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TITLE: Proteomics of Knee Osteoarthritis

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CONTRACTING ORGANIZATION: University of California, Davis

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14. ABSTRACT

MRL/MpJ (super healers) mice have a unique ability to repair wounds and are protected from cartilage degradation subsequent to joint trauma. The hypothesis is that in response to injury, MRL/MpJ mice synthesize proteins that (1) protect the joint from cartilage degradation and/or (2) promote cartilage regeneration. The PIs propose to generate an atlas of the injury-activated proteome in mouse models with varying susceptibility to posttraumatic osteoarthritis (PTOA): (1) C57BL/6; (2) C57BL/6 treated with streptozotocin (STZ), a model of type 1 diabetes; (3) MRL/MpJ (super healers); and (4) STR/ort (spontaneous OA). By conducting comparative proteomics of injured and uninjured joints, the PIs will identify novel protein candidates for further exploration as potential therapies for treating injured joints. The project's specific aims are (1) application of in vivo metabolic labeling to quantify and characterize de novo protein synthesis, cellular proliferation, and mineral apposition in injured joints of mice with varying susceptibility to PTOA and (2) identification of newly synthesized RNA and proteins in the articular cartilage and immune cells of injured knees using a liquid sample interface for the AMS instrument in combination with liquid chromatography-mass spectrometry (LC-MS).

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TABLE OF CONTENTS

INTRODUCTION:

MRL/MpJ (super healers) mice have a unique ability to repair wounds and are protected from cartilage degradation subsequent to joint trauma. The hypothesis is that in response to injury, MRL/MpJ mice synthesize proteins that (1) protect the joint from cartilage degradation and/or (2) promote cartilage regeneration. The PIs propose to generate an atlas of the injury-activated proteome in mouse models with varying susceptibility to posttraumatic osteoarthritis (PTOA): (1) C57BL/6; (2) C57BL/6 treated with streptozotocin (STZ), a model of type 1 diabetes; (3) MRL/MpJ (super healers); and (4) Tlr4 KO (susceptible to PTOA). By conducting comparative proteomics of injured and uninjured joints, the PIs will identify novel protein candidates for further exploration as potential therapies for treating injured joints. The project's specific aims are (1) application of in vivo metabolic labeling to quantify and characterize de novo protein synthesis, cellular proliferation, and mineral apposition in injured joints of mice with varying susceptibility to PTOA and (2) identification of newly synthesized RNA and proteins in the articular cartilage and immune cells of injured knees using a liquid sample interface for the AMS instrument in combination with liquid chromatography-mass spectrometry (LC-MS).

KEYWORDS:

MRL/MpJ; STR/ort; osteoarthritis, post-traumatic osteoarthritis, diabetes, streptozotocin, MetRS, superhealer, chondrocytes, knee joint, anterior cruciate ligament, ACL, de novo protein synthesis, PTOA

ACCOMPLISHMENTS

For the 2nd year of this grant, our main focus has been on conducting tasks associated with Aim 1/Major Task 1 (Sub Aim 1A) of the proposal, following the original tasks and timeline we are highlighting in 'green' subtasks that have been competed, in 'yellow' subtasks that have started and are in progress, and in 'blue' subtasks that have not yet started but will initiate in the next funding period. Also in **'red'** text are some changes to the SOW that have been implemented to overcome some challenges or improve upon the original plan.

Specific Aim 1.	Timeline (months)	Status	Site 1 (LLNL)	Site 2 (UCD)
Major Task 1. (Sub Aim 1A): Quantify *de novo* protein synthesis in injured joints	**1-~~24~~(48)**			
Subtask 1.1. Obtain IACUC/ACURO approval; breed MRL/MpJ, C3H, **Tlr4 KO, Trem2 KO**, and C57BL/6 cohorts.	1-12	completed	Breed animals	
Subtask 1.2. Induce traumatic OA in 10 week old MRL/MpJ, C3H/HeJ, **Tlr4 KO, Trem2 KO** mice. Mice with receive AHA/^{14}C-threonine; 336 mice will be used (2 genotypes x 24 mice per group x 7 time points).	3-~~15~~(48)	In progress	Prepare cohorts of animals, transport to UCD for injury	336 animals will be injured, return animals to LLNL post injury
Subtask 1.3. Sample collection from 336 animals from Subtask 1.2	3-~~15~~(48)	In progress	Dissect joints, extract proteins	microCT (168 scans)
Subtask 1.4. Induce type 1 diabetes in 6 week old C57BL/6 mice for 4 weeks.	7-9	completed	Administer STZ	
Subtask 1.5. Induce traumatic OA in 10 week old C57BL/6 and STZ mice. Mice with receive AHA/^{14}C-threonine; 336 mice will be used (2 treatments x 24 mice per group x 7 time points).	10-18	completed	Prepare cohorts of animals, transport to UCD for injury	336 animals will be injured, return animals to LLNL post injury
Subtask 1.6. Sample collection from 336 animals from Subtask 1.5	10-18	completed	Dissect joints, extract proteins	microCT (168 scans)

Subtask 1.7. AMS analysis to quantify ^{14}C-threonine levels in injured and uninjured animals	10-~~24~~(48)	In progress	^{14}C measurements by AMS	
Subtask 1.8. BONCAT analysis	10-~~24~~(48)	In progress	Click-chemistry; quantification	
Subtask 1.9. FUNCAT/Histological analysis	10-~~24~~(48)	In progress	Embed, section, visualize proteins	Embed, section, visualize proteins
Milestone 1: IACUC/ACURO Approvals				
Milestone 2: Complete Sample Collection for STR and MRL strain				
Milestone 3: Complete Sample Collection for diabetic mice				
Milestone 4: Complete Proteomic Analysis for 1, 3, 5 and 7 days post injury				
Milestone 5: Complete Proteomic Analysis for 14, 21 and 42 days post injury				
Milestone 6.1: Manuscript #1 describing injury-induced phenotypic and molecular changed in T1D mice Rios-Arce ND, Murugesh DK, Hum NR, Sebastian A, Jbeily EH, Christiansen BA, **Loots GG**. Preexisting Type 1 Diabetes Mellitus Blunts the Development of Posttraumatic Osteoarthritis. ***JBMR Plus***. 2022 May;6(5):e10625. doi: 10.1002/jbm4.10625. eCollection 2022 May. PubMed PMID: 35509635; PubMed Central PMCID: PMC9059474.				
Milestone 6.2: Manuscript #2 review article Rios-Arce ND, Hum NR, **Loots GG**. Interactions Between Diabetes Mellitus and Osteoarthritis: From Animal Studies to Clinical Data. ***JBMR Plus***. 2022 May;6(5):e10626. doi: 10.1002/jbm4.10626. eCollection 2022 May. Review. PubMed PMID: 35509632; PubMed Central PMCID: PMC9059469.				

What was accomplished under these goals?

Subtask 1.1. Amend IACUC/ACURO approval to include Tlr4 KO and Trem2 KO mice. Initiate breedings.

Completed.

Subtask 1.2. Induce traumatic OA in 10 week old MRL/MpJ, C3H/HeJ, Tlr4 KO, Trem2 KO mice.

We have completed the characterization of the CH3 strain and compared it to C57BL/6, manuscript is in preparation.

Subtask 1.3. Sample collection from 336 animals from Subtask 1.2

Injuries are continuing to collect samples, stockpile them. Full sets with necessary controls and the required biological replicates to ensure rigor and statistical significance are being stockpiled. We require 5 animals per timepoint, per genotype, per injury, per treatment (PBS vs AHA), therefore for 1 experiment, per strain we need 20 animals per gender per timepoint. Proteomic analysis has initiated and we anticipate to completed the analysis this upcoming year. Proteomic analysis data has been generated for 1, 3, and 7 days post injury, we will complete all time points in the coming year and anticipate to prepare 1 manuscript describing these results.

Subtask 1.4. Induce type 1 diabetes (T1D) 6 week old C57BL/6 mice for 4 weeks.

Completed in year 1.

Subtask 1.5/1.6. Induce traumatic OA in 10 week old C57BL/6 and STZ mice.

Completed in year 1. Manuscript (#1) and review article (#2) were published this year.

Subtask 1.7. AMS analysis to quantify 14C-threonine levels in injured and uninjured animals

This work has initiated, samples are still being collected and quantified, analysis will be concluded in the 4th year.

Subtask 1.8. BONCAT analysis

Ongoing

Subtask 1.9. FUNCAT/Histological analysis

Histological analyses have been completed for T1DM, C57BL6, Tlr4 KO and MRL

Major Tasks 2 work will initiate in the next fiscal year. STR/Ort strain will be replaced with C3H/HeJ.

Major Task 2. (Sub Aim 1B) Quantify cell proliferation in injured joints	**13-~~27~~(48)**			
Subtask 2.1. Breed necessary cohorts of MRL/MpJ, C3H/HeJ, Tlr4 KO, Trem2 KO, and C57BL/6.	13-18 **24-48**	In progress	Breed animals	
Subtask 2.2 Induce traumatic OA in 10 week old MRL/MpJ, C3H/HeJ, STZ and C57BL/6 mice. Mice with receive ^{14}C-thymidine; 336 mice used (4 genotypes x 12 mice per group x 7 time points).	16-24 **24-48**	In progress	Prepare cohorts of animals, transport to UCD for injury	336 animals will be injured, return animals to LLNL post injury
Subtask 2.3. Sample collection from 336 animals from Subtask 2.2	16-24 **24-48**	In progress	Dissect joints, extract DNA	
Subtask 2.4. AMS analysis to quantify ^{14}C-thymidine levels	19-27 **24-48**	In progress	^{14}C measurements by AMS	
Milestone 7: Complete Sample Collection for MRL, STR, STZ and B6 mouse strain				
Milestone 8: Complete AMS analysis				
Major Task 3. (Sub Aim 1C): Quantify mineral apposition in injured joints:	**19-33**			
Subtask 3.1. Breed necessary cohorts of MRL/MpJ, C3H/HeJ, Tlr4 KO, Trem2 KO, and C57BL/6 cohorts.	19-24	completed	Breed animals	
Subtask 3.2 Induce traumatic OA in 10 week old MRL/MpJ, STR/ort mice, Tlr4 KO, Trem2 KO, STZ and C57BL/6 mice. Mice with receive ^{45}Calcium. 192 mice will be used (4 genotypes x 12 mice per group x 4 time points).	22-30	completed	Prepare cohorts of animals, transport to UCD for injury	192 animals will be injured, return animals to LLNL post injury
Subtask 3.3. Sample collection from 192 animals from Subtask 3.2.	22-30	completed	Dissect joints, extract DNA	microCT (192 scans)
Subtask 3.4. LC analysis to quantify ^{45}Calcium- levels in injured and uninjured animals	25-33	completed	Measure ^{45}Ca by liquid scintillation	
Milestone 9: Complete Sample Collection for MRL, STR, STZ, Tlr4 KO and BL6 mouse strain				
Milestone 10: Complete microCT				
Milestone 11: Manuscript #3 describing injury induced cellular proliferation and osteophyte formation in Tlr4 KO is in preparation.				

What was accomplished under these goals?

Subtask 2.1. Breed necessary cohorts of mice. This fiscal year we have added 2 additional strains of mice, Tlr4 KO, Trem2 KO, based on results we generated from single cell sequencing data generate in year 2. These mice were purchased from Jackson Labs, were bred, injured and data has been collected on PTOA phenotypes.

Ongoing

Subtask 2.2/2.3/2.4

Ongoing

Subtask 3.2/2.3/2.4. In this subtask mice were bred, injured and bones and joints were examined by microCT for structural changes. We found that ^{45}Ca data was not an improvement above microCT, therefore we proceeded to complete the analysis by microCT only.

Completed

Specific Aim 2

Specific Aim 2.	Timeline	Status	Site 1 (LLNL)	Site 2 (UCD)
Major Task 4. (Sub Aim 2A): Characterize the injury-induced transcriptome and proteome in the articular cartilage.	**10-48**			
Subtask 4.1. Breed necessary cohorts of ***Ai9; Col2-ER-Cre; MetRS*** animals.	10-48	in progress	Genotype and breed animals	
Subtask 4.2 Induce traumatic OA in 10 week old ***Ai9; Col2-ER-Cre; MetRS*** mice. Mice with receive ^{14}C-threoninine and ANL. 432 mice will be used (2 genotypes x 24 mice per group x 9 time points).	16-24	In progress	Prepare cohorts of animals, transport to UCD for injury	432 animals will be injured, return animals to LLNL post injury
Subtask 4.2A. Conduct scRNA-seq on articular chondrocytes of uninjured and injured C57Bl/6.	12-24	Completed	Prepare cohorts of animals, transport to UCD for injury, isolate single cell, conduct RNA-seq and computational analyses	Injure animals, return animals to LLNL post injury
Subtask 4.2C. Conduct scRNA-seq on articular chondrocytes of uninjured and injured MRL.	24-36	Completed	Prepare cohorts of animals, transport to UCD for injury, isolate single cell, conduct RNA-seq and computational	Injure animals, return animals to LLNL post injury

			analyses	
Subtask 4.2B. Conduct scRNA-seq on immune cells of uninjured and injured C57Bl/6.	12-24	Completed	Prepare cohorts of animals, transport to UCD for injury, isolate single cell, conduct RNA-seq and computational analyses	Injure animals, return animals to LLNL post injury
Subtask 4.2D. Conduct scRNA-seq on immune cells of uninjured and injured MRL.	24-36	Completed	Prepare cohorts of animals, transport to UCD for injury, isolate single cell, conduct RNA-seq and computational analyses	Injure animals, return animals to LLNL post injury
Subtask 4.3. Sample collection from 432 animals from Subtask 4.2.	16-36	Completed	extract proteins, isolate RNA	
Subtask 4.4. AMS analysis to quantify ^{14}C-threonine levels in injured and uninjured animals	36-48	In progress	^{14}C measurements by AMS	
Subtask 4.5. BONCAT analysis	36-48	In progress	Click-chemistry; quantification	
Subtask 4.6. FUNCAT/Histological analysis	36-48	In progress	Embed, section, visualize proteins	Embed, section, visualize proteins
Subtask 4.7. LC-MS/MS analysis	36-48	In progress	Protein identification	
Milestone 12: Complete Sample Collection for *Col2-ER-Cre; MetRS- no longer utilize these mice*				
Milestone 13: Complete Chondrocyte-Specific RNA-seq analysis for C57Bl/6 (scRNA-seq now) **Manuscript #4:** Sebastian A, McCool JL, Hum NR, Murugesh DK, Wilson SP, Christiansen BA and Loots GG. Single-Cell RNA-Seq Reveals Transcriptomic Heterogeneity and Post-Traumatic Osteoarthritis-Associated Early Molecular Changes in Mouse Articular Chondrocytes. ***Cells*** 2021 June 10(6):1462. DOI: 10.3390/cells10061462. **Manuscript #5 (Book chapter accepted):** McCool JL, Hum NR, Sebastian A, Loots GG. Isolation of Murine Articular Chondrocytes for Single Cell RNA or Bulk RNA Sequencing Analysis. *Methods in Molecular Biology* book entitled "Cartilage Tissue Engineering" Editors Prof. Martin Stoddart, Dr. Angela Armiento, Dr. Elena Della Bella. Springer. To be published in 2022.				
Milestone 14: Complete Chondrocyte-Specific Proteomic Analysis				
Milestone 15: Manuscript #6 describing injury-mediated chondrocyte specific protein and gene expression				
Major Task 5. (Sub Aim 2B): Characterize the injury-induced transcriptome and proteome in the immune system.	**13-48**			
Subtask 5.1. Breed necessary cohorts of ***Ai9; Csf1r-Cre; MetRS***	13-25	In progress	Genotype and breed animals	

animals. This task has been modified and original strains will be used here.				
Subtask 5.2 Induce traumatic OA in 10 week old ***Ai9; Csf1r-Cre; MetRS*** mice. Mice will receive 4TU, ^{14}C-threoninine and ANL. 432 mice (2 genotypes x 24 mice per group x 9 time points). This aim has been modified—UPRT/4TU will not be used and the RNA-seq task will be replaced with single cell sequencing	19-27	In progress	Prepare cohorts of animals, transport to UCD for injury	432 animals will be injured, return animals to LLNL post injury
Subtask 5.3. Sample collection from 432 animals from Subtask 4.2. ScRNA-seq has been conducted on uninjured joints	19-27	In progress	Extract proteins, isolate RNA	
Subtask 5.4. Breed MRL/MpJ animals.	19-25	In progress	Breeding	
Subtask 5.5. Injure MRL/MpJ animals, administer AHA+^{14}C-threonine. 104 mice (12 mice x 9 time points)	19-25	In Progress	Transport cohorts to UCD for injury	104 animals will be injured, return to LLNL
Subtask 5.6. Sort macrophages and T-cells from joints, isolate proteins	21-48	In progress	FACs, protein extraction	
Subtask 5.7. AMS analysis to quantify ^{14}C-threonine levels in injured and uninjured animals	36-48	In progress	^{14}C measurements by AMS	
Subtask 5.8. BONCAT analysis	36-48	In progress	Click-chemistry; quantification	
Subtask 5.9. FUNCAT/Histological analysis	36-48	In progress	Embed, section, visualize proteins	Embed, section, visualize proteins
Subtask 5.10. LC-MS/MS analysis	36-48	In progress	Protein identification	
Subtask 5.11. IHC validation (based on scRNA-seq and protein analysis)	31-48	In progress	Visualization of C57Bl/6 and MRL/MpJ protein expression	
Milestone 16: Complete Sample Collection for *Ai9; Csf1r-Cre; MetRS—no longer utilize these mice*				
Milestone 17: Complete Immune-Specific RNA-seq analysis (scRNA-seq now)				
Milestone 18: Complete Immune-Specific Proteomic Analysis				
Milestone 19.1: Manuscript #7 Complete Immune-Specific RNA-seq analysis for C57Bl/6 (scRNA-seq now)-describe all immune cells, in a time course, post injury Sebastian A, Hum NR, McCool JL, Wilson SP, Murugesh DK, Martin KA, Rios-Arce ND, Amiri B, Christiansen BA, **Loots GG.** Single-cell RNA-Seq reveals changes in immune landscape in post-traumatic osteoarthritis. ***Front Immunol***. 2022 Jul 29;13:938075. doi: 10.3389/fimmu.2022.938075. eCollection 2022. PMID: 35967299				
Milestone 19.2: Manuscript #8 Complete Immune-Specific RNA-seq analysis for C57Bl/6 (scRNA-seq				

now)-focus on detailed characterization of neutrophils, manuscript is currently in preparation
Milestone 19.3: Manuscript #9 describing injury-mediated immune specific protein and gene expression, highlighting MRL/MpJ specific proteins that may contribute to PTOA resistance is in preparation

What was accomplished under these goals?

Subtask 4.1/2. Breed necessary cohorts of ***Ai9; Col2-ER-Cre; MetRS*** animals. Because the genetic model is restrictive to C57Bl/6 strain only, and is not applicable to other strains, and because the most meaningful comparisons are between susceptible and resistant strains, we have decided to pivot this subtask and take a new approach, where FACS is utilized to sort out chondrocytes from different strains based on cell surface markers identified by scRNA-sequencing. Here we will continue generating or purchasing all mouse strains listed in subtask 1.1.

In progress.

Subtask 4.2A-D. Conduct scRNA-seq on articular chondrocytes of uninjured and injured C57Bl/6 and MRL.

Completed, this work resulted in 1 published manuscript (#4) and 1 published methodology book chapter (#5)

Subtask 4.3/7. For the reason stated above in Subtask 4.1, we will no longer be using these mice, but use the parent strains. Animals are injured as planned, joints are digested, chondrocytes are purified, proteins are isolated, identified and quantified by LC-MS/MS. Samples are being stockpiled and will be examined in the upcoming year.

In progress.

Subtask 5.1/2. Breed necessary cohorts of ***Ai9; Csf1r-Cre; MetRS*** animals. Because the genetic model is restrictive to C57Bl/6 strain only, and is not applicable to other strains, and because the most meaningful comparisons are between susceptible and resistant strains, we have decided to pivot this subtask and take a new approach, where FACS is utilized to sort out individual immune subpopulations from different strains based on cell surface markers identified by scRNA-sequencing. Here we will continue generating or purchasing all mouse strains listed in subtask 1.1. In particular this past year we have focused on two types of immune cells, macrophages and neutrophils.

In progress.

Subtask 5.3. Conduct scRNA-seq on immune cells of uninjured and injured C57Bl/6 and MRL.

Completed, this work resulted in one published manuscript (#7) and one manuscript that is pending review (#8)

Subtask 5.4-5.11. Samples are being stockpiled and will be examined in the upcoming year. Special focus will be on macrophages and neutrophils, since those are the subpopulations that seem to differ the most, by RNA-seq between C57Bl/6 and MRL/MpJ.

In progress.

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

At UC Davis, one postdoctoral scholar (Hailey Cunningham) and one Ph.D. student (Sophie Orr) are being trained on research related to this project. Dr. Cunningham is continuing to develop her technical and professional skills while she mentors and instructs Sophie Orr (who is starting her third year as a Ph.D. student) in research methodology related to this project, including non-invasive knee injury, survival surgery, and micro-computed tomography. Both trainees regularly attended weekly research seminars in the UC Davis Orthopaedic

Research Laboratory and the Department of Biomedical Engineering. Both trainees will also continue to attend the two premier bone and cartilage national meetings, ORS and ASBMR, every year during their training. Dr. Cunningham's professional goal is to transition to a position in science policy or advocacy, and she is currently pursuing opportunities in that area. Sophie Orr's training goals are: 1.) Didactic training in biomedical engineering, orthopaedic research, and molecular and cell biology; 2.) Hands-on instruction in analysis methodology, statistical analysis, and experimental design; 3.) Professional development that leads to a career in an area related to space biology.

How were the results disseminated to communities of interest?

Abstracts Submitted during this funding period:

McCool, JL, Sebastian, A, Hum, NR, Murugesh, DK, Wilson, SP, Amiri, B, Christiansen, BA, Loots, GG (2021). ***Characterizing Immune Cell Infiltration in the Murine Joint Microenvironment after Traumatic Knee Injury***. Oral Poster Presentation. American Society for Bone and Mineral Research, Sept 11-15th 2022 – (presenter: Jillian McCool)

- ASBMR 2022 Annual Meeting Young Investigator Award Recipient: $1,000 honorarium
- University of California Merced GSA Travel Award

Mendez, M, Wilson, SP, Murugesh, DK, Hum, NR, Jbeily, EH, Sebastian, A, Christiansen, BA, Loots, GG (2021). ***Tlr4 Deficiency Accelerates Post-Traumatic Osteoarthritis Development in Mice.*** Poster Presentation. American Society for Bone and Mineral Research, Sept 11-15th 2022 – (Presenter Melanie Mendez)

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

Main focus for the next period will be to complete **Subtask 4 and 5**.

IMPACT:

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

Our Sebastian et al. manuscript has 13 citations,

Sebastian A, McCool JL, Hum NR, Murugesh DK, Wilson SP, Christiansen BA and Loots GG. Single-Cell RNA-Seq Reveals Transcriptomic Heterogeneity and Post-Traumatic Osteoarthritis-Associated Early Molecular Changes in Mouse Articular Chondrocytes. ***Cells*** 2021 June 10(6):1462. DOI: 10.3390/cells10061462.

And our Rios-Arce et al manuscript already has 3 citations.

What was the impact on other disciplines?

We anticipate that the single cell RNA-seq chondrocyte methods book chapter we will publish in *Methods in Molecular Biology* book entitled "Cartilage Tissue Engineering' will become a standard protocol for purifying chondrocytes from articular cartilage in many laboratories that plan to employ single cell RNA-seq for molecular profiling. We are already getting many inquiries about sharing our data and our protocols for cell isolation.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report

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

Breeding *Ai9; Csf1r-Cre; MetRS* and *Ai9; Col2-ER-Cre; MetRS* to get sufficient cohorts has been very time consuming, and the yield of proteins from the ANL labeling has been very low, making it difficult to obtain accurate proteomics readings. We have decided to amend this approach by using non-transgenic, original strain mice, and employ FACS purification of specific cell populations and then purify and analyze proteins by LC-MS/MS from these samples. This approach has proven successful, and we will utilize this methodology in the next year to complete tasks 4 and 5.

Changes that had a significant impact on expenditures

None

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

None

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals.

None

Significant changes in use of biohazards and/or select agents

None

PRODUCTS:

Publications, conference papers, and presentations

Journal publications from this work:

Published Manuscripts acknowledging federal support by Grants PR180268/PR180268P1

Rios-Arce ND, Murugesh DK, Hum NR, Sebastian A, Jbeily EH, Christiansen BA, Loots GG. Preexisting Type 1 Diabetes Mellitus Blunts the Development of Posttraumatic Osteoarthritis. JBMR Plus. 2022 May;6(5):e10625. doi: 10.1002/jbm4.10625. eCollection 2022 May. PubMed PMID: 35509635; PubMed Central PMCID: PMC9059474.

Rios-Arce ND, Hum NR, Loots GG. Interactions Between Diabetes Mellitus and Osteoarthritis: From Animal Studies to Clinical Data. JBMR Plus. 2022 May;6(5):e10626. doi: 10.1002/jbm4.10626. eCollection 2022 May. Review. PubMed PMID: 35509632; PubMed Central PMCID: PMC9059469.

Sebastian A, Hum NR, McCool JL, Wilson SP, Murugesh DK, Martin KA, Rios-Arce ND, Amiri B, Christiansen BA, Loots GG. Single-cell RNA-Seq reveals changes in immune landscape in post-traumatic osteoarthritis. Front Immunol. 2022 Jul 29;13:938075. doi: 10.3389/fimmu.2022.938075. eCollection 2022. PMID: 35967299

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

McCool JL, Hum NR, Sebastian A, Loots GG. Isolation of Murine Articular Chondrocytes for Single Cell RNA or Bulk RNA Sequencing Analysis. *Methods in Molecular Biology* book entitled "Cartilage Tissue Engineering" Editors Prof. Martin Stoddart, Dr. Angela Armiento, Dr. Elena Della Bella. Springer. To be published in 2022.

Other publications, conference papers, and presentations

McCool, JL, Sebastian, A, Hum, NR, Murugesh, DK, Wilson, SP, Amiri, B, Christiansen, BA, Loots, GG (2021). ***Characterizing Immune Cell Infiltration in the Murine Joint Microenvironment after Traumatic Knee Injury***. Oral Poster Presentation. American Society for Bone and Mineral Research, Sept 11-15th 2022 – (presenter: Jillian McCool)

- ASBMR 2022 Annual Meeting Young Investigator Award Recipient: $1,000 honorarium
- University of California Merced GSA Travel Award

Mendez, M, Wilson, SP, Murugesh, DK, Hum, NR, Jbeily, EH, Sebastian, A, Christiansen, BA, Loots, GG (2021). ***Tlr4 Deficiency Accelerates Post-Traumatic Osteoarthritis Development in Mice.*** Poster Presentation. American Society for Bone and Mineral Research, Sept 11-15th 2022 – (Presenter Melanie Mendez)

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

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Blaine A. Christiansen*
Project Role:	*Partnering PI*
Researcher Identifier (e.g. ORCID ID):	*ORCID ID: 0000-0002-0105-6458*
Nearest person month worked:	1.8
Contribution to Project:	*Dr. Christiansen performed non-invasive knee injuries in mice, and has performed micro-computed tomography imaging and analysis, and advised Dr. Loots's lab personnel on microCT methods*
Funding Support:	*In addition to this award, Dr. Christiansen receives funding support from the NIH – National Institute for Arthritis and Musculoskeletal and Skin Diseases, under award numbers R01 AR071459, R01 AR073772, and R01 AR075013*

Name:	*Hailey C. Cunningham*
Project Role:	*Postdoctoral Scholar*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*9.0*
Contribution to Project:	*Dr. Cunningham performed non-invasive knee injuries in mice, micro-computed tomography imaging and analysis, and statistical analysis. She also serves as the primary research mentor for Sophie Orr, a 3rd year Ph.D. student working in our lab.*
Funding Support:	*In addition to this award, Dr. Cunningham received financial support from the NIH – National Institute for Arthritis and Musculoskeletal and Skin Diseases, under award number R01 AR071459*

Name:	*Sophie Orr*
Project Role:	*Graduate Student Researcher*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2.5*
Contribution to Project:	*Under the guidance of Dr. Cunningham, Sophie performed non-invasive knee injuries in mice and micro-computed tomography imaging and analysis of mouse knee samples.*
Funding Support:	*In addition to this award, Sophie received financial support from the NIH – National Institute for Arthritis and Musculoskeletal and Skin Diseases, under award number T32 AR079099*

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?

Nothing to Report

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

The PI, Dr. Gaby Loots, will be submitting her report in parallel.

QUAD CHARTS:

N/A

APPENDICES:

N/A