Inflammatory and Overuse Tendinopathy Impair Chondrocyte Function in Shoulder Crosstalk

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INTRODUCTION: Shoulder musculoskeletal disease is highly prevalent, with approximately 20% of the population reporting shoulder pain at any given time **[1].** Rotator cuff disease, characterized by inflammation, degeneration, or acute injury of muscles or tendons in the shoulder, is the primary clinical cause of shoulder pain **[2]**. Osteoarthritis of the shoulder (glenohumeral OA) is the second most common cause, affecting more than 30% of the population over the age of 60 **[3]**. These two conditions frequently coexist and are major contributors to shoulder dysfunction. Tendinopathy in the rotator cuff, characterized by chronic matrix degeneration with or without inflammation, frequently develops in response to repetitive overuse **[4]**. While mechanical destabilization of the rotator cuff (e.g. tendon degeneration) is a recognized driver of OA (cartilage degradation), the biochemical crosstalk between degenerative tendon and cartilage in the shoulder remains underexplored **[5]**. To address this, we modeled degenerative tendon phenotypes using cytokine-induced inflammation and mechanical overuse via a tensile loading bioreactor to generate conditioned media (CM). Chondrocytes were treated with tenocyte-derived CM, and their response was assessed through histone modification imaging and gene expression analysis. We hypothesized that inflammatory and overuse CM would impair chondrocyte health and transcriptional capacity. This study aims to determine tendon–cartilage crosstalk as a contributor to shoulder degeneration to ultimately inform future therapeutics.

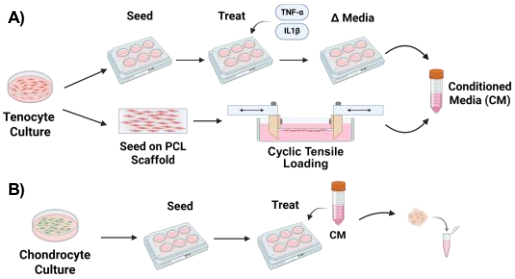

Figure 1: (A) Workflow of inflammatory (top) and overuse (bottom) tendinopathic models to generate conditioned media (CM). **(B)** Chondrocyte culture workflow with CM treatment.

METHODS: Inflammatory tendinopathy model: Juvenile bovine tenocytes were seeded on tissue culture plastic, incubated for 24 hours, and then cultured for 3 days in basal cell growth media (BM, Control) or inflammatory media. Inflammatory media consisted of BM supplemented with both TNFα and IL1β (Sigma) at either 10 ng/mL (10) or 20 ng/mL (20). After 3 days, all media were replaced with BM to create conditioned media (CM) from each group (CM Control, CM 10, CM 20) **(Fig. 1A)**. CM was collected following a 24-hour incubation. **Overuse tendinopathy model**: Tenocytes were seeded onto aligned poly(ε−caprolactone) (PCL) nanofibrous scaffolds and cultured for 2 days in BM. A custom tensile loading bioreactor applied cyclic uniaxial force at 8% strain (at 2 Hz) to seeded scaffolds for 4 hours/day for 1 or 3 days **(Fig. 1A)**. Additionally, unloaded scaffolds cultured in BM served as static controls. CM was collected following static culture (CM Static) and after 1 or 3 days of loading (CM DL 1, CM DL 3), then filtered and spun down. **Chondrocyte treatment**: Juvenile bovine chondrocytes were seeded on tissue culture plastic and incubated for 24 hours in BM, followed by 3-day culture in BM (Control) or CM (CM Control, CM 10, CM 20, CM Static, CM DL 1, CM DL 3) **(Fig. 1B)**. RNA was extracted post-treatment for RT-qPCR analysis. Cells were fixed for immunofluorescence (IF) imaging to assess expression level of histone modifications H3K9ac, a marker of gene activation, and H3K27me3, a marker of gene repression (Invitrogen); fluorescent intensity was quantified using Image-J. Statistical analyses were performed using one-way ANOVA with post-hoc testing.

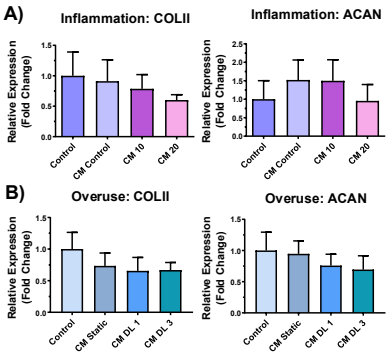

Figure 2: Fold change in collagen II (COLII) and aggrecan (ACAN) in chondrocytes, normalized to GAPDH and Control. **(A)** Cells cultured in BM (Control) or inflammatory conditioned media (CM Control, CM 10 or 20). **(B)** Cells cultured in BM (Control) or overuse conditioned media (CM Static, CM DL 1, CM DL 3). n = 9-20 (technical replicates). Mean ± SD.

RESULTS: RT-qPCR analysis of chondrocytes treated with tenocyte-derived CM from inflammatory and overuse conditions revealed a consistent decreased trend of collagen type II (COLII) and aggrecan (ACAN) expression, both essential proteins for cartilage structure and function **(Fig. 2A, B)**. IF analysis demonstrated epigenetic alterations in chondrocytes exposed to inflammatory CM with reduced H3K9ac (gene activation) and increased H3K27me3 (gene repression) with CM from 10 ng/mL of inflammatory stimulation **(Fig. 3A, B)**. Both H3K9ac and H3K27me3 expression were reduced with CM from Days 1 and 3 of loading, indicating distinct chromatin remodeling patterns associated with overuse. **(Fig. 3C, D).**

DISCUSSION: Our findings demonstrate an interaction between tendinopathic conditioned media and chondrocyte response, suggesting tenocyte-derived biochemical signals can drive both genetic and epigenetic changes in chondrocytes. Reduced expression of COLII and ACAN reflect a shift toward cartilage degradation under both inflammatory and overuse conditions. However, histone modification analysis revealed distinct patterns between tendinopathy models. Inflammatory CM decreased H3K9ac and increased H3K27me3, consistent with transcriptional repression, while overuse-derived CM reduced both activation (H3K9ac) and repression (H3K27me3) marks, suggesting locus-specific disruption of chromatin regulation. CM from lower inflammatory stimulation (CM 10) induced more pronounced histone changes, while CM from higher inflammatory stimulation (CM 20) elicited greater transcriptional responses, suggesting early epigenetic shifts may prime subsequent transcriptional responses. Mechanical loading produced consistent effects at Days 1 and 3, indicating persistent alterations in the genomic and epigenomic state of chondrocytes with overuse. Together, these results suggest that tendon-derived inflammatory and mechanical cues can influence cartilage degeneration through distinct but converging mechanisms. To expand on these findings, future studies will combine inflammatory and overuse conditions, employ ChIP-seq to map locus-specific epigenetic changes, and develop co-culture systems that better recapitulate tendon–cartilage interactions *in vivo*. Such approaches will deepen understanding of tendon–cartilage crosstalk and inform future therapeutic strategies.

SIGNIFICANCE: This study reveals biochemical crosstalk from tendinopathic media alters chondrocyte gene expression and epigenetic state under inflammatory and overuse conditions. These insights highlight tendon–cartilage interactions as contributors to shoulder degeneration and suggest new avenues for developing targeted therapies to prevent or slow the progression of shoulder degeneration.

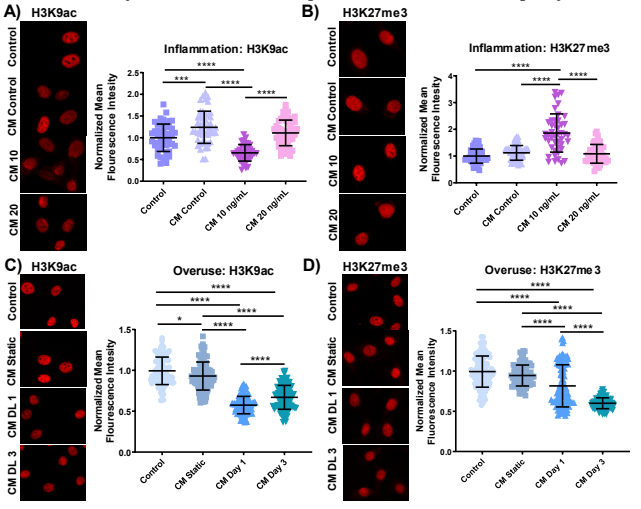

Figure 3: Representative IF images and quantification of **(A)** H3K9ac (n=39-57) and **(B)** H3K27me3 (n=46-57) in chondrocytes cultured in BM (Control), CM Control, or CM from tenocytes treated with 10 or 20 ng/mL of inflammation (CM 10, CM 20). Representative IF images and quantification of **(C)** H3K9ac (n=99-112) and **(D)** H3K27me3 (n=91-104) in chondrocytes cultured in BM (Control), CM Static, or CM from Day 1 or 3 of loading (CM DL 1, CM DL 3). Mean ± SD, *:p≤0.05, ***:p≤0.001, ****:p≤0.0001.

REFERENCES: **[1]** Pope+, *Ann. Rheum. Dis.*, 1997; **[2]** Whittle+, *Ann. Intern. Med.*, 2015; **[3]** Chillemi+, *Arthritis*, 2013. **[4]** Plachel+, *J. Ortho. Res.*, 2019. **[5]** Spiegl+, *KSSTA*, 2015. **[6]** Russo+, *Cells*, 2022.

ACKNOWLEDGEMENTS: This work is supported by the NIH (P50 AR080581) and NSF (CMMI-1548571).