ELSEVIER

Journal of Biomechanics 41 (2008) 1044–1052

JOURNAL OF BIOMECHANICS

www.elsevier.com/locate/jbiomech
www.JBiomech.com

Effects of TGF-β1 and IGF-I on the compressibility, biomechanics, and strain-dependent recovery behavior of single chondrocytes

Eugene J. Koay[a,b], Gidon Ofek[a], Kyriacos A. Athanasiou[a,*]

[a]*Department of Bioengineering, Rice University, MS-142, P.O. Box 1892, Houston, TX 77251-1892, USA*
[b]*Baylor College of Medicine, Houston, TX 77030, USA*

Accepted 5 December 2007

Abstract

The responses of articular chondrocytes to physicochemical stimuli are intimately linked to processes that can lead to both degenerative and regenerative processes. Toward understanding this link, we examined the biomechanical behavior of single chondrocytes in response to growth factors (IGF-I and TGF-β1) and a range of compressive strains. The results indicate that the growth factors alter the biomechanics of the cells in terms of their stiffness coefficient (~two-fold increase over control) and compressibility, as measured by an apparent Poisson's ratio (~two-fold increase over control also). Interestingly, the compressibility decreased significantly with respect to the applied strain. Moreover, we have again detected a critical strain threshold in chondrocytes at ~30% strain in all treatments. Overall, these findings demonstrate that cellular biomechanics change in response to both biochemical and biomechanical perturbations. Understanding the underlying biomechanics of chondrocytes in response to such stimuli may be useful in understanding various aspects of cartilage, including the study of osteoarthritis and the development of tissue-engineering strategies.

Keywords: Chondrocyte; Mechanobiology; Compression; Poisson's ratio; Cartilage

1. Introduction

The biological responses of articular cartilage to mechanical forces play important roles in its normal physiology and disease (Buckwalter et al., 2006). This phenomenon, known as mechanotransduction, may have important ramifications toward understanding and treating debilitating musculoskeletal ailments, including osteoarthritis. For example, researchers have shown both beneficial and detrimental effects of mechanical forces on tissue explants and engineered tissues, using modalities such as direct compression (Aufderheide and Athanasiou, 2006; Ng et al., 2006; Lima et al., 2007), shear (Smith et al., 1995, 2004), and hydrostatic pressure (Smith et al., 2004; Hu and Athanasiou, 2006). Currently, the understanding of what elicits these different responses remains incomplete.

Particularly, vexing is the connection between the cellular biomechanics and biological processes. Toward understanding this vital interplay, it is important to characterize the biomechanics of the chondrocyte and how they change in response to physicochemical stimuli, such as growth factors and mechanical perturbations, as previously discussed (Guilak and Mow, 2000; Guilak et al., 2002, 2006; Shieh and Athanasiou, 2002, 2005; Koay et al., 2003; Leipzig and Athanasiou, 2005; Leipzig et al., 2006; Shieh et al., 2006). Establishing a basic understanding of the mechanical nature of the chondrocyte aids theoretical models of cartilage that consider the cell (Guilak and Mow, 2000) and helps the development of new methodologies to study chondrocyte mechanobiology, such as a 'single-cell' approach (Shieh et al., 2006). This approach entails the use of instruments that can apply discreet forces to single adherent chondrocytes, such as the cytoindenter (Shin and Athanasiou, 1999; Koay et al., 2003), as well as the assessment of a cellular response, such as cartilage-relevant gene expression (Shieh and Athanasiou, 2006). Studies of

*Corresponding author. Tel.: +1 713 348 6385; fax: +1 713 348 5877.
E-mail address: athanasiou@rice.edu (K.A. Athanasiou).
URL: http://www.rice.edu/cartilage (K.A. Athanasiou).

0021-9290/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jbiomech.2007.12.006

single-cell biomechanics help to direct these efforts by providing information on the cellular characteristics of time constants for recovery, compressibility, and mechanical thresholds.

For example, when a possible change in chondrocyte mechanical behavior was identified between 25% and 30% compressive strain (termed the critical strain region), it was postulated that this represented a threshold akin to yield strain. The significance of this critical region remains unclear. However, it is possible that permanent damage may be incurred by the chondrocyte beyond this region. This damage may occur due to high strains or strain accumulation after repeated loading (Shieh et al., 2006), suggesting that this critical region would have important implications in directing appropriate *in vitro* mechanical stimulation regimens as well as for studies of mechanical damage to cartilage. While the cause of this critical region also remains uncertain, it is possible that cytoskeletal components, such as F-actin, rearrange or break down at high strains.

Considering that TGF-β1 and IGF-I increase F-actin levels and thereby increase cell stiffness to two-fold over control (Leipzig et al., 2006), in this study, we tested the hypothesis that these same growth factors would alter the critical strain region. We were also motivated to investigate how these growth factors and mechanical strains affected the compressibility of the cell, as measured by an apparent Poisson's ratio, since current computational models of cartilage assume this characteristic to be constant. Although we have previously observed that the apparent Poisson's ratio does not change with respect to increasing strains (Shieh et al., 2006), we hypothesized that the growth factors, due to their ability to reorganize the cytoskeleton (Gagelin et al., 1995; Boland et al., 1996; Berfield et al., 1997) and alter cellular mechanics (Leipzig et al., 2006), would increase the average apparent Poisson's ratio of the cells compared with control. We also expected that the growth factors would increase cell volume due to these effects on the cytoskeleton. Cell volume and shape are of interest since they are intimately tied to cell function (Urban et al., 1993; Bush and Hall, 2001; Guilak et al., 2002; Kerrigan et al., 2006). To test these hypotheses, single chondrocytes were exposed to IGF-I, TGF-β1, or no growth factors, and cells in each group were subjected to a range of compressive strains from 5% to 60% and their morphology, compressibility, stiffness, and recovery behavior were analyzed.

2. Methods

The basic protocols for this experiment were based on our previous work (Shieh et al., 2006). These methods and a few improvements are briefly explained below. Cell culture supplies were obtained from Invitrogen (Carlsbad, CA, USA) unless specified otherwise.

2.1. Cell isolation and seeding

Articular cartilage was harvested from the fetlock joint of mature steers obtained from the local abattoirs (Doreck and Sons Packing Company, Santa Fe, TX, USA; Kasper's Meat Market, Weimar, TX, USA). Chondrocytes from 13 joints derived from 12 different animals were isolated from the middle/deep region of the tissue as previously described (Shieh et al., 2006). The primary chondrocytes were counted, centrifuged, and resuspended in supplemented DMEM (0.1 mM NEAA, 100 U/ml penicillin/streptomycin, 0.25 μg/ml fungizone) at a concentration of 200,000 cells/ml with either TGF-β1 (10 ng/ml), IGF-I (100 ng/ml), or no growth factor. The chondrocytes were then seeded on 15 mm × 2 mm glass slides, which were placed inside a six-well plate, and incubated for 3 h at 37 °C and 10% CO_2. The 3 h seeding was previously established as a minimal time point that could achieve stable cell adhesion for cellular compression testing (Leipzig et al., 2006).

2.2. Cytocompression

Individual chondrocytes were subjected to a range of strains (~0.05–0.60) using a 50.8-μm diameter tungsten probe (Advanced Probing Systems Inc., Boulder, CO, USA) attached to a modified cytodetacher device, as described previously (Shieh et al., 2006). Glass slides were transferred from a six-well plate to a Petri dish such that the cells could be viewed perpendicular to the original seeding plane using an IMT-2 inverted microscope (Olympus America, Melville, NY, USA) at 400× (Fig. 1). Supplemented DMEM (as above) with 30 mM HEPES and the appropriate growth factor or blank control was added to the dish. Individual cells were brought in close contact (~5–10 μm away) with the probe, and a piezoelectric motor drove the probe, a prescribed distance (12–16 μm) toward the cells at a rate of 4 μm/s. The probe was held at its position for 30 s and then removed from the cell. Chondrocyte recovery was recorded up to 90 s to capture equilibrium.

2.3. Video capture and image analysis

The compression and recovery of each cell were captured on an AVC-D7 CCD camera (Sony Corp., New York, NY, USA) and analyzed, as before (Shieh et al., 2006), using a pixel-to-micron ratio of 7.0.

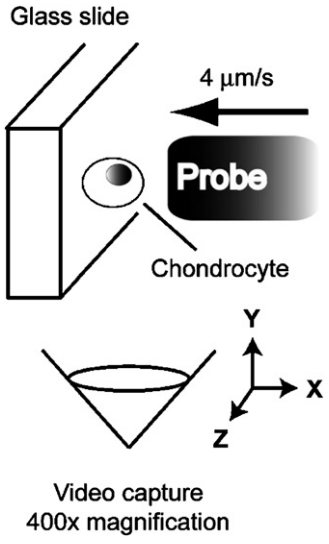

Fig. 1. Experimental setup. Articular chondrocytes were seeded onto a glass slide and exposed to IGF-I, TGF-β1, or no growth factor for 3 h. Individual cells from each group were subsequently compressed by a probe at 4 μm/s to a prescribed distance (12–16 μm). Measurements of the cell were taken before, during, and after the probe was released to provide information regarding cell morphology, biomechanics, compressibility, and recovery behavior. The figure is not drawn to scale.

2.4. Biomechanical properties and characteristics

Cells were approximated as ellipsoids, with rotational symmetry about the x-axis (perpendicular to the plane of seeding). The basic shape of the cell was assumed to remain unchanged through the duration of the compression and recovery events, as we have done before (Shieh et al., 2006). Cell volume was therefore calculated using the equation for an ellipsoid with two identical axes, and the volume of the cell was measured before compression (V_0), immediately after compression (V_1), and at equilibrium recovery (V_r). A normalized volume fraction ($(V_0-V_1)/V_0$) and recovered volume fraction (V_r/V_0) were calculated with these values. In contrast to our previous experiment (Shieh et al., 2006), we assessed applied axial strain (vs. apparent strain) by measuring the difference in probe positions between the initial cell contact and at peak compression, divided by the initial cell height.

The reaction force of the cell onto the probe at peak compression was calculated with cantilever beam theory, using the known properties of the tungsten probe and the deflection of the cantilever (Koay et al., 2003), which was determined by comparing the true displacement of the probe (via subsequent video analysis) with the prescribed piezoelectric displacement. The compressibility (as measured by an apparent Poisson's ratio, v) of the cell, residual strain after compression (ε_r), and characteristic recovery time constant (τ) were determined as described previously (Shieh and Athanasiou, 2005).

2.5. Statistics

Statistical analysis was performed using JMP IN 5.1 (SAS Institute Inc., Cary, NC, USA). Linear regression was used to determine whether the apparent Poisson's ratio, equilibrium stress, normalized volume fraction, recovered volume fraction, and residual strain varied as functions of applied strain, as well as to test the effects of growth factors on these functions. Change point analysis was used to determine if critical levels of the applied strain existed where biomechanical behavior changed, as before (Shieh et al., 2006). Briefly, this entailed performing a series of linear regressions on overlapping segments of data, where the resulting slopes were plotted against the applied strain to look for possible discontinuities. If a discontinuity was apparent, the original data were separated into two subsets, each of which was analyzed with linear regression. An effect was considered significant if $p<0.05$.

3. Results

In this section, treatments will be referred to as control, IGF-I, and TGF-β1. A total of 83 cells were tested and analyzed for this study (26 for control, 26 for IGF-I, and 31 for TGF-β1). Fig. 2 demonstrates how cells were analyzed.

3.1. Chondrocyte size

The different growth factor treatments had significant effects on the morphological measurements of the cell heights (h, $p=0.04$), diameters (dia, $p=0.01$), and volumes (vol, $p=0.005$). Specifically, TGF-β1 cells ($h=11.4\pm1.7\,\mu m$, dia $=11.2\pm1.8\,\mu m$, vol $=795\pm325\,\mu m^3$) had significantly greater dimensions ($p<0.05$) in all three measurements compared with IGF-I cells ($h=10.5\pm1.2\,\mu m$, dia $=10.2\pm0.8\,\mu m$, vol $=600\pm162\,\mu m^3$), while control cells ($h=10.7\pm1.2\,\mu m$, dia $=10.6\pm0.8\,\mu m$, vol $=638\pm137\,\mu m^3$) were not different from either group, with the exception of having less volume than TGF-β1 cells ($p<0.05$).

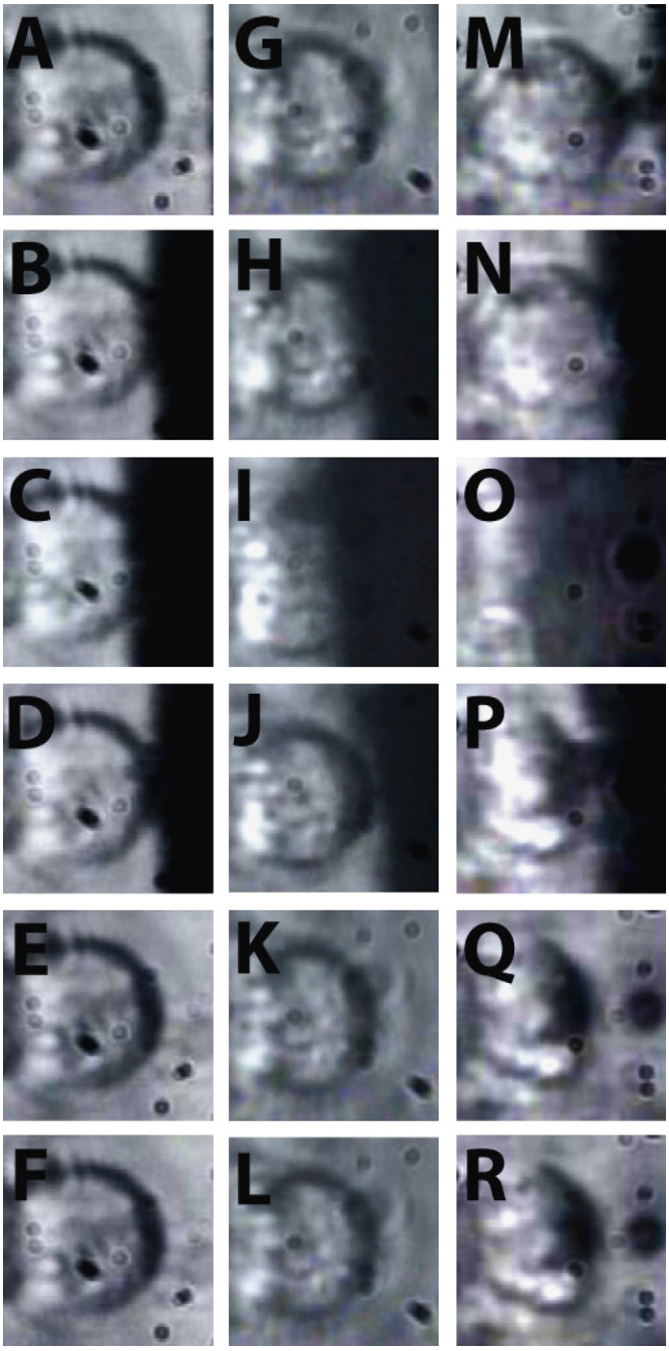

Fig. 2. Cytocompression and cellular recovery behavior. The compression and recovery events were analyzed through video recording. For demonstration, TGF-β1 cells are shown experiencing small (19%) strains (A–F), medium (29%) strains (G–L), and high (52%) strains (M–R). The initial frame (A, G, M), first probe contact (B, H, N), and equilibrium contact (C, I, O) provide information on the applied strain and stress by tracking the movement of the probe and measuring the initial dimensions of the cell. Immediately upon release of the probe (D, J, P), cell dimensions are measured to provide an apparent Poisson's ratio. Additionally, the recovery behavior of the cell is tracked. Shown are cells at 4 s after probe release (E, K, Q) and at equilibrium (F, L, R).

3.2. Compressibility, as measured by an apparent Poisson's ratio

The cells in each group exhibited characteristics of compressible materials, as volume changes were noted as a

function of increasing strain. Plotting the normalized volume fraction (V_{norm}) against the applied strain (ε) showed significant linear correlations for each treatment ($V_{norm} = 0.54\varepsilon - 0.05$ with $R^2 = 0.41$ and $p = 0.0004$ for control, $V_{norm} = 0.30\varepsilon - 0.02$ with $R^2 = 0.71$ and $p < 0.0001$ for IGF-I, and $V_{norm} = 0.31\varepsilon - 0.04$ with $R^2 = 0.45$ and $p < 0.0001$ for TGF-β1).

The apparent Poisson's ratio was significantly higher ($p = 0.0001$) in the growth factor groups compared with control (0.18 ± 0.11 for control, 0.31 ± 0.13 for IGF-I, and 0.30 ± 0.11 for TGF-β1). Additionally, the apparent Poisson's ratio decreased significantly with applied strain for all treatments (Fig. 3, $p = 0.001$ for control, $p = 0.003$ for IGF-I, and $p = 0.003$ for TGF-β1). The rate of decrease in the Poisson's ratio with the applied strain was not statistically different for growth factors and control, but the intercept of the control regression line was significantly less than the growth factors ($p < 0.05$), in line with the finding that the mean Poisson's ratio was less for control.

3.3. Stiffness coefficient

Plotting the equilibrium stress data against the applied strain yielded a stiffness coefficient for each group (Fig. 4). Both growth factor treatments resulted in statistically significant linear correlations between stress and strain ($p = 0.002$ for IGF-I and $p < 0.0001$ for TGF-β1). In contrast, the control cells did not have a significant linear correlation between stress and strain ($p = 0.1$). The linear fits for each group indicated that the growth factor treatments had stiffened the cells compared with control (1.77-fold for IGF-I and 2.03-fold for TGF-β1).

3.4. Recovery behavior

The recovery behavior of the single chondrocytes depended on the applied axial strain for all treatments. Fig. 2 illustrates how the cells recovered over time at different levels of strain. Generally, under all treatments, cells recovered fully and quickly to their original dimensions at low strains. As strains increased, however, the cells in all treatments exhibited a slower recovery response that was not complete, having less height (residual strain) and a lower recovered volume compared with initial values. A general exponential decay function (Shieh et al., 2006) approximated the recovery behavior of the cells in all treatments well with R^2 values between 0.63 and 0.99. Residual strains from the exponential fit agreed well with the measured residual strains from the digital image analysis, with linear coefficients of 0.995 ± 0.005 for control ($R^2 = 0.99$), 1.07 ± 0.007 for IGF-I ($R^2 = 0.97$), and 1.04 ± 0.002 for TGF-β1 ($R^2 = 0.99$). Each line had a significance level of $p < 0.0001$. The time constants (τ) for recovery increased with applied strain (ε) ($\tau = 28.58\varepsilon + 3.32$ with $R^2 = 0.14$ and $p = 0.03$ for control, $\tau = 41.04\varepsilon + 6.00$ with $R^2 = 0.25$ and $p = 0.009$ for IGF-I, $\tau = 48.00\varepsilon - 2.61$ with $R^2 = 0.36$ and $p = 0.0004$ for TGF-β1).

Figs. 5 and 6A–C illustrate how the recovery characteristics of residual strain and recovered volume fraction varied with the applied strain. The residual strain increased with the applied strain in all groups ($p < 0.0001$ for control, $p < 0.0001$ for IGF-I, $p = 0.0006$ for TGF-β1), while the recovered volume fraction changed inversely with the applied strain in all groups ($p < 0.0001$ for all groups).

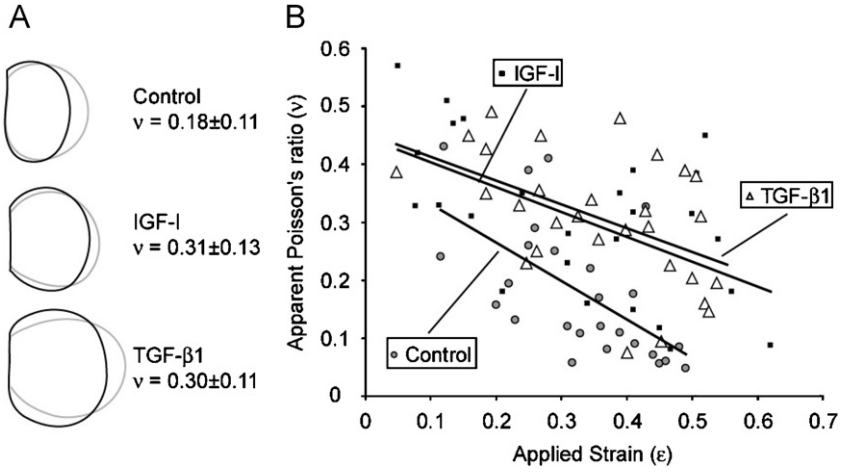

Fig. 3. Changes in apparent Poisson's ratio due to physicochemical stimuli. (A) The apparent Poisson's ratio was measured by measuring the changes in height and diameter of each compressed cell. The growth factors increased the apparent Poisson's ratio compared to control ($p = 0.0001$). For demonstration, superimposed outlines of one cell from each group are shown at a strain of 0.5 before (light gray) and after (black) compression. The cell outlines illustrate the significant effect that the growth factors had on cellular compressibility. (B) The apparent Poisson's ratio (ν) decreased as a function of applied strain (ε) for all treatments, with the intercept of the linear fit for the control cells ($\nu = -0.66\varepsilon + 0.40$, $R^2 = 0.37$ and $p = 0.001$) being significantly different ($p < 0.05$) from both growth factor treatments ($\nu = -0.43\varepsilon + 0.45$, $R^2 = 0.31$ and $p = 0.003$ for IGF-I; and $\nu = -0.41\varepsilon + 0.45$, $R^2 = 0.27$ and $p = 0.003$ for TGF-β1).

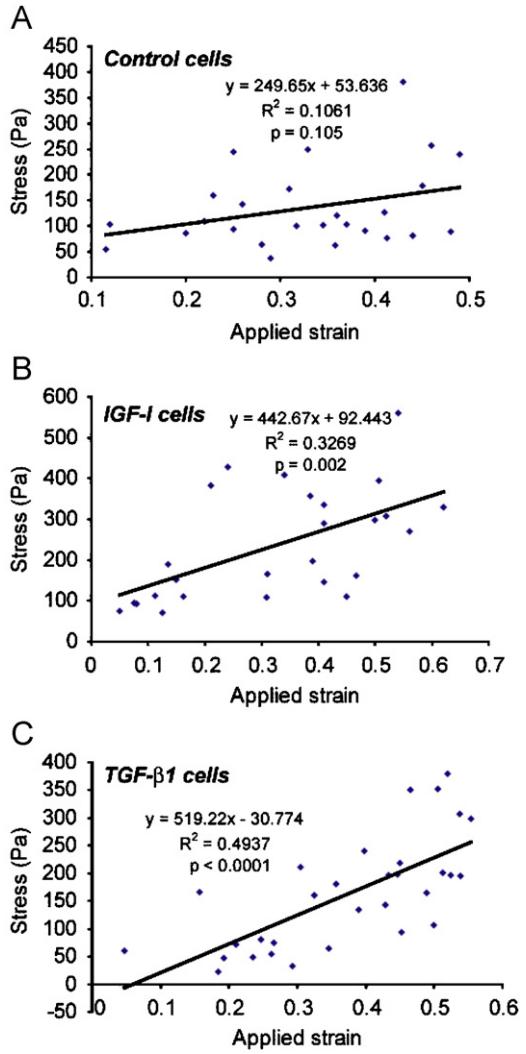

Fig. 4. Cellular stiffness in response to growth factors. Control cells did not have a significant linear correlation between stress and strain. In contrast, equilibrium stress increased significantly with strain when either growth factor was applied, indicating about a two-fold increase in the stiffness coefficient (i.e., the slope) over control.

3.5. Biomechanical behavior changes at $\varepsilon \approx 0.30–0.35$

The change point analyses for residual strain (Fig. 5D–F) and recovered volume fraction (Fig. 6D–F) each as functions of applied strain revealed a discontinuity at an applied strain of 0.30–0.35 for all groups. The linear fits for the data indicated no significant effects of strains less than 0.30 on the recovery behavior of the single chondrocytes in all groups. However, above strains of 0.30, the residual strains and recovered volume fractions exhibited significant linear correlations with the applied strains for all groups ($p<0.05$, Figs. 5 and 6).

4. Discussion

TGF-β1 and IGF-I are widely studied in cartilage research; understanding their effects on the biomechanics and recovery behavior of the chondrocytes can help to develop mechanical stimulation regimens of cartilage and may be relevant to the health and disease of cartilage. Additionally, it is important to understand how cellular biomechanics change with direct compressive strains, since this modality of mechanical stimulation influences cellular processes (Shieh et al., 2006). This study offers several new findings relevant to chondrocyte mechanobiology. First, we show that, with all treatments, the apparent Poisson's ratio decreases with applied strain. Second, TGF-β1 and IGF-I alter the measured Poisson's ratio of the cells compared with control. Finally, we offer evidence that these growth factors do not alter the critical strain of chondrocytes.

The finding that compressibility changes with applied strain (Fig. 3) is a new and important finding, though it was surprising considering previous work (Shieh et al., 2006). Key differences in the methods may account for this discrepancy, which are discussed in detail later. Our new finding suggests that with increasing strains, cells have an impaired ability to translate axial strain to transverse strain. Mechanistically, this change in compressibility may be related to both the greater fluid exudation and cytoskeletal breakdown as applied strains increase. However, the apparent Poisson's ratio measured in this study represents a bulk measurement of the cellular compressibility, whereby changes in individual components of the cell, such as the actin cytoskeleton, can have profound effects on its structural properties. It is well documented that the growth factors in this study cause reorganization of the actin cytoskeleton (Gagelin et al., 1995; Boland et al., 1996; Berfield et al., 1997) through specific signaling molecules, such as focal adhesion kinase and several others (Kim and Feldman, 1998; Edlund et al., 2002; Maddala et al., 2003), that likely relate to enhanced adhesion processes (Loeser, 1997; Shakibaei et al., 1999). These growth factors also increase chondrocyte stiffness (Leipzig et al., 2006). Thus, these changes in cellular structure likely improved the ability of the cell to translate axial to transverse strains, resulting in a higher Poisson's ratio for TGF-β1 and IGF-I cells compared with control (Fig. 3). Theoretical models of cartilage may need to consider the biochemical and biomechanical milieu of the tissue and take into account the observed changes in cellular compressibility. Additionally, this effect on cellular compressibility may be related to the observed detrimental effects on the chondrocyte viability and matrix synthesis due to high-magnitude mechanical stimuli (up to 50% compressive strain) on cartilage explants (Loening et al., 2000; Kurz et al., 2001), as these may reduce cellular volume beyond the point of recovery.

In this study, we also identified a possible change in the recovery behavior of chondrocytes at approximately 30–35% applied compressive strain (Figs. 5 and 6). This critical region is slightly higher than our previous work, which suggested a change in behavior between 25% and 30% apparent strain (discussed below). Physiological strains in femoral head cartilage have been measured to be 2–10% (Armstrong et al., 1979), with native chondrocytes

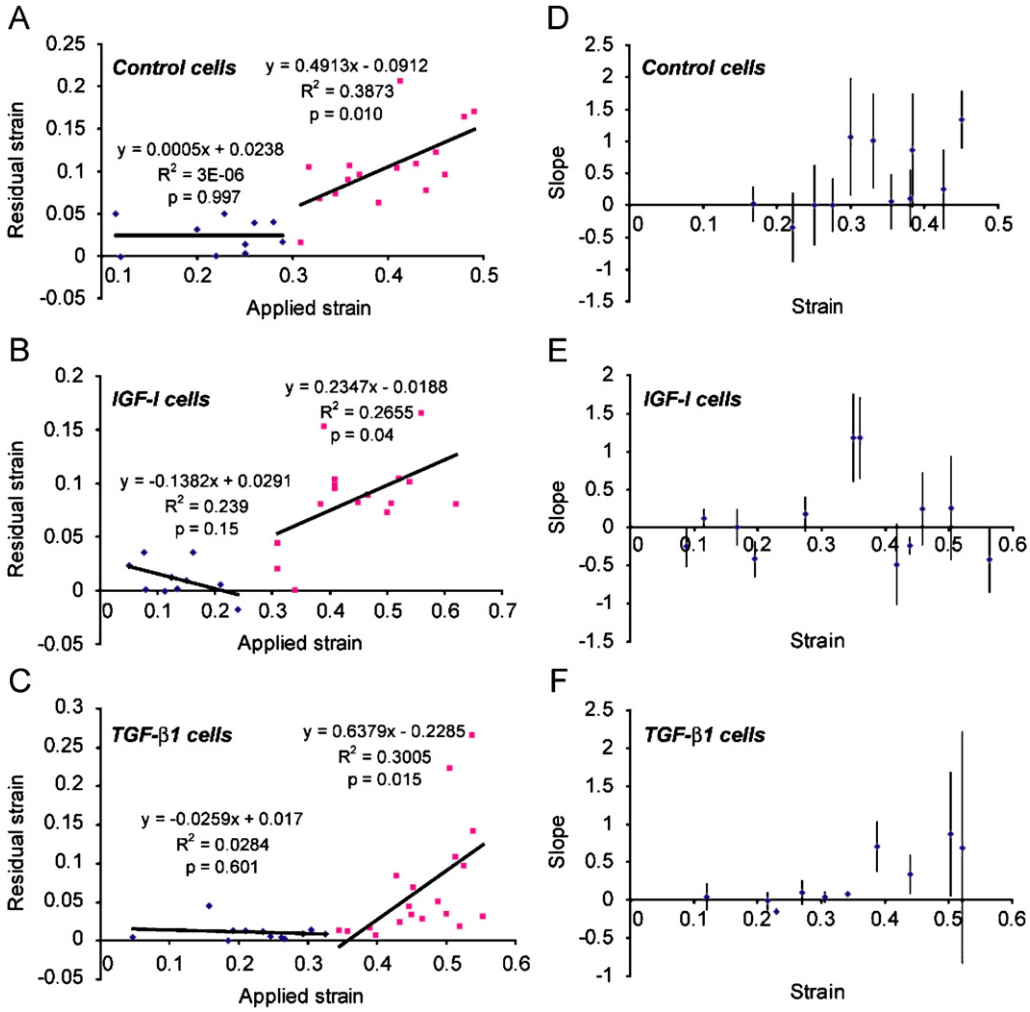

Fig. 5. Residual strain behavior with applied strain. Residual strain (ε_r) significantly increased in control (A), IGF-I (B), and TGF-β1 (C) groups. Change point analysis for each group (D–F) revealed possible discontinuities in all groups at approximately 30% applied strain (ε). Linear fits for each data set above and below this discontinuity are shown. The overall linear fit for control cells was $\varepsilon_r = 0.41\varepsilon - 0.06$ ($R^2 = 0.61$, $p < 0.0001$). IGF-I cells had an overall fit of $\varepsilon_r = 0.24\varepsilon - 0.02$ ($R^2 = 0.61$, $p < 0.0001$). The overall fit for TGF-β1 cells was $\varepsilon_r = 0.27\varepsilon - 0.05$ ($R^2 = 0.34$, $p = 0.0006$).

likely experiencing a two-fold increase in strain compared with the macroscopic tissue (Guilak and Mow, 2000). Though there are differences between species and joints (Athanasiou et al., 1994, 1995, 1998), it is interesting that these estimates of physiological levels of cellular strain fall below the observed critical strain for chondrocytes. A direct link may exist between this critical strain and the determination of chondrocyte behavior toward either degenerative or regenerative/homeostatic processes.

We have previously observed a two-fold stiffening effect of the growth factors with a concurrent increase in F-actin levels (Leipzig et al., 2006). Though we again detected a two-fold stiffening effect of the growth factors, the data show that IGF-I and TGF-β1 did not substantially alter the critical region of strain. Taken together, these results question the role of F-actin in determining the critical region. Future work involving the direct disruption or stimulation of other specific cytoskeletal components should help to elucidate the underlying basis of the critical region. The role of other physical phenomena such as active volume regulation should also be examined since these mechanisms are minimized by the ambient conditions (Bush and Hall, 2001; Guilak et al., 2002) of these experiments. Establishing a fundamental understanding of chondrocyte biomechanics may be relevant to various cartilage processes. For example, changes in chondrocyte volume may be linked to matrix synthesis (Urban et al., 1993).

Improvements to our technique compared with previous work (Shieh et al., 2006) may account for the discrepancy in findings for the compressibility and slight increase in the critical strain, as well as other observations. Serum, which contains a variety of growth factors at unknown concentrations, was judiciously excluded from these experiments so that the effects of the individual growth factors would be isolated. It appears that the media components (i.e., presence or absence of serum and growth factors) affect the biomechanics of the cells, as the stiffness coefficients for the groups was about 50% less than the analogous relaxed moduli measured in our previous work

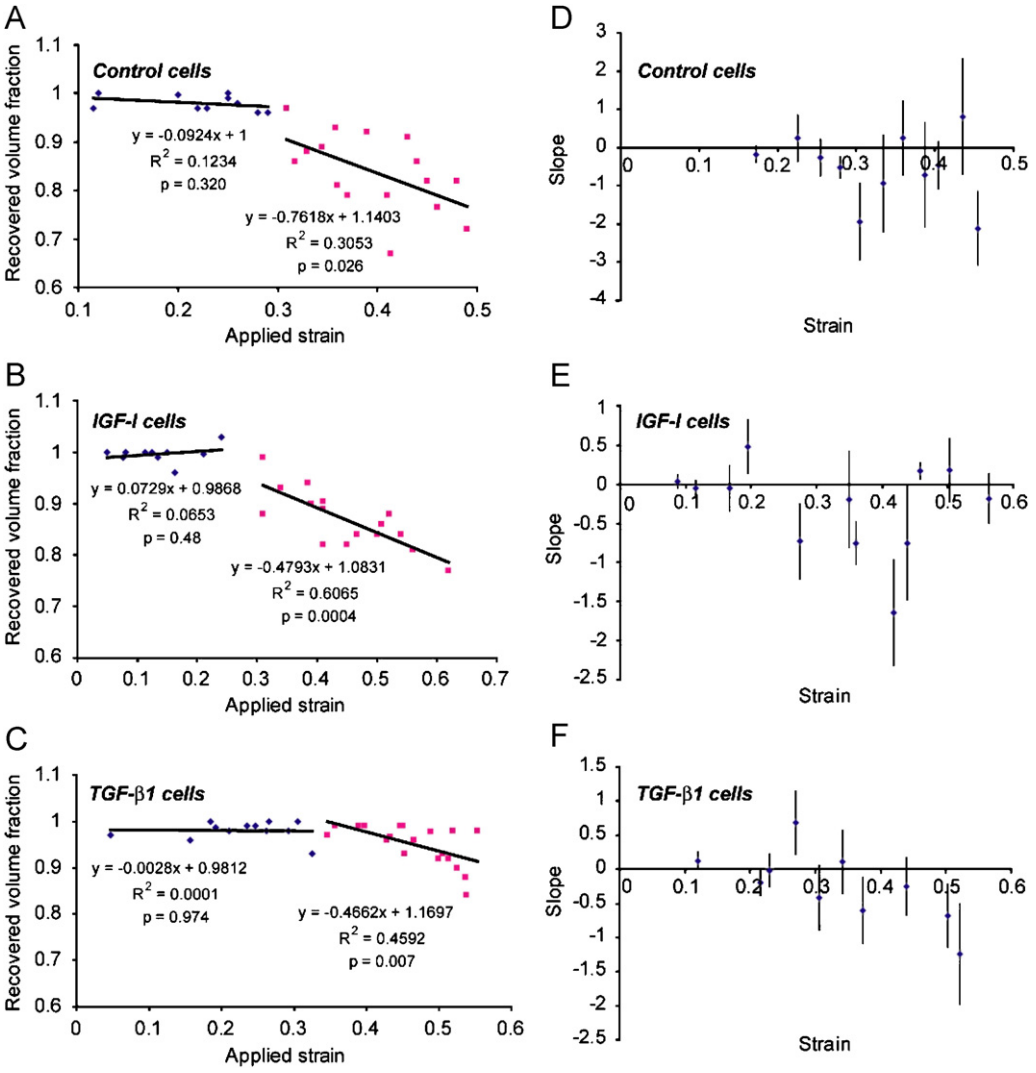

Fig. 6. Recovered volume fraction behavior with applied strain. Recovered volume fraction (V_r) significantly decreased in control (A), IGF-I (B), and TGF-β1 (C) groups. Change point analysis for each group (D–F) revealed possible discontinuities in all groups at approximately 30% applied strain (ε). Linear fits for each data set above and below this discontinuity are shown. The overall linear fit for control cells was $V_r = -0.64\varepsilon + 1.11$ ($R^2 = 0.65$, $p < 0.0001$). IGF-I cells had an overall fit of $V_r = -0.38\varepsilon + 1.04$ ($R^2 = 0.79$, $p < 0.0001$). The overall fit for TGF-β1 cells was $V_r = -0.24\varepsilon + 1.04$ ($R^2 = 0.42$, $p < 0.0001$).

that used serum (Leipzig et al., 2006). Additionally, the mean control Poisson's ratio (0.18) was about 40% less than the published values, while the growth factor groups (~0.30) were similar (Freeman et al., 1994; Shieh and Athanasiou, 2005; Shieh et al., 2006; Trickey et al., 2006). This result highlights the influence of growth factors, added exogenously or through the addition of serum, on the cytoskeleton and its role in determining these cellular properties. These alterations in cellular biomechanics are likely related to the higher recovery time constants observed in this study compared with our previous work (Shieh et al., 2006). It is important to note, however, that we have again observed increases in recovery time constants with applied strains (Shieh et al., 2006), indicating that high-strain, high-frequency stimulation regimens of cartilage may cause irreparable damage to the cell. While relative changes due to growth factors or other treatments within a study can be observed with or without serum, the marked changes in the absolute values of the cellular characteristics again emphasizes the need to consider the environment of the cell when using these values.

Another alteration in this methodology compared to our previous work (Shieh et al., 2006) involved the image analysis. All analysis in this study was based on the applied strain to the cell at equilibrium deformation, as opposed to an apparent strain that was recorded once the platen was removed. The apparent strain would be less than the applied strain and hence account for the differences in critical strain ranges (Shieh et al., 2006). The apparent strain in the previous study was also directly used in the calculation of the apparent Poisson's ratio, whereas our calculations for apparent Poisson's ratio and applied strain in this study were independent. Other important

comparisons include the cell morphologies. Cells in each group largely retained a rounded shape, and the volume measurements ($\sim$700 μm^3) are similar to previously reported values (Bush and Hall, 2001). The finding that TGF-β1 increased cell size ($\sim$25% increase in volume over control) agrees with our prior work, though IGF-I did not alter the size as expected (Leipzig et al., 2006). It is possible that the effects of growth factors on single cells may be different when serum is present.

Our methodology has progressed in several important respects, but has limitations. In particular, the removal of the cell from its physiological environment alters cellular processes (Darling and Athanasiou, 2005). Additionally, two-dimensional recordings of the cell may not fully capture its three-dimensional complexity (Guilak et al., 1995). However, the results of this study are consistent with much of the literature. Moreover, the use of *in vitro* methods to study articular cartilage remains an important tool so long that results are kept in context. It is clear that the isolation of cells from their matrix retains at least some characteristics, including differences in zonal chondrocyte biomechanics (Shieh and Athanasiou, 2005) and osteoarthritic chondrocyte characteristics (Trickey et al., 2000).

In summary, this study examined the effects of TGF-β1 and IGF-I on single chondrocyte morphology, compressibility, biomechanics, and recovery behavior. The results build upon our previous single-cell studies by allowing the measurement of applied strain and the stiffness coefficient as well as the observation of chondrocyte-recovery behavior. Using this methodology we have demonstrated that cell compressibility changes with strain and with growth factors. We have also shown that the critical region of strain does not change with the administration of these growth factors. Characterizing how chondrocytes respond to these physicochemical stimuli and understanding their underlying biomechanics may help elucidate etiologies for osteoarthritis and offer new directions for tissue-engineering efforts.

Conflict of interest

None.

Acknowledgments

We gratefully acknowledge support from the NSF (traineeship for E.J. Koay, DGE-0114264), NIH (traineeship for G. Ofek, 5 T90 DK70121-03 and 5 R90 DK71504-03), and an Osteoarthritis Biomarkers Biomedical Science Grant from the Arthritis Foundation.

References

Armstrong, C.G., Bahrani, A.S., Gardner, D.L., 1979. In vitro measurement of articular cartilage deformations in the intact human hip joint under load. Journal of Bone and Joint Surgery America 61, 744–755.

Athanasiou, K.A., Agarwal, A., Dzida, F.J., 1994. Comparative study of the intrinsic mechanical properties of the human acetabular and femoral head cartilage. Journal of Orthopaedic Research 12, 340–349.

Athanasiou, K.A., Agarwal, A., Muffoletto, A., Dzida, F.J., Constantinides, G., Clem, M., 1995. Biomechanical properties of hip cartilage in experimental animal models. Clinical Orthopaedics and Related Research 316, 254–266.

Athanasiou, K.A., Liu, G.T., Lavery, L.A., Lanctot, D.R., Schenck, R.C., 1998. Biomechanical topography of human articular cartilage in the first metatarsophalangeal joint. Clinical Orthopaedics and Related Research 348, 269–281.

Aufderheide, A.C., Athanasiou, K.A., 2006. A direct compression stimulator for articular cartilage and meniscal explants. Annals of Biomedical Engineering 34, 1463–1474.

Berfield, A.K., Spicer, D., Abrass, C.K., 1997. Insulin-like growth factor (IGF-I) induces unique effects in the cytoskeleton of cultured rat glomerular mesangial cells. Journal of Histochemistry and Cytochemistry 45, 583–593.

Boland, S., Boisvieux-Ulrich, E., Houcine, O., Baeza-Squiban, A., Pouchelet, M., Schoevaert, D., Marano, F., 1996. TGF beta 1 promotes actin cytoskeleton reorganization and migratory phenotype in epithelial tracheal cells in primary culture. Journal of Cell Science 109, 2207–2219.

Buckwalter, J.A., Martin, J.A., Brown, T.D., 2006. Perspectives on chondrocyte mechanobiology and osteoarthritis. Biorheology 43, 603–609.

Bush, P.G., Hall, A.C., 2001. Regulatory volume decrease (RVD) by isolated and in situ bovine articular chondrocytes. Journal of Cellular Physiology 187, 304–314.

Darling, E.M., Athanasiou, K.A., 2005. Rapid phenotypic changes in passaged articular chondrocyte subpopulations. Journal of Orthopaedic Research 23, 425–432.

Edlund, S., Landstrom, M., Heldin, C.H., Aspenstrom, P., 2002. Transforming growth factor-beta-induced mobilization of actin cytoskeleton requires signaling by small GTPases Cdc42 and RhoA. Molecular Biology of the Cell 13, 902–914.

Freeman, P.M., Natarajan, R.N., Kimura, J.H., Andriacchi, T.P., 1994. Chondrocyte cells respond mechanically to compressive loads. Journal of Orthopaedic Research 12, 311–320.

Gagelin, C., Pierre, M., Gavaret, J.M., Toru-Delbauffe, D., 1995. Rapid TGF beta 1 effects on actin cytoskeleton of astrocytes: comparison with other factors and implications for cell motility. Glia 13, 283–293.

Guilak, F., Mow, V.C., 2000. The mechanical environment of the chondrocyte: a biphasic finite element model of cell–matrix interactions in articular cartilage. Journal of Biomechanics 33, 1663–1673.

Guilak, F., Ratcliffe, A., Mow, V.C., 1995. Chondrocyte deformation and local tissue strain in articular cartilage: a confocal microscopy study. Journal of Orthopaedic Research 13, 410–421.

Guilak, F., Erickson, G.R., Ting-Beall, H.P., 2002. The effects of osmotic stress on the viscoelastic and physical properties of articular chondrocytes. Biophysical Journal 82, 720–727.

Guilak, F., Alexopolous, L.G., Upton, M.L., Youn, I., Choi, J.B., Cao, L., Setton, L.A., Haider, M.A., 2006. The pericellular matrix as a transducer of biomechanical and biochemical signals in articular cartilage. Annals of the New York Academy of Sciences 1068, 498–512.

Hu, J.C., Athanasiou, K.A., 2006. The effects of intermittent hydrostatic pressure on self-assembled articular cartilage constructs. Tissue Engineering 12, 1337–1344.

Kerrigan, M.J.P., Hook, C.S.V., Qusous, A., Hall, A.C., 2006. Regulatory volume increase (RVI) by in situ and isolated bovine articular chondrocytes. Journal of Cellular Physiology 209, 481–492.

Kim, B., Feldman, E.L., 1998. Differential regulation of focal adhesion kinase and mitogen-activated protein kinase tyrosine phosphorylation during insulin-like growth factor-I-mediated cytoskeletal reorganization. Journal of Neurochemistry 71, 1333–1336.

Koay, E.J., Shieh, A.C., Athanasiou, K.A., 2003. Creep indentation of single cells. Journal of Biomechanical Engineering 125, 334–341.

Kurz, B., Jin, M., Patwari, P., Cheng, D.M., Lark, M.W., Grodzinsky, A., 2001. Biosynthetic response and mechanical properties of articular cartilage after injurious compression. Journal of Orthopaedic Research 19, 1140–1146.

Leipzig, N.D., Athanasiou, K.A., 2005. Unconfined creep compression of chondrocytes. Journal of Biomechanics 38, 77–85.

Leipzig, N.D., Eleswarapu, S.V., Athanasiou, K.A., 2006. The effects of TGF-beta1 and IGF-I on the biomechanics and cytoskeleton of single chondrocytes. Osteoarthritis and Cartilage 14, 1227–1236.

Lima, E.G., Bian, L., Ng, K.W., Mauck, R.L., Byers, B.A., Tuan, R.S., Ateshian, G.A., Hung, C.T., 2007. The beneficial effect of delayed compressive loading on tissue-engineered cartilage constructs cultured with TGF-beta3. Osteoarthritis and Cartilage 15, 1025–1033.

Loening, A.M., James, I.E., Levenston, M.E., Badger, A.M., Frank, E.H., Kurz, B., Nuttall, M.E., Hung, H.H., Blake, S.M., Grodzinsky, A., Lark, M.W., 2000. Injurious mechanical compression of bovine articular cartilage induces chondrocyte apoptosis. Archives of Biochemistry and Biophysics 381, 205–212.

Loeser, R.F., 1997. Growth factor regulation of chondrocyte integrins. Differential effects of insulin-like growth factor I and transforming growth factor beta on alpha 1 beta 1 integrin expression and chondrocyte adhesion to type VI collagen. Arthritis and Rheumatism 40, 270–276.

Maddala, R., Reddy, V.N., Epstein, D.L., Rao, V., 2003. Growth factor induced activation of Rho and Rac GTPases and actin cytoskeletal reorganization in human lens epithelial cells. Molecular Vision 9, 329–336.

Ng, K.W., Mauck, R.L., Statman, L.Y., Lin, E.Y., Ateshian, G.A., Hung, C.T., 2006. Dynamic deformational loading results in selective application of mechanical stimulation in a layered, tissue-engineered cartilage construct. Biorheology 43, 497–507.

Shakibaei, M., John, T., De Souza, P., Rahmanzadeh, R., Merker, H.J., 1999. Signal transduction by beta1 integrin receptors in human chondrocytes in vitro: collaboration with the insulin-like growth factor-I receptor. Biochemical Journal 342, 615–623.

Shieh, A.C., Athanasiou, K.A., 2002. Biomechanics of single chondrocytes and osteoarthritis. Critical Reviews in Biomedical Engineering 30, 307–343.

Shieh, A.C., Athanasiou, K.A., 2005. Biomechanics of single zonal chondrocytes. Journal of Biomechanics 39, 1595–1602.

Shieh, A.C., Athanasiou, K.A., 2006. Dynamic compression of single cells. Osteoarthritis and Cartilage 15, 328–334.

Shieh, A.C., Koay, E.J., Athanasiou, K.A., 2006. Strain-dependent recovery behavior of single chondrocytes. Biomechanics and Modeling in Mechanobiology 5, 172–179.

Shin, D., Athanasiou, K.A., 1999. Cytoindentation for obtaining cell biomechanical properties. Journal of Orthopaedic Research 17, 880–890.

Smith, R.L., Donlon, B.S., Gupta, M.K., Mohtai, M., Das, P., Carter, D.R., Cooke, J., Gibbons, G., Hutchinson, N., Schurman, D.J., 1995. Effects of fluid-induced shear on articular chondrocyte morphology and metabolism in vitro. Journal of Orthopaedic Research 13, 824–831.

Smith, R.L., Carter, D.R., Schurman, D.J., 2004. Pressure and shear differentially alter human articular chondrocyte metabolism. Clinical Orthopaedics and Related Research 427S, S89–S95.

Trickey, W.R., Lee, G.M., Guilak, F., 2000. Viscoelastic properties of chondrocytes from normal and osteoarthritic human cartilage. Journal of Orthopaedic Research 18, 891–898.

Trickey, W.R., Baaijens, F.P., Laursen, T.A., Alexopolous, L.G., Guilak, F., 2006. Determination of the Poisson's ratio of the cell: recovery properties of chondrocytes after release from complete micropipette aspiration. Journal of Biomechanics 39, 78–87.

Urban, J.P.G., Hall, A.C., Gehl, K.A., 1993. Regulation of matrix synthesis rates by the ionic and osmotic environment of articular chondrocytes. Journal of Cellular Physiology 154, 262–270.