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Unconfined creep compression of chondrocytes

Nic D. Leipzig, Kyriacos A. Athanasiou*

Department of Bioengineering, Rice University, MS 142, P.O. Box 1892, Houston, TX 77251, USA

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Abstract

The study of single cell mechanics offers a valuable tool for understanding cellular milieus. Specific knowledge of chondrocyte biomechanics could lead to elucidation of disease etiologies and the biomechanical factors most critical to stimulating regenerative processes in articular cartilage. Recent studies in our laboratory have suggested that it may be acceptable to approximate the shape of a single chondrocyte as a disc. This geometry is easily utilized for generating models of unconfined compression. In this study, three continuum mechanics models of increasing complexity were formulated and used to fit unconfined compression creep data. Creep curves were obtained from middle/deep zone chondrocytes ($n = 15$) and separately fit using the three continuum models. The linear elastic solid model yielded a Young's modulus of 2.55 ± 0.85 kPa. The viscoelastic model (adapted from the Kelvin model) generated an instantaneous modulus of 2.47 ± 0.85 kPa, a relaxed modulus of 1.48 ± 0.35 kPa, and an apparent viscosity of 1.92 ± 1.80 kPa-s. Finally, a linear biphasic model produced an aggregate modulus of 2.58 ± 0.87 kPa, a permeability of $2.57 \times 10^{-12} \pm 3.09$ m^4/N-s, and a Poisson's ratio of 0.069 ± 0.021. The results of this study demonstrate that similar values for the cell modulus can be obtained from three models of increasing complexity. The elastic model provides an easy method for determining the cell modulus, however, the viscoelastic and biphasic models generate additional material properties that are important for characterizing the transient response of compressed chondrocytes.

Keywords: Chondrocyte; Articular cartilage; Single cell mechanics; Modeling; Unconfined compression; Viscoelasticity; Biphasic theory; Mechanotransduction; Cellular engineering

1. Introduction

Cell biomechanics approaches provide tools for the characterization of important cell and tissue behaviors. In particular, understanding of mechanotransduction or the biological response of cells to their biomechanical environment, would foster further understanding of how cellular responses correlate to tissue level characteristics. The study of mechanotransduction has many implications in biology, engineering and medical science, because it can lead to the elucidation of disease etiologies, and the formulation of repair and tissue engineering strategies. The first step to understanding the phenomenon of mechanotransduction involves characterization of the mechanical environment in and directly around single cells.

Chondrocytes are a prime example where the determination of the material properties of single cells is crucial to understanding their in vivo biomechanical environment (Koay et al., 2003). The mechanical environment of the chondrocyte is vital to the production and maintenance of articular cartilage, as well as for the health of diarthrodial joints. Chondrocytes in loaded articular joints are known to experience a multitude of forces, including hydrostatic, compressive, tensile, and shear forces (Leipzig and Athanasiou, 2004). The proper application of these forces has been shown to be essential for maintenance of the chondrocyte phenotype and for the production of cartilaginous tissue (Buschmann et al., 1995; Vunjak-Novakovic et al., 1999; Lee et al., 2000; Smith et al., 2000). Conversely, abnormal mechanical forces, either from an acute injurious load or the cumulative effect of smaller, repetitive loads, have been demonstrated to lead to altered chondrocyte behavior, resulting in pathological

*Corresponding author. Tel.: +1-713-348-6385; fax: +1-713-348-5877.

E-mail address: athanasiou@rice.edu (K.A. Athanasiou).

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matrix synthesis, increased catabolic activity, and apoptosis (as in osteoarthritis) (Dekel and Weissman, 1978; Radin et al., 1984, 1991; Ehrlich et al., 1987). Previous research strongly supports the hypothesis that chondrocytes respond to their local-stress–strain environment in a temporal and spatially dependent manner. Additionally, the ratio of cell properties to the properties of the surrounding pericellular matrix is a crucial determinant of stress and strain fields (Guilak and Mow, 2000).

Several techniques have been developed for determining mechanical properties of single cells and specifically chondrocytes; for a thorough review please see Shieh and Athanasiou (2002). The current study of chondrocyte biomechanics is quickly leading to an understanding of how these properties change for cartilage homeostasis and disease.

Micropipette aspiration is one of the pioneering techniques used for studying cellular mechanics and continues to be the most widely used. The micropipette technique has been used on numerous cell types and has applied a variety of modeling techniques (Hochmuth, 2000). Studies have utilized a modification of the punch model to determine mechanical properties of bovine aortic endothelial cells (Sato et al., 1987) and of normal and osteoarthritic human chondrocytes (Jones et al., 1999). Other studies have applied the standard linear solid to model the viscoelastic response of single cells (Theret et al., 1988; Sato et al., 1990; Trickey et al., 2000; Guilak et al., 2002).

Another cellular mechanics technique includes atomic force microscopy (AFM), which has seen adaptation from scanning surfaces to the field of cellular mechanics. Several groups have used these devices to resolve mechanical properties of cells (Ricci et al., 1997; Wu et al., 1998; Mathur et al., 2000). However, these properties are often only of local regions of cell since an AFM tip has a diameter on the order of several nanometers. To achieve true microscale testing of cells, in addition to saving the expense of AFMs, several groups have developed custom micromechanical systems. These devices use the same principles of cantilever beam theory that the AFM employs, but at a slightly larger scale. More recently, a microscale device termed the cell-indentation apparatus was developed (Shin and Athanasiou, 1999) which was able to apply ramp controlled indentation to single adherent osteoblast-like (MG63) cells. This device was later modified (Koay et al., 2003), to apply stress-controlled indentation to adherent bovine articular chondrocytes. The creep indentation apparatus was able to determine the material properties of single chondrocytes by application of both a punch and a standard linear solid model.

The ability to model a single cell's response to mechanical loads using a specialized device is the first step towards understanding the mechanical response of a tissue. A microscale unconfined compression device would offer an invaluable tool for mechanically testing single cells and, further, such a device could be utilized to mimic the compressive portion of the in vivo mechanical environment of single cells. The overall goal of this study was to develop a methodology to perform unconfined creep compression on single articular chondrocytes. The first objective of this study was to develop three continuum mechanics models for the unconfined compression of single articular chondrocytes. The second objective was to utilize experimental data to validate these models for obtaining the material properties of single chondrocytes.

2. Methods

2.1. Cell isolation

Articular cartilage was harvested from the distal metatarsal joint of one to two year old heifers obtained from a local abattoir (Lad Pak Inc., Needville, TX). An abrasion technique was used to remove the superficial layer, while preserving the middle/deep zones underneath. Previous work in our laboratory has demonstrated this to be an acceptable technique for isolation of zonal tissue (Darling et al., 2004). The remaining tissue (considered middle/deep zone cartilage) was sliced off and minced into small pieces. The middle deep tissue was digested overnight at 37°C and 10% CO_2 in a solution of 586 U/ml collagenase type 2 (Worthington Biochemical, Lakewood, NJ) in supplemented DMEM containing 10% FBS, 100 U/ml penicillin-streptomycin, 0.25 μg/ml fungizone, 0.1 mM NEAA, 2 mM *L*-glutamine.

2.2. Cell attachment

After digestion, the cell mixture was centrifuged and resuspended in supplemented DMEM to yield a concentration of 10^5 cells/ml. 0.5 ml of the cell suspension was seeded onto a tissue culture treated plastic dish and confined to a 2 cm diameter area using silicone isolators (PGC Scientifics, Gaithersburg, MD) to yield an areal cell density of approximately 1.6×10^4 cells/cm^2. The plates were incubated for 3 h at 37°C and 10% CO_2 prior to compression testing.

2.3. Creep testing

Creep unconfined compression tests were performed using a novel system developed in our laboratory, originally developed for displacement controlled indentation testing of single cells (Shin and Athanasiou, 1999). This device was later modified to perform stress controlled indentation on single cells and termed the

creep cytoindentation apparatus (Koay et al., 2003). This device was designed to apply a constant stress on adherent cells, while employing cantilever beam theory to track the resulting cellular deformation. Originally, the cytoindentation apparatus utilized a probe with a tip diameter of 5 μm. For single cell unconfined compression, the apparatus was modified by using a tungsten probe with a tip measuring 50.8 μm in diameter (Advanced Probing, Boulder, CO).

After cell attachment, the culture dish was filled with media warmed to 37°C and placed into the creep testing apparatus. HEPES buffer (Fisher Scientific, Pittsburg, PA) was added to the media at a concentration of 30 mM. Experiments were conducted under ambient conditions. The experimental set-up for the creep unconfined procedure is shown in Fig. 1. Before creep testing, the 50.8 μm probe is positioned directly above the center of the chondrocyte with the aid of an inverted microscope (Eclipse TS100; Nikon USA, Melville, NY, USA) and two independent micrometers on the stage (M-014; Physik Instrumente, Tustin, CA, USA). Calibration of the cellular creep apparatus was identical to what was originally described (Koay et al., 2003). Due to modifications in the stage set-up the probe did not need to be repositioned for each creep test. As a result, calibration was performed at the start of experimentation and periodically rechecked. Recalibration was required only when the probe was moved.

For creep testing, the experiment began when the probe was ramped down onto the cell. The ramp phase requires time on the order of a tenth of a second to achieve constant force. When the specified test load of 75 nN was achieved, displacement data were recorded for 45 s. The contact stress for each cell was determined using a measurement of the cell diameter via a reticle (Nikon USA, Melville, NY, USA). Contact with the cell was established by simultaneously tracking the displacement of the piezoelectric actuator and the force measured by the deflection of the cantilever, and noting any substantial change in the slope of the force. After each creep test, a measurement of distance to the dish was obtained and compared to the contact distance to determine cell height.

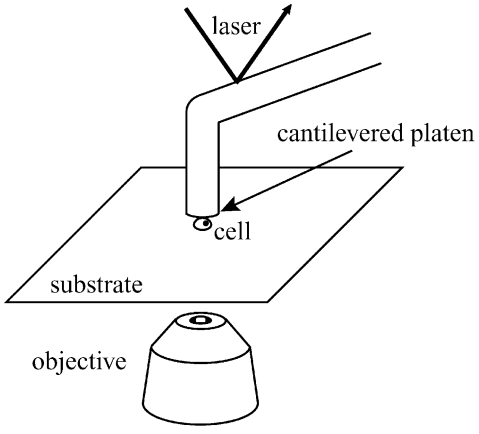

Fig. 1. Unconfined compression experimental set-up. The cantilevered platen is a single 50.8 μm tungsten rod. A piezoelectric translator is used to control the movement at the base of the cantilever, while a laser micrometer records the displacement of the end of the cantilever.

2.4. Continuum mechanics modeling

The idea of modeling a cell as a solid disc was suggested by recent studies in our laboratory using vertical scanning interferometery (Scott et al., 2004). These studies reveal that chondrocytes seeded onto a substrate quickly take on a flattened shape (Fig. 2a), suggesting that it may be acceptable to approximate the shape of a single chondrocyte as a disc. To obtain the material properties of single chondrocytes, three continuum mechanics models of increasing complexity were used to model the creep response of chondrocytes. Each model considered the cell as a disc, under small deformation exposed to an instantaneous and constant load. The extremely short lag time to achieve constant load was not considered in any of the models so that the application of force was treated as a step load. Material properties were obtained using an elastic model, a viscoelastic model, and a linear biphasic model.

For the elastic model, the cell was considered as an isotropic linear elastic solid disc. The model assumed a homogeneous disc, zero body forces, stress free horizontal surfaces, and uniaxial compression. Further simplification is made using the infinitesimal theory of elasticity. As a result of these assumptions, a simple analytical solution can be generated to find the Young's modulus of the cell (E_Y):

$$u_z = \frac{\sigma z}{E_Y}, \tag{1}$$

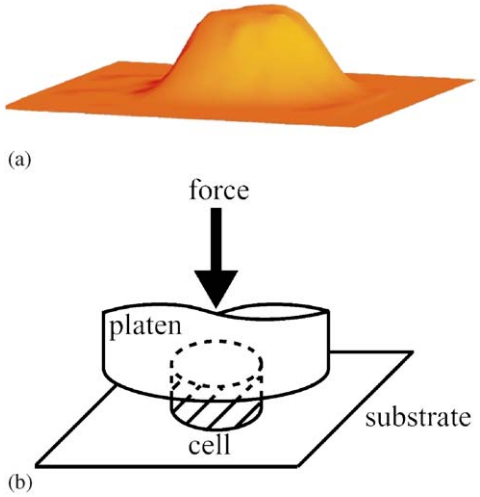

Fig. 2. Unconfined compression model. (a) Vertical scanning interferometry rendering of a chondrocyte seeded onto a glass coverslip. (b) The cell is modeled as a disc under a larger platen.

where u_z is the final deformation, σ the contact stress, and z the initial cell height.

To model the viscoelastic response, a Kelvin model was applied. The Kelvin solid models viscoelastic behavior of a material with a mechanical circuit (Fig. 3). The model includes an elastic element (modulus k_1) arranged in parallel with a Maxwell element, which is composed of a second elastic element (modulus k_2) in series with a viscous element (viscosity coefficient μ).

For viscoelastic modeling, the cell was treated as an isotropic, incompressible, and homogeneous disc. Using these assumptions, the elastic model (Eq. (1)) and the correspondence principle (Fung, 1965); the following solution is generated to describe the viscoelastic creep response of a cell:

$$u_z(r,z(r,o),t)=\frac{2}{3}\frac{\sigma}{E_\infty}z(r,0)\left[1+\left(\frac{\tau_\varepsilon}{\tau_\sigma}-1\right)e^{-t/\tau_\sigma}\right]h(t), \quad (2)$$

where $u_z(r,z,t)$ is the deformation, σ is the contact stress, E_∞ the relaxed modulus, $z(r,t)$ is the cell height, τ_ε the stress relaxation time constant, τ_σ the creep time constant, and $h(t)$ is the unit step function. The instantaneous modulus (E_0) is defined as $E_0=k_1+k_2$ and is related to the relaxed modulus by $E_0=(\tau_\sigma/\tau_\varepsilon)E_\infty$. Using the steady-state profile and comparing the result to Eq. (1) the Young's modulus (E_Y) can be determined by $E_Y=3/2\ E_\infty$. The following relations for the Kelvin model are used to find the intrinsic viscosity (μ):

$$\tau_\varepsilon=\frac{\mu}{k_2},$$

$$\tau_\sigma=\frac{\mu}{k_1}\left(1+\frac{k_1}{k_2}\right), \quad (3)$$

$$E_\infty=k_1,$$

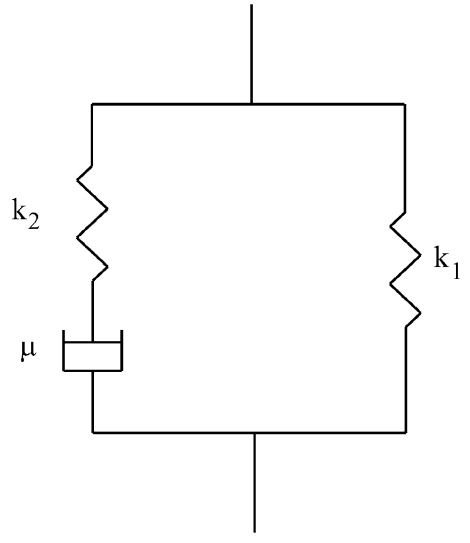

Fig. 3. The mechanical circuit describing the Kelvin model. This model represents a form of the standard linear solid.

Finally, the linear biphasic model for unconfined compression, as developed by Armstrong and colleagues (1984) was applied. This model was adapted from the Kuei, Lai, and Mow (KLM) linear biphasic mixture theory for cartilage (Mow et al., 1980). Armstrong and associates' model was originally used to analyze the unconfined compression of cylindrical discs of articular cartilage between smooth, frictionless, impervious plates. For the given problem it was assumed that the displacement and pressure fields are axisymmetric, and the compressive strain in the axial direction is homogeneous. The final condition implies that $u_z=z\varepsilon_{zz}(t)$, where u_z is the axial deformation, z is the initial cell height, and $\varepsilon_{zz}(t)$ is the axial strain. For further simplification small deformation was assumed. For unconfined creep compression, Armstrong and colleagues formulated the following linear biphasic solution:

$$\varepsilon_{zz}(t)=\frac{-F_0}{H_A\pi r^2}\frac{(1-\nu_s)}{(1+\nu_s)(1-2\nu_s)}\left[1-(1-\nu_s^2)(1-2\nu_s)\sum_{n=1}^{\infty}\frac{4}{9(1-\nu_s)^2\beta_n^2-8(1+\nu_s)(1-2\nu_s)}e^{-\beta_n^2(H_Akt/r^2)}\right], \quad (4)$$

where F_0 is the load, r the radius of the specimen, ν_s the Poisson's ratio of the solid matrix, H_A the aggregate modulus of the solid matrix, k the permeability, and β_n are the roots of the characteristic equation:

$$J_0(x)-\frac{4(1-2\nu_s)}{3(1-\nu_s)}\frac{J_1(x)}{x}=0, \quad (5)$$

where J_0 and J_1 are Bessel functions of the zero and first order.

2.5. Data analysis

Creep experimentation generated three data files for each cell: displacement versus time, force versus time, and force versus displacement. The force versus time data were used to confirm that constant force was achieved at the beginning of creep data acquisition. If it was not, the time at which constant force was reached was used as the starting point for the deformation versus time curve. The force versus displacement data were used to determine the point of contact so that the displacement versus time curve would be in terms of actual cell deformation. For fitting the elastic model, the final deformation was determined by averaging the deformation values over the last second. For the viscoelastic and biphasic models, the resulting creep curves were fitted via the non-linear Levenburg-Marquardt method, using MATLAB 6.5 (The MathWorks, Inc., Natick, MA). Results are reported as mean ± standard deviation.

3. Results

Due to length of set-up, calibration and creep testing, experimentation was performed in two groups from two separate animals. The calibration was checked after approximately every 2–3 creep tests and recalibration was not required during either testing. No significant difference was seen in the results, so the groups were combined for a sample size of $n = 15$. The adherent chondrocytes exhibited a viscoelastic solid creep response to an imposed step load. A typical creep curve and its corresponding force–time curve are shown in Fig. 4a and b, respectively.

The deformation response (Fig. 4a) is characterized by an instantaneous jump followed by a decreasing slope until equilibrium is reached. The fits of the elastic, viscoelastic, and biphasic models are included in Fig. 4a.

The measurement of both the cell diameter and height were important for model fitting. Chondrocytes on tissue culture treated plastic at 3 h post-seeding had a mean cell diameter of $11.36 \pm 0.77\,\mu m$. Cell diameter values were not observed to change during creep testing using the described experimental set-up. The mean height of these cells was $10.79 \pm 2.28\,\mu m$.

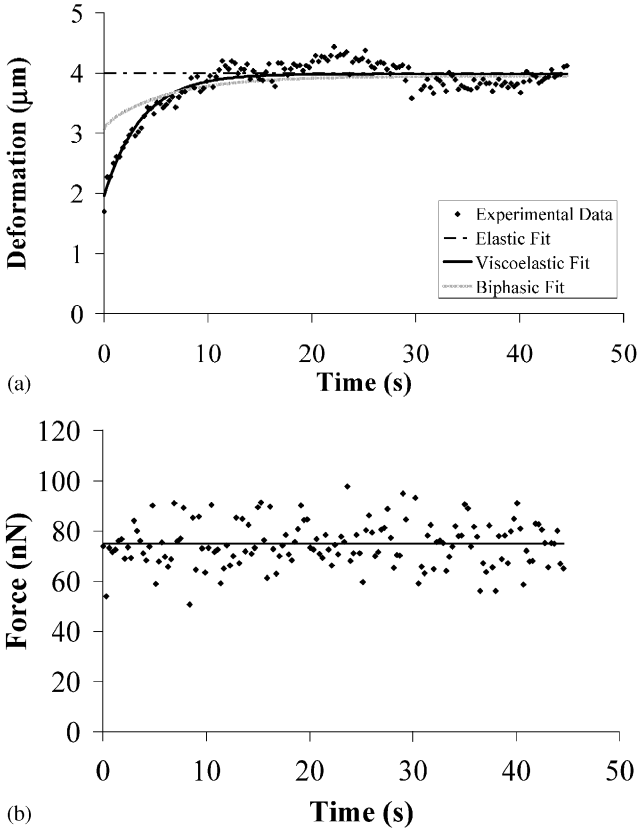

Fig. 4. (a) Creep curve for the unconfined creep compression of an adherent articular chondrocyte and (b) the corresponding force versus time plot.

Table 1
Material properties of bovine articular chondrocytes from three continuum models

Elastic Model (n=15)	
E_Y (kPa)	2.55 ± 0.85
Viscoelastic Model (n=15)	
E_0 (kPa)	2.47 ± 0.85
E_∞ (kPa)	1.48 ± 0.35
μ (kPas)	1.92 ± 1.80
Biphasic KLM Model (n=15)	
H_A (kPa)	2.58 ± 0.87
$k \times 10^{-12}$ (m^4/N-s)	2.57 ± 3.09
v_s	0.069 ± 0.021

The data for each cell were separately fitted to the three continuum models and to generate corresponding sets of material parameters. Table 1 presents the material properties derived from the three models for 15 chondrocytes. The linear elastic solid model yielded an E_Y of 2.55 ± 0.85 kPa. The viscoelastic model generated an E_0 of 2.47 ± 0.85 kPa, an E_∞ of 1.48 ± 0.35 kPa, an $E_Y = 2.22 \pm 0.52$ kPa, and an μ of 1.92 ± 1.80 kPa-s. Finally, the linear biphasic model produced an H_A of 2.58 ± 0.87 kPa, a k of $2.57 \times 10^{-12} \pm 3.09\,m^4$/N-s, and a v_s of 0.069 ± 0.021. Paired student's t-tests demonstrated no significant difference between elastic E_Y and viscoelastic E_Y, between elastic E_Y and H_A, or between viscoelastic E_Y and H_A.

4. Discussion

The results of this study demonstrate the feasibility of mechanically testing single cells using unconfined compression. The three continuum models used in this study generate plausible material properties for chondrocytes as compared to previous studies (Guilak et al., 1999; Jones et al., 1999; Trickey et al., 2000; Guilak et al., 2002; Koay et al., 2003). A comparison of viscoelastic material properties of chondrocytes from this study and previous studies is provided in Table 2.

Comparison to micropipette experiments by Trickey and associates (Trickey et al., 2000), reveals that data from unconfined creep compression yield a higher E_0 and E_∞ and a lower apparent viscosity. This can be attributed to several factors, the most obvious being differences in the testing methodologies. Unconfined compression exposes the entire cell to compressive force, while micropipette experiments use suction to expose a portion of the cell's membrane to tensile forces. It is important to note that the nucleus accounts for part of the cell's response to unconfined compression, whereas it probably has a significantly smaller contribution for the tensile response to micropipette aspiration.

Table 2
Comparison to previous viscoelastic modeling of single articuluar chondrocytes

		Koay et al. (2003)	Trickey et al. (2000)
	Unconfined compression	Indentation*	Micropipette aspiration**
E_0 (kPa)	2.47±0.85	8.0±4.41	0.41±0.17
E_8 (kPa)	1.48±0.35	1.09±0.40	0.24±0.11
μ (kPa-s)	1.92±1.80	1.50±0.92	3.0±1.8
n	15	16	47

*Data from healthy bovine articular chondrocytes.
**Data from healthy human chondrocytes.

Experimentation using micropipette techniques has shown that isolated nuclei from chondrocytes are 3–4 times stiffer than the cell (Guilak et al., 2000). Experiments using AFM have demonstrated similar results in adherent endothelial cells (Mathur et al., 2000). Additionally, cells from two different species and joint locations are compared. Several studies have demonstrated statistically different variation in mechanical properties and biochemical composition for species (Athanasiou et al., 1991,1995), as well as for joint surface and location (Athanasiou et al., 1994,1998; Treppo et al., 2000). These data suggest that cell properties may vary by joint and species. Lastly, differences in mechanical properties could arise from the fact that the unconfined compression is conducted on cells that are adhered to a substratum, whereas micropipette aspiration tests suspended cells. The process of cellular adhesion is known to involve the formation of focal adhesion complexes in conjunction with cytoskeletal remodeling (LeBaron and Athanasiou, 2000). This adhesion process has been demonstrated to produce an overall stiffening of the cell (Wang and Ingber, 1994,1995).

The indentation study by Koay and colleagues (2003) and this study utilized the same species, joint, device, and similar procedures throughout. The difference in testing modalities (indentation versus unconfined compression), as well as the influence of the nucleus, could lead to a higher E_0 for indentation. During their experiments, Koay and associates positioned the indenter over the nucleus before each creep test so that the stiffest part of the cell could be tested. The effect of a stiffer nucleus is dampened by the entirety of the cell during unconfined compression testing. Finally, this study was able to build on the previous work done with the cellular creep apparatus, leading to more efficient experimentation and a better grasp of data processing.

On the whole, the viscoelastic model showed excellent fits for the majority of cells. This model was able to fit the creep region well in most cases. On the other hand, the biphasic model did not fit well the initial creep response. At this point it is important to note that the original unconfined compression studies by Armstrong and associates (1984) did not achieve good agreement between experimental data and the theoretical prediction. This was attributed to significant interfacial shear stress at the boundaries due to surface adhesion and suction effects at the tissue–platen interfaces. This interfacial shear stress was believed to lead to significant confining effects at the specimen surfaces. Brown and Singerman (1986) reported limited accuracy for fitting Armstrong and colleague's model to experimental ramp/stress–relaxation data. The higher peak stresses and longer relaxation times that were observed were ascribed to a substantial underestimate of interstitial fluid transport by the model. Cohen and associates (1998) were able to fully describe the stress–relaxation response of growth plate tissue in unconfined compression, using a transversely isotropic biphasic model. For chondrocytes, it is not known if a single cell's mechanical response can be described by the biphasic theory or if the response to unconfined compression is either isotropic or transversely isotropic in nature. The model by Armstrong et al. (1984) was selected as a starting point for biphasic modeling even though the use of adherent cells in this study may have violated the frictionless platen assumption of the model. However, unlike previous unconfined biphasic compression experimentation, axial displacement is modeled under the condition of constant force. Further, the unconfined compression results of this study fit within the assumptions of small deformation.

When examining the curve fits, there were some cases where the viscoelastic and the biphasic model were in almost perfect agreement. The biphasic theory best modeled the curves where equilibrium was reached more quickly, as characterized by a shorter time constant (τ_σ). This fact is evident in Fig. 5 which presents two creep curves and the corresponding model fits. For Fig. 5a, τ_σ was 2.58 s, while τ_σ for Fig. 5b was 1.21 s.

Fig. 5, combined with Fig. 4 ($\tau_\sigma = 3.17$ s), presents three separate sets of creep data. In all three examples the viscoelastic model shows an excellent fit, while only one excellent fit exists for the biphasic model. These results demonstrate a trend seen for all 15 cells; the quality of fit for the biphasic model increases as τ_σ decreases. Upon further examination of the fitting results, it is evident that a correlation (Fig. 6) exists between τ_σ from the viscoelastic fit and k from the biphasic fit; a shorter τ_σ corresponds to larger k and generally a better fit of the initial creep response.

There are numerous arguments for why a significant enough variation exists in the creep responses of attached chondrocytes to manifest such findings. One possible explanation comes from the fact that after the 3 h seeding time, a varying degree of cell attachment is always observed. It has been established that the extent

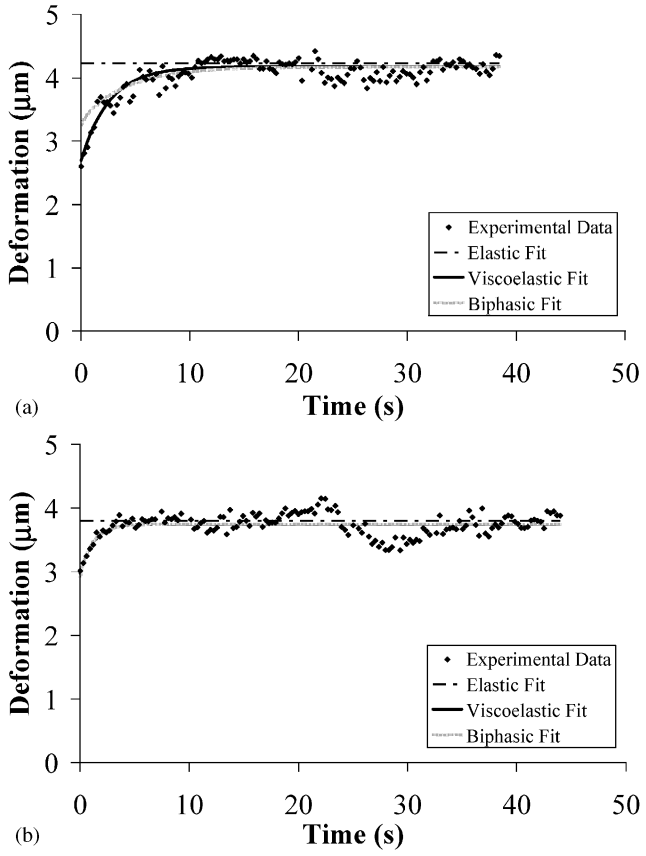

Fig. 5. Creep curves for adherent articular chondrocytes exhibiting different initial responses. Panel (a) represents an intermediate creep response, while panel (b) represents a short creep response.

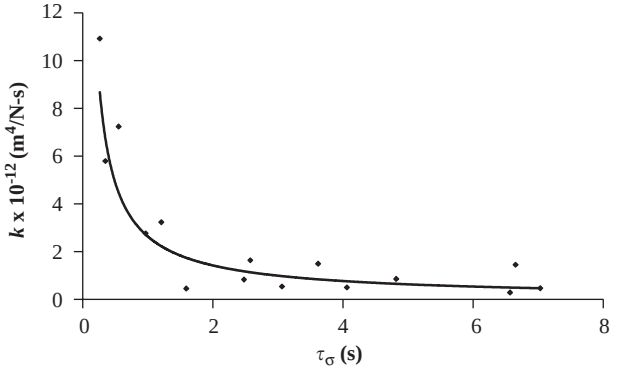

Fig. 6. Plot comparing the permeability (k) versus the creep time constant (τ_σ) for all 15 cells. The permeability is generated from biphasic model fitting, while the time constant is generated from the viscoelastic model fitting.

of cell attachment is a function of the level of cytoskeletal organization (Bradley et al., 1980; Bloom et al., 1999). During the experiments, some cells are seen rolling around, others shake in place when the stage is moved, and others do not seem to move at all. Creep testing only selected the cells that appeared to be firmly adhered. Such chondrocyte adhesive energies have been shown to be 38.9 ± 10.6 nN on glass and 72.6 ± 21.5 nN on fibronectin coated glass (Athanasiou et al., 1999). This experimental variability, as evidenced by the large standard deviations in the mechanical adhesiveness data, could be enough to explain the observed differences in creep behaviors. The fact that the chondrocytes used in this study are isolated from cartilage may also add a degree of variability. Currently the mechanical properties of chondrocytes in vivo have not been characterized directly. Despite this, it is assumed that chondrocytes encased in their extracellular matrix would be stiffer, due to the increased cellular attachment and the subsequent increase of cytoskeletal organization within the cells.

The biphasic curve fitting results point to the fact that a linear biphasic model is not the best choice for modeling the compression of a single chondrocyte. It appears that the linear biphasic model is not able to accommodate the large width in the majority of the experimental creep curves. The width is defined as the difference of the final deformation and the initial deformation after the imposed step load ($u(t=\infty)-u(t=0^+)$). The linear biphasic model generates maximum width when the Poisson's ratio is equal to zero, which could explain the small values of ν_s ($=0.069 \pm 0.021$) obtained in this study. Other biphasic formulations may be more applicable for modeling the compression of single chondrocytes.

The assumptions used to formulate the models used in this study create possible limitations. The most obvious limitation is that the models assume a constant geometry during creep testing. Due to the experimental set-up, cell geometry could only be observed at the start of testing. These geometries are probably not maintained during creep testing, however, they seem to adequately describe the cellular response.

In some creep testing, pure strains slightly greater than 30% were observed. Based on this fact, the small deformation assumption utilized for each model, may lead to significant errors. However, a recent paper by Haider and Guilak (2002) demonstrated that the infinitesimal strain assumption may still be accurate for a viscoelastic halfspace model of micropipette aspiration (which generates cellular strains greater than 30%). More rigorous techniques for improving the cellular unconfined creep apparatus could involve visualization using confocal microscopy during testing as well as finite element analysis.

The results of this study demonstrate that similar values for the cell modulus can be obtained from three models of increasing complexity. The biphasic H_A compares well to E_Y from the elastic model, while the E_∞ from the viscoelastic model is slightly lower in comparison due to the Kelvin model definition. However, the calculated Young's modulus for the viscoelastic model generates an $E_Y = 2.22 \pm 0.52$ kPa which is very

similar to the Young's modulus generated from the elastic model and the aggregate modulus from the biphasic model. These findings demonstrate that the elastic model offers a quick method for determining the cell modulus; however, the viscoelastic and biphasic models generate additional material properties that are important for full characterization of the biomechanical response of chondrocytes. Additionally, this study reveals that the viscoelastic model may be the best choice for modeling the creep responses of adherent chondrocytes to unconfined compression, based upon better fits of the initial response.

The unconfined compression device utilized in this study presents a valuable tool for modeling the creep response of cells attached to substrata. Unconfined compression represents a realistic mechanical environment for articular chondrocytes as compared to the in vivo locale in cartilage. Further, this device offers a plethora of possible uses for the elucidation of the biomechanical and biochemical responses of cells. This study offers the baseline for future work with single articular chondrocytes. Such work could test the combinatory effects of compression and chemical stimuli, substrate or different regimes of force on chondrocytes. It is the hope that the results of such studies will lead to the formulation of successful repair and regeneration strategies for the future engineering of articular cartilage.

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