Mr Diffusion Is Sensitive To Mechanical Loading In Human Intervertebral Disks

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Target audience: Spine surgeons, Diagnostic radiology, Tissue engineering, Tissue regeneration.

Purpose: Although quantitative MR have targeted the assessment of disk composition, water and proteoglycan content as markers of the severity of degeneration, it is the loss of the disks tissues' mechanical function and structural integrity which underlie the degradation of its primary role as a mechanical joint. MR measures of apparent diffusion coefficients (ADC) of water were shown to detect changes in disk physiology and its structure, both *in vivo* and *in vitro*. This study investigated the use of MR diffusion imaging, which allows probing of the spatial microstructure of soft biological tissues, to directly interrogate the loss of dynamic and viscoelastic properties of intact human disks.

Methods: L2-L3 disks were obtained from human donors aged 39, 65, 69, 72 and 81 years.

• *Mechanical testing*: Each disk was immersed in 37°C saline for 4 hours under a 200N constant compression). Using a hydraulic test system (Interlaken, Eden Prairie, MN) it was conditioned (10 compressive load cycles (100 - 300N, 0.5Hz) and mechanically characterized by 1) A dynamic test: 50 cycles of compressive strain (0-9.1%, 1Hz), simulating endplate deformation under daily loads with the displacement and force response recorded (25Hz, LabView V.8.0, NI, TX) and 2) stress-relaxation: A constant displacement, computed to impose 9.1% strain, was applied (4500 seconds) and the change in axial force recorded at 1Hz. Dynamic stiffness, elastic (E1) and viscous (E2) stiffness and viscosity ($\eta 1$) were computed.

• *Magnetic Resonance Imaging*: Each disk was imaged using a custom, MR compatible, mechanical test device located within a 72mm coil (Bruker BioSpin Inc.) situated in a horizontal bore magnet (BioSpec 4.7T, Bruker BioSpin Inc., MA). The disk's caudal-cranial, transverse and anterior-posterior anatomical axes were aligned with Z, X and Y axes of the MR imager, the disk loaded in compression (200N) simulating a sitting condition, for period of 40 minutes and a single axial image obtained at the center of the disk (FOV 60mm, 128*128 matrix, 2mm slice thickness) using the following protocols:- **T2Map**: CPMG sequence with slice selective excitation and refocusing pulses and phase encode rewinders (32 echoes, TR/TE=5000/7..247ms in 7ms spacing, 1 signal average per encoding step). **Diffusion**: diffusion sensitization gradients were applied along three orthogonal directions (gradient configuration of (1,0,0), (0,1,0), (0,0,1)) with four b values (100, 400, 700 and 1000 mm^2/s) computed from the relationship $b=3\cdot(\gamma\delta g)^2\cdot(\Delta-\delta/3)$, ($\Delta$=14ms, δ=8ms) by varying the gradient strength (g), (echo time = 28, repetition = 500)ms. Once this set of measurements was completed, the chamber was retrieved; compression level increased to 700N, simulating a standing load conditions, and the MR experiments repeated after a period of 40 min.

• *Data analysis*: T2Map and diffusion data were computed in Matlab (R14, Mathworks, MA), the annulus and nucleus regions segmented, and the resulting axial images bisected along the disk's transverse and mid-sagittal planes to yield 4 quadrant regions of interest for each tissue. For each MR measurement, a MANOVA repeated measure model (JMP 9.0, SAS, NC) was used to test for change in the mean and standard deviation for the following parameters; loading mode (unload vs. loaded), age, region (nucleus vs. annulus) and spatial location (anterior vs. posterior and lateral vs. medial) as main effects. Age*Loading, Age*region, Loading*region; and were set as cross terms to determine interactions. Post-test Tuckey HSD test was employed to test for significance between individual parameters.

Results: Application of loading caused a significantly lower Mean Diffusivity ((11.3 vs. 10.1)10-4.mm/sec, $p<0.001$) and higher variance (COV: 22.0% vs. 30.1%, $p<0.001$) of diffusivity. No such effect was seen for the T2 values. The NP showed higher T2 (45%) and MD (25%) compared to the AF ($p<0.001$ for both). Under load, however, tissue based differences were observed only for diffusion values with the AF exhibiting significantly lower MD (21%) but higher COV (31.9)% compared to the NP ($p<0.001$). Loading had no significant effect on diffusion anisotropy ($p>0.05$) (Table 1). Degenerative grade was significantly associated with the reduction in MD and T2 ($p<0.001$ for both). MD was significantly lower ($p<0.05$) and its COV higher ($p<0.01$) in the posterior regions of the AF. No such trend was observed for T2 values. ADC values were significantly correlated with the disc dynamic and viscoelastic parameters (Table 1). T2 relaxation significantly correlated with the disk's Dynamic and long term viscoelastic response. MR estimated hydration (based on signal intensity compared saline extrapolated to TE=0), was significantly correlated with the reduction in the disk's dynamic (r^2=0.66, $p<0.05$) and instantaneous (E1: r^2=0.89, $p<0.01$) stiffness.

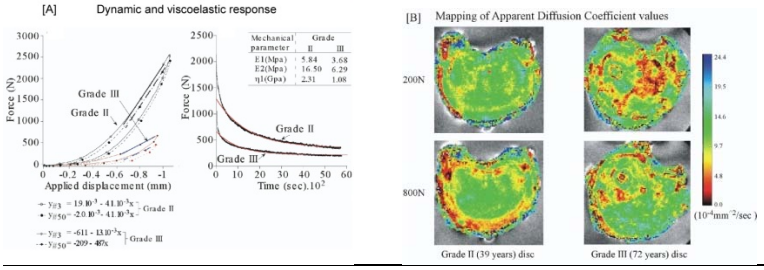

Figure 1. Cyclic and time dependent response [A] and corresponding change in MR diffusivity for a grade II and III discs [B].

Table 1. MR metrics vs. the disc's mechanical response.

MR metric	Mechanical property	Compressive Loading
T2(ms)	Dynamic Stiffness (SD)	r^2= 0.33 P < 0.05
	Instantaneous stiffness (E1)	N.S
	Long term stiffness (E2)	r^2= 0.28 P < 0.05
	Damping coefficient (η)	N.S
ADC.(10-4.mm2/sec)	Dynamic Stiffness (SD)	r^2= 0.44, p <0.01
	Instantaneous stiffness (E1)	r^2= 0.35, p <0.05
	Long term stiffness (E2)	r^2= 0.30, p <0.05
	Damping coefficient (η)	r^2= 0.45, p = 0.01

N.S:Not significant

Discussion: In contrast to the T2, diffusion measures detected the effect of loading on the disk. For both MR metrics, the strong tissue based differences appear to reflect the difference between the highly structured AF vs. the less structured but highly hydrated NP, Fig.1 The increased degenerative state of the disk was associated with loss of tissue definition, decreased variability in the NP and conversely, increased variability in the AF, suggesting a loss of demarcation between these tissues. In effect, the disks appear to become more "cartilaginous", one of the hall marks of disk degeneration. The diffusion values observed in this study, in agreement with those reported clinically, demonstrate our ability to successfully mechanically interrogate the tissue within the MR imager. We also note axis dependent changes in the response of intervertebral disks with increased degeneration. In view of the critical role of the interaction between water molecules and the macro-molecules of the disk in determining its poro-elastic mechanical behavior, noninvasively interrogating this interaction could provide direct information on the disk's mechanical function.

Conclusion: The strong correlations between diffusivity and the rheological assessments of disk mechanics, suggests that MR might permit quantitative assessment of the disk's functional status and its structural integrity.