# Assessment of Extracellular Matrix Degradation in Intervertebral Disc Degeneration by Diffusion Weighted MRS and Chemical Exchange Saturation Transfer

Anna M. WANG[1,2], Adrian Tsang[1,2], and Ed X. Wu[1,2]

[1]*Laboratory of Biomedical Imaging and Signal Processing, Hong Kong, Hong Kong, Hong Kong,* [2]*Department of Electrical and Electronic Engineering, The University of Hong Kong, Hong Kong, Hong Kong, Hong Kong*

**INTRODUCTION:** Intervertebral disc degeneration (IVDD) is the leading cause of low back pain. Glycosaminoglycan (GAG), or proteoglycan (PG) is the critical macromolecule in the extracellular matrix (ECM) in disc nucleus pulposus (NP) (1). Recently, chemical exchange saturation transfer (CEST) imaging has been applied to detect the GAG change/loss in disc NP during IVDD (2). Our recent work has demonstrated that diffusion weighted MRS (DW-MRS) can characterize the diffusion property of the ECM macromolecules in the disc NP (3). We showed that the increase of the macromolecule diffusivity can serve as an early marker for targeting the onset of ECM degradation. In this study, we examined whether the diffusivity increase of GAG in disc NP is more sensitive than the decrease of the GAG content during the ECM degradation. We compared the DW-MRS and CEST methods for longitudinally monitoring the ECM degradation in an ex vivo IVDD model.

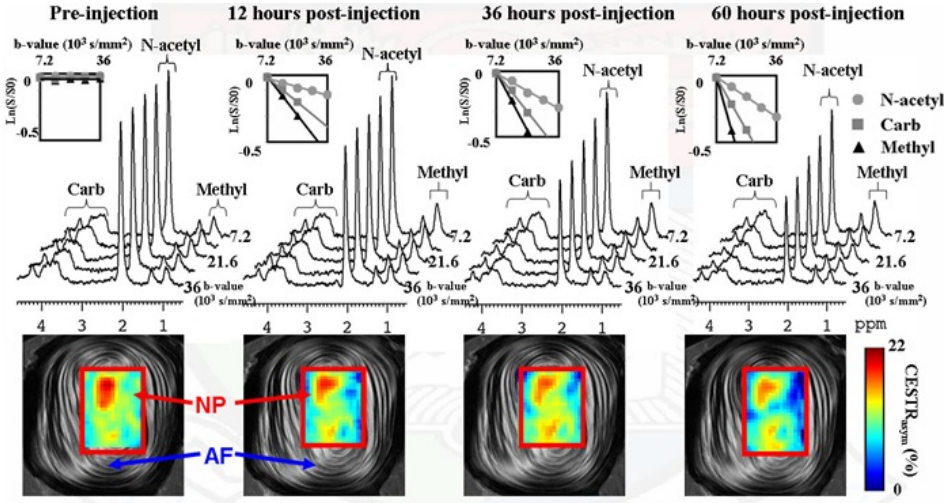


Fig.1 Typical DW spectra and representative $CESTR_{asym}$ maps overlaid on T2 weighted images before and after papain injection. For DW-MRS measurements, the increase of GAG diffusivity was characterized as the ADC increase of the N-acetyl peak. For the CEST measurements, the red rectangle stands for the shimming box and the ROI. The CEST effect was gradually decreasing after papain injection.

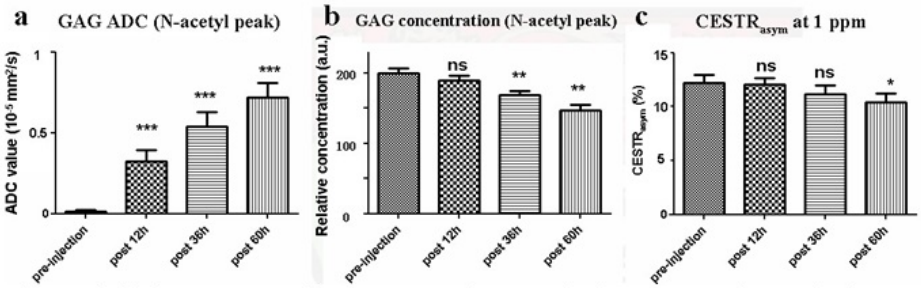


Fig.2 Longitudinal measurements of the GAG ADC and concentration by DW-MRS and CEST (N=6). GAG ADC values calculated from the DW-MRS results (a) drastically increased after papain injection, while the relative concentrations of GAG calculated from DW-MRS results (b) shared a similar slow decreasing trend with the CEST effect (c).

**METHODS:** **Model Preparation:** Six intervertebral discs were harvested from the bovine spine and individually embedded in 5% agarose gel. Papain (1mg) was injected into the NP to induce IVDD according to a previously reported procedure (4). After injection, discs were longitudinally examined over 60 hours. **MR protocol:** All experiments were performed under 7T. For each disc, the NP (500μL) was selected for DW-MRS. A STEAM based single-voxel MRS sequence was implemented (TR/TE/TM= 900/34/110ms, Δ/δ=127/10ms). Macromolecular T2 values were measured in the same voxel. The pulse sequence for CEST imaging consists of a pre-saturation pulse with duration of 4s and power level (B1) of 2.3μT (TE/TR = 64/6000 ms), followed by a spin-echo imaging pulse (RARE) readout. For correcting the B0 inhomogeneity, the WASSR scan (TE/TR = 64/2000 ms) was performed in the same geometry (5). **Data Analysis:** Spectral analysis was performed using JMRUI software. The signals in Carb region (3.5-4.2ppm), N-acetyl region (1.9-2.1ppm) and Methyl region (0.8-1.0ppm) were quantified by AMARES algorithm. Relative GAG concentration was computed as the N-acetyl peak SNR normalized by the voxel volume and corrected by the T2 values individually. The CEST effect was calculated at 1 ppm as the CEST asymmetry ($CESTR_{asym}$): $CESTR_{asym} = (S_{1ppm} - S_{-1\ ppm})/S_{-1ppm}$. The ROI was defined according to the shimming box, which was identical with the corresponding DW-MRS voxel. Results were considered significant when $p<0.05$. (Paired t-test, *p<0.05; **p<0.01; ***p<0.001).

**RESULTS:** Typical DW spectra and representative $CESTR_{asym}$ maps overlaid on T2 weighted images are shown in Fig. 1. Figs. 2a and 2b show the summary of the longitudinal DW-MRS measurements of the GAG ADC and relative concentration corrected by the T2 value in 6 papain-treated discs. Papain-treated discs generally exhibited drastic increase of GAG ADC and slight decrease of the GAG concentration. They arose from the increased GAG mobility and the leakage of the GAG or PG fragment to the surrounding agarose gel, respectively. The decrease of the GAG concentration was also revealed by the CEST result. Fig. 2c shows the gradual decrease of the CEST effect at 1 ppm observed in the same papain-treated samples.

**DISCUSSIONS AND CONCLUSION:** Our previous DW-MRS study of disc samples has showed that the increases of ADCs in different spectral regions are different (Fig. 1). This is because the signals in Methyl and Carb region are partly from the collagen fragments while the signal in the N-acetyl region is dominated by the GAG (or PG). In this study, we focused on the GAG ADC and concentration measurements because of its natural abundance in disc NP and vital role in the disc hydration and IVDD progression. Comparing with signal-voxel MRS, CEST imaging provides the GAG spatial distribution. In Fig. 1, the typical $CESTR_{asym}$ maps show the inhomogeneity of CEST effect in disc NP. GAG concentration decreased from the NP center to the peripheral area. For detecting the GAG loss during the ECM degradation, the results of MRS and CEST experiments shared a similar but slow decreasing trend (Figs. 2b-c), while GAG ADC shows much quicker response to the ECM degradation. At the early stage (12 hours after papain injection), the significant GAG ADC increase was a potent proof that the GAG ADC was more sensitive than GAG concentration measured either by MRS or CEST (Fig. 2a). In this sample study, we observed the increased GAG concentration in the surrounding agarose gel by MRS. We also found that the concentration measurements of GAG could be affected by the model or the sample preparation (not shown), i.e., the volume of surrounding agarose gel. In IVDD patients, the speed of the leakage of GAG is also affected by many factors (6). Moreover, the PH change in the disc during IVDD can also have unpredictable influence on the CEST effect (7). Thus the GAG (or PG) diffusivity measurement, as supported by the present study, constitutes a more sensitive marker for the early ECM degradation during IVDD. Such approach presents an informative means to detect and characterize early IVDD, which is particularly valuable for preclinical investigation of IVDD mechanisms and therapeutic interventions.

**REFERENCES: 1.** Lotz JC. Radiology. 2012;264(1):6-19; **2** Ling W. Proc Natl Acad Sci U S A. 2008;105(7):2266-2270; **3.** Wang AM. Magn Reson Med. 2014; doi: 10.1002; **4.** Zuo J. Magn Reson Med. 2009;62(5):1140-1146; **5.** Kim M. Magn Reson Med. 2009;61(6):1441-1450; **6.** Samartzis D. J Bone Joint Surg Am. 2011;93(7):662-670; **7.** Sun PZ. Magn Reson Med. 2008;60(4):834-841.