Topographical Investigation Of Changes In Depth-wise Proteoglycan Distribution Of Rabbit Articular Cartilage 4 Weeks After Transection Of The Anterior Cruciate Ligament

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Introduction: Anterior cruciate ligament transection (ACLT) is a surgical procedure that is typically used to study osteoarthritis (OA) in animals [1-3]. ACLT in rabbits produce early degenerative changes in knee joint cartilages, which are representative of those changes found in human OA [1]. Previous studies investigating the effect of ACLT on rabbit knee cartilages have shown that proteoglycan (PG) content are already altered at 4 weeks after transection [2, 3]. However, these studies have focused on one, singular, anatomical position in either femoral, tibial or patellar cartilages within a parasagittal plane on these joint surfaces. To our knowledge no information is available on the changes in PG distribution across the femoral condyle (FC) cartilage surface at positions both anteriorly and posteriorly located away from the apex of the posterior curvature on the FC, especially as a function of tissue depth and over a short time period (i.e. 4 weeks) after an ACLT. Therefore, the aim of this study was to explore the topographical PG distribution of cartilage tissue in early OA, as acquired from both the lateral and medial condyles of ACLT and contralateral (CNTRL) rabbit knee joints, 4 weeks post operation.

Methods: Tissue sections of both the lateral and medial FCs were obtained from a previous study in which the knee joints of eight skeletally mature, female New Zealand white rabbits (aged 14 months at the time of euthanasia) were utilized [3]. As previously described, each rabbit was sacrificed 4 weeks following a unilateral ACLT [3]. The FCs from the unilateral ACLT knee joints formed an ACLT group, while the FCs from the other knee formed a CNTRL group. ACLT procedures for rabbits were approved by the Animal Ethics committee at the University of Calgary and the guidelines of the Canadian Council on Animal Care [3]. The knee joint groups were dissected and immersed into PBS and then shipped to Kuopio (Finland) in formalin fixative and were histologically prepared [3]. Briefly, the FCs were fixed in 10% buffered formalin, decalcified in ethylenediaminetetraacetic acid (EDTA), dehydrated in alcohol, treated with xylene, embedded in paraffin, and 3 μm thick sections were stained with Safranin O [3]. The staining enabled us in this current study to determine PG content through optical density measurements [3, 4] and quantify the optical absorbance values through digital densitometry (DD). Two tissue sections per condyle (i.e. both the lateral and medial condyles) from each knee joint were used for the analysis. Low magnification (x1) gray-scale images (Fig. 1a) were taken from the tissue sections of the entire cartilage surface and converted into color maps by using a custom Matlab script (R2007b, The Mathworks Inc., USA). The color maps were then manually line segmented to five to seven segments and 4 sites were selected across each FC (Fig. 1b) to investigate the depth-wise PG content at higher magnification (x5). The apex of the posterior curvature of the cartilage across each FC was selected as site ‘B’ and corresponded to the most load-bearing region on the rabbit FC [5]. The mid-span of the adjacent line segments were named as sites ‘A’, ‘C’ and ‘D’, totaling 4 sites. Site ‘A’ was defined as the position that corresponded with the mid-span of the posteriorly located line segment adjacent to site ‘B’. Site ‘C’ represented the position that corresponded with the mid-span of the anteriorly located line
segment adjacent to site ‘B’. Site ‘D’ represented the position that corresponded with the mid-span of the more anteriorly located line segment adjacent to site ‘C’. Using the higher magnification images, the PG analyses were then performed at these 4 sites. A rectangular area according to the tissue height (from the osteochondral junction to the tissue surface) and a preset width (187.5 μm) was manually selected for every sample (Fig. 1c). Based on successful segmentation of the FCs, the values obtained from seven to eight contralateral (CNTRL) samples from both the medial and lateral condyles, respectively (between 14 and 16 tissue sections, respectively) and eight ACLT samples from both the medial and lateral condyles (16 tissue sections per condyle) had their optical density values averaged together. These values were plotted as a function of normalized tissue depth for each site in the CNTRL and ACLT groups. To ensure that each site, based on the manual segmentation of the low magnification images was performed similar across the CNTRL and ACLT groups, the lineal distances between sites B-A, B-C and B-D were determined. A one way ANOVA test confirmed that the distances between the medial and lateral compartments from the CNTRL and ACLT groups were statistically similar (p > 0.05). Student’s t-tests were then used to determine depth-wise differences in PG content between sites A-D from both the lateral and medial compartments of the CNTRL and ACLT groups. All statistical tests were performed with SPSS (SPSS Inc, Chicago, IL, USA; p < 0.05).

**Results:** In the lateral compartment (Fig. 2), site C had the greatest difference (compared to other sites) between the ACLT and CNTRL groups, with a decrease in PG content due to ACLT, observed up to 48% depth from the cartilage surface. Site D exhibited a smaller difference between the groups (when comparing to site C) and had a decrease in PG content up to 22% depth from the surface. Sites A and B had the smallest differences between the groups, with an observed decrease in PG content up to 20% of tissue thickness. In the medial compartment (Fig. 3), site B experienced the greatest changes due to an ACLT, with a decrease in PG content observed up to 28% of tissue thickness. Site C showed a decrease in PG content up to 25% depth from the tissue surface. Site D experienced a decrease in PG content up to 14% depth from the surface. Site A had the smallest difference between the groups and was observed to have a decrease in PG content only up to 5% depth.

**Discussion:** The current study showed that the decrease in the superficial/middle PG content of cartilage as a result of ACLT (4 weeks after operation) varies both in the anterior-posterior plane and in the medial and lateral femoral condylar compartments. PG content varied from having the smallest changes (due to ACLT) at the posteriorly located site in both the lateral and medial condyles (i.e. site A, Figs. 2&3). The largest changes in PG content were observed in the site anteriorly adjacent to the posterior apex in the lateral condyle (site C, Fig. 2) and in the posterior apex of the medial condyle (site B, Fig. 3). Because an ACLT alters the loading patterns across the lateral and medial condylar surfaces [6], these results suggest that the loss of PG content (and hence the development of early OA) in cartilage varies at different sites on the femoral condyle.

**Significance:** Proteoglycan loss of cartilage as a result of ACLT of rabbit knee joint (early OA) varies differently across the medial and lateral condylar joint surfaces. This is important to consider when using the ACLT model in order to investigate the pathology of OA.
Fig. 1: a) A representative grayscale image of a lateral femoral condyle tissue section from a CNTRL joint; b) a colorized optical density image obtained from Fig. 1a with the ROIs (regions of interest) identified as sites A-D; c) higher magnification images of sites A-D from Fig. 1b.
Fig. 2: PG content from sites A-D in the lateral compartments of the CNTRL and ACLT groups plotted as a function of normalized tissue depth (from the tissue surface to the cartilage-bone junction). O.D. = optical density. Shaded regions indicate statistical differences between groups ($p < 0.05$).
Fig. 3: PG content from sites A-D in the medial compartments of the CNTRL and ACLT groups plotted as a function of normalized tissue depth (from the tissue surface to the cartilage-bone junction). O.D. = optical density. Shaded regions indicate statistical differences between groups ($p < 0.05$).