Quantitative T2 Mapping and Mechanical Testing in an Equine Model of Cartilage Defect Repair

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Introduction Clinical strategies to evaluate damaged articular cartilage and its repair are needed to delay or prevent the onset of osteoarthritis (OA). The implantation of minimally processed concentrated bone marrow aspirate (CBMA) is one cartilage repair approach that has shown promise *in vitro* and in small animal studies. This work has yet to be translated into clinical use, however, partly due to a lack of rigorous large animal trials. A non-invasive evaluation of such techniques is essential. T2 mapping is sensitive to tissue hydration and matrix anisotropy, which have known regional and depth-related variations in humans and horses [1, 2, 3]. The objective of this study, therefore, was to develop a large animal model of cartilage defect repairs comparing CBMA and standard microfracture (MFx), and to rigorously evaluate these treatment modalities after one year of healing. The specific aim was to assess T2 mapping as a surrogate for invasive and destructive tissue analysis.

Methods ***Equine Model:*** Eleven adult horses (7 females, 4 castrated males; mean age 4.5 years) were included. Based on physical examination, lameness evaluation, and radiographs, the animals were deemed free of stifle joint abnormalities. ***Surgical and Post-Surgical Procedures:*** Following anesthesia, each animal underwent a sternal aspiration of 70mL bone marrow, followed by bilateral stifle arthroscopy. Each horse received both treatments (CBMA and MFx); within each horse, the hind limbs were randomized to treatment prior to surgery. In each joint, a 15mm diameter cartilage defect was made in the mid-lateral trochlear ridge. The defects extended to, but did not penetrate, the subchondral bone. In the MFx-treated limbs, 6 microfracture sites were created using an awl within the defect. In the CBMA limbs, the aspirated bone marrow was centrifuged to create a CBMA graft. The horses progressed to free pasture exercise following surgery. 12 months after surgery, the animals were euthanized, and bone and cartilage blocks containing the defects were excised and frozen. ***MR Imaging:*** Multi-slice sagittal 2D T2 mapping images were acquired using a fast spin-echo sequence with 7 echo images (TEs) ranging from 10-80 ms and a repetition time (TR) of 1800 ms [FOV: 12cm; matrix: 384x384; in-plane resolution: 313x313µm; 20 slices; 2mm slice thickness; bandwidth: 325 Hz/pixel; TA: 11 minutes]. Prior to T2-curve fitting, the TE images were down-sampled using cubic interpolation in Matlab (The MathWorks, Natick, MA) to increase the signal-to-noise ratio (SNR), creating an effective in-plane resolution of 416x416µm. T2 maps were generated for a single section from the center of the cartilage defect, and from a "control" area approximately 3mm from the proximal edge of the defect, using MRIMapper software (© Beth Israel Deaconess and MIT 2006) on a Matlab platform. A small full-thickness region of interest (ROI) in the center of the cartilage defect and its corresponding control region were manually segmented. ***Indentation Testing:*** Indentation testing [4, 5] was spatially registered with the center and control sites examined with T2 mapping. At each site, indentation was performed using a 0.8mm diameter spherical indenter to a depth of 100µm. Peak load was normalized to the applied 100µm indentation to obtain the indentation structural stiffness (ISS, N/mm). ***Statistical Analyses:*** Non-parametric Friedman's two-way ANOVA tests (IBM SPSS) were used to assess indentation and T2 data.

Results Neither indentation testing nor T2 mapping showed significant differences between treatment groups in the "control" regions tested ($p \geq 0.50$); the groups were therefore combined for comparison to defect repair tissue analyses. Indentation testing showed a significant difference between control and MFx-treated defect regions ($p=0.03$; Fig. 1), and between control and CBMA-treated defects ($p=0.03$; Fig. 1). Mean T2 map values were significantly different between control and MFx-treated defects ($p<0.01$; Fig. 1), and between control and CBMA-treated defects ($p=0.02$; Fig. 1). Significant differences between MFx- and CBMA-treated defect regions were not demonstrated by either metric ($p>0.99$).

Conclusion After 12 months of healing of MFx or CMAC treatment of cartilage lesions in an equine model, mechanical deficits in repair tissue compared to control cartilage spatially corresponded to significant differences in full-thickness mean T2 values. This result suggests that as cartilage repair techniques are translated into the human clinical setting, T2 mapping may be useful as a non-invasive surrogate for the assessment of repair tissue's functional integrity. While the structural heterogeneity of repair tissue compared to control cartilage is qualitatively obvious from visual inspection of T2 maps, schemes for quantitatively evaluating cartilage T2 beyond full-thickness tissue means may provide more sensitivity to repair tissue status (Fig. 2).

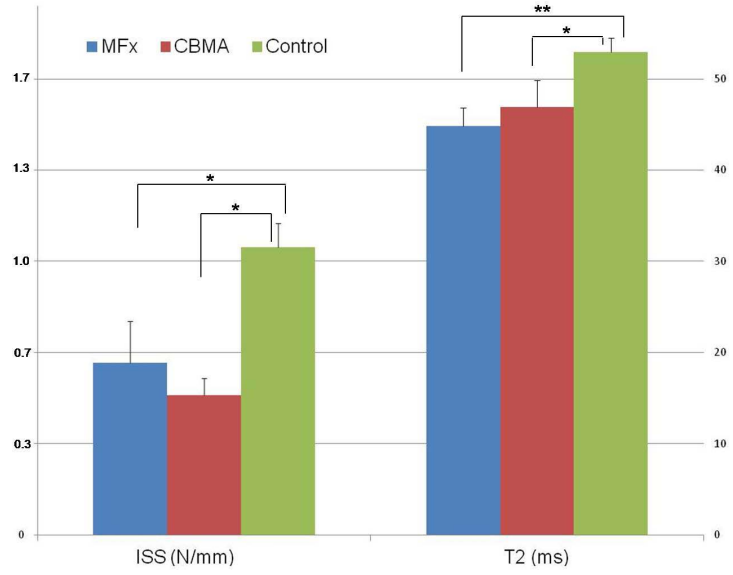
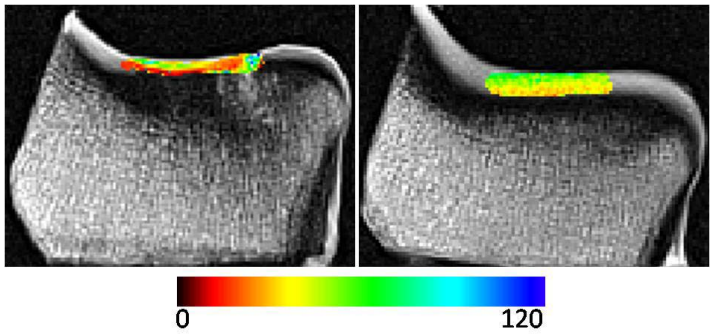


Figure 1 (Left) – Differences in mechanical properties were reflected in corresponding T2 differences. Mean (+SE) Indentation testing (ISS) and Standard T2 values for central defects in the MFx and CBMA groups, and for the surrounding "control" cartilage. * indicates $p<0.05$; ** indicates $p<0.01$.

Figure 2 (Below) – Standard T2 maps from the same explant sample, each with a mean of 46ms. (a.) depicts the center of a treated cartilage defect, while (b.) depicts the organized surrounding cartilage. The maps appear different despite their identical mean Standard T2 values.



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