INTRODUCTION
Articular cartilage is essential for proper joint health and function, providing joint surface lubrication and load transmission across joint surfaces. The cells within articular cartilage – called chondrocytes – are responsible for maintaining the health and integrity of the tissue extracellular matrix (ECM) by synthesizing structural macromolecules. Chondrocyte activity under mechanical loading has been linked to the adaptive/degenerative processes in the joint which lead to osteoarthritis (OA) [1]. The purpose of this study was to investigate the deformation behavior of chondrocytes in their native environment using a novel in situ experimental approach [2] and compare cells from different regions of the knee joint. It was hypothesized that (i) chondrocyte deformation would increase with increasing tissue strain up to a threshold value (approximately 25% nominal compressive strain), but then, for increasing tissue strains, cells would not deform further because of limits imposed by the extra- and peri-cellular matrices (PCM); and (ii) chondrocytes from different regions will deform differently for a given nominal tissue strain.

METHODS
Sample preparation: Intact knee joints were extracted from 6 month old New Zealand White rabbits immediately after sacrifice. Tissue samples were harvested from three regions of the joint: the retropatellar surface (PAT), and the medial and lateral femoral condyles (MFC, LFC). Calcein-AM (Invitrogen, excitation: 488 nm; emission: 515 nm) was suspended in serum-free DMEM at a concentration of 5 uM. The tissue samples were incubated in the calcein-AM solution for 1 – 2 hours at 21°C prior to testing.

Mechanical Testing: After staining, samples were imaged in a specimen holder and tissue thickness was measured using the needle indentation technique. Then, samples were placed in an in situ loading system mounted to the stage of a confocal microscope [2] and immersed in PBS for the duration of testing. A series of static compressive loads were applied to the tissue samples in the following order: 10%, 20%, 30%, 40%, 60%, and 80%, using a custom-designed indentation system at an average rate of 1%/s. The tissue was given 15 minutes to equilibrate after each compressive load was reached. Confocal image sections were recorded before and each load after the tissue had reached equilibrium. Nominal tissue strain, local ECM strain in the superficial zone, and cell deformations were analysed to quantify the overall mechanical response of the tissue. For cell reconstruction, height was defined as the dimension perpendicular to the cartilage surface and width and depth were defined as the two principle axes in the transverse plane (parallel to the cartilage surface).

RESULTS
Cell volume changed with applied loading and these changes varied with joint region (Figure 1). Chondrocytes from patellar cartilage appeared to gain volume at steady-state after compressive loading, whereas cells from the medial and lateral femoral condyles decreased in volume.

DISCUSSION AND CONCLUSIONS
These results provide new insight into the deformation behavior of chondrocytes from different regions of the intact rabbit knee joint. Mechanically, superficial zone cells from patellar chondrocytes appear to behave differently compared to cells from the same zone in medial and lateral femoral condyles (Figure 1). These results are consistent with previous studies reporting major differences in structure and biomechanical properties between patellar and femoral condyle cartilages [3]. We speculate that this is a reflection of the functional requirements of the cartilage tissues and the variable mechanical environment within the knee joint.

REFERENCES

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