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TITLE: Optimizing Muscle Function in Composite Tissue Injuries with Segmental Bone Defects

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CONTRACTING ORGANIZATION: Indiana University, Bloomington, IN

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Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Background: The goal of this proposal is to determine how treatment of a segmental bone defect (SBD) affects function and composition of adjacent muscle in a porcine composite tissue injury (CTI) model. To date, the majority of CTI investigations have focused on the effects of muscle injury on bone healing. In our current ongoing work and have determined that a SBD leads to loss of myogenic proteins, increases in fibrosis, and loss of strength in adjacent uninjured muscle. This opens investigative opportunities to improve muscle function in limbs with CTI. We propose to investigate how two strategies, currently used clinically for SBD management, affect function and composition of the adjacent muscle. Our project is aligned with the PRORP Focus Area: Composite Tissue Regeneration specifically investigating techniques aimed at improving outcomes following high-energy extremity trauma, with a focus on improving neuromuscular recovery following CTI.

We have developed an in vivo CTI model in Yucatan minipigs that includes a tibial diaphyseal SBD and an adjacent volumetric muscle defect (VML). Our model offers a translational vehicle uniquely designed to quantify biologic tissue interactions between muscle and bone. In our most recent work, pigs with SBDs, with or without an adjacent VML injury, lost 50% of muscle torque and had nearly identical adverse changes in muscle protein and fibrous composition three months after injury in muscle adjacent to the SBD. Collectively our data indicate that an untreated SBD is a primary source of muscle deterioration in a CTI injury. In current clinical practice SBDs are typically treated acutely with either 1) staged therapy predicated by sterilizing the SBD with antibiotic-impregnated polymethylmethacrylate (PMMA) followed by delayed bone grafting or 2) with therapies designed to acutely reconstitute bone healing, typically with autogenous bone grafting or bone-morphogenetic protein. It is unknown how acute SBD management strategies affect the function and composition of adjacent muscle.

Objectives and Hypothesis: The Objective of this proposal is to quantify how acute SBD management strategies affect the function and composition of the adjacent muscle in a porcine CTI model. We hypothesize that acute mitigation of a SBD will improve function and composition of adjacent skeletal muscle. We propose to study how a) staged management strategies that involve filling the SBD with an antibiotic loaded polymethylmethacrylate (PMMA) bone cement spacer or b) strategies focused on early accelerated SBD bone healing with bone morphogenetic protein-2 (BMP-2) mitigate collateral injury and dysfunction in adjacent muscle. This hypothesis will be tested in two specific aims.
Specific Aim 1: Quantify changes in muscle function and composition in muscle adjacent to a SBD in pigs treated with an antibiotic loaded PMMA bone cement spacer compared to pigs with an untreated SBD.
Specific Aim 2: Quantify changes in muscle function and composition in muscle adjacent to a SBD in pigs treated with BMP-2 compared to pigs with an untreated SBD or antibiotic loaded PMMA bone cement spacer.

Study Design: We will use our established porcine CTI model. In this study, three groups of pigs will be subjected to a SBD by resecting 25.0 mm of the mid-tibial diaphysis. The SBD is stabilized by dual plating. The first group of pigs (Control) will have no treatment of the SBD. In the second group (PMMA), the SBD will be filled acutely at the time of injury with a PMMA spacer impregnated with Vancomycin and Tobramycin. In the third group (BMP), the SBD will be immediately treated with BMP-2. The primary outcome will be muscle function at three months after injury as measured by maximum isometric torque. Muscle function will be quantified with a custom-fabricated in vivo muscle torque transducer that measures ankle dorsiflexion strength. Pre- and post- operative measurements, made monthly after injury until sacrifice, will be measured both in the surgical leg and in the contralateral uninjured leg. In addition, we will quantify changes in muscle composition through biochemical and histologic analyses. Finally, SBD healing will be quantified by x-ray scoring and computed tomography scanning to interrogate relationships between bone healing and muscle function.

IACUC and ACURO approval have been obtained

15. SUBJECT TERMS

None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	11	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

In this study we will determine how two current clinical methods used at the time of injury to treat a SBD affect adjacent muscle function and composition. In our previous work, we showed that a SBD caused the adjacent muscle to fill with scar tissue that led to loss of 50% of its strength three months after injury. The loss of strength was no different in pigs that had an isolated SBD compared to pigs that had an identical SBD and a direct injury to the muscle. Based on this, we concluded that the SBD was the most important cause of injury and weakness in the adjacent muscle, even more so than the direct muscle injury. Therefore, we designed this study to determine the best way to treat the SBD to minimize collateral muscle injury. We will use our pig model that includes removing a 25 mm (roughly one inch) segment of bone from the middle of the tibia (the shin bone) that is fixed with standardized orthopaedic surgical methods using plates and screws. This model leads to significant injury in the surrounding muscle. In this study, we will create the same SBD. In the first group of pigs, the SBD will be filled with what is done currently in the majority of injuries in humans. The SBD will be filled with a solid spacer made from standard bone cement (the same bone cement we use to secure joint replacements) filled with antibiotics. In a second group of pigs, the SBD will be filled with a bone-morphogenetic protein (BMP-2) which is a protein that speeds up bone healing. Typically, in clinical cases, therapies that accelerate bone healing are withheld until all of the underlying skin and muscle are healed, but it is possible that this may not be the best way to prevent muscle injury. In our initial studies, BMP-2 rapidly accelerated bone healing in the SBD. So here we will test if immediate treatment to accelerate bone healing is better for the adjacent muscle. In the third group of pigs, the SBD will be untreated to replicate the conditions from our previous study. Strength will be measured monthly for three months using a custom pig testing apparatus that measures the peak amount of torque the muscle adjacent to the SBD can produce. The pigs will be euthanized at three months, and we will remove the muscle adjacent to the SBD and do testing to determine concentrations of healthy muscle proteins, proteins that show that the nerves in the muscle are working well, and scar tissue which is detrimental to muscle function.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Bone healing, Segmental bone defect, Volumetric Muscle loss, Muscle healing, Porcine, Pig, BMP-2

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Specific Aim 1: Quantify changes in muscle function and muscle composition in muscle adjacent to a segmental bone defect (SBD) in pigs treated with antibiotic loaded PMMA bone cement spacer compared to pigs with an untreated SBD.	**Timeline**	**Status**
Major Task 1: Administrative and Regulation of Porcine Surgery	Months	
Subtask 1- Obtain Institutional Animal Care and Use Committee (IACUC) approval	0-3	100%

Subtask 2- Establish Data Sharing Agreements and Material Transfer Agreements with USUHS	0-3	33%
Subtask 3- Obtain Animal Care and Use Review Office (ACURO) approval from USAMRDC	3-6	100%
Subtask 4- Secure animal ordering and shipping	3-6	50%
Milestones Achieved: Animals delivered and IACUC and ACURO approval obtained	**6 months**	
Major Task 2: Porcine surgery; Postoperative Strength Testing; Longitudinal x-rays; Euthanasia and Tissue Harvest		
Subtask 1- Perform surgery on 14 pigs (Control and PMMA groups)	6-18	0%
Subtask 2- *In vivo* muscle strength testing and longitudinal x-rays	6-21	0%
Subtask 3- Euthanasia and Tissue Harvest	9-21	0%
Milestones Achieved: Strength testing complete; Longitudinal x-rays complete; Muscle specimens and tibias harvested	**21 months**	
Major Task 3: Muscle Tissue Testing, Computed tomography (CT) scanning, X-ray scoring, and Data Analyses		
Subtask 1- Tissue biochemical testing	24-30	0%
Subtask 2- Tissue Histologic testing	24-30	0%
Subtask 3- CT scanning of tibias for bone defect assessment, Torsional biomechanical testing	24-30	0%
Subtask 4- mRUST scoring of longitudinal x-rays	24-30	0%
Subtask 5- Strength, radiographic (x-ray and CT), and tissue testing analyses	30-36	0%
Milestones Achieved: All strength and tissue testing and radiographic analyses complete	**36 months**	

Specific Aim 2: Quantify changes in muscle function and muscle composition in muscle adjacent to a SBD in pigs treated with bone morphogenetic protein-2 (BMP-2) compared to pigs with an untreated SBD.	**Timeline**	**Status**
Major Task 1: Porcine surgery; Postoperative Strength Testing; Longitudinal x-rays; Euthanasia and Tissue Harvest		
Subtask 1- Perform surgery on 7 pigs (BMP-2 group)	6-18	0%
Subtask 2- *In vivo* muscle strength testing and longitudinal x-rays	6-21	0%
Subtask 3- Euthanasia and Tissue Harvest	9-21	0%
Milestones Achieved: Strength testing complete; Longitudinal x-rays complete; Muscle specimens and tibias harvested	**21 months**	
Major Task 2: Muscle Tissue Testing, CT scanning, X-ray scoring, and Data Analyses		
Subtask 1- Tissue biochemical testing	24-30	0%
Subtask 2- Tissue Histologic testing	24-30	0%
Subtask 3- CT scanning of tibias for bone defect assessment, Torsional biomechanical testing	24-30	0%
Subtask 4- mRUST scoring of longitudinal x-rays	24-30	0%

Subtask 5- Strength, radiographic (x-ray and CT), and tissue testing analyses	30-36	0%
Milestones Achieved: All strength and tissue testing and radiographic analyses complete	**36 months**	
Major Task 3: Dissemination of Results		
Subtask 1- Presentation at national and international meetings	24-36 months	0%
Subtask 2- Manuscript preparation and submission	30-36 months	0%
Subtask 3- Follow up grant preparation	24-36 months	0%
Milestones Achieved: Data in line for publication and secondary funding under review	**36 months**	

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided.

The first six months of the project major activities were Administrative and Regulation of Porcine Surgery. IACUC and ACURO approval have been obtained. Data sharing and Material transfer agreements are being formalized between Indiana University and USUHS; both have been submitted at USUHS and once approved will be sent to Indiana University. A PO for pigs has been initiated, and an animal list for ordering has been requested from our supplier, as we require specific age and weight male, castrated pigs. It is expected that within the month we have completed Major Task 1 for Specific Aim 1 and have an idea of when the porcine surgeries will commence (Subtask 1 of Major task 2).

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

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

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

Within the next reporting period, Major Task 1 will be complete. That just requires ordering appropriate animals and completing the logistics of Data sharing and Material transfer agreements. The next Major Tasks for both Specific Aims 1 and 2 are about the pig survival surgeries. Once we have pigs on site and acclimated, surgeries will commence. After surgeries the planned longitudinal x-rays and muscle strength testing will be performed, followed by euthanasia. We are well acquainted (several publications) on these techniques, so there are no foreseeable hurdles to completing these tasks.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

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

Nothing to Report

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:**

Nothing to Report

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We are in a NCE for a DoD funded pig project at present. We have just completed all the requisite surgeries for that project. Now that they are done, we can proceed forward with the pig surgeries for this project. There were some LARC facilities delays that have been resolved. If there are new LARC facilities delays, there is another large animal research facility nearby that we could pivot to.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

We did not start salary until the project started in Oct 2023. DoD start date was May 2023. So budget is 6 months delayed.

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

Not applicable

Significant changes in use or care of vertebrate animals

IACUC approval date 7/19/2023. ACURO approval 7/28/2023

Significant changes in use of biohazards and/or select agents

Not applicable

6. PRODUCTS

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications.

Nothing to Report

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

Nothing to Report

  Other publications, conference papers and presentations.

Nothing to Report

- **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?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	Roman Natoli, MD, PhD
Project Role:	PI
Researcher Identifier ERA Commons:	RNATOLI
Nearest person month worked:	1
Contribution to Project:	Dr. Natoli is overseeing the project as the PI and has obtained IACUC and ACURO approval. He will perform surgical procedures, data processing, and preparing reports.
Name:	Todd McKinley, MD
Project Role:	Co-Investigator

Researcher Identifier ERA Commons: TMCKINLEY
Nearest person month worked: 1
Contribution to Project: Dr. McKinley will assist Dr. Natoli in the aims of this project including pig surgeries, muscle testing, bone healing scoring, data interpretation, and preparation of abstracts/manuscripts.

Name: Melissa Kacena, PhD
Project Role: Co-Investigator
Researcher Identifier ERA Commons: MKACENA
Nearest person month worked: 1
Contribution to Project: Dr. Kacena will assist Dr. Natoli in the aims of this project including supervise students, data interpretation, and preparation of abstracts/manuscripts.

Name: Christopher Dearth, PhD
Project Role: Co-Investigator/Site PI
Researcher Identifier ERA Commons: DEARTHCL
Nearest person month worked: 1
Contribution to Project: Dr. Dearth is the site PI at USUHS. He will oversee personnel there and assist with data interpretation and preparation of abstracts/manuscripts.

Funding Support: USUHS-Walter Reed National Military Medical Center

Name: Stephen M. Goldman, PhD
Project Role: Co-Investigator
Researcher Identifier ERA Commons: STEPHENGOLDMAN
Nearest person month worked: 1
Contribution to Project: Dr. Goldman will assist Dr. Dearth. He will perform histologic and protein level assessments on post-mortem tissues. He will also assist with data interpretation and preparation of abstracts/manuscripts.

Funding Support: USUHS-Walter Reed National Military Medical Center

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

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Not applicable

QUAD CHART: The quad chart is included as a separate document.

9. APPENDICES:

Not applicable