Augmentation of Chondrocyte Microenvironment With Hyaluronic Acid Hydrogel Reduces Cellular Morphological Changes and Catabolic Activity

Saitheja A. Pucha[1,2], Riley B. Brackin[1,2], Lauren Foster[2], Hicham Drissi[1,2], Thanh N. Doan[1,2], Jay M. Patel[1,2]
[1]*Atlanta VA Medical Center, Decatur GA.* [2]*Department of Orthopaedics, Emory University School of Medicine, Atlanta GA.*
Disclosures: *Jay M. Patel (3B - NovoPedics Inc; 4 – NovoPedics Inc; 4 – Forsagen LLC)*

Introduction: Joint traumatic injuries are increasingly common and often initiate a degenerative cascade in the articular cartilage. Chondrocytes experience both intra- and extra-cellular changes during the initial phases of this process, potentially resulting from and in cytoskeletal changes and catabolic shifts. These changes lead to tissue breakdown, resulting in gradual degradation and progression towards osteoarthritis (OA). Researchers have long attempted to understand and treat this deteriorative process with bioactive factors or cell-based therapies; however, restoration of chondrocyte homeostasis and maintenance of its microenvironment may be critical towards slowing OA [1]. Previously, we developed a tissue-penetrating hyaluronic acid (tp-HA)-based system that reinforced damaged cartilage and prevented its breakdown [2,3], yet the impact of this treatment at the cell scale requires exploration. The purpose of this study was to 1) establish early cellular morphology shifts during degeneration and 2) investigate the cell-scale protective role of our tp-HA hydrogel system.

Methods: Establish Early Cellular Changes: Cartilage explants (juvenile bovine trochlea) were cultured in chemically defined media, without and with IL-1β (10ng/mL) for 2 weeks; IL-1β induction resulted in loss of proteoglycan and increased catabolic gene expression (*previously published*). Explants were stained for collagen-hybridizing peptide (CHP) and CellMask to visualize collagen denaturation and cell morphology, respectively. DAPI staining was also performed to quantify nuclear aspect ratio. tp-HA Gel Influence on Cell-Scale Tissue Breakdown and Morphology: Another set of explants received HA gel treatment at the initiation of the 2-week IL-1β culture period. For this, methacrylated hyaluronic acid (MeHA; 20kDa, ~35% mod, 4% w/v) with LAP photo-initiator (0.05% w/v) was applied to a portion of the cartilage surface, given 5 minutes to diffuse, and photo-crosslinked for 5 minutes with blue light (400-500nm; 25mW/cm^2). Following culture, explants were fixed, sectioned, and stained for: NITEGE (indicator for degraded aggrecan), CellMask, DAPI, and Safranin O/Fast Green (proteoglycan). Confocal images were taken to specifically visualize individual cells and their microenvironments. Biomaterial System for Chondrocyte Morphology and Catabolism: To further investigate how MeHA hydrogel impacts the microenvironment of chondrocytes, juvenile bovine chondrocytes were encapsulated within either gelatin hydrogels (5% methacrylated gelatin; GelMA) or gelatin-HA hydrogels (4% GelMA, 1% MeHA) for 24 hours, followed by IL-1β application (10ng/mL) for 2 hours. Gels were then fixed and stained for NF-κB (inflammatory activation), phalloidin (F-Actin), and DAPI. Cell parameters (area, perimeter, circularity, NF-κB nuclear intensity/translocation) were quantified.

Results: Explants cultured in IL-1β showed little differences in CHP staining (**Fig 1A**), suggesting that the collagen network remained relatively intact. However, chondrocytes in the superficial zone exhibited a more elongated morphology in the IL-1β treated samples, with a significant increase (p<0.0001) in nuclear aspect ratio (**Fig 1B**). Application of our tp-HA gel at the initiation of explant culture mitigated cartilage damage, as evidenced by decreased cell proliferation/spreading, decreased NITEGE staining, and increased Safranin O staining in areas where gel was applied (**Fig 2A**). Upon visualization of individual chondrocytes, we observed that cells treated with tp-HA gel were slightly smaller, less aligned (nucleus and cytoskeleton), showed fewer projections, and exhibited drastically lower pericellular NITEGE staining (**Fig 2B**). Finally, in our GelMA system, chondrocytes exposed to IL-1β experienced considerable increases in NF-κB staining and morphological projections, both of which were alleviated by incorporating MeHA into the initial gel (**Fig 3A**). Quantification of perimeter (**Fig 3B**) and NF-κB nuclear intensity (**Fig 3C**) confirmed the protective impacts of MeHA.

Discussion: This study provides evidence that cellular cytoskeleton and catabolic changes are evident during IL-1β-mediated cartilage deterioration, and that MeHA gel application can mitigate these deteriorative events. In particular, cell elongation and catabolic activation may go hand-in-hand, both of which can be reduced with our HA gel system.

Significance/Clinical Relevance: A tissue-penetrating biomaterial may prevent subtle changes at the cellular level, and serve as a treatment modality to slow, or even prevent, the progression of osteoarthritis.

References: [1] Lauer+, Int J Mol Sci, 2021. [2] Kowalski+, J Tissue Eng Regen Med, 2022. [3] Brackin+, Bioeng, 2023.

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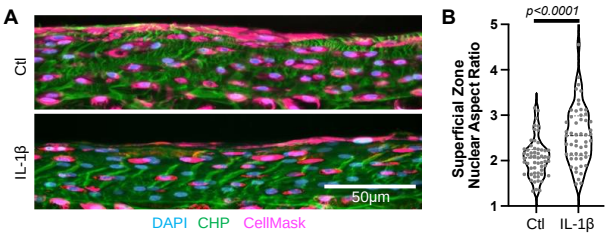

Figure 1. *[A] Superficial zone of cartilage explants cultured in control and IL-1β media for 2 weeks. Stained for DAPI, collagen hybridizing peptide (CHP), and CellMask. [B] Nuclear aspect ratio of cells in each condition.*

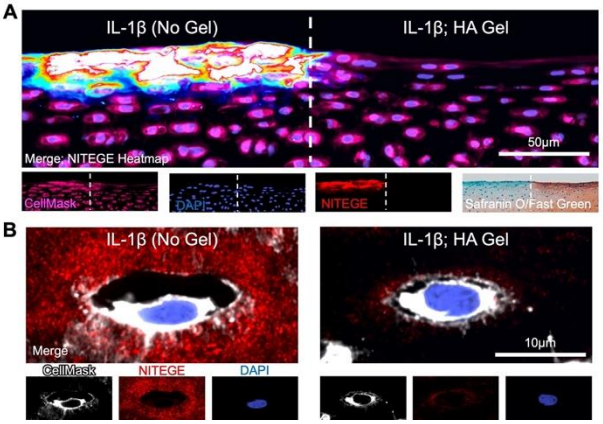

Figure 2. *[A] Cartilage superficial zone in 2-week IL-1β treated explant with MeHA gel applied to a portion of surface at initiation of culture. Stained for CellMask, DAPI, NITEGE, Safranin O/Fast Green. [B] Individual chondrocytes in IL-1β explant areas without and with MeHA application.*

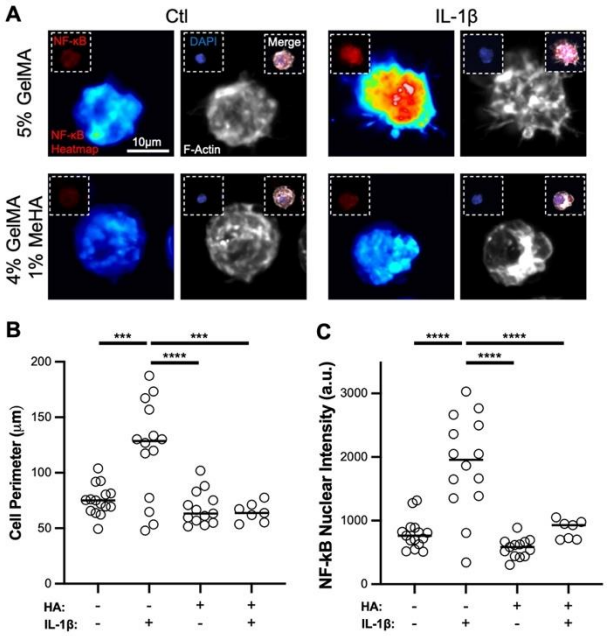

Figure 3. *[A] Chondrocytes in GelMA or GelMA-MeHA gels, without and with 2h IL-1β application. Stained for DAPI, F-Actin, NF-κB. [B] Cell perimeter and [C] NF-κB nuclear intensity. ***, **** represent p<0.001, 0.0001, respectively.*