In Situ Site-specific Determination of Chondrocyte Responses to Deformation in a Mature Rabbit Knee Joint

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Introduction: Previous research has demonstrated that superficial zone chondrocytes in intact rabbit patellar and femoral knee joint cartilage respond differently to deformation [1]. This is important as these joint tissues experience different loading patterns and any change to the way with which the joint tissue is loaded will not only affect its cellular biomechanics, but also the cellular biosynthesis and the mechanobiology of the joint tissue. However, there is no study that has fully examined different contacting surfaces and how chondrocytes from these tissues respond to deformation within the rabbit knee joint. Our hypothesis was that superficial zone chondrocytes from different contacting surfaces in the rabbit knee joint respond differently to deformation. We studied this hypothesis by using an indentation system [2] attached to the stage of a confocal laser scanning microscope (CLSM) for intact rabbit knee joint cartilage attached to its native bone.
Methods: Five skeletally mature female New Zealand white rabbits (13 ± 1 month) were sacrificed and the knee joint tissues were harvested. This procedure was carried out according to the guidelines of the Canadian Council on Animal Care and was approved by the committee on Animal Ethics at the University of Calgary. To achieve flat contacting surfaces for testing, excess non-cartilaginous tissues were stripped of while the joint surfaces and underlying bone were kept intact. The femoral groove tissue was further prepared by carefully removing the lateral aspect, and the medial and lateral tibial plateau compartments were carefully removed from the tibia and were cut into ~6x4 mm cartilage-on-bone blocks. Joint tissues were stained with conjugated fluorescent Dextran at 4°C for 5 hours prior to testing. Samples were mounted with dental cement into a custom built sample holder. Patellae were loaded on the lateral aspect, midway between the proximal and distal poles; groove tissues were loaded midway between the proximal and distal ends. Condyles were loaded on their summits and loading on the lateral and medial plateaus was applied to an area that was shifted 1mm laterally from the block center. All joint tissues were tested within 40 hrs of sacrifice.
CLSM: A custom-designed in situ indentation system [2] mounted to the stage of a confocal laser scanning microscope (LSM 510, Zeiss Inc.) was utilized for testing. A light-transmissible glass indenter (φ = 2mm) mounted on the stage with a x40-magnification objective (40 x 0.8 NA water-immersion objective, Zeiss Inc.) was used to capture images of the same chondrocytes before and after deformation. Before deformation, a tare load was used (0.1-0.2 MPa) to ensure initial contact between the indenter and cartilage surface. A pressure of 2 MPa was applied to the sample at an average speed of 10µm/s. After reaching the desired stress level, the displacement was held constant for 20 min. Image stacks parallel to the cartilage surface (x-z plane 512 x 512 pixels, pixel size 0.41µm x 0.41µm) were obtained with 0.5µm vertical y-axis increments up to 60µm in depth from the cartilage surface. Image stacks were then reconstructed with the Visualization Toolkit 5.2.0 (Kitware Inc.) to render 3D-images of the cells and a code programmed with Python was used to calculate cell volume. Matlab (MathWorks Inc.) was used to calculate cell height, width and depth.
Statistics: After assessing the normality of pooled site-specific data (Kolmogorov-Smirnov test; $p < 0.05$), the percent change in both cell volume and cell dimensions (i.e. height, width and depth) due to deformation were determined for all tissue sites (mean±standard deviation). ANOVA and Tukey Post Hoc tests were performed in order to compare differences among sites ($p < 0.05$).
Results: As a result of cartilage compression, cells from the patellae exhibited a larger volume change with larger changes in both cell height and width when compared to cell volume and cell dimensional changes from femoral groove tissues ($p < 0.05$). Patellae cells also displayed larger volume changes with larger changes in both cell height and width when compared to both cell volume and cell dimensional changes from lateral plateau tissues ($p < 0.05$). Cells from the groove tissues exhibited a smaller volume change and smaller changes in both cell height and width when compared to cell volume and dimensional changes from the lateral condyle ($p < 0.05$). Cells from the lateral condyle displayed a larger cell volume change with larger changes in cell height, width and depth when compared to cell volume and dimensional changes from the lateral plateau ($p < 0.05$). Lateral condyle cells also exhibited larger volume changes and larger changes in cell depth when compared to cell volume and dimensional changes from the medial condyle ($p < 0.05$). Cells from the lateral plateau displayed a smaller volume change with smaller changes in cell height, width and depth when compared to cell volume and dimensional changes from the medial plateau ($p < 0.05$). Cells from the medial condyle exhibited a smaller volume change and smaller changes in cell width and depth when compared to cell volume and dimensional changes from the medial plateau ($p < 0.05$).
Discussion: The current study clearly demonstrated the site-specific deformation responses of chondrocytes from joint tissues within the rabbit knee. When comparing contacting joint tissues within the rabbit knee, it was observed that the cells within the patella underwent greater volume decreases (compared to groove cells) primarily due to a greater decrease in cell height (axial cell strain). Similarly, the cells within the lateral condyles (compared to lateral plateau cells) underwent a greater volume

decrease primarily due to a greater cell height decrease. In both of these aforementioned groups with larger cell volume decreases (compared to their opposite contacting tissue counterpart), substantial axial cell compression was not compensated enough by cell stretching in the lateral direction, amplifying the observed cell volume decreases. In contrast, the cells within the medial tibial plateau underwent greater volume decreases (compared to medial condyle cells) due to smaller cell stretching in the lateral direction. Because cell deformation in cartilage is dependent upon the structure and composition of the surrounding local matrix, one possible explanation of this finding is supportive of the hypothesis that cartilage adapts its matrix constituents to the external mechanical environment that is perceived by the cells within the tissue [3]. Therefore the different functional requirements of the contacting surfaces within the rabbit knee (and therefore the different loading patterns in each site) may be a critical factor that drives cell biosynthesis, tissue mechanobiology and the overall mechanical properties of the tissue [3]. The data presented in this study reveal the important 'baseline' cell response to deformation across different contact sites within the rabbit knee joint, where these site-specific responses may be used to further investigate how altered mechanical loading of the knee joint tissues (e.g. due surgical intervention or other knee joint instabilities) may affect cell deformation and cell biomechanics.

Significance: The results of this study demonstrate site-specific deformation response of chondrocytes within the rabbit knee joint. These findings suggest that the mechanobiological responses of chondrocytes are influenced by site-specific loading requirements of the joint tissues.

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References: [1] Madden et al (2013) J Biomech, 46, 554-60; [2] Han et al (2009) Med Eng Phys, 31, 1038-42; [3] Barker and

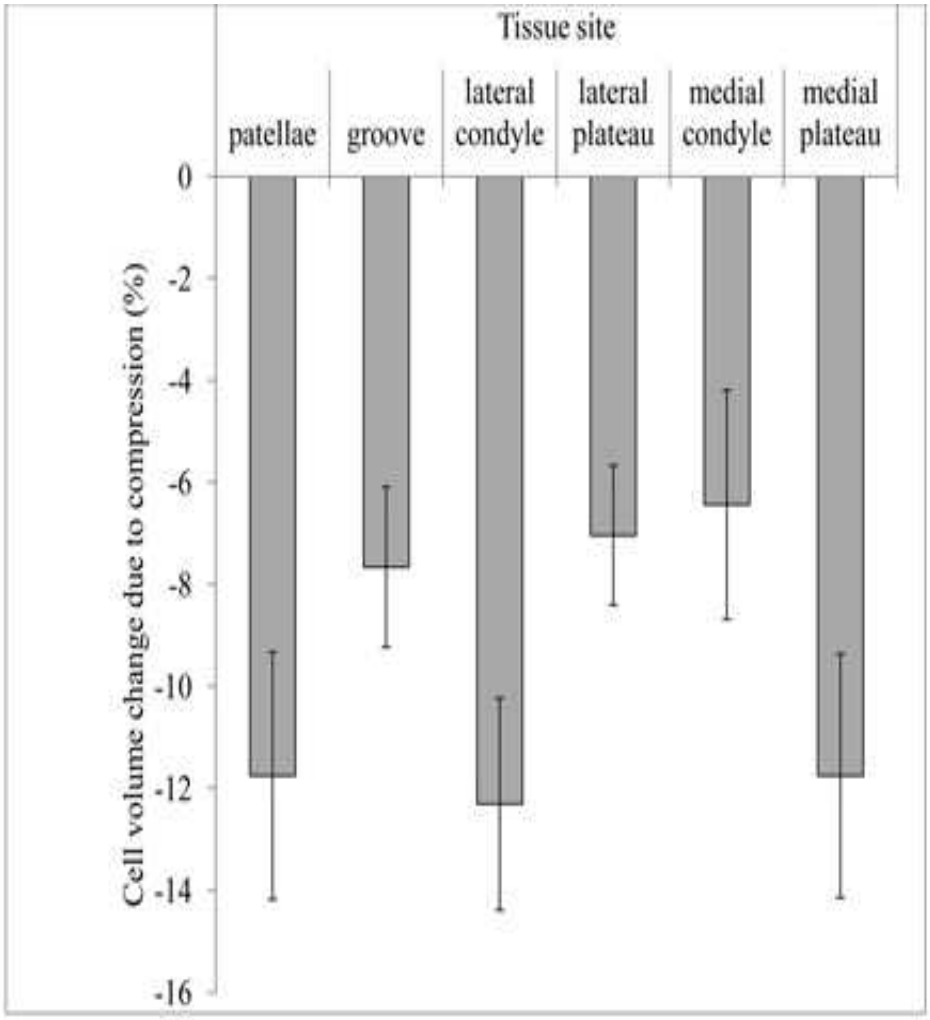

Figure 1. Percent change in cell volumes due to cartilage deformation across various sites within the rabbit knee. The solid black bars denote the significantly different groups ($p < 0.05$).

Table 1. Percent change in cell height, width and depth dimensions due to cartilage deformation across various sites within the rabbit knee. Superscript letters denote statistically significant differences between the groups ($p < 0.05$).

Tissue Site (# of cells)	Deformation-induced changes (%) in cell dimensions (mean ± standard deviation)		
	Height	Width	Depth
Patellae (N =100)	$-20.2 \pm 3.8^{a,b}$	$9.2 \pm 2.3^{i,j}$	4.9 ± 2.6^{r}
Groove (N = 90)	$-14.3 \pm 1.7^{a,c,d,e}$	$4.1 \pm 0.9^{i,k,l}$	4.8 ± 1.0^{s}
Lateral Condyle (N = 100)	$-22.9 \pm 2.1^{c,f}$	$7.2 \pm 1.3^{m,n}$	$7.8 \pm 1.3^{t,u}$
Lateral Plateau (N = 100)	$-12.5 \pm 1.8^{b,f,g,h}$	$3.7 \pm 0.9^{j,m,o,p}$	$3.5 \pm 1.3^{t,v}$
Medial Condyle (N= 100)	$-23.1 \pm 2.4^{d,g}$	$8.8 \pm 1.5^{k,o,q}$	$14.2 \pm 2.0^{r,s,u,v,w}$
Medial Plateau (N= 80)	$-24.9 \pm 2.8^{e,h}$	$12.4 \pm 2.4^{l,n,p,q}$	7.1 ± 2.6^{w}

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