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DIRECT QUANTIFICATION OF INTERVERTEBRAL DISC WATER CONTENT USING MAGNETIC RESONANCE IMAGING

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INTRODUCTION

The intervertebral disc is comprised of the nucleus pulposus (NP) surrounded by the annulus fibrosus (AF). In the healthy disc, both tissues are comprised of primarily water (~85% in the NP, decreasing to ~65% in the outer AF) [1]. Aging and degeneration have been noted by a continuous decrease in NP water content, resulting in altered disc joint mechanics [1]. Previous studies have shown that noninvasive measurements of disc water content, such as T2 mapping, can be used to detect early changes in degeneration [2], which is important for identifying discs for preventative treatment strategies. However, those techniques largely rely on correlations with biochemical composition and do not directly measure water content. Moreover, water content is an important parameter for computational models that include tissue-swelling behavior [3].

Currently, measuring tissue water content is dependent on destructive techniques (e.g., lyophilization) and, therefore, are limited to *ex situ* tissues [1]. Alternatively, magnetic resonance imaging (MRI) has been used to estimate disc water content, as signal intensity depends on the proton density within the tissue [4]. However, MRI signal intensity is dependent on scan-parameters and the concentration of free water molecules in the tissue. However, water molecules that may be rigidly bound to collagen macromolecules (e.g., ~3% of water in the NP and ~10% of water in the AF) do not appear on MRI due to a short T2 time [5, 6]. The objective of this study was to directly measure tissue water content using MRI. NP and AF water contents were measured noninvasively using T2 imaging and compared with traditional techniques. To generate a range of water content for comparison, healthy discs were mechanically dehydrated.

METHODS

Gravimetric water content (GWC), defined as the fraction of water mass divided by tissue mass, is related to volumetric water content (volume fraction, VWC) through tissue and water mass density (ρ, $\rho_{water} = 1 g/cm^3$, Eq. 1). Although GWC is widely used to report water content, MRI signal intensity (SI) correlates with VWC (SI(echo time =0) $\propto$ VWC) or spin density. Specifically, T2 relaxation times are calculated by curve-fitting MR SI versus echo time to an exponential function ($SI = SI_0 \exp(-TE/T2)$, TE: echo time; relaxation rate, R2 = 1/T2). A known tissue mass density is need to directly compare water content calculated by MRI and traditional techniques.

$$GWC = \frac{V_{water}\rho_{water}}{V_{tissue}\rho_{tissue}} = VWC\frac{\rho_{water}}{\rho_{tissue}} = \frac{VWC}{\rho_{tissue}} \quad (1)$$

Bovine caudal spine sections were obtained from a local abattoir to prepare bone-disc-bone motion segments (n = 20). Before testing, samples were thawed and hydrated in 0.15 M phosphate-buffered saline (150 mmol/L 1x PBS) for 24 hours at 4°C and equilibrated to room temperature for one hour prior to imaging.

Before scanning, samples were put into a custom-built plastic compression device (Fig. 1a). Images were first acquired with no load applied. To calculate tissue spin density, T2 relaxation was determined for 2.5 mmol/L gadolinium water, which was placed next to the specimen and served as a phantom reference (Fig. 1a). Each sample was imaged using a 3D Fast Low Angle Shot (FLASH) sequence to record 3D geometric information (7T Bruker MRI machine; FOV = 3.2 X 3.2 X 2.8 cm). Then, samples underwent a 2D scan at the mid-disc height using a T2 Rapid Imaging with Refocused Echoes (RARE) sequence to calculate T2 relaxation times (TEs = 7ms, 21ms, 35ms, 49ms, 63ms, FOV = 5 X 5 X 0.12 cm, slice thickness = 1.2 mm, in-plane resolution = 0.39 mm/pixel). TR was set to 8 sec to enable full relaxation from both the gadolinium water and disc [7]. Then, samples were compressed in 1x PBS for 24 hours at 4°C before re-equilibrating to room temperature to force fluid out of the disc. After dehydration

through mechanical loading, MR imaging was repeated, as described above.

After imaging, samples were quickly unloaded and removed from the device. Explants from the NP center and AF were prepared (6mm in diameter) from a 1-2mm thick transverse slice cut from the mid-disc height. The mass of the hydrated tissue ('wet-mass') was measured on an analytical balance (0.1 mg accuracy). Then, tissue volume was calculated by measuring fluid volume displacement after placing the tissue explant in a bath, according to the Archimedes principle (Fig. 1b) [8] Tissue mass density was calculated by dividing wet-mass over volume. Then, explants were lyophilized for 48 hours to obtain dry mass. GWC was calculated, as defined above.

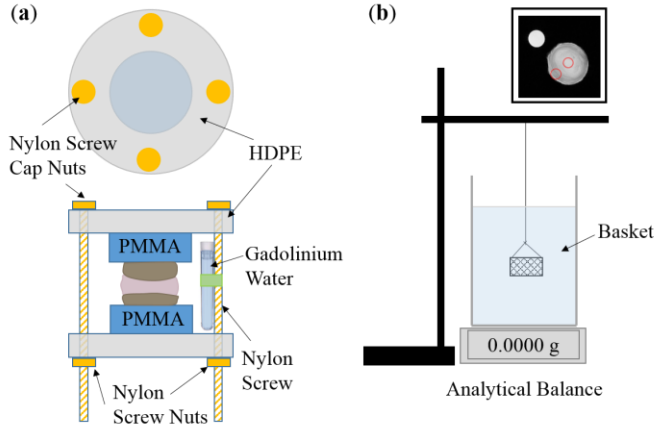

Fig 1: (a) Schematic of plastic compression device with gadolinium water (2.5 mmol/L). HDPE: high-density polyethylene, PMMA: poly (methyl methacrylate). (b) Schematic of tissue volume measurements [12]. Inset: example of T2 scan with circles representing regions of interest for NP and AF.

Exponential models were fit to MR SI versus TE times to calculate T2 and SI_0 on each pixel (*fit* function in Matlab 2018a). Spin density (SD) was calculated by normalizing the tissue SI_0 by SI_0 of gadolinium water. NP and AF region of interest (6 mm in diameter) were selected to calculate averaged SD, T2, and R2 values (Fig. 1 inset). SD and SD normalized by mass density were correlated with GWC. Mass density and R2 were correlated with GWC and SD. Significance was assumed at $p \leq 0.05$.

RESULTS

Three samples were excluded due to damage in the NP (e.g., blood from a fractured endplate). Mechanical compression successfully dehydrated disc samples and generated a wide range of NP GWC (from 0.69 to 0.84). MR spin density was greater than GWC (0.81-0.92) but was positively correlated with GWC ($p < 0.001$; Fig. 2a-open circles). Spin density normalized by mass density (ρ_{tissue}) resulted in a near perfect match with GWC (slope = 1.02, $R^2 = 0.91$, $p < 0.001$; Fig. 2a-black dots). NP mass density (range: 1.07 - 1.17 g/cm^3) and NP relaxation rate (R2) were negatively correlated with both GWC and spin density ($p < 0.001$; Fig. 2b & 2c). NP T2 values (44 – 105 ms) increased with GWC and SD, as expected.

Similar to the NP, AF spin density was positively correlated with GWC ($p < 0.001$; Fig. 2d-circles). Normalizing spin density by mass density resulted in a stronger correlation with GWC ($p < 0.001$), but values were lower than GWC values (Fig. 2d-black dots versus cyan line). Unlike the NP, AF mass density (1.08 - 1.23 g/cm^3) was not correlated with either GWC or spin density ($p > 0.2$, Fig. 2e). AF R2 values were approximately 2X greater than NP R2 values (Fig. 2c & 2f). Correlations between R2 and water content were stronger than correlations with GWC (Fig. 2d vs. 2f).

DISCUSSION

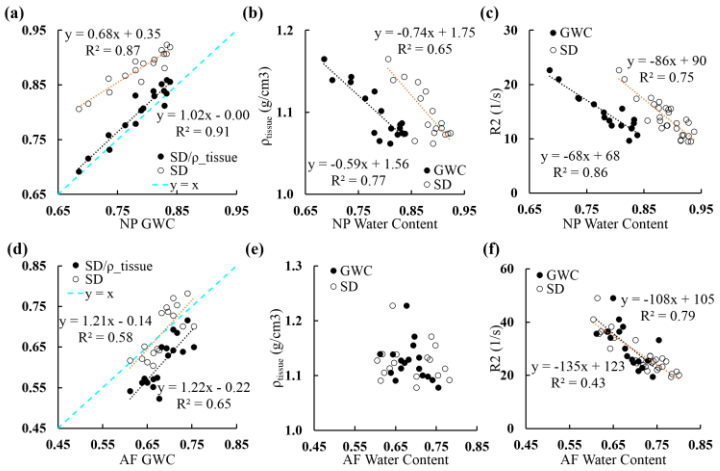

Fig. 2: Results for NP (1st row) and AF (2nd row): (a & d) Spin density (SD) and SD/mass density (ρ_{tissue}) vs. gravimetric water content (GWC). (b & e) Mass density vs. GWC and SD. (c & f) R2 vs, GWC and SD. $p < 0.005$ for (a)-(d) & (f).

Direct measurements of tissue water content are largely limited to destructive methods that require drying out tissue explants. Fast measurements of water content, on the order of minutes (vs. days), using noninvasive techniques is valuable for tracking tissue hydration with loading, disease progression, or biological repair, as tissue hydration affects joint-level mechanics. Quantitative MR parameters, such as T2 or T1ρ relaxation times, are strongly correlated with water or glycosaminoglycan content [2]. However, the relaxation times represent the behavior during the exponential decay in signal intensity, while the y-intercept should be proportional to the direct water content in the tissue (SD ∝ SI at t = 0 ms). In this study, we demonstrated that normalizing spin density by mass density provided excellent agreement between MR measured water content and water content measured through lyophilization.

However, these findings may be limited to homogenous structures, such as the nucleus pulposus and, potentially, articular cartilage. Agreement between MR measures and lyophilization measurements were not as strong for the AF, a fiber-reinforced tissue. This discrepancy is likely due to a higher concentration of bound water molecules in the AF, compared to the NP (~3% in NP and ~10% in AF) [5], where water molecules bound to collagen fibers have T2 values that are too short to be detected in MR imaging [6]. The greater concentration of bound water molecules partially explains the underestimation in MR-related water measurements (Fig. 2d).

To the best of our knowledge, this is the first study to report disc tissue mass density. NP mass density decreased with an increase in water content, but was always greater than 1.0, resulting in spin density values that were consistently larger than GWC. In conclusion, normalizing quantitative MR parameters with mass density or using the empirical formula (GWC vs SD in Fig. 1a) allow for noninvasive measurements of NP water content.

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