Changes in the Local Proteoglycan Content of the Pericellular Matrix are Highly Site-Specific and Related to Cell Deformation Behavior 3 Days after a Partial Meniscectomy in the Rabbit Knee Joint

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Introduction: Chondrocytes (i.e. cells in articular cartilage) maintain articular cartilage health. If knee joint function is impaired, stresses and strains that chondrocytes experience are altered. This, in turn, can alter their synthetic activity and knee joint homeostasis [1]. In our previous study, chondrocyte deformations under indentation loading were observed to be altered 3 days after a partial meniscectomy in a site-specific manner [2]. Proteoglycan (PG) loss from the superficial layers of cartilages was observed in sites where cell deformations were altered [3]. However, the 'global' PG analysis could not explain our cell level findings. We hypothesize that alterations in the PG content of the pericellular matrix (PCM) compared to that of the extracellular matrix (ECM) can explain the chondrocyte behaviors in meniscectomized joint cartilages. To test this hypothesis, the PG content around superficial zone cells was investigated from six different rabbit knee joint locations following partial meniscectomy.

Methods: Fifteen skeletally mature female New Zealand rabbits (13 ± 1 month) were assigned into a control group of five rabbits and a surgical group of ten rabbits [2]. Each knee in the control group was used and one knee from each rabbit in the surgical group was randomly chosen to receive a partial lateral meniscectomy, where ~ 6-10 mm of the anterior horn of the meniscus were removed. Rabbits were sacrificed three days after surgery and had their knee joint tissues harvested from the patella, groove, lateral and medial femoral condyle and tibial plateaus. These procedures were conducted according to the guidelines of the Canadian Council on Animal Care and were approved by the committee on Animal Ethics at the University of Calgary. A confocal microscope with custom indentation system was used to image superficial chondrocyte deformations due to tissue deformation (2 MPa, held for 20 minutes) [2]. Six histological sections (thickness 3 µm) were cut from each site that cells were imaged from. Sections were stained with Safranin-O, which binds to the glycosaminoglycan side chains of the proteoglycans [3]. Images of the sections were captured with a light microscope equipped with a CCD cooled camera using a ×40 objective under monochromatic illumination (492 ± 5 nm).

The superficial zone was determined by cell shape (oblate) and cartilage depth. Optical density (OD) profiles, reflective of PG content, were calculated around selected cells (average 4 cells/section, totaling n = 1440 and n = 1398 for experimental and control groups, respectively) in both horizontal and vertical directions for manually placed regions of interest, extending approximately 6.5 µm into the ECM from the cell edge (figure 1A). Profiles were averaged for each section and normalized to the first point of the ECM (figure 1B). Both normalized profiles and raw absolute value profiles were compared point-by-point between the groups using independent samples Students t-test (at a level of significance of $p < 0.05$). Statistical tests were performed and PG content profiles were calculated with a custom MATLAB script (R2014a, MathWorks Inc., Natick, Massachusetts, United States).

Results: Chondrocyte volumes due to indentation decreased more in experimental group (n = 580) animals compared to the control (n = 570, $p < 0.05$) for the patella, femoral groove and lateral femoral condyle region, whereas smaller volume decreases ($p < 0.05$) were observed for the medial femoral condyle and medial tibial plateau. Lateral tibial plateau cells seemed unaffected by meniscectomy ($p > 0.05$). Absolute PG content around the cells was decreased 3 days after a partial meniscectomy at all tissue sites ($p < 0.05$) except for the lateral tibial plateau ($p > 0.05$). The normalized PG content around the cells in the transverse direction (figure 1B) was significantly higher ($p < 0.05$) in the experimental group animals at all sites except for the lateral tibial plateau ($p > 0.05$). Similar changes were observed in the axial direction (data not shown), with the exception of the femoral groove tissues where no differences were observed between the experimental and control group animals ($p > 0.05$). Elevated levels of PG content extended up to 5 µm into the ECM at the medial condyle and medial plateau regions, whereas elevated PG contents only reached a maximum of 2.5 µm for the remaining sites (figure 1B).

Discussion: In the medial femoral condyle and medial tibial plateau cartilages, meniscectomy caused more excessive changes in the normalized PG content (PCM with respect to ECM) than at any of the other sites. Only at these sites, the chondrocyte volumes were previously reduced less due to indentation than in the control group. Similar cell volume behavior has been observed previously at later stages of osteoarthritis [5]. This may point to an increased level of PG synthesis from chondrocytes, which might indicate that chondrocytes attempt to maintain or repair their surroundings. The patella, groove and lateral femoral condyle tissues had also increased normalized PG content in the PCM, but this did not extend as far into the ECM. This smaller response may indicate that repair at these sites has started, but has not yet reached the ECM, which suggests that the cell response did not progress as fast at these sites and may not necessarily be related to the onset of osteoarthritis. The greater levels of normalized PG content extending further into the tissue in the medial regions also suggests that the collagen network may be disrupted, and that PGs can more readily move into the ECM. It is also important to note that the lateral tibial plateau tissues did not show changes in PG content and that the cell responses observed at this site were similar between the groups.

Significance: Pericellular proteoglycan content of rabbit knee joint cartilages (with respect to that of the ECM) was found to be related to altered cell deformation behavior in a site-specific manner just 3 days after a partial meniscectomy. This finding could indicate an altered biological response of chondrocytes shortly following intervention, which could be targeted with therapeutic drugs to enhance transfer of PGs from the PCM into the ECM.

References: [1] Goldring and Marcu, Arthritis Research & therapy 11, 2009. [2] Fick *et al*. ORS 2014 Annual Meeting, Poster: 1295. [3] Ronkainen *et al*. ESB 2015 Annual Meeting, Abstract: O 187. [4] Kiviranta *et al*. Histochemistry 82, 1985. [5] Turunen *et al*. Osteoarthritis and Cartilage 21, 2013.

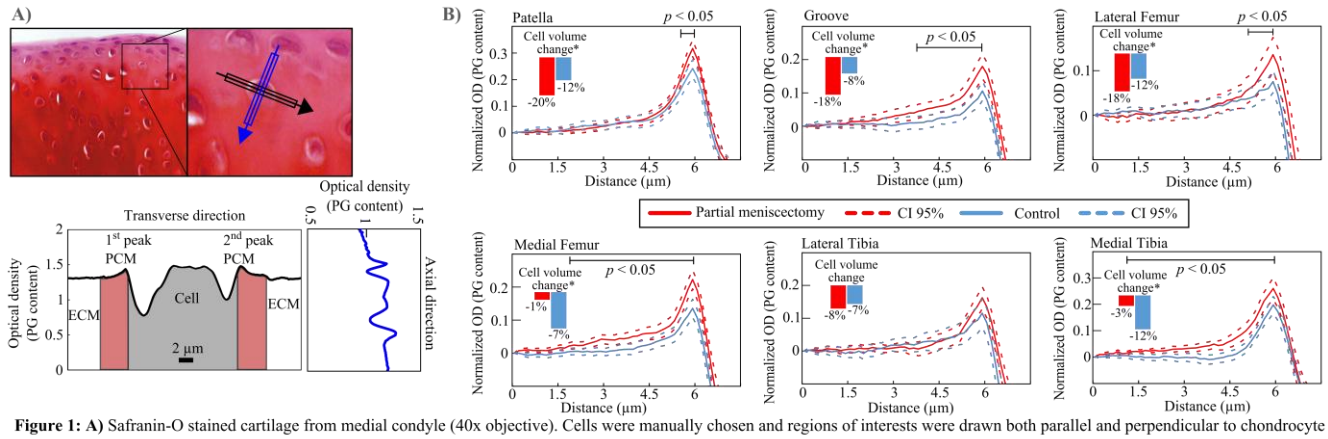

Figure 1: A) Safranin-O stained cartilage from medial condyle (40x objective). Cells were manually chosen and regions of interests were drawn both parallel and perpendicular to chondrocyte main axis (width). Resulting profiles in transverse and axial directions are shown, highlighting the different parts of the cell and surrounding matrix. **B)** Average PG content graphs from the transverse direction for each studied location and previously measured cell volume changes. Shown is the first peak (see A), which corresponds to the middle of the PCM. Shown profiles are normalized to the first point of the ECM. Dashed lines are 95% confidence intervals and black bars and stars (*) denote significant ($p < 0.05$) differences between the two groups.