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Zonal changes in the three-dimensional morphology of the chondron under compression: The relationship among cellular, pericellular, and extracellular deformation in articular cartilage

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Abstract

The pericellular matrix (PCM) is a narrow region of tissue that completely surrounds chondrocytes in articular cartilage. Previous theoretical models of the “chondron” (the PCM with enclosed cells) suggest that the structure and properties of the PCM may significantly influence the mechanical environment of the chondrocyte. The objective of this study was to quantify changes in the 3D morphology of the chondron *in situ* at different magnitudes of compression applied to the cartilage extracellular matrix. Fluorescence immunolabeling for type VI collagen was used to identify the boundaries of the cell and PCM, and confocal microscopy was used to form 3D images of chondrons from superficial, middle, and deep zone cartilage in explants compressed to 0%, 10%, 30%, and 50% surface-to-surface strain. Lagrangian tissue strain, determined locally using texture correlation, was highly inhomogeneous and revealed depth-dependent properties of the extracellular matrix. Compression significantly decreased cell and chondron height and volume, depending on the zone and magnitude of compression. In the superficial zone, cellular-level strains were always lower than tissue-level strains. In the middle and deep zones, however, tissue strains below 25% were amplified at the cellular level, while tissue strains above 25% were decreased at the cellular level. These findings are consistent with previous theoretical models of the chondron, suggesting that the PCM can serve as either a protective layer for the chondrocyte or a transducer that amplifies strain, such that cellular-level strains are more homogenous throughout the tissue depth despite large inhomogeneities in local ECM strains.

Keywords

articular cartilage; pericellular matrix; extracellular matrix; three-dimensional reconstruction; collagen; chondrocyte; type VI collagen; chondrocyte; immunohistochemistry

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INTRODUCTION

Biomechanical factors play an important role in the health and physiology of the synovial joints, and a number of *in vivo* and *in vitro* studies provide evidence that cells in articular cartilage (chondrocytes) respond to different biophysical signals engendered by mechanical loading of the extracellular matrix (ECM) (Guilak et al., 1997). Compression of articular cartilage induces a complex mechanical environment that is characterized by time- and spatially-varying stresses, strains, osmotic and hydrostatic pressures, interstitial fluid flow, and electrokinetic effects (Frank and Grodzinsky, 1987; Lai et al., 1991; Maroudas et al., 1991; Mow and Guo, 2002; Mow et al., 1999). Studies of the site-specific response of chondrocytes to mechanical compression have shown correlations between specific factors in the mechanical environment and the ensuing changes in biosynthetic activity (Buschmann et al., 1995; Kim et al., 1994; Wong et al., 1997), suggesting that chondrocytes respond to local changes in the biophysical environment.

The specific biomechanical factors involved in chondrocyte mechanotransduction are not fully understood. In this respect, theoretical models of cell-matrix interactions in cartilage have provided further insights into the complex stress-strain and fluid flow environments of the chondrocyte (Baer et al., 2003; Baer and Setton, 2000; Guilak et al., 2006; Guilak and Mow, 2000; Haider, 2004; Haider et al., 2006; Upton et al., 2006; Wu et al., 1999) and their variations among the different zones of articular cartilage (Alexopoulos et al., 2005b). These models suggest that the cellular environment is influenced significantly by the relative mechanical properties of the chondrocyte, ECM, and the pericellular matrix (PCM), a narrow tissue region of tissue that completely surrounds chondrocytes. The PCM together with the enclosed cell(s) are termed the “chondron” (Benninghoff, 1925; Poole, 1992), a structural entity that forms a small fraction of the tissue (Quinn et al., 2005) but is hypothesized to play an important role in modulating the biochemical and biomechanical environments of the chondrocytes. The PCM is generally defined by the exclusive presence of type VI collagen (Poole et al., 1991), but also contains other collagens such as types II, IX, XI (Poole et al., 1988; Smith et al., 1989), aggrecan (Poole et al., 1991), and fibronectin (McDevitt et al., 1991; Poole et al., 1990). Because the PCM completely surrounds each chondrocyte, it has been hypothesized that this region serves as a transducer of biomechanical signals (Guilak et al., 2006; Poole, 1992; Szirmai, 1974). In support of these hypotheses, experimental studies have shown that the presence of a newly formed PCM alters the metabolic response of chondrocytes to mechanical loading (Buschmann et al., 1995) and influences the deformation of chondrocytes to mechanical or osmotic stress (Hing et al., 2002; Knight et al., 1998).

While theoretical models of chondron deformation have been developed based on direct measurements of cell, PCM, and ECM mechanical properties, the *in situ* mechanical behavior of the PCM has not been well characterized. Previous studies have used either confocal microscopy (Guilak, 1995; Guilak et al., 1995) or histologic fixation and physical sectioning (Buschmann et al., 1995; Quinn et al., 1998) to study the three-dimensional (3D) deformation behavior of chondrocytes, showing that cell shape and volume are coordinated with local ECM deformation. The objective of this study was to quantify changes in the 3D morphology of the intact chondron *in situ* under different magnitudes of equilibrium compression applied to the ECM. Fluorescence immunolabeling for type VI collagen was used to identify the boundaries of the PCM, and confocal laser scanning microscopy was used to form quantitative 3D models of chondrons in free swelling and compressed cartilage explants. Local tissue strains were measured using texture correlation. These methods were applied to determine zone-specific changes in the shape and volume of the chondrocyte and PCM in relation to local tissue strains, and to test the hypothesis that the relationship between cellular, PCM, and ECM strains vary with zone within the tissue.

MATERIALS AND METHODS

Specimen Preparation and Immunolabeling

Full thickness cylindrical cartilage blocks (5mm diameter) were harvested from the inner one-third region of the medial femoral condyle from knee joints of skeletally mature female pigs (2–3 years old), obtained from an abattoir immediately after sacrifice. For each compressed specimen, an adjacent cartilage explant was prepared as a site-matched, uncompressed control. Only cartilage showing no osteoarthritic changes or fibrillation was used (Collins grade 0).

A consistent orientation was maintained among samples, based on the split-line pattern of the cartilage surface (Below et al., 2002). Specimens were compressed in unconfined compression between polysulfone platens to nominal deformations (surface-to-surface strain) of $10\pm3\%$, $30\pm4\%$, and $50\pm4\%$ ($n=6$ per group). Compressed specimens were equilibrated in phosphate buffered saline for 2 hrs and fixed for 2 hrs in 4% paraformaldehyde within the compression device. Specimens were removed from the device and fixed overnight. Tissue thickness was measured to confirm that the applied compressive deformation was maintained after fixation. Uncompressed control specimens were fixed for equivalent time. Thick frozen sections ($40\mu\text{m}$) were made from each fixed block, oriented along the local split-line direction.

Sections were fluorescently immunolabeled *en bloc* for type VI collagen to delineate the PCM, as described previously (Youn et al., 2006). Specimens were labeled using a primary anti-collagen type VI antibody (RDI-600401108, Fitzgerald) followed by a FITC conjugated fluorescent secondary antibody (RDI-711095152, Fitzgerald).

Chondron Imaging and Three-Dimensional Reconstruction

Chondrons were randomly selected for analysis from each zone (superficial, middle and deep) for both the uncompressed and compressed groups. In the uncompressed specimens, the superficial zone was defined as 0– $50\mu\text{m}$ from the articular surface, the middle zone as 100– $200\mu\text{m}$ from the articular surface, and the deep zone as the lower 50% of the full thickness cartilage. Because articular cartilage shows depth-dependent compressive properties (Schinagl et al., 1997; Wang et al., 2003), the same criteria could not be used following compression. In compressed samples, the superficial chondrons were selected within the top 25 μm depth from the articular surface, and the deep chondrons were selected in the bottom half of the full thickness tissue for all compressions. The middle zone was defined as 100– $150\mu\text{m}$, 80– $120\mu\text{m}$, and 50– $100\mu\text{m}$ from the tissue surface for the 10%, 30%, 50% compression groups, respectively. Chondrons in the deep zone were further divided into two sub-groups depending on the number of chondrocytes enclosed in the chondron ($n=12$ per group).

All chondrons were selected from the central region (radially) of the specimen to avoid inhomogeneous strain fields near the edges of the explant in unconfined compression (Fig. 1). A series of ~50 serial sections (512×512 pixels) was recorded for each chondron at $0.5\mu\text{m}$ intervals using a confocal laser scanning microscope (LSM 510, Zeiss) at excitation/emission wavelengths of 488nm/512nm using a water-immersion objective (63x, 1.3 numerical aperture, Zeiss).

Three-dimensional reconstruction and morphological measurement of chondrons were performed using custom routines, as described previously (Youn et al., 2006). Briefly, images were smoothed using a median filter (to preserve edges), and surface reconstruction was performed using the IsoSurface function in MATLAB, based on the marching cubes algorithm (Guilak, 1994; Guilak, 1995; Guilak et al., 1995).

For each specimen, morphological parameters of the chondron, including surface area, volume, height, medial-lateral (ML) width, and anterior-posterior (AP) width, were calculated from the

reconstructed 3D images (Guilak, 1994). PCM volume was obtained by subtracting the cell volume from the chondron volume.

Cell and chondron height were defined as the maximum distance between the vertical coordinates of all the vertices, and widths as the maximum distance between the coordinates in the ML and AP directions of all the vertices. To determine the principal angle of chondron orientation, all vertices forming the iso-intensity surface contour were screened to identify the two vertices with the maximum distance, forming the principal axis of the chondron. The principal direction was calculated as the direction cosine with respect to a unit vector perpendicular to the cartilage surface.

Local Tissue Strain Measurement using Texture Correlation

Cartilage explants were incubated for 4 hours in Syto-64 (Molecular Probes, Eugene OR) to label the cell nuclei (see Supplementary Figure). Specimens were fluorescently imaged while being compressed using a custom-built micrometer-driven instrument (Guilak, 1995; Guilak et al., 1995). Using a 10x (middle and deep zones) or 20x objective (superficial zone), the ECM was imaged as increasing strains were applied. Local tissue strain was determined using texture correlation analysis (Gilchrist et al., 2006; Gilchrist et al., 2004) using fluorescently labeled cell nuclei as image texture (Wang et al., 2002). Texture correlation uses the inherent patterns in an image to track the displacement of regions from the original to the compressed image (Bay, 1995; Bey et al., 2002; Wall et al., 2006). Both Lagrangian and engineering strains were calculated from the displacement data. Average strains were calculated within each zone, defined as described above.

Statistical Analysis

Statistical comparisons were performed using ANOVA with a Tukey *post hoc* test (STATISTICA, StatSoft, Inc.) test at $\alpha=0.05$. Results are shown as mean $\pm$ s.d.

RESULTS

Compression of the ECM resulted in a nonuniform distribution of local tissue strain. In response to mean applied surface-to-surface engineering strains of $10.6\pm0.70\%$, $30.3\pm1.2\%$, and $50.1\pm1.4\%$, superficial zone strains were $30.0\pm3.0\%$, $48.0\pm4.4\%$, $64.7\pm4.5\%$, middle zone strains were $6.7\pm1.8\%$, $24.6\pm4.0\%$, and $54.6\pm7.8\%$; and deep zone strains were $2.2\pm0.98\%$, $14.1\pm2.6\%$, and $35.4\pm6.4\%$ (Table 1).

Chondrons exhibited significant changes in 3D morphology in each zone, depending upon the level of compression (Fig. 1). Chondron height decreased significantly with compression (Fig. 2a, $p<0.05$). At 10% tissue compression, no significant change was detected in the height of deep zone chondrons as compared to uncompressed controls. For 10%, 30%, and 50% compression groups, the apparent engineering strain of the chondrons calculated from the height change corresponded to 25%, 44%, and 62% in the superficial zone, 18%, 28%, and 46% in the middle zone, and 1.7%, 15%, and 35% in the deep zone, respectively. The corresponding strain of the chondrocytes was 27%, 43%, and 59% in the superficial zone, 21%, 32%, and 44% in the middle zone, and 2.2%, 19%, and 32% in the deep zone. There was no significant change in the ML width of the chondrons under compression except for deep zone chondrons at 50% compression (one-cell chondron, $p<0.05$, Fig. 2b).

Significant decreases were observed in the volume and surface area of the PCM with compression in all zones (Fig. 3, $p<0.001$). Chondrocytes also underwent a significant decrease in volume with increasing compression, except in the deep zone (Fig. 4, $p<0.001$). The surface areas of the PCM and the cell showed similar trends as the volume (data not shown).

In uncompressed specimens, significant zonal differences were observed in the ratio of PCM volume to chondrocyte volume, with a maximum ratio of ~4 in the middle zone (Fig. 5). This trend was present at the 10% compression level also, but the zonal variations of this ratio were not present at 30% or 50% compression. At 50% compression, significant decreases in chondrocyte volume in the superficial zone resulted in PCM to cell volume ratio of ~25 (bar is omitted in Fig. 5 for clarity).

In the deep zone, the volume of the PCM in the two-cell chondrons also showed a significant difference under compression, similar to one-cell chondrons (Fig. 6, $p < 0.05$). However, the normalized ratio of the PCM volume to the chondrocyte volume showed no significant differences in either one-cell chondron or two-cell chondron under compression. Chondrons with two cells had statistically greater PCM volume than one-cell chondrons ($p < 0.05$), while one-cell chondrons had higher normalized ratio of the PCM volume to the chondrocyte volume at all strain levels ($p < 0.05$).

In comparing the relationship among the apparent strains at the cellular, PCM, and tissue levels (Figure 7), large differences were observed in the ratio of cellular to tissue level strains in the different zones, as well as at different strains. At all levels of compression, cell strains were lower than tissue level strains in the superficial zone. Similarly, at 50% compression, cell strains in all zones were lower than the local tissue strains. At 10% and 30% compression, cell strains were greater than tissue level strains in the middle and deep zones.

DISCUSSION

The findings of this study provide direct evidence based on 3D *in situ* measurements that the chondron undergoes significant changes in shape, size, and volume with compression of the cartilage ECM. Importantly, the magnitude of these changes was highly dependent on the zone of origin of the chondron, with larger deformations in the superficial zone as compared to the middle and deep zones, consistent with significantly greater local tissue strains in this region. These findings provide further evidence of the complex and inhomogeneous mechanical environment of chondrocytes within articular cartilage, and support the hypothesis that the PCM plays an important role in modulating the mechanical environment of the chondrocyte.

Of particular note was the finding that the relationship between cellular-level strains and local ECM strains was nonlinear. Specifically, in conditions of high local ECM strain ($>25\%$), the apparent cellular strains were lower than the ECM strains. However, in conditions of lower local tissue strain ($<25\%$), apparent cellular strains were increased. These findings are consistent with theoretical models of the chondron (Alexopoulos et al., 2005b; Guilak and Mow, 2000) in combination with direct measurements of the mechanical properties of the cell (Trickey et al., 2006; Trickey et al., 2000; Trickey et al., 2004) and PCM (Alexopoulos et al., 2003; Alexopoulos et al., 2005c; Allen and Mao, 2004; Guilak et al., 2005). Previous studies have shown that the modulus of the PCM is uniform with tissue depth and similar to that of the superficial zone ECM, but significantly lower than the modulus of the middle or deep zones of cartilage. Taken together, one interpretation of these theoretical and experimental studies is that a potential role of the PCM is to serve as a nonlinear mechanical filter that protects the chondrocyte (i.e., reduces cellular strain) in regions of high local strain such as the superficial zone, but amplifies lower magnitudes of local strain in the middle and deep zone, such that cellular-level strains are more homogenous throughout the tissue depth despite large inhomogeneities in local ECM strains.

Compression of the ECM resulted in changes in height and volume of the cell and PCM that varied significantly with the zone of origin of the chondron. In the superficial zone, changes in PCM height and volume were greater than the nominally applied matrix strain, whereas in

the deep zone, changes in PCM height and volume were less than the applied strains. These findings are consistent with previous measurements of the zonal variations in cell shape and volume with compression (Guilak et al., 1995; Wong et al., 1997), which also showed highest cell deformation in the surface zone and lowest in the deep zone. These studies and others reflect the large inhomogeneities in the compressive mechanical properties of the adult cartilage ECM, with approximately an order of magnitude increase in the compressive modulus the tissue from the superficial zone to the deep zone (Chen et al., 2001; Schinagl et al., 1997; Wang et al., 2001).

The largest changes in cell and chondron morphology were observed in the superficial zone at high strains (50% tissue deformation), with an apparent “collapse” of the cell and PCM in half of the sampled chondrons, as evidenced by contact between the top and bottom surfaces of the PCM. Because of the low compressive modulus of the superficial zone relative to the middle and deep zones, local tissue strains were highest in the superficial zone, exceeding 60%. In previous studies, local strains of ~60% resulted in damage to the chondrocyte membrane as evidenced by leakage of an intracellular fluorescent dye (Guilak et al., 1995). Thus the apparent collapse of superficial zone chondrons at high strain may reflect strain-induced cell damage and possible cell death under these conditions.

Significant zonal variations were observed in the medial-lateral (ML) width of the chondrons with compression. In the superficial zone, little or no change in chondron width occurred. This finding is consistent with micropipette aspiration studies of mechanically isolated chondrons that show the PCM possesses an intrinsic Poisson’s ratio of ~0.04 (Alexopoulos et al., 2005c). However, in the deep zone, the ratio of the change in ML width to height of the chondron was ~0.5. These differences likely reflect zonal differences in the Poisson’s ratio of the ECM (Table 1) (Jurvelin et al., 1997), and suggests that the mechanical behavior of the chondron may be mostly dependent on the local response of the ECM, as opposed to zone-dependent differences in cell or PCM properties (Alexopoulos et al., 2005c; Darling et al., 2006).

An important advance of this study was the application of *en bloc* imaging of the 3D morphology of the chondron (Youn et al., 2006) to study the effects of compression, without extraction or physical sectioning of the tissue. While such approach necessitates the use of separate specimens for control and compressed groups, similar methods using light or transmission electron microscopy have been used successfully in previous studies to quantify the deformation of cells and subcellular organelles (Buschmann et al., 1995; Szafranski et al., 2004), but have not been directly applied to measure changes in PCM morphology. In one study, such compression/fixation techniques, combined with autoradiographic measurements of matrix biosynthesis, showed that the pericellular region showed greatest sensitivity to compression as compared to other matrix compartments (Quinn et al., 1998). Furthermore, the largest changes in autoradiographic grain density occurred in directions perpendicular to that of applied compression, suggesting significant inhomogeneities in local tissue strains. These findings are consistent with the present findings and provide additional evidence of PCM deformation during cartilage compression.

While there is evidence for the presence of other molecules such as collagen types IX or XI in the PCM (Poole, 1997; Poole et al., 1997), the chondron is generally defined by the presence of type VI collagen (Alexopoulos et al., 2005a; Lee et al., 1997; Poole, 1992; Pullig et al., 1999; Sherwin et al., 1999). Nonetheless, because of the clear pericellular distribution of type VI collagen in normal cartilage, the measurements in this study would be expected to directly reflect the deformation behavior of the pericellular region of the cell, if not that of the “chondron” *per se*.

In summary, our findings suggest that the chondron and PCM undergo significant deformation *in situ* in response to compression of the ECM. The zonal variations in the mechanical response of the chondron do not appear to be due to zonal differences in PCM properties (Alexopoulos et al., 2003; Alexopoulos et al., 2005c; Guilak et al., 2005), but rather seem to result from significant inhomogeneities in ECM properties, which reflect different relative moduli of the ECM and PCM with depth. Taken together with previous zone-specific finite element models of cell-matrix interactions in cartilage, these findings suggest that the PCM plays a biomechanical role in cartilage by regulating the local stress-strain and fluid flow environments of the chondrocyte in a zone-dependent manner (Alexopoulos et al., 2005b), supporting previous hypotheses of a “protective” role of the PCM for the chondrocyte (Poole et al., 1987). Future studies of the deformation behavior of the chondron with aging or disease may provide new insights into the influence of this structure on physiology and the pathology of articular cartilage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Alexopoulos LG, Bonaldo P, Guilak F. Accelerated development of osteoarthritis in col6a1 knockout mice: The biomechanical role of the type VI collagen in articular cartilage. *Transactions of the Orthopaedic Research Society* 2005a;29:393.
- Alexopoulos LG, Haider MA, Vail TP, Guilak F. Alterations in the mechanical properties of the human chondrocyte pericellular matrix with osteoarthritis. *Journal of Biomechanical Engineering* 2003;125:323–333. [PubMed: 12929236]
- Alexopoulos LG, Setton LA, Guilak F. The biomechanical role of the chondrocyte pericellular matrix in articular cartilage. *Acta Biomaterialia* 2005b;1:317–325. [PubMed: 16701810]
- Alexopoulos LG, Williams GM, Upton ML, Setton LA, Guilak F. Osteoarthritic changes in the biphasic mechanical properties of the chondrocyte pericellular matrix in articular cartilage. *Journal of Biomechanics* 2005c;38:509–517. [PubMed: 15652549]
- Allen DM, Mao JJ. Heterogeneous nanostructural and nanoelastic properties of pericellular and interterritorial matrices of chondrocytes by atomic force microscopy. *Journal of Structural Biology* 2004;145:196–204. [PubMed: 14960370]
- Baer AE, Laursen TA, Guilak F, Setton LA. The micromechanical environment of intervertebral disc cells determined by a finite deformation, anisotropic, and biphasic finite element model. *Journal of Biomechanical Engineering* 2003;125:1–11. [PubMed: 12661192]
- Baer AE, Setton LA. The micromechanical environment of intervertebral disc cells: Effect of matrix anisotropy and cell geometry predicted by a linear model. *Journal of Biomechanical Engineering* 2000;122:245–251. [PubMed: 10923292]
- Bay BK. Texture correlation: a method for the measurement of detailed strain distributions within trabecular bone. *Journal of Orthopaedic Research* 1995;13:258–267. [PubMed: 7722763]
- Below S, Arnoczky SP, Dodds J, Kooima C, Walter N. The split-line pattern of the distal femur: A consideration in the orientation of autologous cartilage grafts. *Arthroscopy* 2002;18:613–617. [PubMed: 12098122]
- Benninghoff A. Form und bau der Gelenkknorpel in ihren Beziehungen Zur Funktion. Zweiter Teil: der Aufbau des Gelenkknorpels in seinen Beziehungen zur Funktion 1925;2:783.
- Bey MJ, Song HK, Wehrli FW, Soslowsky LJ. A noncontact, nondestructive method for quantifying intratissue deformations and strains. *Journal of Biomechanical Engineering* 2002;124:253–258. [PubMed: 12002136]

- Buschmann MD, Gluzband YA, Grodzinsky AJ, Hunziker EB. Mechanical compression modulates matrix biosynthesis in chondrocyte/agarose culture. *Journal of Cell Science* 1995;108:1497–1508. [PubMed: 7615670]
- Chen SS, Falcovitz YH, Schneiderman R, Maroudas A, Sah RL. Depth-dependent compressive properties of normal aged human femoral head articular cartilage: relationship to fixed charge density. *Osteoarthritis and Cartilage* 2001;9:561–569. [PubMed: 11520170]
- Darling EM, Zauscher S, Guilak F. Viscoelastic properties of zonal articular chondrocytes measured by atomic force microscopy. *Osteoarthritis and Cartilage* 2006;14:571–579. [PubMed: 16478668]
- Frank EH, Grodzinsky AJ. Cartilage electromechanics--II. A continuum model of cartilage electrokinetics and correlation with experiments. *Journal of Biomechanics* 1987;20:629–639. [PubMed: 3611138]
- Gilchrist CL, Witvoet-Braam SW, Guilak F, Setton LA. Measurement of intracellular strain on deformable substrates with texture correlation. *Journal of Biomechanics*. 2006in press, Epub
- Gilchrist CL, Xia JQ, Setton LA, Hsu EW. High-resolution determination of soft tissue deformations using MRI and first-order texture correlation. *IEEE Trans Med Imaging* 2004;23:546–553. [PubMed: 15147008]
- Guilak F. Volume and surface area measurement of viable chondrocytes in situ using geometric modelling of serial confocal sections. *J Microsc* 1994;173(Pt 3):245–256. [PubMed: 8189447]
- Guilak F. Compression-induced changes in the shape and volume of the chondrocyte nucleus. *Journal of Biomechanics* 1995;28:1529–1542. [PubMed: 8666592]
- Guilak F, Alexopoulos LG, Haider MA, Ting-Beall HP, Setton LA. Zonal uniformity in mechanical properties of the chondrocyte pericellular matrix: Micropipette aspiration of canine chondrons isolated by cartilage homogenization. *Annals of Biomedical Engineering* 2005;33:1312–1318. [PubMed: 16240080]
- Guilak F, Alexopoulos LG, Upton ML, Youn I, Choi JB, Cao L, Setton LA, Haider MA. The pericellular matrix as a transducer of biomechanical and biochemical signals in articular cartilage. *Annals of the New York Academy of Sciences* 2006;1068:498–512. [PubMed: 16831947]
- Guilak F, Mow VC. The mechanical environment of the chondrocyte: a biphasic finite element model of cell-matrix interactions in articular cartilage. *Journal of Biomechanics* 2000;33:1663–1673. [PubMed: 11006391]
- Guilak F, Ratcliffe A, Mow VC. Chondrocyte deformation and local tissue strain in articular cartilage: a confocal microscopy study. *Journal of Orthopaedic Research* 1995;13:410–421. [PubMed: 7602402]
- Guilak, F.; Sah, RL.; Setton, LA. Physical regulation of cartilage metabolism. In: Mow, VC.; Hayes, WC., editors. *Basic Orthopaedic Biomechanics*. Lippincott-Raven; Philadelphia: 1997. p. 179-207.
- Haider MA. A radial biphasic model for local cell-matrix mechanics in articular cartilage. *SIAM Journal on Applied Mathematics* 2004;64:1588–1608.
- Haider MA, Schugart RC, Setton LA, Guilak F. A mechano-chemical model for the passive swelling response of an isolated chondron under osmotic loading. *Biomechanics and Modeling in Mechanobiology* 2006;5:160–171. [PubMed: 16520959]
- Hing WA, Sherwin AF, Poole CA. The influence of the pericellular microenvironment on the chondrocyte response to osmotic challenge. *Osteoarthritis and Cartilage* 2002;10:297–307. [PubMed: 11950253]
- Jurvelin JS, Buschmann MD, Hunziker EB. Optical and mechanical determination of Poisson's ratio of adult bovine humeral articular cartilage. *Journal of Biomechanics* 1997;30:235–241. [PubMed: 9119822]
- Kim YJ, Sah RL, Grodzinsky AJ, Plaas AH, Sandy JD. Mechanical regulation of cartilage biosynthetic behavior: physical stimuli. *Archives of Biochemistry & Biophysics* 1994;311:1–12. [PubMed: 8185305]
- Knight MM, Lee DA, Bader DL. The influence of elaborated pericellular matrix on the deformation of isolated articular chondrocytes cultured in agarose. *Biochimica et Biophysica Acta* 1998;1405:67–77. [PubMed: 9784610]
- Lai WM, Hou JS, Mow VC. A triphasic theory for the swelling and deformation behaviors of articular cartilage. *Journal of Biomechanical Engineering* 1991;113:245–258. [PubMed: 1921350]

- Lee GM, Poole CA, Kelley SS, Chang J, Caterson B. Isolated chondrons: a viable alternative for studies of chondrocyte metabolism in vitro. *Osteoarthritis and Cartilage* 1997;5:261–274. [PubMed: 9404471]
- Maroudas A, Wachtel E, Grushko G, Katz EP, Weinberg P. The effect of osmotic and mechanical pressures on water partitioning in articular cartilage. *Biochimica et Biophysica Acta* 1991;1073:285–294. [PubMed: 2009281]
- McDevitt CA, Marcelino J, Tucker L. Interaction of intact type VI collagen with hyaluronan. *FEBS Letters* 1991;294:167–170. [PubMed: 1756855]
- Mow VC, Guo XE. Mechano-electrochemical properties of articular cartilage: their inhomogeneities and anisotropies. *Annual Review of Biomedical Engineering* 2002;4:175–209.
- Mow VC, Wang CC, Hung CT. The extracellular matrix, interstitial fluid and ions as a mechanical signal transducer in articular cartilage. *Osteoarthritis and Cartilage* 1999;7:41–58. [PubMed: 10367014]
- Poole, CA. Chondrons: the chondrocyte and its pericellular microenvironment. In: Kuettner, KE.; Schleyerbach, R.; Peyron, JG.; Hascall, VC., editors. *Articular Cartilage and Osteoarthritis*. London: Academic Press, New York; 1992. p. 201–220.
- Poole CA. Articular cartilage chondrons: form, function and failure. *Journal of Anatomy* 1997;191:1–13. [PubMed: 9279653]
- Poole CA, Flint MH, Beaumont BW. Chondrons in cartilage: ultrastructural analysis of the pericellular microenvironment in adult human articular cartilages. *Journal of Orthopaedic Research* 1987;5:509–522. [PubMed: 3681525]
- Poole CA, Flint MH, Beaumont BW. Chondrons extracted from canine tibial cartilage: preliminary report on their isolation and structure. *Journal of Orthopaedic Research* 1988;6:408–419. [PubMed: 3357089]
- Poole CA, Gilbert RT, Herbage D, Hartmann DJ. Immunolocalization of type IX collagen in normal and spontaneously osteoarthritic canine tibial cartilage and isolated chondrons. *Osteoarthritis and Cartilage* 1997;5:191–204. [PubMed: 9219682]
- Poole CA, Honda T, Skinner SJ, Schofield JR, Hyde KF, Shinkai H. Chondrons from articular cartilage (II): Analysis of the glycosaminoglycans in the cellular microenvironment of isolated canine chondrons. *Connective Tissue Research* 1990;24:319–330. [PubMed: 2376132]
- Poole CA, Matsuoka A, Schofield JR. Chondrons from articular cartilage. III. Morphologic changes in the cellular microenvironment of chondrons isolated from osteoarthritic cartilage. *Arthritis & Rheumatism* 1991;34:22–35. [PubMed: 1984777]
- Pullig O, Weseloh G, Swoboda B. Expression of type VI collagen in normal and osteoarthritic human cartilage. *Osteoarthritis and Cartilage* 1999;7:191–202. [PubMed: 1022218]
- Quinn TM, Grodzinsky AJ, Buschmann MD, Kim YJ, Hunziker EB. Mechanical compression alters proteoglycan deposition and matrix deformation around individual cells in cartilage explants. *Journal of Cell Science* 1998;111:573–583. [PubMed: 9454731]
- Quinn TM, Hunziker EB, Hauselmann HJ. Variation of cell and matrix morphologies in articular cartilage among locations in the adult human knee. *Osteoarthritis and Cartilage* 2005;13:672–678. [PubMed: 15970445]
- Schinagl RM, Gurskis D, Chen AC, Sah RL. Depth-dependent confined compression modulus of full-thickness bovine articular cartilage. *Journal of Orthopaedic Research* 1997;15:499–506. [PubMed: 9379258]
- Sherwin AF, Carter DH, Poole CA, Hoyland JA, Ayad S. The distribution of type VI collagen in the developing tissues of the bovine femoral head. *Histochem J* 1999;31:623–632. [PubMed: 10579632]
- Smith GN Jr, Hasty KA, Brandt KD. Type XI collagen is associated with the chondrocyte surface in suspension culture. *Matrix* 1989;9:186–192. [PubMed: 2506410]
- Szafranski JD, Grodzinsky AJ, Burger E, Gaschen V, Hung HH, Hunziker EB. Chondrocyte mechanotransduction: effects of compression on deformation of intracellular organelles and relevance to cellular biosynthesis. *Osteoarthritis and Cartilage* 2004;12:937–946. [PubMed: 15564060]
- Szirmai, JA. The concept of the chondron as a biomechanical unit. In: Hartmann, F., editor. *Biopolymer und Biomechanik von Bindegewebsystemen*. Academic Press; Berlin: 1974. p. 87

- Trickey WR, Baaijens FPT, Laursen TA, Alexopoulos LG, Guilak F. Determination of the Poisson's ratio of the cell: Recovery properties of chondrocytes after release from complete micropipette aspiration. *Journal of Biomechanics* 2006;39:78–87. [PubMed: 16271590]
- Trickey WR, Lee GM, Guilak F. Viscoelastic properties of chondrocytes from normal and osteoarthritic human cartilage. *Journal of Orthopaedic Research* 2000;18:891–898. [PubMed: 11192248]
- Trickey WR, Vail TP, Guilak F. The role of the cytoskeleton in the viscoelastic properties of human articular chondrocytes. *Journal of Orthopaedic Research* 2004;22:131–139. [PubMed: 14656671]
- Upton ML, Guilak F, Laursen TA, Setton LA. Finite element modeling predictions of region-specific cell-matrix mechanics in the meniscus. *Biomechanics and Modeling in Mechanobiology*. 2006in press
- Wall ME, Weinhold PS, Siu T, Brown TD, Banes AJ. Comparison of cellular strain with applied substrate strain in vitro. *Journal of Biomechanics*. 2006in press, Epub
- Wang CC, Chahine NO, Hung CT, Ateshian GA. Optical determination of anisotropic material properties of bovine articular cartilage in compression. *Journal of Biomechanics* 2003;36:339–353. [PubMed: 12594982]
- Wang CC, Guo XE, Sun D, Mow VC, Ateshian GA, Hung CT. The functional environment of chondrocytes within cartilage subjected to compressive loading: a theoretical and experimental approach. *Biorheology* 2002;39:11–25. [PubMed: 12082263]
- Wang CCB, Hung CT, Mow VC. Analysis of the effects of depth-dependent aggregate modulus on articular cartilage stress-relaxation behavior in compression. *Journal of Biomechanics* 2001;34:75–84. [PubMed: 11425083]
- Wong M, Wuethrich P, Buschmann MD, Eggli P, Hunziker E. Chondrocyte biosynthesis correlates with local tissue strain in statically compressed adult articular cartilage. *Journal of Orthopaedic Research* 1997;15:189–196. [PubMed: 9167620]
- Wu JZ, Herzog W, Epstein M. Modelling of location- and time-dependent deformation of chondrocytes during cartilage loading. *Journal of Biomechanics* 1999;32:563–572. [PubMed: 10332619]
- Youn I, Choi JB, Cao L, Setton LA, Guilak F. Zonal variations in the three-dimensional morphology of the chondron measured in situ using confocal microscopy. *Osteoarthritis and Cartilage* 2006;14:889–897. [PubMed: 16626979]

Figure 1.

(left) Fluorescence confocal images of cartilage tissue blocks immunolabeled for type VI collagen at 0%, 10%, 30%, and 50% compression. Significant inhomogeneities in tissue deformation were observed at the periphery of the explant, so all specimens were taken from the center of the tissue block. (Right) Typical shapes of chondrons in each zone under compression. Chondrons exhibited significant changes in height, shape, and volume in each zone, depending upon the applied compressive strain levels.

Figure 2a

Figure 2b

Figure 2.

Changes in chondron height (a) and medial-lateral (ML) width (b) under compression. In each zone, chondrons exhibited significant decreases in height with compression. However, no significant change was found in the ML width under compression except the deep zone chondrons at 50% compression (data is for one-cell chondrons only in deep zone) (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$).

Figure 3.

Changes in PCM volume with compression. PCM volume was significantly decreased with compression in each zone (data is for one-cell chondrons only in deep zone). (* $p < 0.001$, ** $p < 0.0001$)

Figure 4.

Changes in chondrocyte volume under compression. Chondrocyte volume was significantly decreased in all zones except the deep zone (data is for one-cell chondrons only in deep zone). At 50% compression, the chondrocyte boundary was undetectable in four out of eight chondrons in the superficial zone (* $p < 0.0005$, ** $p < 0.001$ uncompressed group vs. 50% compression group in the middle zone).

Figure 5.

The ratio of PCM volume to chondrocyte volume in different zones under compression. In uncompressed specimens, a significant zonal difference in the PCM to cell volume ratio was observed (* $p < 0.05$). No changes in this ratio were observed except at 50% compression, where the superficial zone PCM to cell volume ratio was increased to 25 (**bar is omitted in the figure for clarity). Data is for one-cell chondrons only in the deep zone.

Figure 6.

Changes in the volume of the PCM for deep zone chondrons with one cell or two cells. PCM volume in two-cell chondrons also showed a significant difference under compression, similar as in one-cell chondrons (* $p < 0.0001$, ** $p < 0.05$). The normalized ratio of the PCM volume to the chondrocyte volume showed no significant difference in either one-cell chondron or two-cell chondron under compression.

Figure 7.

The relationship between local ECM strain, and apparent PCM and cellular strain in different zones at increasing magnitudes of tissue compression. The apparent strain at the ECM, PCM, and cell level is displayed as engineering strain [$\epsilon = (h - h_0)/h_0$] and as finite deformation strain [$e = (\lambda^2 - 1)/2$, where $\lambda = h/h_0$, h is the deformed height and h_0 is the original height. Strains are displayed as positive for clarity. In conditions of high local ECM strain ($>25\%$), the apparent cellular strains were lower than the ECM strains. However, in conditions of lower local tissue strain ($<25\%$), apparent cellular strains were increased relative to local ECM strains.

Table 1
Local values of ECM compressive strain measured using texture correlation at surface-to-surface deformations of 10%, 30%, and 50%. Negative values indicate compressive strain. The Poisson's ratio for each zone was determined from the ratio of compressive to lateral engineering strain at 10% applied compression.

Zone	10%		30%		50%		Poisson's Ratio
	Lagrangian Strain	Engineering Strain	Lagrangian Strain	Engineering Strain	Lagrangian Strain	Engineering Strain	
Surface	-0.25±0.02	-0.30±0.032	-0.36±0.02	-0.48±0.04	-0.43±0.02	-0.65±0.05	0.03±0.009
Middle	-0.06±0.02	-0.07±0.02	-0.21±0.03	-0.25±0.04	-0.38±0.04	-0.55±0.08	0.12±0.04
Deep	-0.02±0.01	-0.02±0.01	-0.12±0.02	-0.14±0.03	-0.27±0.04	-0.35±0.06	0.12±0.03