

MECHANICAL LOADING OF IN SITU CHONDROCYTES IN A LAPINE RETROPATELLAR CARTILAGE AFTER ANTERIOR CRUCIATE LIGAMENT TRANSECTION

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INTRODUCTION

Early osteoarthritis (OA) changes the structural integrity of articular cartilage and results in softening, swelling, fibrillation and fissuring of the tissue [1]. These changes are thought to alter the mechanical environment of chondrocytes, thereby affecting chondrocyte mechanical response [2]. Although chondrocyte mechanics and biology have been studied in early OA, these studies were performed in isolated chondrocytes or for *in situ* cartilage preparations using histological analyses [3, 4]. Chondrocyte mechanics in OA tissue have not been measured in intact articular cartilage in real time. Therefore, the purpose of this study was to quantify chondrocyte mechanics in intact articular cartilage in healthy and early OA tissue.

METHODS

Patellar cartilage from twelve knees of 15 months old, skeletally mature female New Zealand white rabbits was used for chondrocyte mechanics analysis. Four knees were harvested nine weeks following Anterior Cruciate Ligament (ACL) transection, and the corresponding intact contralateral controls were harvested at the same time. Four additional knees were harvested from normal (ACL intact) rabbits. Retropatellar cartilages were grouped according to experimental (ACL-transected, $n = 4$) and control (contralateral, $n = 4$ and normal controls, $n = 4$), and were harvested with the intact patellae. Fluorescein conjugated dextran (excitation: 488nm, emission: 500nm. Molecular Probes, OR, USA) was suspended in DMEM (Dulbecco's Modified Eagle's Medium, Gibco, OR, USA) at a concentration of 0.8 mg/ml (0.26 mM). The patella was incubated in the dextran solution for 4-8 hours at 4°C prior to fluorescent confocal imaging.

Two MPa surface pressure was applied to the mid region of the medial side of the retro-patellar cartilage using a round glass indenter (diameter = 2 mm) at an average speed of 6 $\mu\text{m/s}$. Once the desired pressure was reached, the indenter displacement was held constant for 20 min when a steady state was reached [4]. Optical sections were recorded at before and after loading using a spacing of 0.5 μm in the z (optical) direction. The indentation system based on confocal microscope was developed specifically for the purpose of quantifying chondrocyte mechano-biology in the intact cartilage and has been described in detail previously [5] (Figure 1).

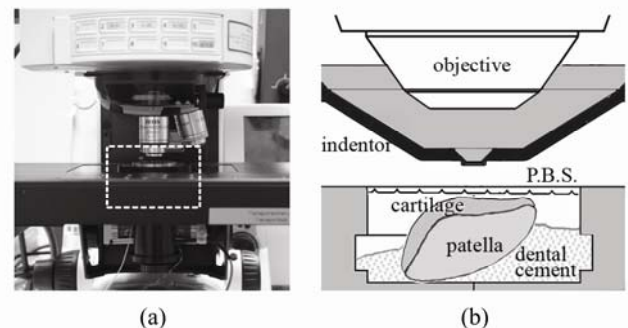


Figure 1: Custom-designed indentation system; (a) indentation system on confocal microscope, (b) schematic illustration for the area marked with the dashed line in (a).

Twelve cells from each patella (for a total of 48 cells each from experimental, contralateral and normal joints) were used for cell morphology analysis. Cells were located in the superficial zone of the rabbit retropatellar cartilage between the articular surface and 40 μm depth. Cell widths and depths were defined along the major and minor axis of the cross section taken perpendicular to the cell height, respectively.

After indentation testing, retropatellar cartilages in all three groups were assessed by histological analysis and graded according to the Mankin scoring system.

RESULTS AND DISCUSSION

Gross Morphology: Experimental joints showed symptomatic signs of early OA. Mankin scores for the proximal region of the retropatellar surface were significantly greater for the experimental compared to the contralateral and normal control tissues ($P < 0.05$), while they were not different for the middle and distal regions. Average cartilage thickness in the experimental joints ($803 \pm 139 \mu\text{m}$) was significantly greater than the contralateral ($674 \pm 47 \mu\text{m}$) and normal joints ($646 \pm 79 \mu\text{m}$) ($P < 0.05$).

Tissue Deformation: Average compressive tissue strains for 2 MPa surface pressure loading were similar for the experimental ($16 \pm 7\%$), contralateral ($16 \pm 3\%$) and normal tissue ($17 \pm 6\%$). However, average axial local extracellular matrix (ECM) strains were significantly greater for the experimental tissue ($38 \pm 8\%$) than the contralateral ($27 \pm 10\%$) and normal tissues ($28 \pm 8\%$) (Figure 2, $P < 0.05$). The average axial local ECM strains were also greater than the average cell strains in all experimental group tissues (Figure 2, $*** P < 0.005$). Average transverse ECM strains were also greater for the major and minor directions ($14 \pm 6\%$ and $4 \pm 4\%$, respectively) in the experimental compared to the contralateral ($8 \pm 6\%$ and $0 \pm 2\%$, respectively) and normal tissues ($5 \pm 4\%$ and $1 \pm 2\%$, respectively) (Figure 2, $P < 0.05$).

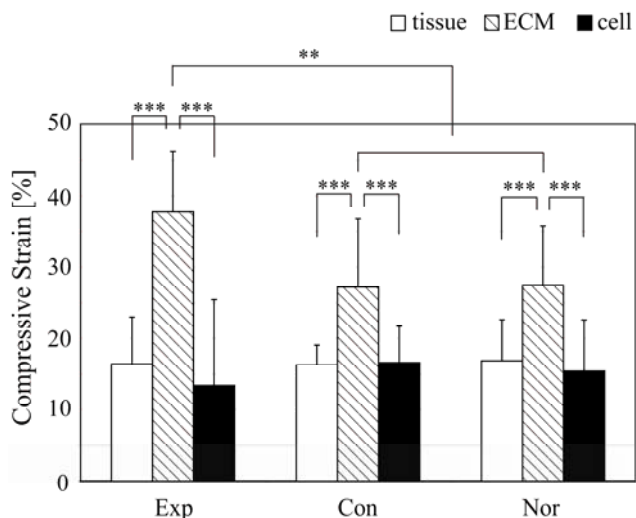


Figure 2: Compressive nominal tissue strains, extracellular matrix strains, and cell strains for

retropatellar cartilage from experimental, contralateral and normal knees ($n = 4$, $** P < 0.05$, $*** P < 0.005$).

Cellular Deformation: There was no significant difference in the average axial cell strains for the 2 MPa load application between the experimental ($13 \pm 12\%$), contralateral ($17 \pm 5\%$) and normal groups ($15 \pm 7\%$). However, average increases in cell width were greater in the experimental ($13 \pm 10\%$) than the contralateral ($7 \pm 13\%$) and normal joint cartilages ($6 \pm 6\%$) ($P < 0.05$, $P < 0.005$, respectively). Cell depth changes were similar in all three groups: $8 \pm 13\%$, $7 \pm 14\%$ and $6 \pm 6\%$ for the experimental, contralateral, and normal tissues, respectively. Following loading, average cell volumes increased in the experimental joints ($8 \pm 24\%$, $P < 0.005$), while they decreased in the contralateral ($-8 \pm 10\%$, $P < 0.005$) and normal joints ($-8 \pm 8\%$, $P < 0.05$). Before loading, average cell volumes in the experimental ($352 \pm 88 \mu\text{m}^3$) and contralateral joints ($349 \pm 66 \mu\text{m}^3$) were significantly greater than those observed in the normal joints ($302 \pm 73 \mu\text{m}^3$) ($P < 0.005$). Following loading, average cell volumes increased in the experimental joints ($8 \pm 24\%$, $P < 0.005$), while they decreased in the contralateral ($-8 \pm 10\%$, $P < 0.005$) and normal joints ($-8 \pm 8\%$, $P < 0.05$).

CONCLUSIONS

Based on the results of this study, we conclude that chondrocyte deformations following controlled loading of retropatellar cartilage differs qualitatively and quantitatively between normal and early OA cartilages.

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