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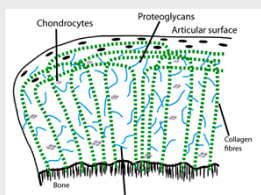
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INTRODUCTION   Diseases of articular cartilage (AC), such as osteoarthritis (OA), impact up to a quarter of the Australian population. The avascular nature of cartilage and its limited ability to self-repair makes early diagnosis and treatment of OA an important factor in reducing the healthcare burden, particularly for an aging population. Our work aims to develop a robust MRI methodology for evaluation of cartilage ECM within an individual joint for early diagnosis of OA. As an extension to our work on water diffusion and relaxation times in isolated cartilage to interrogate the molecular hydrodynamics of water in AC [1], we aimed to investigate the metabolite distribution within the cartilage matrix and the influence of water on metabolite signals, using solid state NMR.

BACKGROUND   Articular cartilage, an avascular connective tissue lining articulating surfaces of the long bones, is comprised predominantly of extracellular biopolymers. Healthy adult human cartilage is 2 – 4 mm thick and contains a sparse population of chondrocytes within an extracellular matrix of collagen (15 - 20%), proteoglycans (3 – 10%) lipids (1 – 5%) and water (65 – 80%). Understanding of the characteristics responsible for the load bearing efficiency of AC and the factors leading to its degradation are incomplete.

Whilst DTI shows the structural alignment of collagen in AC and T2 relaxation measurements [1] suggest that the average director of reorientational motion of water molecules depends on the degree of alignment of collagen in AC [2], the need for AC structural integrity makes solid state NMR an ideal tool to study the metabolic profile and chemical interactions involved in functional AC. We examined the contribution of water in different functional 'compartments' using ^{1}H-MAS, ^{13}C-MAS and ^{13}C-CPMAS NMR of bovine patellar and femoral cartilage incubated in D_2O to remove free water. Previous reports have described the metabolite profile in AC using MAS NMR [3-6] but the influence of freezing and thawing on the metabolite distribution has not been reported. Spectra recorded from fresh cartilage were compared with those from frozen tissue.

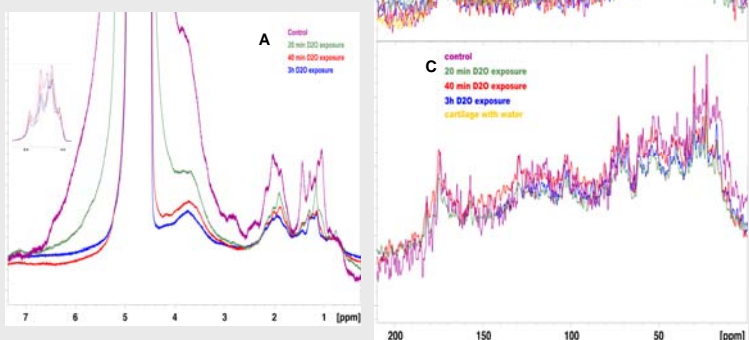Schematic illustration of collagen composition and fibre alignment in articular cartilage.

Adapted from Scheidt HA, et al., Biopolymers. **93** (2010) 520-32

Cartilage origin   The development of specific cartilage characteristics such as chondrocyte morphology and collagen alignment occurs in response to the load experienced by the joint. Given these structural and functional differences, we assessed cartilage isolated from the femur and patella of bovine knee joints to determine whether this was also associated with a different metabolite profile. Peaks were simulated for a ^{13}C-MAS spectrum of patella using DMFIT software [7] and the resultant peaks assigned according to published chemical shifts (table and A). Panel B shows ^{13}C-MAS NMR spectra. Amino-acid signals dominated ^{1}H spectra (C).

position [ppm]	T_2(ms)	Assignment
181.6	1.71	Glu C=O
178	1.06	C=O
174.3	0.88	C=O
170.7	1.24	C=O
168.2	1.06	C=O
157.6	1.27	Arg C_δ
129.5	0.88	ring carbons
124.5	0.88	sideband
120.8	0.9	sideband
103	0.88	GAG
81.29	0.78	GAG
76	0.82	GAG
72.93	1.2	GAG
70.8	1.3	Hyp C_γ
67.59	0.79	Thr
62.01	0.87	Val, Thr
59.4	1.03	Pro C_α, Hyp C_α
54.16	0.47	Ala C_α, Hyp C_β, Arg, Glu
47.54	0.49	Pro C_δ
41.97	0.73	Gly C_α, Arg
37.77	0.69	Hyp C_β
34	1.27	Glu
30.5	0.89	Pro C_β, Val
28	1.38	Arg, Glu
25.4	1.09	Pro C_γ
22.85	1.22	GAG
20.15	1.32	2x Val, Thr
17.3	1.27	Ala C_β
11.35	0.48	no assignment

Evidence for water compartmentation ^{1}H MAS NMR spectra of cartilage were dominated by a large water signal, with asymmetric spinning side bands. The asymmetry of the spinning sidebands is consistent with the presence of anisotropic water environments within cartilage. The inset peaks show the second spinning side band on each side of the water peak.
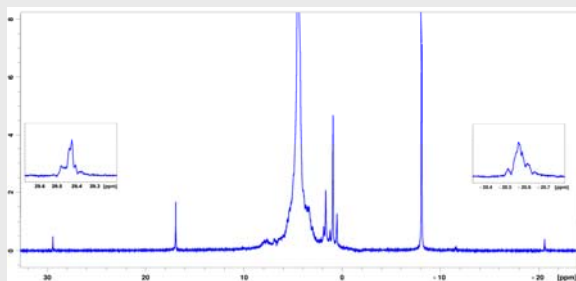
Water replacement in cartilage   Cartilage samples were incubated for up to 3 hours in D_2O prior to measurement of ^{1}H (A) and ^{13}C MAS NMR spectra. "Dehydration" by exchange of D_2O for water resulted in a reduction of signal intensity in ^{1}H (A) and ^{13}C-CP-MAS (B) measurements but not single pulse ^{13}C measurements (C), indicating that water protons are participating in the polarization transfer.
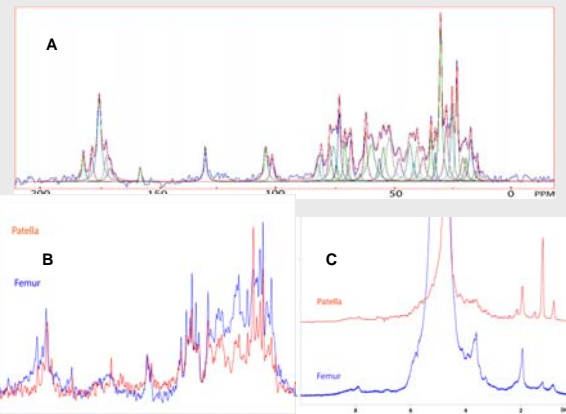

Storage effects   Many studies of cartilage report that tissue was frozen and 'stored until required' after collection of samples from an abattoir. When cartilage was soaked in D_2O and the ^{13}C NMR spectrum of the supernatant recorded, there was a difference in metabolites released into the incubation solution, depending on whether or not the tissue had been frozen. The figure below shows the differences in the profile of metabolites released from fresh (A), compared to cartilage frozen for short-term (1 week - B) and long-term (6 months - C) storage at -20˚ C. The duration of freezing did not alter the metabolites released into D_2O, but the act of freezing did. In structural and functional studies, the differences in metabolite release following freezing may alter cartilage performance or account for differences between studies.
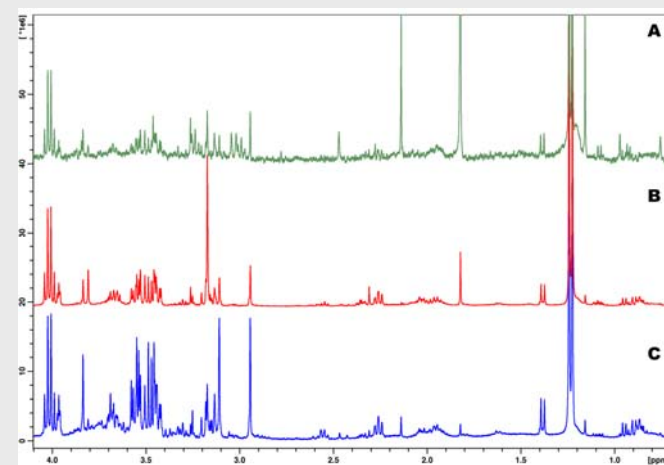
Conclusions   MAS-NMR measurements of ^{1}H and D_2O exchange provide evidence for water compartmentation within cartilage. Replacement of mobile tissue water with D_2O results in residual signal from hydrogen in bound environments which could include non-exchanging water.

MAS-^{13}C NMR measurements of patella and femur cartilage show different metabolic profiles. This may reflect the functional performance of the cartilage but also provides a window into the changes that may be produced by load bearing.

Freezing of cartilage for even short periods of time at -20˚ C results in changes to the diffusional egress of metabolites from the matrix into surrounding solution. This may not be important for structural studies of cartilage but the potential loss of metabolites due to frozen storage should be considered when cartilage function is examined.

References

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