

Spatial Proteoglycan Content of Chondrocyte Microenvironment is Greater in Very Early Post-Traumatic Osteoarthritis Compared to the Neighboring Matrix in Site and Zone-Dependent Manner

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INTRODUCTION: Joint injuries can lead to tissue inflammation and to the development of post-traumatic osteoarthritis (PTOA) [1]. It is currently believed that a pericellular matrix (PCM) and territorial extracellular matrix (ECM), the so-called cell microenvironment, surrounding chondrocytes have a significant role in regulating cell mechanotransduction and tissue health [2]. Furthermore, it has been suggested that the proteoglycan (PG) content of the PCM degrades later than that of the surrounding ECM in early PTOA, possibly due to upregulated biosynthesis of the chondrocytes [3]. In a previous study, we observed changes in cell deformation of superficial zone chondrocytes in lateral and medial femoral condylar and patellar cartilage, but not in lateral and medial tibial plateau (LTP, MTP) and femoral groove (FGR) cartilage, following physiologically relevant loading in a rabbit model of early PTOA [4]. In the following study [5], we analyzed the PG content of the cell microenvironment of lateral and medial femoral condylar and patellar cartilage and observed that the PG content of ACLT cartilage degraded at similar rates with the ECM in the superficial zone compared to controls while the degradation progressed slower in the middle zone cartilage. Here, our objective was to analyze the PG content of the cell microenvironment of LTP, MTP, and FGR cartilage to further elucidate the understanding of how very early PTOA modulates the PG content of the cell microenvironment.

METHODS: Samples: In this work, we reanalyzed histological sections of rabbit knee cartilage, previously studied in [4] and [5], extending the analysis to the cell microenvironment of knee cartilage sites (LTP, MTP, and FGR) omitted in the previous studies. PTOA was induced in the test subjects with unilateral anterior cruciate ligament transection (ACLT) surgery. Operated ACLT ($N=8$) and contralateral CL ($N=8$) knees were collected 2 weeks post-surgery together with samples from unoperated control animals (CNTRL, $N=8$). LTP, MTP, and FGR cartilages were examined. The experimental protocol was approved by the Committee on Animal Ethics at the University of Calgary. **Workflow:** From each site and sample, three 3 μm thick histological sections were prepared from the main load-bearing areas and stained with Safranin-O. The superficial and middle cartilage zones were determined from adjacent unstained sections of CNTRL group samples using polarized light microscopy. Finally, on average 130 viable and isolated cells were imaged per measurement group in each cartilage site and zone with digital densitometry (pixel size: $0.43 \times 0.43 \mu\text{m}^2$) to determine the optical density (an estimate of the PG content) of the cell microenvironment (PCM and territorial ECM). **Analysis:** The optical density (PG content) profiles were calculated using a rectangular region of interest (ROI, height: 6 μm , width: extended 20 μm into the ECM from the cell edge) aligned parallel to the cell width (Figure 1, A). First, the maximum PG content was calculated from the cell microenvironment near the cell (distance from cell edge < 5 μm , region containing the presumed PCM). Second, the profiles were normalized to the last point of the profile (located in the ECM), to determine differences in the PG content between the cell microenvironment and ECM. A linear mixed-effect model was used to compare the maximum values and normalized profiles between all groups (significance level: $p < 0.05$).

RESULTS: Superficial zone: The maximum PG content of the cell microenvironment was 26% smaller in the ACLT group compared to the CNTRL group in both LTP and MTP cartilage (Figure 1, B). On the other hand, the maximum PG content in the CL group was 22% and 19% greater compared to the CNTRL group in MTP and FGR cartilage, respectively, and was also greater compared to the ACLT group at each site. Normalized PG content of the ACLT group was observed to be greater in LTP and FGR cartilage compared to the CL and CNTRL groups, while MTP cartilage exhibited no clear differences between the groups (Figure 1, C-E). The normalized PG content of the CL group was smaller near the cell edge compared to the CNTRL group in LTP cartilage. **Middle zone:** The maximum PG content was 9%, 11%, and 17% smaller in the ACLT group compared to the CNTRL group in LTP, MTP, and FGR cartilage, respectively. The maximum PG content of the CL group was observed to be greater only in MTP cartilage, on average 23%, compared to the CNTRL group. The maximum PG content in the CL group was greater compared to the ACLT group for each site. The normalized PG content was greater in the ACLT group compared to the CNTRL group in LTP and FGR cartilage and compared to the CL group at all sites (Figure 1, F-H). The normalized PG content of the CL group did not significantly differ compared to the CNTRL group, except only in MTP cartilage, where the CL group had a smaller normalized PG content compared to the CNTRL group.

DISCUSSION: We observed that after two weeks post-ACLT, the PG content of the cell microenvironment of ACLT knees was significantly smaller in superficial and middle zones of LTP, MTP, and FGR cartilage compared to the CNTRL animals. This was consistent with our previous findings for the lateral and medial femoral condyle and patella [5]. We also observed that the normalized PG content of the cell microenvironment was greater in the ACLT groups compared to the CNTRL groups for the superficial and middle zones in LTP and FGR cartilage. This finding differs from our previous results in medial and tibial femoral condylar and patellar cartilage [5] where the major changes were mostly seen in the middle zone. The normalized PG content did not differ between the CL and CNTRL groups in the middle zone, except in the MTP cartilage, where the normalized PG content of the CNTRL group was greater compared to the CL group. This difference may be caused by spontaneous OA observed in the CNTRL group rabbits [4] ($\text{OARSI}_{\text{CNTRL}} = 9$; $\text{OARSI}_{\text{ACLT}} = 5$; $\text{OARSI}_{\text{CL}} = 5$). This also suggests that the ACLT-related increase in the normalized PG content may originate from changes in joint loading.

SIGNIFICANCE/CLINICAL RELEVANCE: Our observations suggest that chondrocytes upregulate PG biosynthesis to the cell microenvironment in very early PTOA and the extent of this change seems to be cartilage site and zone-dependent, possibly due to different local loading environments within the tissue.

REFERENCES: [1] Lieberthal *et al.*, *Osteoarthr. Cartil.*, 23:1825-1834, 2015. [2] Zhao *et al.*, *J Cell Mol Med.*, 24:5408-5419, 2020. [3] Ojanen *et al.*, *PLoS One*, 13(4): e0196203, 2018. [4] Ojanen *et al.*, *J. Biomech.*, 98:109450, 2020. [5] Hiltunen *et al.*, *Trans. Orthop. Res. Soc.* 48:1421, 2023.

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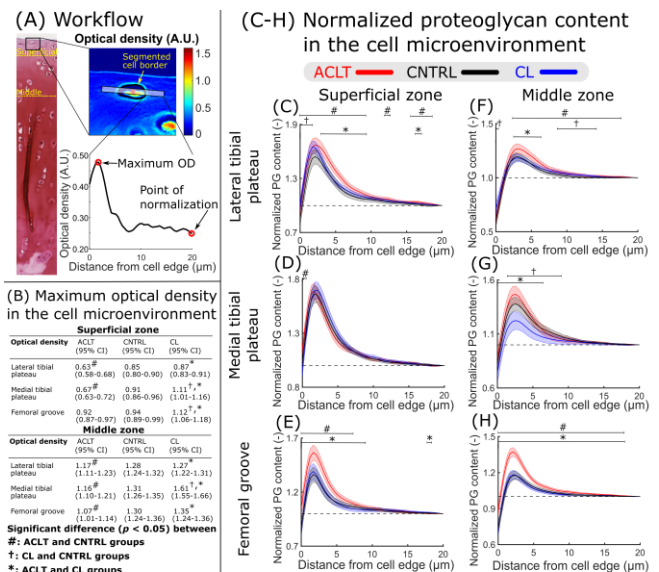


Figure 1. (A) Light microscopy image from a lateral tibial plateau control group sample, optical density (OD) image of a superficial zone cell, and a right side OD profile of the cell microenvironment; **(B)** Maximum pericellular OD values (PG content) with 95% confidence intervals for all locations (lateral tibial plateau, medial tibial plateau and femoral groove), zones (superficial and middle) and groups (operated (ACLT), control (CNTRL) and contralateral (CL)); **(C-H)** Normalized mean proteoglycan (PG) profiles (optical density profiles measured with digital densitometry) for all locations (lateral tibial plateau, medial tibial plateau and femoral groove) and zones (superficial and middle), and groups (operated (ACLT, red), control (CNTRL, black) and contralateral (CL, blue)). Shaded areas correspond to 95% confidence intervals.