Intrinsically Disordered Peptide P6 Alters Subchondral Bone Response in a Preclinical Mouse Model of Post-Traumatic Osteoarthritis

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DISCLOSURES: AMS serves on committees for AO, OTA, AAOS, and ORS. MR serves on committees for ISFR ORS. The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this study, except for ØØ who is the inventor of the hydrogel used. The intellectual property rights and commercial rights to the material are owned by Nupep AS, where ØØ is the main shareholder.

INTRODUCTION: Post-traumatic osteoarthritis (PTOA) is a debilitating condition that arises after joint injuries such as anterior cruciate ligament (ACL) rupture or meniscal damage, leading to pain, loss of function, and progressive joint degeneration. Unlike idiopathic osteoarthritis, PTOA develops earlier in life, particularly in young and active patients, representing a major unmet clinical need (1). Current clinical strategies are largely palliative, focusing on symptom management rather than modifying disease progression. Emerging evidence highlights subchondral bone remodeling as a critical early event in PTOA pathogenesis, with alterations in bone architecture driving cartilage degeneration and joint failure. Thus, targeting the bone-cartilage unit is an attractive approach for disease modification. Intrinsically disordered peptides (IDPs) are a novel class of biomolecules that lack a fixed three-dimensional structure under physiological conditions but can participate in diverse signaling and structural functions. Our proline-rich IDP, P6, has demonstrated potential to promote chondrogenesis and modulate bone repair, suggesting utility in preserving joint health following injury. We aimed to evaluate the effect of P6 delivered in a hyaluronic acid (HA) hydrogel on subchondral bone remodeling in a murine model of isolated ACL rupture. We hypothesized that intra-articular administration of HA+P6 would beneficially alter subchondral bone response and attenuate early features of PTOA progression.

METHODS: All procedures were performed at UC Davis in compliance with ARRIVE guidelines and approved by the Institutional Animal Care and Use Committee (IACUC). A total of 48 C57BL/6J male mice were randomized into experimental groups (n=8 per group per time point). Post-traumatic osteoarthritis was induced via isolated anterior cruciate ligament (ACL) rupture in the right hind limb using a controlled overload device (Bose ElectroForce 3200). Mice were assigned to three groups: 1) sham, 2) hyaluronic acid hydrogel (HA), and 3) HA + P6 hydrogel. Intra-articular injections were performed immediately following ACL rupture. Animals were sacrificed at 3 and 12 weeks post-injury. Knee joints were harvested for micro-computed tomography (μCT) analysis of the knee site to assess subchondral bone morphology. Additional tissues were processed for histology and immunohistochemistry (IHC) to evaluate cartilage and bone structure. Blood was collected via cardiac puncture at harvest, plasma isolated by centrifugation, and cytokine levels quantified using a 13-plex BioLegend mouse proinflammatory panel on a BD Fortessa 18-color flow cytometer. Quantitative outcomes included trabecular bone volume, thickness, separation, and surface area-to-volume ratio. Data were analyzed using two-way ANOVA with GraphPad Prism 8 (GraphPad Software, San Diego, CA). Statistical significance was defined as *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.

RESULTS:
Our μCT data revealed distinct alterations in trabecular bone morphology following ACL rupture. At 3 weeks, the sham group exhibited significantly greater trabecular thickness, lower surface area-to-volume ratio, and fewer trabecular objects compared to both HA and HA+P6 groups (*P < 0.05). These differences persisted across time points, suggesting altered bone remodeling in untreated injury. In contrast, the HA and HA+P6 groups demonstrated improved trabecular architecture, with reduced separation and more favorable bone morphometric indices at both 3 and 12 weeks. Across all groups, trabecular separation decreased between 3 and 12 weeks, consistent with ongoing bone adaptation. Planned analyses including bone mineral density quantification, pro-inflammatory cytokine profiling, and histological/IHC assessment are underway to determine whether these structural changes correlate with cartilage protection and systemic immune modulation.

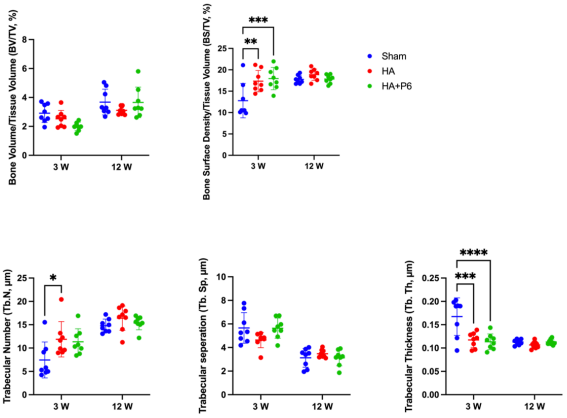

Figure 1. Micro-CT analysis of subchondral bone 3 and 12 weeks after ACL rupture. Parameters include trabecular thickness, separation, surface area-to-volume ratio, and object counts across sham, HA, and HA+P6 groups. Significant differences are indicated (*P < 0.05, **P < 0.01, ***P < 0.001).

DISCUSSION: This study demonstrates that intra-articular HA and HA+P6 treatments modulate subchondral bone remodeling following ACL rupture in a murine model of PTOA. Sham animals displayed unfavorable trabecular characteristics, including excessive thickening and reduced trabecular number, consistent with maladaptive bone remodeling after joint injury. In contrast, HA and HA+P6 groups showed more balanced trabecular architecture, with reduced separation and improved morphometric indices, suggesting a potential protective effect on early joint homeostasis. Although HA alone has been used clinically as a viscosupplement with limited structural benefit, the addition of the intrinsically disordered peptide P6 may provide added capacity to influence bone quality and cartilage–bone crosstalk. Ongoing analyses of bone mineral density, histology, and inflammatory cytokines will clarify whether these structural changes translate into cartilage protection and reduced PTOA progression

SIGNIFICANCE/CLINICAL RELEVANCE: Post-traumatic osteoarthritis lacks disease-modifying therapies. Our results suggest that peptide-enhanced HA hydrogels can modulate subchondral bone remodeling after joint injury, offering a potential injectable strategy to preserve joint health and delay osteoarthritis progression.

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