Macrophage Diversity in the Stiffening and Fibrotic Synovial Niche following Joint Injury

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INTRODUCTION: Osteoarthritis (OA) is a debilitating condition characterized by degeneration of multiple joint tissues. While OA is traditionally referred to as "non-inflammatory", inflammation is now recognized as an active component in OA pathogenesis and the synovium is a major site of inflammatory changes[1]. Macrophages (MΦ) are key cellular mediators of synovial inflammation and recent studies suggest that distinct MΦ clusters occupy specific niches within the synovium. Namely, tissue-resident CX3CR1$^+$ macrophages of the intima are derived from a monocyte-independent lineage and form a tight-junction-mediated barrier that physically restricts inflammatory progression[2]. In contrast, MΦ from the subintimal niche include infiltrating, monocyte-derived MΦs as well as distinctive monocyte-independent interstitial resident subsets[2]. While diverse synovial MΦ populations and their genetic/functional signatures have been well characterized in the context of rheumatoid arthritis (RA), macrophage heterogeneity in OA is not yet well defined[3]. In addition, while soluble factors (such as chemokines and cytokines) are known to regulate MΦ polarization and function, less is known regarding how biophysical cues might regulate these processes. Notably, in OA, the progressive fibrosis of the synovium results in substantial remodeling of the extracellular matrix. ***The goal of this study was to interrogate how the dynamic physical environment in synovium modulates the behavior and function of synovial MΦs in a commonly used murine model of OA.***

METHODS: All animal procedures were IACUC approved. At 12 weeks of age, male, wild-type C57BL/6J mice underwent surgical destabilization of the medial meniscus (DMM) on the right knee to induce OA. Sham surgery was performed on contralateral knees. At 4- and 8- weeks post-DMM, unprocessed knee joints were harvested and directly embedded in OCT. AFM-nanoindentation was performed using microspherical tips (R= 12.5μm; k=0.6 N/m) on cryosectioned knee joints to measure micromodulus E_{ind} (N=10 animals/group; N≥18 indentations/animal)[4]. For single cell RNA-sequencing (scRNA-seq; 10X Genomics), synovial cells were isolated from DMM- and sham- operated knee joints (N=15/group) 4-weeks post-surgery via collagenase digestion and the CD45$^+$ cell population was enriched using magnetic cell separation. scRNA-seq data was processed using Seurat[5]. Statistical analysis was performed using Wilcoxon matched-pairs signed rank and $p<0.05$ was considered statistically significant. Paraffin processed knee joints were stained for H&E.

RESULTS: (**Fig 1**) H&E histology confirmed fibrotic remodeling of the synovial ECM 4-weeks post-DMM. (**Fig 2A-C**) Quantitative mechanical characterization of the synovial ECM likewise corroborated fibrotic remodeling in DMM-operated knees at 4-weeks post-surgery, with a median ~3.1 fold increase in synovial stiffness compared to sham surgical controls. (**Fig 2D-F**) AFM at 8-weeks post-surgery showed persistent synovial fibrosis at this later stage of OA pathogenesis. (**Fig 3A**) scRNA-seq revealed multiple populations of CD45$^+$ immune cells in both sham and DMM synovial tissue with unique gene expression profiles (**Fig 3B**). Notably, four unique MΦ clusters were identified across conditions, including CX3CR1$^+$ lining MΦs and their precursors, namely, MHCII$^+$ interstitial MΦs. (**Fig 3C**) The proportion of each MΦ cluster was similar in both the sham and DMM-operated conditions. (**Fig 3D**) Differential gene expression analysis highlighted the fact that, out of 32,285 genes that were detected in the sequencing experiment, very few genes were differentially expressed between congruent MΦ clusters in sham and DMM.

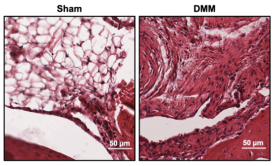


Fig 1. Representative H&E images of sham- and DMM- operated synovium (anterior tibial) 4- weeks post-surgery.

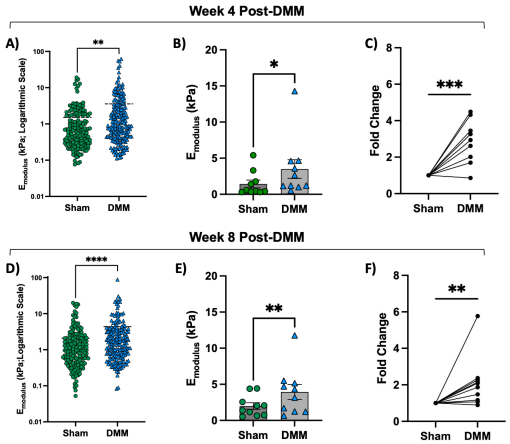
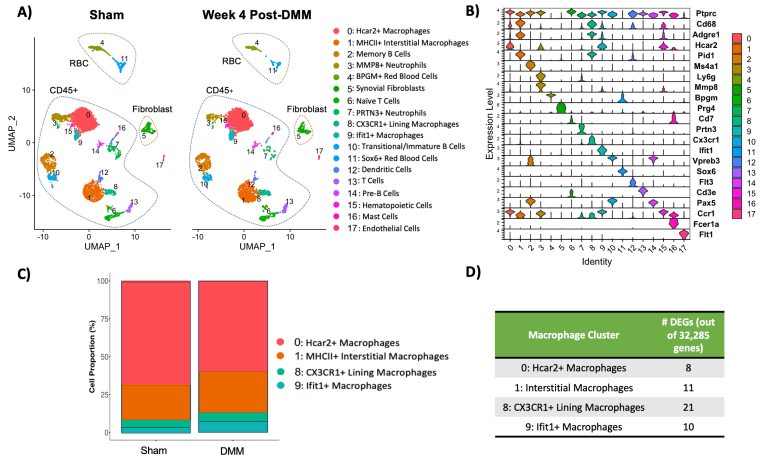


Fig 2. Mechanical characterization of murine synovium after DMM: *(A&D) Indentation modulus of murine synovial tissue in DMM- and sham- operated knees. (B&E) Average stiffness of the synovium for each animal. (C&F) Fold change in synovial E_{ind} for DMM-operated knees relative to contralateral sham controls.*



Macrophage Cluster	# DEGs (out of 32,285 genes)
0: Hcar2+ Macrophages	8
1: Interstitial Macrophages	11
8: CX3CR1+ Lining Macrophages	21
9: Ifit1+ Macrophages	10

Fig 3. Transcriptomic analysis of CD45+ sorted synovial cell phenotypes: *(A) UMAP projection of CD45+ sorted cells isolated from the synovium of sham- and DMM- operated knees. (B) Violin plots of synovial cell cluster marker genes. (C) Proportional breakdown of synovial macrophage subtypes in each condition. (D) Summary of the total number of DEGs between sham and DMM cells from the same cluster.*

DISCUSSION: Recent advances in single-cell omics have facilitated the discovery of distinct synovial MΦ populations. In the context of RA, studies identified discrete phenotypic MΦ clusters in healthy, inflamed, and remitting joints, with both morphological and spatial re-organization with disease[3]. Namely, a report by Culemann et al. suggested that the onset of inflammation in RA results in a rapid spatial reorientation of CX3CR1$^+$ lining MΦs that abrogates their cell-cell contact and disintegrates the tight-junction-mediated barrier formed by these MΦs in homeostasis. Accordingly, we hypothesized that inflammatory processes in OA resulting from DMM surgery would induce phenotypic and transcriptomic shifts in the synovial MΦ populations. Given that MΦs are mechanosensitive cells that integrate both biophysical and biochemical signals to tune cellular behavior, we further hypothesized that the dynamic synovial microenvironment in OA may play a role in modulating synovial MΦ behavior. While our data do support that the murine synovium stiffens by a factor of three within 4 weeks of DMM surgery (while the sham did not), our single-cell sequencing revealed that sham- and DMM- operated knees had comparable transcriptomic profiles, proportions and subsets of synovial immune cells at this timepoint. This suggest that, at 4-weeks post-surgery, the surgical insult involved in both sham- and DMM- procedures is the main driver of initial inflammatory infiltration. Further studies at a later timepoint in this model—when inflammation due to the surgical insult has subsided—are needed to delineate the chronic inflammatory signatures of OA-related injuries. Additional analysis of the non-CD45+ cell populations (synovial fibroblasts) and naïve controls may likewise provide additional insight.

SIGNIFICANCE: These findings highlight the fact that the sham- and DMM- operated knee joints induce comparable synovial inflammatory phenotypes at the early stages of this model. Consistent with Moradi et al, our results suggest that DMM- and sham- surgeries should be viewed as OA-inducing and non-OA-inducing injuries respectively, providing an opportunity to understand how this acute inflammatory response impacts the chronic divergent clinical outcomes when combined with mechanical instability[6].

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REFERENCES: [1] Scanzello+ 2011, [2] Culemann 2019+ [3] Alivernini+ 2020 [4] Kwok+ 2023 [5] Knights+ 2023 [6] Moradi+ 2019