Analysis of the Metabolic Response of Cartilage Tissue to Injury and Inflammation in vitro

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Disclosures: A. Stoker: None. K. James: None. F. Pfeiffer: None. K. Kuroki: None. C. Bozynski: None. J. Cook: 1; Arthrex. 3B; Arthrex. 3C; Schwartz Biomedical. 5; Arthrex, Zimmer, Synthes.

Introduction: Post-traumatic osteoarthritis (PTOA) is a common sequelae to traumatic joint injury. Biomechanical insult to articular cartilage incites pro-inflammatory responses of varying degrees depending on several variables, including severity of impact and number of tissues affected. The activation of this inflammatory cascade can result in increased production of degradative enzymes and apoptosis within the affected tissues, driving the degradation of the tissue and development of PTOA. Further, it is theorized that a similar injuries applied to an OA joint will be more severe due to compromised tissue biomechanical properties and presence inflammation associated of OA. Therefore, understanding how the combination of cartilage injury and tissue inflammation affects the tissues metabolism after impact can give insight into how PTOA develops clinically.

Methods: Tissue collection: All procedures were approved by the IACUC. Cartilage tissue was harvested from 8 dogs euthanatized for reasons unrelated to this study and 6mm cartilage explants were created (n=6/dog).

- Tissue culture: An Instron 8821S servo-hydraulic testing machine was used to apply a single impact load to the tissue. The cartilage explant was placed in a stainless steel well (6mm diameter by 2.54mm deep) and a 3.9mm diameter flat punch attached to the ram was used to measure the thickness of the explant. The thickness measurement was used to calculate the parameters to apply a 0%, 25%, or 75% strain impact at 100mm/sec based on our previous work. After impact tissues were cultured with or without recombinant canine (rc)IL-1β (0.1ng/ml). One explant from each dog was placed into the following treatment groups: 1) NEG Control (0-C), 2) 25% (25-C), 3) 75% (75-C), 4) 0% + rcIL-1β (0-I), 5) 25% + rcIL-1β (25-I), 6) 75% + rcIL-1β (75-I). Explants were cultured in 1ml of media for 21 days. Media was changed every 3 days and collected for biomarker assessment. On day 21 tissue explants were collected for evaluation of cell viability and matrix composition.

- Tissue Analysis: Explants were evaluated for viable cell content using fluorescent staining. Viable cell density was calculated as the number of viable cells divided by the area of the tissue analyzed. Half of the explant was digested and proteoglycan (GAG) content was determined using the DMMB assay. The other half of the tissue explant was used for histologic analysis using the modified Mankin score.

- Media Biomarker Analysis: Culture media from days 3, 6, and 9 were evaluated for Prostaglandin E2 (PGE2); Nitric Oxide (NO); Matrix Metalloproteinase (MMP)-1, -2, -3 and -13; total MMP activity; and IL-6, IL-8, MCP-1, and KC using commercially available assays.

- Data Analysis: Statistical significance was determined using a one-way ANOVA for data between groups and a repeated measures ANOVA to assess biomarker levels over time with significance set at p<0.05.

Results: Tissue GAG (Figure 1A): The mean tissue GAG content decreased with impact severity in from 0-C to 25-C to 75-C. However there was not a significant difference between the impacted groups (25-C and 75-C) and the control (0-C). The tissue GAG content in the 0-I, 25-I, and 75-I groups were all
significantly lower than the 0-C group after 21 days of culture. Further, the 25-I group was significantly lower than the 25-C group.

Histological evaluation (Figure 1B): Histological evaluation of the cartilage tissue found increasing scores, indicative of pathologic tissue changes, with increasing tissue impact. Treatment of tissue with IL-1β was additive to the tissue damage observed histologically. The histology score in the 75-I group was significantly higher than the 0-C group on day 21 of culture.

Cell Viability: The viable cell density of the 25-C, 75-C, and 75-I groups were significantly lower than the 0-C control after 21 days of culture. The viable cell density of the 75-I group was significantly lower than the 0-I and 25-I groups after 21 days of culture.

MMP Concentration: The media concentrations of MMP-1, MMP-2, MMP-3 and MMP-13 were not significantly different between the 25-C and 75-C groups and the 0-C control at any time point tested. The 0-I, 25-I, and 75-I group all had significantly higher MMP-1, MMP-3, and MMP-13 levels compared to the 0-C, 25-C, and 75-C groups at all time points tested. The concentration of MMP-2 was not significantly different between any of the groups tested.

General MMP Activity (Figure 1C): The level of general MMP activity was not significantly different between the 25-C and 75-C groups and the 0-C control at any time point tested. The 0-I, 25-I, and 75-I group all had significantly higher level of MMP activity compared to the 0-C, 25-C, and 75-C groups at all time points tested.

Cytokine Concentration: The concentration of MCP-1, IL-8 (Figure 1D), and KC in the 0-I, 25-I, and 75-I were all significantly higher than the 0-C, 25-C, and 75-C groups on all time points tested. The concentration of IL-6 was significantly higher in the 0-I, 25-I, and 75-I groups compared to the 0-C, 25-C, and 75-C groups on day 3. After day 3, the concentration decreased significantly in the 0-I, 25-I, and 75-I groups, but was still significantly higher on day 6 compared to the 0-C, 25-C, and 75-C groups.

PGE2 and NO Concentration: The concentration of NO in the 0-I, 25-I, and 75-I were all significantly higher than the 0-C, 25-C, and 75-C groups on all time points tested. The production of PGE2 was significantly higher on day 3 than day 6 and 9 for all test groups. Further, the mean concentration of PGE2 was higher in the 75% strain groups and the 75-C was significantly higher than the 0-C and the 75-I was significantly higher than the 0-I group. On day 3. However, after day 3, there was not a significant difference between any of the groups for PGE2 production.

Discussion: These data indicate that a single traumatic load applied to the cartilage was able to disrupt the tissue, as indicated by the histology data, and cause a significant level of cell death, as indicated by the decrease in viable cell density. Further, the mean histological scores increased, and tissue GAG content decreased in a load dependent manner. However, these changes were not significantly different than the 0-C controls, and the single impact alone was not enough to drive a sustained inflammatory or degradative response by the tissue as indicate by the concentration of biomarkers in the media. This indicates that a single tissue impact is not enough to drive the inflammatory and degradative responses associated with PTOA development. The addition of IL-1β to the model resulted in a significant increase in histologic scores, cytokine, NO, and degradative enzyme production, as well as a significant decrease in tissue GAG content and viable cell density. This indicates that inflammation associated with healing other tissues, like synovium and bone, may help drive the development of PTOA after injury. Additionally, injuries to joints that are already developing OA, and have a low level of inflammation in the joint, may develop PTOA at a faster rate than the same injury to a patient without OA. Ongoing
studies in our laboratory are aimed at determining the effect of post-injury load and inflammation on metabolism of the cartilage tissue.

**Significance:** These data indicate that a single impact injury to the cartilage tissue does not result in the significant sustained inflammatory and degradative response by the tissue associated with PTOA progression. Therefore, other factors like subsequent tissue loading and inflammation from surrounding tissues make a significant contribution to the development and progression of PTOA.

**Figure 1** – Tissue GAG (A) and Histology Scores (B) on day 21 of culture, and media general MMP Activity (C) and IL-8 concentration (D) on days 3, 6, and 9 of culture. *=significantly higher than NEG control at that time point.