The Effects of Mechanical and Osmotic Stress on Intact and PG-Depleted cartilage.

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Introduction It is a common practice in the literature to simulate the effects of mechanical pressure on the articular cartilage by subjecting the tissue to osmotic stress. The aim of this study was to compare the effects of mechanical and osmotic stress on the collagen fiber architecture in native and proteoglycan (PG) depleted cartilage. The anisotropic motion of the water molecules in the vicinity of the aligned collagen fibers results in ^{2}H quadrupolar splitting for articular cartilage equilibrated in deuterated saline (1). The quadrupolar splitting, ν_Q, is a measure for the fraction of water molecules interacting with the collagen fibers and the alignment of the fibers relative to the magnetic filed. ^{2}H in-phase double quantum filtered (IP-DQF) spectroscopic imaging (1,2) is a convenient non destructive method to obtain the spatial distribution of the deuterium quadrupolar interaction.

Materials and Methods Cartilage bone plugs, 7-8 mm in diameter were excised from bovine femoral condoyles. All plugs were equilibrated in deuterated saline, wiped dry and immersed in fluorinated oil. For the mechanical pressure two plugs, separated by a Teflon spacer, were placed surface to surface, in a heavy walled NMR tube. In this way the physiological situation is mimicked. A home built pressure device with a series of weights was used (3). For the osmotic stress we used polyethyleneglycol solute (PEG, Mw 20,000 in 150 mM NaCl/D_2O). For PG depletion, intact cartilage was equilibrated in a solution of 1 mg/ml trypsin in PBS for 12 h, at 25^{o}C. A mechanical load of 0.5 MPa for intact and 0.12 MPa for depleted results in the same shrinkage of the cartilage. The same is true for osmotic stress when we use 20% PEG and 10% PEG solutions for intact and depleted samples respectively. Moreover the shrinking caused by 20% PEG is similar to that caused by 0.5 MPa.

Results In ^{2}H one-dimensional IP-DQF spectroscopic MRI of articular cartilage, at least two pairs of satellite transitions are observed near the bone and in the radial zone. The quadrupolar splittings decrease towards the transitional zone and after a certain point only one pair of satellite is observed.

We have studied both intact and depleted cartilage-bone plugs. In intact cartilage, mechanical load of 0.5 MPa results in the disappearance of the large splitting near the bone (Fig. 1, 15%), as previously reported by Shinar et al. (1). A similar result is obtained for PG-depleted cartilage subjected to mechanical load of 0.12 MPa - a pressure that leads to a similar degree of compression. A striking difference between intact and depleted cartilage is observed at depths of 40% and 60% from the bone. For the intact cartilage only one pair of quadrupolar split satellite is observed, while two pairs are clearly resolved for the depleted sample.

We have also applied osmotic stress to intact and depleted cartilage, and in this case were able to measure the quadrupolar splitting at a few orientations of the plug relative to the external field (Fig.2). The quadrupolar splitting depends on the geometric factor ($3\cos^2\theta - 1$), where θ is the angle between the director of the quadrupolar interaction and the magnetic field. Unlike the case of mechanical compression under osmotic stress the large quadrupolar splitting, near the bone, did not disappear for both intact and depleted cartilage. Also in both depleted and intact samples, the orientation dependence of the quadrupolar splitting that was observed before the applied osmotic stress is no longer evident. For example, osmotic stress applied to depleted cartilages at depth of 60% from bone, results in the appearance of two pairs of satellites that are independent of the orientation relative to the magnetic field.

Conclusions The effect of PG depletion on the ^{2}H ν_Q was found to be minimal, indicating no role of the PG on the collagen fiber architecture. Neither the splittings nor their orientation dependence changed. This is in line with the fact that also T_2 values in intact and depleted plugs are very similar. The observation that under mechanical load the quadrupolar splittings at 40%-60% from bone do not vanish for the depleted cartilage may be an indication of higher degree of order or dehydration compared with the case of the intact cartilage. As has been shown before (4) there is a pronounced difference between mechanical load and osmotic stress for intact cartilage, this is also true for the depleted cartilage. Under osmotic stress in both cases the orientation dependence is lost.

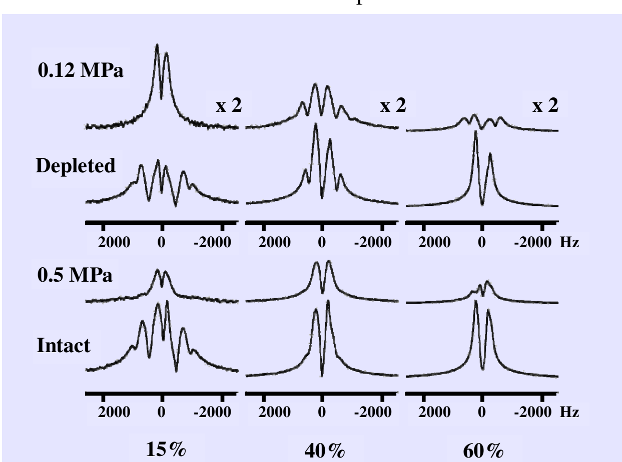

Fig. 1 – The effect of mechanical load on intact and depleted cartilage-bone plugs at depth of 15%, 40% and 60% from the bone.

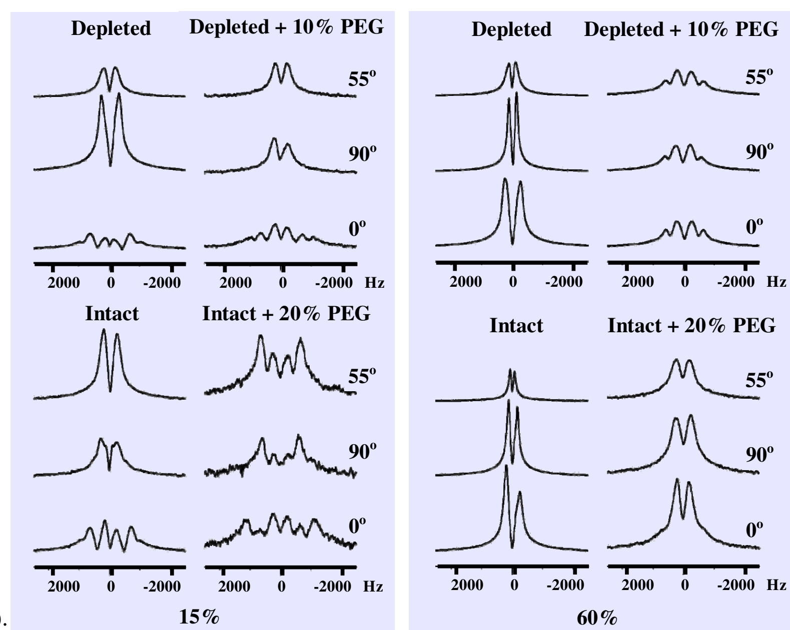

Fig. 2 – The effect of osmotic stress on intact and depleted cartilage-bone plugs, at depths of 15% and 60% from bone, at three angles (0^o, 90^o, 55^o) between the direction of the collagen fibers near the bone and B_0.

References: 1. H. Shinar et al., Magn. Reson. Med., 48, 322 (2002). 2.U. Eliav et al., J.Magn. Reson.,137, 295 (1999). 3. K. Keinan-Adamsky et al., J Ortho Res, 23:109 (2005). 4. H. Shinar et al, ISMRM 11th Meeting, 55 (2003).