Structure-function of the intervertebral disc assessed via MR elastography, quantitative MRI, and rheometry

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INTRODUCTION: Degeneration of the intervertebral disc (IVD) is associated with low back pain and is characterized by progressive changes in the tissue's composition and corresponding changes in material properties [1-5]. Magnetic resonance elastography (MRE) is a non-invasive imaging technique that allows the relative assessment of shear material properties in-vivo and has been previously used to demonstrate increases in in-vivo IVD shear stiffness with progressing degeneration [5]. However, it remains unclear how close the relative MRE measures of IVD shear modulus are compared to those derived from torsional mechanical testing. Furthermore, correlations of quantitative MRI metrics reflective of glycosaminoglycan (GAG) and water content have been correlated with functional compressive properties [6-7]; however, the structure-function relationship associated with shear properties remains unclear. The objectives of this study were to: **1)** compare MRE-derived and rheometry-derived shear modulus in both homogenous agarose gels and NP tissue and **2)** correlate MRE and rheological measures of NP tissue with composition and quantitative MRI.

METHODS: Agarose: Initial validation was conducted in agarose (2%, 3%, & 4% (w/v)) allowing testing of a controllable homogenous material. Agarose samples were cast in a ~120mm x 120mm x 30mm plastic container and scanned with a T1ρ and T2 mapping sequence using a 3T MR scanner (Siemens). Subsequently, a spin-echo echo-planar imaging MRE sequence was performed at 80 and 100Hz. Shear stiffness was calculated using a principal frequency analysis algorithm as previously described [5]. Separate agarose samples (N=9/%agarose, 20mm Ø x 1.5mm plugs) cast simultaneously as those for MRE were used for shear mechanical testing on a rheometer (TA Instruments). The loading protocol consisted of equilibrating at 10% axial strain for 600s followed by a frequency sweep from 1-100Hz at a rotational strain of 0.05%. **Tissue:** For validation on tissue, IVDs were isolated from the C1/2 and C2/3 level from 16 bovine tails, placed in dialysis tubing (cutoff 1 kDa), and underwent equilibrium dialysis for 48 hours in one of two osmotic conditions: 5% and 25% (g/mL) PEG in 0.15 mol/L NaCl (N=15/%PEG). These PEG solutions exert osmotic pressures of 0.027 and 0.565 MPa, respectively, and modify the tissue's hydration [8]. The IVDs were then embedded in 2% agarose for MRI/MRE analysis. After the scans, the NP region was excised, and mechanical testing samples were prepared (8mm Ø x 2mm thick). IVD samples were preloaded and underwent a frequency sweep as described above. **Structure-Function:** Samples were then lyophilized and weighed for dry weights. %Water content was calculated (wet weight – dry weight)/wet weight x 100%. After the tissue was lyophilized, ~5mg of dry tissue was digested with proteinase K, and the GAG content of all samples was assessed via the DMMB assay. ANOVA and univariate regression analyses were conducted with R (version 4.3.1) to look at the effects of testing method, %agarose or %PEG, and frequency. Correlations between parameters were also assessed.

RESULTS: Agarose: MRE-derived complex shear modulus ($|G^*|_{MRE}$) measurements were significantly greater for 4% and 3% samples compared to 2% at 80Hz and 100Hz ($p<0.001$) (Fig 1A). Rheometry-derived complex shear modulus ($|G^*|_{Rheo}$) measurements increased with concentration and were different between all agarose concentration (i.e., 4%, 3% and 2%) and all frequencies ($p\leq0.05$) (Fig 1A). |G*| values derived from both imaging and mechanical testing techniques demonstrated a frequency dependence with higher complex shear stiffness values determined at 100Hz compared to 80Hz. There was a significant positive correlation between MRE- and rheometry- derived |G*| at 80Hz ($r=0.95$, $p<0.001$) and 100Hz ($r=0.75$, $p<0.05$). T1ρ and T2 relaxation times of agarose increased with increasing agarose percentage and were negatively correlated with complex shear modulus derived from both techniques ($r<-0.87$, $p<0.05$). **Tissue:** Both MRE- and rheometer- derived complex shear moduli were significantly greater for NP tissue equilibrated in 25% PEG compared to 5% PEG at both 80Hz and 100Hz ($p<0.05$) (Fig 1B). |G*| values derived from both imaging and mechanical testing techniques demonstrated a frequency dependence with higher complex shear stiffness values determined at 100Hz compared to 80Hz. There was no significant correlation between MRE- and rheometry- derived |G*|. **Structure-Function Correlations:** T1ρ and T2 relaxation times had significant negative correlations with MRE- and rheometry-derived shear stiffnesses ($r<-0.40$, $p<0.05$). GAG/wet weight was negatively correlated with T1ρ and T2 values and positively correlated with shear stiffness ($r>0.58$, $p<0.05$). % Water content was positively correlated with both T1ρ and T2 values ($r>0.93$, $p<0.05$) and negatively correlated with shear stiffness ($r<-0.48$, $p<0.05$) (Fig 1C).

DISCUSSION: Collectively, both MRE and rheometry observed the same viscoelastic effects in both agarose and tissue, although the relationship between MRE and rheometry in NP tissue was less directly correlated. Agarose was used to validate MRE and mechanical measurements in a controllable homogenous material. An increase in |G*| with increasing agarose percentage and a significant correlation between measurements was observed from both techniques. In NP tissue, tissue hydration was modified in order to induce a range of stiffness values for evaluation. Both MRE and rheometry measurements observed the same overall effects with tissue of lower hydration having an increased complex shear modulus. Additionally, both techniques exhibited the expected frequency dependence in agarose and NP tissue ($|G^*|_{100Hz} > |G^*|_{80Hz}$). However, in NP tissue, the magnitude of the shear modulus values from MRE were significantly lower than those derived from rheometry. This is likely influenced by technical limitations of the rheometer with smaller diameter NP samples compared to agarose ($Ø_{NP}$ = 8mm / $Ø_{Agarose}$ = 20mm) making them more susceptible to inertial effects. The difference in boundary conditions applied during MRE and rheometry may have also contributed to this difference in magnitude, especially as mechanical testing required compressing the samples prior to measurements where MRE did not. Despite this limitation, all mechanical tests occurred within the linear viscoelastic region and results demonstrated no evidence of sample slippage. In both agarose and tissue, the more hydrated samples had a significantly lower shear modulus, and NP tissue exhibited strong negative correlations between %water content and shear modulus measurements. These hydration associated changes in shear modulus is consistent with previous studies that used either MRE [5] or rheologic [2-4] measurements on healthy and degenerated IVD tissue and found that healthy NP tissue, with a higher water content, exhibited lower shear stiffness values compared to degenerated NP tissue. The negative correlations between GAG/wet weight and shear modulus measurements are similar to a previous study that demonstrated a negative trend between GAG/dry weight and compressive aggregate modulus [1]. T1ρ and T2 relaxation times were strongly correlated with water content and shear stiffness suggesting that MRI/MRE can inform composition and the mechanical behaviors of IVD tissue.

SIGNIFICANCE: Overall, MRE provides a relative measurement of tissue shear properties capable of assessing hydration-induced changes. This study also demonstrates that composition, assessed via direct measurement or non-invasive MRI mapping, influences the shear behaviors of NP tissue and helps explain the complex relationship between structure and function within IVDs.

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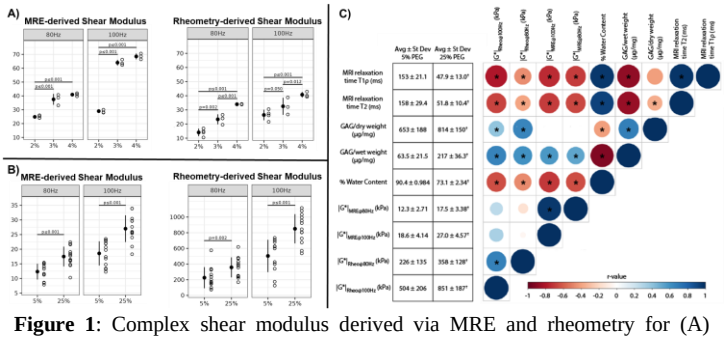

Figure 1: Complex shear modulus derived via MRE and rheometry for (A) agarose gels (B) nucleus pulposus tissue (NP). (C) Correlations between MRE- and rheometry-derived mechanical properties, quantitative MRI parameters, and compositional measures for 5% PEG and 25% PEG equilibrated NP tissue. Values are mean ± SD. †$p<0.05$ compared against 5% PEG samples. Circle size and color corresponds to p-values and correlation coefficient (r), respectively. *$p<0.05$.