Assessment of Porcine Intervertebral Disc Specimen pH via Chemical Exchange Saturation Transfer (CEST) MRI

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Introduction: Intervertebral disc (IVD) degeneration is considered one of the underlying factors of low back pain [1]. The IVD is highly avascular. Its nutrients must diffuse from peripheral capillaries and its energy is mainly generated by anaerobic glycolysis [2]. When the IVD cells are metabolically taxed, lactate (Lac) is accumulating, the pH drops and and this has been shown to cause a number of metabolic changes to the cell of the IVD [3]. Studies have linked low pH and loss of glycoaminoglycan (gag) in patients IVDs with discogenic back pain [4]. The accumulation of Lac and a decrease in pH seem to be initiating steps in disc degeneration and discogenic back pain. Therefore MRI methods to measure pH in IVD would be valuable in research and clinical settings. gagCEST, which detects the amount of gag by off-resonance irradiation of the exchangeable hydroxyl protons [5], may also be the sensitive to pH, due to the sensitivity -OH to pH [6]. In the first part of the present study two questions are investigated: (i) whether gagCEST is pH dependent and (ii) whether gagCEST can be used to detect pH changes in an IVD. It also has been shown previously that the DIACEST contrast agent Iopromide could be used to measure the extracellular pH in tumors by detecting two pH dependent amide groups using CEST [7]. Therefore the pH measurement using Iopromide is independent of the local concentration of the molecule. In the second part of the present study CEST MRI is used to investigate if pH changes in the IVD are detectable using Iopromide.

Material and Methods: *Phantoms*: A chondroitin sulfate solution (Sigma-Aldrich, St. Louis, MO) was diluted with H_2O to 200 mM and separated in eight 5mm diameter NMR tubes, which were titrated with NaOH/HCl to obtain a range of pH values: 5.66, 5.96, 6.10, 6.49, 6.76, 7.07, 7.5 and 7.86. In a second set of seven 5mm NMR tubes a 200mM solution of Iopromide (Ultravist 370, Bayer Healthcare) was titrated to a range of pH values of: 5.96, 6.18, 6.40, 6.70, 6.88, 7.12 and 7.48. The pH values were measured using a pH meter. *Specimens*: Porcine lumbar spine samples were obtained from a U.S. Department of Agriculture-approved slaughterhouse (Baigio Artisan Meats, Oakland, CA) 5-6h after slaughter from 2- to 5-month-old piglets. The pH of the porcine IVDs was manipulated using a 1M-lactate solution, which was injected into the IVDs. *MRI*: Imaging was performed on 7T Agilent horizontal MR scanner equipped with a 400mT/m gradient system using a 38mm diameter ^{1}H quadrature birdcage coil. A pulsed CEST preparation module in combination with a single slice turbo spin-echo imaging sequence (TR=3s, TE_{eff}=35.5ms, FOV=40x40mm^2, matrix=64x64) was used for Z-spectra acquisition. The CEST preparation module consisted out of 30 Gaussian pulses (pulse-length=100ms, bandwidth=25Hz (FWHM)) and B_1=0.75µT. Z-spectra were acquired form −1kHz to +1kHz (for chondroitin and specimens) and -3kHz to +3kHz (for Iopromide and specimens) around the water resonance with a spectral resolution of 25Hz. For B_0-correction WASSR spectra were acquired and the asymmetry of the magnetization transfer rate (MTR_{Asym}) was calculated [8].

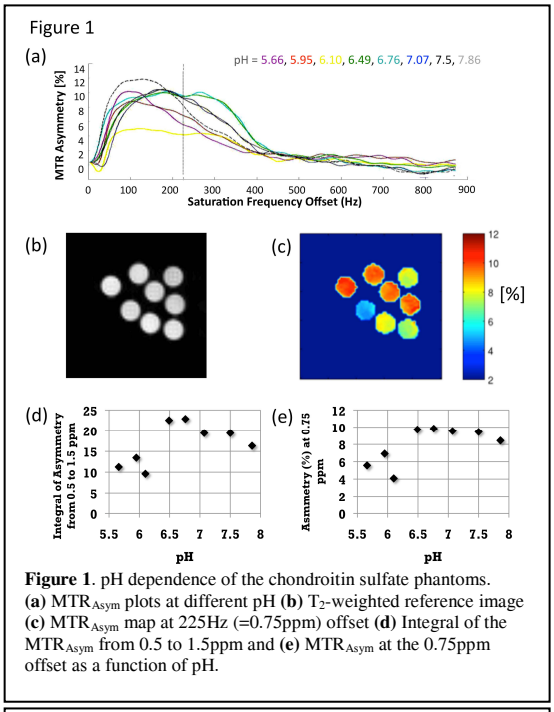

Figure 1. pH dependence of the chondroitin sulfate phantoms. **(a)** MTR_{Asym} plots at different pH **(b)** T_2-weighted reference image **(c)** MTR_{Asym} map at 225Hz (=0.75ppm) offset **(d)** Integral of the MTR_{Asym} from 0.5 to 1.5ppm and **(e)** MTR_{Asym} at the 0.75ppm offset as a function of pH.

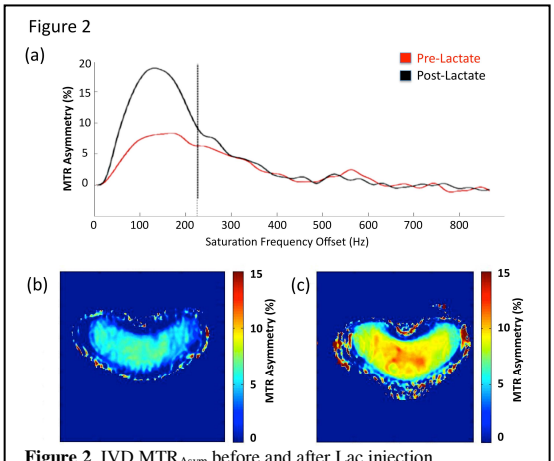

Figure 2. IVD MTR_{Asym} before and after Lac injection. **(a)** MTR_{Asym} plots **(b)** MTR_{Asym} image at 225Hz (=0.75ppm) offset before and **(c)** after Lac application.

Results: Fig. 1a shows the MTR_{Asym} plots from the eight different pH chondroitin sulfate phantoms. An intensity- and shape-based pH dependence of hydroxyl group can be observed. Fig. 1b shows the T_2-weighted reference image of the phantom, Fig 1c the MTR_{Asym} map at an offset of 220Hz (=0.75ppm) from the water resonance. The intensity of MTR_{Asym} at this offset is pH dependent. Fig. 1d displays the integral of the MTR_{Asym} (from 0.5ppm to 1.5ppm offset), Fig 1d the MTR_{Asym} value at the offset of 0.75ppm as a function of pH. The hydroxyl groups of the chondroitin sulfate show non-linear pH dependence. Fig. 2a shows the MTR_{Asym} plot from a porcine IVD before and after Lac application. After Lac injection the MTR_{Asym} of the hydroxyl-groups is increasing, which can also be seen in the MTR_{Asym} maps at the offset of 0.75ppm before (Fig. 2b) and after (Fig. 2c) Lac injected in the disc. Fig 3a shows the Z-spectra of the different pH Iopromide phantoms. The NH-groups at 4.2ppm and 5.6ppm are pH-dependent, which results in the expected linear relation when the logarithm of the peak amplitudes is plotted against the pH (Fig 3b). The pH drops from pH=7.07 before to pH=6.88 after Lac is injected in the IVD.

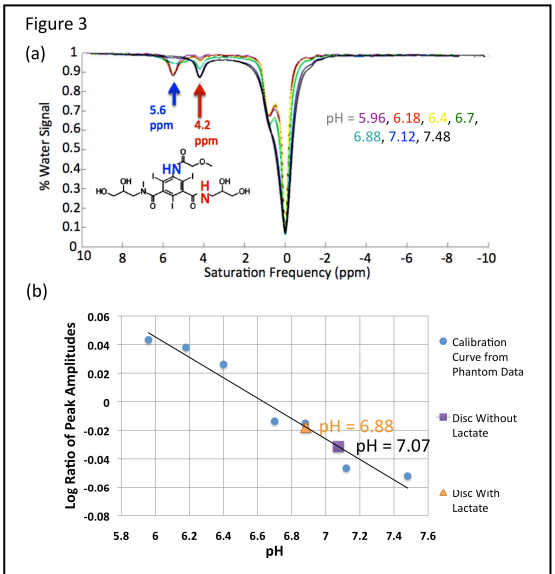

Figure 3. **(a)** Z-spectra of Iopromide phantoms at different pH. **(b)** The logarithmic ratio of the two NH- groups of Iopromide has a linear regression that correlates with pH. The IVD pH values using Iopromide before and after Lac injection are also shown.

Discussion: gagCEST, which uses the irradiation of the hydroxyl protons of gag, could be a technique to detect pH changes in IVD *in vivo* (if the gag concentration is known). The shown pH dependence can help to understand gagCEST data from IVDs. Iopromide is a promising molecule to measure pH in IVDs (independent from the local concentration), but must be applied directly (invasively) into the IVDs.

References:

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[8] Kim et al., Magn Reson Med 2009; 61:1441-50