

Sodium MRI of Intervertebral Disc Degeneration

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Objective: To establish sodium MRI as a non-invasive method for measuring intervertebral disc proteoglycan content

Introduction:

Intervertebral disc (IVD) degeneration is a common and sometimes debilitating condition, incurring tremendous financial cost on society. The initial stage of IVD degeneration involves the breakdown of proteoglycans (PG) in the nucleus pulposus (NP) region of the disc[1]. Currently, IVD degeneration is usually diagnosed using T_2 -weighted proton MRI, which is not sensitive to changes in PG content. Previous studies have demonstrated that sodium MRI can be used to non-invasively analyze PG content in the articular cartilage [2]. Sodium MRI directly measures the Na^+ concentration ($[\text{Na}^+]$) of the tissue, which is the primary cation in the NP region of the IVD. The Na^+ cations are attracted by the fixed-charge density on the PG. Thus sodium MRI can be used to estimate the PG concentration ($[\text{PG}]$) of IVD. The purpose of this study is to establish sodium MRI as a non-invasive method for measuring IVD [PG], by comparing $[\text{Na}^+]$ map obtained using sodium MRI and 1,9-dimethylmethylen blue (DMMB) PG assay results.

Materials and Methods:

Two fresh veal lumbar spines were obtained from a local abattoir within a few hours of slaughter. From each spine specimen, the last two caudal discs on the posterior side were surgically harvested. MRI was performed on a 3T Siemens Trio clinical MRI scanner. Tissue samples were placed inside a custom-made low-pass quadrature birdcage RF coil tuned to sodium resonance frequency at 3T. The dimensions of the RF coil were 17cm in diameter and 12.5cm long, and contained 16 struts. The two receiver ports were inductively coupled to the coil and oriented 90° relative to each other. Five 10% agarose gel phantoms containing 100mM, 150mM, 200mM, 250mM, and 300mM $[\text{Na}^+]$ were imaged alongside each specimen for later $[\text{Na}^+]$ calibration. The vendor's 3D FLASH pulse sequence was used to acquire all sodium images. Imaging parameters were as follows: TE/TR = 6/30 ms, flip angle = 90°, FOV = 15 x 15 cm, matrix size = 128 x 128, slices = 128, slice thickness = 1.2 mm, BW = 60 Hz/Pixel, signal average = 75. These parameters were chosen to obtain a minimum signal-to-noise ratio (SNR) of 15:1 for an isotropic voxel size of 1.2mm³. The isotropic voxel size allowed us to extract 2D slices from the 3D image in any orientation. The sodium signals from IVD and phantoms were corrected separately for T_1 and T_2 decays. A calibration curve of phantom sodium MRI signal and their known $[\text{Na}^+]$ was used to compute $[\text{Na}^+]$ maps of the IVD. After imaging, IVDs were isolated via sharp dissection. A series of ordered 4-mm diameter punches were harvested from the NP for subsequent DMMB assay, the punch removal sites were indicated in Fig 1. The discs were photographed against a dark background to record the position of the removed punches. These photographs were used later to generate image masks for reporting $[\text{Na}^+]$ values from the exact region where the punches were located. The wet weight of the punches was determined prior to digestion using papain. Digested solutions were diluted and determination of sulfated-glycosaminoglycan content was performed using 1, 9-dimethylmethylen blue (DMMB) in microplate reader assay. PG concentration was calculated from a standard curve of shark chondroitin sulfate C. All data processing and analysis were carried out using algorithms developed with MATLAB software (Mathworks, Natick, MA). Photographs of each dissected disc were interpolated to the same spatial resolution as the sodium MR images (1.2 mm²). Intensity thresholding of the photographs resulted in binary masks showing the region of interest (ROI) where punches were removed. The location of each ROI was used to report average $[\text{Na}^+]$ from the maps. An automatic co-registration routine applied step-wise in-plane rotation and translation while optimizing the linear regression fit of $[\text{Na}^+]$ map ROIs and the PG assay results.

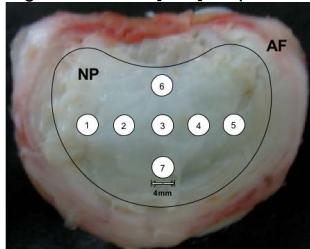


Fig 1. Photo of actual dissected disc with the punch removal sites marked.

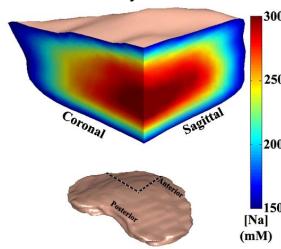


Fig 2. A 3D rendering of an intervertebral disc on the bottom. Colored $[\text{Na}^+]$ maps are overlaid on an anterior cutaway section, marked by the dotted line.

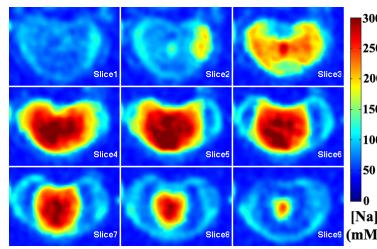


Fig 3. Nine consecutive axial slices of a disc $[\text{Na}^+]$ map. Each slice is 1.2 mm thick.

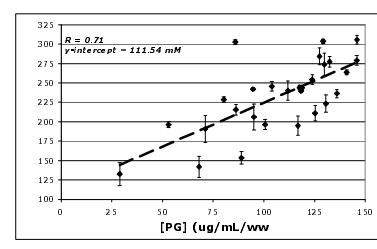


Fig 4. Scatter plot of ROI $[\text{Na}^+]$ values measured with sodium MRI and ROI $[\text{PG}]$ values measured with DMMB assay. A linear regression fit was applied to the scatter plot. $[\text{Na}^+]$ StDev was graphed as error bar.

Results:

A 3D rendered representation of a disc was shown in Fig 2, along with an anterior cutaway section depicting both coronal and sagittal planes of the $[\text{Na}^+]$ color map. The dashed lines showed where the cutaway section was extracted from the whole disc. A series of consecutive axial $[\text{Na}^+]$ color maps were shown in Fig 3. The $[\text{Na}^+]$ maps in all three primary planes showed inhomogeneity of $[\text{Na}^+]$ distribution within the disc NP. The center of the NP typically had the highest $[\text{Na}^+]$, and $[\text{Na}^+]$ decreased radially away from the NP center. This $[\text{Na}^+]$ gradient allowed us to elucidate the relationship between $[\text{Na}^+]$ measured by sodium MRI and $[\text{PG}]$ measured by DMMB assay. A plot of $[\text{Na}^+]$ measured by sodium MRI vs. $[\text{PG}]$ measured by PG assay produced a positive linear trend with a significant correlation coefficient of 0.71, as shown in Fig 3. The linear regression fit's y-intercept of 111.54 mM represented $[\text{Na}^+]$ at a zero PG concentration, which corresponded to the serum $[\text{Na}^+]$ that surrounds the IVD.

Conclusions:

In conclusion, our results demonstrated the feasibility of quantifying disc $[\text{Na}^+]$ and $[\text{PG}]$ using sodium MRI. We found a strong linear correlation between $[\text{Na}^+]$ measured by sodium MRI and $[\text{PG}]$ determined using DMMB assay. To the best of our knowledge, this is the first validation of sodium MRI as a method for assessing IVD $[\text{PG}]$ non-invasively, and thus we demonstrated that sodium MRI has the potential to be used in a clinical setting for diagnosing the depletion of PG typical of initial stage of IVD degeneration. In the near future, we propose to conduct *in vivo* sodium MRI on a series of human subjects with age ranging from 22–65 years, which would provide some insight into IVD $[\text{PG}]$ change due to age-induced degeneration.

1. Urban, J.P. and J.F. McMullin, *Swelling pressure of the lumbar intervertebral discs: influence of age, spinal level, composition, and degeneration*. Spine, 1988. **13**(2): p. 179-87.
2. Shapiro, E.M., et al., *²³Na MRI accurately measures fixed charge density in articular cartilage*. Magn Reson Med, 2002. **47**(2): p. 284-91.