Center-corrected gagCEST Assessment of Intervertebral Disc Degeneration

B. Zhang[1], X. Xu[1], J-S. Lee[1,2], G. Navon[3], R. R. Regatte[2], and A. Jerschow[1]
[1]Department of Chemistry, New York University, New York, NY, United States, [2]Department of Radiology, New York University School of Medicine, New York, NY, United States, [3]School of Chemistry, Tel Aviv University, Tel Aviv, Israel

Introduction The intervertebral disc (IVD) degeneration is an irreversible process which is thought to be a factor strongly associated with degenerative disc disease and lower back pain. The IVD consists of two anatomical regions: the nucleous pulposus (NP) and the annulus fibrosus (AF). Glycosaminoglycan (GAG) is one of the major structural components in discs and the changes of [GAG] in NP are believed to indicate the early stages of disc degereration. It has been reported that GAG concentration can be assessed via the Chemical Exchange Saturation Transfer (gagCEST) approch [1]. Here, we demonstrate *in vitro* that the center-corrected gagCEST method represents a good assessment tool for GAG of IVD tissue specimens. In contrast to previous tissue studies, the current work also shows that interpolated center correction for B_0 compensation, inspired by the WASSR approach [2], is an essential procedure to insure reproducible and consistant results.

Method Five NP samples were obtained from bovine IVD samples. The samples underwent depletions by trypsin solution at different time lengths, followed by equillibrating in PBS. CEST effect and ^{23}Na NMR spectra were measured after each depletion at 11.7 T on a Bruker Advance Spectrometer. The concentration of N-acetyl is calculated based on ^{1}H NMR measurements and a standard 25 mM Chondroitin Sulfate reference solution. Fixed Charge Density (FCD) was derived from sodium concentration using ideal Donnan equilibrium equation [3]. In addition to the CEST z-spectra, direct water saturation spectra were also acquired. These spectra reflect the absolute water frequency in a specific B_0 field and were used as the reference to center the CEST spectra [2].
CEST ratio was calculated by the equation: $CEST=[M_{sat}(-\delta)-M_{sat}(+\delta)]/ M_{sat}(-\delta)$.

Results and Discussion Fig.1 (a) shows representative data from five NP depletion series. Each sample was depleted consecutively with certain steps until reaching the overall 7 min depletion time. Different colors and symbols represent different depletion series. A good correlation between –OH CEST and FCD can be observed, indicating CEST is a promising tool for detecting GAG concentration changes at different degenerative stages. One of the five depletion series is shown in Fig.1 (b). The decrease of the CEST effect could be clearly seen when the sample is depleted from 0 min to 7 min. The CEST effect is negligible after 7 minutes' trypsin treatment (pink line). Fig.2 (a) shows the correlation between N-acetyl concentration, assessed from ^{1}H spectroscopy, and the CEST effect. Overall we show that there is a good linear correlation between FCD and gagCEST on one hand and N-acetyl concentration and gagCEST on the other. Therefore we conclude that gagCEST can be used for assessing the GAG content of NP in IVD specimens.

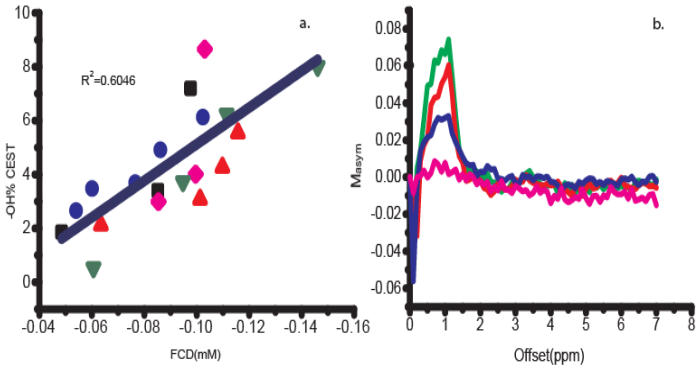

Fig. 1 NP trypsinization series. (a) CEST effect as a function of FCD with a total of five depletion series (b) one of the five depletion series showing the decrease of M_{asym} at 1.1 ppm downfield from water (-OH) with increasing depletion time. The depletion time was 0 min (green), 1 min (red), 5 min (blue) and 7min (pink).

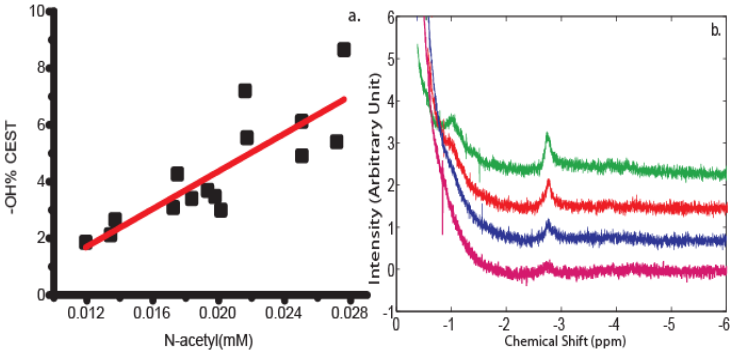

Fig. 2 (a) CEST *Vs.* N-acetyl concentration. Four NP samples were measured. (b) ^{1}H spectra showing five depletion stages of one NP sample. Only region of N-acetyl peak (2.8 ppm upfield from water) is shown. The colors indicate depletion times as descried in Fig. 1. R^2=0.6983

Conclusion We demostrated the validation of center-corrected gagCEST approach to assess GAG concentration in the NP of intervertebral disc *in vitro*, which provides possible solution of detecting early stages of degenerative disc. There is a linear correlation between OH-gagCEST and FCD measured from sodium NMR spectroscopy as well as N-acetyl concentration and gagCEST. These IVD tissue results may also prove useful in advancing *in vivo* assessment of the disc.

Acknowledgement The work is supported by a US-Israel BSF grant no. 2007157 and NIH 1R21 AR055724

References

1. W. Ling, R. R. Regatte, G. Navon, A. Jerschow, *Proc Natl Acad Sci U S A* **2008**, 105, 2266-2270.
2. M. Kim, J. Gillen, B.A. Landma, J. Zhou, P.C. van Zijl. *Magn Reson Med* **2009**, 61, 1441-1450.
3. L.M. Lesperance, M.L. Gray, D. Burstein. *J Orthop Res* **1992**; 10, 1-13.