# ALTERATIONS IN THE MECHANICAL PROPERTIES OF THE HUMAN CHONDROCYTE PERICELLULAR MATRIX WITH OSTEOARTHRITIS

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**Introduction:** In articular cartilage, chondrocytes are surrounded by a pericellular matrix (PCM), which together with the chondrocyte have been termed the chondron [1]. This region is characterized by the presence of type VI collagen and increased proteoglycan concentration relative to the extracellular matrix. While the specific function of the PCM is not fully understood, it has been hypothesized to play important roles in regulating the biomechanical, biophysical, and biochemical signals that the chondrocyte perceives during normal joint activity [1,5,7]. A more thorough understanding of the mechanical properties of the PCM would provide important insights into the potential biomechanical function of the chondron as a discrete entity in articular cartilage. Furthermore, previous studies have reported changes in the distribution and amount of collagen VI in osteoarthritic (OA) cartilage, suggesting that the biomechanical function of the PCM may be altered with disease. The goal of this study was to test the hypothesis that the biomechanical properties of the PCM vary with depth from the cartilage surface and are altered with OA. Using a newly developed microaspiration technique, chondrons were extracted from normal and osteoarthritic cartilage. The Young's modulus of the PCM was determined using the micropipette aspiration technique in combination with a recently developed theoretical model that represents the chondron as an elastic, compressible layer overlying an elastic half-space (Fig.1) [2].

**Methods:** Chondrons were mechanically isolated from full thickness articular cartilage of human femoral heads at the time of joint replacement surgery (N=73 chondrons from 13 donors, ages: 19-75 yr). Chondrons were extracted from two different zones (surface and middle/deep) by applying suction pressure to the cartilage surface with a glass pipette. Chondrons were classified as osteoarthritic ('OA') or non-osteoarthritic ('non-OA') based on a semi-quantitative histology grading scale from 0 (normal) to 20 (OA) of the cartilage from which they were extracted. The average grades for non-OA and OA cartilage were 4.5±3.1 and 15.8±2.1, respectively. The elastic properties of chondrons were measured using a new axisymetric layered elastic half-space model for the micropipette aspiration technique (Fig.1) [2]. The primary advantage of this model is that it accounts for different mechanical properties in two distinct regions of the chondron, a peripheral layer (i.e., PCM) and a substrate (i.e., chondrocyte). This model significantly reduces errors introduced by neglecting the thickness of the PCM [2], and thus can be applied to quantify the mechanical properties of the PCM of isolated chondrons.

**Results:** The mean Young's modulus of the PCM of chondrons from non-OA cartilage was $E_{non\text{-}OA}$=66.5±23.3 kPa. With OA, the Young's modulus of the PCM was significantly reduced to $E_{OA}$=41.3±21.1 kPa ($p<0.05$). No zonal variation was found in the Young's modulus of PCM of chondrons from OA or non-OA cartilage (Fig. 2).

**Discussion:** Our findings provide direct evidence of a loss of the biomechanical properties of the PCM in OA. The mean Young's modulus of the PCM was found to be ~40% lower in OA specimens as compared to the non-OA controls. According to a previous study that has utilized multiscale finite element methods to model the biomechanics of the chondron within articular cartilage [5], the mechanical properties of the PCM can have a dramatic influence on the local stress-strain and fluid flow environment of the chondrocyte *in situ*. Based on the present findings, this finite element model suggests that chondrocytes may experience significantly different biomechanical signals due to alterations in the PCM with OA. The reduced stiffness of the PCM with OA may reflect degenerative changes though enzymatic cleavage of collagen by metalloproteinases [3].

While significant differences exist in the extracellular matrix mechanical properties from the surface zone of cartilage to the deep zones, we found no significant difference in the elastic properties of the PCM from surface to deep zones. Based on our results and on a study by Schinagl et al. [4], in the superficial zone the PCM has the same stiffness with the extracellular matrix ($E_{PCM}/E_{ECM} \cong 1$). In the deep zone, however, the extracellular matrix may be more than one order of magnitude stiffer than the PCM ($E_{PCM}/E_{ECM} \cong 0.05$). Based on previous finite element studies [5], the local strain that the chondrocyte perceives is amplified when $E_{PCM}/E_{ECM} < 1$. Thus in the deep zone, strain amplification is more pronounced than in the superficial zone. This phenomenon may represent an intrinsic mechanism for amplifying mechanical signals at the deep layers of articular cartilage, where tissue level strain magnitudes may be relatively small.

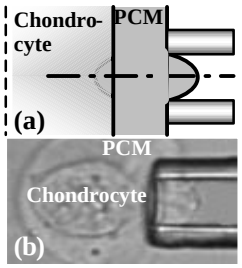


*Figure 1a: The mathematical model represents the chondron as a layer overlying a half-space. 1b: Micropipette aspiration of mechanically isolated chondron.*

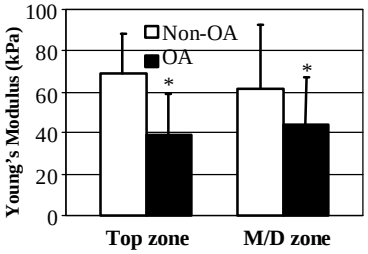


*Figure 2: The Young's modulus of the PCM is altered with OA but does not vary with depth from the cartilage surface (*p<0.03 vs. non-OA PCM).*

In contrast to previous studies on enzymatically isolated chondrons [6] which reported a mean Young's modulus of ~1.5 kPa for the PCM [7], our findings suggest that the Young's modulus of the mechanically isolated PCM is nearly 50 times larger than that of the enzymatically isolated chondrons. This difference is most likely attributable to the effects of enzymatic isolation on the properties of the PCM. These findings are consistent with previous studies examining the deformation behavior of enzymatically and mechanically isolated chondrons embedded in an agarose matrix [8], and suggest that the Young's modulus of the mechanically isolated chondrons is greater than the Young's modulus of enzymatically isolated chondrons and in excess of the Young's modulus of the agarose (25kPa).

A unique aspect of this study was the development of a new chondron isolation technique, which is based on extraction of chondrons directly from the cartilage by applying suction pressure using a small diameter pipette. This technique requires minimal tissue preparation, and can be used to extract chondrons from precise sites (i.e., zones) of the cartilage. Compared with the homogenization technique [1], our method is fast and easy and yields a large number of chondrons with a smaller amount of debris.

Increasing evidence suggests that the chondron is a distinct functional compartment in articular cartilage and serves to regulate the mechanical environment of the chondrocytes [5,8]. The mechanical properties of the PCM determined in this study can be applied in several models of articular cartilage to further examine the mechanical microenvironment of chondrocytes. In addition, the development of an easy chondron extraction technique will further facilitate the investigation of this unique structure in articular cartilage.

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**References:** [1] Poole CA et al., 1988, J Orthop Res, 6, pp. 408-19. [2] Alexopoulos LG et al.,2001 ASME Adv Bioeng, Vol.51. [3] Poole AR, et al., 1993, Agents & Actions - Supplements, 39, pp. 3-13. [4] Schinagl RM et al., 1997, J Orthop Res, 15, pp. 499-506. [5] Guilak F & Mow VC, 2000, J Biomech, 33, pp. 1663-73. [6] Lee GM et al., 1997, Osteoarthritis and Cartilage, 5, pp. 261-74.[7] Guilak F, 2000, Biorheology, 37, pp. 27-44. [8] Knight MM et al., 2001, Biochim Biophys Acta, 1526, pp. 141-6.

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