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TITLE: Low-Intensity Vibration as a Treatment for Traumatic Muscle Injury

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Fort Detrick, Maryland 21702-5012



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**6. AUTHOR(S)**
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CHICAGO IL 60612-4305

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

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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT (~200 words of most sig finding during this period)**

Traumatic musculoskeletal injuries are among the most common injuries experienced during military combat. Poor healing of traumatic muscle injuries is associated with impaired muscle function, joint stiffness and loss of mobility. Our long-term goal is to develop a device and treatment protocol that provide a safe, inexpensive, and easy to apply treatment that will help to restore normal muscle and joint function to injured military personnel. In this report, we provide preliminary data indicating a trend towards improved healing with LIV. We observed a trend towards a larger fiber area and increased angiogenesis in muscles from LIV-treated mice vs. controls. We have initiated additional experiments to follow up on these findings. Furthermore, initial in vitro studies in macrophages (Mp) demonstrated that these cells are responsive to the LIV signals and that LIV downregulates the expression of pro-inflammatory markers and upregulates the expression of pro-healing markers in Mp. Findings from continued work on this project will provide insight into the potential for LIV as a non-invasive and simple treatment for improving muscle healing, thereby reducing joint stiffness and increasing mobility of polytrauma patients.

**15. SUBJECT TERMS**
Skeletal muscle repair, low-intensity vibration, monocytes/macrophages, endothelial precursor cells, angiogenesis, myogenesis

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
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| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRMC |
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## 1. INTRODUCTION

Traumatic skeletal muscle injuries typically result in impaired muscle function, joint stiffness and loss of mobility, which, in turn, results in significant costs for rehabilitation, loss of time for work and reduced combat readiness. Unfortunately, effective treatments for improving the recovery of muscle function and joint mobility are lacking. The proposed Idea Development study is an early-stage investigation into a novel treatment of traumatic muscle injuries – mechanical stimulation via low-intensity vibration (LIV). Mechanical stimulation has an anabolic effect on musculoskeletal tissues, and mechanical stimulation via LIV has been shown to accelerate bone regeneration. Our preliminary data indicate that LIV reduces fibrosis and enhances muscle fiber growth following traumatic muscle injury in mice. Our data also indicate that LIV increases numbers of monocytes and macrophages (Mo/Mp) and endothelial precursor cells (EPC) in the blood; these cells are known to promote healing of muscle injuries. Thus, the central hypothesis of this study is that LIV improves healing of traumatic muscle injuries by increasing the activity of Mo/Mp and EPC in damaged muscle. We will address this hypothesis in three Specific Aims: First, we will determine the effectiveness of locally applied versus whole-body LIV for improving angiogenesis and muscle regeneration and reducing fibrosis. Second, we will determine the role of bone marrow-derived cells (BMDC) in LIV-induced improvements in muscle healing. Third, we will identify specific cells that detect and transduce the LIV signal. If successful, LIV would provide an innovative, non-invasive and simple treatment for improving muscle healing and thereby reducing joint stiffness and increasing mobility of polytrauma patients.

## 2. KEYWORDS

Skeletal muscle repair, low-intensity vibration, monocytes/macrophages, endothelial precursor cells, angiogenesis, myogenesis

## 3. ACCOMPLISHMENTS

### What were the major goals of the project?

The major goals of this project were divided into three Specific Aims:

1. Determine the effectiveness of locally applied versus whole-body low-intensity vibration (LIV) for improving muscle regeneration following traumatic injury.
2. Determine the role of bone marrow-derived cells (BMDC) in LIV-induced improvements in muscle healing.
3. Identify specific cells that detect and transduce the LIV signal.

## Timeline and Cost

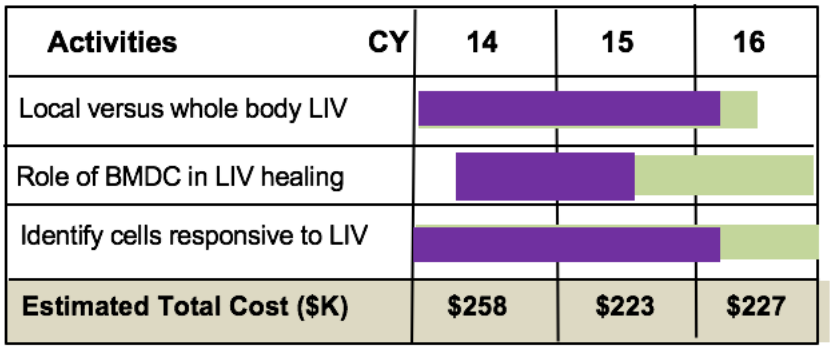



**Figure 1. Timeline for completion of Specific Aims and associated estimated costs for each calendar year.** The green bars denote the timeline for each of the three aims and the percentage of the aim completed is indicated in purple.

### What was accomplished under these goals?

Specific Aim 1

A. Assessment of Volumetric Muscle Loss (VML) injury using MRI
   i. A new model of volumetric muscle injury was established in our laboratory. A 3mm biopsy punch is used to remove a significant portion of the lateral gastrocnemius muscle (% mass removed to be determined). This new technique has been proven to be consistent and amenable to assessment using MRI. It is also more representative of traumatic muscle injury with volumetric muscle loss (VML). We have begun collaborations with Dr. Kara Spiller, Drexel University, to test the effectiveness of combined therapy with tissue engineering scaffolds and LIV treatment on recovery from VML injuries.
   ii. We have completed our first experiment using the punch model of VML injury along with MRI analysis. Eight mice were injured and either treated with whole-body LIV at 0.2g and 90 Hz or handled identically without LIV treatment. T2 images, which capture edema and inflammation, shows obvious changes within the injured muscle over time from 2 to 22 days post injury. Unfortunately, using this model of traumatic muscle injury, LIV is not showing any significant differences from the control group when looking at area, volume, or T2 signal intensity measurements (Figure 2-6). This could mean that this particular model creates too severe of an injury that cannot be improved with LIV alone and/or the MRI analysis is not sensitive enough to detect those differences. All in all, we are excited about the development of our new model of VML injury and the usefulness of MRI analysis.

Figure 2: T2 Signal Intensity over days 3, 7, and 14. T2 Signal Intensity is a measure of edema and inflammation. Values have been normalized to the signal intensity of the uninjured contralateral leg. Reduction of the signal intensity occurs over the time course.

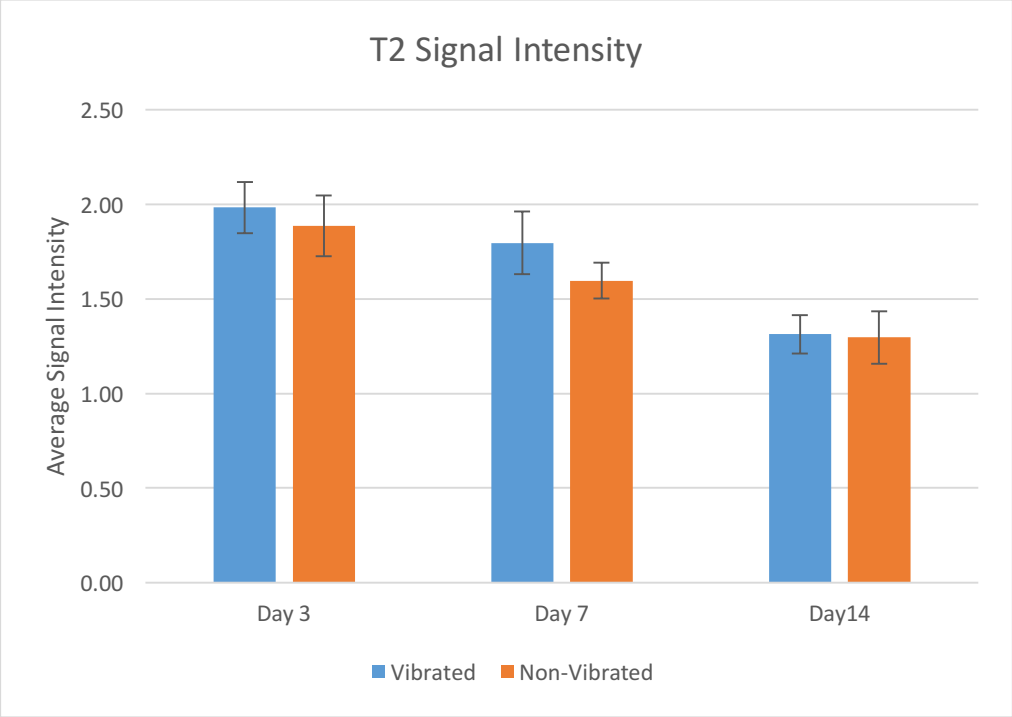



Figure 3: Percent Injured Area over days 3, 7, and 14. Values were calculated by dividing the injured area by the entire area of the injured gastrocnemius muscle. The injury as detected by T2 signal intensity decreases from day 3 to day 14. Reduction of the injured area occurs over this time course.

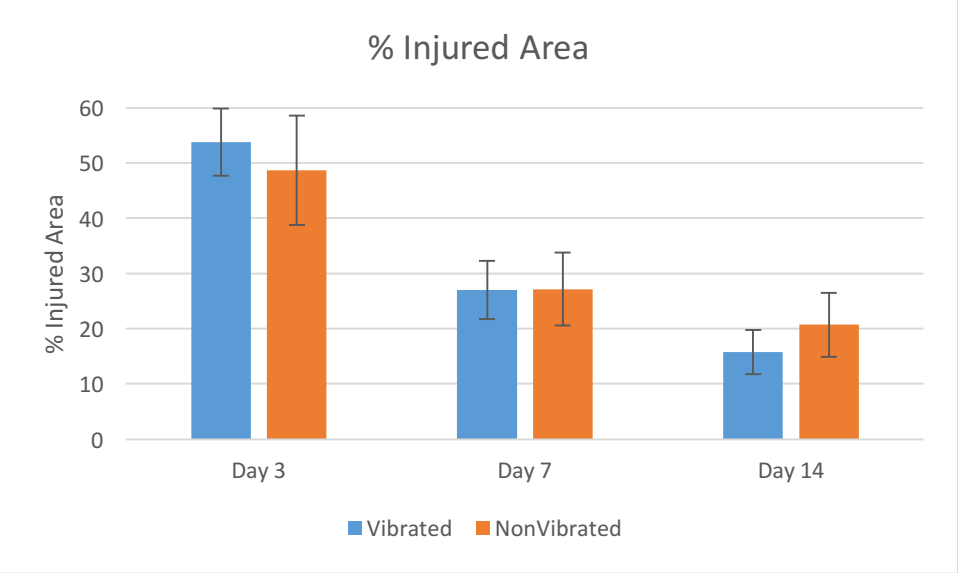


Figure 4: Area of the injured gastrocnemius. Values were obtained by measuring the area of the injured gastrocnemius on corresponding representative images. Selected images were determined based on anatomical landmarks. Day 3 shows obvious increased volume due to inflammation that subsides by day 7 and 14.

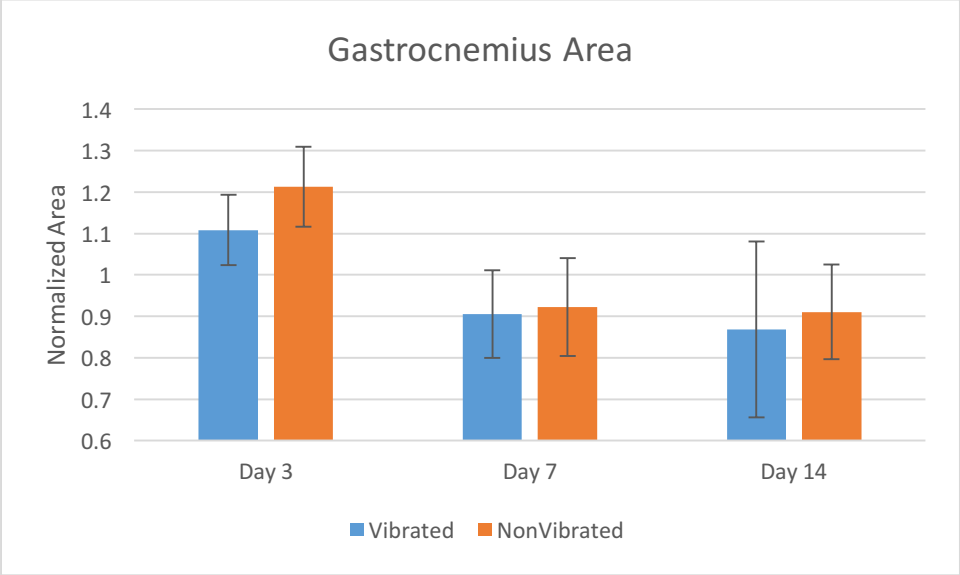



Figure 5: Injured Muscle Volume. Values were normalized to the uninjured contralateral leg.

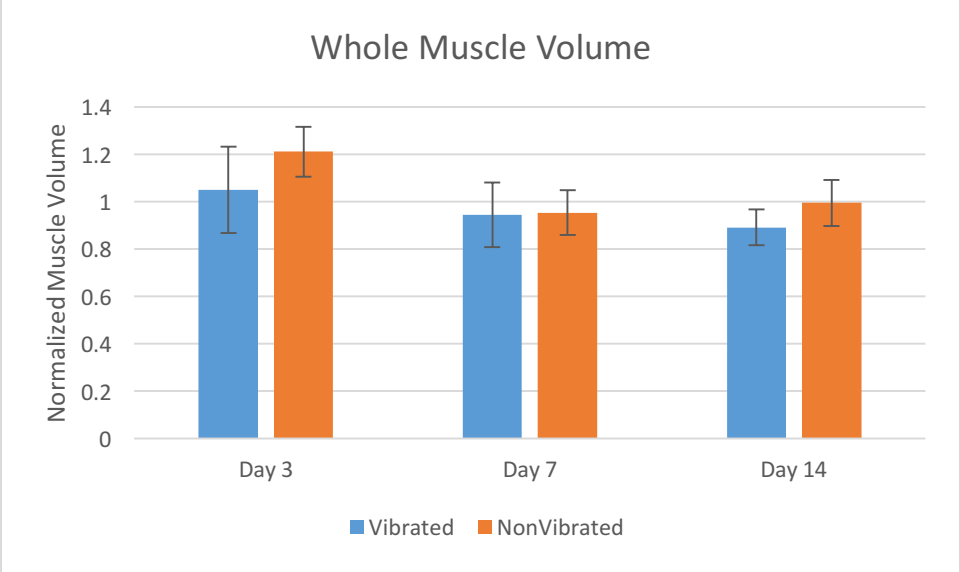
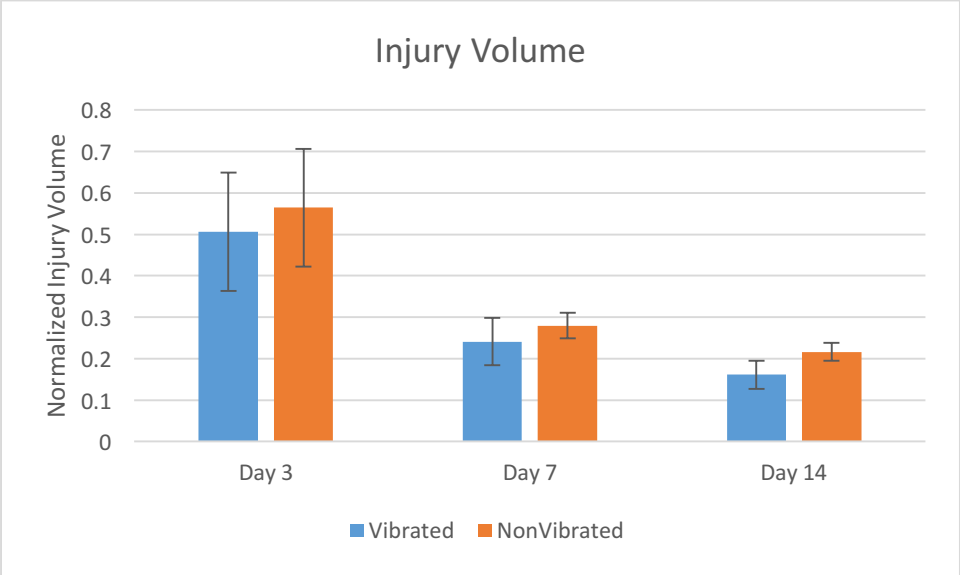


Figure 6: Injury Volume. Values were normalized to the uninjured contralateral leg.




iii. We have completed histological analysis of muscles harvested on day 23 post-injury from both LIV-treated and sham-treated mice.

a. No significant differences were detected for % damaged area, % regenerating area, % normal area, fiber diameter or number of fibers (Figures 7-9).

b. There was a significant difference between the LIV group and control for fiber area (Figure 10), which is an opposite effect from previous experiments using the laceration model. Thus, the positive effect of LIV on muscle fiber area maybe limited to non-volumetric injuries. However, we continue to work with Dr. Kara Spiller, Drexel University, to develop a collagen-based scaffold to test the effectiveness of LIV in combination with tissue engineering scaffolds on VML injuries. These scaffolds will be engineered to enhance macrophage and myoblast activity and we hypothesize that in combination with LIV, healing outcomes will improve in the VML model. This has been proposed in a grant submitted in August of 2017 to the Department of Defense as the expansion award based on data generated by the present grant. We will pursue this hypothesis in 3 specific aims.

1. Specific Aim 1 is to determine whether scaffolds designed to induce different macrophage phenotypes improve angiogenesis and nerve and muscle regeneration resulting in improved muscle and limb function.
2. Specific Aim 2 is to determine whether incorporating HGF into the scaffold that produces the most M1-like and most M2-like macrophage phenotypes, respectively, in Aim 1, further enhances myoblast infiltration and muscle fiber formation in the scaffold and has synergistic effects with the macrophage modulating effects of the scaffold, resulting in improved muscle and limb function.

3. Specific Aim 3 is to determine whether applying LIV as a rehabilitation modality further enhances macrophage activity and angiogenesis, nerve and muscle regeneration as well as muscle and limb function.

c. Muscles were analyzed for collagen deposition using Masson's trichrome. Measurements were taken for % area stained, staining intensity, and staining intensity variation and no significant differences were detected between LIV and control (Figures 11-13).

d. Staining for CD31 and F4/80 were performed. No significant difference was found in CD31 staining between treatment and control. F4/80 staining was not intense enough at day 23 to warrant analysis. This most likely means that staining for F4/80 is more appropriate at earlier time points.

Figure 7: Muscle Regeneration at day 23 post-VML injury.

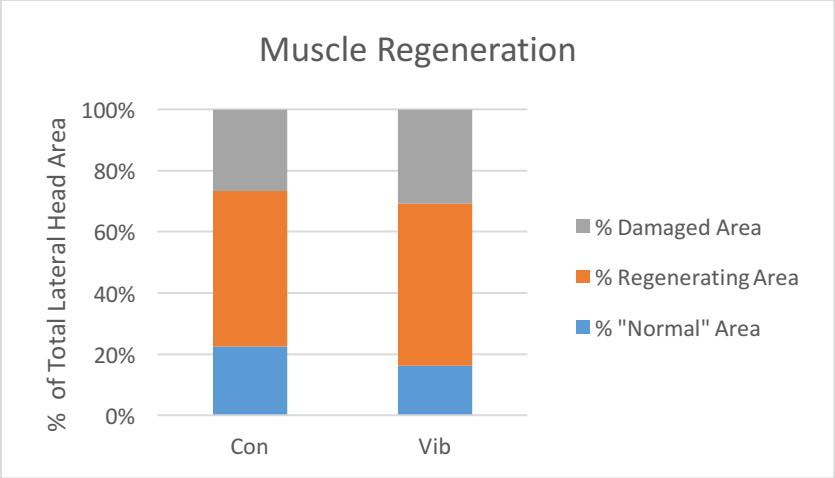


Figure 8: Average Myofiber diameter at day 23 post-VML injury.

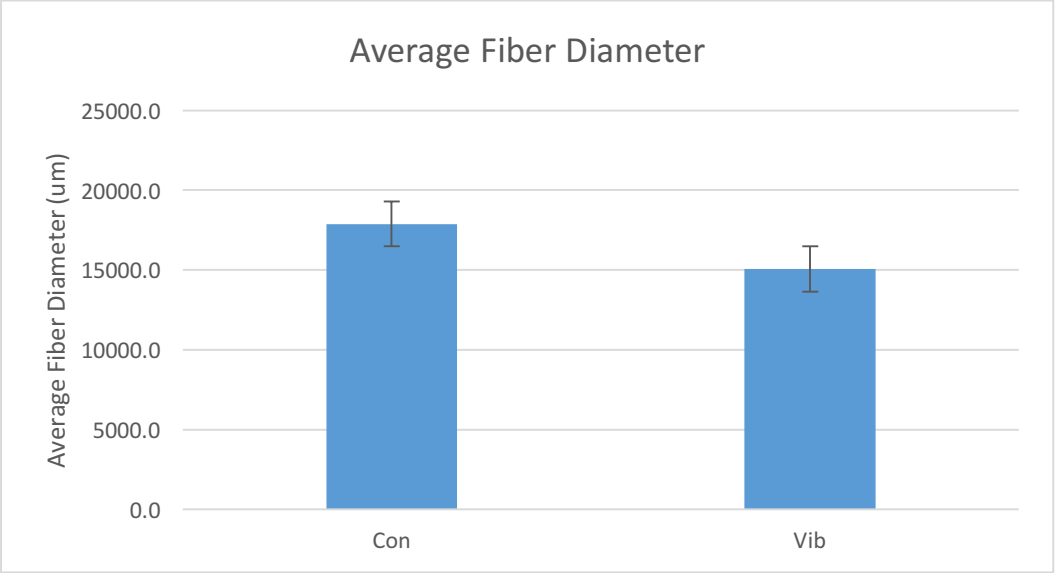



Figure 9: Number of myofibers per $mm^2$ at day 23 post-VML injury

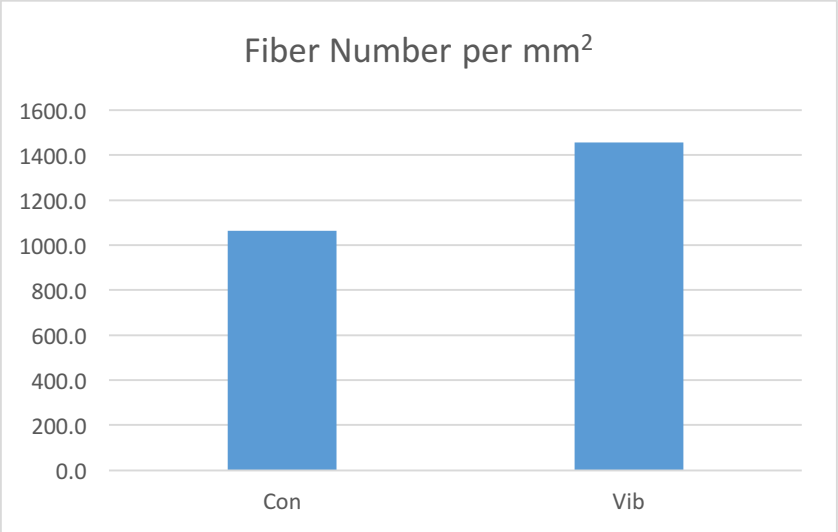


Figure 10: Average Myofiber Area at day 23 post-VML injury

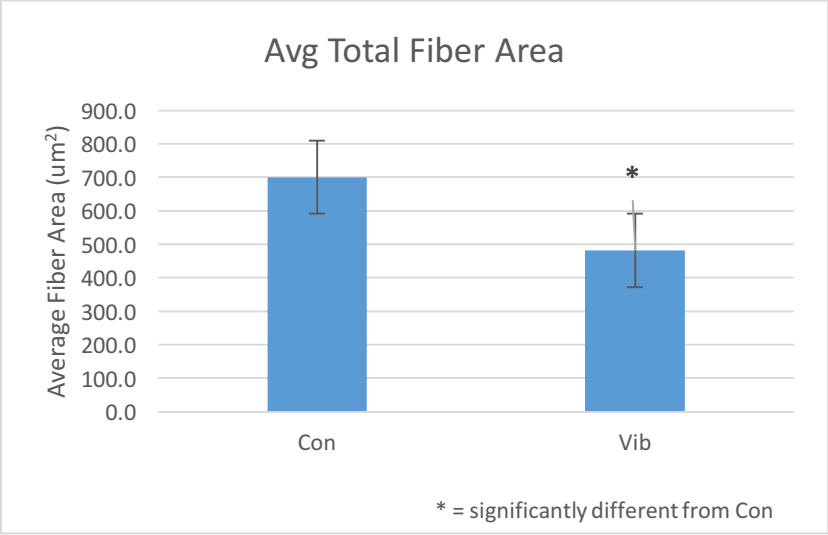



Figure 11: Percent Area Stained for Collagen at day 23 post-VML injury

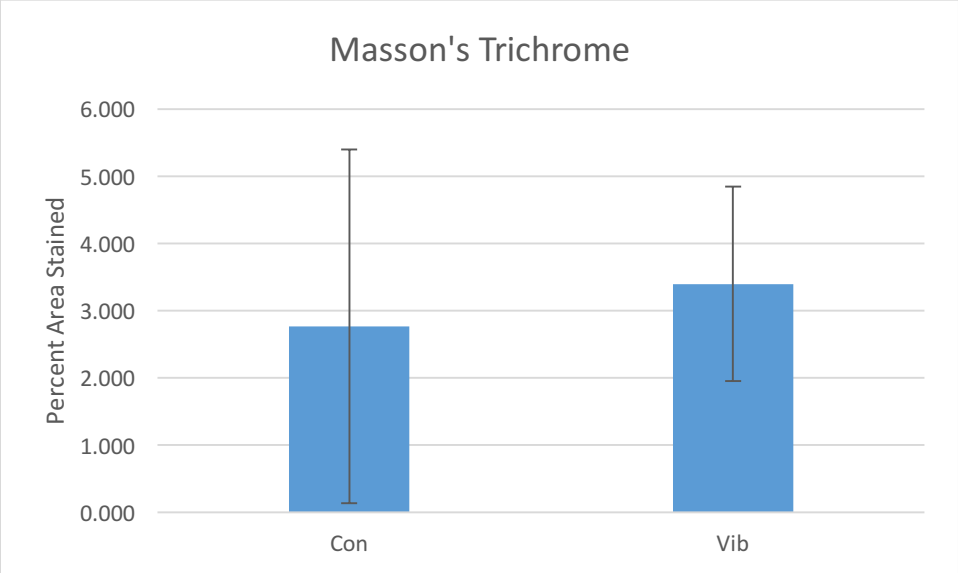


Figure 12: Collagen Staining Intensity at day 23 post-VML injury

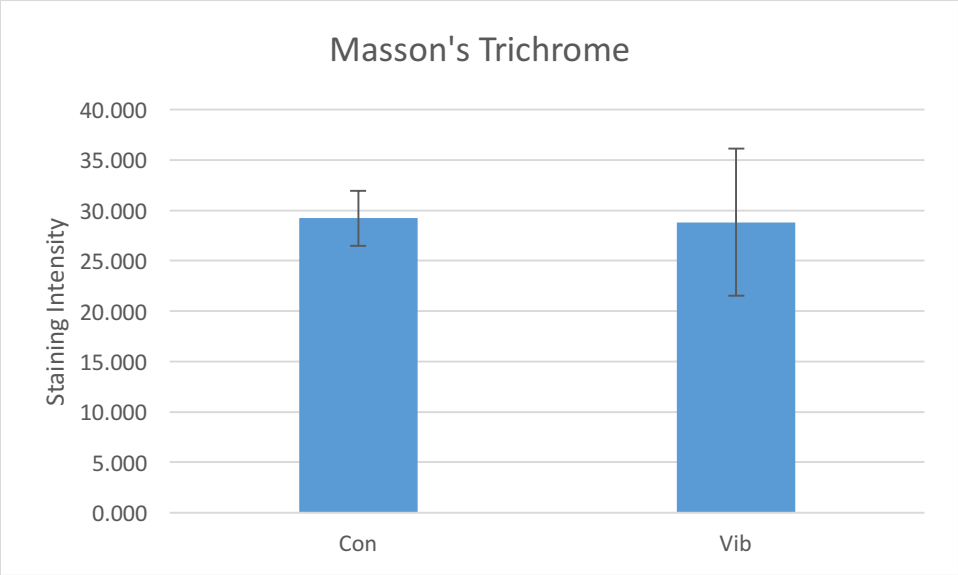
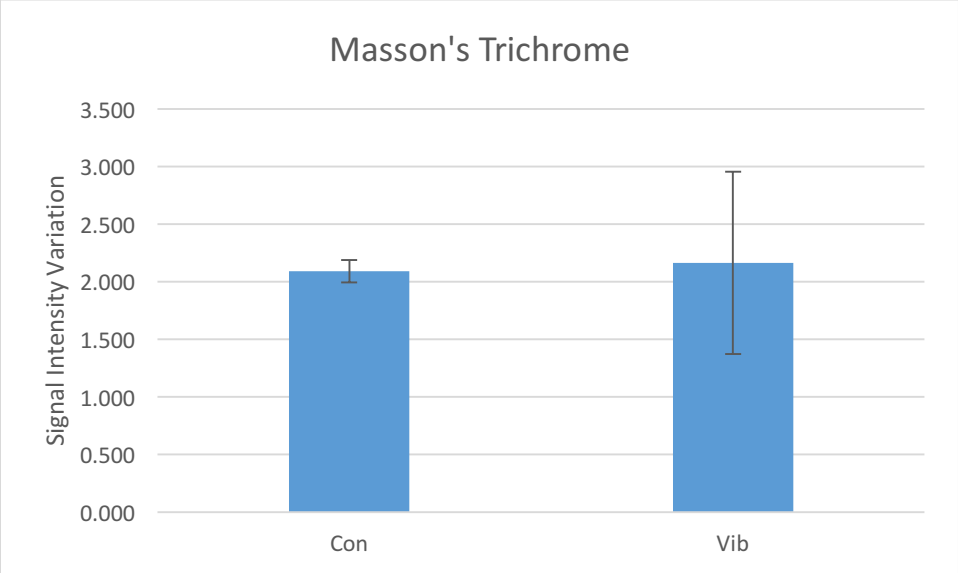



Figure 13: Collagen Staining Intensity Variation at day 23 post-VML injury



B. Effect of Local LIV on laceration injury

i. We have begun an experiment exploring the effects of local LIV on muscle healing using our laceration model of injury. Twelve mice were given laceration injuries. Six mice are being treated with local LIV for 14 days and 6 mice are being treated identically without LIV. Due to the number of mice that fit on the machine, we are performing 2 trials with 6 mice each. We are currently performing histological analysis. We are measuring morphological characteristics such as fiber area, fiber diameter, % damaged tissue, % regenerating tissue, and % normal tissue, as well as collagen deposition, new blood vessel formation, and macrophage accumulation. Figures 14 and 15 show images of the local LIV device applying a signal to the legs of the mice.

ii. We are working in conjunction with Dr. Onur Bilgen to develop a local, wearable vibration device. Dr. Bilgen is an expert on smart materials that can convert electrical energy to mechanical energy and vice versa. A prototype has been developed and we are currently exploring the most appropriate applications for this device. We plan to use this device in experiments in the future.

Figure 1: Local LIV device with mice being treated

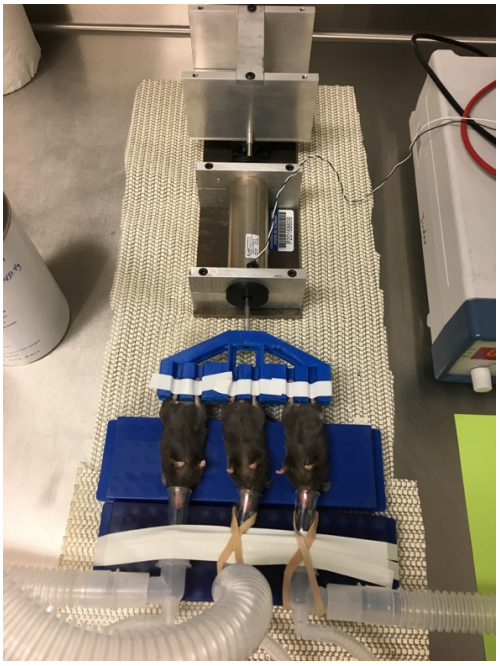
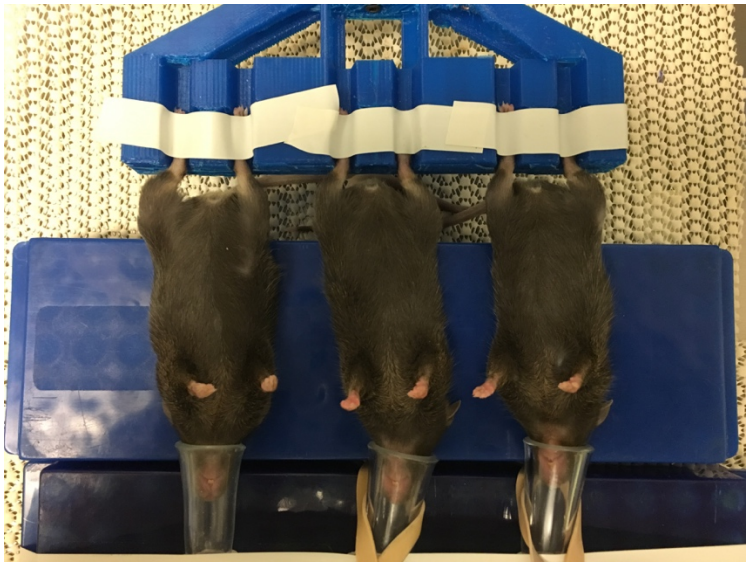


Figure 2: Closer view of mice on Local LIV device



Specific Aim 2

In the upcoming year, under the recently approved no cost extension, we will be performing experiments to determine the role of bone marrow-derived cells (BMDC) in LIV-induced improvements in muscle healing. Experiments will be performed to determine whether LIV increases mobilization and homing of monocytes/macrophages (Mo/Mp) and hematopoietic stem and precursor cells (HSPC) following traumatic muscle injury. This will be done by collecting cells from bone marrow, blood and damaged muscle from mice in Specific Aim 1, measuring numbers of Mo/Mp and HSPC using fluorescent cell sorting (FACS) analysis. We will also double-stain muscle cryosections from Specific Aim 1 to corroborate FACS analysis and to identify the specific locations of Mo, Mp, and HSPC in the damaged muscles. Experiments will also be performed to determine whether LIV enhances the pro-angiogenic and pro-healing phenotype of Mo/Mp and HSPC following traumatic muscle injury. This will be done by RNA isolation and real time PCR to assess expression of a panel of genes associated with angiogenesis, muscle regeneration and fibrosis (e.g. VEGF, HGF, IGF-1, TGF-$\beta$1, CTGF).

Specific Aim 3

In the upcoming year, under the recently approved no cost extension, we will be performing experiments to identify specific cells that detect and transduce the LIV signal. First, we will determine whether LIV directly alters expression of pro-healing or pro-inflammatory genes in cultured Mp. This will be done by generating cultures of bone marrow-derived Mp, subjecting them to LIV signals, and measuring expression of genes associated with angiogenesis, regeneration, and fibrosis (e.g. VEGF, FGF-2, HGF, IGF-1, TGF-$\beta$1, CTGF) by real time PCR. Another set of experiments will involve generating cultures of bone marrow-derived Mp, subjecting them to LIV signals, and collecting conditioned medium. We will assess proliferation and differentiation in the absence and presence of conditioned medium from Mp that had been left unstimulated or stimulated with LIV.

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

Nothing to report

**How were the results disseminated to communities of interest?**

Nothing to report

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

During the next reporting period, we plan the following:

1. We will initiate experiments for Specific Aim 1, Major Task 1, Subtasks 3 and 4, performing laceration injuries and applying optimized LIV signals locally to injured site or systemically via whole body vibration and assessing muscle regeneration and fibrosis in the injured muscles.
2. We will also continue experiments for Specific Aim 2, Major Task 1, Subtask 1, determining whether LIV increases mobilization and homing of bone marrow derived cells (BMDC).
3. We will continue experiments for Specific Aim 3, Major Task 1, Subtask 1, determining whether LIV directly induces expression of pro-healing genes in BMDC, as well as Specific Aim 3, Major Task 2, Subtask 1, determining whether LIV directly induces secretion of growth factors associated with angiogenesis, regeneration, and fibrosis. (Subtask 4).
4. We will initiate experiments for Specific Aim 3, Major Task 2, Subtask 4, determining whether LIV effects the proliferation or differentiation of C2C12 muscle cells.

## 4. IMPACT

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

Traumatic musculoskeletal injuries are among the most common injuries experienced during military combat and recovery from these injuries is typically prolonged and incomplete, leading to impaired muscle function, joint stiffness and loss of mobility. Unfortunately, effective treatments for improving the recovery of muscle structure and function and consequent joint mobility are lacking. Our long-term goal is to develop a device and treatment protocol that provide a safe, inexpensive, and easy to apply treatment that will help to restore normal muscle and joint function to injured military personnel. The proposed animal and cell culture studies will help to identify the optimal methods for delivery of LIV signals to the damaged muscle and will begin to elucidate the mechanisms by which LIV signals improve healing. If successful, LIV would provide an innovative, non-invasive and simple treatment for improving muscle healing and thereby reducing joint stiffness and increasing mobility of polytrauma patients.

**What was the impact on other disciplines?**

Nothing to report

**What was the impact on technology transfer?**

Nothing to report

**What was the impact on society beyond science and technology?**

Nothing to report

## 5. CHANGES/PROBLEMS

**Changes in approach and reasons for change**

Nothing to report

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

Nothing to report

**Changes that had a significant impact on expenditures**

Nothing to report

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

Nothing to report

## 6. PRODUCTS

**Publications, conference papers, and presentations**

A paper was submitted for publication using the data from the experiments performed in the previous grant year and has been reviewed by the Journal of Functional Morphology and Kinesiology (JFMK). Minor revisions were made and the revised manuscript has been resubmitted. These data show that, compared to non-LIV control mice, myofiber cross-sectional area was larger in mice treated with two different LIV protocols (90 Hz/0.2 *g* and 45 Hz/0.4 *g*). Minimum fiber diameter was also larger in mice treated with LIV of 90 Hz/0.2 *g*. There was also a trend toward a reduction in collagen deposition at 45 Hz/0.4 *g* ($p = 0.059$). These findings suggest that LIV may improve muscle healing by enhancing myofiber growth and reducing fibrosis (Specific Aim 1, Major Task 1, Subtask 3 and 4).

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

Nothing to report

**Technologies or techniques**

Nothing to report

**Inventions, patent applications, and/or licenses**

Nothing to report

**Other Products**

Nothing to report

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**

| | |
|---|---|
| **Name:** | Timothy Koh |
| **Project Role:** | PI |
| **Researcher Identifier (e.g. ORCID ID):** | 0000-0001-6549-7060 |
| **Nearest person month worked:** | 1 academic month, 1 summer month |
| **Contribution to Project:** | Oversaw all aspects of the study including the in vivo LIV experiments and other activities at UIC |

| | |
|---|---|
| **Name:** | Stefan Judex |
| **Project Role:** | Co-I (SBU) |
| **Researcher Identifier (e.g. ORCID ID):** | 0000-0002-4511-1535 |
| **Nearest person month worked:** | 2 summer months |
| **Contribution to Project:** | Oversaw in vitro experiments at SBU and worked with machine shop at SBU to manufacture device for local application of LIV |

| | |
|---|---|
| **Name:** | Norifumi Urao |
| **Project Role:** | Co-I (UIC) |
| **Researcher Identifier (e.g. ORCID ID):** | 0000-0001-9750-8406 |
| **Nearest person month worked:** | 3 calendar months |
| **Contribution to Project:** | Oversaw MRI data collection and analysis. |

| | |
|---|---|
| **Name:** | Thomas Corbiere |
| **Project Role:** | PhD Student |
| **Researcher Identifier (e.g. ORCID ID):** | 0000-0001-5408-0024 |
| **Nearest person month worked:** | 6 calendar months |
| **Contribution to Project:** | Performed analyses for Specific Aim 1 and was trained to perform in vivo and in vitro experiments going forward. |

| | |
|---|---|
| **Name:** | Aaron Damato |
| **Project Role:** | Graduate Student (SBU) |
| **Researcher Identifier (e.g. ORCID ID):** | 0000-0003-0557-0849 |
| **Nearest person month worked:** | 4 calendar months |
| **Contribution to Project:** | Will continue in vitro experiments on macrophages for Specific Aim 3. |
| **Funding Support:** | Biomedical Engineering Department at SBU and National Aeronautics and Space Administration |

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

**What other organizations were involved as partners?**

**Organization name:** Stony Brook University

**Location:** Stony Brook, New York

**Partner's contribution to the project:** Dr. Judex completed the manufacturing of a device to deliver LIV locally to injured tissue and to accomplish the tasks in Specific Aim 3. The initial in vitro experiments for Specific Aim 3 in macrophages were completed at SBU.