AWARD NUMBER: W81XWH-20-2-0057

TITLE: Therapeutic small molecule and timed limb stabilization strategies to prevent complications of extremity trauma and enhance return to duty

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CONTRACTING ORGANIZATION: UT Southwestern Medical Center, Dallas, TX

REPORT DATE: October 2021

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE October 2021	**2. REPORT TYPE** Annual Report	**3. DATES COVERED** 30Sep2020-29Sep2021

4. TITLE AND SUBTITLE

Therapeutic small molecule and timed limb stabilization strategies to prevent complications of extremity trauma and enhance return to duty

5a. CONTRACT NUMBER W81XWH-20-2-0057

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The central goal of this project is to change the Standard Practice Guidelines for extremity trauma in a PFC scenario to prevent the 2 main barriers to return to duty: IRI and HO. Specifically, we will target mechanotransduction through a readily deployable therapeutic and an easily followed mobilization protocol. Specifically, we will evaluate the efficacy of therapeutic strategies that target mechanotransduction through focal adhesion kinase (FAK) and YAP/TAZ signaling as well as a clinically relevant post-injury immobilization protocol to prophylax against IRI organ injury and HO. Our overall goal is to generate a paradigm shift in our approach to patients with severe extremity trauma and tourniquet use to include an easily deployable orally bioavailable therapeutic and a synergistic early limb immobilization strategy to mitigate the systemic effects if IRI and to prevent HO. We hypothesize that mechanotransductive signaling is critical for IRI and HO and that pharmacologic or occupational therapy based inhibition of the FAK pathway will prevent trauma-induced HO and limit IRI organ dysfunction.

15. SUBJECT TERMS
Heterotopic ossification, mechanotransduction, trauma, healing, immobilization, focal adhesion kinase (FAK), YAP/TAZ, ischemia reperfusion injury (IRI)

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	23	

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1. INTRODUCTION:

Heterotopic ossification (HO) is a condition in which ectopic bone forms inappropriately in the soft tissue following large surface area burns and severe musculoskeletal trauma resulting in reduced range of motion and quality of life. Battlefield injuries result in a higher prevalence of HO compared to civilian injuries, with occurrences in greater than 70% (range 65-95%) of blast-related extremity amputations. HO is known to affect sites that experience mechanical strain, such as joints. Current strategies to prevent HO lack standardization of mechanotransductive-based treatments and mobilization and demonstrate low efficacy or infeasibility. Our group demonstrated a central role of mechanotransduction and mechano-sensitive proteins, focal adhesion kinase (FAK) and YAP/TAZ signaling, in promoting the formation of trauma-induced HO, implicating these as promising targets for potential therapeutic interventions. While novel technologies are under development that allow for immediate limb stabilization, the timing and mechanism behind these therapeutic approaches are not known. Our group also developed a burn plus Achilles tenotomy (BT) mouse model which consistently results in the formation of HO. Therefore, using our well-validated BT mouse model, we aim to characterize the effectiveness of FDA-approved YAP/TAZ inhibitor, Verteporfin, as well as durations of injured limb immobilization as potential therapeutic strategies to minimize traumatic HO formation.

2. KEYWORDS:

Heterotopic ossification, mechanotransduction, trauma, healing, immobilization, focal adhesion kinase (FAK), YAP/TAZ, ischemia reperfusion injury (IRI)

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Regulatory Tasks:
Subtask 1: IACUC/ACURO Approval
Status: UTSW IACUC protocol number: 2020-102985 approved 2020-10-19. ACURO protocol number: OR190048

Specific Aim 1: *To demonstrate that pharmacological FAK and YAP/TAZ inhibitors mitigate extremity trauma-induced IRI and HO formation using validated PFC relevant extremity trauma models.*
Status: In progress Y1Q4

Subtask 1: Burn/tenotomy (traumatic HO model) performed in C57BL/6J mice with or without time administration of pre-clinical orally available FAK inhibitor (Defactinib) and FDA approved YAP/TAZ inhibitor (Verteporfin) for microCT HO bone volume assessment.

Status: In Progress Y1Q4

Subtask 2: Blast/amputation/ischemia reperfusion injury in Sprague Dawley rats with or without administration of FAK inhibitor (Defactinib) or YAP/TAZ inhibitor (Verteporfin) for microCT HO bone volume assessment and IRI end organ damage assessment (kidney, liver, endothelial cells, muscle, DAMPS).
Status: In progress Y1Q4

Subtask 3: Burn/tenotomy (traumatic HO model) performed in C57BL/6J mice with or without time administration of pre-clinical orally available FAK inhibitor (Defactinib) and FDA approved YAP/TAZ inhibitor (Verteporfin) for histomorphometry, histology, and transcriptional profiling.
Status: In Progress Y1Q4

Subtask 4: Blast/amputation/ischemia reperfusion injury in Sprague Dawley rats with or without administration of FAK inhibitor (Defactinib) or YAP/TAZ inhibitor (Verteporfin) for histomorphometry, histology, and transcriptional profiling.
Status: In progress Y1Q4

Milestone: Completion of FAK and YAP/TAZ inhibitor studies and data analysis by end of month 20. Preparation and submission of peer-reviewed manuscripts.
Status: Not started Y1Q4

Specific Aim 2: *Validate the ability of brief, timed injury-site stabilization of high-risk joints to mitigate HO and joint contractures.*
Status: In progress Y1Q4

Subtask 1: Assess HO formation by microCT after burn/tenotomy with single joint immobilization weeks 0-1, 0-2, 0-3 or -0-1+FAK inhibitor treatment.
Status: In progress Y1Q4

Subtask 2: In vivo timed assessment of immobilization weeks 0-1, 0-2, 0-3 or -0-1+FAK inhibitor treatment by in vivo fluorescence microscopy, flow cytometry, single cell RNA sequencing.
Status: In progress Y1Q4

Subtask 3: In vivo timed assessment of immobilization weeks 0-1, 0-2, 0-3 or -0-1+FAK inhibitor treatment on muscle function and gait analysis in mice.
Status: In progress Y1Q4

Milestone: Completion of IRI and HO studies and data analysis by end of 24th month. Preparation and submission of peer-reviewed manuscripts.
Status: In progress Y1Q4

What was accomplished under these goals?

Specific Aim 2: Validate the ability of brief, timed injury-site stabilization of high-risk joints to mitigate HO and joint contractures.

Subtask 1: Assess HO formation by microCT after burn/tenotomy with single joint immobilization weeks 0-1, 0-2, 0-3 or -0-1+FAK inhibitor treatment.
Status: In progress Y1Q3

1a: Assessment of HO formation by microCT after BT plus joint immobilization weeks 0-1, 0-2, 0-3 post-injury.

Methods:

BT Injury: Male 8–10-week-old BL6 mice were housed at standard conditions with 5 mice per group. Mice were anesthetized with inhaled isoflurane. Dorsal hair was shaved and a partial thickness burn injury was created on the dorsal surface that comprised ~30% of total body surface area. The mouse also received a full thickness tenotomy of the Achilles tendon and closure of the incision site with a single Vicryl suture (burn/tenotomy model; BT). Mice were treated with post-operative analgesia and monitored per our standard protocols.

Immobilization: Immediately following BT trauma, the injured limb in some groups was immobilized using a stiff Velcro strip secured to the base of the leg. The Velcro was gently wrapped around the length of the leg and secured at the base of the foot while the surgeon stabilized the leg in a fully outstretched position. Mice were monitored frequently to ensure proper immobilization has been achieved and the Velcro apparatus replaced as needed over the duration of immobilization (as indicated). Following the immobilization step mice were allowed free ambulation until they reached 9 weeks post-BT. Previous iterations of immobilization studies were achieved by inserting the injured limb into a retrofitted 1.5 mL Eppendorf tube which was secured at the base of the leg. These methods have been optimized for mouse comfort and immobilization efficiency. In either case, control mice ('mobilized'/'mob') are allowed free use of their injured limb and ad lib ambulation.

MicroCT Imaging and Analysis: Mice were imaged using a high-resolution micro-computed tomography (microCT) on post-operative week 9. Same scan setting as previous studies from our group were used. Images were processed and quantified using our standard protocols.

Results: Immediately following BT injury mice were either allow free mobilization of their injured limb ('mob') or the injured limb was immobilized for 1, 2, or 3 weeks, followed by a period of re-

ambulation. Quantitative volumetric measurement of ectopic bone was calculated at 9-weeks post-BT injury by micro-CT. (Fig 1A). All timepoints of immobilization were sufficient to significantly reduce traumatic HO formation in all regions (Fig 1B).

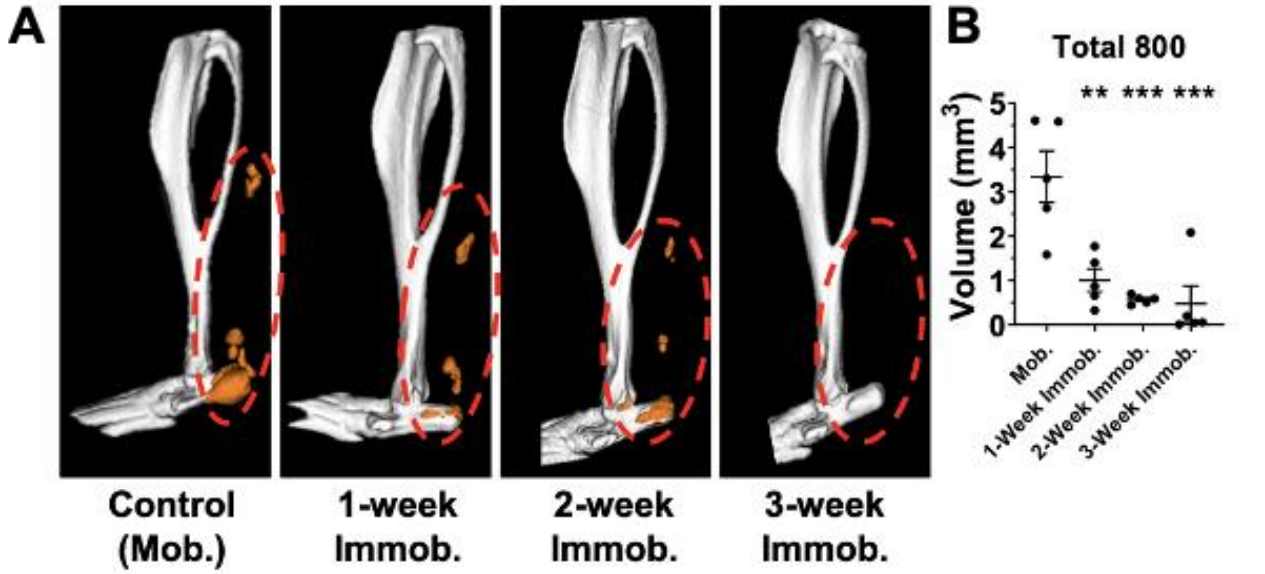

Figure 1: A) Representative microCT images and B) volumetric microCT quantification of ectopic bone from all ('total') regions of HO development at 9-weeks post trauma in after 0-3 weeks of limb immobilization. HO is visualized in orange pseudo-color. Statistical analyses performed by student's t-test followed by Mann-Whitney U-test vs mob control. Wide bar represents the mean and narrow bar represents SEM. **p<0.005, ***p<0.005, n=5 mice per group

Discussion/Conclusions: These results confirm our previous findings that immobilization is an effective strategy to prevent HO formation following traumatic injury. By including various timepoints of immobilization we can implicate the first week as a critical time for these disruptive, mechanical interventions and show that the greatest effect size can be seen during this time. Longer durations of immobilization appear to have enhanced HO-reduction though these longer time points may be sub-optimal for war-time implementation. Considering this, future work will focus primarily on the one-week immobilization time point and will also incorporate pharmacological inhibitors to achieve even greater reduction of HO following trauma.

Subtask 2: *In vivo* timed assessment of immobilization weeks 0-1, 0-2, 0-3 or -0-1+FAK inhibitor treatment by in vivo fluorescence microscopy, flow cytometry, single cell RNA sequencing.
Status: In progress Y1Q3

2a: Assessment of disruption of mechano-sensing and ECM remodeling pathways in immobilization weeks 0-1, 0-2, 0-3, and 2-3 by fluorescence microscopy
Previously we showed that BT plus 1 week of immobilization resulted in disorganization of the collagen matrix which led to increased adipogenic differentiation of MPCs in the HO anlagen at the expense of osteo/chondrogenic differentiation.(Huber et al., 2020) While these studies implicated

the role of YAP/TAZ and FAK, the manner in which these intracellular players sense changes in the local extracellular mechanical environment is not well understood. Discoidin Domain Receptor 2 (DDR2) is a collagen-induced receptor tyrosine kinase that activates downstream ECM remodeling and cell proliferation pathways. We sought to elucidate whether DDR2 is a potential transducer of collagen organization signals to MPCs, thereby implicating DDR2 as a potential novel mechanosensory protein target for investigative therapeutic inhibition. Additionally, we sought to assay the effects of longer durations of immobilization (2 or 3 weeks) as well as delayed immobilization (immobilization initiated at week 2) on ECM composition and organization.

Methods:

Animals: (see above)

BT Injury: (see above)

Immobilization: (see above)

Immunofluorescence (IF): Injured limbs were taken after one week post BT or post BT + immobilization, fixed for 24 hours in 4% PFA and decalcified for 6 weeks in 15% EDTA. Following OCT embedding and sectioning, 10-20 micron sections from the HO anlagen site of the distal portion of the Achilles tendon of were stained with the following antibodies: anti-PDGFRa, anti-DDR2 and 1:1000 Hoechst 33342. Antibody labeled sections were imaged on confocal microscope. Each site was imaged on all channels and overlaid before examination. Collagen fibers visualized via second harmonic generation (SHG) and anisotropy quantified using the FibrilTool ImageJ plugin.(Boudaoud et al., 2014) Images only adjusted for brightness and contrast identically across comparison groups for clarity as needed.

Histology: Safranin O and Hematoxylin staining conducted following standard protocols on injured limbs from mice that underwent BT or BT with the indicated durations of immobilization. All samples harvested at 3 weeks post-injury.

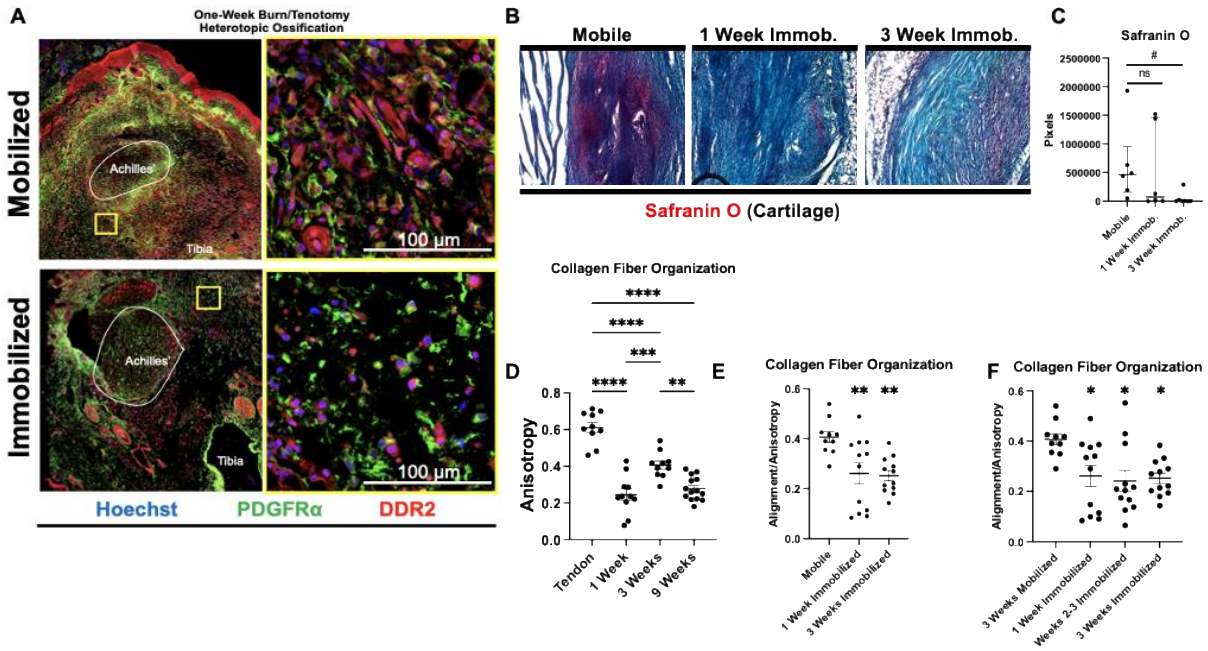

Figure 2: A) Representative micrographs from axial sections of the injury site 1 week post-BT stained for PDGFRα (MPC) and DDR2 (ECM binding protein). Regions of interest are highlighted in yellow. B) Representative micrographs of longitudinal sections of the injury site at indicated time points stained with Safranin O (cartilage) and hematoxylin counterstain. C) Quantification of Safranin O positive pixels using (n=3 mice/group, 1-2 images/group). #$p<0.05$ by Kruskal-Wallis Non-Parametric ANOVA and Dunn's Multiple Comparison Post-Hoc Test. Quantification of collagen fiber anisotropy D) in healthy tendon vs post-injury tendon at the indicated time points, harvested at indicated time points. Quantification of collagen fiber anisotropy E) in mobilized injured tendon vs 1- or 3-week immobilized injured tendon , collected at 3 weeks post injury and F) in mobilized injured tendon vs 1-, weeks 2-3 only, or 3-weeks immobilized injured tendon. D-F) *$p<0.05$, **$p<0.005$, ***$p<0.0005$, ****$p<0.0001$ by non-parametric ANOVA.

Results:

Qualitative assessment of IF revealed that in BT-injured mice the rate of colocalization of antibodies labeling the MPC marker PDGFRa with DDR2 labeling antibodies was decreased following 1 week of immobilization when compared with mobilized counterparts (Fig 2A) indicating decreased overall DDR2 protein expression following immobilization. Safranin O staining quantification (Fig 2B & C) 3 weeks post injury revealed that 1 and 3 weeks of immobilization were sufficient to reduce chondrogenesis at the injury site. Assessment of collagen alignment, measured by anisotropy score, revealed that injury alone at all time points tested (1, 3, and 9 weeks) reduced collagen anisotropy (Fig 2D). Interestingly, 1 and 3 weeks of immobilization of injured limbs further significantly decreased collagen anisotropy (Fig 2E). Furthermore, delayed immobilization (initiated at week 2) reduced collagen anisotropy as efficiently as 3 full weeks of immobilization (Fig 2F).

Discussion/Conclusions: Our findings suggest that DDR2 may be a key player in mechanosensation following tendon injury and subsequent immobilization. The specific expression of DDR2 on PDGFRα^+ MPCs, which are known to contribute to aberrant healing in trauma-induced

HO, further suggests that this protein may make a suitable alternative target for inhibition to prevent trauma-induced HO. Previously we demonstrated that 1 week of immobilization resulted in MPC preferential differentiation to adipocytes. It is well-understood that traumatic HO follows an endochondral process, however whether immobilization is sufficient to alter chondrogenic differentiation remains unknown. The reduced chondrogenesis observed via Safranin O staining suggests that the entire osteogenic process is reduced by immobilization. Importantly, for this experiment mouse tissues were harvested at 3 weeks post injury, even for the 1-week immobilized group, which suggests immobilization has long-lasting effects on MPC differentiation even after re-ambulation. In our previous manuscript we found that collagen misalignment occurred after 1 week of immobilization post-BT and that this was sufficient to drive MPC differentiation to adipocytes which led to significant reduction of HO. The collagen anisotropy measurements presented herein revealed that immobilization initiated on week 2 was sufficient to induce collagen anisotropy. Considering that in Fig 1, 3 full weeks of immobilization led to the most significant reduction in HO formation following traumatic injury and that delayed immobilization and 3 full weeks had the same level of collagen anisotropy, these data suggest that delayed immobilization may have the same HO-reduction efficacy as 3 full weeks of immobilization.

2b: Assessment of immobilization weeks 0-1 on MPC stromal function and injury-niche signaling by scRNAseq.

Preliminary data revealed no significant difference in macrophage number at the injury site with and without 1 week of immobilization. However, the possibility remains that immobilization may lead to changes in the immune milieu regarding both quantity of other cell types and their function which we have shown can have a profound impact on the progression of HO.(Sorkin et al., 2020) In support of this idea, there is a growing body of literature that suggests that mechanical/ECM changes in the MPC niche microenvironment can impact the secretion profile of MPCs, which are known to direct immune cell recruitment and activation both in vitro and in vivo, in addition to their differentiation which we see in our models.(Huber *et al.*, 2020) Exploring the effect of immobilization on injury-site MPCs interaction with immune cells via secretory pathways may reveal novel mechanosensitive gene targets and expand options for drug development for injury-repair.

Methods:

Animals: (see above)

BT Injury: (see above)

Immobilization: (see above)

Single Cell RNA Sequencing and Analysis: Tissues were collected at day 7 post BT with and without immobilization. Tissues were digested in collagenase and processed for single-cell RNA sequencing (scRNA-seq) using 10X sequencing and analyzed downstream using Seurat as previously described. For the purposes of the analysis that is presented here, clusters were grouped as Mesenchymal Progenitor Cells (MPCs; clusters 1, 4, 7, 13, 23, and 25). Clusters 1, 4, 7, 13, 23, and 25 were grouped in subsequent analysis and labeled Mesenchymal Progenitor Cell (MPC). KEGG pathway overrepresentation analysis and Gene Set Enrichment Analysis (GSEA) were run for the combined MPC cluster combined.

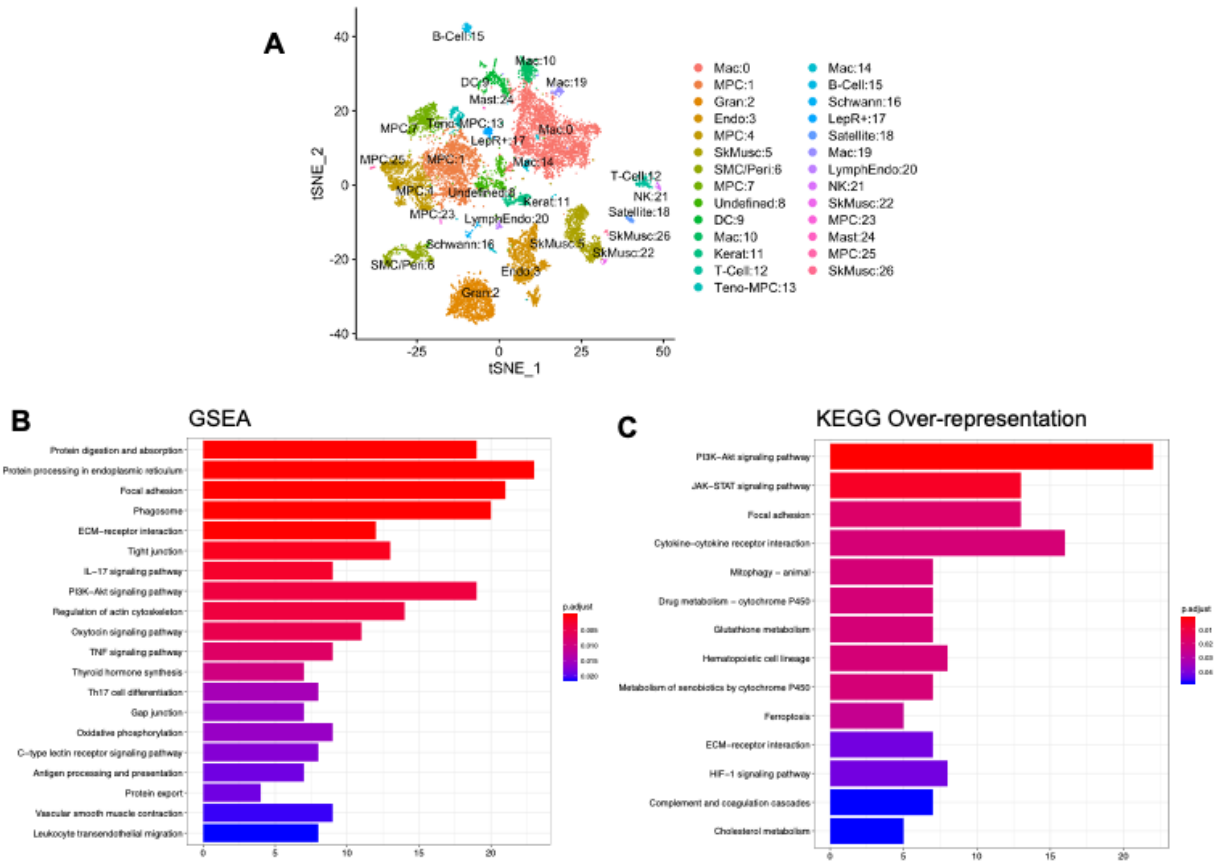

Figure 3: A) Clustering analysis of the scRNAseq data set. B) GSEA and C) KEGG pathways from all MPC clusters combined in BT + immobilized vs BT.

Results: Single cell RNAseq clustering revealed 6 subclusters of cells that can be classified as 'MPCs', cells thought to contribute to HO formation following traumatic injury (Fig 3A). GSEA revealed terms related to immune cell regulation such as 'IL-17 signaling pathway', 'TNF signaling pathway', and 'antigen processing and presentation' were enriched in MPC clusters combined from 1-week immobilized mice vs mobilized (Fig 3B). Similarly, KEGG pathway analysis showed terms 'cytokine-cytokine receptor interaction' and 'hematopoietic cell lineage' (Fig 3C), both of which contain many genes related to MPC interaction with immune cells, were enriched in immobilized MPCs vs mobilized.

Discussion/Conclusions: These enriched terms support the hypothesis that immobilization alters the MPC signaling milieu in a manner that may alter the recruitment or identity/function of immune cells to the injury site. Much more work is needed to explore this idea and elucidate key genes that may be used as targets for trauma recovery intervention.

Subtask 3: *In vivo* timed assessment of immobilization weeks 0-1, 0-2, 0-3 or -0-1+FAK inhibitor treatment on muscle function and gait analysis in mice.

3a: Assessment of passive range of motion (ROM) of injured and uninjured limbs following 0-1, 0-2, or 0-3 weeks of immobilization

An important goal of these studies is assessing the potential for return to normal function of the injured limb. Concerns initially were present regarding possible joint contractures because of the immobilization, these must be balanced by a need to effectively mitigate the development of HO following trauma. To investigate these concerns and assess the utility of limb immobilization for return to function, we have planned more thorough gait analyses. As an accessible auxiliary data point we sought to study the passive ROM of the injured limb at different time points of immobilization and following removal of the immobilizers.

Methods:

Animals: (see above)

BT Injury: (see above)

Immobilization: (see above)

Range of Motion: To perform the range of motion testing, mice were anesthetized and placed in a custom-built harness that allowed for extension of hind limbs. A 40g weight was attached to a string with a clip, and the clip was gently placed on the mouse's hind paw. The weight extends the ankle to its full range of motion and the ankle was digitally photographed. Using ImageJ, the angle of the ankle was measured by marking points at the knee, ankle, and mid-paw. Means were compared by Student's T-Test assuming equal variance at alpha= 0.05. Means and standard deviations are displayed.

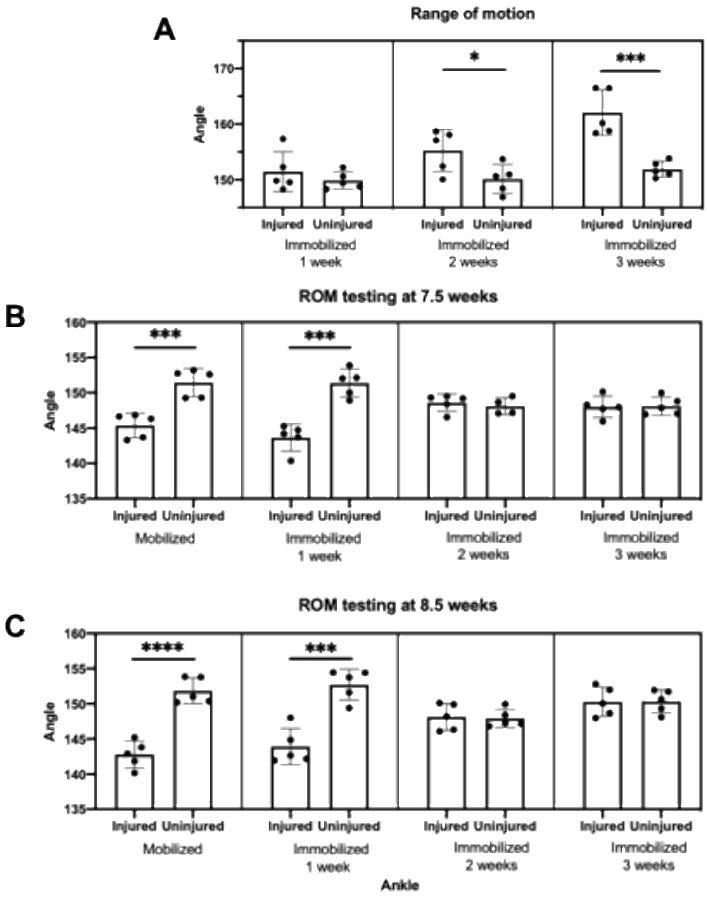

Figure 4: Mice (n=5/time point) were subjected to burn tenotomy and immobilized with a Velcro immobilizer at time of injury. At 1-, 2-, and 3-weeks post-injury, immobilizers were taken off and the ROM of both injured and contralateral uninjured ankle was measured (A). Alternatively, mice were allowed to ambulate after immobilizer removal to 7.5 weeks (B) and 8.5 weeks post-injury (C) at which point ROM was tested. Means were compared by Student's T-Test assuming equal variance at alpha= 0.05. Means and standard deviations are displayed.

Results: At initial timepoints (2- and 3-weeks post-injury) immobilization increases ROM of the injured ankle above that of contralateral control (Fig. 4A). ROM between the injured (and formerly immobilized limb) is not significantly different from the contralateral uninjured limb at 7.5 weeks (Fig 4B) and 8.5 weeks (4C) post-injury for both 0-2 weeks and 0-3 weeks immobilization.

Discussion/Conclusion: These data suggest that effects on mouse gait may be apparent immediately after immobilizer removal due to muscle atrophy but may be restored to the uninjured state as strength is regained in the immobilized limb during re-ambulation. Importantly, these data indicate that from a restoration of joint function standpoint, 2 weeks of immobilization may be sufficient and that decreased range of motion due to potential contractures is not present after longer time points of immobilization.

USUHS:

Specific Aim 1: ***To demonstrate that pharmacological FAK and YAP/TAZ inhibitors mitigate extremity trauma-induced IRI and HO formation using validated PFC relevant extremity trauma models.***

Subtask 4: Blast/amputation/ischemia reperfusion injury in Sprague Dawley rats with or without administration of FAK inhibitor (Defactinib) or YAP/TAZ inhibitor (Verteporfin) for histomorphometry, histology, and transcriptional profiling.

Progress: Our team at USUHS worked towards putting an IACUC protocol in place in Yr1 Qtr 4. The protocol (SUR-21-069) was approved by the IACUC committee as of 26 OCT 2021 and we are awaiting ACURO approval before commencing experiments.

What opportunities for training and professional development has the project provided?

This proposal has enabled trainees to attend seminars across the UTSW and USUHS campus. Additionally, it has supported trainees to attend national conferences including the ASBMR and Plastic Surgery Research Council.

How were the results disseminated to communities of interest?

Dr. Levi has presented his work to burn units locally and nationally to share the importance of loading and range of motion on heterotopic ossification.

What do you plan to do during the next reporting period to accomplish the goals?

As discussed in the Y1Q3 report, in response to the poor efficacy of both Defactinib (Y1Q2) and Verteporfin (Y1Q3) in reducing HO, additional studies exploring alternative mechanotransductive pathway inhibitors will be undertaken though more focus is planned for Aim 2 in the upcoming quarter. Additionally, as mentioned previously it is possible that immobilization in conjunction with administration of these inhibitors may have a synergistic effect. Therefore, the initially proposed time course of 1 week of immobilization plus either Defactinib or Verteforfin treatment may still be attempted. While issues surrounding pharmacologic inhibition of FAK and YAP/TAZ are addressed, in the next quarter we will continue addressing Aim 2, Subtask 2 by working to complete the immobilization time course IHC, scRNAseq, and flow cytometry studies.

Exploring other mechanisms by which immobilization reduces HO formation following traumatic injury is an important route towards uncovering other potential downstream pharmacologic treatment strategies. We will explore our hypothesis that immobilization alters immune cell recruitment, angiogenesis and lymphangiogenesis and/or function at the injury site. We will use our day 7 immob vs mob scRNAseq dataset to determine if there are prominent changes in immune cell numbers within each cluster as well as perform subcluster analysis to see if immune cell function/phenotype is altered. Additionally, proof of principle in vitro functional assays aimed at quantifying changes to cytokines secreted by injury site-derived primary MPCs in different mechanical/ECM environments may be initiated.

Because the data presented in Figs 2 and 4 demonstrate that week 2 is a critical and sufficient time point for immobilization-induced collagen misalignment and potential preservation of joint function we will continue to address Aim 2, Subtask 1 by performing delayed immobilization studies and assessing HO formation by microCT. We will also investigate potential routes for following up on our promising ROM data with gait analysis. We have identified movement tracking programs that may be suitable for detailed assessment of changes to gait/limb use in injury recovery with and without periods of immobilization. We will begin optimizing program, housing, and camera setups for these studies in the next quarter.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Within our burn unit at UTSW, we do not allow aggressive passive range of motion in burn patients during the first two weeks after their burn injury.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes to approach and reasons for change.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

Nothing to report

  Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

1. Amy L. Strong, M.D., Ph.D.[1], **Benjamin Levi, M.D**.[2], John Mares, B.S.[3], Thomas A. Davis, Ph.D.[3], Philip J. Spreadborough, M.D., Ph.D.[3]. Prolonged Tourniquet use following blast related lower extremity injuries increase Heterotopic Ossification in a pre-clinical model. PSRC, Virtual June 7-11, 2021
2. Chase A. Pagani, BA[1], Amy L. Strong, MD/PhD[2], Nicholas Livingston, BA[1], Yuxiao Sun, PhD[1], Geoffrey E. Hespe, MD[2], Johanna Nunez, MD[1], Nicole Patel, BS[2], Amanda K. Huber, PhD[2], Chunxi Ge, PhD[2], Renny Franceschi, PhD[2], **Benjamin Levi, MD[1]**. Genetic- and Mobilization-Based Alterations in Matrix Alignment to Mitigate Aberrant Cell Fate Determination, PSRC, Virtual June 7-11, 2021
3. **Benjamin Levi, MD**. ACS 2017 Clowes Memorial Research Career Development Award Recipient Presentation, American College of Surgeons, Virtual October 10-12,2021

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Benjamin Levi
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	1
Contribution to Project:	Planning of experimentation.
Name:	Eva Gabriela Baylon
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	4
Contribution to Project:	Was the project lead planning of experimentation.
Name:	Yuxiao Sun
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	3
Contribution to Project:	Planning and execution of experimentation.
Name:	Spencer Barnes
Project Role:	Biostatistician
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	5
Contribution to Project:	Single-cells RNA sequencing analyses
Name:	Jessica Medrano
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	1
Contribution to Project:	Planning and execution of experimentation.

USUHS

Name:	Thomas Davis
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	1
Contribution to Project:	USU Project management and oversight
Name:	Cassie Rowe
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	1
Contribution to Project:	Planning of experimentation
Name:	Elsa Ronzier
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	1
Contribution to Project:	Planning of experimentation.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: