Morphological changes of chondrocytes in compressed articular cartilage using polarized light microscopy

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***Abstract*— The orientation and shape of chondrocytes in compressed articular cartilage were studied quantitatively by polarized light microscopy (PLM) at 0.34μm pixel-resolution. The morphological changes of the cell shapes, the cell orientation, and the territorial matrix surrounding the chondrocytes in articular cartilage were quantified.**

Keywords: Chondrocyte, articular cartilage, compression, polarized light microscopy

I. INTRODUCTION

Articular cartilage, which is a thin layer of connective tissue covering the end of bones in synovial (diarthrodial) joints, distributes repetitive loads when human/animal is in motion. The molecular compositions of articular cartilage are water and a solid matrix of primarily collagen fibrils (type II) and proteoglycans (aggrecans of glycosaminoglycans, GAGs). The tissue is strongly anisotropic because the collagen fibrils in articular cartilage forms a structural matrix that has three orientational zones cross the thickness (depth) of this thin layer of tissue (Fig 1): the superficial zone (SZ) where collagen fibrils are oriented parallel to the articular surface, the transitional zone (TZ) where collagen fibrils are oriented randomly and the radial zone (RZ) where collagen fibrils are oriented perpendicular to the articular surface [1-2].

Compressive properties of articular cartilage are attributed to the closely-packed negatively-charged sulfate and carboxyl groups in GAGs. To ensure electroneutrality, GAGs preferentially attract positive ions and the water protons, which generate an osmotic pressure that must be delicately balanced by the restraining force of the collagen matrix. An external load will elevate local compressive stress and ***force*** some water out of the GAG matrix until the increased repulsion from the more compact GAGs ultimately reaches a new equilibrium with the external load. Such behaviors are best described by some theoretical models of articular cartilage such as the biphasic theory, which considers collagens/proteoglycans as solid and water/small-ions as fluid. The fluid phase can redistribute itself under loading, thus altering hydrostatic pressure within the tissue.

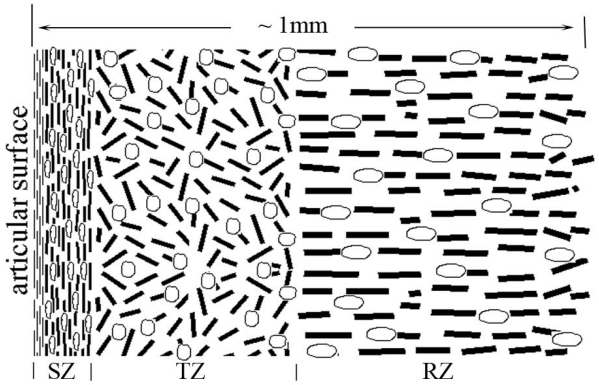

Fig 1 The orientation of collagen fibrils (lines) and chondrocytes in articular cartilage

The most important characteristic of articular cartilage as a load-bearing tissue is its depth-dependent compressive modulus across the thickness of the tissue – the superficial zone is the softest part of the tissue and the radial zone is the hardest part of the tissue. Several theoretical and experimental studies [3-10] examined the effects of mechanical loading on the organization of the collagen fibrils in the cartilage matrix. For example, the histological zones can be re-organized under different strains and the collagen fibrils can become crimped under strain.

Different aspects of articular cartilage can be studied by different microscopic imaging techniques, such as various electron microscopes for ultra-structural visualization [9, 10], microscopic MRI for the correlation with clinical MRI [2, 4, 5], light microscopy for morphological identification [2, 3, 7, 8], and Fourier-transform infrared microscopy for chemical composition [8]. Among these imaging techniques, Polarized light microscopy (PLM) is probably the most commonly used technique in biomedical labs in order to describe the morphological structure in articular cartilage. A quantitative version of PLM was developed and validated in our lab in cartilage research [2]. Based on the optical property of the collagen fibrils, this PLM technique can construct two-dimensional images of the optical retardation and angular orientation of articular cartilage quantitatively. The retardation maps in this technique illustrate the localized order of the collagen fibrils in the tissue. A larger value in retardation indicates that the collagen fibrils are more ordered (hence a large birefringence). The angle maps in this technique represent the pixel-averaged orientations of the collagen fibrils in the tissue. This technique has been used extensively in our lab for over ten years to study the morphology of cartilage when the tissue is healthy and lesioned.

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The aim of this project was to examine morphological changes in compressed articular cartilage, especially the changes in the orientation and shape of the chondrocytes at different tissue depths, by quantitative PLM at high magnification (0.34μm pixel resolution).

II. Materials and Methods

Specimen preparation

Thirty cartilage-bone blocks were harvested from the central load-bearing surface of a humeral head, which came from a healthy and mature dog sacrificed for an unrelated experimental study. This animal belonged to a group of similar dogs that has been studied extensively in our lab for over ten years by different imaging and non-imaging tools. Before the static loading, the cartilage specimens were bathed in physiological saline with 1 % protease inhibitor cocktail (P2714 Sigma, St. Louis, MO).

Twenty-five tissue blocks were compressed at 0%, 16.8%, 30.3% and 50% strains. The unconfined compression was performed by placing each block (about 1.5 × 2 × 5 mm) in a Hoffman clamp and compressed by means of screws. The compressed blocks remained in the clamps during the entire histological process to ensure the preservation of the local environments in the compression state. The clamp was removed in the paraffin container just before the specimen was sectioned. The unstained sections were placed on the MirrIR slides (Indianapolis, IN) [7].

PLM experiments

A 12-bit CCD camera is mounted on a Leica polarized light microscope (Leica Microsystems Wetzlar, Germany), which has a pixel resolution 0.34μm and 2.72μm when a 40x and 5x objective is used respectively. Circularly polarized light with a liquid crystal compensator is used in this system, which allows the compensation of birefringent elements of any fibril orientation in cartilage. Two quantitative images are calculated for each tissue section, the optical retardance (in unit of nm) and angular orientation (in unit of degree) [2]. In a previous study, these tissue sections were imaged at a low magnification (2.72μm per pixel) for its general morphological features [8]. In this project, the same tissue sections were studied at high magnification (0.34μm per pixel), which enables the detailed calibration of the fine structure of collagen fibrils surrounding the individual chondrocytes.

Image analysis

To determine the morphology of the chondrocytes, the long and short axes of the cells and the angle of the long axis of individual cells were measured quantitatively. The coordinates of the cells were also measured, which were converted into the relative tissue depth (0 = articular surface, 1 = tidemark line). The open-source software ImageJ was used to analyze the cells (the National Institutes of Health, Bethesda, MD).

III. Results

Fig 2 shows the angle maps from these cartilage specimens when a 40x objective was used. The average thickness of articular cartilage at 0% strain (uncompressed) was 658 ± 40 μm. Since the field of view at the high resolution is small, each full depth image of articular cartilage requires the acquisition of up to seven images. These individual images were pieced together digitally in computer [11]. The consistency in the color maps testifies the reliability and reproducibility of this PLM technique. In the angle map of the unloaded tissue, the collagen fibrils close to the surface have a nominal orientational difference of 90° from the fibrils deep in the tissue, which was represented by the color blue and red in the image. When the tissue is compressed, the blue region increased its relative portion, which represents a ***larger*** portion of the surface tissue now has the collagen fibrils that are parallel with the articular surface. (In the retardance map (data not shown), the unloaded specimen reaches a minimum at a normalized depth of ~ 0.1 from the articular surface, marking the center of the transitional zone that has the most random orientation.)

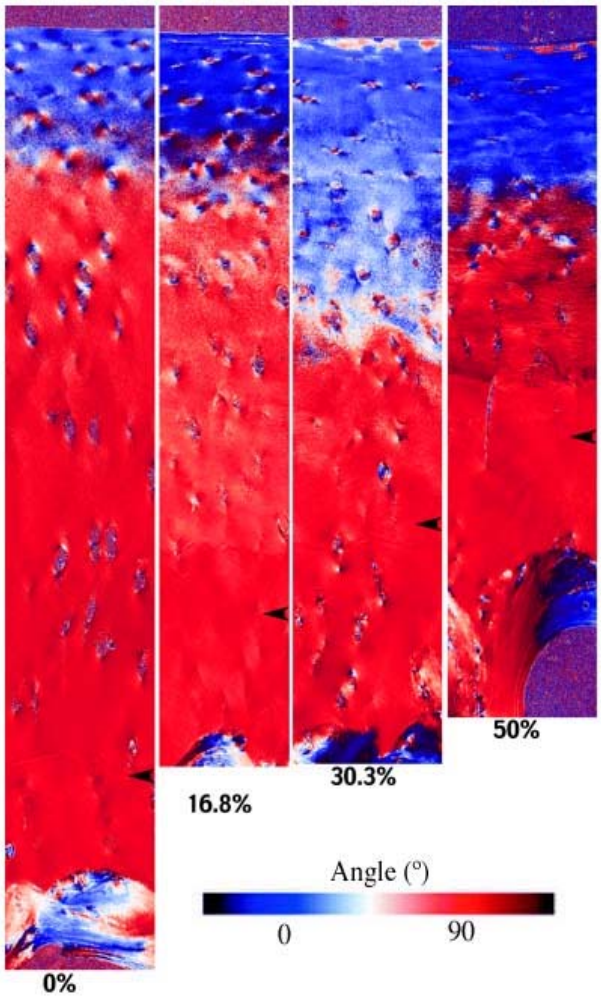

Fig 2 The angle images at 40x objective (0.34μm per pixel). The arrowheads indicate the tide mark line in the tissue

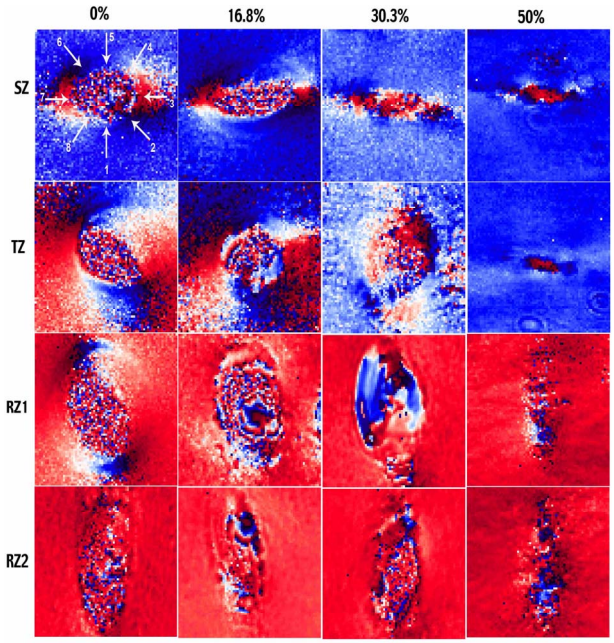

Fig 3 The angle images of chondrocytes from different zones. In the first image (SZ and 0% strain), the arrows indicate where the angle values were measured around the chondrocyte. SZ: Superficial zone, TZ: Transitional zone, RZ1: the beginning of the radial zone, RZ2: the end of the radial zone.

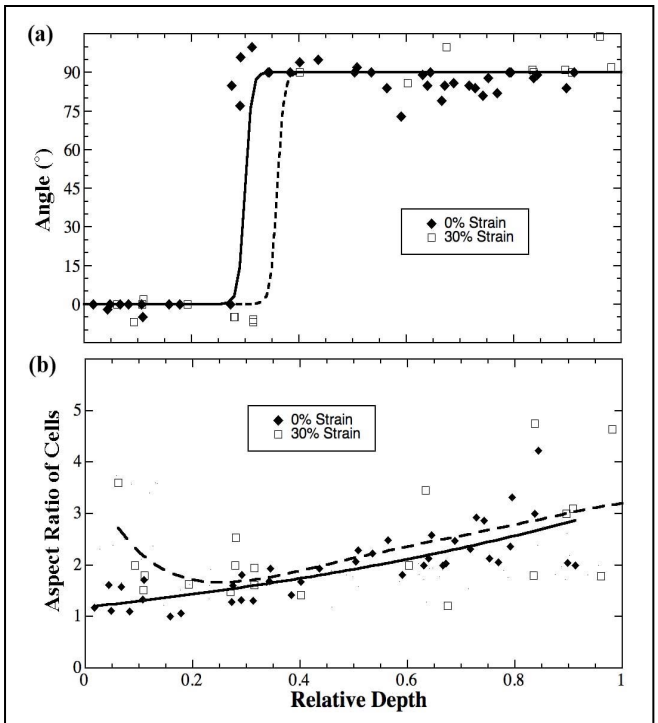

Fig 4 The orientation (a) and aspect ratio (b) of the chondrocytes.

Several cells from each histological zone of differently compressed tissues were selected and shown in Fig 3. The random angles inside the elliptical-shaped cells illustrate the fact that these regions in the histologic sections have no ordered fibrils (inside the cells). The retardation values in the immediately cell-adjacent areas were higher than other far-away areas in cartilage (data not shown), which indicates that the adjacent region has higher orientational order in the collagen fibrils (the ordered fibrils in the territorial matrix). Since the collagen fibrils have neither a head nor a tail, the angle values vary between 0° to 180° around the cells twice.

The morphology of the chondrocytes were quantified and plotted according to each cell's relative location along the tissue depth (0 = articular surface, 1 = tidemark line), shown in Fig 4a as the orientation of the cells and in Fig 4b as the aspect ratio of the cells. The orientation of the cells measures the orientation of the long axis of the cell relative to the cartilage surface. It is clear in Fig 4a that the cells rotate by approximately 90˚ at a relative depth of 0.3, and that this rotation of cells occurs in a deeper tissue when the tissue is compressed. In our previous work that measures the fibril orientation along the tissue depth, similar changes in the fibril orientation have also been observed [2, 7]. This result shows that the orientation of the cells closely follow the orientation of the nearby fibrils in articular cartilage.

The aspect ratio of the chondrocytes (Fig 4b) is the ratio of the long axis over the short axis of the cell measurement. For most of the deep tissue, the aspect ratio of the cells had a similar trend, increasing gradually as the depth. The most striking difference of the aspect ratios at different strains occurred near the surface of the tissue – the values increases as the strain increases. This result illustrates the fact that articular cartilage has a depth-dependent mechanical stiffness [3], where the softest part being the surface portion and the hardest part being the deep tissue. When one compresses the tissue, the surface cells were compressed the most from its usual oval shape, which increases the aspect ratio of the cells in the measurements (Fig 4b).

The "cocoon fibrils" in the territorial matrix surrounding several cells under various strains were also examined in detail, by measuring eight adjacent locations surrounding each cell, labeled as location #1 to #8 in Fig 3. In the superficial zone (Fig 5a), the fibril angles in the territorial matrix when the tissue is not compressed have two circular variations or oscillations between 0˚ to 180˚ continuously (the angle calculation in our PLM technique falls into the range of 0 – 180˚ because the collagen fibrils have neither head nor tail). With the increase of the strain, this fibril oscillation reduced its 'magnitude', reflecting the flattening of the cells. In the transitional zone (Fig 5b), the fibrils also had a similar large oscillation at the zero or low strains but lost its oscillatory when the strain increases to above 30%. In the radial zone (Fig 5c), since the cells are now oriented 'vertically' between the well-organized radial zone fibrils (see the uniform red color in Fig 3, which represents the values of the 90˚ angle), there was no clear periodic difference in the fibril orientation surrounding the chondrocytes for RZ.

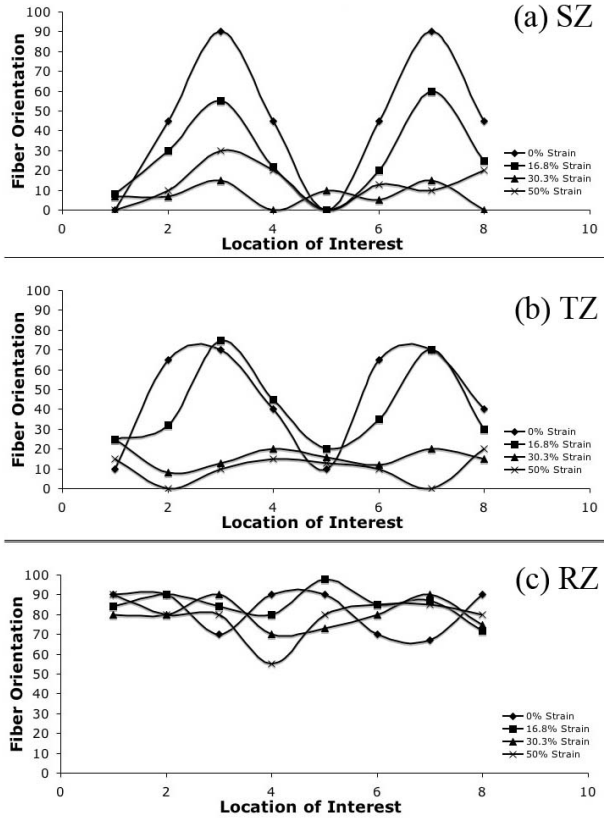

Fig 5 Orientation of the territorial fibrils

IV. DISCUSSIONS AND CONCLUSION

In this project, the deformation of the collagen matrix in articular cartilage was studied by PLM at 0.34μm pixel resolution. Since the superficial zone cartilage is softer than the transitional zone and radial zone cartilage [3, 7], it gets compressed the most at a given strain. At the same strain, the radial zone tissue, being the most stiff part of the tissue, will get compressed the least. The deformation of collagen matrix will result unavoidably in the depth-dependent reorganization of the individual zone thicknesses. Our previous studies indicate that as a function of strain, the thickness of the (optical) 'superficial zone' expands at the expense of the (optical) 'radial zone' [4, 5].

This project has studied the territorial matrix of the chondrocytes, which consist of the collagen fibrils organized as ordered circular 'cocoons' around the clusters of chondrocytes in cartilage. One observation in this project is that the long axis of the cells closely follows the orientation of the local collagen fibrils. This similarity in the local orientations results in the change of the cell orientation between the superficial and radial zones. In addition, the shape of the cell was found to change differently at different strains - more changes in the superficial zone than in the radial zone, which agrees with the measurement of the depth-dependent tissue modulus in cartilage.

In conclusion, our results demonstrate that even though PLM does not have the resolution to visualize individual fibrils, this imaging technique is capable of detecting a group of collagen fibrils such as the circular 'cocoons' (territorial matrix of collagen fibrils) surrounding the chondrocytes. We have illustrated in this project the quantification of the morphological changes in compressed articular cartilage, in terms of the changes of cell shapes, cell orientation and fibril orientation surrounding the chondrocytes. These quantitative measurements could provide important information in clinical diagnosis when one examines the lesioned tissue, which will have a damaged (altered) fibril structure.

ACKNOLEDGEMENT

E Cicek thanks the Scientific and Technological Research Council of Turkey (TUBITAK) BIDEP-2219 for grant and Professor Y Xia for hosting him at Oakland University during his sabbatical. Y Xia is supported by the R01 grants from the National Institutes of Health (AR045172, AR052353). The authors are grateful to Drs. C Les and H Sabbah (Henry Ford Hospital, Detroit) for providing the canine specimens, and to Daniel Mittelstaedt for assistance.

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