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

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). 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. Recently, we observed abnormal cell deformation under cartilage loading in the superficial zones of lateral and medial femoral condyles (LFC, MFC) and patella (CL) in early PTOA, which could not be explained by changes in the collagen structure or proteoglycan (PG) content of the extracellular matrix (ECM). This led us to investigate if the abnormal cell deformation could be explained by increased resistance to the ECM changes in the PCM. 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 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 CL, and we evaluated changes in the superficial zone cell morphology before and after loading (Fig. 1, A.1, 40-70 viable cells). Proteoglycan content: Samples were then prepared from the main load-bearing areas, fixed in formalin, decalified, dehydrated, and embedded in paraffin. A total of 3 histological levels were analyzed microscopically (3 µm thick) per sample, stained with Safranin-O to quantitatively estimate the PG content. The superficial zone of each cartilage preparation 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) 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 the 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 α = 0.05.

RESULTS: We observed that the pericellular PG contents were 27.8% (LFC), 28.1% (MFC), and 32.2% (CL) 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 in the pericellular PG content was greatest in ACLT group of LFC, followed by 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 possible link 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. This may be explained by the lower pericellular PG content in ACLT 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 at each location and in the pericellular PG content was greatest in ACLT group of LFC, followed by 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 possible link with the change in cell width.

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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