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# STRUCTURAL INTEGRITY OF EXTRACELLULAR MATRIX INFLUENCES THE MECHANICAL BEHAVIOUR OF IN-SITU CHONDROCYTES

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## SUMMARY
Cartilage lesions change the microenvironment of cells and may accelerate cartilage degradation through catabolic responses in chondrocytes. In this study, we investigated the effects of structural integrity of extracellular matrix (ECM) on chondrocytes by comparing the mechanics of cells surrounded by an intact ECM with cells close to a cartilage lesion using experimental and numerical methods. Experimentally, 15% unconfined compression was applied to bovine cartilage tissues using a light-transmissible compression system. Target cells in the intact ECM or near lesions were imaged by dual-photon microscopy. Changes in cell morphology (n= 32 for both ECM conditions) were quantified. A two-scale (tissue level and cell level) Finite Element (FE) model was also developed. A 10% unconfined compression load was applied to a non-linear, biphasic tissue model with the corresponding cell level models studied at different radial locations from the center of the tissue in the transient phase and at steady state. Experimental and theoretical results indicated that cells near lesions deform less than chondrocytes in the intact ECM at steady state. Transient responses showed that cells near lesions experienced large (10 %) tensile strains in the direction of tissue thickness. Previous experiments showed that tensile strain causes an up-regulation of digestive enzyme gene expressions. Therefore, we propose that cartilage degradation near tissue lesions may be due to the tensile strains applied to cells, thus leading to an up-regulation of catabolic factors.

## INTRODUCTION
Osteoarthritis is a joint disease associated, among many other things, with lesions of the articular cartilage [1]. These lesions are thought to accelerate the progression of articular cartilage degradation, and are known to change the structural environment of cells. The mechanical behavior of chondrocytes depends significantly on the structural integrity of its environment [2, 3]. There is evidence of digestive enzyme accumulation exclusively in regions close to cartilage lesions [4], and support that these proteolytic enzymes are released from chondrocytes near tissue lesions. Therefore, we hypothesized that the structural integrity (or lack thereof near cartilage lesions) of the ECM in the vicinity of chondrocytes affects the mechanics of cells, and in turn the biosynthetic response of chondrocytes. The objective of this study was to determine the mechanics of in-situ chondrocytes for different conditions of the ECM: (i) chondrocytes surrounded by an intact ECM; and (ii) chondrocytes close to cartilage lesions. We hypothesized that chondrocytes near lesions deform more than cells in the intact ECM, resulting in catabolic responses originating in cells near tissue lesions.

## METHODS
*i. Experiment*
6mm diameter full-thickness cartilage-bone samples were harvested from the medial side of bovine metatarsal-phalangeal joints (n=6) using a round-shaped punch. Cells in the cartilage specimens were positively stained with calcein AM (8μM, Molecular Probe, USA) and carboxyfluorescein diacetate, succinimidyl ester (0.9mM, Molecular Probe, USA) for 30 minutes. After staining, samples were washed three-times in dye-free phosphate buffered saline (PBS) prior to fixation in specimen holders using dental cement. Tissue thickness was determined by needle indentation [5]. The osteochondral samples were compressed in a custom-made piezo-electrically driven compression system mounted onto the stage of a laser scanning microscope (LSM 510, Zeiss Inc. Germany) that allows for imaging of live cells in their native environment [5]. A 15% unconfined compression test was performed at 0.5%/s. The nominal strain was maintained for 20 minutes, followed by load removal. Image stacks were acquired at the center of the tissue (intact ECM condition) at three time points: before compression, 20 min following the onset of compression, and 20 minutes after load removal. Following the loading protocol, the cylindrical tissue was cut vertically into two-halves and the same 15% nominal tissue strain was applied. Cells located within 150 μm of the cutting edge (lesioned ECM condition) were imaged using the imaging protocol described above. Chondrocyte images (N=32 for both boundary conditions) were obtained with dual-photon excitation microscopy using a 40×/0.8 NA water immersion objective (Zeiss Inc., Germany). A series of planar images (pixel size: 0.41μm x 0.41μm; bit-depth: 8) were acquired along the objective axis at intervals of 1μm. Images were taken from the superficial zone of the cartilage samples. Three-dimensional (3D) reconstruction of chondrocytes was performed using a custom-written code (VTK, Kitware Inc., USA) for cell morphology analysis [6]. Changes in paired cells' separation and orientation (measured by the rotation angles of an axis defined by the paired cells) were measured to determine local tissue strains and changes in axial and transverse plane tissue orientation. Local tissue compressive strains were calculated by dividing the change in vertical separation of two cells by the initial vertical separation of these cells [5].

*ii. Finite Element (FE) Modeling*

A two-scale FE model [7, 8] was used. The macro-scale (tissue level) model was taken as a 6mm-diameter osteochondral explant, with a 1mm-thick cartilage layer on top of a 2mm-thick subchondral bone. The cartilage was modeled as a biphasic material with a hyperelastic, isotropic, non-homogeneous solid phase [9], and a strain-dependent permeability [8, 9]. The subchondral bone was assumed as homogeneous, biphasic, and linearly elastic. The material properties of the cartilage were modeled as a function of normalized depth, $\xi$ ($\xi$=0 cartilage-bone interface; $\xi$=1 articular surface) [7]. The micro-scale (cell level) model is made up of a chondron (cell surrounded by the pericellular matrix (PCM)) embedded in an ellipsoidal ECM. Chondrocytes were modeled as oblate revolution ellipsoids, surrounded by a cell membrane and PCM. The surrounding ellipsoidal ECM was constructed to match the empirically-derived volumetric fraction of chondrocytes as a function of tissue depth, $\xi$ [3]. Cells and cell membranes were assumed to be isotropic, homogeneous, linear and biphasic continua [2, 3]. The ECM and PCM layers were assumed to be non-linear, isotropic, homogeneous, and biphasic. The permeability of the cells and PCMs was assumed to be strain-dependent [8]. An axisymmetric model was used to represent the macro-scale model, while the micro-scale model was constructed based on plane-strain theory. An unconfined compression test was performed on the tissue model by allowing radial displacement of the cartilage while fixing the subchondral bone at the boundary. The articular surface of the tissue model was then subjected to a nominal strain of 10% at a strain rate of 0.33 %/s. The pore pressure was assumed to be zero at the articular surface and at the lateral boundaries to allow for free fluid flow at these places. Cells in the superficial zone ($\xi$ = 0.90) were studied at different radial locations, r (r =0 represents cells in the intact ECM while r=0.97 represents cells near lesions). The time-varying solid displacements and fluid pressures at each node of the tissue model were used as boundary conditions for the cell level model.

## RESULTS AND DISCUSSION

In contrast to expectation, both tissue and cells near lesions deformed less than chondrocytes surrounded by an intact ECM (Table 1, Fig. 1) at steady state. Results of the FE approach confirmed the experimental findings (Fig. 1). The smaller deformation experienced by cells near lesions compared to cells in the intact ECM may be due to the unrestricted radial displacement and radial fluid flow at the site of lesion, which decreased the stresses in the tissue close to lesions, thereby resulting in decreased cell deformations. The radial bulging of the tissue near lesions during compression was thought to expose the tissue in these regions to high shear strains. However, tissue near lesions showed smaller changes in orientation angles (Table 1), suggesting that cell shear strains were also lower near tissue lesions compared to those in the intact ECM [2, 3].

Table 1. Local tissue (superficial zone) deformations during unconfined compression. The label "*" indicates significant changes in local tissue behavior from the initial state and "†" indicates significant differences in tissue response between the two ECM conditions.

| Superficial Zone Tissue | | Tissue Deformation | |
|---|---|---|---|
| | | Intact ECM | Near Lesion |
| **Displacement (μm)** | Axial | *,† -6.6 ± 0.5 | * -5.0 ± 0.4 |
| | Transverse | * 1.3 ± 0.2 | * 1.3 ± 0.1 |
| **Strain (%)** | Axial | *,† -45.2 ± 1.4 | * -35.2 ± 2.0 |
| **Orientation Change ($^0$)** | Axial | *,† 16.3 ± 1.4 | * 9.1 ± 1.1 |
| | Transverse | * 4.1 ± 1.4 | 2.3 ± 0.5 |

The transient response of cells (measured at peak tissue strain), calculated using FE analysis, showed cell deformation patterns similar to those observed at steady state, with the exception that cells near lesions experienced tensile strains of about 10% in a direction perpendicular to the articular surface which was not observed in cells in the intact ECM (Figure 1). We suspect that the tensile strains may be caused by the high radial fluid flow and the tissue deformation towards the lesions in the compromised ECM. Also, the vast increase in tensile strains at the cell level compared to the tissue level (10% in cells vs 1% in tissue) is likely caused by the huge difference in material stiffness between cells and tissue (tissue is ~ 140 times stiffer than cells) which makes the cells behave like voids.

Previous studies found that cells exposed to tensile strains of various magnitudes (0.3% - 23%) led to an up-regulation of digestive enzyme gene expression [10, 11], which was not matched by the up-regulation of the corresponding inhibitors, thus leading to a catabolic cell response [10]. Therefore, we suggest that the gradual tissue degradation near tissue lesions may be the consequence of tensile strains on chondrocytes near lesions during daily joint loading. The altered mechanical signals received by the cells lead to the up-regulation of catabolic enzymes that gradually digest the articular cartilage.

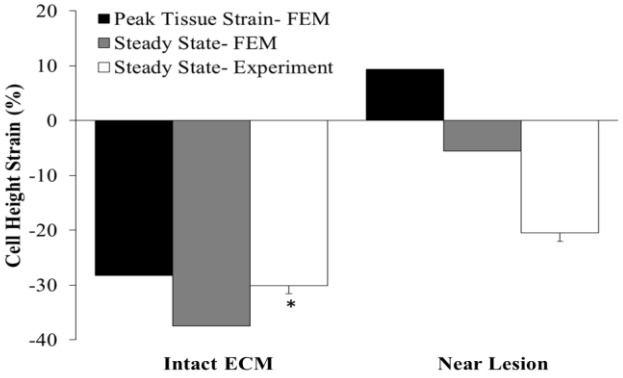


Figure 1. Cell compressive strains obtained experimentally and numerically for cells in the intact ECM and near lesions. The label '*' shows significant differences in height strain in intact ECM compared to lesioned ECM.

## CONCLUSIONS

We conclude (i) that cells deform differently in the intact ECM compared to near tissue lesions, (ii) that these mechanical differences lead to differences in biosynthetic responses of the cells, and (iii) that the tensile strain in the transient phase of cell deformation near cartilage lesions may be the primary trigger for the catabolic responses observed near cartilage lesions.

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