AWARD NUMBER: W81XWH-18-1-0352

TITLE: Neurotization to Improve Graft Reinnervation and Recovery Following Severe Muscle Injuries

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CONTRACTING ORGANIZATION: Virginia Commonwealth University

REPORT DATE: August 2020

TYPE OF REPORT: Annual Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
Aug 2020	Annual Report	08/01/2019-07/31/2020

4. TITLE AND SUBTITLE

Neurotization to Improve Graft Reinnervation and Recovery Following Severe Muscle Injuries

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0352

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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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 Materiel 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

Extremity soft tissue trauma can result in permanent loss of skeletal muscle mass and denervation, posing a significant clinical challenge in the military. Clinical options are to either neglect the wound, expecting fibrosis to develop, or to perform surgery and fill the muscle void with a local autologous muscle graft. The development of non-contractile tissue (mainly fibrosis) in the muscle injury is typically observed in cases where neural innervation is irrecoverable and muscle function is severely impaired. Interruption of the intramuscular neural connections in these devastating injuries is a serious regenerative obstacle that is rarely considered. We demonstrate here that muscle force is recovered only slightly through the use of DMM and autograft, confirming what typically occurs functionally in VML injuries. When we investigated histological data we indeed showed that our findings support the hypothesis that severing those intramuscular neural connections potentially impairs muscle regeneration. In this project, we determined that DMM and autograft are sufficient to support some new muscle fiber growth and satellite cell activity. Furthermore, we demonstrated positive AChR-gamma and NCAM staining in DMM treated sites and autograft treated sites. In addition, DMM sites had more intense staining for AChRs compared to autograft. Collectively, these data suggest that these intramuscular neural connections are important in maintaining contractile properties of muscle fibers and could potentially regulate muscle regeneration. Future studies will demonstrate whether improving innervation within the existing muscle or in the graft area will improve muscle regeneration.

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

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

An abstract shall be provided in Block 14 and shall state the purpose, scope, and major findings and be an up-to-date report of the progress in terms of results and significance. Abstracts will be submitted to the Defense Technical Information Center (DTIC) and shall not contain proprietary information. Subject terms are keywords that may have been previously assigned to the proposal abstract or are keywords that may be significant to the research.

TABLE OF CONTENTS

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

Extremity soft tissue trauma, in particular, VML injury, results in permanent loss of skeletal muscle mass, posing a significant clinical challenge. Treatments for these devastating injuries are to stabilize the wound by either allowing fibrosis to occur or to engraft autologous tissue. Although some autologous grafts can support limb salvage, functional recovery rates are often low (8%)[36,37]. As a consequence, these grafts typically remodel into non-contractile tissue after a long healing and reparative process [38,39]. Despite post-operative efforts to rehabilitate the muscle deficit using physical therapy, patients continue to experience functional losses. In total, these surgeries and rehabilitation cost the United States $600 billion (2016 data) each year [37,2].

The rapid degeneration of muscle fibers following traumatic muscle injury seems to be related to disuse and loss of trophic support and feedback normally provided by intact axons. Unlike most cells in the body, muscle fibers function as a syncytium of fused nucleated cells. In cases of muscle trauma, these multinucleated muscle fibers are lost in the injured area. Initially, muscle injury activates a pool of quiescent, regenerative satellite cells that migrate to the injured site, differentiate into myoblasts, and fuse with existing myofibers to bolster their reduced size. However, lack of muscle regeneration and re-innervation in the traumatized area subsequently negates these temporary mechanisms, leaving muscle to promote reparative features like fibrosis. With time, degeneration occurs, and fibrosis develops in the traumatized area. In addition, distally affected muscle fibers lose the intramuscular connections that are important in motor unit recruitment during contraction. Fibrosis, denervation, and lack of muscle regeneration collectively contribute to muscle weakness. Eventually, the injured muscle is completely replaced with fibrotic adipose tissue with no hope of recovery.

Reduced motor function is directly associated with the development of non-contractile tissue (mainly fibrosis) in the muscle injury area. This suboptimal reparative state can be ascribed to a combination of inflammatory mediators, overactive connective tissue cells, dysfunctional regenerative processes, and denervation both in the zone of injury and distal to the defect that limit de novo regeneration. As such, achieving functional gains will require treatment modalities that favor de novo muscle regeneration over fibrosis.

Skeletal muscle regeneration is strongly influenced by environmental factors such as extracellular matrix (ECM). We have developed a decellularized muscle matrix (DMM) that supports de novo fiber formation within the central region of the DMM graft in a rodent VML model. These types of models are harsh regenerative environments where few de novo muscle fibers form in the graft area. Instead, excessive collagenous matrix forms, which is theorized to impair muscle regeneration and subsequent innervation. Use of DMM provides a cell-free ECM with the proteins and structure necessary to regenerate skeletal muscle and has the potential to reduce the amount of fibrotic scar that develops. Considering the effects of denervation muscle atrophy, we sought to determine whether atrophy continued to be a contributing factor that abrogates muscle regeneration, and whether we could overcome this co-morbidity using neurotization strategies to re-innervate newly regenerating and denervated muscle.

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

Volumetric Muscle Loss; Denervation; Atrophy; Neurotization; Regeneration

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.

The major goals of this project are to: 1. Examine denervation markers following a severe muscle defect created in a rat gastrocnemius (100% complete). 2. Semi-quantitatively assess muscle staining to determine degree of denervation following muscle graft surgeries (100% complete). 3. Neurotize muscle grafts using peroneal and tibial nerve grafts (100% complete). 4. Assess histology, immunostained sections, and protein levels in those animal studies (25% complete) 5. Test muscle function following neurotization (100% complete). 6. Determine ryanodine receptor and sarcoplasmic reticulum calcium ATPase levels (0% compete).

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 progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Specific Aim 1. Determine if decellularized and autologous muscle grafts are denervated in rat gastrocnemius.

Major Goals: 1. Examine denervation markers following a severe muscle defect created in a rat gastrocnemius, and 2. Semi-quantitatively assess muscle staining to determine degree of denervation following muscle graft surgeries.

Prior work demonstrated that skeletal muscle defects repaired with decellularized muscle matrix improved muscle force output compared to empty defects, and there was no evidence of re-innervation in newly regenerated muscle fibers within the graft area or around the graft area. From those studies performed in Aim 1 of this proposal, we determined that no de novo muscle fibers that formed due to decellularized muscle or autologous muscle treatments were innervated.

Gene analysis using Nanostring

In addition to performing histology and immunohistochemistry, we assessed gene expression using lysates from VML decellularized muscle and autograft treatments. Quality RNA was successfully obtained from graft sites, and those RNA were sent off for Nanostring analysis. Results from that analysis are shown below, which included both muscle and neural gene markers. We report full genetic analysis using those gene panels and we will focus on some select differentially expressed genes within those cohorts.

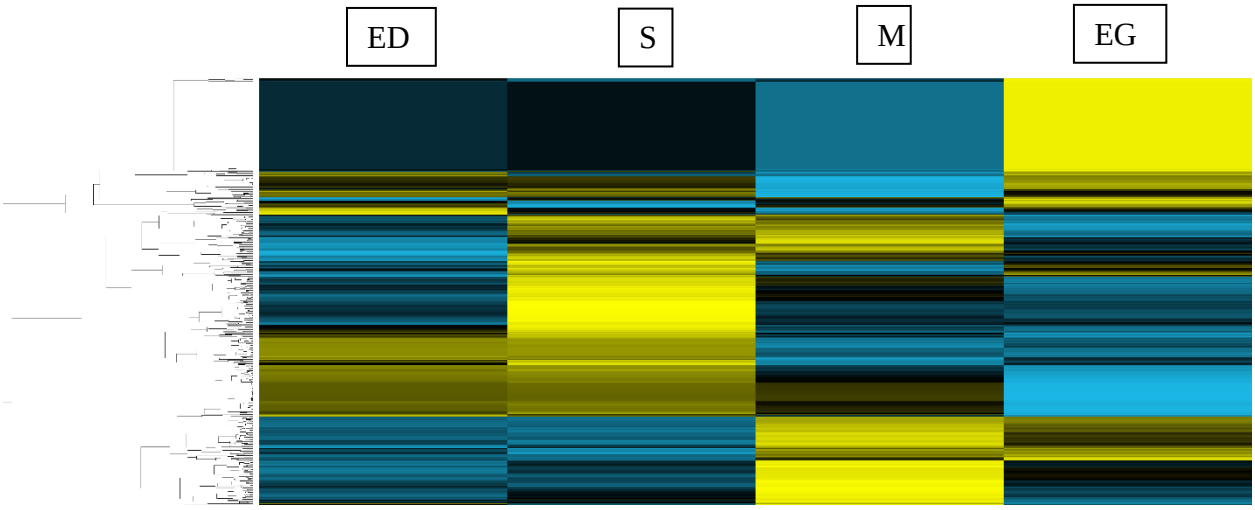


Figure 1. Nanostring analysis of VML injuries versus sham where VML injuries were treated with DMM (EG – empty graft) or autograft (M – muscle). Those animals were compared to no treatment (ED – empty defect) and sham (S).

mRNA levels in VML animals appeared different than sham animals. Furthermore, VML animals treated with decellularized muscle grafts (EG) showed some unique differences from VML animals that received no treatment (ED). More, VML animals that received an autograft (M) were similar to EG groups as well, but also displayed some unique differences from ED. These data according to the heat map showed that treating a VML injury resulted in different expression levels from leaving the injury to undergo fibrosis.

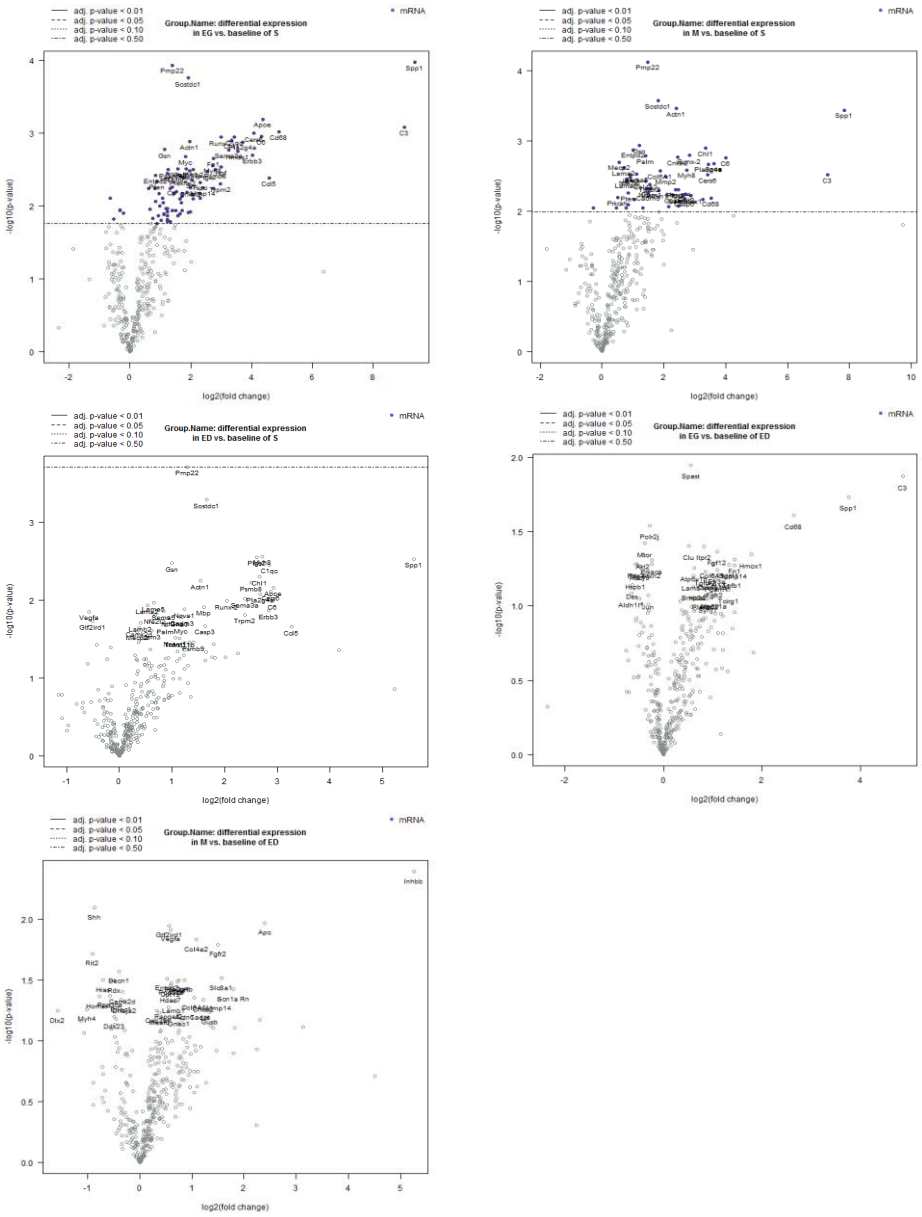


Figure 2. Volcano plots of differentially expressed genes in DMM, autograft, untreated, and sham animals.

Analysis of fold changes in mRNA levels were compared to sham, where we determined that both DMM and autograft groups shared most differentially expressed genes. However, some of the levels for those shared genes were different between treatments. When we compared VML treatments to no treatment, we determined that DMM and autograft shared less genes, indicating that the different treatment modalities elicited different responses. Importantly, we determined that both DMM and autograft were pro-myogenic, demonstrating elevated levels of Myh8, a gene that encodes for new muscle fiber formation, and Pax7, a gene marker of satellite cells. In addition, other extracellular matrix conditions that would favor regeneration were elevated in DMM treated animals such as

Col6a1 and Col4a1 which encode for basal lamina proteins and are known to stimulate muscle regeneration. *More, we determined that Ncam1 gene levels in DMM and autograft were elevated compared to sham, confirming our immunohistochemical staining and that muscle graft treatment sites remained denervated.*

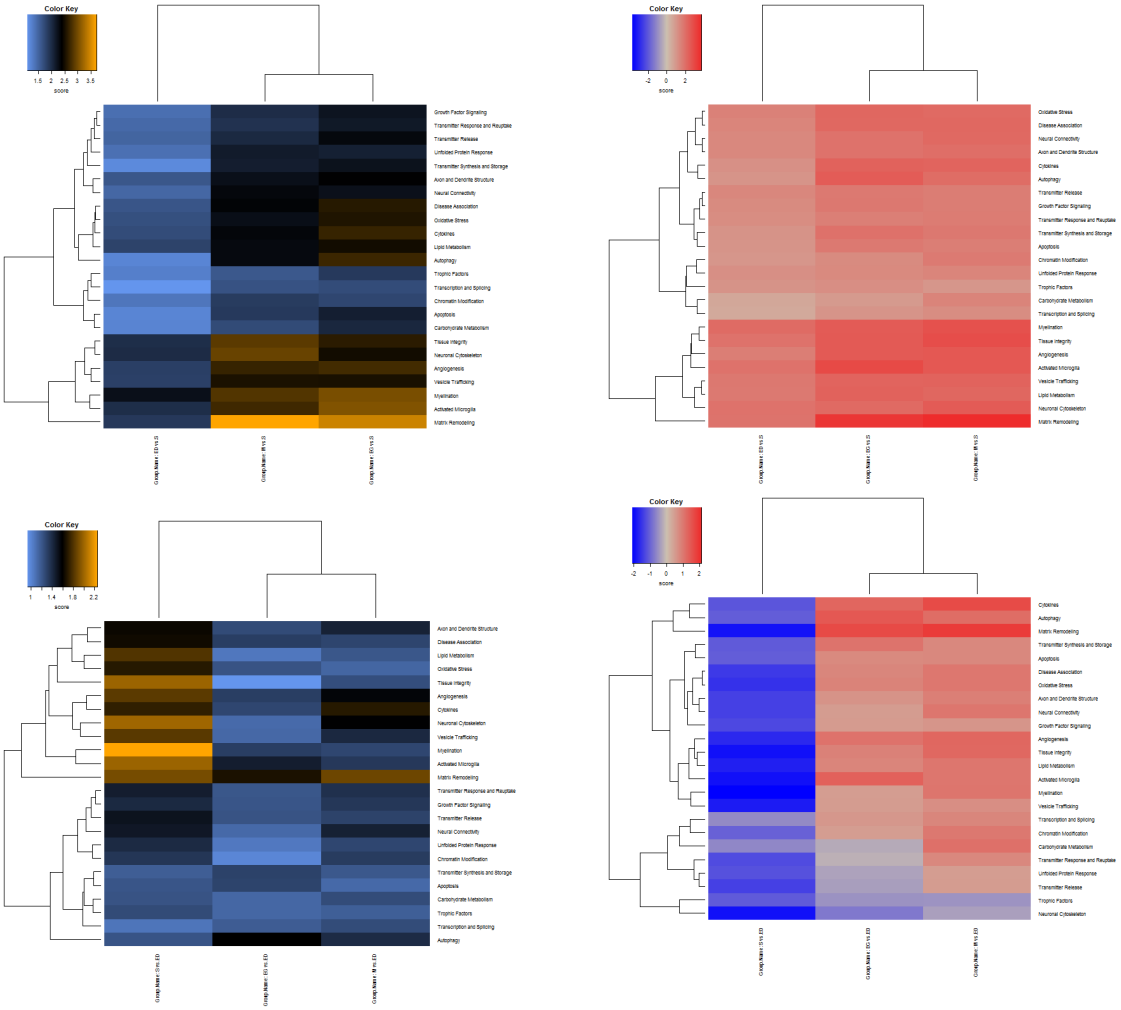


Figure 3. Global significance scores to summarize differentially expressed genes and the extent of differential expression in VML animals compared to sham.

When we examined neural markers, we determined that genes encoding for axon and dendrite structure like Apoe, Spp1, and Chl1 were upregulated in DMM and autograft treated animals. In addition, genes encoding for myelination were high such as Pmp22 in both DMM and autograft animals, and trophic signaling for neurotrophin-3 was upregulated in DMM treated sites.

Interestingly, global significance scores in Figure 3 showed that neural connectivity, vesicle trafficking, myelination, and angiogenesis related genes were higher in VML treated animals compared to sham. In addition, those same gene sets in untreated animals were downregulated when compared to sham, suggesting that treatment alone aided in improving the neural microenvironment. Not surprisingly, matrix remodeling expression was highest in treated animals.

When we explored global significance scores VML treated animals and sham versus untreated animals we determined that neural connectivity and myelination were highest in autograft animals compared to no treatment. In addition, transmitter release genes were higher in autograft than when DMM was compared to no treatment. Taken together these data indicate that while DMM displays a pro-myogenic and pro-neurogenic environment it remains less so than when host muscle is used.

Specific Aim 2. Determine if muscle graft neurotization using peroneal or tibial axon sources affects re-innervation following injury.

Major goals: 3) Neurotize muscle grafts using peroneal and tibial nerve grafts. 4) Assess histology, immunostained sections, and Western blot. 5) Muscle function tests following neurotization. 6) RyR and SERCA protein assessment.

Neurotization Experiments

As it stands now, in accordance with the Statement of Work, we have completed Major Task 3, we are currently working on Major Task 4, we have completed Major Task 6, and Major Task 6 is being assessed in conjunction with immunostaining from Major Task 4. We report here our current results.

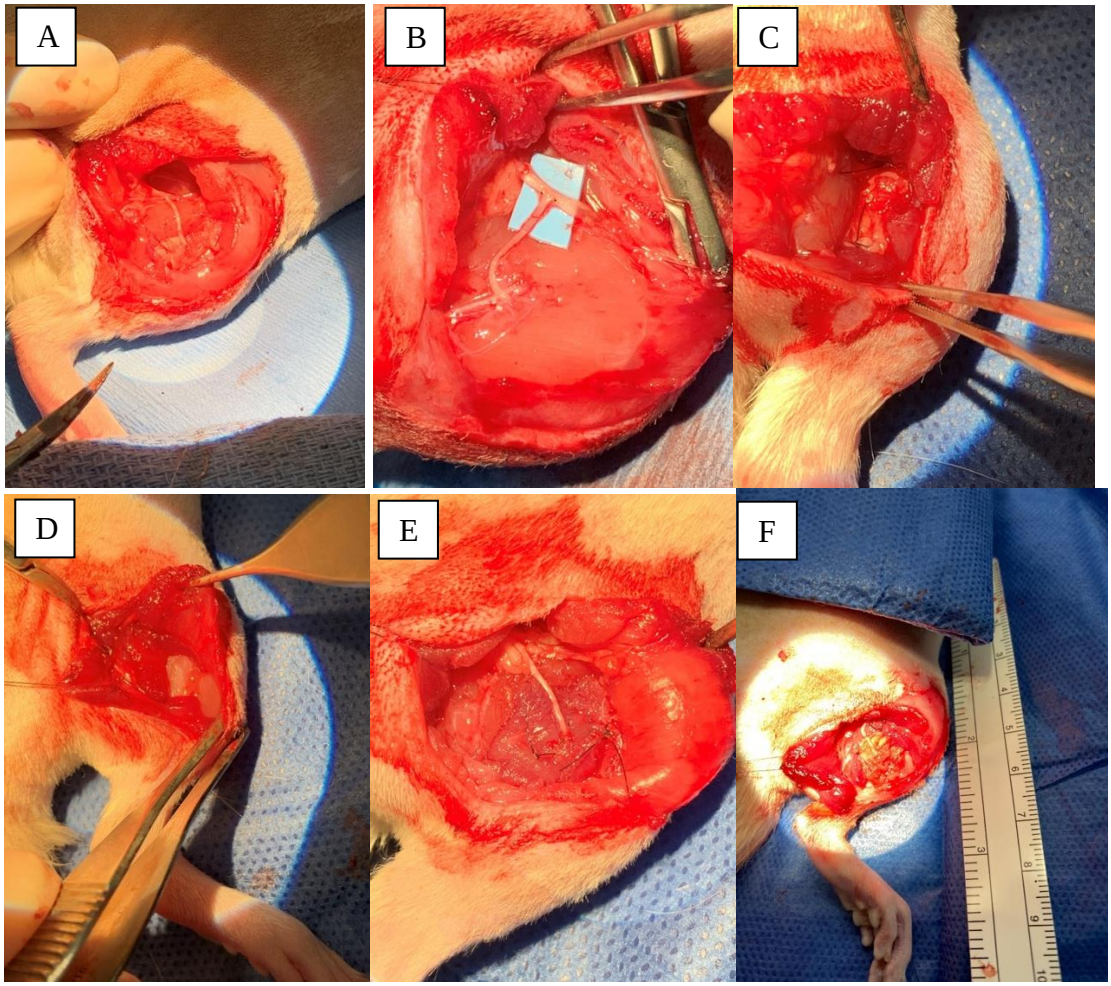


Figure 5. Neurotization surgeries completed.

Figure 5 demonstrates example images from neurotization surgeries where either DMM or autograft were implanted into the muscle defect. Neurotization was performed by either using a peroneal swing over graft or an end-to-side coaptation procedure using the peroneal nerve as the graft and the tibial nerve as the nerve used to donate axons to the peroneal graft. (A) shows a peroneal swing over prior to graft implantation, (B) shows an end-to-side procedure prior to graft implantation, (C) shows the graft alone with the peroneal nerve cut and embedded into the wall of the biceps femoris, (D) shows an empty defect with the same nerve embedment procedure as in C, (E) shows a peroneal swing over after autograft implantation, and (F) shows a peroneal swing over after DMM implantation.

Muscle force was recorded throughout the duration of this experiment at 2, 4, and 8 weeks by stimulating the posterior crural muscles.

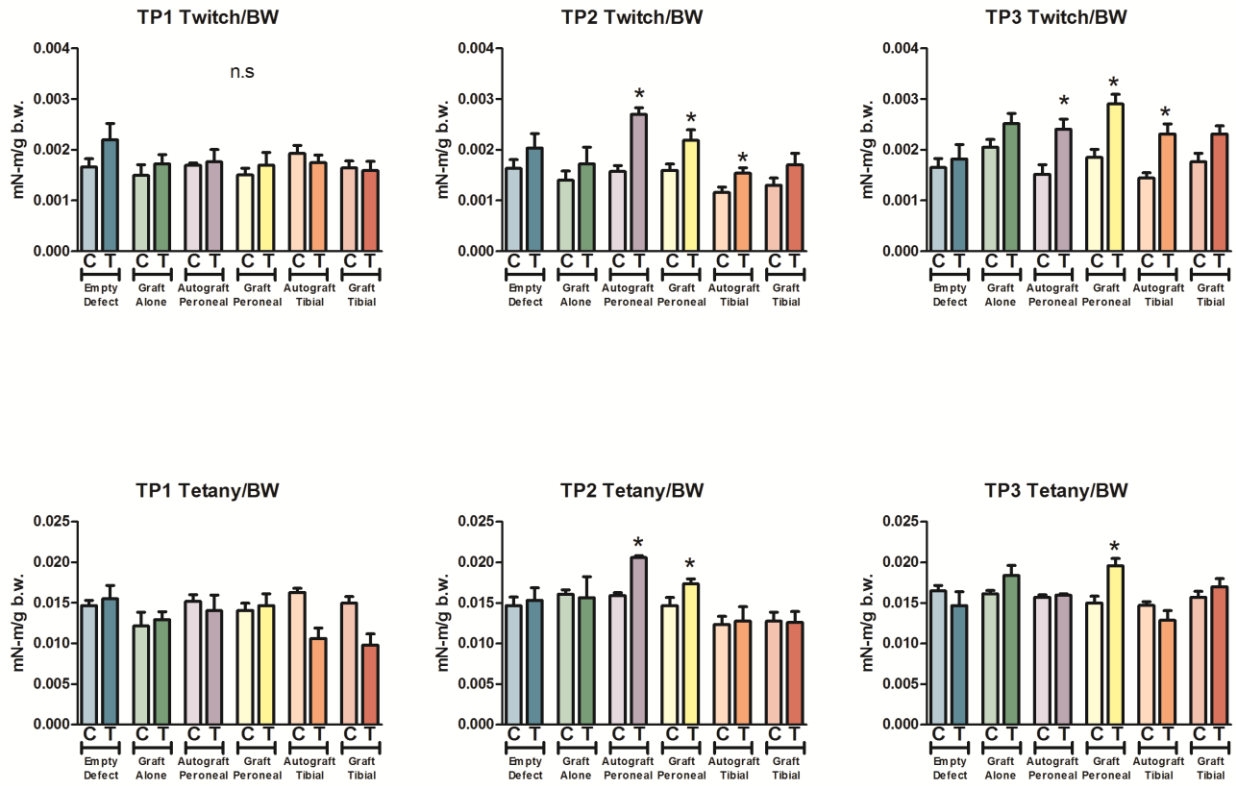
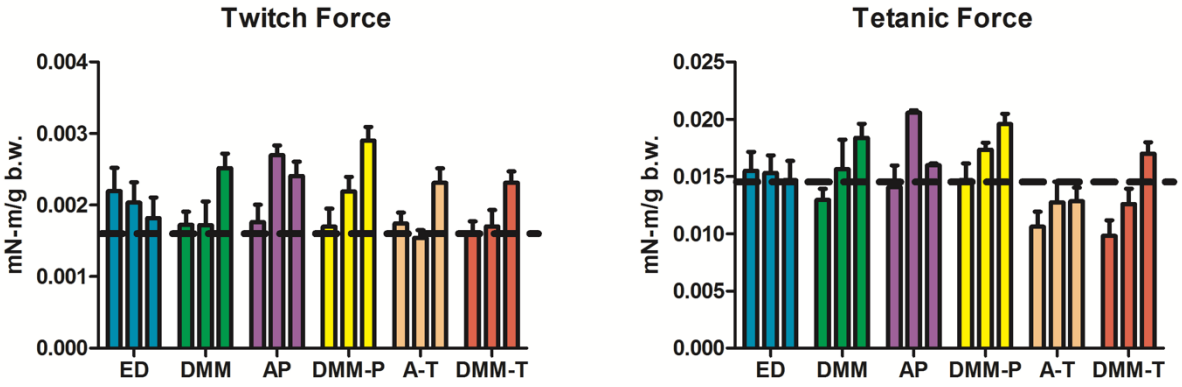


Figure 6. In vivo specific muscle force generated over 8 weeks compared to contralateral (uninjured) leg. * indicates a significant difference from control legs ($p<0.05$).

Muscle twitch and tetanic force were measured for all treatment legs and their contralateral control legs. Data demonstrated that both autograft and DMM treated legs with the addition of a peroneal swing over produced far better force than DMM grafts alone, or graft treated legs with a tibial nerve neurotization. More, DMM treated with a peroneal nerve produced higher force levels for both twitch and tetanic force measurements compared to control. Finally, it appeared that twitch force improved due to neurotization in the same manner as DMM graft alone with a sudden increase in force at week

8. This suggests tibial nerves did not adequately contribute to muscle fiber activation. Additional measurements were also determined such as ½ relaxation time, max rate of contraction, time to ½



max contraction, and force-time integration. Data are not shown however to preserve space, but those data will be reported in the final manuscript.

Figure 7. Representation of measured twitch and tetanic force over 8 weeks as compared to control leg. Dashed bar represents the average control leg. *Note that empty defect legs while they did not display a significant reduction in force production, force did not change over time.*

<u>Muscle-Nerve Cross-talk</u>

In addition to working toward the effects of neurotization on muscle force production and muscle regeneration, we began experiments to start understanding the cross-talk between muscle and nerves using myoblasts, myotube, schwann cells, and motor neurons. We report here these most recent cross-talk experiments. According to our preliminary results, myoblasts respond to Schwann cell conditioned media to upregulate cellular motility, while there appears to be little effect on myotubes once they have formed. Alternatively, myoblasts do not appear to have a significant effect on Schwann cells; however, myotube conditioned media appears to upregulate Sox10, suggesting a reparative Schwann cell phenotype.

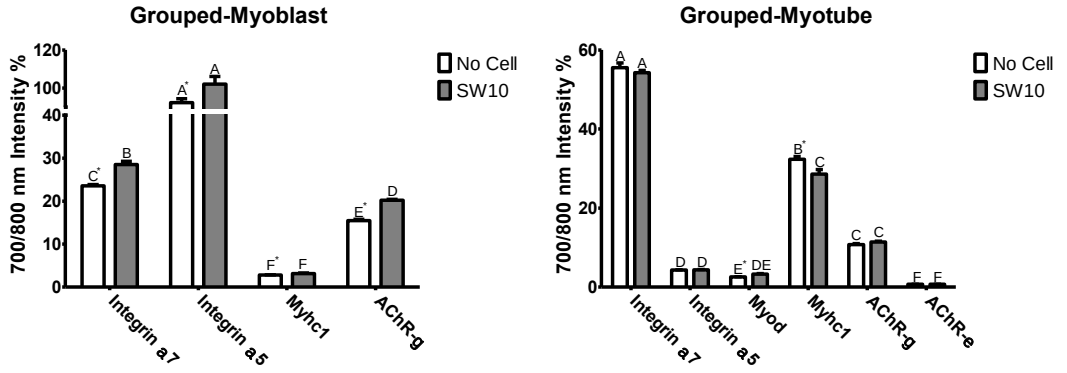


Figure 8. Myoblasts and myotubes treated with Schwann cell conditioned media.

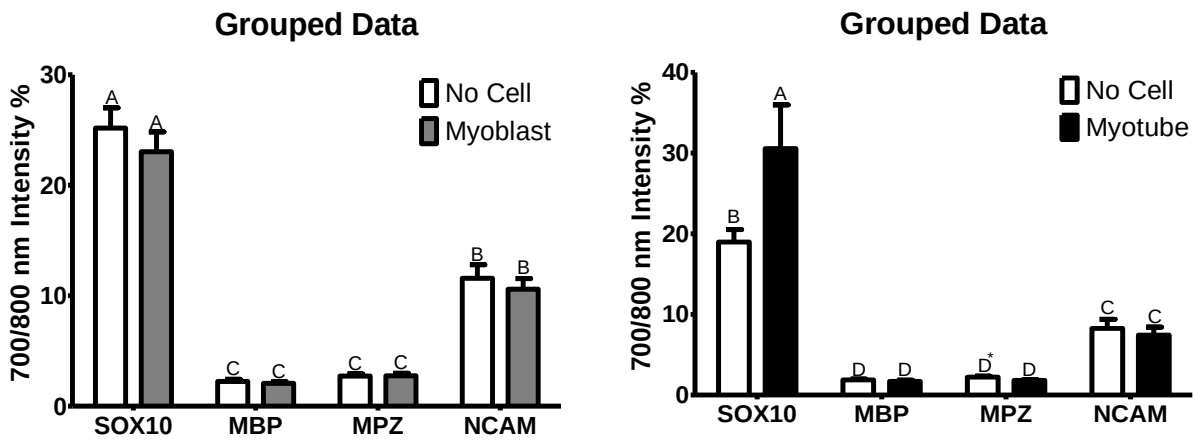


Figure 9. Schwann cells treated with myoblast or myotube conditioned media.

Conclusions: Collectively, data indicate that DMM and autogaft implants are indeed denervated and are actively upregulating markers of muscle regeneration. Furthermore, neurotization studies showed that peroneal nerve anastomosis exhibited better outcomes likely due to reduced surgical times. Tibial nerve anastomosis was more difficult and time consuming considering that we needed to create an epineurial window first and suture into window using an end-to-side anastomosis technique. On the other hand, peroneal nerve anastomosis simply cutting the peroneal nerve and implanting it directly into the muscle injury. Force analysis showed that neurotizations improved force output compared to control legs. As we continue to collect data, we will be able to determine whether neurotization improves regeneration.

Stated goals not met:

1. Milestone #1 in progress and estimated to be completed in November
2. Histomorphometry (estimated to complete in December/January)
3. Examine immunohistochemical staining (estimated to complete in January)
4. Western blot (estimated to complete in November/December)
5. Nanostring analysis for all neurotization groups. (estimated to complete in November)

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, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The project has provided the ability to train graduate students (both PhD and Masters), undergraduate students, and medical students. While training amongst these students varied depending on their specific contribution to the study, the ultimate goal was to expand their skillsets in multiple biological assessments and outcome measures such as surgery, animal care, force analysis, gene data and analysis, rodent anatomy, histological sectioning and staining, immunohistochemical staining, and protein analysis. Furthermore, while recently our department acquired funding to train undergraduates using an NSF-REU. These training opportunities will be used in the second summer of this project to better understand the interactions between muscle fibers and motor neurons.

This project also provided an ability to develop professionally as well. The PI attended several conferences using funds from both this grant and from startup money. Specifically, the PI attended Biomedical Engineering Society, Orthopaedic Research Society, Society for Biomaterials, and Military Health System Research Symposium. In addition, the PI became involved in grant workshops available at VCU during this project period, using data collected from this study to apply for future funding at both NIH and DoD.

How were the results disseminated to communities of interest?
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.

The PI recently attended an event with Henrico County high school and middle school teachers to discuss the findings from these studies and inform them about the research occurring at VCU BME.

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

In the next reporting period (quarter 1 of year 3), we plan to finish histological assessment and be near completion of genetic results and analysis. We plan to finish milestone #1. We plan to have western blotting and immunohistochemical staining started but not fully complete.

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?
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).

The impact of this project thus far indicates that intact, distal muscle fibers and fibers regenerated using decellularized muscle grafts are indeed denervated. The implications of this knowledge suggest that the effects of denervation muscle atrophy need to be considered when treating a volumetric muscle loss wound. Furthermore, gene data from freshly isolated tissue confirms this. When animals were treated using neurotization, there appeared to be improved muscle force output. This suggests that more motor units are being activated than under control conditions and suggests that innervation may play a role in these results.

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.

Initial results from neurotization suggests that clinical treatment of muscle injuries with allogenic or autogenic graft material may need to include neurotization strategies.

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

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Data from Aim 1 of this project demonstrates a possibility that any VML graft implanted into the muscle wound site will need to develop an additional strategy to address denervation. In addition, these findings could affect the way we prepare our grafts, possibly leading to a patent. More, our preliminary results appear favorable toward use of neurotization. Whether direct electrical stimulation is the main cause of increased motor unit recruitment, or trophic factors are playing a role in increased force production needs to be assessed.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS: *The PD/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. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

COVID-19 was problematic for efficient assessment of histological specimens. The Isaac lab shut down during the pandemic and recently opened back up in the beginning of August. Thus, we are making progress toward our additional tasks but were delayed due to the pandemic.

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.

Delays in histological staining are actively being resolved with the reopening of labs.

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.

Nothing to report.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No human subjects are used in this study.

No significant changes.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **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; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report. Publication is in preparation and should be out for publication within the next 2 months.

  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).*

Publication is in preparation.

  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.

MHSRS, ORS

- **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.*

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: *Michael J. McClure*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0002-5428-5270
Nearest person month worked: *0.3*
Contribution to Project: *Regulatory process, supervising studies, performed surgeries.*

Name: *Barbara D. Boyan*
Project Role: *Co-PI*
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0002-9642-0311
Nearest person month worked: *0.01*
Contribution to Project: *Involved in experimental design for animal studies and is actively engaged in data analysis.*

Name: *Jonathan E. Isaacs*
Project Role: *Co-PI*
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0002-7791-6725
Nearest person month worked: *0.01*
Contribution to Project: *Involved in experimental design for the animal studies.*

Name: *Geetanjali Bendale (replacement for Satya Mallu)*
Project Role: *Co-investigator*
Researcher Identifier (e.g. ORCID ID): Not available
Nearest person month worked: *0.05*
Contribution to Project: *Involved in muscle force tests and histology.*

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.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*