IN-SITU CHONDROCYTE DEFORMATION IN ENDSTAGE OSTEOARTHRITIC (OA) ARTICULAR CARTILAGE

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INTRODUCTION
Chondrocytes and their nuclei deform throughout the depth of articular cartilage in proportion to the magnitude of applied compression (Clark et al., 2000a, Guilak et al., 1995). Furthermore, deformation alters the biosynthetic activity of normal chondrocytes in vitro (Wong et al., 1997). Little is known about how compression influences the magnitude and local variation of chondrocyte deformation in situ, and if these parameters change with disease. Our aim was to evaluate variations in chondrocyte deformation throughout intact specimens of OA cartilage subjected to uniform static compression.

METHODS
One skeletally mature male cat (mass 8.3kg) was studied, the first of a series of longterm animals. Anterior cruciate ligament transection (ACL-T) was carried out on the experimental hindlimb (Herzog et al., 1993) and the animal was allowed free movement until sacrifice at 57 months post ACL-T. Hindlimbs were removed immediately after sacrifice and inspected for degeneration before the experimental femur and patella were prepared for loading. A cylindrical, 1mm diameter, flat, non-porous indentor was used to apply a local surface pressure of 15MPa (value typical of cat gait) to the middle of the specimen at a rate of 4µm/s. The cartilage was allowed to relax before being immersed in ruthenium hexammine trichloride fixative solution (Clark et al., 2000a) for 2 hours. Full thickness osteochondral blocks (3mm x 1mm) were harvested from the same anatomical sites in experimental and contralateral specimens. These samples were then embedded and 0.6µm thick sections were cut and stained with toluidine blue for light microscopic examination. One section from the center of each indent (OA indent) and a further section 1mm away in the posterior/anterior direction (OA control) was photographed and the resulting images analyzed using computer software. Tissue depth, cell aspect ratio (height/width) and volumetric fraction (using point counting) were evaluated.

RESULTS
The results from this experiment are shown together with the results obtained from an identical experiment (n=3) using healthy articular cartilage (Clark et al., 2000a) for comparison. Inspection of the hindlimbs revealed a grossly normal contralateral knee and a degenerated experimental joint (Clark et al., 2000b). The articular cartilage of the experimental patella was thickened (Figure 1), and the matrix staining intensity was

Figure 1: Histological slides of (a) healthy and (b) experimental OA patellar articular cartilage. Both sections cut at 0.6µm thickness, stained with toluidine blue and magnified 25x.
decreased in the top 40% of patella cartilage indicating a decrease in proteoglycan concentration. These changes were not observed in the experimental femur.

Static compression decreased the aspect ratio of chondrocytes throughout the depth of both tissues (Figure 2). This decrease was similar for OA and healthy femoral articular cartilage however differed both in magnitude and tissue depth penetration in patellar articular cartilage. Due to the rounded chondrocytes in the surface zone of experimental patella tissue, the magnitude of aspect ratio change was more than double that found in healthy tissue in this zone. Furthermore, the tissue depth penetration of aspect ratio change is 80% in healthy patella cartilage though only 50% in OA tissue.

![Figure 2](image_url)  
**Figure 2**: Graphs of linearly transformed chondrocyte aspect ratio (CAR) \(y = 3.33 \times \ln[\text{CAR}] + 10.22\) as a function of cartilage depth for (a) femoral groove and (b) patella tissues. Each graph compares healthy and OA tissue in the unindented control and indented state. Values of CAR have been averaged (±SD) within 5 or 10% bins throughout the cartilage depth so that n>10 cells for each bin.

![Figure 3](image_url)  
**Figure 3**: Graphs of chondrocyte volumetric fraction as a function of cartilage layer for (a) femoral groove and (b) patella tissues. Each graph compares healthy and OA tissue in the unindented control and indented state. Values of volumetric fraction for the healthy tissue have been averaged over three subjects and are shown with their standard deviations.
Inspection of Figure 3 reveals a decrease in chondrocyte volumetric fraction in the surface layer of both indented and unindented femoral and patellar cartilage with OA. This decrease is most pronounced (60%) in the patella tissue (Figure 3b). The middle and deep layers of femoral and patellar articular cartilage appear to be affected to a lesser extent.

DISCUSSION

The results of this study indicate that, in loaded patella OA cartilage, chondrocytes near the articular surface flatten more while those in the deeper layer are flattened less than chondrocytes in healthy tissue. It has been shown that the elastic modulus of isolated human chondrocytes is not altered with endstage OA disease (Jones et al., 1999) which, in view of our results, implies that the cartilage matrix properties dictate the in situ deformation behaviour of chondrocytes. We speculate that changes in chondrocyte volumetric fraction, cartilage thickness, collagen fiber orientation, and collagen and proteoglycan concentration are primary determinants of the local articular cartilage strain response to load. Thus, the significant alterations in the mechanical and architectural properties of OA cartilage influence the deformation, and presumably the metabolic response of chondrocytes in situ. Our results also suggest that the adaptation of articular cartilage with the development of OA is dependent upon its position within the knee joint. Specifically, patella articular cartilage showed significant changes in depth, proteoglycan concentration and chondrocyte aspect ratio with OA, however these adaptations were not present in the femoral groove tissue. We further speculate that it is the differences in the mechanical and architectural properties of cartilage across the knee joint and thus the strain response, and the metabolic response of the chondrocytes that give rise to this site specific adaptive behavior. For example, differences in cartilage thickness, chondrocyte volumetric fraction and collagen fiber and proteoglycan concentration between femoral groove and patella cartilage may play a role.

REFERENCES


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