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Zonal Uniformity in Mechanical Properties of the Chondrocyte Pericellular Matrix: Micropipette Aspiration of Canine Chondrons Isolated by Cartilage Homogenization

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Abstract—The pericellular matrix (PCM) is a region of tissue that surrounds chondrocytes in articular cartilage and together with the enclosed cells is termed the chondron. Previous studies suggest that the mechanical properties of the PCM, relative to those of the chondrocyte and the extracellular matrix (ECM), may significantly influence the stress–strain, physicochemical, and fluid-flow environments of the cell. The aim of this study was to measure the biomechanical properties of the PCM of mechanically isolated chondrons and to test the hypothesis that the Young's modulus of the PCM varies with zone of origin in articular cartilage (surface vs. middle/deep). Chondrons were extracted from articular cartilage of the canine knee using mechanical homogenization, and the elastic properties of the PCM were determined using micropipette aspiration in combination with theoretical models of the chondron as an elastic incompressible half-space, an elastic compressible bilayer, or an elastic compressible shell. The Young's modulus of the PCM was significantly higher than that reported for isolated chondrocytes but over an order of magnitude lower than that of the cartilage ECM. No significant differences were observed in the Young's modulus of the PCM between surface zone (24.0 ± 8.9 kPa) and middle/deep zone cartilage (23.2 ± 7.1 kPa). In combination with previous theoretical biomechanical models of the chondron, these findings suggest that the PCM significantly influences the mechanical environment of the chondrocyte in articular cartilage and therefore may play a role in modulating cellular responses to micromechanical factors.

Keywords—Cartilage, Cell, Mechanics, Mechanical properties, Osteoarthritis, Pericellular, Collagen, Micropipette aspiration, Biomechanics, Chondron, Collagen type VI.

INTRODUCTION

The mechanical environment of the chondrocyte is one of several important environmental factors that influence the normal balance between synthesis and degradation of the articular cartilage extracellular matrix (ECM).[24] Previous studies have shown that compression of cartilage explants alters the cellular metabolism in a time and spatially varying manner that is correlated with specific parameters in the local mechanical environment of the chondrocytes.[9,16,20,29,42,49,52] These findings suggest that local biophysical factors in the microenvironment of each cell are responsible for governing chondrocyte response to matrix compression. Therefore, detailed information on the stresses and strains that the individual cell is exposed to during cartilage compression would improve our understanding of the sequence of events involved in the transduction of mechanical signals at the cellular level.[13,17,36,50,53]

Within the ECM, chondrocytes are surrounded by a narrow region termed the pericellular matrix (PCM) that together with the chondrocyte is referred to as the chondron.[8,40,44] This region is primarily characterized by the presence of type VI collagen and increased proteoglycan concentration relative to the ECM.[11,39,41] The functional role of this complex structural unit is not known, although the fact that the PCM completely surrounds the cell suggests that it influences the biochemical and biomechanical interactions between the ECM and the chondrocyte. For this reason, there has been considerable speculation that the PCM plays a role in regulating the biomechanical environment of the chondrocyte.[12,15,37,38,40,44]

To explore such hypotheses, previous studies have utilized analytical and finite element models to predict the mechanical environment of the chondrocytes within the ECM and to determine the potential influence of the PCM. The results of these studies have shown that the mechanical environment of the chondrocytes is highly dependent on the relative biomechanical properties of the ECM, PCM, cells,[2,17] the cell volume fraction,[53] and cell shape.[6,7,18] In other studies, analytical models of a chondron subjected to

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cyclic loading suggest that the transmission of mechanical signals to the cell is particularly sensitive to the modulus and permeability of the PCM.[21]

Chondron and chondrocyte morphology may vary substantially from an elongated and flattened shape at the articular surface to a more rounded morphology and columnar arrangement in the deep zone of cartilage.[19,40,45] In addition, there is an abundance of data demonstrating substantial variations in the structure, composition, and mechanical properties of the cartilage ECM with depth.[5,10,33,43,51] Recently, a novel microaspiration technique was developed to isolate viable chondrons that were mechanically tested using micropipette aspiration.[1] The results of this study show that the PCM of human chondrocytes has an elastic Young's modulus of ~70 kPa, which is significantly decreased by ~40% in osteoarthritic cartilage. Using a biphasic model, the PCM was found to have a Young's modulus of 40 kPa, a hydraulic permeability of 4.2×10^{-17} m^4/Ns, and a Poisson's ratio of 0.04.[3] In other studies, chondrons isolated by mechanical homogenization were found not to deform measurably when compressed in an agarose gel with a stiffness of 25 kPa, suggesting that the modulus of the PCM is greater than this value.[31] Despite the widespread use of homogenization techniques for extracting chondrons, the mechanical properties of chondrons isolated by this technique have not been measured directly, particularly with respect to their zone of origin in the tissue.

The aim of this study was to test the hypothesis that the mechanical properties of chondrons isolated from the superficial zone of cartilage differ from those isolated from the middle and deep zones. Chondrons were isolated from the articular cartilage of the femoral condyles of canine knees using an established homogenization technique.[40] To determine the Young's modulus of the PCM, data from testing the chondrons in a micropipette aspiration experiment were evaluated with three different analytical elastic models of the chondron as an incompressible, elastic half-space,[46] as a layered, compressible, elastic half-space,[1] and as a compressible, elastic layer corresponding to the assumption of negligible mechanical stiffness of the chondrocyte (i.e., shell model).[1] Mechanical properties of the PCM were compared using the three analytical models, and differences in PCM properties were evaluated between chondrons isolated from the surface or middle/deep zones of the cartilage.

METHODS

Knee joints ($N = 16$) were harvested from skeletally mature beagle dogs immediately following sacrifice. These tissues were acquired as discarded waste from an unrelated study approved by the Duke University Institutional Animal Care and Use Committee, and no animals were sacrificed for the present study. All joints appeared normal macroscopically and showed no fibrillation, discoloration, or other signs of osteoarthritis or other joint disease. The articular cartilage was removed from the femoral condyles of the joints using a razor cutting device that removes 150–200 μm of tissue per cut. Chondrons were mechanically isolated from the surface (top 200 μm) or middle/deep zones (pooled remaining thickness) of the articular cartilage using a sequential homogenization technique adapted from a protocol developed previously.[40] Briefly, 1 g of cartilage was placed in 1 ml Dulbecco's phosphate-buffered saline (PBS; Gibco, Grand Island, NY) and homogenized for 5 min at 3500 rpm (PRO Scientific Inc., Monroe, CT). The supernatant was removed and passed through a 70 μm filter to remove large debris, and the process was repeated. The filtrate was centrifuged at 50 g for 15 min to form a pellet.

The mechanical properties of the PCM were measured on individual chondrons using the micropipette aspiration technique.[1,26,46] Isolated chondrons were suspended in 1 ml of PBS and placed in a specially designed chamber on an inverted optical microscope. The flattened tip of a small glass micropipette was brought in contact with a chondron and a series of five to eight steps in pressure (1 kPa each) were applied to the PCM using a small glass pipette (~6 μm diameter) (Fig. 1). The length of PCM aspiration vs. time was determined from optical imaging of the experiment recorded for 5 min at each step, which represented an equilibrium deformation.

To determine the Young's modulus of the PCM, the experimental length–pressure data were analyzed using three different theoretical models of the micropipette aspiration technique (Fig. 2). First, the chondron was represented as an isotropic, incompressible, elastic half-space.[15,27,46] The

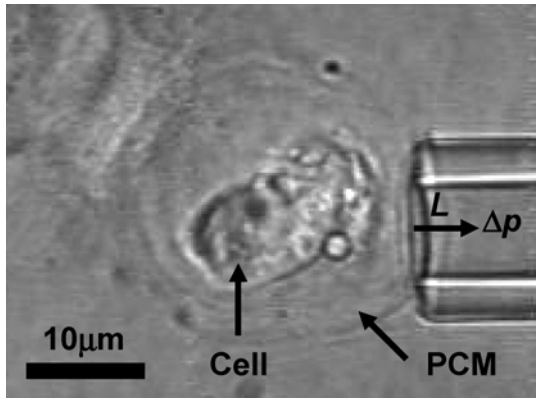

FIGURE 1. Micropipette aspiration of an isolated chondron. Isolated chondrons were suspended in a specially designed chamber on an inverted optical microscope. The flattened tip of a small glass micropipette was brought in contact with a chondron, and a series of five to eight steps in pressure (1 kPa each) were applied to the PCM using a small glass pipette (~6 μm diameter) and allowed to equilibrate, and the length of PCM aspiration vs. applied pressure was used in combination with different theoretical analyses to determine the Young's modulus of the PCM.

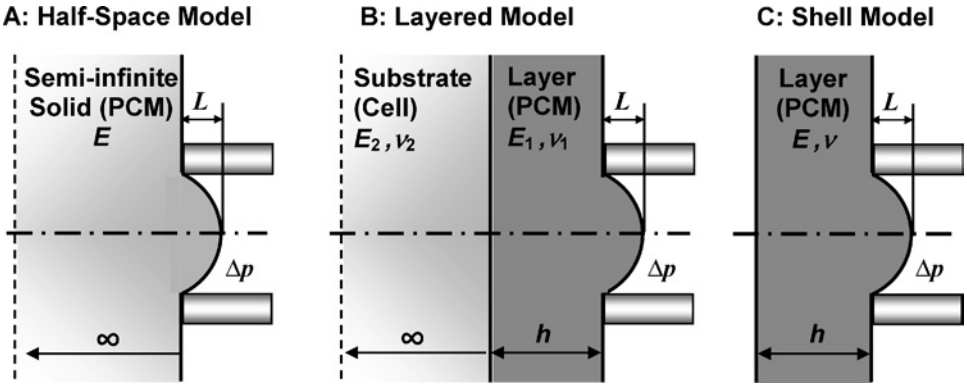

FIGURE 2. Mathematical models used for the quantification of the mechanical properties of the pericellular matrix. In model A, the PCM was represented as an isotropic, incompressible, and elastic half-space. In model B, the PCM was represented by an isotropic, compressible, and elastic layer of thickness *h* which overlies an elastic and compressible half-space (i.e., the chondrocyte). Model C is a subcategory of model B where $E_1/E_2 \rightarrow \infty$. This model is based on the assumption of zero stiffness for the chondrocyte, resulting in a model of the PCM as a shell of thickness *h*.

Young's modulus of the PCM (E_{PCM}) was determined using the slope of the equilibrium length of PCM projection (L), normalized to the inner micropipette radius (r_i), plotted against the applied aspiration pressure (Δp), and calculated using linear regression.

$$E_{PCM} = \Phi \frac{3 r_i \Delta p}{2\pi L}, \tag{1}$$

where Φ is a "wall parameter" that depends on the inner and outer radii of the micropipette and was set to $\Phi = 2.1$ for the range of micropipette sizes in this study.[46]

Second, the chondron was represented by an isotropic, compressible, elastic layer (the PCM) overlying an elastic and compressible half-space representing the chondrocyte.[1] The primary advantage of this layered model is that it accounts for the thickness of the PCM and the different mechanical properties in two distinct regions of the chondron. The Young's modulus was again determined from the slope of the applied negative pressure (Δp) vs. the normalized equilibrium length (L/r_i) of PCM projection using the following equation[1]:

$$E_{PCM} = 2C(1 + \nu_{PCM}) \frac{\Delta p}{L/r_i}, \tag{2}$$

where ν_{PCM} is the Poisson's ratio of the PCM and C a function of the following four dimensionless constants that depend on the material and geometric parameters: the Poisson's ratio of the cell (ν_{cell}), the normalized PCM thickness measured optically ($h^*_{PCM} = h_{PCM}/r_i$), the Poisson's ratio of the PCM (ν_{PCM}), and the Young's modulus ratio (E_{PCM}/E_{cell}). The constant C was determined for assumed values of E_{cell} of 0.36 kPa, ν_{cell} of 0.43,[14,48] and ν_{PCM} of 0.04,[3] based on prior findings of cell and PCM mechanics. From knowledge of the measured PCM thickness, h_{PCM}, the constant C depends only on the Young's modulus ratio E_{PCM}/E_{cell} that was determined implicitly from Eq. (2) to calculate E_{PCM}.

Third, the chondron was modeled as described above for the compressible, layered half-space but under the assumption of zero stiffness for the chondrocyte (i.e., when $E_{PCM}/E_{cell} \rightarrow \infty$), resulting in a model of the chondron as a shell of thickness h_{PCM}.[1] The Young's modulus of the PCM (E_{PCM}) is given by the equation

$$E_{PCM} = 2C(h^*_{PCM})(1 - \nu_{PCM})(1 + \nu_{PCM}) \frac{\Delta p}{L/r_i}, \tag{3}$$

where $C(h^*_{PCM})$ is a function of the normalized PCM thickness.[1] Values for E_{PCM} were determined for an assumed value of ν_{PCM} of 0.04.[3]

Statistical analysis was performed using a two-factor analysis of variance (ANOVA) to test for differences in the elastic modulus between surface and deep zone chondrons using the three different models.

RESULTS

Following mechanical homogenization, ~3–5 intact chondrons were retrieved from each gram of tissue. Chondron yields were similar from surface or middle/deep specimens ($n = 22$ chondrons from five donors). Chondrons did not exhibit major differences in shape or size based on zone, although cells in the surface zone chondrons were generally flatter and possessed a thinner PCM than in middle/deep chondrons (Fig. 3).

Chondrons exhibited linear deformation behavior in response to a series of step increases in pressure (Fig. 4). The average value of r_i for all micropipettes employed was $2.9 \pm 0.3\ \mu$m, and the average value of h_{PCM} was $3.3 \pm 1.5\ \mu$m. Using the half-space model, chondrons from surface and middle/deep cartilage exhibited mean Young's moduli $E_S^{\text{half-space}} = 10.8 \pm 4.3$ kPa and $E_{M/D}^{\text{half-space}} = 12.1 \pm 3.9$ kPa (Fig. 5).

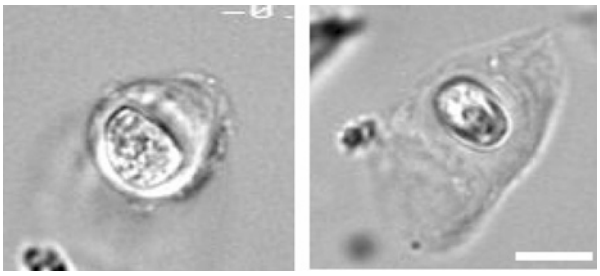

FIGURE 3. Images of chondrons isolated from the surface (left) and middle/deep (right) tissues. Chondron shape was not quantified in the present study, but in general, no major differences were observed in shape or size based on zone. Cells in the surface zone chondrons were generally flatter and possessed a thinner PCM, while cells in middle/deep chondrons had a larger PCM and were sometimes found in columns. Scale bar equals 15 μm.

If the geometry and compressibility of the PCM were taken into account using the layered half-space model of the chondron, the mean Young's modulus of the PCM was found to be significantly higher (24.0 ± 10.9 kPa in the surface zone and 23.2 ± 7.1 kPa in the middle/deep zones) in comparison with the half-space model ($p < 0.001$, ANOVA). With application of the shell model, the mean Young's modulus of the PCM was found to be similar to the layered half-space model (25.1 ± 11.5 kPa in the surface zone and 23.6 ± 7.3 kPa in the middle/deep zones; $p > 0.75$, ANOVA). While differences were apparent in mechanical properties among models, there was no evidence of significant differences in PCM mechanics between the zones.

DISCUSSION

The results of this study provide direct measurements of the biomechanical properties of the native PCM of articular chondrocytes isolated by mechanical homogenization, which is the most commonly used method for extracting chondrons from cartilage.[25,31,40] Our findings indicate that the Young's modulus of the PCM (~23 kPa) is significantly higher than that of the chondrocytes but 1–2 orders of magnitude lower than that of the cartilage ECM. In combination with previous theoretical models of cell–matrix interactions in cartilage, our findings suggest that the mismatch in properties of the chondrocyte, PCM, and ECM may have an important influence on the mechanical environment of the chondrocyte and support the hypothesis that the function of the PCM is biomechanical in nature.

An important finding of this study was the observation of no significant differences in PCM properties between the surface and middle/deep zones, although significant differences have been shown in the mechanical properties of ECM from the surface zone of cartilage to the deep zones. On the basis of the previous finite element studies,[17] a mismatch of the Young's modulus between PCM and ECM by a factor of 10 ($E_{PCM}/E_{ECM} = 0.1$) has been shown to amplify local strains by 50% in the vicinity of the chondrocyte. Because the Young's modulus of cartilage increases with depth,[10,19,43,51] the ratio E_{PCM}/E_{ECM} becomes significantly smaller and the strain amplification is likely more pronounced. This phenomenon may represent a mechanism whereby mechanical signals are amplified under conditions where tissue level strain magnitudes may be relatively small, i.e., the deep zone, while cell level strain magnitudes may be similar to or smaller than tissue strains in the surface zone. Taken together with previous studies of the properties of the cartilage ECM[19,43,50] and PCM,[1,3,31] our findings suggest that a potential role of the chondron is to provide for a more uniform mechanical environment for the cell from the surface to the deep zones, despite large inhomogeneities in ECM properties and local tissue strain.[2]

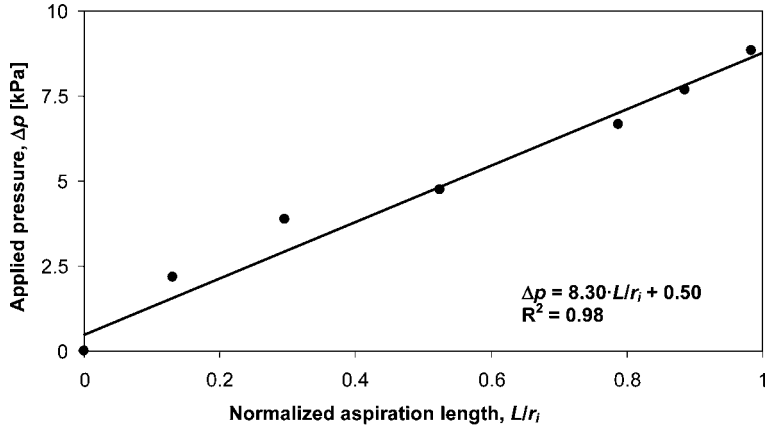

FIGURE 4. Typical equilibrium response of the pericellular matrix (PCM) subjected to a series of step increases in aspiration pressure Δp. The Young's modulus of the PCM is determined from the slope of the length vs. pressure curve, as described in the Methods section.

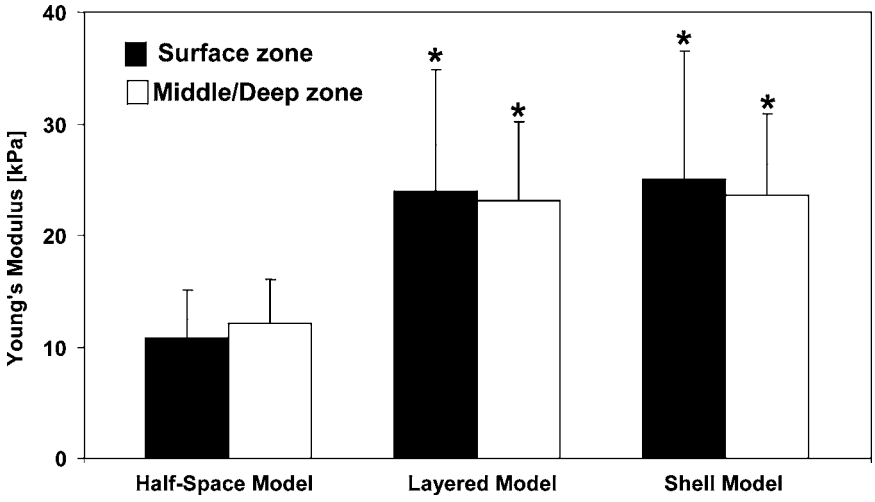

FIGURE 5. Young's moduli of the PCM calculated using three different models: the half-space model, the layered model, and the shell model. Significant differences were observed between the layered model and the half-space model (*$p < 0.001$ vs. the half-space counterpart) as well as between the shell model and the half-space model (*$p < 0.001$ vs. the half-space counterpart). No significant differences were found between surface and middle/deep zones.

An important consideration in the theoretical modeling of micropipette aspiration of the chondron is the incorporation of the layered geometry of the chondron and the compressibility of the PCM in the model. Significant differences were found in the apparent modulus of the PCM using the different models, and the study suggests that neglecting PCM thickness and compressibility in an incompressible, elastic half-space model[46] may lead to underestimation of the elastic modulus of the PCM by over 50%.[1] However, both the layered model and the shell model yielded similar results, consistent with previous reports of the relatively low stiffness of the chondrocyte relative to that of the PCM.[14,32,48] These findings are consistent with a previous study that used finite element modeling of pipette aspiration to account for differences in the thickness of different layers when determining the mechanical properties of soft tissues.[4] Future studies may wish to use such numerical methods to incorporate more complex aspects of the geometry and constitutive behavior of the chondron, such as viscoelasticity, large deformation behavior, and contact boundary conditions in the micropipette aspiration experiment.[3,4,22,23,47]

The findings of this study are generally consistent with previous measurements of the Young's modulus of the PCM of human chondrons, extracted by microaspiration ($E_{PCM} \approx 65$ kPa with the layered or shell models and $E_{PCM} \approx 43$ kPa using the half-space model)[1]; although it is important to note that the present study examined canine chondrons, while previous studies were performed on human chondrons. However, these results are in contrast to our previous studies on enzymatically isolated human chondrons[15,27] or canine chondrons,[35] which reported a Young's modulus of ~1–2 kPa for the PCM. In these previous studies, no differences were observed in the viscoelastic properties of enzymatically isolated canine chondrons between surface and middle/deep zones.[35] This difference of over an order of magnitude between enzymatically isolated and mechanically isolated chondrons is most likely attributable to degradation of other PCM components by collagenase, which allows for enzymatic isolation of chondrons[34] due to the resistance of type VI collagen to this enzyme. The loss of mechanical properties in enzymatically isolated chondrons suggests that collagenase-digestible macromolecules in the PCM, such as type II collagen, play an important role in determining the mechanical properties of the PCM. Nonetheless, a potential limitation of this work is that mechanical homogenization of the cartilage yields a very low fraction of viable chondrons, raising the issue that damage may be occurring to the PCM during the isolation process or that this technique preferentially selects for more "robust" chondrons that have greater strength or other failure properties. Further studies would be required to perform direct comparisons of the different chondron isolation methods.

Our findings are also consistent with previous studies examining the deformation behavior of enzymatically and mechanically isolated chondrons embedded in an agarose matrix.[30,31] These studies showed that enzymatically isolated chondrons initially deformed significantly as the agarose matrix was deformed but appeared to stiffen with time in culture. Mechanically isolated chondrons, however, did not deform measurably, suggesting that they possessed a compressive modulus higher than that of the agarose (~25 kPa).

In this study, the mechanical stiffness of the chondrocyte was assumed to be significantly lower than that of the PCM,

to allow for simplification of the analytical solutions used to determine the modulus of the PCM.[1] This assumption is consistent with several studies that have directly measured the elastic and viscoelastic properties of the chondrocyte and have shown its Young's modulus to be 1–2 orders of magnitude lower than that of the PCM.[14,28,32,47] However, it is important to note that this study did not measure viscoelastic or biphasic properties of the PCM,[3] which may also have a significant influence of the local stress–strain and fluid flow environments of the cell.[17,21]

In summary, our findings provide further support for the biomechanical role of the PCM and chondron in articular cartilage and suggest that the mismatch of the PCM and ECM stiffness modulates the stress–strain environment of the chondrocyte. Despite depth-dependent variations in cell morphology and ECM mechanics, there was no evidence of a difference in the elastic properties of the PCM between chondrons isolated from surface and middle/deep zones articular cartilage. The methods developed in this study may provide a means of more accurately characterizing alterations in the mechanical environment of the chondrocyte *in situ* under physiological and pathological conditions.

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