In-situ observation of T2 and ADC changes in cartilage during collagen depletion

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Introduction:

Osteoarthritis, one of the most common arthritic diseases in the adult population world wide, is a slowly progressive disorder of the articular cartilage and the underlying bone. That degradation process is characterised by thinning and destruction of the cartilage and can be simulated by treating the cartilage with an enzymatic solution and selectively depleting one, or more of its constituents (collagen, proteoglycans and water). This study investigates the changes in porcine patellar cartilage during collagen depletion. Two parameters were used to investigate the *in-situ* changes in cartilage during enzymatic collagen depletion. The spin-spin relaxation parameter T_2 was used to monitor the enzyme activity (changes in the cartilage chemical environment) and the Apparent Diffusion Coefficient (ADC) was used to monitor structural changes in the cartilage (changes in restriction to free water mobility).

Materials and Methods:

Two patellae were excised from fresh cadaveric porcine knees provided by the local abattoir. They were submerged in an enzymatic solution containing 0.25 mg/ml of collagenase and maintained at 37±2ºC during the entire experiment, permitting penetration of the enzyme through the cartilage surface only. The chamber was placed inside the magnet and regularly scanned within a period of 16 hours. The experimental set-up can be seen in Figure 1 where the patella is submerged in a cylindrical cavity (B) filled with the enzymatic solution (A) surrounded by a water jacket (C) to maintain the desired temperature. All the images were acquired using a Magnex 4.7 T, 16 cm diameter horizontal bore actively screened magnet (Magnex, Scientific, Oxford, UK) linked to a Bruker Pharmascan console operating with Paravision 2.1.1 software (Bruker Medizin Technik, Karlsruhe, Germany). A self shielded magnetic field gradient set of 90mm diameter with a maximum gradient strength of 293 mT/m was used in conjunction with a 54 mm diameter sine spaced probe. A standard Carr-Purcell-Meiboom-Gill (CPMG) sequence was used to measure the changes in T_2 during enzymatic degradation using the following parameters: TR/TE = 2000/10.39 msec, 12 echoes and 195 µm isotropic in-plane resolution. A Stimulated Echo Diffusion Weighted (STE-DW) sequence was used to measure the Apparent Diffusion Coefficient (ADC) changes with the following parameters: TR/TE = 2000/16.5 msec, Δ/δ=30/2.5 msec, b=10.547, 1004.085 s/mm^2 optimised using Cramer-Rao[1]. The CPMG and STE-DW images were acquired every two hours to monitor the degradation process of the cartilage *in-situ*. The images were processed using algorithms based on the Nelder Mead simplex search method to obtain both, the T_2 and the ADC maps. The cartilage was segmented from the rest of the maps using a Simplex Mesh Diffusion Snakes (SMDS) algorithm[2] (Figure 1 D). Following the structural classification suggested by Mow et al[3], cartilage pixels were grouped in three different categories: the superficial tangential zone (STZ), the middle zone (MZ) and the deep zone (DZ), according to their distance from the cartilage-enzymatic solution interface.

Results and Conclusions:

This work demonstrates the *in-situ* changes in T_2 (Figure 2) and ADC (Figure 3) during collagen depletion. We observed that the depletion process has 3 distinct phases: in the first phase (0-5h), T_2 is reduced across all three layers without significant changes in ADC. In the second phase (5-13h), the T_2 decay persists and the ADC shows a significant change, indicating a change in water restriction inside all three layers. In the third phase (>13h), T_2 continues decreasing within the MZ and DZ layers, however, it remains almost constant for the STZ. Significantly, the ADC converges to the same value for the MZ and DZ layers, indicating a similar level of water restriction after the 14th hour.

These results could further suggest that during the first phase of degradation, there is a strong reaction of enzymes attacking the collagen fibres, as reflected by the changes in T_2, however few collagen fibres are removed, as no significant ADC changes are detected. During the second phase, the enzyme's attack continues, with collagen fibres also being removed, as reflected by the significant changes in ADC. During the third phase, there is not much enzyme activity in the STZ as the collagen fibres are already removed. On the other hand, the enzyme activity continues in MZ and DZ layers where a similar level of restriction to water mobility is reached.

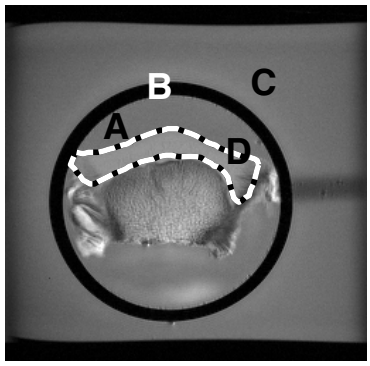

Figure 1: Spin Echo image illustrating the experimental set-up. Patella submerged in a collagenase enzymatic solution (A), contained in a cylinder (B) and surrounded by a water jacket at 37 ºC (C). The white dashed band (D) indicates the segmentation of the cartilage.

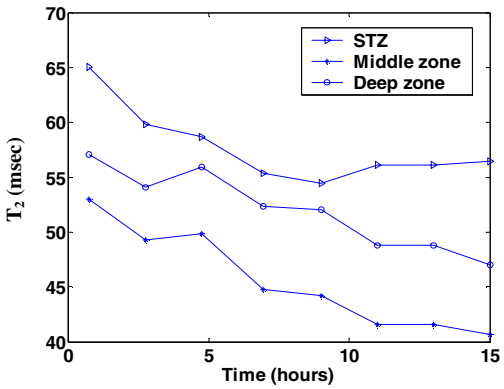

Figure 2: Changes in T_2 during collagen depletion for each of the three structural layers of articular cartilage.

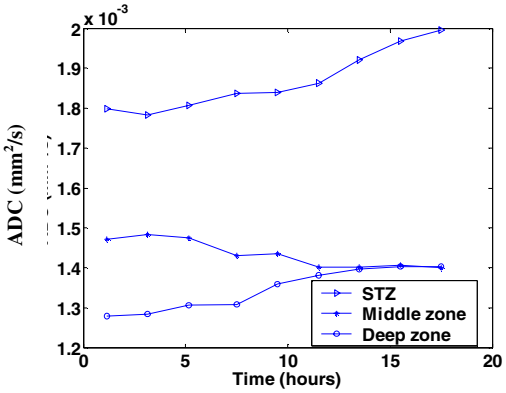

Figure 3: Changes in ADC during collagen depletion for each of the three structural layers of articular cartilage.

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