Introduction

Functional competence of articular cartilage is mainly determined by the organization, content and interactions of collagen, proteoglycans (PGs) and extracellular fluid. Characterization of cartilage constituents in vivo is essential for the detection of early stages of degenerative joint disease (osteoarthritis).

In articular cartilage, type II collagen forms a highly organized, anisotropic three-dimensional fibrous network, where the fibril orientation changes depthwise from the surface to subchondral bone. It is suggested that T2 relaxation is closely related to fibril arrangement [1,2]. In this study, a quantitative spatial approach was conducted to verify the relationship between T2 and collagen fibril orientation as measured by quantitative polarized light microscopy, a method for analysis of tissue structural organization.

Methods

Osteochondral plugs from healthy articular cartilage (n=9) were prepared for MR microscopic and polarized light microscopic (PLM) analyses from patellae of 1 to 3-year-old bulls, one sample per patella.

The MR microscopic measurements were performed at 9.4T with a 16mm high resolution spectroscopy probe. Cartilage T2 maps were obtained from spin-echo measurements with TR/TE=2500/14, 24, 34 and 44ms, 1mm slice thickness and 20mm FOV with 2562 resolution at 25±1 °C. Cartilage surface was oriented normal to the B0 field. For each sample, a T2 profile across cartilage thickness was calculated from a 1mm wide area.

After MR measurements, samples were fixed in formalin, dehydrated and mounted in paraffin. For each sample, 5µm-thick microscopic sections were cut and PGs were digested with hyaluronidase to reveal the collagen network. Samples were analyzed in four vertically randomized orientations using a computer assisted polarized light microscope to obtain a mean optical retardation (OR) profile of collagen from cartilage surface to subchondral bone. OR of polarized light, i.e. birefringence, is known to reflect sensitively the degree of collagen orientation [3].

Results

The intermediate cartilage zone is characterized by non-ordered collagen fibrils. Therefore, a maximum T2 and minimum optical retardation are observed in this zone. These landmarks were used to match spatial T2 and high resolution OR data for quantitative comparison.

A structural similarity was observed between T2 maps and PLM images (Fig. 1). After reducing spatial PLM resolution to match MR resolution, a reproducible inverse relation between T2 relaxation and OR was detected (Fig. 2A). Consequently, a linear positive correlation between T2 and OR was established in each sample (Fig. 2B, with 3 60. A second T2 maximum was often observed in the deep cartilage zone, which was systematically detected also by 1/OR maximum in PLM. This extra lamina is located near the cartilage-subchondral bone-interface and may associate with collagen fibrils bending around the hypertrophied chondrocytes. This demonstrates that the collagen network structure in the deep zone may diverge from the conventional conception of highly parallelly organized collagen fibrils.

Although a perfect relationship does not exist between cartilage T2 and PLM, the spatial agreement of these two totally different techniques reveals that T2 changes in normal articular cartilage reflect sensitively architectural changes in collagen fibril arrangement.

Discussion

Maximum contrast between T2 laminae is observed when cartilage surface is aligned at 0 or 90° in reference to B0. This starting point gives potential to observe the possible relationship between T2 and the birefringence of oriented tissue.

The results reveal a close relationship between MR and PLM imaging modalities. Experimental evidence is presented that cartilage T2 follows sensitively collagen fibril arrangement. Both techniques reflect primarily changes in collagen orientation, but secondary contributors may also exist. Only moderate correlation between the reduced T2 and 1/OR data suggests that it may be inaccurate to quantitatively predict one parameter by using the other.

Fig. 1: PLM image with original high resolution (A), OR (B) and 1/OR (D) from a representative sample.

Fig. 2: (A) T2 and 1/OR profiles as a function of thickness from cartilage surface, and (B) T2 plotted as a function of 1/OR for a representative sample.

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References


1Department of Anatomy, University of Kuopio; 2Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital; 3NMR Research Group, A. I. Virtanen Institute for Molecular Sciences, University of Kuopio, Kuopio, Finland.