Temporal Proteomic Profiling of Post-Traumatic Osteoarthritis Identifies the Optimal Therapeutic Window for Anti-inflammatory Cell Therapy

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Introduction: Post-traumatic osteoarthritis (PTOA) arises after joint injury through an acute inflammatory cascade that progresses toward chronic tissue degeneration. Despite extensive study, the early molecular events that determine disease trajectory remain poorly defined, limiting development of effective disease-modifying interventions. Recognizing PTOA as a disorder of the *whole joint*, the synovium plays a significant role in cytokine and metabolic environment that drives cartilage damage. This study aimed to map the temporal proteomic response of rat synovium following destabilization of the medial meniscus (DMM) injury and to define the optimal therapeutic window for anti-inflammatory cell therapy, for example macrophage mediated.

Methods: Synovial tissues were collected from DMM-operated and contralateral knees at 1, 3-, 7-, 10-, and 14-days post-surgery. Quantitative label-free proteomics was performed, and proteins were analyzed for differential expression, pathway enrichment, and macrophage-associated trends. Statistical and visualization analyses included PCA, hierarchical clustering, KEGG enrichment, volcano plots, and temporal trend mapping of key pathways.

Results: Global proteomic clustering (PCA and hierarchical heatmap) revealed a distinct proteomic signature separating early (Days 1–3) from later (Days 7–14) post-injury time points, confirming a rapid injury-induced molecular shift (**Fig. 1A-B**). Cytokines and signaling proteins (**Fig. 1C**), including IL6, NFκB, STAT family members, and TGFB1, peaked within the first 3 days, capturing the acute inflammatory cytokine storm. Pathway enrichment analysis demonstrated strong up-regulation of acute inflammatory signaling, including chemokine, cytokine-cytokine receptor, JAK-STAT, complement, and Toll-like receptor pathways, which peaked between Days 1–3 and declined by Day 7 (**Fig. 1D**). Pathway-specific analysis demonstrated distinct temporal regulation following DMM injury: arachidonic acid metabolism and complement/coagulation cascades peaked during Days 1–3, indicating early inflammatory activation, whereas ECM–receptor interaction proteins increased after Day 7, marking the transition toward matrix remodeling and tissue repair (**Fig. 1E**). The trajectory of macrophage-associated proteins (**Fig. 1F**) revealed a clear polarization pattern, with M1-related activity dominating initially and M2-related proteins increasing from Day 7 onward, indicating the transition from inflammation toward repair. Interestingly, proteomic comparison of contralateral limbs between Day 14 and Day 1 revealed distinct molecular alterations (**Fig. 1G**). Although less extensive than in the injured joints, several proteins showed significant regulation. These findings suggest that DMM injury elicits a systemic or compensatory proteomic response in distant joints, likely mediated by circulating inflammatory factors and altered mechanical loading resulting from post-injury gait adaptation.

Discussion: Temporal proteomic profiling of the DMM model delineates a clear molecular chronology following injury, consisting of an acute inflammatory phase (Days 1-3), a transitional immune-metabolic phase (Day 7), and a later reparative remodeling phase (Days 10-14). The gradual reduction of inflammatory signaling and increase in M2-associated proteins suggest a shift toward resolution and tissue repair. These observations point to the period between Days 5-10 as a potentially favorable window for evaluating iMac-M2 therapy, when modulation of the inflammatory environment may best support joint recovery. The systemic molecular changes detected in contralateral joints further indicate that joint trauma triggers both local and organism-wide responses, emphasizing the need for therapeutic approaches that address coordinated immune and metabolic regulation rather than localized inflammation alone.

Significance/Clinical Relevance: This study maps the molecular progression of synovial inflammation and repair following DMM injury, mirroring early PTOA. Targeting the transition between inflammation and early repair may help limit degeneration and slow PTOA progression when designing therapeutic strategies. These findings support the development of timing-optimized macrophage-based therapies such as iPSC-derive macrophages and highlight systemic effects in contralateral joints that further refine approaches for cell-based intervention.

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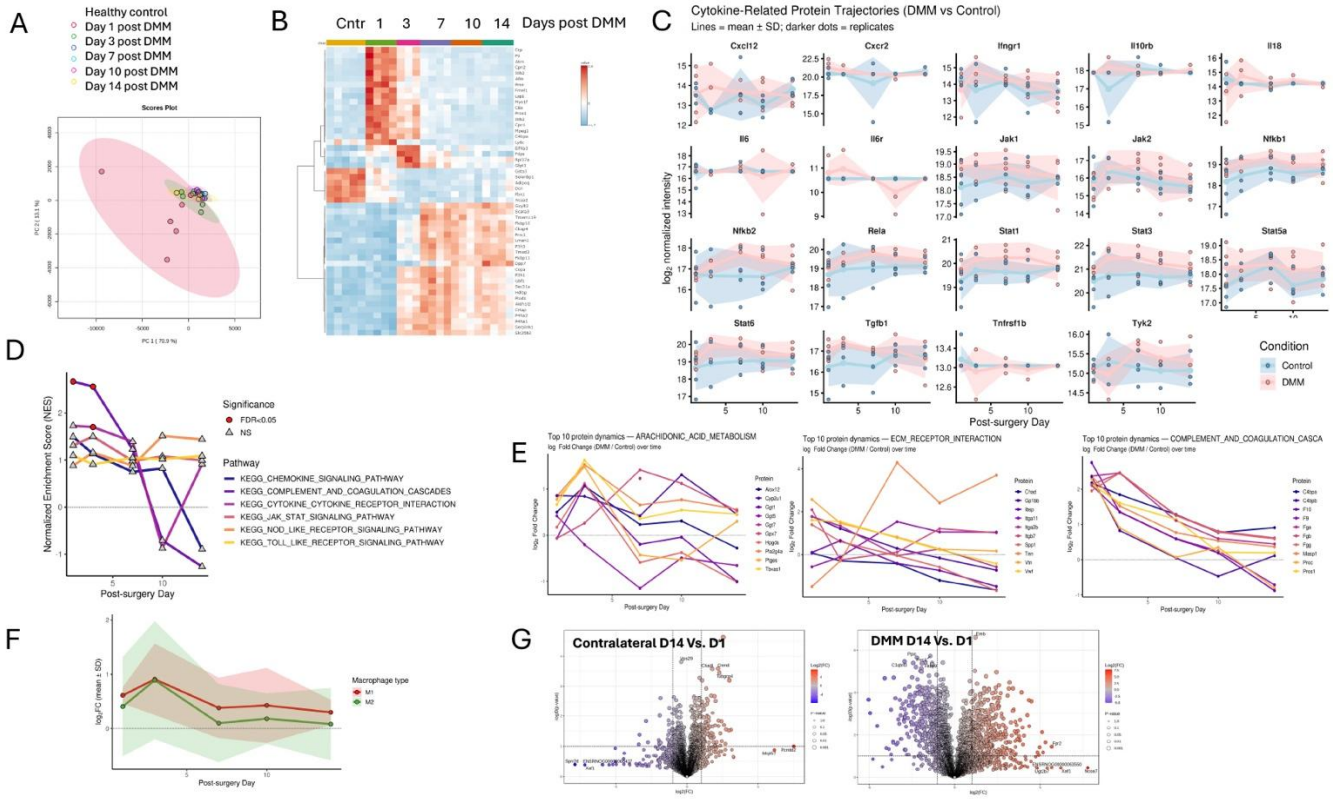

Figure 1: Temporal proteomic landscape of synovial response after DMM injury. (**A–B**) PCA and heatmap show distinct clustering and early proteomic divergence across timepoints. (**C**) Cytokine-related protein trajectories showing early up-regulation of IL-6, NFκB, STAT, and TGFB1 signaling. (**D**) KEGG enrichment analysis indicating transient activation of chemokine, cytokine-receptor, JAK–STAT, complement, and Toll-like receptor pathways at Days 1–3 (NES from GSEA, DMM vs contralateral) (**E**) top 10 protein dynamics showing activation of ECM–receptor interaction, complement/coagulation cascades, and arachidonic-acid metabolism. (**F**) M1 vs M2 macrophage markers dynamics demonstrated an early M1 peak followed by gradual M2 increase. (**G**) Volcano plots comparing Day 14 vs. Day 1 in DMM and contralateral joints show ongoing molecular remodeling and a modest systemic proteomic response beyond the directly injured joint.