Viscoelastic Properties of the Cell Nucleus

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Mechanical factors play an important role in the regulation of cell physiology. One pathway by which mechanical stress may influence gene expression is through a direct physical connection from the extracellular matrix across the plasma membrane and to the nucleus. However, little is known of the mechanical properties or deformation behavior of the nucleus. The goal of this study was to quantify the viscoelastic properties of mechanically and chemically isolated nuclei of articular chondrocytes using micropipet aspiration in conjunction theoretical viscoelastic model. Isolated nuclei behaved as viscoelastic solid materials similar to the cytoplasm, but were 3–4 times stiffer and nearly twice as viscous as the cytoplasm. Quantitative information of the biophysical properties and deformation behavior of the nucleus may provide further insight on the relationships between the stress–strain state of the nucleus and that of the extracellular matrix, as well as potential mechanisms of mechanical signal transduction. © 2000 Academic Press

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Under normal physiological circumstances, cells of the body are exposed to a complex and diverse environment of mechanical stresses and strains due to passive deformation and active behavior. Within the musculoskeleton, tissues such as the articular cartilage lining the joints of the body may be exposed to cyclic stresses with peak magnitudes exceeding 10 MPa (1). Cells of the body are not only able to withstand such stress environments, but in fact respond to mechanical stress and other biophysical factors related to mechanical stress (e.g., strain, fluid flow and pressure, electrokinetic effects) as "signals" to regulate their gene expression and metabolic activity. The mechanisms of mechanical signal transduction at the cellular level are not fully understood, and may involve activation of ion channels, G-proteins, or other transmembrane signaling pathways (2–4). More recently, it has been hypothesized that one pathway which mechanical stress may regulate cellular activity at the genome is through a direct physical connection from the extracellular matrix across the plasma membrane and to the nucleus (5, 6).

In support of this hypothesis, previous studies have shown evidence of cytoskeletal connections between the extracellular matrix and the nucleus via integrin receptors (5), as well as coordination of nuclear shape with that of the cell via the cytoskeletal proteins (7). Other studies have demonstrated that nuclear pore size and the rate of nucleocytoplasmic transport may depend on cell and nucleus shape during cell attachment (8, 9). Specific to articular cartilage, the nuclei of chondrocytes change shape and volume with physiologic magnitudes of extracellular matrix compression (10), and nuclear shape is coordinated with alterations in the biosynthesis of matrix components such as aggrecan (11).

Taken together, these various studies support the hypothesis that mechanical deformation of the nucleus plays a role in signal transduction. To directly test such a hypothesis, it would be important to have an accurate knowledge of the stress–strain state of the cell and nucleus. However, a quantitative description of the stress and strain environment at the cellular and subcellular levels may be difficult or even impossible to measure in certain circumstances. In this regard, theoretical models of cellular mechanics can provide insight on the biophysical factors which influence cell physiology, and a measure of the biomechanical properties of the cell nucleus would be important for theoretical models of cell mechanics or cell-matrix interactions (12, 13). A number of studies have utilized micromechanical techniques to measure the properties of the cytoplasm (e.g., 14–22), but little or no quantitative information is available on the deformation behavior or mechanical properties of the cell nucleus. Preliminary studies using micropipet aspiration with a

viscoelastic fluid model have suggested that the inhomogeneities in the properties of neutrophils may be due to significantly different nucleus stiffness as compared to the cytoplasm (15). More recently, scanning probe microscopy has revealed inhomogeneities in the properties of adherent cells which have been attributed to differences in cytoplasmic and nuclear properties (20).

The goal of this study was to test the hypotheses that the cell nucleus behaves as a viscoelastic solid, and that it possesses biomechanical properties that are distinct from those of the cytoplasm. The nuclei of primary chondrocytes were isolated using both mechanical (microdissection using micropipet aspiration) and chemical (detergent) methods, and direct quantitative measurements of the viscoelastic properties of cells and nuclei were made using the micropipet aspiration technique in conjunction with a viscoelastic solid model of the cell nucleus.

MATERIALS AND METHODS

Isolation and culture of cells. Unless otherwise noted, reagents and chemicals were purchased from GibcoBRL (Grand Island, NY). Knee joints of 2-year-old pigs ($N = 4$) were obtained from a local abattoir. Under aseptic conditions, the joints were dissected and explants of articular cartilage was harvested from the femoral condyles. Chondrocytes were isolated from the extracellular matrix using previously described methods (23). Briefly, the tissue was minced and incubated in wash media containing 1320 PUK/mL of pronase (Calbiochem, San Diego, CA) and 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY) for 1 hour at 37°C, 95% air, 5% CO_2. The tissue pieces were centrifuged and rinsed in wash media twice. The tissue pellet was then resuspended in wash media containing 0.4% collagenase (Worthington, Freehold, NJ) and 10% FBS (Gibco) for 3 hours at 37°C, 95% air, 5% CO_2. Following the incubation, the cell suspension was filtered through a sterile nylon filter (70 μm) (Becton Dickinson, Franklin Lakes, NJ) to remove extracellular debris. Cell viability were determined using a trypan blue exclusion assay and was found to be greater than 95% in all cases. The cells were resuspended at a density of 10^6 cells/ml in 1.2% (w/v) alginate (Alginic Acid, Sigma Chemical) dissolved in 150 mM NaCl. Beads were formed by dropwise addition of the alginate into 102 mM $CaCl_2$, as described previously (23) and allowed to cure for 10 min. The beads were rinsed three times with 150 mM NaCl and cultured overnight in culture medium consisting of Ham's F-12 medium with 10% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 1 μg/ml Fungizone.

Immediately prior to testing, the alginate beads were dissolved in a 55 mM sodium citrate and 50 mM sodium chloride solution to release the cells. The cells were then suspended in Dulbecco's phosphate-buffered saline (without Ca^{2+} or Mg^{2+}) at room temperature containing 0.1% bovine serum albumin. Approximately 1 ml of cell suspension was placed in a custom-built chamber designed to allow for the entry of two micropipets from the sides (16). A coverslip (No. 1), coated with Sigmacote, was used as the bottom of the chamber, providing a short, optically clear path between the objective lens and the cells. All experiments were performed between 1 and 3 days following cell isolation.

Micropipet manipulation. Micropipet micromanipulation and aspiration were performed using techniques similar to those described previously (16, 17, 19). Micropipets were made by drawing glass
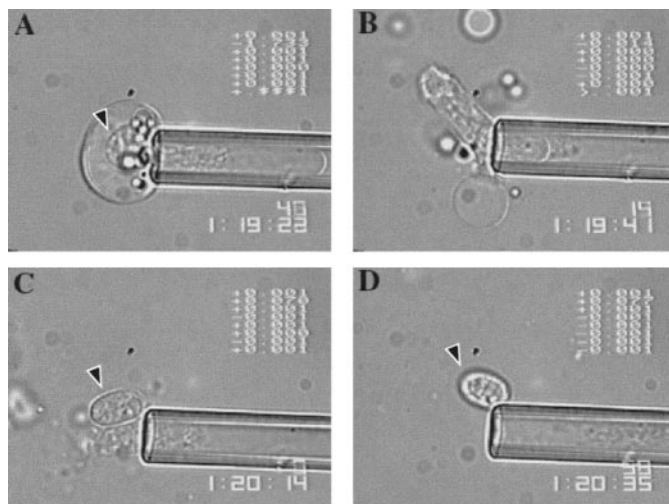
FIG. 1. Mechanical isolation of the cell nucleus using micropipet aspiration. A glass micropipet with an inner diameter of 4 to 6 μm was used to completely aspirate the cell repeatedly until the membrane ruptured. The micropipet was then used to aspirate the nucleus in order to physically extract and separate it from the surrounding cellular debris. Arrowheads indicate the nucleus.

capillary tubes (A-M Systems, Inc., Everett, WA) with a vertical pipet puller (Model 700C, David Kopf Instruments, Tujunga, CA) and were then fractured on a custom-built microforge to an inner radius (*a*) of 2–3 μm for nuclear isolations and cell aspiration testing, or *a* = 1–2 μm for nuclear aspiration testing. A range of micropipet diameters was used to maintain a constant ratio of cell or nucleus diameter to micropipet diameter. Micropipets were coated with Sigmacote (Sigma Chemical) to prevent cell adhesion during testing.

Mechanical isolation of nuclei. Chondrocytes were suspended in the testing buffer in a chamber on an inverted video microscope. A glass micropipet of radius *a* = 2–3 μm was used to repeatedly aspirate the cell completely until the membrane ruptured (Figure 1). The micropipet was then used to aspirate the nucleus in order to physically extract and separate it from the surrounding cellular debris.

Chemical isolation of nuclei. Nuclei were chemically isolated by treatment with detergent (0.1% IGEPAL CA-630 in phosphate-buffered saline), followed by centrifugation at 500*g* for 20 min. to separate the nuclei from the cellular debris. The nuclei were then suspended in a testing buffer consisting of 0.75% bovine serum albumin in Hank's balanced salt solution with 15 mM Hepes.

Viscoelastic testing. Micropipet 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 Corp., Northridge, CA) with a resolution of 0.1 Pa. The initial diameter of each cell or nucleus was obtained by averaging the measured values of the vertical and horizontal diameter before aspiration.

A tare pressure of 10 Pa was first applied to the cell or nucleus surface for 60 s to achieve an initial test geometry and to ensure a complete pressure seal. A test pressure (ΔP) of 100 to 500 Pa was then applied to the cell or nucleus and images of the specimen aspiration into the micropipet were recorded at video rates (60 fields/s) with a CCD camera (Cohu, San Diego, CA) through a brightfield microscope (Nikon, Melville, NY), using a 60× oil immersion objective (numerical aperture = 1.4) (Nikon) and a 10× wide field
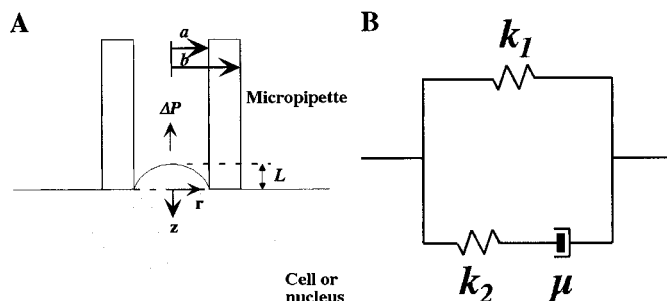
FIG. 2. Theoretical model of the micropipet aspiration test. (A) The cell or nucleus was represented as a homogeneous half-space under small deformation with an applied uniform axisymmetric pressure from the micropipet (19). (B) A three-parameter viscoelastic model (standard linear solid) was used to represent the material behavior of the cell.

eyepiece (Edmund Scientific Co, Barrington, NJ). The applied pressure magnitude and the time were recorded simultaneously on S-VHS videotape using a digital multiplexer (Vista Electronics, Ramona, CA). The initial diameter of each cell was obtained by averaging the measured values of the vertical and horizontal diameters before it was drawn into the micropipet. Initial cell volume was calculated from the cell diameter assuming a spherical geometry. The length (L) of cell protrusion into the micropipet 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.

Theoretical modeling of micropipet aspiration. The viscoelastic mechanical properties of the cell were determined from the experimental pressure and cell deformation data using a theoretical model (19) which represents the cell as a homogeneous half-space under small deformation with an applied uniform axisymmetric pressure from the micropipet (Figure 2A). A three-parameter viscoelastic model (standard linear solid) was used to represent the material behavior of the cell (Figure 2B), assuming intrinsic incompressibility and isotropy (Poisson's ratio = 0.5) (19). The governing equations for this model were solved for the elastic parameters (k_1 and k_2) 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 micropipet, assuming a boundary condition of no axial displacement of the cell at the micropipet end. For this "rigid punch" model, a dimensionless parameter Φ was used to incorporate the effect of the wall thickness of the micropipet (21). For the micropipet 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. The radial and axial coordinates were set to zero to obtain a simple equation for the displacement $L(t)$ at the surface of the cell in the center of the micropipet, which were solved by nonlinear regression to obtain k_1, k_2, and μ

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

where

$$\mu = \frac{\tau \cdot k_1 k_2}{k_1 + k_2}. \qquad [2]$$

These elastic constants k_1 and k_2 are related to standard elasticity coefficients by the following relationships

$$E_0 = \frac{3}{2}(k_1 + k_2), \quad E_\infty = \frac{3}{2} k_1, \qquad [3]$$

where E_0 is the instantaneous Young's modulus and E_∞ is the equilibrium Young's modulus.

Statistical analysis. The micropipet aspiration technique was used to determine the viscoelastic properties of the intact chondrocytes ($n = 15$), chemically isolated nuclei ($n = 15$), and mechanically isolated nuclei ($n = 20$). A multivariate analysis of variance (MANOVA) with a Student–Newman–Keuls *post hoc* test was used to test for differences in diameter, k_1, k_2, $k_1 + k_2$, and μ between cells and the mechanically and chemically isolated nuclei. Statistical significance was reported at the 95% confidence level ($P < 0.05$). Statistical analysis was performed using the Statistica software package (Statsoft, Inc., Tulsa, OK). All results were reported as mean $\pm$ one standard deviation.

RESULTS

Both chemical and mechanical isolation techniques yielded whole intact nuclei with little or no remnants of cytoplasmic material, as visualized by differential interference contrast (DIC) microscopy (Figure 3A) or fluorescent labeling of nuclei acids (Figure 3B) or the nuclear membrane (Figure 3C).

Cells and their nuclei displayed creep behavior typical of a viscoelastic solid, characterized by an initial jump in displacement with the application of step pres-
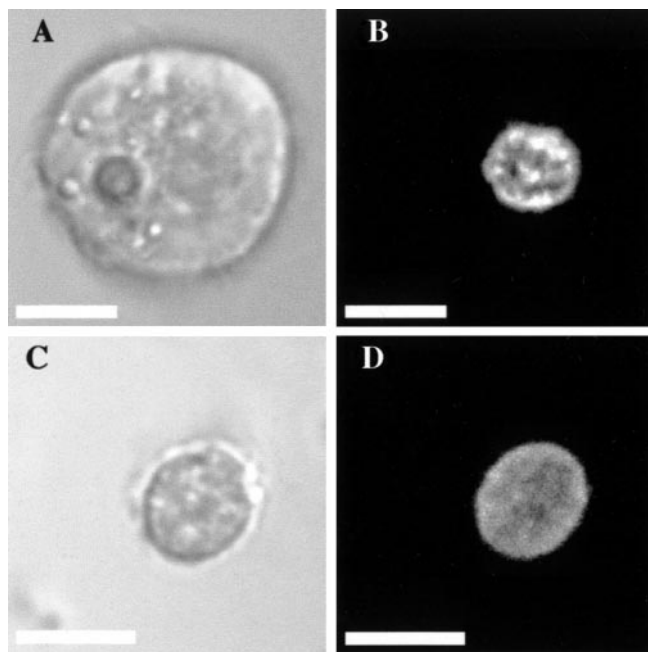
FIG. 3. Micrographs of intact cells and isolated nuclei. (A) Differential interference contrast image of intact chondrocyte. (B) Fluorescent confocal image of the same cell labeled with acridine orange, a marker of nucleic acids. (C) DIC image of a chemically isolated nucleus and (D) acridine orange labeling of the same nucleus. Both chemical and mechanical isolation techniques yielded whole intact nuclei will little or no remnants of the cytoplasm. Scale bars = 5 μm.
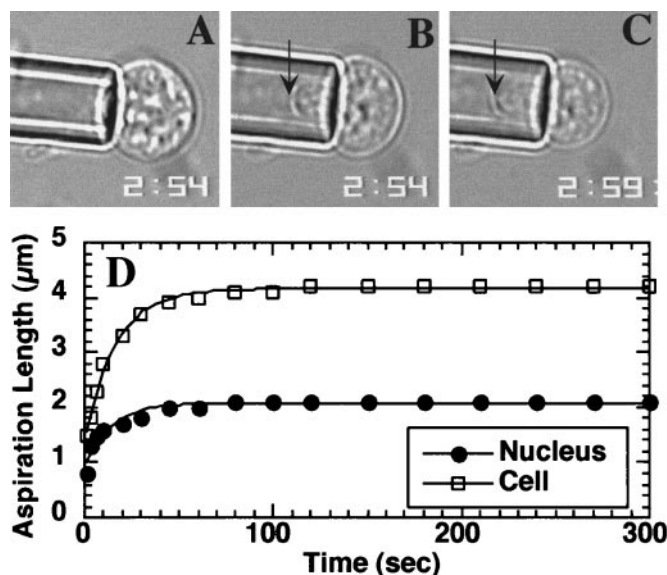
FIG. 4. Micropipet aspiration and viscoelastic creep behavior of cells and nuclei. Cell and nuclear behavior was characterized by an initial jump in displacement with the application of step pressure, followed by asymptotic creep displacement of the edge of the nucleus (arrows) until equilibrium. (A) Resting state of nucleus at time $t = 0$; (B) transient deformation at time $t = 10$ s after application of test pressure; (C) equilibrium state at $t = 300$ s after application of test pressure; (D) nonlinear regression curve fits to an exponential rise function were excellent, with a mean correlation coefficients of $R^2 = 0.93$–0.99. Data points represent experimental measurements and lines represent theoretical curve fits.

sure, followed by asymptotic creep displacement until equilibrium (Figures 4A–4C). Overall, nonlinear regression curve-fits to an exponential rise function were excellent, with a mean correlation coefficient of $R^2 = 0.96$ (Figure 4D).
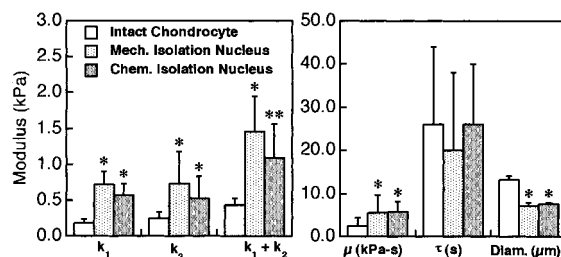
Highly significant differences were observed in all measured mechanical properties of cells as compared to mechanically or chemically-isolated nuclei (Figure 5). Chondrocyte nuclei exhibited instantaneous and equilibrium elastic moduli that were approximately 3 times that of the cytoplasm in intact cells. Nuclei also exhibited an apparent viscosity that was more than twice that of intact chondrocytes. The mechanically isolated nuclei also exhibited a significantly higher equilibrium modulus as compared to chemically isolated nuclei. No significant differences were observed in the exponential time constant (τ).

DISCUSSION

The findings of this study indicate that the nucleus behaves as a viscoelastic solid material, similar to that of the chondrocyte cytoplasm. However, nuclear properties were distinct from those of the cell cytoplasm, and nuclei were significantly stiffer and more viscous

than intact chondrocytes. These data provide important evidence of the constitutive behavior and viscoelastic properties of the cell nucleus. In this manner, a direct, quantitative measurement of the biomechanical properties of the nucleus has important implications regarding theoretical models of cell mechanics which have generally assumed cells to be homogeneous in properties. This finding is in general agreement with previous confocal microscopy studies of intact cartilage which showed that chondrocyte nuclei deform less than intact cells under the same loading conditions (10). The large differences between cytoplasmic and nuclear properties suggest that the nucleus may provide a major contribution to the inhomogeneity, and thus, the apparent mechanical properties of the cell (15).

In this study it was found that the chondrocyte nucleus behaved as a viscoelastic solid. This conclusion was based on the theoretical model of a viscous fluid drop with an elastic cortical shell being drawn into a micropipet (22), which would predict complete aspiration of the nucleus into the pipet beyond a threshold deformation (i.e., $L/a = 1$) (24). However, aspiration of the cell into the micropipet beyond this threshold length suggests that the nucleus is exhibiting behavior characteristic of a solid rather than that of a viscous fluid with cortical tension. In the present study, chondrocytes and their nuclei exhibited an initial "jump" in deformation with applied pressure, followed by a slow asymptotic entry into the micropipet up to two times their radii (Figure 4), which is behavior that is characteristic of a viscoelastic solid. The equilibrium Young's modulus (E_∞) of the nucleus was found to be on the order of 1 kPa, which is consistent with an elastic modulus of 1–5 kPa reported for isolated eukaryote chromosomes studied by micromanipulation and micropipet aspiration (25) as well as previous reports on microdissected or osmotically isolated neutrophil nuclei (15).

FIG. 5. Mechanical properties of cells and mechanically or chemically isolated nuclei. Chondrocyte nuclei exhibited instantaneous and equilibrium elastic moduli that were 3–4 times greater and an apparent viscosity that was more than 2 times greater than that of intact chondrocytes. The mechanically isolated nuclei also exhibited a significantly higher equilibrium modulus as compared to chemically isolated nuclei, *$P < 0.05$ vs intact cells, **$P < 0.05$ vs mechanically isolated nuclei. Results are presented as mean $\pm$ SD, $n = 15$–20 per group.

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

Several mechanisms have been proposed whereby signal transduction may be regulated by mechanical deformation of the nucleus (5, 6). Changes in nuclear shape or volume may alter the properties of the pore complex of the nuclear membrane, which regulates the transport of nucleic acids, proteins, and ions between the cytoplasm and nucleus (26). Previous studies have demonstrated that the rate of transport of nucleoplasmin-coated gold particles into the nucleus, as well as the functional size of the nuclear pores, was greater in cells which had a more flattened shape (8). Alternatively, mechanical signals may have a direct effect on intranuclear transcription or translation processes. The evidence for this hypothesis is still indirect, but it has been suggested that mechanical perturbations of nuclear matrix proteins may influence gene expression by exposing or obscuring molecular binding sites during mRNA transcription or through changes in DNA conformation (5).

To model such phenomena, recent studies have described cells and their nuclei as elastic "tensegrity" structures which assume that specific components of the cell are in tension while others bear compressive stresses (27, 28). These models have provided an intriguing paradigm which has been able to describe many of the behaviors of adherent cells. Our findings further suggest that in such a paradigm, the intrinsic viscoelasticity of the nucleus may play a significant role in the biomechanical interactions between the nucleus, cytoskeleton, and extracellular matrix. In the future, a more thorough understanding of the mechanics of the nucleus may require an investigation of the physical properties of the nucleic acids and nuclear matrix proteins, as well as their structural arrangement within the nucleus (25, 29).

The "microdissection" method used to mechanically isolate the cell nucleus allowed direct testing of the nucleus while minimizing the confounding effects of the cell membrane, cytoskeleton, and cytoplasm. It is important to note that mechanically-isolated nuclei exhibited significantly higher stiffness as compared to an established procedure for chemical isolation of cell nuclei. The source of this differences is not clear, but may be due to partial disruption of the nuclear matrix or membrane which has been observed during chemical isolation (30). Each of the two isolation procedures has specific advantages. The mechanical isolation technique provides a direct correlation of cell and nuclear properties and possibly less damage to the nucleus but is difficult to perform on large numbers of cells. Chemical isolation can be performed on a large number of cells simultaneously, but its effects on nuclear properties are not fully understood and are sensitive to the concentration and time of exposure to the detergent.

Knowledge of the viscoelastic properties of the nucleus also may have important implications in the study of mechanical signal transduction. Our findings indicate that the intact nucleus behaves as a viscoelastic solid, with properties that are distinct from those of the cytoplasm. Previous studies suggest that mechanical factors may play an important role in the regulation of cell activity. In this regard, quantitative information on the deformation behavior and mechanical properties of the nucleus could provide new insight on the relationship between the stress-strain state of the nucleus and that of the extracellular matrix.

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