Introduction

Recent µMRI studies found three MRI regions across the depth of articular cartilage, with each region having some unique characteristics [1]. The observation of these distinct MRI regions based on T2 relaxation profile is analogous to the identification of histological zones in conventional histology based on the collagen fiber orientation. These µMRI findings demonstrated that it is now possible to study the tissue structures and molecular interactions in individual zones of the tissue using µMRI non-invasively.

The goal of this study is three fold. Firstly, we want to establish a set of quantitative criteria to subdivide the tissue as seen in MRI into the MRI ‘zones’. Secondly, we want to measure not only the retardation of the histological sections but also the angular orientation of the collagen fibers in the tissue using PLM quantitatively. Thirdly, and most importantly, we want to establish the correlation between the non-invasive imaging by µMRI and the conventional histological imaging by PLM. We want to establish this correlation accurately, at the highest possible MRI resolution, and based on the same piece of tissue.

Materials and Methods

Six cartilage-bone plugs were harvested from the right shoulder joint of a two-year old healthy beagle. Quantitative T2 experiments were carried out using a Bruker AMX 300 NMR spectrometer with a 7T/89mm magnet and micro-imaging accessory. The in-plane pixel resolution, which was across the depth of the cartilage tissue, was 13.7 µm and the slice thickness in MRI was 1 mm. Other µMRI details have been described earlier [1, 2]. After the µMRI experiments, the specimens were cut right at the location of the imaging slice so that the same plane that was just examined by µMRI could be examined by histology. Conventional paraffin embedding was used to prepare the specimens. 10 to 14 unstained sections were prepared for each specimen. These sections were studied using a digitized polarized light microscope that has a configuration similar to those described in literature [3, 4]. The pixel resolution in PLM was 2.72µm and the slice thickness in PLM was 6µm.

Results

A classic laminar appearance of the cartilage tissue was found in the MRI proton images for all six plugs. 1D T2 profiles were extracted from the 2D images and shown here. It is clear that T2 in cartilage is anisotropic, depth-dependent, and orientation-dependent. In particular, we noticed that the T2 profiles have a distinct line shape of an asymmetrically bell-shaped curve.

For each specimen, 2D images of angle and retardation were calculated for all histological sections using PLM. The next figure shows both the 1D retardation and the angle profiles from the same location where the MRI T2 profiles were acquired. These profiles of angle and retardation in cartilage have a number of distinct features. In particular, (a) In the transitional zone, the retardation reaches a non-zero minimum. The first derivative of the angle profile has a peak that is precisely located at the minimum of the retardation profile. (b) In the radial zone, although the retardation values increase gradually and near linearly from the minimum in the transitional zone to the maximum at the deep part of the radial zone, the averaged orientation of the collagen fibers at this resolution is relatively constant. (c) The facts that the retardation is not zero at the transitional zone and that the angle is a continuous function in the transitional zone suggest that the collagen fibers in the transitional zone are not entirely random but have a residual order.

Discussions and Conclusions

We have developed a set of criteria to analyze the cross-sectional profiles of the cartilage tissue in both µMRI and PLM. Applying these criteria to our data has yielded the quantitative correlation of the zones in µMR images and in PLM images based on the same specimens. We have shown that the three histological zones of cartilage based on collagen orientation are statistically identical to the µMRI regions based on the T2 relaxation characteristics. µMRI has important potential to characterize the tissue properties and to elucidate fundamental mechanisms in cartilage at the molecular level non-invasively.

Acknowledgments

This work is supported in part by a Research Excellence Fund in Biotechnology from Oakland University, an instrument endorrsment from R. B. and J. N. Bennett, and an ROI grant (AR45172) from NIH (YX).

References


**Cornell University, Ithaca, NY 14853.