Inflammation-Induced Chromatin-Speckle Crosstalk Drives Zone-Specific Nuclear Remodeling in Meniscus Cells

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INTRODUCTION: The meniscus exhibits distinct zonal heterogeneity in cellular phenotype, matrix composition, and mechanical environment, which collectively determines its functional integrity and repair potential **[1]**. Inflammation plays a pivotal role in meniscus degeneration by altering cellular phenotypes, promoting catabolic enzyme expression, and disrupting extracellular matrix (ECM) homeostasis that impair tissue repair **[2]**. However, how inflammatory cues alter nuclear organization and epigenetic regulation across these zones remains poorly understood. Nuclear speckles (NSs), dynamic and membrane-less subnuclear condensates enriched in SRSF2, SRRM2, and SON, serve as critical hubs for RNA splicing, retention, and stability **[3]**, contributing to transcriptional regulation. Their reorganization under stress suggests that early nuclear remodeling could precede and potentially predict tissue-level degeneration [**3**]. Despite their emerging roles in gene regulation, the function and remodeling of nuclear speckles in meniscus cells during inflammation or degeneration have never been investigated. Thus, in this study, we investigate how inflammatory stimulation induces zone-specific chromatin and nuclear speckle remodeling in meniscus cells, leading to distinct transcriptional and regenerative responses between inner and outer zone populations.

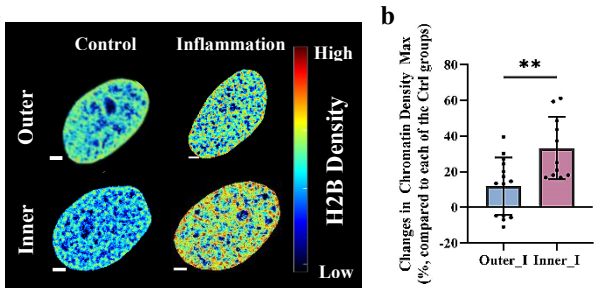

Figure 1. STORM imaging analysis. (a) Heat maps showing cytokines-induced H2B chromatin condensation in outer and inner meniscus cells (scale bar: 2 μm). (b) Quantitative analysis of changes in H2B chromatin condensation following inflammatory stimulation [n>10 nuclei/group, **: p<0.01, I: inflammation groups co-treated with TNF-α and IL-1β (10 ng/mL each, 72 h)].

METHODS: Outer and inner human meniscus cells were isolated from the same human donor (male, 48 years, BMI 26.1). Passage 1 cells were cultured on 8-well chambered coverglass slides for 24 hours, followed by co-treatment with tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) (10 ng/mL each) for 72 hours to induce inflammatory stress. Chromatin architecture was evaluated by super-resolution STORM imaging (ONI) of histone-H2B (H2B) [**4**]. Nuclear speckle organization was evaluated by immunofluorescence (IF) staining of SRSF2 (splicing factor) and SRRM2 (structural backbone protein). Colocalization coefficients were measured using the BIOP JACop plugin in Fiji. Statistical analysis was performed using one-way ANOVA (n>10 nuclei/group for STORM; n>300 NSs/group for IF).

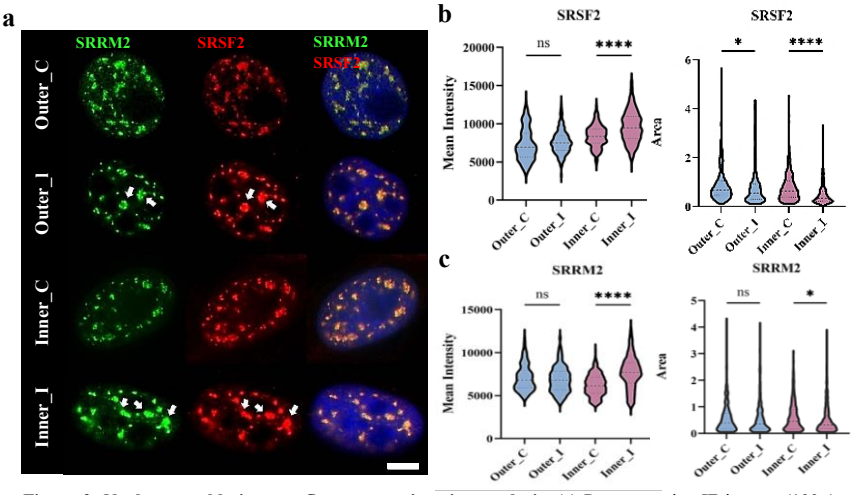

Figure 2. Nuclear speckle immunofluorescence imaging analysis. (a) Representative IF images (100×) showing condensed nuclear speckles in cytokines-treated cells (scale bar = 5 μm). (b-c) Quantitative analysis of mean intensity and area of SRSF2 and SRRM2 signals [n>300 NSs/group, ****: p<0.0001, *: p<0.05, C: Ctrl, I: Inflammation groups].

RESULTS: STORM imaging of H2B revealed distinct zone-specific chromatin responses to inflammatory stress. Inner zone meniscus cells showed significantly greater chromatin condensation compared to outer cells following the cytokine treatment (p<0.01) (**Fig. 1a-b**). Immunofluorescence analysis demonstrated parallel zone-dependent nuclear speckle remodeling. While outer cells showed modest clustering of speckles, inner cells demonstrated pronounced speckle condensation and aggregation under inflammation conditions (**Fig. 2a-b**). Pearson's correlation analysis indicated enhanced SRRM2-SRSF2 colocalization in inner cells, suggesting enhanced molecular compaction within condensed speckles (**Fig. 3a-c**).

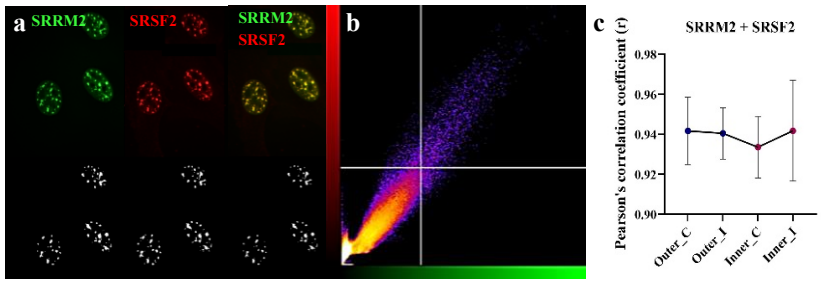

Figure 3. Colocalization analysis of SRRM2 and SRSF2. (a) Binarized images generated using the BIOP JACoP plugin in Fiji. (b) Representative scatter plot showing strong pixel-to-pixel correlation between SRRM2 and SRSF2 signals. (c) Quantification of Pearson's correlation coefficients across control (C) and inflammation (I) groups.

DISCUSSION: This study demonstrates that inflammatory stimulation induces distinct zone-specific nuclear remodeling in human meniscus cells, characterized by chromatin condensation and nuclear speckle reorganization. Super-resolution imaging revealed that inner zone cells, which experience limited vascularity and higher susceptibility to degradation, exhibited interestingly markedly enhanced chromatin compaction and nuclear speckle condensation under inflammatory stress compared to outer zone cells. Chromatin condensation is a hallmark of nuclear stress and degeneration, often restricting transcription and silencing repair programs [**4**]. Enhanced SRRM2–SRSF2 colocalization in inner zone cells further suggests tighter molecular packing within nuclear speckles, reflecting altered RNA processing and transcriptional regulation. Such condensation-driven aggregation may thus represent a structural reorganization that traps transcriptional machinery, contributing to transcriptional imbalance and mRNA retention [**5**]. These two events appear to be coupled as chromatin becomes more condensed, nuclear speckles aggregate more tightly, resulting in increased colocalization among multiple speckle markers (SRRM2, SRSF2). This correlation likely reflects a physical constraint, where condensed chromatin limits nuclear space for transcription and promotes clustering of splicing components. In contrast, outer cells maintained more canonical nuclear organization, which may preserve transcriptional fidelity and repair potential. Together, these findings suggest that inflammation-driven nuclear remodeling may serve as an early indicator of zone-dependent degeneration, highlighting chromatin–speckle dynamics as a critical epigenetic mechanism underlying meniscus pathology.

SIGNIFICANCE/CLINICAL RELEVANCE: Inflammation-induced chromatin-speckle remodeling represents an early nuclear marker of meniscus degeneration. These insights establish a mechanistic link between nuclear architecture and tissue pathology, offering potential targets for therapeutic intervention.

REFERENCES: [1] Bhan 2020, *Cureus*; [2] Stone+ 2013, *Osteoarthritis Cartilage*; [3] Faber+ 2022, *J Cell Sci.*; [4] Heo+ 2022, *Nat. Biomed. Eng.*; [4] Misteli 2020, *Cell*; [5] Mclntyre + 2025, *Nat Commun*.

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