^{2}H Double Quantum Filtering NMR Can Determine Degenerative Changes in the Human Nucleus Pulposus of the Intervertebral Disc

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INTRODUCTION

Each tissue in the body has different forms of water within the cellular and extracellular matrix that can be broadly categorized as bound, intermediately bound and free water. Little is documented of detailed water structure of normal or degenerated nucleus pulposus of the intervertebral disc. For tendon, double quantum filtering (DQF) has been utilized as a method for examining bound water. In this work, we utilize ^{2}H DQF with nuclear magnetic resonance spectroscopy (NMR) as a sensitive probe of the water dynamics in nucleus pulposus tissues. The total water present in nucleus is dependent, in part, on the charge density of sulfated glycosaminoglycans (GAGs) on proteoglycan (aggrecan) molecules, which change in structure and reduce in quantity with degeneration of the nucleus pulposus of the disc leading to a reduction in water content (1), although the bond state of this water has not been well-described. Our hypothesis was that the bound water (as determined by DQF NMR) increases with degeneration of the nucleus pulposus while the overall hydration level decreases. The implications of this work are that more specific structural changes associated with the nucleus pulposus tissue might lead to more accurate and earlier diagnosis of degenerative disc.

METHODS

Twenty-six nucleus pulposus samples with different degenerative grades (Thomason Grade 1-5) were obtained from different levels from ten human cadaveric thoracic and lumbar spines (15-80 y/o). The specimens were allowed to exchange water in a ^2H_2O environment with care taken to prevent swelling behavior by immersing the specimen in a dialysis membrane (1,000 MW) in a 0.12 g/mL polyethylene glycol solution[2]. All NMR spectra and relaxation curves were acquired on a 9.4 T Tecmag Discovery solid-state NMR spectrometer (^{2}H frequency of 61.4 MHz), equipped with a triple resonance MAS probe. The data were collected at 23 ^{0}C (+/- 0.2^0). The build-up rates and the creation times for the maximum DQF signal, τ_{max}, were measured by acquiring spectra while varying the creation time τ. The data were processed in NTNMR. Fitting of the relaxation data was performed in IgorPro.

RESULTS

Figure 1 shows the ^{2}H DQF peak height normalized with respect to the single quantum spectrum height as a function of the creation time, τ. Older and more degenerative specimens showed shorter creation times than younger specimens. Shorter creation times are consistent with the reduced water mobility on the time scales of the DQF experiment and with stronger residual quadrupolar interactions[2]. The increased intensities shown with older and with more degenerative nucleus samples represent more tightly bound water than those of the younger and less degenerate samples. In Figure 2, the creation time for maximum DQF signal is plotted versus specimen age. This graph shows a nearly linear relationship between τ_{max} and the specimen's age for all twenty six samples, with the τ_{max} decreasing monotonously from 20 to 3.2 ms when the age increases from 15 to 80 years.

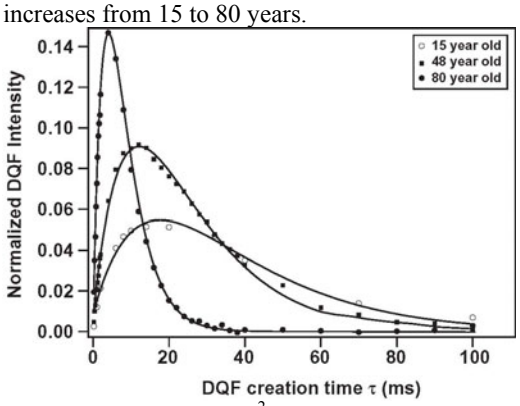

Figure 1: Normalized ^{2}H DQF peak height of nucleus pulposus as a function of creation time τ.

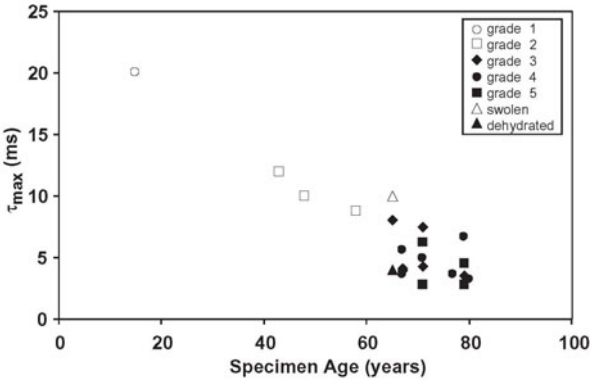

Figure 2: Creation time τ_{max} for the maximum DQF signal in human nucleus pulposus as a function of specimen's age.

DISCUSSION

^{2}H DQF NMR spectroscopy is a sensitive probe of the water dynamics in nucleus pulposus tissues. τ_{max} correlates strongly with the age and degenerative grade of the human cadaveric nucleus pulposus specimens. The normalized DQF buildup rates are also dependent on the age of the samples. Structural changes associated with the nucleus pulposus directly affect the water dynamics as measured via the ^{2}H DQF NMR spectroscopy and might be useful indicators of degenerative changes in the disc tissue.

REFERENCES

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