THE EFFECT OF FINITE COMPRESSIVE STRAIN ON CHONDROCYTE VIABILITY IN STATICALLY LOADED BOVINE ARTICULAR CARTILAGE

Chahine, N O; Wang C CB; Kelly T N; Elbeshbeshy R; Mauck R L; Hung C T; +Ateshian, G A
Department of Biomedical Engineering, Columbia University, New York, NY

INTRODUCTION Several recent studies have reported that compressive loading of articular cartilage leads to increased cell death [1,2], preferentially in the superficial zone [3,4]. Similarly, confined and unconfined compression studies of the depth-dependent properties of articular cartilage have shown that the tissue exhibits significantly higher compressive strains in the superficial zone than in the deep zone under equilibrium loading [5,6,7]. Based on these results, it is the hypothesis of this study that chondrocyte death under static loading correlates with the magnitude of equilibrium compressive strain. To test this hypothesis, cylindrical bovine cartilage explants were cut into equal parts, with one half tested for cell viability after prolonged static loading, and the other tested under similar loading conditions to determine the depth-dependent finite deformation strain field. Quantitative imaging tools were used to correlate depth-dependent strain fields against staining for dead cells.

METHODS Sample Preparation: Full thickness osteochondral plugs (Ø 4mm) were harvested from the carpometacarpal joint of 3-4 month old calves and cultured in high glucose DMEM with buffers, amino acids, and 10% FBS at 37° C and 5% CO_2. On the day of testing, the subchondral bone was excised and each explant was divided in half using a custom cutting device. One half of each sample was used for loading while the other served as a free swelling control. Loading: Unconfined static compression was applied to 80% of original tissue thickness (applied at ~2.5 μm/sec) for duration of 12 hours (n=6) in a tissue culture incubator. Cell Viability: After removal of load and 1 hour of equilibration, cellular viability was assessed using the Live/Dead Assay Kit (Molecular Probes). Full thickness fluorescence images of the tissue were acquired using an inverted microscope (Olympus IX-70). Intensity analysis was performed using NIH Image to determine the distribution of dead cells (red nuclei) through the entire thickness. Strain Measurement: Free swelling samples from the viability assay were tested in an unconfined compression microscopy device and imaged prior to deformation [8]. Uniaxial compression was applied in 10% increments from 0% to 80% and imaged at equilibrium at each increment. Strain analysis was performed using optimized digital image correlation [8] for each pair of consecutive images. The cumulative compression at each point through the tissue depth was represented by the stretch ratio (λ=current length/original length) where the stretch ratio λ is related to the axial strain, ε, by the expression $\varepsilon = 1 - \lambda$.

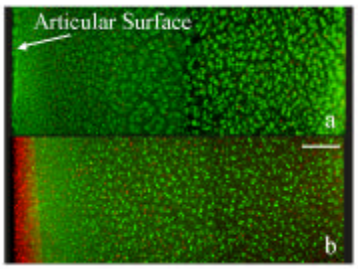

Figure 1: Live / Dead staining of (a) control & (b) loaded explants; Bar =100 μm.

RESULTS Sample preparation and incubation induced uniformly distributed and minimally detectable cell death (figure 1a). Uniaxial compression applied for 12 hours caused an increase in cell death primarily in the superficial zone (figure 1b). The average distribution of cell death for samples compressed to 80% strain for 12 hrs. is shown in figure 2. Cell death increased to a maximum intensity near the articular surface and thereafter sharply decreased to 10% of its peak intensity over a narrow superficial region (10.4% ± 0.3% of the sample thickness, figure 2). The intermediate and deep zone showed a generally less intense and more uniform cell death distribution when compared to the superficial zone. The stretch ratio increased nonlinearly from the articular surface (λ=0.23±0.04) to the deep zone (λ=0.45±0.08). A typical correlation between stretch ratio and cell death intensity is shown in figure 3, confirming that cell death occurs in a narrow range of stretch ratios with an upper bound of λ=0.26±0.05.

DISCUSSION The higher compression observed in the superficial zone relative to the middle and deep zones is consistent with previous results in the literature [5,6,7], though the magnitude of compression is significantly greater in this finite deformation study (~77% compressive strain in the superficial zone, ~55% in the deep zone). Furthermore, the depth-dependent strain inhomogeneity appears to be less pronounced under finite deformation than at smaller strains. In contrast, the overwhelming majority of dead chondrocytes are concentrated in a narrow region of the superficial zone consisting of ~10% of the full thickness (figure 2) where the compressive strain varies over a small range, ~74%-77% (figure 3). These results suggest that cells remain viable over a wide range of compressive strains, but when the stretch ratio drops below a threshold value (λ<0.26, corresponding to a compressive strain >74%), there is a very steep correlation between cell death and the magnitude of compression. This interpretation is supported by the fact that the stretch ratios found in the superficial zone correspond to such high levels of compaction as to exclude most interstitial fluid, potentially compromising the transport of nutrients and waste products. An alternative interpretation of these results is that superficial zone chondrocytes are more vulnerable to loading than elsewhere in the tissue. Indeed, it has been shown that when compressed in agarose, chondrocytes isolated from the superficial zone deform to a greater extent than those from the deep zone [9]. The relative softness of these superficial zone cells may make them more sensitive to high strains, and result in increased cell death in this region. To our knowledge, this basic science study represents the first attempt to measure the depth-dependent strain field in articular cartilage under finite deformation, and to correlate it with cell death, providing insight into the factors governing cell viability. It is important to note that the prolonged static loading applied on these explants is not physiologic and the results of this study should not be extrapolated to *in situ* loading conditions in living joints. However, they may serve to interpret comparable basic science studies of the biosynthetic response of articular cartilage.

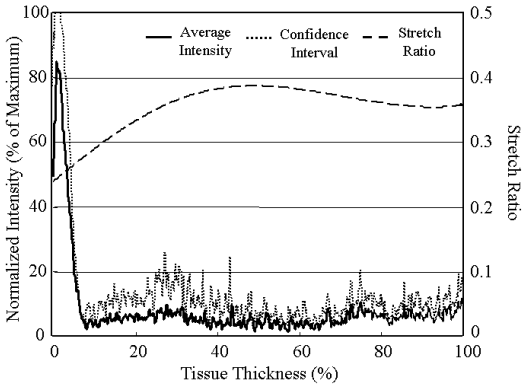

Figure 2: Average intensity of dead cells and stretch ratio vs. tissue thickness (articular surface= 0% thickness)

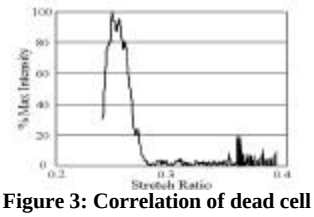

Figure 3: Correlation of dead cell intensity and stretch ratio

ACKNOWLEDGEMENTS This study was supported by the National Institutes of Health (AR46532, AR46568).

REFERENCES 1) Chen, et al., 2001, Trans ORS, 26: 259. 2) Quinn, et al., 2001, JOR, 19(2):242-9 3) Lucchinetti, et al., 2002, OA and Cart, 10(1):71-81.. 4) Ewers, et al., 2000, Trans ORS, 25:107. 5) Guilak, et al., 1995, JOR, 13(3):410-21. 6) Schinagl, et al., 1996, ABME, 24(4):500-12. 7) Wang, et al., 2001, ASME-BED, 50. 8) Wang, et al., 2002, JBE, in press. 9) Lee, et al., 1995, In Vitro Cell Dev Biol Anim., 31(11):828-35.