and penicillin/streptomycin for 1–3 days until testing. Immediately before testing, cells were released from the alginate with 55 mM sodium citrate and 50 mM sodium chloride. Cells were suspended in an isoosmotic buffer for testing (Hank's balanced salt solution with 0.1% bovine serum albumin). NaCl or deionized water was added to the isoosmotic solution (303 mOsm) to achieve hypoosmotic (153 mOsm) or hyperosmotic (466 mOsm) testing solutions. Media osmolality was measured using a freezing-point depression osmometer (Advanced Instruments, Needham Heights, MA). All experiments were performed at room temperature (~21°C). At room temperature, cells reached a steady-state volume within 5 min of osmotic challenge, but exhibited little or no volume recovery for up to 30 min. In all experiments, cells were exposed to osmotic challenge or control solution for 15 min before testing or fixation.

Cell size and morphology

Quantitative measurements of cell diameter were recorded in different osmotic environments using video light microscopy coupled with a digital caliper system (Vista Electronics, Ramona, CA). The diameter of each cell was obtained by averaging the measured values of the vertical and horizontal diameters. Cell volume and apparent surface area were calculated from cell diameter assuming that cells were spherical. Qualitative measures of cell and membrane morphology were made using scanning electron microscopy (SEM). Cells were placed on 12-mm glass disks treated with poly-L-lysine (Sigma Chemical, St. Louis, MO). The cells were allowed to settle and adhere for 30 min, then exposed to osmotic challenge for 15 min before being fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (Electron Microscopy Sciences, Fort Washington, PA) for 1 h at room temperature. The cells were washed in DMEM-H medium and postfixed with 1% OsO_4 in a veronal acetate buffer (pH 7.4). To prevent shrinkage, the cells were further fixed in freshly prepared 1% tannic acid (Sigma Chemical) as described previously with 50 mM cacodylate buffer, pH 7.4 for 1 h and followed by another hour of fixation in 0.5% uranyl acetate in deionized water (McCarthy et al., 1985; Wollweber et al., 1981). A series of acetone dehydrations was performed and the cells were air-dried in tetramethylsilane (Electron Microscopy Sciences). A conductive paint was used to attach the cell-covered glass disks to aluminum stubs and the cells were then sputter-coated with gold and palladium. Specimens were observed in a scanning electron microscope (Model 501, Philips, Mahwah, NJ) and stored in a desiccator before and after imaging.

Apparent membrane area

Approximately 1 ml of cell suspension was placed into a specially designed chamber that allowed for the entry of the micropipette from the side (Hochmuth, 2000). The chamber was mounted on a translating stage that allowed the entire chamber and solution to be rapidly replaced as a micropipette held a single cell stationary in the viewing field of the microscope. A coverslip (No. 1), coated with Sigmacote (Sigma Chemical), was used as the bottom of each chamber, providing a short, optically clear path between the objective lens and the cells. All experiments were performed on passage 0 cells, 1–3 days after cell isolation. Micropipettes were made by drawing glass capillary tubes (A-M Systems, Everett, WA) with a vertical pipette puller (Model 700C, David Kopf Instruments, Tujunga, CA) and were then fractured on a custom-built microforge to an inner diameter (a) of 7–11 μm. Micropipettes were coated with Sigmacote to prevent cell adhesion during testing.

Chondrocytes ($n = 58$) were held stationary with a glass micropipette in a chamber containing isoosmotic solution. The micropipette was then inserted inside a large micropipette (~50 μm diameter) to minimize perturbations to the cell during transfer to a new chamber (Evans, 1989). The chamber was rapidly replaced by an adjacent chamber containing deionized water, and the larger micropipette was then removed. Cell swelling was recorded at video rates (60 fields/s) until lysis using a charge-coupled device camera (Model XC-77, Hamamatsu Photonic System, Bridgewater, NJ) through a bright-field microscope (Leitz Diavert, Rockleigh, NJ), using a 100× oil immersion objective of 1.25 N.A. (Carl Zeiss, Thornwood, NY). Cell diameter was measured as the mean of the horizontal and vertical diameters and was measured from videotape using a video dimensional analysis system (Vista Electronics, Ramona, CA). Cell volume and surface area were calculated from the cell diameter assuming a spherical geometry. The apparent "excess" membrane area was calculated as the maximum change in the cell surface area between the isoosmotic solution and deionized water.

Osmotic activity

A linear model of the ideal osmotic swelling behavior was fit to the cell volume (V) (normalized to the volume in isoosmotic solution) and the osmolality of the suspending medium (P) (normalized to the isoosmotic osmolality) (Lucke and McCutcheon, 1932; Ponder, 1948; Ting-Beall et al., 1993). Assuming that the osmotic activity within the cell remains constant, then $V/V_o = (R/P) + (1 - R)$, where R (the Ponder's value) represents the chemical activity of the intracellular water relative to the isoosmotic state.

Viscoelastic properties

Micropipette aspiration and micromanipulation were performed using techniques similar to those described previously (Evans and Hochmuth, 1976; Guilak, 2000; Hochmuth, 2000; Jones et al., 1999; Sato et al., 1990; Trickey et al., 2000b). A range of micropipette diameters was used to maintain a constant ratio of micropipette diameter to cell diameter of ~0.44. In previous studies, we have developed numerical models of the micropipette aspiration test that show there is little influence of the test geometry on the predicted response of the cell if the ratio of micropipette diameter to cell diameter is maintained between 0.3 and 0.5 (Haider and Guilak, 2000).

Micropipette aspiration of the cell was performed by applying a pressure gradient to the cell surface using a custom-built adjustable water reservoir, and pressures were measured directly with an in-line pressure transducer (Validyne Engineering, Northridge, CA) with a resolution of 0.1 Pa. A tare pressure of 10 Pa was first applied to the cell surface for 60 s to achieve an initial test geometry and to ensure a complete pressure seal. A single step change in pressure (ΔP) of 100–500 Pa was then applied to the cell, and images of cell aspiration into the micropipette were recorded at video rates (60 fields/s) as described previously. The applied pressure magnitude and the time were recorded on the monitor using a digital multiplexer (Vista Electronics). The length (L) of cell protrusion into the micropipette as a function of time was measured from videotape at a resolution of ± 0.2 μm with a video dimensional analysis system (Vista Electronics) in synchronization with the time and applied pressure readings.

The viscoelastic mechanical properties of the cell were determined from the experimental pressure and cell deformation data using a theoretical model (Sato et al., 1990) that represents the cell as a homogeneous half-space under small deformation with an applied uniform axisymmetric pressure from the micropipette. A three-parameter viscoelastic model (standard linear solid) was used to represent the material behavior of the cell, assuming intrinsic incompressibility of the cell (Poisson's ratio = 0.5) (Guilak, 2000; Sato et al., 1990; Trickey et al., 2000b). The governing equations for this model were solved for the parallel and series elastic parameters (k_1 and k_2, respectively) and the apparent viscosity (μ) of the cell as a function of the radial (r) and axial (z) coordinates, the applied pressure (ΔP), the displacement (L) at the cell surface, and the inner (a) and outer (b) radii of the micropipette, assuming a boundary condition of no axial displacement of the cell at the micropipette end. For this "rigid punch" model, a dimensionless parameter Φ is used to incorporate the effect of the wall thickness of the micropipette (Theret et al., 1988). For the micropipette geometries used in the present study, a value of $\Phi = 2.1$ was applicable for the entire range of values of a and b used in our experiments.

A nonlinear regression analysis was used to obtain the viscoelastic coefficients k_1, k_2, and μ from the measured displacement $L(t)$ at the surface of the cell and the applied pressure ΔP using the following equations:

$$L(t) = \frac{\Phi a \Delta P}{\pi k_1}\left[1 - \frac{k_2}{k_1 + k_2}\, e^{-t/\tau}\right], \qquad (1)$$

where

$$\mu = \frac{\tau \cdot k_1 k_2}{k_1 + k_2}. \qquad (2)$$

F-actin distribution

Chondrocytes were plated on coverslips in plastic chambers (Lab-Tek II Chamberslides, NalgeNunc International, Naperville, IL) for 30 min. Cells were fixed in 3.7% (w/v) paraformaldehyde solution of the same osmolality for 10 min at room temperature. After washing twice with phosphate-buffered saline, cells were extracted with chilled acetone for 5 min at −20°C, followed by a 20 min labeling in 3.3 units/coverslip of fluorescently labeled phalloidin (Oregon Green 488 phalloidin, Molecular Probes, Eugene, OR) with 1% bovine serum albumin in phosphate-buffered saline. The samples were protected from light and air-dried before mounting with antifade medium (Prolong, Molecular Probes). F-actin labeling in the cells was visualized using confocal laser scanning microscopy (LSM 510, Carl Zeiss). Images were obtained through an inverted fluorescent microscope (Axiovert 100M, Carl Zeiss) with a C-Apochromat, 63×, water immersion, 1.2-NA objective lens. Argon-ion laser excitation intensity (488 nm) was set to 10% of full power and images were recorded at an emission wavelength of 515 nm on an 8-bit intensity scale.

Statistical analysis

A multivariate analysis of variance (MANOVA) with a Student-Newman-Keuls post hoc test was used to test for differences in cell diameter, volume, k_1, k_2, $k_1 + k_2$, and μ among the different osmolalities. Statistical significance was reported at the 95% confidence level ($P < 0.05$). Linear correlation analysis was used to examine the relationship between osmolality and cell volume for the Ponder's analysis. Statistical analysis was performed using the Statistica software package (Statsoft, Tulsa, OK). All results are reported as mean ± one standard deviation.

RESULTS

Cell size and morphology

In isoosmotic medium, chondrocytes exhibited a spherical shape with numerous membrane ruffles and microvilli (Fig. 1). In hypoosmotic medium, the cells remained spherical and swelled significantly, exhibiting a relatively smooth membrane. In hyperosmotic medium, cells decreased in volume and showed an apparent increase in the size of membrane ruffles. Statistical analysis showed that cell diameter and cell volume were significantly increased in hypoosmotic media and decreased in hyperosmotic media ($P < 0.001$, Fig. 2).

Excess membrane area

Cells exposed to deionized water swelled monotonically until lysis. The mean cell diameter increased from 12.6 ± 1.8 μm to 19.0 ± 2.1 μm at lysis ($P < 0.00001$), corresponding to an increase in apparent surface area from 509 ± 145 μm^2 to 1150 ± 240 μm^2 ($P < 0.00001$), and an increase in cell volume from 1110 ± 480 μm^3 to 3720 ± 1110 μm^3 ($P < 0.00001$). The "excess" membrane area, or

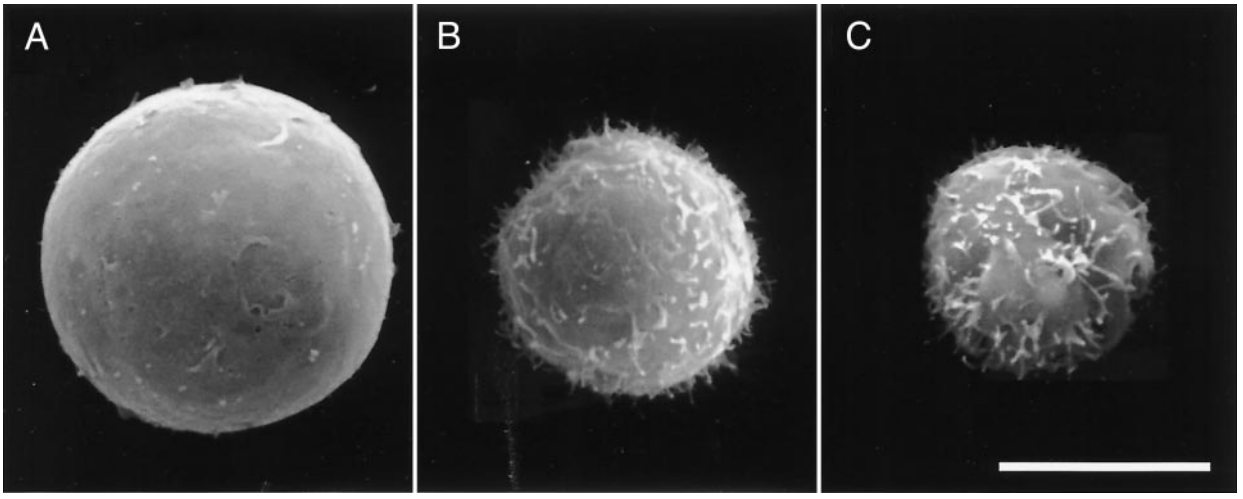

FIGURE 1 SEM of the effects of osmotic stress on chondrocyte morphology. (*A*) In hypoosmotic medium (153 mOsm), chondrocytes swelled significantly, exhibiting a relatively smooth plasma membrane. (*B*) In isoosmotic medium (303 mOsm), chondrocytes exhibited numerous membrane ruffles and microvilli. (*C*) Hyperosmotic medium (466 mOsm) decreased cell volume with an apparent increase in membrane ruffling. Scale bar = 10 μm.

the maximum surface area at lysis, was 234 ± 49% of the initial area ($P < 0.0001$, $n = 58$, Fig. 3).

Osmotic activity

Cell size changed monotonically in response to osmotic stress, and no active volume regulation was observed during the 15-min test period. In a Boyle Van't Hoff plot, a linear relationship was observed between normalized chondrocyte volume V/V_o and the inverse osmolality relative to the isoosmotic state ($1/P$) over a range of osmotic stress (Fig. 4). The slope of this curve R (the Ponder's value), was found to be 0.61 ($R^2 = 0.99$).

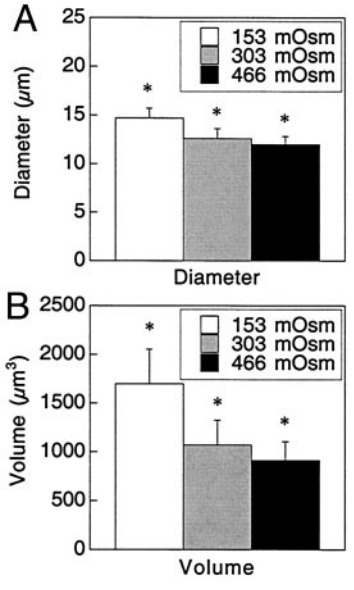

FIGURE 2 Effects of the osmotic environment on chondrocyte diameter and volume. Alterations in the osmotic environment of the cells caused significant changes in (*A*) cell diameter and (*B*) cell volume. *$P < 0.0001$ vs. other osmolalities.

Viscoelastic properties

Chondrocytes exhibited viscoelastic solid creep behavior in response to a step increase in pressure. Hypoosmotic stress significantly decreased the instantaneous and equilibrium elastic moduli and the apparent viscosity (μ) of the cell in comparison to the isoosmotic control and the hyperosmotic cases ($P < 0.001$ by MANOVA, Fig. 5). Hyperosmotic stress, however, did not significantly affect cell properties relative to the isoosmotic condition. The relaxation time (τ), which is related to the moduli and to μ by Eq. 2, was not significantly affected by hyper- or hypoosmotic stress.

F-actin distribution

Significant changes were observed in the distribution of F-actin in response to hypoosmotic stress, but not hyperosmotic stress (Fig. 6). In control cells, F-actin was distributed primarily in a narrow region at the cortex of the cell. With hyperosmotic stress, a similar distribution was observed. After hypoosmotic stress, F-actin was distributed evenly throughout the cell and showed no localization to the cortex.

DISCUSSION

The findings of this study indicate that acute changes in the osmotic environment have a strong influence on the viscoelastic mechanical properties as well as the morphology of the chondrocyte. These results suggest that changes in the osmotic environment in situ, secondary to mechanical com-

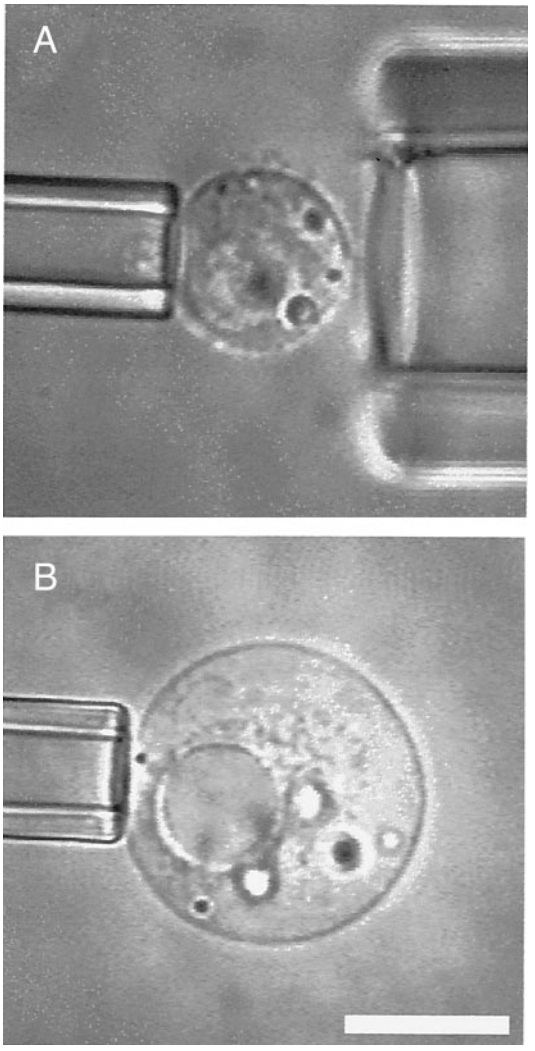

FIGURE 3 Determination of the apparent surface area of the chondrocyte membrane. Isolated chondrocytes were rapidly transferred from a chamber containing isoosmotic medium (*A*) to a chamber containing deionized water (*B*). Chondrocytes exposed to deionized water swelled rapidly until the plasma membrane was completely unfolded and the cell lysed. The maximum apparent membrane area at lysis was found to be 234 ± 49% that of the apparent membrane area in the isoosmotic state. Scale bar = 10 μm.

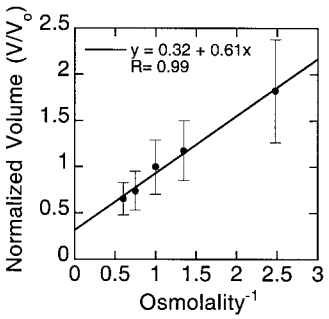

FIGURE 4 Ponder's plot for chondrocytes. Normalized cell volume was found to be linearly related to the reciprocal of the extracellular osmolality (Boyle van't Hoff relationship), suggesting that chondrocytes behave as "ideal" osmometers. The osmotically active volume fraction of intracellular water was determined to be 61% of the cell volume. Each datapoint represents the mean ± SD of n = 135–150 cells, N = 3–4 experiments.

pression of the cartilage extracellular matrix, may have a significant influence on the mechanical properties of the chondrocyte. Hypoosmotic swelling caused a significant increase in cell diameter and volume, leading to cell lysis with exposure to deionized water, whereas hyperosmotic stress caused significant cell shrinkage. These findings are consistent with the changes in chondrocyte volume observed in situ with mechanical or osmotic stress of the matrix (Bush and Hall, 2001; Guilak, 1995; Guilak et al., 1995).

Chondrocytes exposed to deionized water swelled until lysis. Under these conditions, the apparent cell surface area increased on average by a factor of 2.34 before rupture, suggesting that chondrocytes possess a significant amount of "excess" membrane area in the isoosmotic state. Presumably, this membrane area is contained in the many folds and ruffles that are observed by SEM in the isoosmotic state (Fig. 1). This characteristic is similar to that of neutrophils, which will swell to 2.1 times their normal surface area before lysis (Ting-Beall et al., 1993). The presence of significant excess membrane area in the isoosmotic state, coupled with the fact that the plasma membrane has a relatively low bending and shear moduli as compared with its areal modulus (Heinrich and Waugh, 1996), suggests that the chondrocyte may undergo relatively large deformations in situ without exposing the cell membrane to significant stress. This information may be useful in the interpretation of studies of cellular signaling that have implicated membrane stress or stretch as a potential mechanism of mechanotransduction (Guilak et al., 1999; Lee et al., 2000). Additionally, this conclusion provides support for the assumption used for theoretical models of cell-matrix interactions (Gu et al., 1997; Guilak and Mow, 2000; Mow et al., 1999) as well as for micropipette analysis of single cells (Jones et al., 1999; Trickey et al., 2000*b*) that the chondrocyte membrane does not structurally contribute to the mechanical properties of the cell at small strains.

Normalized cell volume was found to be linearly related to the reciprocal of the extracellular osmolality (Boyle van't Hoff relationship), suggesting that chondrocytes behave as "ideal" osmometers under passive conditions. It is important to note that these experiments were performed at room temperature, where little or no volume regulation is observed in chondrocytes (Urban et al., 1993). However, the influence of temperature on their viscoelastic properties is unknown. The osmotically active volume fraction of intracellular water was determined to be 61% of the cell volume. Previous studies on bovine chondrocytes have reported a similar osmotically active volume fraction of 59% measured on populations of cells using a Coulter counter (McGann et

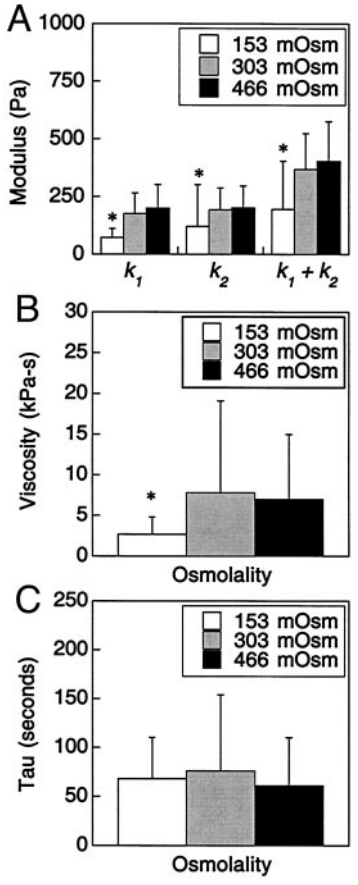

FIGURE 5 Effects of osmotic environment on chondrocyte viscoelastic properties. Alterations in the osmotic environment of the cells caused significant changes in cell viscoelastic properties. (*A*) The instantaneous modulus ($k_1 + k_2$) and the equilibrium modulus (k_1) were significantly decreased by hypoosmotic stress but were unaffected by hyperosmotic stress. (*B*) Hypoosmotic stress, but not hyperosmotic stress, also significantly decreased the apparent viscosity μ of the chondrocytes. (*C*) No changes were observed in the relaxation time (τ) because of osmotic stress. $^*P < 0.005$.

al., 1988). These findings are generally consistent with an osmotically active fraction of 77% previously determined for neutrophils (Ting-Beall et al., 1993), 74% for lymphocytes (Devireddy et al., 1998), and 75% for pancreatic islet cells (de Freitas et al., 1997).

In response to a step change in pressure, chondrocytes exhibited 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. Significant decreases were observed in the material coefficients (k_1, k_2, $k_1 + k_2$, and μ) of the chondrocyte between the hypoosmotic solution as compared with the iso- and hyperosmotic solutions. This finding indicates that the mechanical properties of chondrocytes are coupled to their physicochemical state, and that hypoosmotic stress has a distinct effect on the cell as compared with no discernible effect of hyperosmotic stress. This finding is in contrast with the behavior of neutrophils, which exhibit an increase in moduli and apparent viscosity with increased osmolality, but exhibit little change in their viscoelastic properties with decreased osmolality (Sung et al., 1982). The explanation for this difference may be the fact that neutrophils behave mechanically as a liquid drop with a persistent cortical tension (Evans and Yeung, 1989; Hochmuth, 2000), whereas chondrocytes behave as viscoelastic solids over a large range of strains (Hochmuth, 2000; Trickey et al., 2000a, 2000b). In this regard, the swelling behavior associated with hypoosmotic stress would be expected to increase the cortical tension of neutrophils and therefore the apparent viscoelastic coefficients (Sung et al., 1982). This hypothesis is further supported by a recent finding that the apparent cortical tension of neutrophils increases by stretching the membrane either by osmotic swelling or mechanical dilation (Albarran et al., 2000).

The changes observed in chondrocyte properties may simply be attributable to changes in the apparent concentration and three-dimensional organization of cytoskeletal proteins with altered cell volume; however, the different responses to hypo- and hyperosmotic stress suggest a more complex alteration in the structure of cytoskeletal elements or other intracellular proteins. Our findings showed a significant change in the distribution of F-actin within the cell after hypoosmotic stress, consistent with dissociation of the actin cortex (Fig. 6). This observation is consistent with previous studies showing that cell-swelling because of hypoosmotic stress decreases F-actin content in Ehrlich ascites tumor cells (Pedersen et al., 1999). Other studies have shown that hypoosmotic stress may cause rapid dissociation of F-actin microfilaments in PC12 cells (Cornet et al., 1994). F-actin is the predominant cytoskeletal protein that determines the elastic and viscoelastic properties of chondrocytes, and disruption of the F-actin with cytochalasin D decreases all three viscoelastic coefficients of chondrocytes, as measured by micropipette aspiration (Trickey et al., 2000a). Our study supports the hypothesis that the observed changes in chondrocyte viscoelastic properties with hypoosmotic stress are attributable to a dissociation or redistribution of the F-actin cytoskeleton. The mechanisms leading to this event are not fully understood but potentially involve a transient increase in the intracellular concentration of Ca^{2+} (Erickson and Guilak, 2001; Richelme et al., 2000). These findings suggest that changes occurring in the physicochemical environment of the chondrocyte in situ because of deformation of the extracellular matrix may directly influence the cytoskeletal structure and viscoelastic properties of the cell, therefore altering the biomechanical interactions between the chondrocyte and its extracellular matrix (Guilak and Mow, 2000). It is important to note, however, that the present studies were performed on isolated cells and the response of chondrocytes to osmotic stress in situ may be altered by physical interactions between the actin cytoskel-

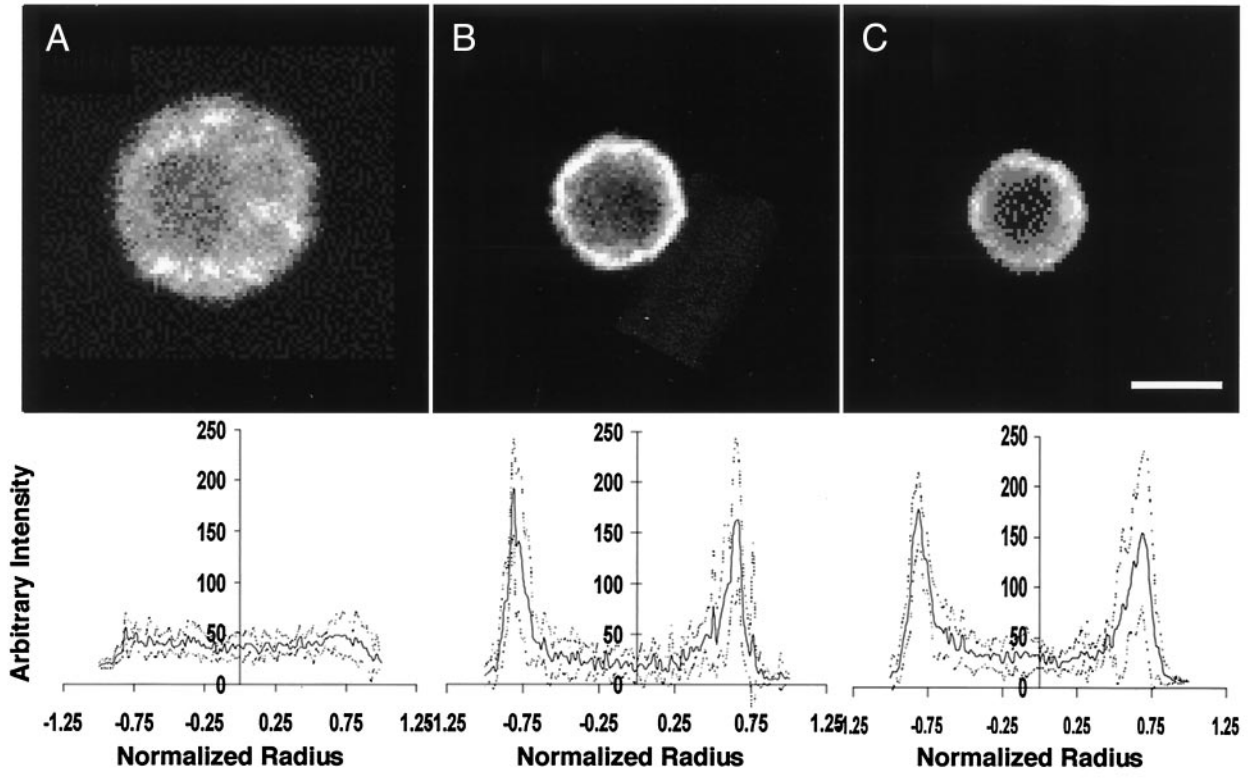

FIGURE 6 Reorganization of the F-actin cytoskeleton in response to hypoosmotic stress. Profiles of fluorescence intensity were recorded and averaged across the centers of 10 cells at each osmolality (mean ± SD). The actin cytoskeleton was found to dissociate and disperse after exposure to hypoosmotic stimulation (*A*) when compared with the isoosmotic control (*B*). Exposure to hyperosmotic stress did not alter F-actin distribution (*C*). Scale bar = 10 μm.

eton and the extracellular matrix through transmembrane proteins (Low et al., 1997).

Theoretical models of the mechanical and physicochemical response of the chondrocyte are particularly valuable in that they may be used to predict biophysical quantities and relationships that are difficult or impossible to measure experimentally. The osmotic and viscoelastic properties measured in this study will potentially be of value to models that seek to predict the coupled stress-strain, electrical, and physicochemical environments of cells (Gu et al., 1997; Guilak and Mow, 2000; Mow et al., 1999). Additionally, an understanding of the biophysical and osmotic properties of chondrocytes is important for the optimization of cell viability during cryopreservation (McGann et al., 1988), where significant osmotic gradients may be generated across the cell membrane. Further study of the transient and active responses of the cell to osmotic stress will improve our understanding of the biophysical pathways involved in mechanical signal transduction.

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