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TITLE: Repair of Traumatized Muscle Tissue for Improvement of Musculoskeletal Healing

PRINCIPAL INVESTIGATOR: Dr. Todd McKinley

CONTRACTING ORGANIZATION: Trustees of Indiana University
Indianapolis, IN 46202-5130

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Todd McKinley, MD

E-Mail: tmckinley@IUHealth.org

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Trustees of Indiana University
980 Indiana Ave RM 2232
Indianapolis, IN 46202-5130

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Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT – 200 Words unclassified summary

We are pursuing how skeletal muscle autografting improves muscle function and bone healing in a swine model of a segmental bone defect. Minipigs have been subjected to a 25mm diaphyseal bone defect in the mid-tibia stabilized with double plating and a customized scaffold. Experimental animals have an adjacent 7.0 g muscle defect. Functional muscle testing is performed monthly until sacrifice with a customized testing machine. We have had a reaction to our scaffold resulting in nonunions in the control animals. We are repeating the control animals with the isolated bone defect by eliminating the scaffold. The experimental design will continue once the control group has been successfully re-established.

15. SUBJECT TERMS – Key Words or phrases ID major concepts in the report
Volumetric muscle loss; bone defect, swine model; skeletal muscle autografting;

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	15	**19b. TELEPHONE NUMBER** *(include area code)*

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1. INTRODUCTION

We are investigating skeletal muscle autografting in a miniature swine model to determine how well muscle grafting improves bone healing and restores muscle function. The purpose of the investigation is to establish efficacy of muscle grafting in a pre-clinical model to translate into human use. We are using a miniature swine model that employs a 25 mm segmental bone defect that leads to delayed bone healing in tandem with a 7.0 g adjacent skeletal muscle defect in the anterior compartment.

2. KEYWORDS:

Bone defect; volumetric muscle loss; skeletal muscle autografting; nonunion; mangled limb

3. ACCOMPLISHMENTS:

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task 1: Evaluate VML of Fracture Healing
Major Task 2: Evaluate Muscle healing after open fracture
Major Task 3: Interrogate immune and osteogenic signaling
Major Task 4: Evaluate effect of minced graft on fracture healing
Major Task 5: Evaluate minced graft mediated muscle tissue regeneration on functional muscle recovery
Major Task 6: Interrogate the effect of minced graft muscle repair on acute immune and osteogenic signaling

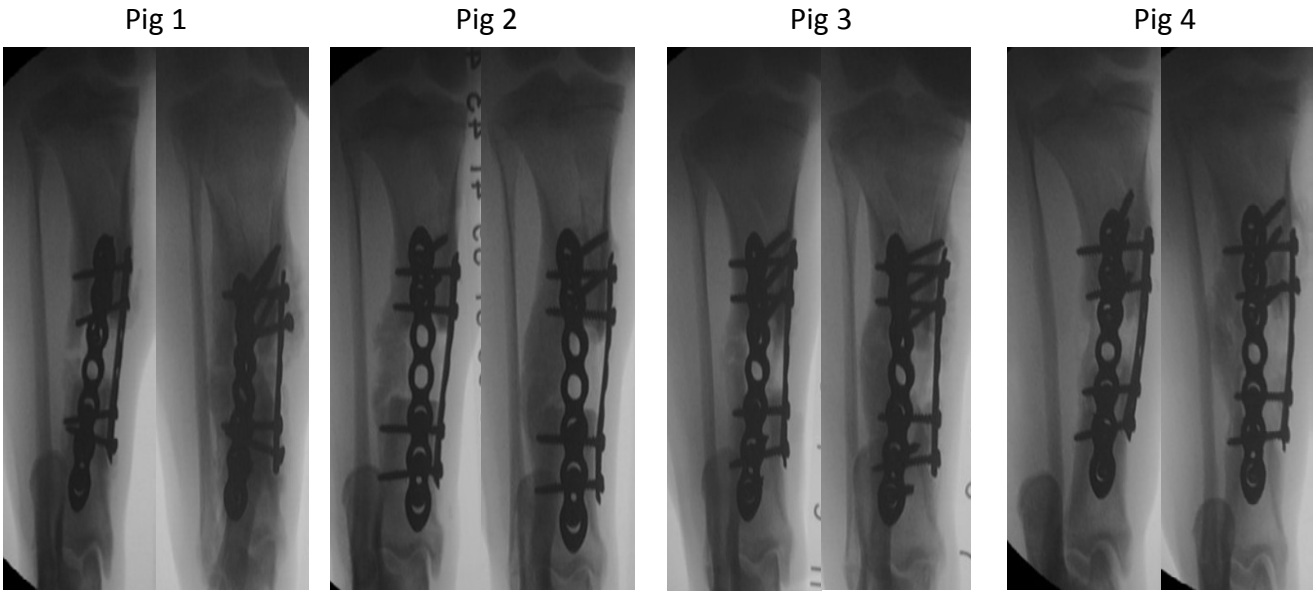
What was accomplished under these goals?

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. As the project

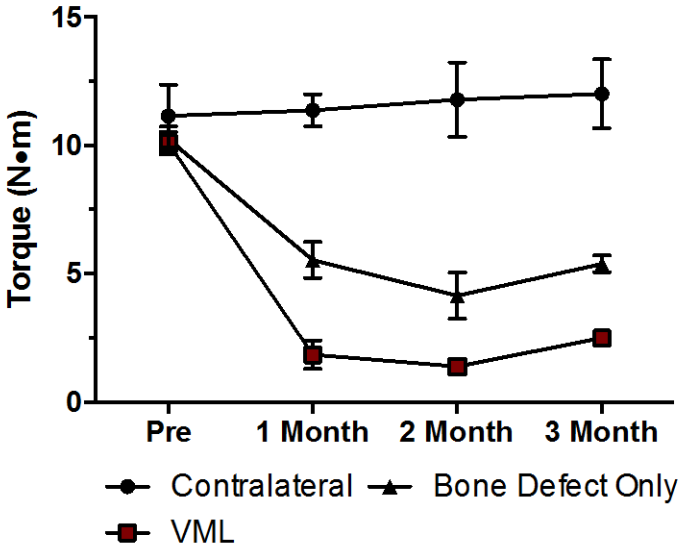
Major Task 1: We have operated on three negative control bone defect specimens and five specimens with bone defect and VML. In all eight animals, the pigs had a reaction to our scaffold spacer and around one month after surgery, the wounds opened up and the scaffold was spit out of the wound in pieces. All eight animals have been sacrificed and all eight have developed nonunions of the bone defect in the tibia.

After it was discovered that the scaffolds were being rejected, I (the PI) reported this to our scientific officer, Dr. Behrsing (at the MHSRS conference in August) and put the experiment on hold prior to operating on any further animals. Subsequently, I investigated our scaffolds as this was not a problem in our previous studies. The current scaffolds were cultured and none cultured positive except for one colony of coagulase negative staph on one culture. The clinical appearance is very consistent with foreign body reaction.

Presumably, the scaffolds were manufactured as they were two years ago in another study, but in face of all of the scaffolds being rejected, we are working under the assumption that this is a foreign body rejection. In addition, in our previous study, the primary purpose of the scaffold was to facilitate delivery of a biologic agent which is not part of this study. Therefore, we are moving forward on re-establishing our segmental bone defect negative control model without inserting the scaffold. I have secured internal funding from our department and medical school to repeat the experiments on the eight pigs that we have used to date. We plan on operating on one pig initially with a bone defect only and follow the first pig a minimum of 8 weeks until we operate on a second bone defect only pig . We will use our previous pigs from our previous experiment as a template to judge healing. The time course of the bone-defect only animals from our previous study is depicted below are four pigs from our previous study



Major Task 2: We have performed serial in vivo muscle testing on pigs with bone defects with or without an adjacent muscle defect. Data were captured just prior to injury and then monthly for four months. Below are data demonstrating how the bone defect and combined bone and muscle defect have affected muscle strength.



Major Task 3: Samples obtained from the wound have been processed and are currently in our -80 freezer. We plan on moving forward with analysis even though the specimens eventually rejected the scaffold.

Major Task 4 – 6: Nothing to report as we have not embarked on muscle autografting yet.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars,

We several undergraduate, graduate, and medical students who have been integral in conducting the experiment. They have been trained in in vivo muscle testing methods, in harvesting the wound site aspirates and in all tissue harvest methods at sacrifice. In addition, one of the students is maintaining the data base.
Our senior medical student has been scrubbing the surgeries and being instructed in surgical technique. He will be pursuing an Orthopedic residency.
The majority of our students attended the ORS Midwest Regional Symposium.

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We are repeating our bone defect only surgeries to re-establish our negative control model. Once successful, we will repeat the bone and muscle defect positive control animals and then do the experimental group with the muscle autograft.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?

If successful, we will have produced a product that will be positioned for a Phase I clinical trial for a clinical problem, Volumetric Muscle Loss, that currently has no successful options for treatment.

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Our primary problem is described above with the scaffold reaction. Accordingly, we are embarking on repeating the bone defect only specimens without any spacer. We will operate on individual pigs and follow them to ascertain the model is successful before re-embarking on the experiment.

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

See above.

Changes that had a significant impact on expenditures

None to report. We are financing the repeat negative and positive (bone and muscle defect without any treatment.

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

Our unanticipated outcome was the uniform scaffold rejection by the pigs. We have performed a repeat operation without the scaffold and will follow individual pigs to determine healing under the new conditions.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

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

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal;*

Nothing to report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Peters AW; Corona BT, Milto AJ; Brinker A; Tucker A; Savaglio M; Adam G; Ganesh V; Gunderson Z; Childress P; Natoli RN; McKinley TO; Kacena MA. In vivo muscle testing of a porcine tibia fracture model. Orthopaedic Research Society Midwest Regional Symposium. Ann Arbor, MI. August 2018.

Milto AJ; Peters AW; Brinker A; Tucker A; Savaglio M; Adam G; Ganesh V; Gunderson Z; Childress P; Natoli RN; McKinley TO; Kacena MA. Evaluation of a critical sized defect in a porcine model. Orthopaedic Research Society Midwest Regional Symposium. Ann Arbor, MI. August 2018

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

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

Technologies or techniques

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

Other Products

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Our swine in vivo muscle testing machine has been working without difficulty. This was originally developed by Corona (Investigator on this grant) and colleagues and used for preliminary swine work. We have used it extensively in this study and it has yielded accurate results and is a remarkably effective muscle force transducer for this and future studies.

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:	Todd McKinley
Project Role:	Principal Investigator
Person Month Worked:	2
Contribution:	In charge of the entire investigation; conducting all surgeries
Funding:	na

Name:	Roman Natoli
Project Role:	Investigator
Person Month Worked:	2
Contribution:	Conducting all parts of the investigation: all surgeries
Funding:	na

Name:	Benjamin Corona
Project Role:	Investigator
Person Month Worked	2
Contribution	Muscle testing; developing muscle autograft methods; all analysis
Funding:	na

Name:	Melissa Kacena
Project Role:	Investigator
Person Month Worked	2
Contribution	Study design; data analysis
Funding:	na

Name:	Alex Brinker
Project Role:	Graduate Student
Person Month Worked	6
Contribution	Technical support for all surgeries, postop care, data acquisition
Funding:	na

Name:	Aamir Tucker
Project Role:	Medical Student
Person Month Worked	6
Contribution	Technical support for all surgeries, postop care, data acquisition
Funding:	na

Name:	Michael Savaglio
Project Role:	Medical Student
Person Month Worked	5
Contribution	Technical support for all surgeries, postop care, data acquisition
Funding:	na

Name:	Gremah Adams
Project Role:	Graduate Student
Person Month Worked	4
Contribution	Technical support for all surgeries, postop care, data acquisition
Funding:	na

Name:	Venkat Ganesh
Project Role:	Graduate Student
Person Month Worked	2
Contribution	Technical support for all surgeries, postop care, data acquisition
Funding:	na
Name:	Zachary Gunderson
Project Role:	Medical Student
Person Month Worked	2
Contribution	Technical support for all surgeries, postop care, data acquisition
Funding:	na
Name:	George Kolettis
Project Role:	Medical Student
Person Month Worked	2
Contribution	Technical support for all surgeries, postop care, data acquisition
Funding:	na
Name:	James Fischer
Project Role:	Medical Student
Person Month Worked	2
Contribution	Technical support for all surgeries, postop care, data acquisition
Funding:	na
Name:	Austin Winniger
Project Role:	Medical Student
Person Month Worked	2
Contribution	Technical support for all surgeries, postop care, data acquisition
Funding:	na

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

None

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: Attached

9. APPENDICES: N/A

Repair of Traumatized Muscle Tissue for Improvement of Musculoskeletal Healing
OR160094
W81XWH-17-1-0626

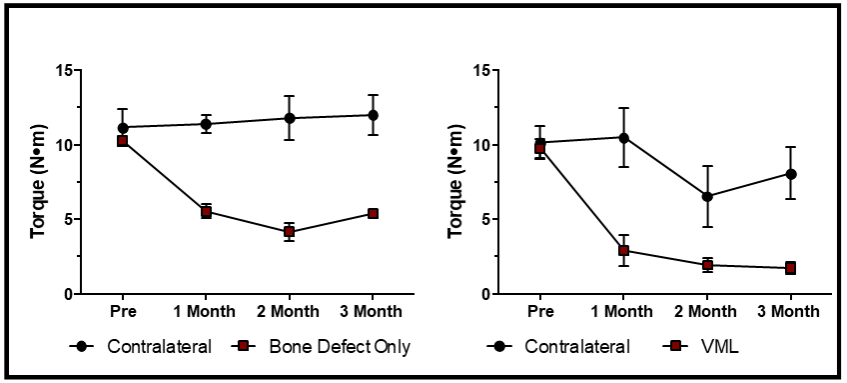
PI: Dr. Todd McKinley, M.D. **Org:** Indiana University **Award Amount:** $499,044

Study/Product Aim(s)

Study/Product Aim(s) & Approach

Establish an unprecedented level of care for open fracture:

- Treat traumatized muscle for the improvement of fracture healing and prolonged functional outcomes.
- Establish immunomodulatory role of muscle repair for improved 'musculoskeletal' healing
- A porcine open fracture model will be used for this investigation that involves a segmental tibia defect and volumetric muscle loss injury.
- Extensive preclinical rodent testing supports the use of autologous muscle tissue grafts for Volumetric Muscle Loss



Strength testing in vivo demonstrates definitive loss of strength in the pigs with Muscle defects (right) compared to bone defect only pigs (left).

Timeline and Cost

Activities CY	17	18	19	
Perform surgeries				
Impact on Fracture Healing				
Impact on Muscle Healing				
Dissemination/Transition				
Estimated Budget ($K)	**$000**	**$450**	**$50**	**$000**

Updated: (October 28th , 2018)

Goals/Milestones

CY17 Goal –

☑Get IACUC and ACURO approvals

CY18 Goals –

☐Perform all pig surgeries and collect all tissues and images for analysis.

ONGOING - Spacer reaction has resulted in modulating the negative control model and we have eliminated the spacer. We will do sequential negative control animals and confirm that they heal as predicted prior to embarking on repeat positive controls (bone and muscle defects) and experimental specimens (bone and muscle defect with muscle autografting.

CY19 Goal –

Re-establish successful control model and proceed with experiments.

Budget Expenditure to Date: Actual Expenditure as of 09/29/2018 = $291,135.07 in salary expenses, pigs, materials and supplies.

Projected Expenditure: Continue with salary and pig per diems.