A Single Instance of Joint Overloading Results in Persistent Changes to the Synovial Cell Landscape

Kevin Burt[1,2], Vu Nguyen[2], Yasaman Moharrer[1], Joel Boerckel[1], Robert Mauck[1,2], Carla Scanzello[1,2,3]
[1]Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA; [2] CReATE Motion Center, CMC VA Medical Center; Philadelphia PA;
[3]Division of Rheumatology, University of Pennsylvania, Philadelphia, PA.

Disclosures: KGB (N) - kevin.burt@pennmedicine.upenn.edu, VN (N), YM (N), JB(N), RLM (8), CRS (8)

INTRODUCTION: Contrary to the initial characterization of OA as a non-inflammatory arthritis, joint inflammation is now a widely accepted disease feature. Thus, evaluating inflammatory responses during the onset of OA is fundamental. Surgical destabilization models have been widely utilized for their ability to reproducibly create progressive articular pathology that mimics OA pathology in human disease. However, the acute impacts of the traumatic surgical approach on surrounding soft tissues (i.e., synovium) are difficult to decouple from the instability in terms of disease-propagating inflammation. Utilizing methods in spatial proteomics and transcriptomic sequencing, we previously showed that closed joint overloading induces milder early synovial inflammation compared to the severe inflammatory synovial environment produced by the DMM surgical approach[1]. Expanding upon this work, **we hypothesized that repeated injurious loading would induce a more severe disease pathology, similar to surgical DMM, and that a single instance of loading would result in a milder end-stage disease pathology.**

METHODS: *Surgical OA model*: Destabilization of the medial meniscus (DMM) surgery was performed to induce OA in skeletally mature (12-wk old) male C57BL/6 (WT) mice[1,2]. *Nonsurgical loading OA model:* Injurious loading was performed on male WT mice via either a single loading event (40 cycles, 9N max load, 0.1Hz), or repeated loading 3X weekly for 2 weeks[1,3,4]. Contralateral non-loaded knees were used as controls. *Histopathology (n=5-6):* Evaluation of cartilage damage[5] and synovitis[6] was performed on Toluidine Blue and HE stained sections, respectively. *Pain analysis (n=15):* Knee hyperalgesia was recorded using a pressure application measurement device, measuring withdrawal threshold (capped at 450g). *Imaging Mass Cytometry (IMC) (n=5-6*): Paraffin-embedded sections underwent heat-mediated antigen retrieval and overnight incubation with a 24-marker multiplex panel of metal-conjugated antibodies, followed by incubation with Intercalator-Ir nuclear stain, and imaging using a Hyperion Imaging System (Standard Biotools). *Spatial protein expression and cellular phenotype analysis:* Single cell masks were created using the nuclear stain (deepcell.org). IMACytE software was used for t-distributed stochastic neighbor embedding (t-SNE) dimensionality reduction analysis to normalize data and perform cluster analysis[7]. *Statistical analysis:* T-tests (Holm-Sidak post-hoc) compared outcomes between models, and two-way ANOVA (Tukey post-hoc) compared models and time, $p<0.05$ considered significant.

RESULTS: Histopathological analysis revealed that both single- and multi-loading injury produced the same extent of cartilage damage, with no significant difference in histological scores across the joint at any time point up to 12-wks post-loading (**Fig. 1A**). Notably, DMM produced a similar level of cartilage damage 8-wks post-DMM, when compared to both loading groups at 12-wks (**Fig. 1A**). While DMM resulted in significant increases in knee hyperalgesia by 8-wks post-surgery, injurious loading produced no significant change to knee withdrawal threshold values (**Fig. 1B**). Probing synovial histopathology at the earlier 2-wks post-loading time point, multi-loading produced a significant increase in cellularity ($p=0.010$) and fibrosis ($p=0.002$) compared to the single-loading group (**Fig. 1C,D**). However, both loading groups exhibited significantly lower histopathologic evidence of synovitis at 2-wks when compared to surgical DMM at that time point (**Fig. 1C,D**). By 12-wks, both loading regimens produce similarly low levels of synovitis, comparable with which is observed in 8-wks post DMM (**Fig. 1C,D**). Spatial proteomics (IMC) revealed a single instance of loading upregulates a population of synovial lining monocytes ($CD45^+,CD14^+$) with high expression of the cell structural protein vimentin (Vim)[1]. These cells are present at 2-wks following loading and are sustained up to 12-wks (**Fig. 2A**). At 12-wks, spatial phenotyping revealed 9 unique cellular clusters, with the single loading group having a significant increase in the Vim^{High} monocyte (Cluster 2, $p=0.042$), in addition to macrophage ($CD45^+,F4/80^{low}$ Cluster 6, $p=0.015$) and fibroblast clusters ($CD45^-,aSMA^+,Col1/2^+$, Cluster 8, $p=0.027$) (**Fig. 2B,C,D**). Spatial mapping of clusters across the synovium revealed the increased Vim^{High} monocyte (Cluster 2) and macrophage (Cluster 6) clusters within the loaded synovium (arrows, **Fig. 2E**), localized near a $CD31^{high}$ vascular cluster (Cluster 5) (hollow arrows, **Fig. 2E**).

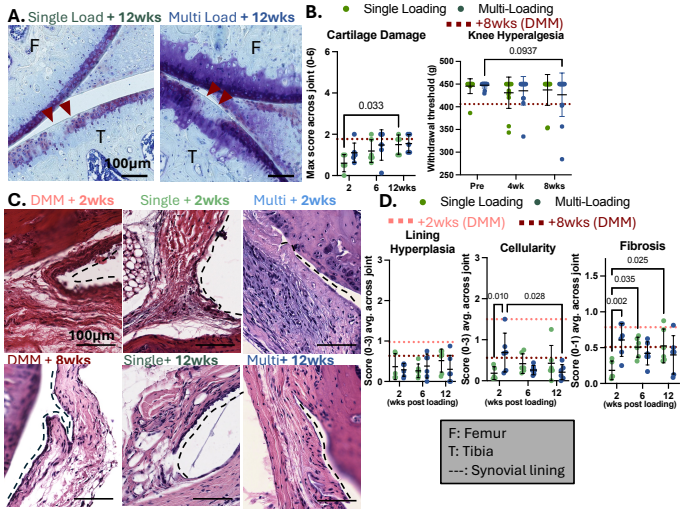

Figure 1: *(**A**) Safranin O (cartilage) stained knee sections. (**B**) Max OARSI cartilage score across the knee joint, and knee hyperalgesia measured as the withdrawal threshold (g). (**C**) H&E-stained knee joint synovium. (**D**) Average synovial inflammation scores across sub-categories.*

DISCUSSION: Contrary to our original hypothesis, we found that both single (1X) and multiple (6X) injurious loading events produce similar late-stage cartilage degeneration and synovial inflammatory histopathology. However, the single loading regimen resulted in a slower progressing disease pathology. Interestingly, the single injurious loading event also produced *highly similar* late-stage histopathologic disease features to the well-established surgical DMM model of PTOA; however, 1X loading *did not* recapitulate the late-stage knee hyperalgesia that is well established within the DMM model. Notably, the surgical DMM model produced robust early synovitis (2-wks), compared to the low levels of early synovial inflammation following a single loading event. Spatial immunophenotyping via IMC revealed an early (2-wks) and sustained (12-wks) loading-activated synovial lining monocyte population, in addition to various monocyte, macrophage, and fibroblast cell clusters that were present up to 12-wks following a single loading event.

SIGNIFICANCE: Our results highlight the differences in disease progression across surgical and nonsurgical OA models and reveal the lasting influence of injurious loading on knee joint synovium within a model of minimal knee pain, suggesting possible homeostatic mechanisms of synovial lining monocytes, as well as providing a potential model for testing immune-focused synovial therapeutics within a PTOA model of non-surgical overload injury.

REFERENCES: [1]Burt+, ORS Annual Meeting (2025), [2]Burt+, BioRxiv (2025), [3]Cho+, NBM (2015), [4]Poulet+, ArthRhuem (2011), [5]Glasson+, OAC (2010), [6]Obeidat, OAC (2024), [7]Somarakis, IEEE Trans Vis (2020). **ACKNOWLEDGEMENTS:** Funding-I01 BX004912(VA BLR&D), R01 AR075737(NIAMS), IK6 RX003416(VA RR&D), CReATE Motion Center(I50 RX004845).

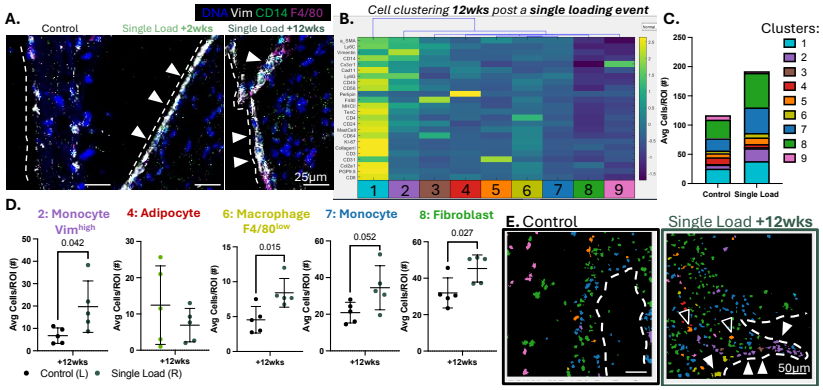

Figure 2: *(**A**) Selected IMC marker expression within synovial ROIs. (**B**) Marker expression heatmap within clusters and breakdown of cell populations across groups. (**D**) Cell number analysis within clusters. **E**) Cell cluster assignment within synovium. Synovial lining = white-dashed line.*