Differences in Osteoarthritic Cartilage Biomarker Content Based on Biomechanical Properties of the Tissue


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Introduction: Osteoarthritis (OA) is a multifactorial disease resulting in significant changes to the structure and biomechanical properties of articular cartilage. OA is a disease of the entire joint organ in which inflammatory and degradative processes contribute to its development and progression. However, the relationships among changes in articular cartilage biomechanical properties and concentrations of inflammation- and degradative enzyme-related proteins in the cartilage have not been fully characterized. Therefore, this study was designed to characterize differences in clinically relevant biomarkers in OA cartilage based on the aggregate modulus (Ha) and permeability (K) of tissues recovered from patients undergoing total knee arthroplasty (TKA). It was hypothesized that there would be moderate (0.5-0.69) to strong (r≥0.7) significant correlations among cartilage tissue biomechanical properties (Ha, K, and the ratio of Ha:K) and concentrations of targeted biomarkers extracted from the cartilage. Specifically, we postulated that the concentrations of pro-inflammatory, pro-degradative, and bone turnover-related biomarkers in OA cartilage would increase significantly as the biomechanical properties of the tissues deteriorate. Characterizing these mechanistic relationships has the potential to elucidate specific targets for intervention to mitigate the development and progression of OA.

Methods: Tissue recovery: With IRB approval (IRB#1208392) and informed patient consent, resected femoral condyle (FC) and tibial plateau (TP) articular surfaces that would otherwise be discarded were recovered from patients (n=14, 11F, age: 65.9±9.2 Y, BMI: 35.6±5.8) undergoing TKA for symptomatic knee OA. Osteochondral explant (6mm, n=47) was created from the medial and lateral hemiplates of the TP (n=25) and medial and lateral FCs (n=22) and processed for cartilage biomechanical testing. Biomechanical testing: The explants underwent confined creep compression (10N, 5 min) in PBS, and the stress relaxation curve was used to calculate the Ha and K of the cartilage tissue. Tissue processing: After testing, the explant was cut in half and half was stored at ~80°C for protein extraction, while the other half was formalin fixed for histological assessment. Histology: H&E-stained sections of each explant was used to measure the thickness of the cartilage portion of the explant. Tissue Protein Extraction and Testing: Protein was extracted from the cartilage tissue using the T-Per protein extraction reagent with protease inhibitors. The protein content of the extract was determined using the BCA assay, and the concentration of leptin, adiponectin, adipisin, CRP, MMP-1, MMP-2, MMP-3, MMP-9, TIMP-1, TIMP-2, TIMP-3, TIMP-4, GRO-α, MCP-1, MCP-3, IL-6, IL-8, MIP-1α, VEGF, OPG, OPN, and SOST was determined using commercially available Luminex assays. Statistical Analysis: Biomarker concentrations were standardized to protein content and log transformed for analysis. A Pearson correlation was performed between biomarker concentrations, Ha, K, and Ha:K. Samples were grouped based on the Ha, K or Ha:K value (1-4), with higher group number indicating higher property value (fig. 1). Significant (p<0.05) differences between groups for each biomarker was determined using one-way ANOVA and Tukey post-hoc. A two-way ANOVA was performed using the Ha and K groups to determine significant differences for each biomarker based on the interaction of the Ha and K groups.

Results: Correlations among Tissue Biomarker Content and Tissue Biomechanical Properties: When all samples were grouped together, no moderate to strong correlations were observed between tissue biomechanical properties and protein content. For FC samples there was a moderate negative (r≤-0.5) correlation for Ha to adiponectin and FC. For TP samples there was a moderate positive (r≥0.5) correlation for Ha to MMP-13 and TIMP-4, and a moderate (r≤-0.5) negative correlation for the Ha:K to MCP-1. Differences based on Ha groups (fig. 2): For all samples, MMP-2 was significantly lower in Ha group 1 than 3. For TP samples, MMP-2 and TIMP-1 were significantly lower in Ha group 1 than 2-4 and 2-3, respectively. For FC samples, MMP-2 was significantly lower in Ha group 2 than 3. Differences based on K groups (fig. 2): For all samples, adiponectin was significantly lower in K group 1 than 3. For FC samples, resistin was significantly lower in K group 3 than 2. Differences based on Ha:K groups (fig. 2): For all samples, adiponectin was significantly lower in Ha:K group 4 than 2, resistin was significantly lower in Ha:K group 3 than 4, and MMP-2 was significantly lower in Ha:K group 1 than 3. For TP samples, adiponectin was significantly lower in Ha:K group 3 than 4, and MMP-2 was significantly lower in Ha:K group 1 than 2. Differences based on interaction of Ha and K groups (fig. 3): For all samples, leptin was significantly higher in Ha and K group 1 than group 4 when samples were both in group 1. However, when Ha and K group increased leptin was higher in groups 3 and/or 4 compared to groups 1 and/or 2. For TP samples, MCP-1 was significantly higher in Ha group 4 across K groups, but MCP-1 was only significantly higher in K group 4 samples only in Ha group 4.

Discussion: The data from this study indicate that the relationships among OA cartilage tissue biomechanical properties (Ha, K) and inflammatory and degradative enzyme-related protein content are multifaceted and complex, and that differences in tissue inflammatory and degradative enzyme related protein concentrations appear to be more related to changes in tissue aggregate modulus than tissue permeability. A lower level of tissue MMP-2 was consistently observed in cartilage samples with a lower aggregate modulus, indicating that cartilage MMP-2 decreases as cartilage tissue degradation progresses. Further, the concentration of MCP-1 in TP cartilage had a moderate negative correlation to the Ha:K ratio, and the interaction between Ha and K groups indicated that samples with higher Ha had higher MCP-1 compared to other groups when the K of the tissue was also high, potentially indicating that changes in tissue MCP-1 occur with tissue permeability increases. However, further study is required to define these complex relationships between osteoarthritic cartilage biology and biomechanics. Ongoing studies in our lab are aimed at expanding this data set towards the goal of determining clinically important relationships between tissue biomechanics, inflammation, and degradation that govern the development and progression of OA.

Significance: Further characterization of the relationships between osteoarthritic cartilage biology and biomechanics will elucidate specific targets for intervention to mitigate the development and progression of OA.