T_2-weighted-MRI and Dielectric Spectroscopy to investigate collagen structure behaviour during cartilage dehydration

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Introduction: Cartilage aging, associated to a reduction in the tissue water content, is one of the leading risk factor for the developing of osteoarthritis. Recent investigations have demonstrated that the dehydration of the cartilage matrix does not affect the collagen network [1] whose structure is based on a triple-stranded helix of polypeptide chains, resembling a rod of a diameter of about 15 Å and length of about 3000 Å. The packing of these rods results in assemblies in form of microfibrils. Channels between adjacent microfibrils are available for water and water can be adsorbed in these channels because of its capability to form rather strong hydrogen bonds with the polar groups residing on collagen, particularly the peptide C=O and NH groups. In addition, other hydrogen bonds are possible between neighbouring water molecules. Since the energy of these hydrogen bonds is strongly negative (about -5 kcal/mol), a water molecule network is expected and a chain structure is then the most likely. Collagen fibers in cartilage influence the water T_2 magnitude through the magic angle effect [1,5] and the structural arrangement of water molecules trapped in collagen fibers can be analysed by means of dielectric spectroscopy [2]. The aim of this study was to investigate the mechanism of cartilage microstructural damage due to dehydration, with a special regard to the collagen fibers, by means of quantitative T_2-weighted-MRI and radiowave dielectric spectroscopy measurements as a function of time.

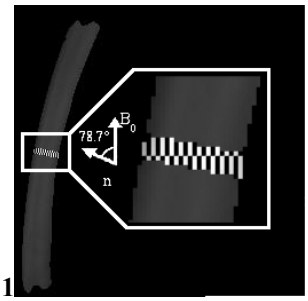
Methods: T_2-weighted images were obtained from fresh femoral cartilage samples, extracted from 8 month-old calves. Experiments were carried out on a Bruker Avance 400 MHz NMR spectrometer, equipped with a micro-imaging probe. Images resolution was 60X60 mm^2, with 1 mm slice thickness. Multislice-Multiecho (MSME) sequence was used with echo-times from 4 ms to 64 ms (namely 16 echoes, with a constant 4 ms delay) and TR=2000 ms. The dielectric properties of cartilage (the permittivity ε and the electrical conductivity σ) have been measured in the frequency range from 100 Hz to 100 MHz by means of Hewlett-Packard mod.4292A, in conjunction with a sample holder (Dielectric test fixtures, Hewlett-Packard mod. 16451B). Dielectric spectroscopy and MRI measurements have been carried out at room temperature (T=23±1°C) over an extended period of time (from 3 to 193 hours for MRI measurements, and from 1 to 410 hours for dielectric spectroscopy measurements). The real and the imaginary parts of the measured complex impedance were converted into the permittivity ε and the electrical conductivity σ through a calibration procedure employing dielectric materials of known dielectric properties. Samples contamination and water desorption were reduced encasing the samples in appropriate boxes.

MRI data were pre-processed using a homemade program written in MATLAB. Each image was aligned and then smoothed with a 2D isotropic Gaussian smoothing function performed by SPM, with a FWHM of 1.2 mm (i.e. FWHM corresponds to two voxels). In order to obtain an appropriate mask of the data, a segmentation of the cartilage on the first echo time (TE=4 ms) for each T_2-weighted image was made. A semi-automatic segmentation eliminates the signal of bone and background noise. T_2-maps were obtained from 16 images, processed with a homemade MATLAB program, in order to obtain in each voxel a fit of experimental data, according to: $S(\mathrm{T}) = S_1 \exp(-\mathrm{T_E}/\mathrm{T_2}) + S_0$. Fitting results were obtained using the Levenberg-Marquardt algorithm. In order to display T_2 values as a function of both cartilage zones and dehydration time, data were displayed as a function of the distance from the bone-cartilage junction. The contribution to the measured permittivity ε as a result of the water molecule dipolar reorientation, at least to a first approximation, can be written as:

$$(\varepsilon - 1) = 4\pi \frac{N}{V} \frac{\mu^2}{k_B T} \frac{1+p}{1-p}, \qquad (1)$$

where p is the probability of hydrogen bond formation, μ the dipole moment of a water molecule and N/V its concentration in the sample. $k_B T$ is, as usual, the thermal energy. In the present case, we assume μ=1.8 D and N/V of the order of $1.6 \bullet 10^{22}$ molecule/cm^3 (corresponding to an initial water content of the order of 50% of the hydrated collagen).

Results: Fig.1 shows an example of MRI data elaboration. Starting from an obtained T_2-weighted image, the program selects a series of ROIs within the same area and parallel to cartilage surface, extracting the mean T_2 values and their standard deviations from a mean of voxels' values. Fig.2 shows T_2 values as a function of the distance from the bone-cartilage junction for different time of dehydration (i.e. at different times from slaughtering). T_2 trend as a function of cartilage location remains unchanged in the time interval from 60 to 105 hours. Moreover from 3 to 104 hours, no reduction in cartilage thickness was observed. T_2 values decrease in radial zone from 3 to 36 hours, and in the intermediate zone from 3 to 61 hours. T_2 values seem to remain constant in the superficial zone in the time interval from 3 to 105 hours. After 105 hours, T_2 values decrease in the radial zone and a thickness reduction of the intermediate zone is observable. Furthermore, T_2 values gradually decrease after 104 hours in all cartilage zones. Equation (1) allows us to evaluate the probability p of hydrogen bonds formation in water channels. Fig.3 shows the values of p as a function of time of dehydration. p varies from 0.6 to 0.3, in the time interval from 1 to 410 hours from the slaughtering, showing anyhow a non-monotonous behaviour. Indeed, from 1 to 4 hours, from 6 to 137 hours and from 197 to 410 hours, the value of p value remains constant.



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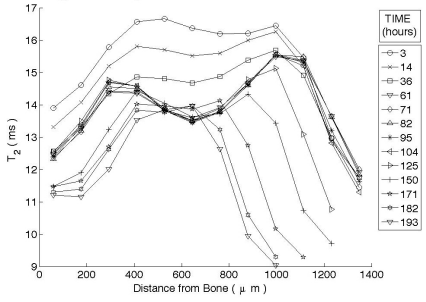

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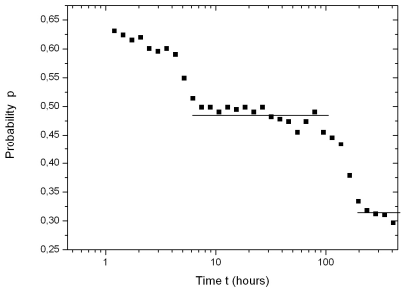

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Discussion: The decrease of p as a function of time can be interpreted as an increase of the fraction of the broken hydrogen bonds, i.e., the progressive loss of the zig-zag chains in the channels. Instead, T_2 values are characterized by the contribution of two different water molecules pools: the first is due to anisotropic rotating water molecules [4,5] and the second is due to feeble interacting water. As a consequence, variation of T_2 values is due to variation in concentration of these two compartments. In conclusion, both techniques provide indirect information on the structural cartilage changes. From dielectric spectroscopy, it is clear that the loss of water chains in collagen channels occurs with three characteristic times, resulting in the three regions observed in Fig.3. Similarly, variation of T_2 values as a function of dehydration time shows different behaviours during the same three time ranges. Therefore, it is possible to conclude that: from 3 to 60 hours the loss of feeble interacting water is the main process due to cartilage dehydration; from 60 to 105 hours no evident changes occur in collagen network. Finally, from 105 to 200 hours, two processes could be assumed: loss of feeble interacting water pool and structural modification of collagen; the second process results evident from the observation of hydrogen-bounded water molecules (detected with Dielectric Spectroscopy) and from the observation of anisotropic rotating water pool (detected with T_2-weighted-MRI). In particular, after 105 hours, the thickness reduction and the different variation of T_2 values between radial zone and intermediate and superficial zones are supposed to be clear indices of structural collagen network disruption.

Conclusions: These observations can help to elucidate the ability of T_2 maps to detect different damages in cartilage and can explain T_2 behaviour as a consequence of cartilage dehydration.

References: [1]G.Zernia,D.Huster,NMR Biomed. 19,1010–1019(2006). [2]W.Grunder,NMR Biomed.19,855-876(2006). [3]H.J.C.Berendsen et al., Biopolymers 18,47-57(1979). [4]C.Migchelsen,H.J.C.Berendsen,J.Chem.Phys.59(1),296-305 (1973). [5] R.Reddy et al.,Magn.Res.Med.52,1103-1109(2004).