and from this automatically to quantify a bone structure marker strongly associated with the presence of OA. The advantages of this approach are the use of low cost MRI equipments and a fully automatic computer-based framework, which makes the developed marker well suitable for large longitudinal clinical studies.

150 QUANTITATIVE AND HISTOPATHOLOGICAL ANALYSIS OF OSTEO-ARTHritic CARTILAGE USING MRI, HRMAS SPECTROSCOPY AND IMMUNO-HISTOCHEMISTRY


Purpose: Osteoarthritis (OA) involves cartilage degradation resulting from biochemical changes. In this study we perform ex-vivo T1-rho weighted MRI followed by high resolution magic angle spinning (HRMAS) NMR spectroscopy on 11.7 T using knee articular cartilage obtained from OA patients during total knee replacement (TKA) surgery. HRMAS ensures tissue integrity and allows immuno-histochemistry (IHC) and histology of the same cartilage samples following spectroscopy. We use immuno-histochemistry and histology to show that the biochemical changes measured with HRMAS correlate with the histopathology of cartilage, and thus validate that HRMAS is a viable and powerful addition to our capabilities to study early and late OA, and it complements the T1-rho relaxation map acquired using ex-vivo MRI that depicts regions of increased cartilage degradation in OA specimens.

Methods: Osteoarthritic samples were excised from OA patients (n=4, range = 57–81 years) undergoing TKA during surgery. In case of each patient lateral and medial femoral condyles were acquired, flash-frozen in dry ice, then immediately stored at ~80°C until experiments. The condyles were mounted and secured on a grid (Fig. 1) after thawing to obtain T1-rho MRI of the ex-vivo specimens using a 3 Tesla GE scanner. Semi-automatic segmentation software developed in-house was used to segment the cartilage overlying the condyles and the mean±SD of T1rho was determined in each case. 8 cartilage punches were acquired per patient using a 3 mm punch resulting in a total of n=24 punches. The samples were then analyzed using HRMAS NMR spectroscopy using a Carr Purcell Meiboom Gill (CPMG) pulse sequence to determine the biochemical composition of the samples. Following HRMAS, histologic (hematoxylin/eosin and Safranin-O/Fast Green) and immunohistochemical evaluation were performed on the same samples using antibodies that recognize aggrecan cleavage neoepitopes G1-NITEGE (reflecting activities of ADAMTS) and G1-VDIPEN (reflecting activities of MMPs) as well as those that recognize the collagen-II cleavage neoepitopes (GPQG).

Fig. 1. OA knee cartilage/bone specimen fixed on a plastic grid for MRI ex-vivo scan.

Results: Figure 2(A) shows a representative segmented ex-vivo T1-rho weighted MRI color map of the lateral inferior femoral condyle of an OA patient. The mean±SD of T1-rho for the segmented cartilage was (60.4±18.45) ms. The corresponding HRMAS spectra of the punch sample shown in 2(A) is indicated in Figure 2(B), with well resolved spectral peaks including N-acetyl (reflecting proteoglycan concentration) and alanine (reflecting collagen concentration). Figure 3 shows the H&E staining, Safranin-O staining and the immuno-histochemical labeling of anti-NITEGE of the same punch sample as in Figure 2) stained using (A) H&E, (B) Safranin-O, (C) anti-NITEGE.

Fig. 3. Histological/immunohistochemical sections of cartilage (the same punch sample as in Figure 2) stained using (A) H&E, (B) Safranin-O, (C) anti-NITEGE.

Conclusions: This study is a multi-modality approach to understand the biochemical changes involved in OA. The use of HRMAS spectroscopy after performing ex-vivo T1-rho MRI allows the correlation of T1-rho relaxation with biochemical profiles of cartilage as determined by HRMAS. The fact that cartilage samples remain intact after HRMAS provides the ability to perform histo-pathological analysis of the same cartilage samples. Quantitative analysis of T1-rho values, HRMAS spectral quantification and histology/IHC staining grading will be performed with data collected from this study. This technique provides a validation of spectroscopy approach and helps establish potential biomarkers of the cartilage degradation process in OA.

151 COLLAGEN BIOMARKER RESPONSE TO ACUTE JOINT INJURY IN A NON-TERMINAL ANIMAL MODEL OF OSTEOARTHRITIS

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Purpose: Biomarkers of cartilage metabolism have potential to identify early stages of osteoarthritis (OA), before clinical evidence is present, and thus facilitate early diagnosis and therapy. The purpose of this study was to identify synovial fluid (SF) and serum biomarker changes that occur after acute joint injury using an equine model of early OA. We hypothesized that collagen biomarkers would identify early metabolic changes that occur secondary to acute osteochondral (OC) injury.

Methods: Twenty-two clinically and radiographically normal age- and sex-matched Quarter Horses were randomly divided into 2 groups: (1) horses (n=11) that had an OC fragment created arthroscopically on the dorsomedial aspect of the first phalanx in one randomly selected metacarpophalangeal (MCP) joint and a sham operation in the contralateral joint at week 0; and (2) unoperated exercise control horses (n=11). All horses were exercised on a high-speed treadmill 5 days/week from week 2 to week 16. Blood was collected from the jugular vein and SF samples were collected without lavage from both MCP joints of all horses at baseline (week 0), week 2, 8, and 16. Commercially available collagen degradation (CTXII [IDS/Nordic], C2C, C12C [IBEX]) and synthesis (CPII [IBEX]) ELISA assays were used to analyze SF and serum samples. All assays were previously validated for equine use. Data was assessed for normality and outliers were removed from further analyses. A repeated measures ANOVA with a Tukey’s test for multiple comparisons was used for all analyses. P≤ 0.05 was considered significant. All procedures were approved by institutional animal care and use committees.