Solid-state MAS NMR Measurements of Intact Articular Bovine Cartilage

R Mark Wellard[1], Alf Pawlik[2], Sabrina Barheine[2], and Konstantin I Momot[3]
[1]QUT, Brisbane, Qld, Australia, [2]QUT, [3]QUT, Australia

Articular cartilage (AC), an avascular connective tissue lining articulating surfaces of the long bones, comprises extracellular biopolymers. In functionally compromised states such as osteoarthritis, thinned or lost AC causes reduced mobility and increased health-care costs. Understanding of the characteristics responsible for the load bearing efficiency of AC and the factors leading to its degradation are incomplete.

Objectives DTI shows the structural alignment of collagen in AC [1, 2] and T_2 relaxation measurements suggest that the average director of re-orientational motion of water molecules depends on the degree of alignment of collagen in AC [3]. Information on the nature of the chemical interactions involved in functional AC is lacking. Our aim was to characterize intact cartilage using magnetic resonance spectroscopy. The need for AC structural integrity makes solid state NMR an ideal tool to study this tissue.

Method: We examined the contribution of water in different functional 'compartments' of cartilage using solid-state ^{1}H-MAS, ^{13}C-MAS and ^{13}C-CPMAS NMR of bovine patellar cartilage, before and after incubation in D_2O to allow removal of freely exchangeable water. This was to enhance detection of NMR signal from protons from the extracellular matrix of cartilage.

Results: ^{1}H-MAS spectra signal intensity was reduced due to H/D exchange without a measureable redistribution of relative signal intensity. Chemical shift anisotropy was estimated by lineshape analysis of multiple peaks in the ^{1}H-MAS spinning sidebands. These asymmetrical sidebands suggested the presence of multiple water species in AC (Figure 1). Therefore, water was added in small aliquots to D_2O saturated AC and the influence of H_2O and D_2O on organic components was studied with ^{13}C-MAS-NMR and ^{13}C-CPMAS-NMR. Signal intensity in ^{13}C-MAS spectra showed no change in relative signal intensity throughout the spectrum. In ^{13}C-CPMAS spectra, displacement of water by D_2O resulted in a loss of signal in the aliphatic region due to a reduction in proton availability for cross-polarization.

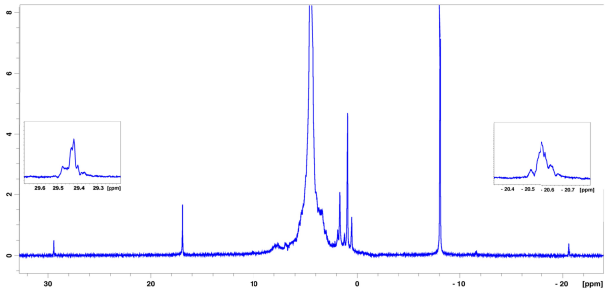

Figure 1: solid-state MAS NMR spectrum of cartilage showing asymmetrical sidebands associated with different water environments.

In vitro studies of articular cartilage often utilize frozen tissue samples despite the effects of freezing and thawing on cartilage not being well described. We examined the impact of freezing and thawing on metabolite mobility within cartilage using ^{13}C NMR by soaking cartilage in D_2O to enable measurement of mobile metabolites. We found that independent of storage time, freezing resulted in increased signals in the chemical shift range of 3.4 to 3.6ppm and a large reduction in the lipid signal at 1.2 to 1.3ppm corresponding to proteoglycans [4].

Conclusions: Our results complement dehydration studies of cartilage using osmotic manipulation [5] and demonstrate components of cartilage that are in contact with mobile water. They also suggest that reliable metabolite profiles are only obtained from fresh tissue samples.

1. Meder, R., et al., Diffusion tensor imaging of articular cartilage as a measure of tissue microstructure. Osteoarthritis and cartilage, 2006. 14(9): p. 875-81.
2. de Visser, S.K., et al., Anisotropy of collagen fibre alignment in bovine cartilage: comparison of polarised light microscopy and spatially resolved diffusion-tensor measurements. Osteoarthritis and cartilage, 2008. 16(6): p. 689-97.
3. Momot, K.I., J.M. Pope, and R.M. Wellard, Anisotropy of spin relaxation of water protons in cartilage and tendon. NMR in biomedicine, 2010. 23(3): p. 313-24.
4. Shet, K., et al., High-resolution magic angle spinning NMR spectroscopy of human osteoarthritic cartilage. NMR in biomedicine, 2011.
5. Zernia, G. and D. Huster, Collagen dynamics in articular cartilage under osmotic pressure. NMR Biomed, 2006. 19(8): p. 1010-9.