Proteoglycan Content of the Cell Microenvironment Regulates Cell Deformation in Healthy Cartilage and This Is Impaired in Early Post-Traumatic Osteoarthritis

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INTRODUCTION: Joint injuries often involve continuous low-grade inflammation that may lead to increased chondrocyte catabolic processes, and possibly to the development of post-traumatic osteoarthritis (PTOA)[1]. Chondrocytes are surrounded by the pericellular (PCM) and territorial matrix regions, and this cell microenvironment has a significant role in regulating chondrocyte mechanotransduction and cartilage health[2]. Recently, we observed abnormal cell deformation under cartilage loading in the superficial zones of lateral and medial femoral condyles (LFC, MFC) and patella (PAT) in early PTOA, which could not be explained by changes in the collagen structure or proteoglycan (PG) content of the extracellular matrix (ECM)[3]. This led us to investigate if the abnormal cell deformation could be explained by increased PG content in the cell microenvironment relative to the ECM, possibly due to increased PG biosynthesis during early PTOA[4]. Here, we examined if the relationship between cell deformation and PG content of the cell microenvironment is altered in early PTOA.
METHODS: Unilateral anterior cruciate ligament transection (ACLT) surgery was performed on a random knee of skeletally mature female New Zealand white rabbits (*N*=8, study approved by the Committee on Animal Ethics at the University of Calgary). Two weeks post-surgery both the operated (*N*=8, ACLT) and contralateral (*N*=8, CL) joints were collected. Age-matched control knee joints (*N*=8, CNTRL) were also collected from randomly selected left or right knees of unoperated rabbits. **Cell deformation:** In the previous study[3] conducted on the same samples, confocal microscopy imaging with simultaneous *in situ* indentation testing was conducted on the intact joint cartilage surfaces of LFC, MFC, and PAT to evaluate the changes in the superficial zone cell morphology before and after loading[3] (Fig. 1, A.1, 40-70 viable cells). **Proteoglycan content:** Samples were then prepared from the main load-bearing areas, fixed in formalin, decalcified, dehydrated, and embedded in paraffin. A total of 3 histological sections (3 µm thick) per sample were stained with Safranin-O to quantitatively estimate the PG content. The superficial zone of each cartilage location was defined based on the average depth-wise collagen orientation angle profile of the CNTRL group using polarized light microscopy. In this study, microscopic digital densitometry (pixel size: 0.43×0.43 µm^2) was used to analyze the PG content (optical density, scaled from 0 to 3000) of the chondrocyte microenvironment (the PCM and surrounding ECM). The PG content of the chondrocyte microenvironment was averaged from a rectangular region of interest (ROI, height: 6 µm) that laterally extended 20 µm from the cell edge towards the ECM (Fig. 1, A.2, 30-50 viable cells). **Analysis:** The PG content of the cell microenvironment, presumably in the PCM, was taken as the maximum value from the averaged PG profile up to 5 µm from the cell edge. Extracellular PG content was obtained from the point 20 µm away from the cell edge. The PG content of the PCM with respect to the ECM was analyzed to highlight their relative difference. **Statistics:** A linear mixed-effects model was used to compare the pericellular and relative PG content between the animal groups in each cartilage location, and the PG content of the PCM and the ECM for each animal group. Pearson correlation coefficient was determined to investigate the animal-wise associations between the changes in the chondrocyte morphology and the measured pericellular, extracellular, and relative PG contents. The level of statistical significance was set to $\alpha = 0.05$.
RESULTS: We observed that the pericellular PG contents were 27.8% (LCF), 28.1% (MFC), and 32.2% (PAT) smaller in the ACLT group when compared with the CNTRL group (Fig. 1, B.1-B.3, darker-colored boxes). The relative PG content was elevated in the ACLT group of LFC and MFC, and the CL group of MFC when compared with the CNTRL group. Moreover, the pericellular PG content was greater than that of the ECM (dim-colored boxes) at each location and animal group. The pericellular PG content was associated with a smaller loss in cell volume in the CNTRL group of LFC (Fig. 1, C.1). In contrast, for the ACLT group of the same location, the relationship was lost (Fig. 1, C.2). A similar behavior was also observed at other cartilage surfaces. Linear relationships between the PG content of the cell microenvironment and change in cell morphology were mostly observed in the CNTRL groups, with individual correlations observed in the ACLT group of PAT cartilage (Fig. 1, C.3). Interestingly, greater PG content of the cell microenvironment was linked to a smaller loss in cell surface area in the CNTRL group of LFC. In contrast, the relationship was reversed in the ACLT group of PAT. In the CNTRL group of MFC, a negative relationship between the change in chondrocyte height and PG content of the cell microenvironment was observed, where a lower PG content was associated with a greater loss in cell height due to cartilage compression. In the CNTRL groups of both MFC and PAT, the extracellular PG content was seen to have a positive relationship with the change in cell width.
DISCUSSION: In the ACLT group of LFC and PAT cartilage, we previously reported a smaller cell volume loss due to loading when compared to the CNTRL and CL groups, respectively[3]. This may be explained by the lower pericellular PG content[5] we observed in the ACLT groups. In the LFC, the greater relative PG content could also have limited cell deformation. Additionally, in the ACLT group of PAT, the smaller PG content in the PCM was associated with an increase in cell surface area that may have translated to the smaller loss in overall cell volume. It has been suggested that higher pericellular PG content leads to a greater loss in cell volume following mechanical loading[5]. In contrast, we observed an opposite relationship between the pericellular PG content and the change in cell volume in the CNTRL group of the LFC. Though the animal-wise loss in cell volume was substantially greater in the CNTRL group of the LFC (-39% to -9%) as compared with the ACLT group (-18% to -2%), the observed relationship was surprising. This may suggest that in healthy cartilage, though the loss in cell volume was greater, the PG content of the cell microenvironment may restrict cell deformation and the relationship between the two cannot be analyzed only through a group-wide mean. These findings suggest that the PCM regulates cell deformation in healthy tissue according to local tissue composition (closely related to local stiffness) and that this regulation is disturbed during early PTOA (e.g., due to impaired force transduction between the matrix and the cell[6] or change in cell phenotype[7]).
SIGNIFICANCE/CLINICAL RELEVANCE: In healthy cartilage, the PG content of the cell microenvironment of superficial zone chondrocytes may regulate chondrocyte deformation during loading, a function that seems to be impaired in early PTOA.
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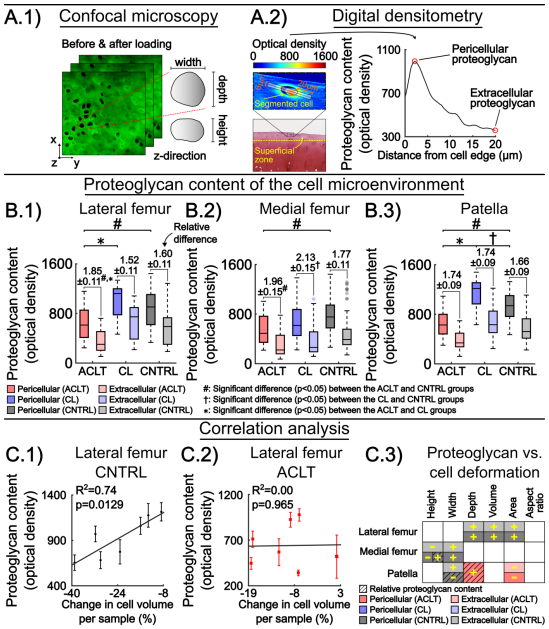

Figure 1. (A.1) Confocal microscopy imaging of a cell before and after loading and the measurement of cell width, depth, height, area, and volume; (A.2) light microscopy imaging of superficial cells, optical density (i.e., proteoglycan) image of a chosen cell, and analyzed values from the ROI; (B.1-B.3) proteoglycan content in the chondrocyte microenvironment in lateral and medial femoral condyle, and patella; (C.1-C.2) proteoglycan content of the pericellular region (±SEM) as a function of the change in cell volume in the CNTRL and ACLT groups of the lateral femoral condyle; (C.3) the observed positive (+) and negative (-) correlations between proteoglycan content of the cell microenvironment and change in cell morphology.