Articular cartilage under static load: A quantitative polarized light microscopic imaging study correlated with micro-MRI (μMRI) findings

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Introduction

Articular cartilage is a load-bearing tissue, which has a very ordered collagen ultrastructure that maintains the tissue shape and provides unique meshwork that strengthens the tissue. Under mechanical load, a deformation in the collagen network in articular cartilage is expected. The understanding of the apparent and ultrastructural changes of cartilage in the loading state will be highly valuable in revealing the tissue adaptability to external loading and in assessing joint abnormalities. Recently, a microscopic MRI (μMRI) T_2 anisotropy technique was used to investigate ultrastructural changes in compressed cartilage [1,2]. We found a significant increase in the Superficial Zone (SZ) thickness, defined mainly from the collage fiber orientation, at a mean of 20% reduction of total thickness due to compression. In this study, a quantitative polarized light microscopy (PLM) technique was used to investigate the ultrastructure changes in collagen fiber organization, and to validate the ability of μMRI to detect subtle changes in loaded tissue.

Methods

Thirteen osteochondral specimens were excised from the central load-bearing region of a humeral head obtained from two healthy mature beagles. Eight specimens were compressed at different levels of strain and were prepared for histology while compressed, and five specimens were served as control. Two specimens were used in the previous MRI study. The static compression was performed with placing cartilage-bone plugs in Hoffman clamps and exercising different forces by the means of metal screws. The compressed specimens were in the clamps during the entire histological process (fixation, decalcification, paraffin processing, and embedding) to ensure the preservation of the deformation of the local matrix in its compressed state.

Quantitative polarized light microscopy images from the histological sections were constructed at 5× and the 2-D images were the optical retardance and the average angular orientation of the collagen fibers in cartilage tissue, with 2.72 μm/pixel resolution. For data analysis, five to ten histological sections were processed for each specimen, and 1-D profiles from the angle and the retardance image were extracted from several regions of interest. The final 1-D profiles were the averaged profiles across the available sections from each specimen.

Results

Figure 1 shows 1-D profiles extracted from selected regions of interest of (a) 2D angle maps (the arrows point to the expansion of SZ) and (b) retardance maps at different values of strain (the black arrows point to the tide-mark.) the angle profile demonstrates a profound change at the orientation of fibers along the cartilage depth, more significantly at 30% strain. As compression introduced into the tissue, more fibers are aligned along the articular surface near the surface (the SZ thickness increased), in agreement with the shift of T_2 peak away from the cartilage surface observed previously in μMRI results [1]. Also, at 30% strain, an apparent second "transitional" zone is formed within the original transitional zone (in 0.2-0.5 depth range). Furthermore, the retardance profile shows no significant change from 0% to 10% strain, and an increase in overall profile at 30% strain. Because retardation is directly related to the birefringence in the tissue, an increase in retardance suggests a more compact and coherent packing among the fibers.

Figure 2 shows the relative thickness of the SZ, from a previous μMRI results (n=7) [1, 2] and the current PLM analysis versus strain value. The relative thickness of SZ is shown to be dependent of strain as it increases above 10% from both 2D MR T_2 maps and PLM maps (r^2=0.796 and r^2=0.539 for MRI and PLM, respectively), suggesting several subtle modifications in cartilage fiber organization, which essentially defines the histological zones. However, we could not observe correlated significant changes in other zones at strain values less that 20% (data not shown).

Discussions

Our μMRI and PLM results demonstrate that mechanical compression above physiological strain level can have profound effects on the orientation of macromolecules, mainly collagen fibers. The increase in SZ thickness in PLM correlates to our nondestructive μMRI results. This shows that μMRI, coupled with T_2 anisotropy, can detect changes in ultrastructural network of cartilage nondestructively. μMRI T_2 analysis of cartilage under mechanical stress has the potential to detect the adaptability of cartilage in response to compression within and above physiological strain level, which could become extremely useful in characterizing the subtle changes in joint diseases induced by excessive loading such as sport injury.

Figure 1a:

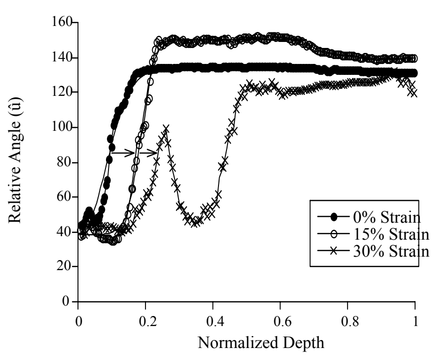


Figure 1b:

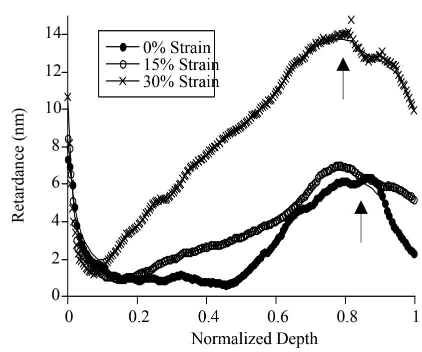
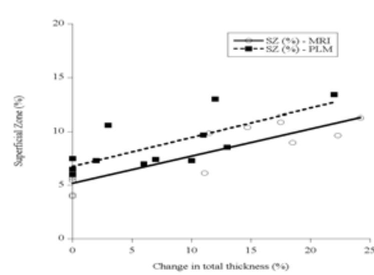


Figure 2:



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References

[1] Alhadlaq H. and Xia Y. *Osteoarthritis Cartilage* 2004; 12:887-894.
[2] Alhadlaq, H and Xia Y. *JMRI* 2005; 22:665-673.