Short-Term Mechanical Stimulation Modulates Young and Aged Human Articular Chondrocyte Redifferentiation

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INTRODUCTION: Articular cartilage (AC), essential for smooth joint movement, faces challenges upon injury, including extracellular matrix (ECM) degradation, chondrocyte senescence and mechanical overloading, which lead to post-traumatic osteoarthritis (PTOA). Mechanical loading, reflecting the physiological joint environment, is a well-known key factor of chondrocyte redifferentiation through modulation of chondrocytes gene expression, ECM synthesis, and mechanotransduction pathways [2]. Chondrocytes at different stages of aging exhibit distinct responses to mechanical loading due to variations in their remodeling and maintenance capacity. Understanding the complex roles of specific mechanosignaling mechanisms in healthy and OA cartilage can identify processes that may be therapeutically targeted to interrupt pathological pathways. The primary objective of this study was to investigate the mechanisms by which mechanical loading modulates the phenotype of young healthy and aged chondrocytes. We investigated the role of mechanical cues in regulating mechanotransduction pathways and chondrocyte redifferentiation, comparing young and aged cells to reveal aged-associated impairments in mechanosignaling that can inform the design of targeted cartilage repair strategies. Simultaneous longitudinal loading and daily measurement of mechanical properties was achieved using a novel ultrasound technique, tensile acoustic rheometry (TAR) [3].

METHODS: Hydrogels containing HACs (20M cells/mL; male, 19 and 74 years) were crosslinked with 0.3% Irgacure 2959 under 365 nm UV (28 mW/cm²) for 60 s [4]. Cyclic loading was applied longitudinally from day (D) 1–5, and Young's modulus was measured daily using the TAR system [3]. Cell viability was assessed on D0 and 7 via LIVE/DEAD® staining and confocal imaging. qPCR was performed on D0, 1, 5, and 7 for key chondrogenic, hypertrophic, and mechanotransductive genes. Immunofluorescence (IF) was performed on D5 to visualize the protein expression. Two-way ANOVA or mixed models with Tukey's post-hoc test were used for statistical analysis.

RESULTS SECTION: Young chondrocytes exhibited enhanced the expression of chondrogenic markers under loading but the expression of hypertrophic markers also increased. In contrast, aged chondrocytes displayed minimal changes in the expression of chondrogenic genetic and protein markers, while loading markedly reduced the expression of hypertrophic markers to the level of to the level of the young free-swelling samples (**Fig. 1A, B**). Moreover, mechanotransduction-related genes were more robustly upregulated in young cells under loading, with activation persisting throughout the loading period, whereas aged cells showed modest transcription responses. IF results showed age-dependent signaling, with TGFβ1/SMAD1/5/9 signaling pathway predominantly activated in young cells and TGFβ1/SMAD2/3 pathway in aged cells (**Fig. 2**). Cyclic loading significantly enhanced cartilage matrix stiffness by 17%, with cumulative effects resulting in the highest modulus by D5 (**Fig. 3**).

DISCUSSION: This study reveals the effects of mechanical stimulation on both young and aged chondrocytes. When subjected to loading, aged chondrocytes exhibit altered chondrogenic and hypertrophic responses, highlighting disease-associated differences in mechanosignaling that affect phenotype maintenance. Interestingly, the predominance of TGFβ1/SMAD2/3 signaling in aged chondrocytes suggests a shift in mechanotransduction favoring anti-hypertrophic effects, consistent with reports that SMAD2/3 activity counteracts hypertrophic differentiation and maintains cartilage homeostasis.

SIGNIFICANCE/CLINICAL RELEVANCE: We investigated the role of mechanical loading in shaping chondrocyte fate through mechanotransduction and identified age-dependent differences in mechanotransduction that have direct implications for cartilage repair. These findings highlight distinct signaling mechanisms in young versus aged chondrocytes, deepening our understanding of cartilage mechanobiology and informing the optimization of mechanical and biomaterial-based strategies for cartilage repair.

REFERENCES:
[1] Choi, J et al., *J Cell Physiol*, 233:1913-1928, 2018
[2] Tan, A & Hung, C et al., *Stem Cells Transl Med*, 6:1295-1303, 2017
[3] Li, W et al., *Biomaterials*, 321:123325, 2025
[4] Carrion, B et al., *Adv Healthc Mater*, 5:1192-1202, 2016
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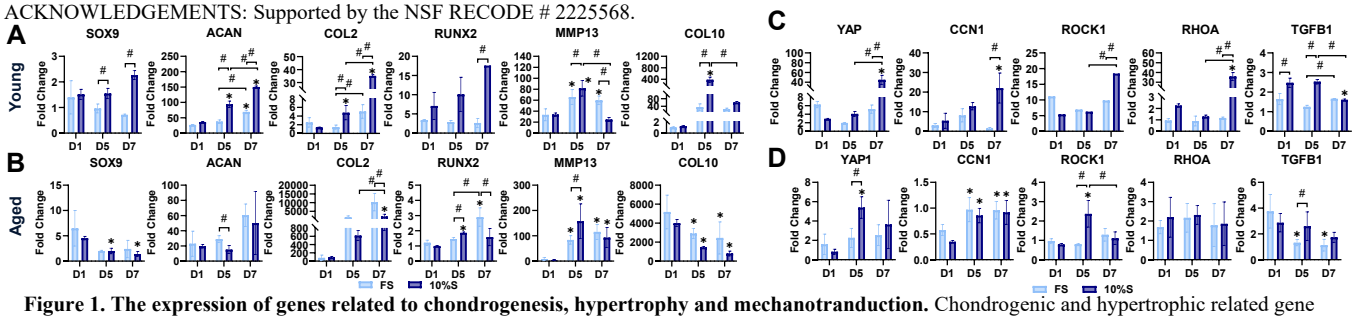

Figure 1. The expression of genes related to chondrogenesis, hypertrophy and mechanotranduction. Chondrogenic and hypertrophic related gene expression of (A) young and (B) aged cells. Mechanotransduction related gene expression of (C) young and (D) aged cells. FS=Free-swell, S=Strain.

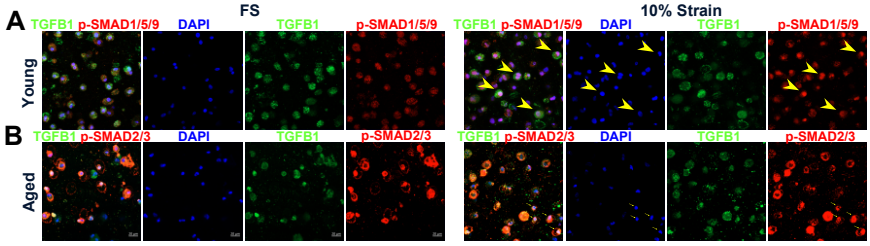

Figure 2. IF staining of signaling pathway activation in young and aged chondrocytes. (A) TGFβ1/SMAD1/5/9 signaling in young chondrocytes. (B) TGFβ1/SMAD2/3 signaling in aged chondrocytes. Scale bars indicate 20μm.

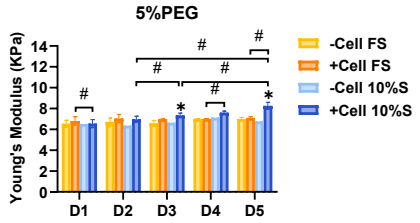

Figure 3. Mechanical property of 5% PEG hydrogel. * indicates statistical significance of the specified condition compared to D1. # indicates statistical significance between specified groups.