

Correlation of Meniscal T2* with Multiphoton Microscopy and Changes of Articular Cartilage T2 in an Ovine Model of Meniscal Repair

Matthew F Koff¹, Lisa A Fortier², Scott A Rodeo³, Parina Shah¹, Bethsabe Romero⁴, Sarah Pownder², Rebecca Williams⁵, Suzanne Maher⁶, and Hollis G Potter¹

¹Department of Radiology and Imaging - MRI, Hospital for Special Surgery, New York, New York, United States, ²College of Veterinary Medicine, Cornell University, Ithaca, New York, United States, ³Department of Orthopaedic Surgery, Hospital for Special Surgery, New York, New York, United States, ⁴Department of Molecular Medicine, Cornell University, Ithaca, New York, United States, ⁵Department of Biomedical Engineering, Cornell University, Ithaca, New York, United States, ⁶Department of Biomechanics, Hospital for Special Surgery, New York, New York, United States

Introduction. The menisci of the knee increase contact area between distal femoral and proximal tibial articular surfaces and also aid in joint stability. Previous reports have found a correlation between meniscal tears and osteoarthritis [1], emphasizing the need of meniscal reparative surgery to preserve meniscus function and subsequent joint health [2]. However, the poor sensitivity and qualitative nature of clinical meniscal healing evaluation precludes accurate decisions about return to activities of daily living.

Magnetic resonance imaging (MRI) is commonly used to evaluate meniscal repairs, but short meniscal T2 values preclude acquisition of sufficient signal intensity when using standardized imaging techniques. Ultra-short echo (UTE) sequences display image contrast within the meniscus and allow for quantitative T2* calculation [3]. We have previously correlated T2* values of repaired menisci with corresponding mechanical strength of the repair site as well as Safranin-O fast green stained histologic sections [4] and found a good *qualitative* relationship between T2* values and histologic assessment. The purpose of this study was to evaluate the technique of T2* mapping using UTE imaging as a biomarker of meniscal integrity, by correlation with *quantitative* histologic methods, and to determine the effect of meniscal repair on post-operative cartilage T2 values.

Methods. In this IACUC approved study, a vertical, longitudinally oriented tear, 15-20 mm in length, was created surgically in the anterior horn of the medial meniscus in 28 sheep, and was repaired with vertical mattress sutures. The animals were euthanized at 3 time points (8 each at 0, 4, 8 mo.) **MR Imaging:** Performed on surgical and contralateral limbs (GE Healthcare, Waukesha, WI): **2D-FSE:** TE:20ms, TR:500ms, FOV:12cm, Matrix:512x480, 1.3mm thick, BW: ±62.5kHz, NEX:2; **2D-UTE:** TE:0.3,5,4,10,6,16.4ms, TR:350ms, Flip Angle:45°, FOV:12cm, Matrix:512x512, Radial Spokes: 1001, 2mm thick, BW: ±100kHz, NEX:2. **2D-T2 Mapping [5]:** TE:7.3-58.1ms (8 echoes), TR:1000ms, FOV:12cm, Matrix:384x256, 2mm thick, BW: ±62.5kHz, NEX: 2. Custom written software was used to calculate meniscal T2* values. T2 values of femoral and tibial cartilage were calculated for load bearing regions. Tibial cartilage was further segmented into anterior and posterior regions. **Multiphoton Microscopy (MPM):** The knees were disarticulated after imaging and histological samples from 8Mo animals were taken for MPM, collecting second harmonic generation (SHG) and autofluorescence (AF) channels simultaneously to evaluate meniscal healing (780nm excitation with near UV and broad blue emission filters, respectively). The SHG and AF image intensities, are proportional to fibrillar collagen content and collagen crosslinking, respectively, and were calculated by normalizing for input power. The SHG images were also subjected to 2D image autocorrelation to measure image heterogeneity, with high correlation values (ACD) indicating a more homogeneous structure. **Statistics:** A Wilcoxon Rank Sum test was performed to detect differences of SHG, AF and ACD between Non-OP and Tear limbs. Spearman correlation was performed to assess the relationship between meniscal T2* value and corresponding SHG, AF and ACD value. A two-way ANOVA was performed to detect differences of cartilage T2 by Type (Non-Op or Tear Limb) and Time (Zero, 4Mo, 8Mo). Analysis for tibial cartilage also included Region (anterior or posterior). Multiple comparison tests were performed when significance was found. Significance was set at p<0.05.

Results. Meniscal MPM: Significant differences of SHG (p=0.04), AF (p=0.03), and ACD (p=0.02) was found between Non-Op and Tear limbs indicating reduced collagen content, reduced collagen crosslinking and greater heterogeneity of Tear limbs (Fig 1 C,D, Fig 2 C,D). Significant correlations were found between meniscal T2* and SHG (r=-0.53, p=0.03) and AF (r=-0.54, p=0.03). **Femoral Cartilage:** T2 of Tear limbs was significantly longer than Non-Op limbs, p=0.005. T2 of Tear limbs at time Zero was similar to 8 Mo, and significantly shorter than 4 Mo, p<0.0001. No effect of Time was found for Non-Op limbs. **Tibial Cartilage:** T2 of Tear limbs was significantly longer than Non-Op limbs, p<0.0001. Overall T2 of time Zero limbs was similar to 4Mo limbs, but longer than 8 Mo limbs. The T2 of 4Mo limbs were similar to 8Mo limbs. The anterior region had longer T2 than posterior region, p<0.0001. Anteriorly, T2 in Tear limbs was longer than Non-Op limbs, p<0.0001, with no changes over time, p=0.36. Posteriorly, T2 of Tear limbs was similar to NonOp limbs, p=0.15 and T2 values were similar at 4Mo and 8Mo, and both time points were shorter than time Zero, p=0.037.

Discussion. The quantitative meniscal T2* in a meniscal repair model correlated with MPM measures of meniscal integrity and demonstrated greater disorganization of the meniscus in the Tear limb. The direct MPM measurements of meniscal collagen content and collagen organization confirms UTE T2* data as a quantitative, objective, and indirect measure of meniscal integrity. Differences of cartilage T2 were also detected, indicating degeneration of the articular surface as a result of the imposed meniscal repair and loss of meniscal function. Furthermore, the increased anterior tibial cartilage T2 values may indicate an altered loading pattern in the joint resulting in a change of the biomechanical loading environment. These data lend strong support to the use of T2 and T2* applications to clinical meniscal repair and the assessment of risk for the development of osteoarthritis.

References. 1. Ding C *et al.*, J Rheumatol 34, (2007) 2. Fithian DC *et al.*, CORR, (1990) 3. Gatehouse PD *et al.*, Br J Radiol 77, (2004) 4. Koff MF *et al.*, Proc ISMRM 2011, 568 5. Maier CF *et al.*, J MRI 17, (2003). **Acknowledgements.** This project was supported by NIH/NIAMS grant RC1-AR058255. Institutional research support was provided by General Electric Healthcare. The authors thank Drs. Saadiq El-Amin, Cliff Voigt and Demetris Delos for study assistance and Dr. Atsushi Takahashi for assistance with the UTE pulse sequence.

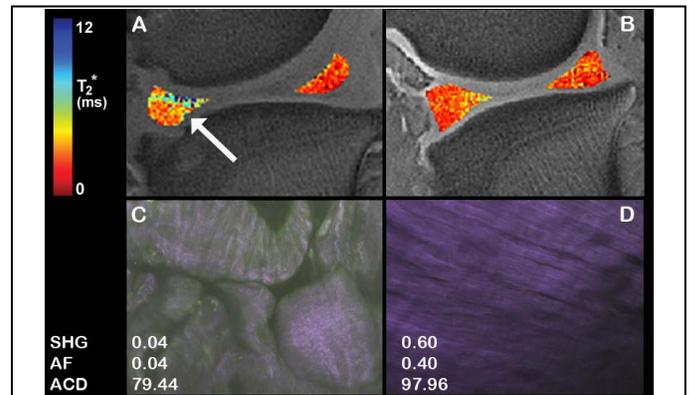


Figure 1. Representative sagittal medial meniscal T2* maps and corresponding MPM numerical and image data. Tear limbs had focal increases in T2* (A) and disrupted collagen fibers in MPM imaging (C). Non-Op limbs had less heterogeneity of T2* (B) and highly ordered collagen structure (D).

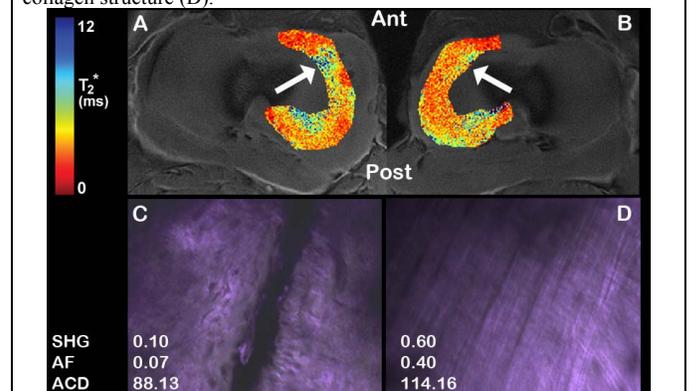


Figure 2. Axial medial meniscal T2* maps and corresponding MPM image and numerical data. Tear limbs had heterogeneous T2* maps (A, arrow) and frank tears on MPM (C). Non-Op limbs had T2* maps of lower magnitude (B) and an overall ordered collagen structure (D).