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## PERICELLULAR MATRIX DEFORMATIONS *IN SITU* UNDER MECHANICAL COMPRESSION

<sup>1</sup>Sang Kuy Han, <sup>2</sup>Ryan Madden, <sup>2</sup>Walter Herzog

<sup>1</sup>Fischell Department of Bioengineering, University of Maryland, MD, U.S.A.

<sup>2</sup>Human Performance Laboratory, University of Calgary, AB, Canada; email: sangkuy.han@hotmail.com

### SUMMARY

The pericellular matrix (PCM) in the chondron, which is the structural network surrounding chondrocytes, has been widely studied to understand its functional role for chondrocyte mechanics under mechanical compression. The purpose of this study was to investigate PCM deformations in their natural environment using an *in situ* experimental approach designed for real-time observation of live cell deformations. In agreement with previous histology studies, our results demonstrate that the PCM plays a non-linear mechanical damping role for cell deformations under mechanical compression. The findings of the present study suggest that the PCM plays a unique mechanical damping role to prevent excessive cell deformations when cartilage is experiencing large compressive strains.

### INTRODUCTION

The health and integrity of the cartilage extracellular matrix is maintained by chondrocytes, the cells in articular cartilage, which synthesize structural macromolecules. The mechanical environment of chondrocytes plays an important role in the biosynthetic activity of cells and the health of the tissue and joint. Given the three orders of magnitude difference in material properties between chondrocytes (0.7 kPa, chondrocytes from cartilage of human knees, hips, ankles, and elbows [1]) and extracellular matrix (ECM, 0.4 MPa, patellofemoral groove of adult bovine [2]), one would expect cell deformations to be much higher than those in the surrounding ECM. However, experimental studies suggest that the magnitude of chondrocyte deformation is much smaller than expected based on the material properties of ECM and isolated cells [3,4,5]. Therefore, it seems that chondrocytes are protected from large deformations *in situ*. One such protective mechanism has been associated with the chondron, a structural network surrounding cells [6].

Chondrons consist of a chondrocyte and an associated dense fibrous structure wedged between cell and extracellular matrix, the so-called pericellular matrix (PCM) [6]. Partial degradation of the PCM was associated with increased cell deformations for given loading conditions compared to cells with perfectly intact chondrons, while they deformed less than isolated cells [7]. These findings suggest that the PCM plays an important role in the control of chondrocyte deformation. Mechanical deformations of PCM have been investigated by pipette aspiration and histology on fixed

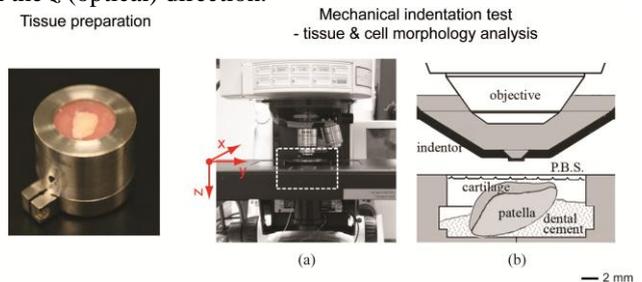
tissue samples [4,8]. However, it is not understood how the PCM deforms *in situ* under mechanical loading. Therefore, the purpose of this study was to investigate the pericellular matrix deformations in their natural environments using an *in situ* experimental approach designed for real-time observation of live cell deformations.

### METHODS

#### Sample preparation:

Patellar cartilage from twelve knees of 15 months old, skeletally mature female New Zealand white rabbits was used for chondrocyte mechanics analysis. Fluorescein conjugated dextran (excitation: 488nm, emission: 500nm. Molecular Probes, OR, USA) was suspended in DMEM (Dulbecco's Modified Eagle's Medium, Gibco, OR, USA) at a concentration of 0.8 mg/ml (0.26 mM). The patella was incubated in the dextran solution for 4-8 hours at 4°C prior to fluorescent confocal imaging ( $N = 4$  patellae).

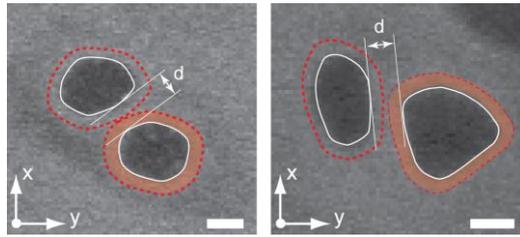
Mechanical compression test: Two MPa surface pressure was applied to the mid region of the medial side of the retro-patellar cartilage using a round glass indenter (diameter = 2 mm) at an average speed of 6  $\mu\text{m/s}$  (Figure 1). Once the desired pressure was reached, the indenter was held for 20 min when steady state was reached. Optical sections were recorded before and after loading using a spacing of 0.5  $\mu\text{m}$  in the  $z$  (optical) direction.



**Figure 1:** Sample preparation and indentation tests. Tissue preparation: patella fixed in specimen holder. Indentation test: (a) photo of the actual indentation system on the x-y stage of a microscope. (b) schematic illustration for the area marked with the dashed line in A.

PCM deformation measurement: Since there is no fluorescent dye for PCMs surrounding live cells in fresh cartilage tissue, it was assumed that the deformations between two adjacent chondrocytes which are less than 5

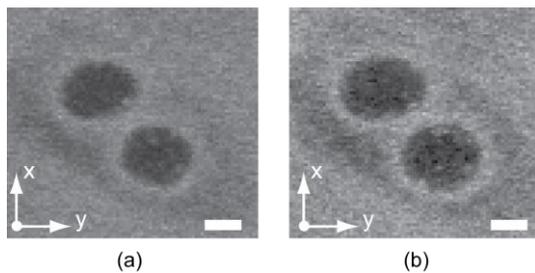
microns apart on the x-y focal plane (lateral plane) would represent the PCM deformations (Figure 2,  $n = 7$  paired cells). Local tissue strain, cell and PCM deformations were calculated from the superficial zone cartilage.



**Figure 2:** Typical examples of selecting two cells in the x-y focal plane for measuring PCM deformations, denoted as “d”. White lines indicate the boundary of cells. Red dashed lines indicate the assumed boundary of PCM. Red shaded areas indicate the PCM area. Scale bar = 5  $\mu\text{m}$ .

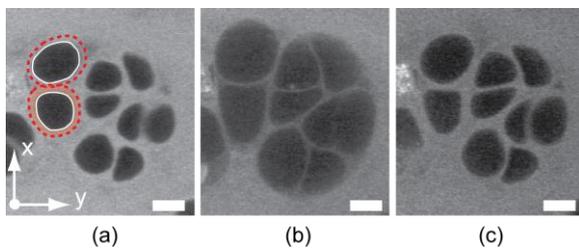
## RESULTS AND DISCUSSION

In response to the average axial local tissue strain of 28% in the superficial zone, cell deformations in the lateral plane (9.7%) differ from the surrounding PCM deformations (-11.4%) (Figure 3, Table 1). However, the absolute amounts of cell and PCM deformations were in the same range.



**Figure 3:** Typical example of cell and PCM deformations. (a) before loading. (b) after loading. Scale bar = 5  $\mu\text{m}$ .

For large tissue deformations (36% axial local compressive tissue strain), the absolute amount of PCM deformations started to differ considerably from chondrocyte deformations (Figure 4, Table 1\*).



**Figure 4:** Cell and PCM deformations for large tissue deformations. (a) before loading, White lines indicate the boundary of cells. Red dashed lines indicate the assumed

boundary of PCM. Red shaded area indicates the PCM area. (b) after loading. (c) during recovery. Scale bar = 10  $\mu\text{m}$ .

In agreement with previous histology studies [4], our results demonstrated that PCM plays a non-linear mechanical damping role for cell deformations under mechanical compression. For large tissue deformations (a 36% of axial compressive tissue strain), the absolute amount of PCM deformations became substantially larger than the 28% of local tissue deformations (Figure 4, Table 1). In general, one would expect that PCM regions between adjacent cells expand in the lateral plane under axial tissue compression. However, surprisingly PCM regions between adjacent cells decreased in the lateral plane under tissue compression. Thus, we anticipate that the PCM limits excessive cell deformations in the presence of large tissue strains. We suspect that the non-linear damping of the PCM also affects the biosynthetic activity and homeostasis of chondrocytes for various mechanical loading conditions of cartilage. Further studies will be aimed at understanding the mechanical role of the PCM in osteoarthritic tissue.

Since there is no fluorescent dye for PCMs surrounding live cells in fresh cartilage tissue to date, measuring the deformations between adjacent chondrocyte which are separated by twice the average PCM thickness can be used to investigate PCM deformations.

## CONCLUSIONS

We investigated pericellular matrix deformations of live cells in their natural environment for the first time. The results of this study suggest that the PCM plays a unique mechanical damping role to prevent excessive cell deformations when cartilage is experiencing large compressive strains.

## ACKNOWLEDGEMENTS

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Axial local tissue strain	Cell	PCM
- 27.5 $\pm$ 8.3	9.7 $\pm$ 4.5	- 11.4 $\pm$ 11.8
- 35.6 $\pm$ 0.7*	30.5 $\pm$ 17.0	- 59.7 $\pm$ 10.6

**Table 1:** Comparison between cell and PCM deformations in the lateral plane at the given axial local tissue strain. Data are shown as mean  $\pm$  s.d [%].