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The deformation behavior and mechanical properties of chondrocytes in articular cartilage

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Summary

Introduction: Chondrocytes in articular cartilage utilize mechanical signals to regulate their metabolic activity. A fundamental step in determining the role of various biophysical factors in this process is to characterize the local mechanical environment of the chondrocyte under physiological loading.

Methods: A combined experimental and theoretical approach was used to quantify the in-situ mechanical environment of the chondrocyte. The mechanical properties of enzymatically-isolated chondrocytes and their pericellular matrix (PCM) were determined using micropipette aspiration. The values were used in a finite element model of the chondron (the chondrocyte and its PCM) within articular cartilage to predict the stress-strain and fluid flow microenvironment of the cell. The theoretical predictions were validated using three-dimensional confocal microscopy of chondrocyte deformation *in situ*.

Results: Chondrocytes were found to behave as a viscoelastic solid material with a Young's modulus of approximately 0.6 kPa. The elastic modulus of the PCM was significantly higher than that of the chondrocyte, but several orders of magnitude lower than that of the extracellular matrix. Theoretical modeling of cell-matrix interactions suggests the mechanical environment of the chondrocyte is highly non-uniform and is dependent on the viscoelastic properties of the PCM. Excellent agreement was observed between the theoretical predictions and the direct measurements of chondrocyte deformation, but only if the model incorporated the PCM.

Conclusions: These findings imply that the PCM plays a functional biomechanical role in articular cartilage, and alterations in PCM properties with aging or disease will significantly affect the biophysical environment of the chondrocyte.

Key words: Cartilage mechanics, Pericellular matrix, Mechanotransduction, Micropipette aspiration, Confocal microscopy, Type VI collagen.

Introduction

The mechanical environment of the chondrocytes is an important factor which influences the health and function of the diarthrodial joint. Chondrocytes in articular cartilage utilize mechanical signals in conjunction with other environmental and genetic factors to regulate their metabolic activity. This capability provides a means by which articular cartilage can alter its structure and composition to meet the physical demands of the body. A number of different approaches have been used to investigate the role of physical stimuli in regulating cartilage and chondrocyte activity, ranging from in-vivo studies to in-vitro experiments at the cellular and molecular level (reviewed in [1–4]). However, the sequence of biomechanical and biochemical events which regulate mechanical signal transduction by the chondrocytes has not been fully dicephered. A more thorough understanding of this regulatory pathway would provide important insight into the normal biological processes responsible for maintaining the cartilage extracellular matrix (ECM), as well as the pathologic processes which lead to degenerative joint diseases such as osteoarthritis.

The chondrocyte perceives its mechanical environment through complex biological and biophysical interactions with the cartilage ECM. This matrix consists of several distinct regions, termed the pericellular, territorial, and interterritorial matrices [5]. The bulk of the tissue is made up of the interterritorial matrix, which consists primarily of water dissolved with small electrolytes (Na^+, Cl^-, Ca^{2+}, etc.). The remaining solid portion is

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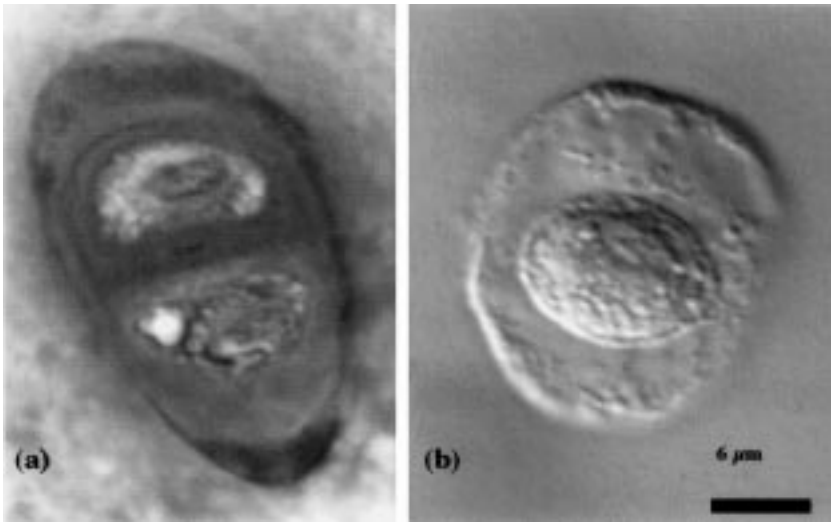

FIG. 1. Light microscopy images of chondrons. (a) Toluidine blue staining of a section of human articular cartilage indicating the presence of a proteoglycan-rich pericellular region. (b) Differential interference contrast microscopy of an enzymatically isolated chondron from human articular cartilage.

composed of collagen (mainly type II) and the proteoglycan aggrecan [6], with smaller molar amounts of other collagens, proteoglycans, proteins and glycoproteins. Fibrillar collagen forms a dense, crosslinked network and provides primarily the tensile and shear properties of the tissue [7–9]. Aggrecan contains keratan sulfate and chondroitin sulfate glycosaminoglycan chains which consist of numerous carboxyl and sulfate groups which become negatively charged when dissolved in the interstitial fluid. The presence of these charges gives rise to repulsive forces and osmotic gradients so that a swelling pressure exists within the tissue [10, 11]. This swelling pressure will influence the hydration state of the tissue, as well as the mechanical response to compressive loading or deformation.

Chondrocytes are surrounded pericellularly by a distinct tissue region which contains large amounts of type VI collagen and a high concentration of proteoglycan. The chondrocyte together with this pericellular region and a surrounding capsule have been termed the 'chondron' (Fig. 1) [12–15]. Some investigators have referred to this region as the 'pericellular' matrix, while others include it in the definition of the 'territorial' matrix. In this paper, for simplicity we refer to the entire region surrounding the chondrocyte as the 'pericellular matrix' (PCM) and to the remaining cartilage tissue as the ECM. The structure, molecular architecture and metabolism of the chondron unit have been studied recently using both mechanical [16] and enzymatic [17] techniques for isolating them from the ECM. The functional significance of this distinct structural unit is as of yet unknown. Because the PCM completely surrounds the chondrocyte, it is likely that any signals that the chondrocyte perceives, either biochemical or biophysical, are influenced by this region. Indeed, there has been considerable speculation that the primary function of the chondron is biomechanical in nature [14, 15, 18].

Because of the structural and compositional characteristics of articular cartilage and the intrinsic coupling between the mechanical and physiochemical properties of the tissue [10], it has been difficult to achieve a complete understanding of the mechanical signal transduction pathways used by chondrocytes. Fundamental to this issue is an understanding of the mechanical environment of the chondrocytes within the articular cartilage ECM. For example, compressive loading of the cartilage ECM exposes the chondrocytes to spatially- and time-varying stress, strain, fluid flow and pressure, osmotic pressure, and electric fields [10, 11, 19]. The relative contribution of each of these factors to the regulation of chondrocyte activity is an important consideration which is being studied by a number of investigators (see review in [1]).

A fundamental step in determining the role of various factors in regulating chondrocyte activity is to characterize the biophysical environment within the tissue under physiological conditions of mechanical loading, in order to be able to reproduce specific aspects of this environment in different model systems. New, non-invasive microscopy

techniques have provided important measurements of the in-situ deformation behavior (i.e., shape and volume changes) of living chondrocytes during compression of the cartilage matrix. However, the signal(s) which the chondrocyte perceives may also involve other biophysical parameters related to the local stress-strain, fluid flow, osmotic environment, or fixed charge density. In this regard, theoretical models of the microenvironment of the chondrocyte are particularly valuable since they can provide quantitative predictions of many biophysical factors of interest which cannot be measured experimentally. These models require information on the mechanical properties and constitutive behavior of the chondrocytes as well as those of the various tissue structures within articular cartilage. Theoretical predictions can be experimentally validated using information on the deformation behavior of the tissue at a cellular level. In this paper, we review a number of studies which have sought to characterize the mechanical environment of the chondrocyte by quantifying chondrocyte properties and deformation behavior and theoretically modeling the cell within its pericellular and extracellular matrices. These studies have combined theoretical methods such as constitutive modeling and finite element analysis, as well as novel biomechanical and microscopic experimental systems. The ultimate goal of these studies has been to elucidate the sequence of biomechanical and biochemical events through which mechanical stress influences chondrocyte activity in both health and disease.

The in-situ deformation behavior of chondrocytes

One of the first steps in understanding the biophysical environment of the chondrocyte during cartilage deformation has been to visualize and quantify the deformation behavior of the chondrocyte *in situ*. The first reported study of this nature was made using light microscopy with Nomarski imaging of a cut cartilage section which was compressed up to 30% using a specially designed loading instrument [20]. This study provided qualitative evidence that chondrocytes in articular cartilage undergo large changes in shape and intercellular spacing as the ECM is deformed. Chondrocytes were found to recover their morphology upon removal of compression and to have a stiffness that was less than or equal to that of the surrounding tissues. Furthermore, collagen fiber orientation was shown to change with compression and recover upon removal of compression.

Quantitative measurements of chondrocyte deformation have been more difficult, and were first reported using a novel system in which Swarm rat chondrosarcoma cells were compressed within a clear agarose gel [21]. The time-dependent response of the cells was quantified in two dimensions with video microscopy, and showed that chondrocytes exhibited significant changes in shape and cross-sectional area with compression. These changes in cross-sectional area were assumed to indicate a mechanically-induced change in cell volume. The quantitative measurements were combined with a finite element analysis (described below) to calculate the mechanical properties of the individual cells.

More recently, techniques have been developed to accurately quantify the morphology of viable chondrocytes within their natural ECM using confocal scanning laser microscopy [22]. The confocal microscope allows three-dimensional imaging of fluorescently-labeled cells [23] or subcellular structures [24] within a tissue explant. In these experiments, volumetric images of chondrocytes were recorded in three different zones of an osteochondral explant prior to compression and at equilibrium following application of a 15% surface-to-surface compressive displacement (Fig. 2). Custom-written geometric modeling algorithms [22] were used to quantify changes in height, width, volume, surface area, and shape factor in individual cells. Significant decreases in cell height of 26%, 19%, and 20% and decreases in cell volume of 22%, 16%, and 17% of control values were observed in the surface, middle and deep zones, respectively. These findings indicate that chondrocyte shape and volume are integrally linked to ECM deformation, supporting the hypothesis that chondrocytes undergo significant deformation during in-vivo loading of the joint.

The process by which changes in the shape or volume of the chondrocyte are converted to an intracellular signal may involve different membrane and cytoskeletal pathways. One hypothesis suggests that a cytoskeletal link between the ECM and the cell nucleus may serve as a direct physical connection by which mechanical signals could be converted to an intracellular message [25]. This hypothesis is supported indirectly by a study in which three-dimensional confocal microscopy and fluorescent labeling of nucleic acids were used to quantify the deformation behavior of the chondrocyte nucleus with compression of the cartilage matrix [24]. Unconfined compression of osteochondral explants to a 15% surface-to-surface strain resulted in a significant decrease of chondrocyte height and volume by 14% and 11%,

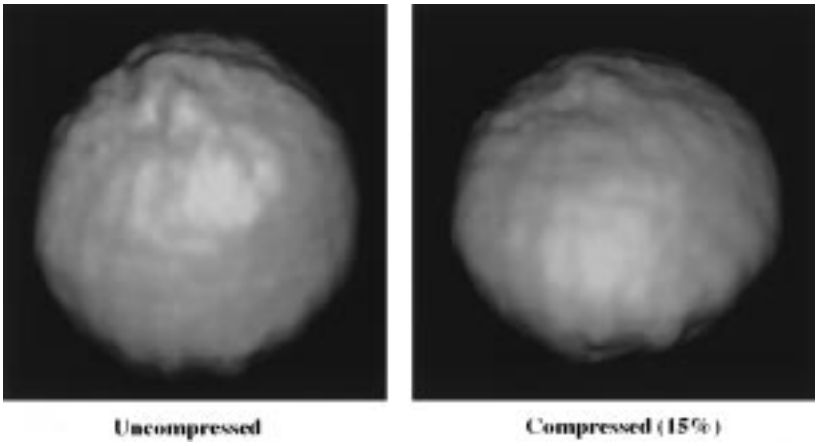

FIG. 2. Chondrocyte deformation measured *in situ* using 3D confocal microscopy. Confocal reconstructions of surface zone chondrocytes from canine patellofemoral groove cartilage prior to compression (left) and at equilibrium following a 15% surface-to-surface displacement of the tissue (right). Chondrocytes were imaged with a confocal scanning laser microscope by introducing a dextran-bound fluorescein dye into the media. The dye was excluded from intact cells, forming a negative, high-contrast fluorescent image of the cell-matrix boundary. Geometric reconstruction and quantitative morphometry were performed using previously developed techniques. Significant decreases in chondrocyte height and volume were observed with compression of the ECM (compression was applied vertically as shown in the figure). All changes were found to recover upon removal of compression.

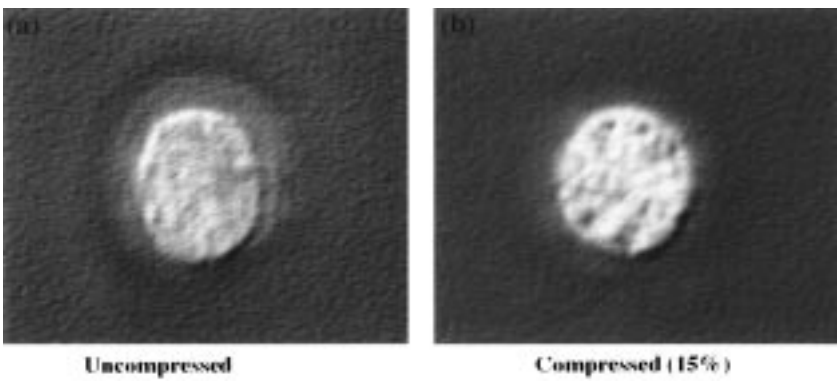

FIG. 3. Digitally enhanced confocal image of a chondrocyte nucleus prior to (left) and after (right) compression of the ECM. Chondrocyte nuclei were labeled *in situ* with acridine orange, a fluorescent dye which binds to nucleic acids. Chondrocyte nuclei were observed to deform in a coordinated manner with the chondrocyte and ECM during compression. Pre-treatment of the tissue with cytochalasin D altered the relationship between matrix deformation and nuclear height and shape, but not volume. Reproduced with permission from [1].

respectively, and of nuclear height and volume by approximately 9% and 10%, respectively (Fig. 3). Disruption of the actin cytoskeleton using cytochalasin D altered the relationship between matrix deformation and the decrease in nuclear height and shape, but did not affect the corresponding volume changes. The overall morphology and deformation behavior of the chondrocytes were not affected by cytochalasin treatment. These results suggest that the actin cytoskeleton plays an important role in the link between compression of the ECM and deformation of the chondrocyte nuclei, and imply that chondrocytes and their nuclei undergo significant changes in shape and volume *in vivo*.

The findings of these confocal microscopy experiments are supported by studies which utilized stereological techniques to quantify chondrocyte and nucleus morphology in cartilage which was been compressed and then histologically fixed. Reductions in cell and nucleus volume and radii in the direction of compression were measured which were in approximate proportion to the reduction in cartilage thickness [26, 27]. Compression also resulted in an increase in the mean cell density within the tissue. Cell and nucleus dimensions perpendicular to the direction of compression did not change significantly.

The presence of newly formed tissue constituents seems to have a significant effect on the

deformation behavior of chondrocytes in an artificial matrix [28]. Chondrocytes seeded in a 3% agarose gel were shown to deform in a coordinated manner with the gel. After 6 days in culture in the presence of 20% bovine serum, chondrocytes deformed significantly less and exhibited a viscoelastic recovery behavior upon removal of compression.

Chondrocyte deformation and the regulation of cartilage metabolism

There is significant evidence that chondrocytes perceive and respond to changes in their shape and volume as signals for regulating their metabolic activity and gene expression. It has long been known that chondrocyte shape has a strong influence on phenotypic expression, as evidenced by the tendency of chondrocytes to express a more fibroblastic phenotype in monolayer culture [29] as compared to three-dimensional culture systems [30, 31]. On this basis, it has been hypothesized that a change in cell shape (e.g., deformation) serves as a regulator of chondrocyte metabolism. Most studies investigating the effects of cellular deformation have used isolated chondrocytes in monolayer culture, as it is difficult to eliminate the effects of other physical factors *in situ*.

For example, several studies have examined the response of chondrocytes to tensile stretch using two-dimensional cell culture systems [32–34]. In one model of cyclic tensile stretch, the collagen matrix generated by epiphyseal chondrocytes in high density culture was stretched at strains of 5.5% at 0.2 Hz, and significant increases were observed in aggrecan synthesis rates following 24 hrs of applied strains [32]. In other studies, tensile strains of 10% applied to an elastin membrane at 1 Hz resulted in a 2–3 fold increase in the rates of aggrecan synthesis in chick sternal chondrocytes [33]. Agitation of the substrate had similar effects on cellular activity as stretch, implying that fluid motion may have contributed to the observed effects [33]. Other studies have also observed a significant increase in aggrecan synthesis by rat costal chondrocytes as induced by tensile deformation of an underlying substrate, although the magnitude of applied strain was not reported [34]. It is important to note, however, that even if the strain in the substrate is precisely characterized in studies such as these, the relationship between substrate strain and cellular strain may be complex.

Compression of the cartilage matrix also induces a decrease in cell and nuclear volume [24, 26]. The mechanism for chondrocyte volumetric decrease may be related to mechanical and osmotic effects associated with matrix compression [35], or may be an active cellular response to loading [21]. Such changes in volume exert a strong effect on chondrocyte activity, both in isolated cells and explant culture [36]. Volumetric changes in mammalian cells have been associated with mechanical transduction and signaling through the transport of ions and organic compounds (e.g., taurine, glutamate, creatine) [37]. Cell volume increases significantly by 30–40% when chondrocytes are removed from the ECM [36]. Furthermore, chondrocytes *in situ* will shrink or swell in proportion to changes in the ionic composition of the ECM and exhibit active volume recovery mechanisms in response to osmotic shock [35]. Uncoupling direct cellular deformation from ion-specific osmotic activity changes may be difficult in these studies, since the physicochemical environment may also be altered by the factors used to change osmotic pressure. Alternatively, changes in cell volume may initiate signaling through the accompanying deformation of the cell membrane and cytoplasm.

In an attempt to isolate the effects of cellular deformation from other factors associated with matrix deformation, techniques have been utilized to examine intracellular ionic changes during physical micro-manipulation of single cells [38]. Direct perturbation of the chondrocyte membrane with a glass micropipette has been shown to cause a rapid increase in the concentration of cytosolic calcium ion, an intracellular second messenger. Stretch-activated ion channel blockers such as gadolinium or amiloride significantly attenuated the peak increase of calcium concentration in deformed cells, suggesting that this calcium signaling was regulated through stretch of the chondrocyte membrane. These findings provide support for the hypothesis that chondrocytes have the ability to transduce isolated deformations in the absence of other matrix-related effects.

Furthermore, evidence for the role of chondrocyte deformation was provided in experiments which used glutaraldehyde fixation and quantitative autoradiography of compressed and radiolabeled cartilage disks to correlate changes in cell and nuclear morphology with the spatial localization of newly synthesized aggrecan [26]. Compression of cartilage explants resulted in reductions in cell and nucleus volume and height, as well as a coincident reduction in aggrecan synthesis, which were in approximate proportion to the reduction in tissue thickness. In other studies using this technique, a significant correlation was observed between the magnitude of local tissue strain and

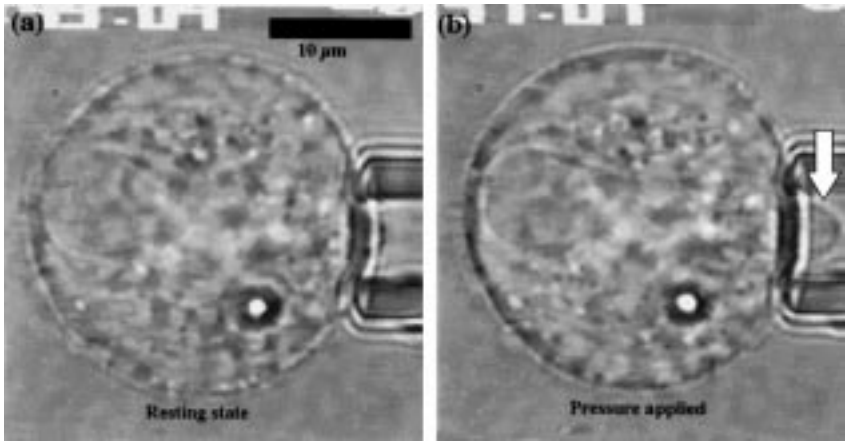

FIG. 4. Video microscopy images of the micropipette aspiration test prior to (a) and following (b) application of pressure. A series of prescribed step pressures were applied to an isolated chondrocyte using a glass micropipette (~5 μm diameter) and the ensuing deformation of the cell into the pipette was quantified (arrow). A linear regression of the equilibrium deformation and the applied pressure was used to calculate the elastic Young's modulus, and an exponential regression of the creep behavior was used to calculate the viscoelastic time constant of the cell.

the decrease in proteoglycan synthesis rates associated with static compression of cartilage [27]. These studies provide further scientific support, though indirect, for the hypothesis that shape and volume changes of the chondrocyte play a role in mechanical signaling.

Quantification of the mechanical properties of chondrocytes

To fully understand the local stress–strain and fluid flow environment of individual cells, it is necessary to develop theoretical models which incorporate chondrocytes within the ECM, since many of the biophysical parameters which may be influencing chondrocyte behavior cannot be measured non-invasively. For such models to provide useful and accurate information, however, it is necessary to know the mechanical properties of the ECM and PCM, the mechanical properties of the chondrocytes, and the geometry of the chondrocytes.

One of the first measurements of the mechanical properties of a chondrocytic cell line was performed by measuring the deformation of chondrosarcoma cells compressed in a 2% agarose matrix [21]. Strains of 5, 10, and 15% were applied to the cell-agarose composite, and video images of the cell were recorded from initial loading to equilibrium. The experimentally-measured deformation behavior of the cells was matched to that predicted by a standard linear viscoelastic finite element model of the experimental configuration. The experimental and theoretical models showed the best agreement when the elastic modulus of the chondrocyte was assigned to be that of the surrounding agarose gel (4.0 kPa) with a Poisson's ratio of 0.4. Viscoelastic analysis showed that the chondrocytes contributed a significant viscoelastic component to the behavior of the composite, in comparison with the agarose gel alone.

Direct measurements of the mechanical properties of primary chondrocytes from normal and osteoarthritic cartilage and human chondrosarcoma cells have been performed recently using the micro-mechanical testing method of micropipette aspiration [39–41]. With this technique, small aspiration pressures are applied to a single isolated cell, and the ensuing transient deformation behavior of the cell into the micropipette is quantified using video microscopy [42, 43]. In these experiments, controlled pressures were applied to single chondrocytes using a glass micropipette (radius $a = 2.75$ μm) in increments of 10 Pa, and the deformation at equilibrium was measured at each pressure step (Fig. 4). The equilibrium Young's modulus of the chondrocyte (E) was calculated from a linear regression of deformation (L) vs. pressure (ΔP) using the formula $E = 3a\Delta P\Phi/2\pi L$, where Φ is a parameter based on the wall thickness of the micropipette. Chondrocytes were found to behave as a viscoelastic solid with a mean equilibrium modulus of 0.6 kPa, which is approximately 3 orders of magnitude lower than that of the cartilage ECM. The relaxation time constant (t_σ) ranged from 33 s for osteoarthritic cells to 65 s for non-osteoarthritic, suggesting that viscoelastic effects may play a role in defining the mechanical response of chondrocytes under stress. Furthermore, osteoarthritic chondrocytes exhibited an

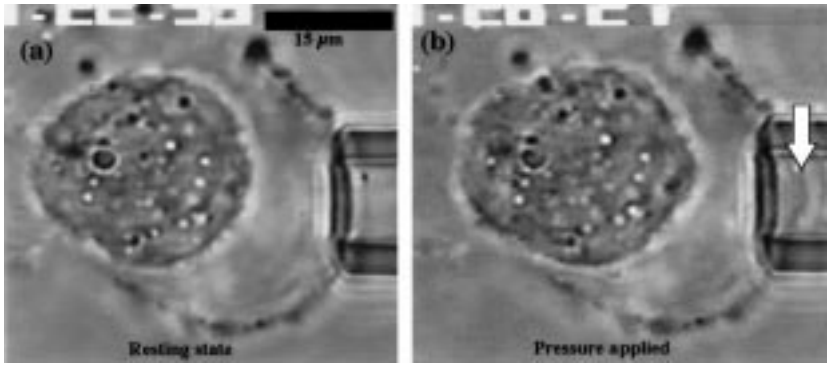

FIG. 5. Micropipette aspiration to determine the mechanical properties of the PCM prior to (a) and following (b) application of pressure. Chondrons were isolated from the ECM using an enzymatic digestion protocol. A series of step pressures were applied to the PCM and the resulting deformation was quantified with video microscopy (arrow). A linear regression of the equilibrium deformation and applied pressure was used to calculate the Young's modulus of the PCM.

instantaneous modulus which approximately was twice that of non-osteoarthritic cells. These findings are in general agreement with the studies of Freeman and co-workers on chondrosarcoma cells in agarose [21], although the elastic modulus of primary human chondrocytes was lower than that of the Swarm rat chondrosarcoma cells.

In a separate set of experiments, the volume of chondrocytes was measured before and after they were drawn completely into a micropipette [40, 41]. A significant decrease in cell volume was observed after complete aspiration of the cell into the pipette, which corresponded to a *decrease in cell diameter* by an average of 32%. Normal cells exhibited an 11% decrease in volume, while osteoarthritic cells exhibited a significantly greater volume decrease of 20%. These findings suggest that chondrocytes from osteoarthritic cartilage may have altered volume regulation capabilities and further emphasise the need to separate the effects of different physical factors such as osmotic and mechanical stress on chondrocyte response.

Micromechanical properties of the pericellular matrix

To accurately model the physical signals to which the chondrocyte is exposed during loading, it is necessary to incorporate as much detail of the micromechanical structure of the tissue and cells as possible. The chondrocyte is surrounded by a thin region of tissue termed the PCM, which together with the chondrocyte and an enclosing capsule is termed the chondron. Chondrons have not received much attention because the previous method of isolation used a multistep mechanical homogenization procedure which gave a low yield (1–2%) of viable chondrons [14]. More recently, Lee and co-workers have developed a simple enzymatic procedure for isolating chondrons, which provides viable yields of 80% [17, 44]. Chondrons have been shown to be rich in proteoglycans and collagen types II, VI, and IX and are defined primarily by the presence of type VI collagen [13]. In osteoarthritis, the area of the chondron is greatly enlarged and the incidence of chondrocyte proliferation is increased [16, 45].

Chondrons were isolated from non-osteoarthritic and osteoarthritic human hips and knees using an enzymatic digestion protocol [17]. Using the micropipette aspiration test described above, a series of step pressures were applied to the PCM of isolated chondrons in increments of 100 Pa, and deformation measurements were made at equilibrium at each pressure step (Fig. 5). The Young's modulus of the PCM was calculated from a linear regression of deformation vs. pressure [42]. The mean modulus of the PCM from non-osteoarthritic cartilage (1.54 kPa) was approximately 40% higher than that of osteoarthritic cartilage (1.09 kPa) [40]. This trend was not statistically significant in preliminary studies, possibly due to the variability caused by pooling specimens from different joints and from the simplified geometric assumption of the analysis. The modulus of the PCM of enzymatically-isolated chondrons was found to be significantly greater than that of the chondrocytes, but still 2–3 orders of magnitude lower than that of the cartilage ECM [46]. It is important to note that a limitation of these studies is that these properties may reflect changes in the PCM properties incurred during the enzymatic isolation.

Table I
Equilibrium elastic moduli of the structural components of cartilage

Structure	Modulus (Non-OA)	Modulus (OA)	Reference
Extracellular matrix (tension)	5–20 MPa	1–5 MPa	[7, 8, 57]
Extracellular matrix (compression)	0.5–1.0 MPa	0.25–0.5 MPa	[46, 51]
Pericellular matrix	1.5 kPa	1.1 kPa	[40, 58]
Human chondrocyte	0.6 kPa	0.6 kPa	[40, 41]
Chondrosarcoma (Swarm rat)	4.0 kPa	—	[21]
Chondrosarcoma (105KC)	0.19 kPa	—	[39, 40]

Theoretical modeling of chondrocyte-matrix interactions

To determine the local stress–strain and fluid-flow environment of the chondrocytes within cartilage, theoretical models have been developed of the chondrocyte and its surrounding PCM (i.e., the chondron) within articular cartilage [47–50]. Because of the complex geometry and constitutive behavior involved in this problem, numerical methods such as finite element analysis have generally been used to solve the theoretical models of cell–matrix interactions. These models can provide quantitative predictions of time-varying mechanical fields in the vicinity of the chondrocyte under dynamic loading of the cartilage matrix. The input parameters required for such models are the geometry and intrinsic mechanical properties of the chondrocyte, the PCM, and the ECM, as well as the time history of the traction and displacement boundary conditions which are applied to the cartilage matrix. While some of these input parameters can be prescribed for a specific experimental configuration, other parameters, such as the properties of the chondrocyte and PCM, must be measured experimentally. Additionally, the experimentally-measured deformation behavior of chondrocytes within cartilage can be used as a validation of the theoretical modeling.

With knowledge of the mechanical properties and geometry of the chondrocyte and its PCM (Table 1), theoretical models have been able to provide important information on the local mechanical environment of the chondrocyte. One such model [49] has utilized the linear biphasic theory for articular cartilage [51] in a penalty method finite element formulation [52] to quantify the transient stress-strain and fluid flow environments of the chondrocyte during compression. This model represented the various components of cartilage (chondrocytes and the pericellular and extracellular matrices) as biphasic media whose viscoelastic behavior is governed predominantly by interactions between solid and fluid phases [51]. An important consideration in this model is the large difference in the geometric scales between the level of the tissue explant and the level of the individual cell (i.e., two orders of magnitude). By dividing the analysis into two separate problems, a multiple scaling algorithm was used to calculate the stress-strain environment in the vicinity of the cell. A coarse finite element mesh of the macroscopic scale problem (i.e., a cartilage explant under compression) was solved using standard methods [53]. The predicted fluid velocities and solid displacements within a defined region of the tissue were used as the kinematic boundary conditions for a separate microscopic scale mesh which incorporated the geometry and distinct mechanical properties of a chondrocyte (or group of chondrocytes) with a PCM. In this manner, the local mechanical environment in the vicinity of the chondrocyte could be calculated, assuming that the chondrocytes do not appreciably contribute to the overall mechanical properties of the cartilage tissue.

This technique was first used to examine the effects of the relative mechanical properties of the chondrocyte and ECM, as well as those of cell shape and intercellular spacing, on the mechanical environment of the chondrocyte [49]. Results of this parametric study indicate that the mechanical environment of the chondrocyte is time-varying and spatially non-uniform (Fig. 6). Furthermore, the stress–strain environment of the chondrocytes are dependent on cell shape and the relative properties of the chondrocyte and the ECM. This model predicts that under physiologic magnitudes of matrix strain [54], the peak strains at the chondrocyte-matrix interface may be 50–100% higher than the nominal strains in the tissue, due to the difference in mechanical stiffness between the chondrocyte and the matrix. This finding has important implications in regard to studies which seek to duplicate the physiologic strain environment of the chondrocyte in an in-vitro cell culture experiment [32–34, 38].

More recently, an analytical solution to the biphasic inclusion problem has been developed

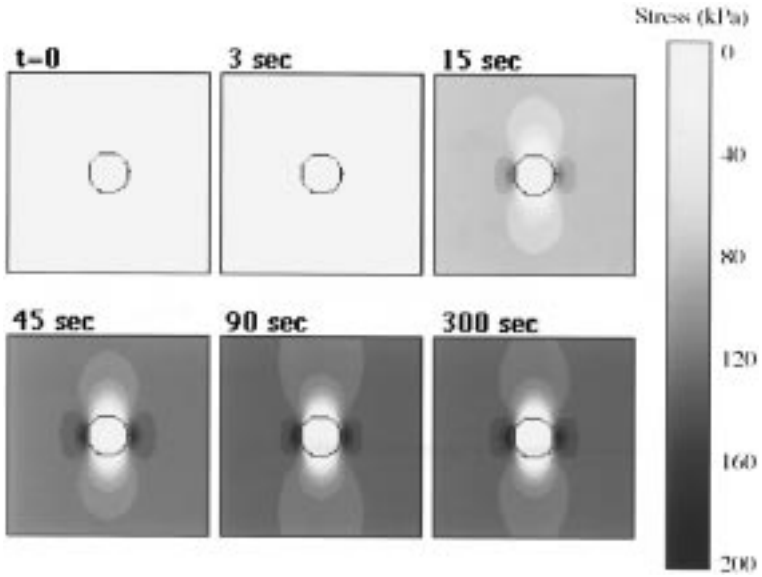

FIG. 6. Predictions of solid stress in the microenvironment of the chondrocyte. A multi-scale finite element model was developed to predict the mechanical environment of the chondrocyte during creep compression of a cartilage explant, where the aggregate modulus of the chondrocyte equals 0.7 kPa and that of the ECM equals 0.7 MPa. Early time points are characterized by predominantly hydrostatic loading in the ECM and a uniform stress environment around the chondrocyte. At equilibrium, the solid matrix is entirely responsible for load carriage, and stress concentration are present at the cell-matrix interface due to the mismatch in the elastic properties of the chondrocyte and surrounding tissue. These findings emphasize the complex and nonuniform biophysical environment that the chondrocyte is exposed to during compressive loading of the cartilage ECM.

for the configuration of an articular cartilage explant in confined compression [47]. The results of this study confirm and extend those of the finite element analysis [49], indicating that the stress–strain and fluid-flow environments of the chondrocyte are non-uniform and dependent on the relative properties of the chondrocyte and matrix. Of particular interest was the observation that changes in proteoglycan synthesis rates in response to compression paralleled the time-dependent changes in the mechanical environment of the chondrocytes. This finding suggests that, as time progresses, the chondrocytes are responding to different mechanical stimuli within the ECM (e.g., hydrostatic pressure, matrix deformation) [47].

The finite element formulation of this theoretical model has also been used to examine the role of the PCM on the mechanical environment of the chondrocyte [49, 55]. Chondrocytes were modeled with a surrounding PCM of varying size and properties, as measured experimentally by confocal microscopy and micropipette techniques. These studies suggest that the viscoelastic properties of the PCM can have a significant effect on the mechanical environment of the chondrocyte. Of particular interest is the finding that the mechanical environment of the chondrocyte will be significantly altered in osteoarthritis due to the altered structure (increased size) of the PCM and the decreased elastic modulus of the extracellular and pericellular matrices. Preliminary studies which combine experimental measurements and theoretical models predict that with osteoarthritis, the chondrocyte will undergo strains which are 50–75% lower in magnitude as compared to normal cartilage subjected to the same deformation. These findings indicate that the mechanical signals which the chondrocyte perceives are significantly altered in osteoarthritis, and provide a working hypothesis for examining potential initiating and progressive factors in degenerative joint disease.

To assess how the mechanical state of the ECM interacts with the chondrocyte, the components of surface traction at the cell membrane have been calculated from the stress tensor acting within the tissue and the unit outward and tangent normals of the cell–matrix boundary [50, 56]. This analysis showed that the magnitude of surface traction induced by fluid pressure in the tissue varies significantly with time and with site within the explant, but may be relatively uniform across the boundary of the cell. In contrast, surface tractions induced by the interaction of the cell with the solid extracellular (and pericellular) matrix were found to vary significantly across the cell boundary. Of particular interest is the finding that the component of normal stress may vary from tensile to

compressive across the cell boundary, emphasizing the complexity of interpreting data on the response of chondrocytes to compressive loading of the ECM.

Comparison of these theoretical models with experimental measurements of chondrocyte deformation show good agreement, but only if the theoretical model includes the geometry and appropriate mechanical properties of the PCM [55]. The discrepancy between the experimental data and the theoretical predictions which neglect the presence of a PCM suggests that the PCM plays an important role in determining the mechanical stress environment and deformation behavior of the chondrocyte. This finding further supports the concept of a functional mechanical role for the chondron. For example, it has been hypothesized that the chondron provides a 'protective' effect for the chondrocytes during loading through an 'adaptive water loss from pericellular matrix proteoglycans' [14]. Others have suggested that the chondron serves as a mechanical transducer which influences the biomechanics of chondrocyte-matrix interactions [18, 55]. Future studies which combine theoretical modeling of the chondron in the ECM with direct measurements of the morphologic and metabolic response of chondrocytes to loading may provide the information necessary to validate specific hypotheses on PCM function.

Conclusions

In summary, these studies have provided new information on the fundamental biomechanical interactions between chondrocytes, the PCM and the ECM, and therefore have opened a number of new avenues for future studies. From these studies, it is apparent that chondrocytes will undergo significant changes in shape and volume under normal physiological conditions, and that non-uniform and time-varying stress, strain, fluid flow and pressure fields act on the chondrocytes *in situ*. Presumably, these deformations are involved in the regulation of chondrocyte metabolism in response to mechanical loading of the joint. Through finite element studies which incorporate experimentally-measured chondrocyte mechanical properties and geometry, it is apparent that the mechanical environment of the chondrocyte is strongly influenced by the structure and properties of the chondrocytes, the PCM, and the ECM. Thus the changes in pericellular and ECM properties which may occur in osteoarthritis suggest that the mechanical environment of the chondrocytes is significantly altered with disease. Furthermore, these theoretical models suggest that the PCM may serve an important biomechanical role in articular cartilage. Together, these studies provide the framework for a number of further studies on the role of mechanical factors in regulating the health and maintenance of articular cartilage.

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