# Relaxation Parameters of N-acetyl in Healthy and Osteo-Arthritic Cartilage - An High Resolution Magic Angle Spinning (HR-MAS) Study

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## Abstract

Osteoarthritis (OA) is a multi-factorial degenerative joint disease that results in degradation and gradual loss of articular cartilage. Proteoglycan loss and alteration in the cartilage has been considered an important marker for detecting and measuring the progress of OA. The N-acetyl resonance observed in HR-MAS spectra arises from the proteoglycan content in cartilage and is the focus of this study. We aim to detect the changes in the mobility of the N-acetyl moiety due to the degradation process that occurs in osteo-arthritic cartilage as reflected in the relaxation parameters of the entity ($T_1$ and $T_2$). An increase in $T_2$ relaxation time is observed in case of ex-vivo human osteo-arthritic cartilage samples.

## Introduction

Osteoarthritis (OA) is a disease characterized by articular cartilage degeneration. Proteoglycan loss and alteration in cartilage has been considered an important marker for understanding OA and monitoring the disease [1]. NMR spectroscopy is useful to providing direct measurement of biochemical changes in tissues. Furthermore, High-resolution magic angle spinning (HR-MAS) NMR spectroscopy provides the partial attenuation of the dipolar coupling and chemical shift anisotropy effects in intact tissues, such as cartilage, that result in broad resonances. Previous studies in bovine cartilage have shown that the N-acetyl resonance located at 2.04ppm in the HR-MAS (500 MHz) spectra arises from the PG content in cartilage [2]. We have previously reported decreased N-acetyl in human OA cartilage [3] when compared to healthy cartilage as shown here in figure 1. In this HR-MAS study we measure the difference in the relaxation parameters ($T_1$ and $T_2$) of healthy and OA cartilage as observed in ex-vivo human cartilage samples.

## Materials and methods

Three OA cartilage samples were harvested from 3 patients who underwent Total Knee Arthroplasty (TKA) surgeries; and 3 healthy (control) samples were extracted from NDRI cadavers using 3.5 mm biopsy punches from the lateral inferior femoral condyle. The samples were flash frozen at -80˚C for storage. The samples were scanned in a 500MHz Varian INOVA spectrometer in a 30µL zirconium rotor which was spun at a rate of 2.25 KHz. The reference used was 0.75 mass % 3-(trimethylsilyl) propionic-2, 2, 3, 3-d4 acid (TSP in $D_2O$). The 1-D HR-MAS NMR spectra were acquired at a temperature of 1˚C. The $T_1$ relaxation time was measured by using an inversion recovery pulse sequence with variable delay times in the range 0.125 -3.2 sec, a total repetition time of 8 sec (>5 times $T_1$) and an acquisition time window of 2 s (>5 times $T_2$). The $T_2$ relaxation time was measured using the Carr-Purcell-Meiboom-Gill (CPMG) sequence that was rotor synchronized (i.e., τ delay = n x (spin rate)$^{-1}$, where n is an even number) with echo-times TE ranging from 0 – 500 ms. A spectral bandwidth of 20 KHz was used in the above experiments. Both acquisition pulse sequences were preceded by a water pre-saturation pulse to minimize the water signal at 5 ppm and to ensure a flat baseline. The NMR spectra were processed using the ACD Labs 1D NMR processor (ver. 8.0). The 1D FID's were apodized with an exponential function, Fourier transformed, phase corrected, baseline corrected and referenced to TSP at 0 ppm. The $T_1$ and $T_2$ curve fitting was performed using the curve fitting toolbox in MATLAB. A two-tailed T-Test was performed to determine whether there was a statistically significant difference between the relaxation parameters of OA and healthy cartilage.
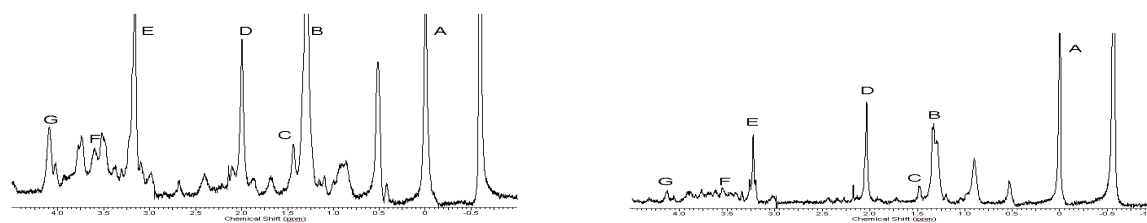
## Results



Figure 1(a)

Figure 1(b)

Figure 1(a) and (b) Representative HRMAS spectra of healthy and OA cartilage respectively indicating a decrease in the intensity of the N-acetyl peak in case of OA

| | $T_1$ (ms) | $T_2$ (ms) |
|---|---|---|
| CONTROL | 356 ± 15 | 38.16 ± 8.34 |
| OA | 365 ± 15 | 65.16 ± 7.18 |

Figure 2. $T_1$ and $T_2$ relaxation times of the N-acetyl resonance of OA and healthy (control) cartilage. Values provided are mean ± SD

Figure 2 shows the mean and standard deviation of the relaxation times namely $T_1$ and $T_2$ for control and osteo-arthritic cartilage. A statistically significant increase in $T_2$ relaxation time was observed in case of OA cartilage samples when compared to controls (P<0.05)

## Discussion and Conclusion

An increase in $T_2$ relaxation time of the N-acetyl resonance is observed in OA cartilage compared to controls. This can be attributed to the increase in mobility of the N-acetyl moiety and other components in cartilage as a result of the degradation in osteo-arthritis. The knowledge of the relaxation time constants is also useful in determining the timing parameters for other HRMAS studies that employ more sophisticated spectral acquisition techniques for 1-D and 2-D NMR wherein the knowledge of relaxation parameters is important in achieving accurate spectral information. HRMAS is a powerful non-destructive tool for identification of spectroscopic markers of early cartilage degeneration in osteoarthritis.

**References:**
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