

AWARD NUMBER: W81XWH-20-2-0010

TITLE: Optimization of an Acute Care Strategy to Stabilize Composite Tissue Injuries and Facilitate Improved Functional Outcomes for Accelerated Return to Duty

PRINCIPAL INVESTIGATOR: Todd McKinley MD

CONTRACTING ORGANIZATION: Indiana University

REPORT DATE: AUGUST 2023

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE AUGUST 2023		2. REPORT TYPE ANNUAL		3. DATES COVERED 1AUG2022 - 31JUL2023	
4. TITLE AND SUBTITLE Optimization of an Acute Care Strategy to Stabilize Composite Tissue Injuries and Facilitate Improved Functional Outcomes for Accelerated Return to Duty				5a. CONTRACT NUMBER W81XWH-20-2-0010	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Todd McKinley MD; Christopher Dearth				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
E-Mail: tmckinley@iuhealth.org				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Indiana University				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 In this study, we hypothesized that treating a volumetric muscle loss (VML) defect with a customized muscle void filler will improve bone healing and muscle strength recovery in pigs subjected to VML with or without an adjacent segmental bone defect (SBD). We are testing the customized void filler in minipigs with isolated VML defects and in pigs with composite tissue injuries (CTI) that include the VML with a SBD. Control animals will have either a VML or CTI injury but no void filler. Experimental animals will have either a VML or CTI injury and will have the muscle defect fill with void filler at the time of the injury. All pigs will have muscle autografting 28 days after the original injury. To date, 7 CTI and 3 VML controls have been enrolled. Six (3 CTI and 3 VML) specimens have proceeded to euthanasia. All animals have tolerated surgery. Muscle testing and bone healing are comparable on our first six specimens to our previous work. Additional pigs will be delivered in December. Biochemical and histological analyses are pending.					
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			19b. TELEPHONE NUMBER (include area code)
U	U	U	UU	8	USAMRDC

TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	5
2. Keywords	5
3. Accomplishments	5
4. Opportunities for Training and Professional Development	6
5. Dissemination of Results	6
6. Plans for Next Reporting Period	6
7. Impact; Changes/Problems; Products	7
8. Personnel	7
9. Appendices	NA

1. INTRODUCTION

In this project, we will be testing how muscle void fillers improve muscle function and bone healing in a porcine model of composite tissue injury (CTI). This work is collaborative between the swine surgeons at Indiana University (Drs. Natoli, Kacena, and McKinley) and the muscle researchers at Uniformed Services University (Drs. Goldman and Dearth). The initial specific aims, specifically developing the void filler are near completion at USUHS. We are now ready to move into porcine testing at Indiana University. We will use our porcine CTI model to test our hypotheses.

2. KEYWORDS:

Composite tissue injury, volumetric muscle loss, segmental bone defect, tibia fracture, muscle void filler, nonunion

3. ACCOMPLISHMENTS:

Major Task 1: Regulation: DONE

Subtask 1: Obtain IACUC approval: DONE

Subtask 2: ACURO Approval: DONE

Major Task 2: Surgical Procedure for VML with and without experimental treatment: ONGOING

Subtask 1: Pig acquisition and acclimation. To date, we have had 10 pigs delivered from Sinclair Bio Resources (Auxvasse, MO). All 10 pigs have had surgery. We have contacted Sinclair Bio Resources (Auxvasse, MO) and have finalized delivery of eighteen additional pigs to be delivered the week of December 11-15, 2023. All pigs at the time of delivery will be in our target age range.

Subtask 2: Surgical Procedure. As stated, we have done surgery on 10 pigs to date that includes the original operation in which pigs either are subjected to an isolated volumetric muscle loss operation (VML) or are subjected to a composite tissue injury (CTI) that includes the VML injury combined with a segmental bone defect (SBD). All ten pigs have successfully tolerated the second operation that occurred 28 days after the initial operation at which time they had a minced muscle autograft (MMA) obtained from the ipsilateral quadriceps muscle which was implanted into the region of the VML defect. To date, all 10 pigs have populated the control groups that have not received the VML void filler developed by Dr. Dearth and his laboratory at USU Walter Reed. In these control pigs, scar tissue that has formed in the 28-day interval between the injury and muscle grafting procedures was evacuated and the defect was filled with the MMA.

Six pigs have made it to the 4-month euthanasia time point and have been successfully euthanized with appropriate collection of tissue samples at the time of euthanasia. This includes three CTI and three VML pigs. An additional four CTI pigs have had both operations and will be undergoing 2-month testing on October 9, 2023. All tissue samples are in storage for biochemical and histologic analysis.

Subtask 3: General Postoperative Monitoring with muscle testing and imaging. To date, six pigs (3 VML and 3 CTI) have completed muscle testing and imaging (see attached Figures). An additional four CTI pigs have had preoperative and 1 month muscle testing and 1 month imaging.

Muscle Testing

1. CTI pigs strength deficits (compared to preoperative values prior to injury) measured 78% at 1 month, 75% at two months, 71% at 3 months and 66% at 4 months after injury.
2. VML pigs strength deficits (compared to preoperative values prior to injury) measured 62% at 1 month, 61% at two months, 63% at 3 months and 59% at 4 months after injury.

The number of specimens is too small for any statistical analysis. VML specimens are mildly stronger than CTI specimens. Compared to our previous experiment, (McKinley TO et al. Minced muscle autografting improves bone healing but not muscle function in a porcine composite injury model. J Orthop Res. 2023 Sep;41(9):1890-1901) the CTI compare very favorably. In this original study, strength deficits at one month in a group of seven pigs identical to the CTI pigs in this current study measured 67%.

Bone Healing Imaging. We have completed all imaging on three CTI pigs and have one-month imaging on an additional four CTI pigs. Individual cortical scores and overall mRUST scores are detailed in the attached figures. In summary, one of the three original CTI pigs healed at the 4 month mark and the other two pigs developed nonunions. In the four CTI pigs that are actively in the protocol, two have strong evidence at one month imaging that they are developing nonunions evidenced by hardware loosening, one pig has some early callus but it is minimal, and the final pig has abundant callus formation. In our previous work, the overall mRUST score at 3 months in seven pigs similar to the CTI pigs in the current study was 9.4. In our current study, at three months the average mRUST score is 9.0 which compares favorably. Likewise, in our original experiment, 2 of 7 CTI pigs healed with 5 nonunions. To date in the current experiment, one pig has healed, two had nonunions, an additional two have highly likely nonunions and two are indeterminate. Again, these data compare favorably with our previous work.

Major Task 3: Tissue Harvest and Analysis: ONGOING

Subtask 1: Serum Analysis. All serum has been collected and stored but no analysis has occurred.

Subtask 2: Muscle protein analysis. All muscle samples have been collected and stored but no analysis has occurred.

Subtask 3: Bone protein and histologic analysis. All bone samples have been collected and stored but no analysis has occurred.

4. OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT

We have six medical students actively working on the porcine surgery project. We have a postdoctoral PhD student working on the project also.

5. DISSEMINATION OF RESULTS TO THE RESEARCH COMMUNITY

The muscle void filler data were presented at the MHSRS 2023 meeting. Andrew Clark (our student at USUHS) was awarded the Young Investigator Award for this presentation. We are too early for any manuscript preparation.

6. PLANS FOR THE NEXT REPORTING PERIOD

We will complete all of the porcine operations and move the entire group through the experimental queue to euthanasia by June/July of 2024. Imaging and muscle testing will be complete by then. Our biochemical analyses will be initiated once all pigs have been euthanized. This will be done by Drs. Dearth and Goldman at USUHS.

We have already received funding for the next iteration of the experiment (Optimizing Muscle Function in Composite Tissue Injuries with Segmental Bone Defects; Natoli RM Principal Investigator; 2022 PRORP Applied Research Award). We will embark on this testing as the current test is winding down.

7. IMPACT

Nothing to report

8. CHANGES/PROBLEMS

Nothing to report

9. PRODUCTS

Nothing to report

10. PERSONNEL

Name:	Todd McKinley
Project Role:	PI
ORCID ID	0000-0001-6354-7685
Nearest person month worked:	1.2
Contribution to Project:	Site PI; orchestrating porcine operations
Funding Support:	PRMRP W81XWH-20-2-0010

Name:	Roman Natoli
Project Role:	Investigator
ORCID ID	0000-0002-4182-3244
Nearest person month worked:	0.6
Contribution to Project:	Will be participating in pig surgeries
Funding Support:	PRMRP W81XWH-20-2-0010

Name:	Melissa Kacena
Project Role:	Investigator

ORCID ID	0000-0001-7293-0088
Nearest person month worked:	0.6
Contribution to Project:	Getting surgeries coordinated with students
Funding Support:	PRMRP W81XWH-20-2-0010

What other organizations were involved as partners? Uniformed Services University (Drs. Christopher Dearth and Stephen Goldman)

Location of Organization: USUHS

Partner's contribution to the project: Collaborators on whole project. They are developing the void filler

Financial support; PRMRP: W81XWH-20-2-0010