Zonal Differences in the Deformation Characteristics of the Cartilage Chondron

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Chondrocytes in articular cartilage are surrounded by a narrow pericellular matrix (PCM), which together with the enclosed cell(s) are termed the “chondron”. Although the precise function of this tissue region is unknown, previous studies provide indirect evidence that the PCM plays an important role in governing the local mechanical environment of chondrocytes. The goal of this study was to quantify the zonal differences in the strain and Poisson’s ratio of the chondron in situ under compression, using immunolabeling of type VI collagen coupled with fluorescence confocal microscopy. Up to 30% tissue strain, most compressions were found to be concentrated at the superficial and middle zones which are relatively soft. Apparent Poisson’s ratio of the PCM and cell was about 0.1 in the superficial and middle zone and 0.5 in the deep zone. The zonal variations in the mechanical response of the PCM and cell do not appear to be due to zonal differences in PCM and cell properties, but rather seem to result from significant inhomogeneities in extra-cellular matrix (ECM) properties, which result in different relative stiffnesses of the ECM and PCM with depth. These findings suggest that the PCM plays an important biomechanical role in cartilage by regulating the local stress-strain and fluid flow environments of the chondrocyte in a zone-dependent manner.

Key words: Extra-cellular matrix, Peri-cellular matrix, Chondrocyte, Type VI collagen, Immunohistochemistry

INTRODUCTION

Articular cartilage is the avascular connective tissue that covers the ends of bones in diarthrodial joints and provides a nearly frictionless bearing surface. The articular cartilage extracellular matrix (ECM) is heterogeneous in composition and structure, and is classified into different zones based on distance from the cartilage surface (superficial, middle, and deep). The ECM also exhibits significant heterogeneity based on distance from the chondrocytes and is classified into distinct regions termed the pericellular matrix (PCM), territorial matrix, and interterritorial matrix. The interterritorial matrix constitutes the largest fraction of cartilage matrix, consisting mostly of type II collagen and aggregating proteoglycan (aggrecan) molecules. The PCM is a narrow region of tissue that completely surrounds chondrocytes, which together with the enclosed cell(s), have been termed the “chondron”.1 The PCM contains many of the same molecular constituents as the other matrix regions, including collagen types II, IX and XI, aggrecan, and fibronectin, but is generally defined by the unique presence of type VI collagen in normal cartilage.2,3

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Preferential pericellular concentration of type VI collagen suggests that it would mediate matrix interactions and mechanotransductions between the chondrocyte and the ECM. During normal joint activity, chondrocytes maintain the structural composition of the ECM and PCM through the incessant anabolic and catabolic turnovers.

The mechanical environment of chondrocyte together with biochemical factors plays an important role on the maintenance of healthy cartilage. A variety of biomechanical factors affecting the cartilage regeneration has been quantified in vivo and in vitro. These include static and dynamic stress,4,5 shear stress,6,7 intermittent hydrostatic pressure8,9 and indirect effects of loading such as interstitial fluid flow10,11 streaming potential12,13 and osmotic pressure.14,15 Mechanical stimulus exerted on the articular joint surface passes through ECM and PCM and is transmitted to the chondrocyte which converts the biomechanical signal to an intracellular biochemical response. Therefore, the knowledge about the mechanical performances of each component is critical in determining the force transmitted to the chondrocytes during joint loading.

In particular, the structural property of PCM would play an important role on the signal transduction because it transmits a deformation from the ECM to the chondrocyte. However,
little is known about the function of the PCM even though mechanical properties of the bulk PCM was identified for cultured chondron in agarose and for mechanically isolated chondrons from normal and osteoarthritic (OA) tissue. And also, the mechanism through which the presence of PCM might influence cell micro-deformation is not fully understood. Mechanical properties of PCM relative to those of the ECM may greatly affect the stress-strain state in the mechanical micro-environment of the chondrocytes and accordingly the signal transduction upon loading.

While theoretical models of PCM deformation have been developed based on direct measurements of cell and PCM mechanical properties, the in situ mechanical behavior of the PCM has not been well characterized. The objective of this study was to quantify the zonal differences in the strain and Poisson’s ratio 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 PCM and chondron, and a series of fluorescent confocal images were recorded and reconstructed to form quantitative three-dimensional models of the cell and PCM in free swelling and compressed cartilage explants. These methods were used to examine the hypothesis that matrix compression induces changes in the three-dimensional morphology of the PCM in a manner that depends on the zonal site of the chondron.

**MATERIALS AND METHODS**

**Specimen Preparation**

For a compression test, full thickness cylindrical cartilage blocks of 5 mm in diameter were harvested from the inner one-third region of medial femoral condyle from knee joints of skeletally mature pigs (2-3 years old) obtained from a local abattoir. Cartilage blocks near by each harvested region for the compression test were also prepared for the uncompressed control specimen.

Cylindrical blocks were cut slightly on the edge along the split-line pattern to ascertain the orientation, and unconfined compressions of 10%, 30%, and 50% were applied (N=6, each group) with a simple compression device. The compressed specimens were fixed in 4% paraformaldehyde within the compression device to minimize the recovery, followed by overnight treatment removed from the compression device. The uncompressed control blocks were fixed overnight immediately after the harvest. Frozen sections (40 µm) of full thickness cartilage were sliced from each block, oriented along the local split-line direction.

**Immunohistochemistry for Type VI Collagen**

To image in situ chondrons in the compressed and uncompressed cartilage specimens, the immunohistochemistry procedure developed for type VI collagen immunostaining in the chondron morphology study was used with a slight modification in treatment time. Frozen sections (40 µm) of full thickness cartilage from each block were rinsed in PBS and digested with 0.1 M Trypsin-EDTA at 37°C for 3 hrs to restore immunoreactivity to tissue antigens. After rinsed in TBS, sections were then blocked in 10% normal donkey serum (RDI-NSDNY, Fitzgerald) diluted in assay buffer (0.1% Bovine Albumin Solution (BAS) in 0.1 M Tris-Buffered Saline (TBS), BIO-RAD) at room temperature for 3 hrs and were rinsed in TBS again. The affinity purified primary antibody (anti-collagen type VI raised in rabbit, RDI-600401108, Fitzgerald) was diluted to 1:50 in assay buffer and samples were incubated in it at 4°C overnight. After rinsed in TBS, samples were incubated in the secondary antibody (FITC conjugated anti-rabbit, RDI-711095152, Fitzgerald) at a dilution 1:4 in a dark room for 3 hrs. Sliced specimens were then rinsed again in TBS and fixed with a water base mounting media (95-9943, Zymed) for confocal microscope.

**Chondron Sampling and Imaging**

For the analysis of zonal variation of PCM under compression, a total of 12 chondrons were randomly selected from each superficial, middle, and deep zone for the uncompressed and compressed groups (18 porcine knee joints for the uncompressed group and 6 porcine knee joints for each compressed group) except chondrons in the superficial zone at 50% compression (n=8). In the uncompressed specimen, the superficial zone was defined as 0-500 µm from the articular surface, the middle zone as 100-200 µm from the articular surface, and the deep zone as the lower 50% of the full thickness cartilage. In the case of the compressed specimens, it is difficult to quantitatively define each zone because the articular cartilage shows a depth-dependent response under compression. In this study, the superficial chondrons were selected right below the articular surface and the deep chondrons below half of the full thickness cartilage for all compressions. Chondrons in the middle zone were selected from different regions, depending on the level of compression. The middle zone was defined as 100-150 µm from the articular surface, 200-200 µm for the 30% and 50% compression groups, respectively. All the chondrons were selected from the central region of the specimen because unconfinned compression can give rise to a rotational effect in addition to the compression effect for chondrons in the peripheral region of the specimen. For each selected chondron, a three-dimensional image was recorded at 488 nm using 30-50 serial sections of 512 x 512 pixels at an interval of 0.5 µm using a confocal laser scanning microscope (LSM 510, Zeiss) with a water-immersion objec-
Three-Dimensional Reconstruction of Serial Confocal Images

Three-dimensional reconstruction and morphological measurement of chondrons were also performed by the procedures applied to the chondron morphology study, using a custom program and graphic user interface in MATLAB. To facilitate the extraction of the morphological parameters of the chondrons, true colored confocal images (16 bit, 512 × 512 pixels) were converted to gray scale (8 bit) with a range of 0-256 and then smoothed using a median filter, which simultaneously reduces noise but preserves edges in an image. The IsoSurface function in MATLAB used for isointensity surface rendering was based on the marching cubes algorithm [2], by which a smooth isointensity surface of the inner and outer boundaries of PCM was created using a large numbers of triangular patches. After converting the vertex coordinates on the reconstructed surface from pixels to real scaled values (µm), all vertex coordinates and surface connectivity matrix were stored for further computation of height and width of chondron.

Determination of Morphological Parameters for PCM and Cell

To analyze the zonal variations of PCM and cell under compression, the morphological parameters such surface height and widths (medial-lateral (ML) and anterior-posterior (AP)) were extracted from the reconstructed 3-D images. The heights of the PCM and the cell 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. Deep zone and surface zone chondrons whose major axes were less than 10° from the vertical axis or horizontal axis, respectively, were selected to minimize error in extracting the height and widths of the PCM and the cell. To determine the principal angle of chondron orientation, all the vertices forming the isointensity surface contour were screened to find the two vertices with the maximum distance, which forms the major axes of the chondron. All statistical analyses for the morphometric variations of the PCM and the cell under compression were performed using ANOVA and post-hoc test (STATISTICA, StatSoft, Inc.) with Tukey honest significant difference test at α=0.05.

Poisson’s Ratio of Whole Cartilage Tissue

For the calculation of the Poisson’s ratio of the whole cartilage tissue, the ML width was measured at a depth of 25 µm, 100 µm for the superficial and middle zone for all compressions, respectively. For the deep zone, it was measured at the position which showed the maximum lateral expansion for all compressions.

Numerical and Experimental Calibration

To assess errors involved in the modeling algorithm, a series of parametric studies were performed using a digitally defined rectangular prism and sphere. Pre-defined images (rectangle or circle with a 1µm interval) were generated and three-dimensional objects (rectangular prism or sphere) were reconstructed using the current algorithm. The parameters such as height, width of sphere were compared to the measurements of these values performed by three-dimensional reconstruction algorithm.

For the experimental calibration of the algorithm, latex micro-spheres (18.0 ± 0.5 µm) were embedded in 2% agarose gel, scanned with an interval of 0.5 µm, and reconstructed in the customized MATLAB code. Tests were performed to identify the possible errors resulting from inhomogeneous fluorescence and photobleaching, in addition to determining the threshold (isointensity) value and also to confirm the accuracy of the slicing interval.

RESULTS

The whole cartilage tissue was shown to have Poisson’s ratio of less than 0.1 at the superficial and middle zone, about 0.5 at the deep zone (Figure 1). Using the polarized microscope, the collagen fiber at the deep zone was clarified to preserve the vertical direction at 10% strain, which means that most compression is localized at the superficial and middle zone at small tissue strain of 10%.

The chondrons in each zone were shown to undergo a significant decrease in the height under compression (Figure 2a, p<0.05). At small compression of 10%, there was no significant change in the height of the deep zone chondron, com-

![Figure 1. Deformed whole cartilage at each tissue strain and representative directions of the collagen fiber.](image-url)
pared to that of the uncompressed chondron in that zone. At this compression level, the deformation didn't fully transmitted into the deep zone and most changes in the height were concentrated at the chondrons in the superficial and middle zone which is relatively soft. Apparent vertical strains of the chondrons calculated from the height change correspond to 25%, 44%, 62% in the superficial zone and 18%, 28%, 46% in the middle zone and 17%, 15%, 35% in the deep zone for 10%, 30%, 50% compression groups, respectively.

However, there was no significant change in the ML width of the chondrons under compression except the deep zone chondrons at 50% compression (Figure 2b, p<0.05). Apparent Poisson's ratio of the chondrons obtained from the changes in the height and ML width was less than 0.1 in the superficial and middle zone at all strain levels, even if the ML width change of the chondrons was not significant in those zones. However, the deep zone chondron at 50% compression, which showed the significant difference in both height and ML width at that compression level, was found to have the apparent Poisson's ratio of 0.53.

The chondrocytes in each zone were also shown to undergo a significant decrease in the height under compression (Figure 3a, p<0.05). As in the chondron at small compression of 10%, there was also no significant change in the height of the deep zone chondrocytes, which means that most changes in the cell height were concentrated at the superficial and middle zone. Apparent vertical strains of the chondrocytes calculated from the height change correspond to 27%, 43%, 59% in the superficial zone and 21%, 32%, 44% in the middle zone and 2.2%, 19%, 32% in the deep zone for 10%, 30%, 50% compression groups, respectively.

As in the case of the chondron, there was no significant change in the ML width of the chondrocytes under compression except the deep zone chondrocytes at 50% compression (Figure 3b, p<0.05) which was shown to have the apparent Poisson's ratio of 0.51. Apparent Poisson's ratio of the chondrocytes obtained from the changes in the height and ML width was about 0.1 in the superficial and middle zone at all strain levels, even if the ML width change of the chondrocytes was not significant in those zones.

**DISCUSSION**

The findings of this study provide direct evidence based on three-dimensional *in situ* measurements that the chondron undergoes significant changes in the morphology with compression. Importantly, the magnitude of these changes was highly dependent on the zone of origin of the chondron, with larger deformations in the surface zone as compared to the middle and deep zones. These findings provide further evidence of the complex and inhomogenous mechanical environment of chondrocytes within articular cartilage, and provide additional evidence for a potential biomechanical role for the chondrocyte PCM.

The Young's modulus of ECM is known to increase with depth from 0.1 MPa to 2 MPa, however, the Young's moduli of the PCM and the chondrocyte to be uniform with depth through the cartilage (70 kPa : PCM, 0.4 kPa — 4 kPa : chondrocyte),16,17 If the articular cartilage is treated as a fibered composite material with soft inclusion, the soft inclusions
increase the stiffness of the composite in the fiber direction, and reduce the stiffness of the composite in the direction normal to the fibers.\textsuperscript{10)} Thus, considering the chondrocyte shape and fiber direction at each zone, the articular cartilage is stiffened horizontally at the superficial zone and vertically at the deep zone. The present result for the height change of the PCM and cell shows the corresponding deformation characteristics under the tissue compression that most compression is concentrated at the superficial and middle zone at small tissue strain.

Mechanical compression of the ECM resulted in changes in height of the cell and PCM that varied significantly with the zone of origin of the chondron. In the surface zone, changes in PCM and cell height were greater than the nominally applied matrix strain, whereas in the deep zone, changes in PCM and cell height were less than the applied strains. These findings are consistent with previous measurements of the zonal variations in cell shape with compression,\textsuperscript{10} 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 surface zone to the deep zone.\textsuperscript{10} It is important to note, however, that the degree of this inhomogeneity may vary significantly with the age, species, and site of the tissue.

In particular, major changes in the chondron morphology were observed in the surface zone at high strains (50% tissue deformation), with an apparent “collapse” of the cell and PCM in half of the sampled chondrons. Because of the low compressive modulus of the surface zone relative to the middle and deep zones, it would be expected that local tissue strains exceeded 50% in the surface zone. In previous studies, it was found that local strains of ~60% resulted in rupture of the chondrocyte membrane as evidenced by leakage of an intracellular fluorescent dye. Thus the apparent collapse of surface zone chondrons at high strain may reflect strain-induced cell damage and possible cell death.

One limitation of the current approach is that, while the surface-to-surface deformation on the specimen is carefully prescribed, the ECM strain field is nonuniform with depth due to inhomogeneity of the ECM properties. Thus it is not possible to apply inverse methods to finite element models of the chondron in cartilage in order to determine the mechanical properties of the ECM and PCM.\textsuperscript{1,5,6}

Significant zonal variations were observed in the medial-lateral (ML) width of the chondron and cell with compression. In the surface zone, little or no change in chondron and cell width was observed, suggesting an apparent PCM and cell Poisson’s ratio of 0.1 or less. This finding is consistent with micropipette aspiration studies of mechanically isolated chondrons that showed a Poisson’s ratio of ~0.04.\textsuperscript{15} However, in the deep zone, the apparent Poisson’s ratio of the chondron and cell was ~0.5. This apparent discrepancy may be due to similar zonal variations in the Poisson’s ratio of the ECM, and suggests that the mechanical behavior of the chondron may be mostly dependent on the local response of the ECM.

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, but rather seem to result from significant inhomogeneities in ECM properties, which result in different relative stiffnesses 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 zonedependent manner. 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.

REFERENCES


