High-resolution DTI to study articular cartilage dehydration

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Introduction Diffusion tensor imaging (DTI) has already been successfully used to investigate both collagen orientation (1-2) and proteoglycan degeneration (3). Cartilage aging is one of the leading risk factor for developing osteoarthritis (4), and it is generally associated with a tissue reduction of water content. Some studies demonstrate that dehydration of cartilage matrix does not affect the collagen network. The purpose of this study is to investigate, using high resolution DTI, the occurrence of structural variations and protein desegregation in the cartilage matrix as a consequence of de-hydration.

Methods DTI and T2-waighed images were obtained from femoral cartilage of five 8-12 month-old bovines. Cartilage-bone plugs were maintained in contact with air at room temperature during the experiments, in an uncorked 10mm test-tube. All acquisitions were performed using a Bruker Avance 400 system operated at 9.4T, and equipped with a microimaging probe (maximum gradient strength of 1200mT/m). Mean diffusivity (MD), fractional anisotropy (FA), apparent diffusion coefficient (ADC) and T2 were measured from each sample, at one day intervals, for five consecutive days, in order to monitor changes related to the dehydration process. Image resolution was 60x60μm^2 and slice thickness was 1mm for all sequences. T2-weighted images were performed using a multislice-multiecho (MSME) sequence with echo-times ranging from 4 ms to 64 ms (namely 16 echoes with a constant interval of 4ms) and a TR=2000 ms. To perform DTI protocol, diffusion-weighted pulsed-gradient stimulated-echo sequence (PGSTE) was employed, with TE/TR =18/2000 ms (see for example fig. 1A). Diffusion-sensitizing gradients along 6 non-collinear directions with b=0 s/mm2, b= 400 s/mm^2 and b= 1000 s/mm^2 were used. MD and FA maps were obtained using FSL software package (Functional MRI of the Brain Software Library). Data were pre-processed using home-made programs written in MATLAB. Each image was aligned and smoothed (2 pixel Gaussian smoothing). Then, a segmentation algorithm based on the b=0 s/mm^2 images was applied to obtain an appropriate mask for each set of data. Specifically, a semi-automatic segmentation eliminates the signal of bone and background noise, to optimize the calculation of the diffusion tensor (performed by FSL) on cartilage signal only. Smoothing reduces the contrast between adjacent voxels, thus minimizing artifacts arising from typical DTI acquisitions (5). MD, FA and T2 images were successively post-processed by a home-made MATLAB program to obtain a fit of experimental data by nonlinear least squares Levenberg-Marquardt. Selecting a set of n mm2 ROIs in three cartilage locations, MD, FA and T2 values were derived from each correspondent map ,Then, a home-made program was used to correlate these values with cartilage thickness, from superficial to deep zones (see fig.1B).

Results and Discussion T2 data show a continuous decrease of T_2 values as function de-hydration times. Conversely, from DTI maps, two different behaviors could be recognized (fig.1C and 1D). During the first 48hrs a decrease of MD and an increase of FA was observed. These data match with a reduction of water content and a consequent increase of collagen fibril density. Surprisingly, a singular pattern was found from 48 to 60hs, with an increase of MD and a decrease of FA.

As a consequence, we recognized two different dynamic behaviors during cartilage de-hydration. One trivial (due to loss of water and collagen ri-arrangement) and one non-trivial which can be explained by proteoglycans degeneration. In fact, hydration level of cartilage matrix has no effects on the collagen (6), while a disruption of proteoglycan molecules is more likely to contribute to the effect we observed (7). Bio-chemical experiments underlined that degeneration of proteoglycan occurs through de-attachment of glycosamminoglicans (GAG). Degeneration of proteoglycans may produce a higher GAG mobility (and so higher water molecules mobility). As a consequence the de-attachment of GAG determines an increase of MD and decrease of FA

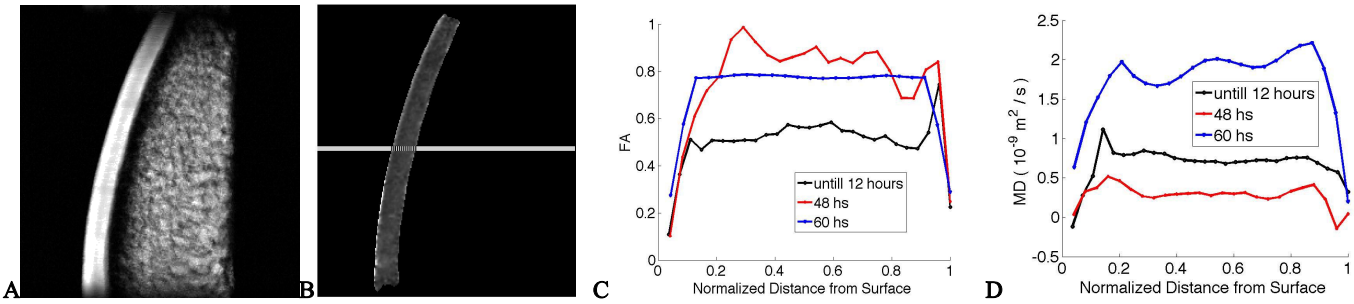

Fig 1: Example of data elaboration and DTI results. Starting from Diffusion Weighted Images (**A**), the home-made program segments and calculates FA and MD maps. Then, it automatically selects a series of ROIs with the same area (**B**), thus resulting in the two different graphs reported in **C** and **D**. FA results as a function of the normalized distance from cartilage surface are shown in C, while MD results as function of the normalized distance from cartilage surface are shown in D, at 3 different times of dehydration: 12, 48 and 60 hours.

Conclusions Preliminary results show that DTI can recognize variations of cartilage matrix structure which are not detected by T2 images. DTI monitoring of cartilage de-hydration provides useful information about GAG de-attachment from proteoglicans. Specifically, strong de-hydration of cartilage implies a process of destruction of GAG molecules. These observations could be useful to investigate the pathophysiological processes underling osteoarthritis, and to develop a new approaches for an early diagnosis.

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