

AWARD NUMBER: W81XWH-20-1-0336

TITLE: Understanding and Enhancing the Regenerative Capacity of Skeletal Muscle to Trauma by Targeting Muscle-Nerve Synergy

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CONTRACTING ORGANIZATION: UNIVERSITY OF MICHIGAN
ANN ARBOR MI 48109-1340

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13. SUPPLEMENTARY NOTES 14. ABSTRACT: Background: Poor healing after lower-limb extremity trauma represents an enormous medical problem (\$400B / year ascribed to trauma in CONUS and >24M limited duty days in 2005) and recent conflicts in Iraq and Afghanistan have emphasized the prevalence of lower-limb extremity trauma (up to 78 percent of medical disability discharges). These injuries present debilitating consequences, which have been shown to result in pronounced disabilities ranging from declines in limb function, to development of osteoarthritic pathology and delayed or elected limb amputation. Moreover, the effects of lower-limb extremity trauma have significantly reduced Department of Defense (DoD) readiness and performance and as each force begins to downsize, the importance of sustaining Warfighter readiness and recovery from trauma is a priority. Objective/ Hypothesis: While efforts to develop treatments that hasten and improve healing for lower-limb skeletal muscle injuries are ongoing, their development has been inherently limited due largely to our lack of understanding of the basic processes involved in the healing process. Efficient and appropriate repair and regeneration of skeletal muscle is mediated by a pool of muscle stem cells (MuSCs) called satellite cells, which activate, proliferate and differentiate and fuse to form new multinucleated myofibers. After regeneration of myofibers, function must be established by re-formation / attachment of a neuromuscular junction (NMJ). The NMJ connects the axon of a motor neuron to a muscle fiber and is responsible for excitation / contraction coupling and voluntary motor function. The intricate interaction between MuSCs and the NMJ niche is not fully understood and as such, how neural control influences response to trauma remains an open question. Specific Aims and Study Design: The overarching purposes of this project are to understand how the regenerative capacity of MuSCs are altered when the NMJ is disrupted and to utilize this niche to enhance MuSC-based therapies against traumatic injuries. Accordingly, we will perform three specific aims: Aim 1) Identify intrinsic factors in muscle stem cells that modulate muscle repair and regeneration after disruption to the neuromuscular junction. We have developed an animal model that permits study of NMJ disruption and MuSC response and will use this model to administer severe trauma followed by isolation of purified populations of MuSCs using flow cytometry.					
15. SUBJECT TERMS NONE LISTED					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 21	19a. NAME OF RESPONSIBLE PERSON USAMRMC
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TABLE OF CONTENTS

Page

1. Introduction
2. Keywords
3. Accomplishments
4. Impact
5. Changes/Problems
6. Products
7. Participants & Other Collaborating Organizations
8. Special Reporting Requirements
9. Appendices

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

The repair and regeneration of severely damaged soft tissues such as skeletal muscle remains a substantial clinical challenge and relatively few treatments exist. The overarching purposes of this project are to understand how the regenerative capacity of muscle stem cells are altered when the neuro-muscular junction is disrupted and to utilize this niche to enhance muscle stem cell-based therapies against traumatic injuries.

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

Neuromuscular Junction, Satellite Cells, Motor Neurons, Regeneration, Single Cells, Signaling

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: Establish mechanistic insights into the intrinsic molecular mechanisms and signaling pathways that couple skeletal muscle regeneration and neural control (14 months or 11/21).

Major Task 2: Evaluate how manipulations to the NMJ can influence MuSC transplantation and functional regeneration following traumatic injury (15 months).

Major Task 3: Evaluate whether co-delivery of potent neurotrophic factor and MuSCs via an engineered biofunctional hydrogel synergistically augments regenerative capacity and functional recovery following traumatic injury (12 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. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Task 1 – Key Experiments

Isolation and characterization of muscle stem/satellite cells (MuSC) before and after nerve trauma.

- Contrasted MuSC contributions to myofibers after denervation and muscle injury using lineage tracing of MuSCs
- Compared MuSC characteristics from control (sham) and denervated muscle.
- Myogenic activity (proliferation, differentiation) of MuSCs from control (sham) and denervated muscle.
- Assess protein synthesis pathway in control and denervated myoblast.
- Comparison of muscle regeneration *in vivo*

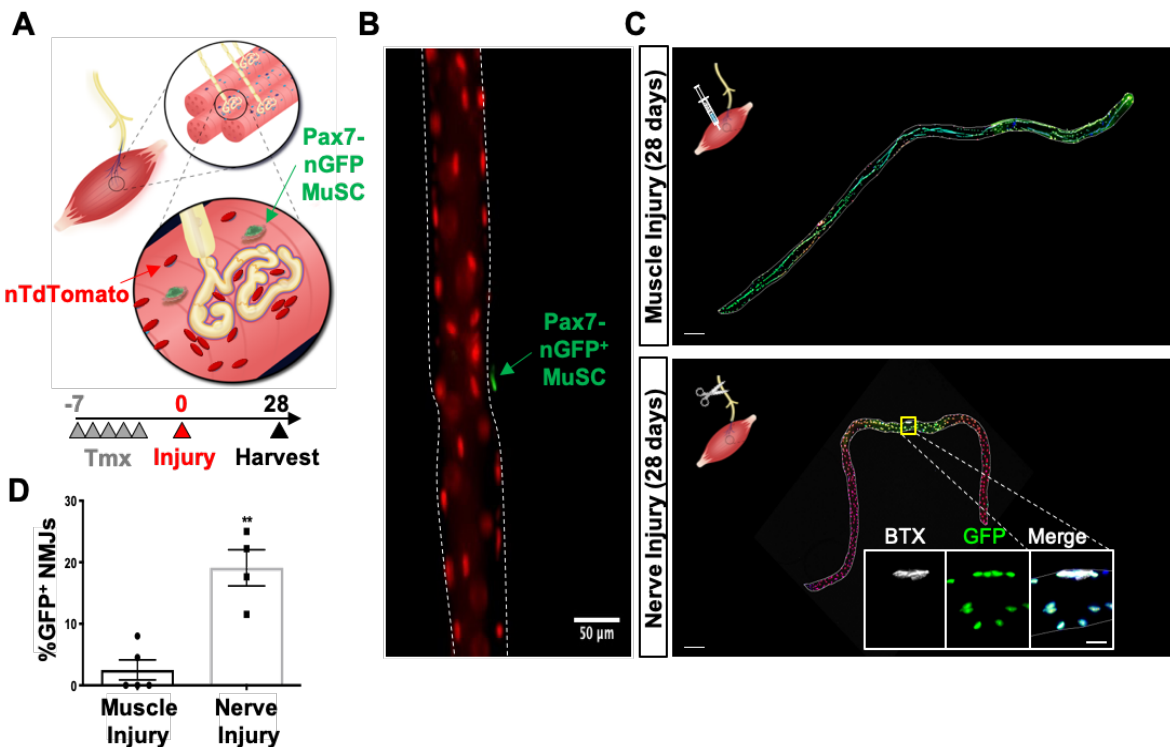
Summary of major findings

- Denervation in young mice induces engraftment proximal to the neuro-muscular junction
- Denervation in young mice increases myogenic activity of MuSCs *in vitro*
- Mild nerve injury stimulates muscle regeneration *in vivo*.

Peripheral nerve injury induces alterations in muscle stem cells.

To examine how muscle stem cell (MuSC) fate and myogenic progression are modified in response to perturbation of motor neurons, we employed sciatic nerve transection (SNT) injury. We utilized a MuSC lineage tracing system (Pax7^{CreER/+}-Rosa26^{nTnG/+}; P7^{nTnG}) whereby all nuclei contain a red fluorescent protein and after administration of tamoxifen, Pax7⁺ MuSCs and their progeny are indelibly labeled with a nuclear green fluorescent protein (nGFP, **Figure 1a**). We contrasted acute and specific perturbation of motor neurons using sciatic nerve transection (SNT), with intramuscular injection of barium chloride (BaCl₂) in extensor digitorum longus (EDL), a muscle primarily composed of Type II fibers in mice. Consistent with previous observations, imaging 5 days after tamoxifen administration, only Pax7⁺ MuSCs are initially nGFP labelled along the length of isolated myofibers, and devoid of precocious nGFP expression in myonuclei (**Figure 1b**). Examination of single myofibers 28 days after SNT or BaCl₂ injection revealed variations in MuSC-derived contribution of nGFP. As expected, BaCl₂-induced myofiber degeneration results in contribution of MuSC-derived progenitors and centrally located myonuclei along the entire length of the regenerated myofiber (**Figure 1c**). In contrast, after SNT, MuSC derived myonuclei (indicated by nGFP⁺) were confined at or near young NMJ myofiber regions (<250µm from the NMJ, **Figures 1c-d**). These results are consistent with previous reports whereby MuSC depletion leads to loss of myonuclei proximal to young NMJs after SNT. While we cannot discount that existing synaptic myonuclei incorporate nGFP after MuSC engraftment, diffusion of molecules near the NMJ is limited to prevent synaptic transcription in myonuclei outside of the junctional area. Taken together, these results show MuSCs are sensitive to NMJ disruptions, and in such contexts, fuse near NMJs.

Figure 1. Denervation induces muscle stem cell actions proximal to the neuromuscular junction. A) Schematic of neuromuscular junction (NMJ) from Pax7^{CreER/+}-Rosa26^{nTnG} mice, which display red fluorescent protein (RFP) in their nuclei and following administration of tamoxifen, Pax7⁺ MuSCs (labeled with green arrow) and their progeny are labeled with a nuclear green fluorescent protein (nGFP). B) Representative immuno-fluorescence image of single myofiber isolated from extensor digitorum longus (EDL) muscle showing GFP expression exclusively in MuSCs and red nuclei. Scale=50 μ m. C) After muscle injury through barium chloride (BaCl₂) injection (top), myofiber degeneration resulted in contribution of MuSC derived progenitors and centrally located myonuclei along the entire length of the regenerated myofiber. After sciatic nerve transection (SNT), MuSC derived myonuclei were confined at or near NMJ myofiber regions. Magnified inset images show NMJs (Acetylcholine receptor: AChR labeled with α -bungarotoxin: BTX) with nGFP⁺ nuclei. Scale bar for myofibers = 200 μ m for inset = 25 μ m. n = 5 muscles for both injury types and 20-30 myofibers counted from each isolated muscle. D) Quantification of fraction of nGFP⁺ nuclei underneath and near synapses compared to all positions on myofibers, where **p < 0.01 using two-sided t-test.



Nerve injury stimulates myogenesis and protein synthesis *ex vivo*

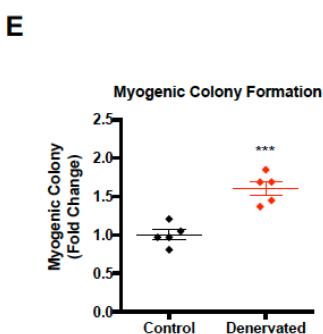
To examine changes in muscle stem cell (MuSC) dynamics following nerve injury, MuSCs were FACS-purified 7 days after sciatic nerve crush (SNC) injury (axonotmesis), in which motor neurons undergo Wallerian degeneration while the epineurium is left intact, and evaluated for myogenic changes. RNA-seq data showed that denervated MuSCs maintained similar levels of early MuSC activation markers (i.e., *Pax7*, *CD34*, *Sdc4*, *Myf5*), whereas cell cycle regulators, myogenic differentiation, and myofibril assembly markers, such as *Myogenin*, *Myf6*, *Myh1*, *Acta1*, *Tnnt1*, and *Myoglobin*, were significantly upregulated compared to contralateral controls (**Figure 2A**). These results further support the notion that denervated MuSCs are transcriptionally primed to engage in myogenesis, similar to the transition from G₀ to G_{alert} active quiescent state.

Next, to validate an enhanced myogenic activity of denervated MuSCs, we assessed proliferation and differential potential. As expected, when cells were cultured *ex vivo* for 3 days, MuSCs from muscle that underwent nerve injury showed an approximately 2-fold increase in expansion, as measured by total myoblast number (**Figure 2C**). Consistent with a higher frequency of myoblasts, denervated MuSCs exhibited increased proliferation as measured by incorporation of 5-ethynyl-2'-deoxyuridine (EdU) following 24 hours pulse-chase (**Figure 2D**). Furthermore, when single MuSC were seeded and grown in 96-well plates, MuSCs from nerve-injured muscle formed colonies with up to 50% increased efficiency as compared to control cells, indicating that denervated muscle contains a substantially higher pool of functional regenerative cells (**Figure 2E**). To further validate the improved myogenic activity of denervated MuSCs, we next measured the differentiation of these cells by reducing serum concentration. When an equal number of myoblasts were seeded in a 2% serum condition, MuSCs from denervated muscle formed multinucleated, myosin heavy chain (MHC) expressing myotubes at a much faster rate compared to control cells (**Figure 2B, bottom**). Likewise, the rate of fusion was significantly greater for myoblasts from denervated muscle. To verify the fusion capacity, we isolated control MuSCs from cyan fluorescence protein (CFP) and yellow fluorescence protein (YFP) transgenic mice, and denervated MuSCs from TdTomato transgenic mice. An equal number of myoblasts in 3 different colors were randomly seeded and allowed to differentiate for 5 days, then the fluorescence of myotubes was quantified. When CFP⁺ (control) and YFP⁺ (control) myoblasts were differentiated, cyan, green, and yellow myoblasts fused randomly, myotubes with 3 fluorescence were evenly distributed, 33%, 23%, and 44%, respectively. In stark contrast, when CFP⁺ (control) and TdTomato⁺ (denervated) myoblasts were seeded together, denervated myoblasts exhibited a higher tendency to fuse with each other (61%, TdTomato⁺) and only 14% were fused to control myoblasts (magenta⁺ - CFP + TdTomato), suggesting denervated myoblasts were fusing at a higher rate compared to control myoblasts (CFP) (**Figure 2F**).

Since activated MuSCs are characterized by metabolic changes associated with increased biosynthesis of macromolecules, and these changes are coordinated through the mammalian target of rapamycin (mTOR) signaling pathway, we used a Luminex multiplex assay to evaluate anabolic protein synthesis. In concert with upregulation in genes associated with muscle formation and increased myogenesis, the phosphorylated (activated) insulin receptor substrate 1 (IRS-1), AKT, PTEN, mTOR, TSC2, S6K, RPS-6 protein contents were significantly elevated in myotubes derived from nerve-injured mice compared to myotubes from sham controls (**Figure 2G**). Taken together, these data further validate that SNC injury enriches MuSC population toward myogenesis progression.

Figure 2. Nerve injury stimulates satellite cell proliferation, differentiation, and protein synthesis ex vivo

(A) Heatmap of key differentially expressed genes associated with satellite cell identity, cell-cycle, and myogenic differentiation. (B) Representative images of FACS purified MuSCs proliferation (top) and differentiation (bottom) in culture. Scale bar 200 μ m. (C) Quantification of total satellite cells per cluster 48 hours after seeding. *** $p < 0.001$, Mean $\pm$ SEM, $n = 3$ biological replicates. (D) Quantification of proliferating satellite cells 48 hours after seeding as measured by EdU⁺ and DAPI⁺ cells. (E) Single cell clonal expansion assays of sorted satellite cells from control or denervated mice. *** $p < 0.001$, Mean $\pm$ SEM. (F) Representative images of myogenic fusion assay from control (cyan fluorescent protein and yellow fluorescence protein) or denervated (TdTomato) mice (top). Scale bar 50 μ m. Quantification of fusion rate in percentage (bottom). Values are mean of duplicate experiments in 3 biological replicates (G) Luminex multiplex assay for protein synthesis pathway (phosphorylated IRS1, IGF1R, IR, AKT, mTOR, TSC2, RPS6, PTEN, and S6K) following 5 days in culture. * $p < 0.05$, ** $p < 0.01$, Mean $\pm$ SEM, $n = 4$ (Scale bars; (B) 200 μ m, (F) 50 μ m).



Major Task 2 – Key Experiments

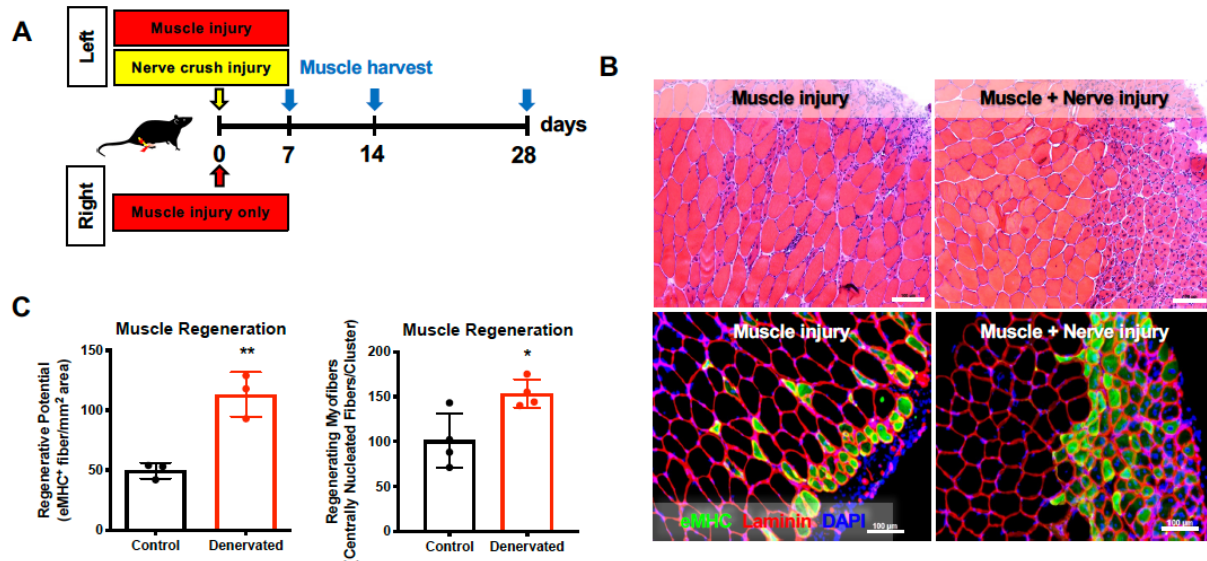
Isolation and characterization of muscle stem/satellite cells (MuSC) before and after nerve trauma.

- Hematoxylin and Eosin (H&E) staining of cross-sectioned muscle tissue before and after nerve injury.
- Immunofluorescence (IF) detection of regenerating neurons, NMJs, muscle stem cells and muscle fibers
- Quantification of muscle regenerative potential at multiple time points using histological and IF imaging

Nerve perturbation promotes a positive signaling microenvironment that augments muscle regeneration

We postulated that the positive enrichments in MuSC function induced by nerve injury would result in concomitant enhancements in muscle regeneration. Thus, we tested an injury response from a composite injury, in which SNC was combined with muscle cryo-injury to tibialis anterior (TA) and compared regenerative outcomes with muscle cryo-injury only in the contralateral TA. Muscle regeneration was evaluated at three time points (7, 14, and 28 days) after injury (**Figure 3A**). In all time points analyzed, regenerative activity, as measured by the number of centrally nucleated myofibers and embryonic myosin heavy chain (eMHC) positive fibers, was significantly greater in nerve and muscle composite injury compared to muscle injury only group (**Figures 3B and 3C**). Hematoxylin and Eosin (H&E) staining of cross-sectioned muscle tissue.

Figure 3. Nerve regeneration synergistically enhances skeletal muscle regeneration *in vivo* (A) Schematic diagram of experimental design measuring muscle regeneration *in vivo*. (B) H&E (*top*) and immunofluorescence staining (embryonic myosin heavy chain (eMHC), laminin, DAPI (*bottom*)) cross-section from TA muscle of 7-day cardiotoxin injury (right leg) and cardiotoxin + sciatic nerve pinch injury (left leg). (C) Quantification of muscle regeneration assessed by the eMHC⁺ and centrally nucleated myofibers, ** $p < 0.01$, Mean $\pm$ SEM.



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.

Nothing to report

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.

We published results from Major Task 1 in eLife, which is an open access journal. We also have deposited sequencing datasets on the Gene Expression Omnibus (GEO) and are accessible to the community for download. We will also present these results at University seminars during the next fiscal year.

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.

Major Task 1: Establish mechanistic insights into the intrinsic molecular mechanisms and signaling pathways that couple skeletal muscle regeneration and neural control (14 months or 11/21).

In the next year, we will perform sequencing of myonuclei in response to denervation to glean molecular mechanisms that promote re-innervation and can enhance muscle regeneration after trauma.

- Sequence MuSC-derived myonuclei and compare to existing myonuclei before and after neural injury
- Use fluorescence imaging to assess regeneration after trauma

Major Task 2: Evaluate how manipulations to the NMJ can influence MuSC transplantation and functional regeneration following traumatic injury (15 months).

In the next year, we will perform modulation through the NMJ to enhance muscle stem cell engraftment and improve regenerative potential

- Prime MuSC through NMJ and transplant into regenerating muscle
- Use fluorescence and intravital imaging to assess engraftment efficiency

Major Task 3: Evaluate whether co-delivery of potent neurotrophic factor and MuSCs via an engineered biofunctional hydrogel synergistically augments regenerative capacity and functional recovery following traumatic injury (12 months).

In the next year, we will assess the response of MuSCs to exposure to neurotrophic factors (BDNF and GDNF) and incorporate these proteins into our bioactive hydrogels for muscle transplantation.

- Determine MuSC responses to neurotrophic factors
- Incorporate neurotrophic factors into hydrogels and deliver to injured muscle.

4. IMPACT: *Describe 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).

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 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.*

Nothing to report

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:*

None

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.

None

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.

None

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

None

None

Significant changes in use of biohazards and/or select agents

None

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).*

- 1) Larouche, J. et al. Muscle Stem Cell Response to Perturbations of the Neuromuscular Junction Are Attenuated With Age. *eLife* **10**, e66749 (2021).
- 2) Choi, J. et al. Regenerating motor neurons prime muscle stem cells for myogenesis by enhancing protein synthesis and mitochondrial bioenergetics. *Being prepared for resubmission* (2021).

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).*

None

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.*

None

- **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.

<https://www.nobel.bme.umich.edu/>

<https://www.janglabgatech.org/>

- **Technologies or techniques**

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

None

- **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.

None

- **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.*

None

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”.

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.

Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

Name: Paula Fraczek
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 12
Contribution to Project: Ms. Fraczek has administered nerve trauma on transgenic animals, imaged single myofibers to glean muscle stem cell contributions and worked on isolating myonuclei from muscle for profiling.

Name: Mahir Mohiuddin
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 12
Contribution to Project: Mr. Mohiuddin performed denervation surgery and histological analyses of muscle on transgenic mice. He also participated in maintain and genotyping mice used in the study.

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.

No

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.*

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.*