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

PRINCIPAL INVESTIGATOR: Dr. Timothy Koh

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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.					
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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

Activities	CY	14	15	16
Local versus whole body LIV				
Role of BMDC in LIV healing				
Identify cells responsive to LIV				
Estimated Total Cost (\$K)		\$258	\$223	\$227

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

In the Statement of Work for this project, the goal of Specific Aim 1 is to determine the effectiveness of locally applied versus whole-body low-intensity vibration (LIV) for improving muscle regeneration following traumatic injury. During this second year, progress has been made on many Subtasks related to Major Task 1. With the departure of Dr. Eileen Weinheimer-Haus, Mr. Thomas Corbiere was recruited and hired to fill her position. After Mr. Corbiere was trained on the muscle injury technique, he initiated analysis of the tissue samples from the replicate experiment that was performed to follow up on the preliminary trends seen in the first cohort of mice aiming to optimize the whole body LIV signal. For these experiments, mouse gastrocnemius muscles were subjected to laceration injury and then mice were subjected to daily bouts of whole-body LIV at 0.2 g and 90 Hz or handled identically without LIV treatment for controls. Fourteen days after injury, muscles were harvested and healing was assessed. In our preliminary results from cohort 1 as reported in the previous annual report, no statistically significant differences in healing outcomes were found between LIV-treated mice and non-LIV control mice. While we did not see a statistically significant difference between treatment groups, there were trends towards a larger fiber area with mice receiving the 0.2 g at 90 Hz LIV compared to controls. Cohort 2 showed a significant increase in muscle fiber area (Figure 2a). Fiber diameter also showed a trend towards increasing with LIV, although not significantly (Figure 2b). The percentage of normal area showed a significant increase with vibration (Figure 2d). This data set also showed close to significant decreases (p -value = 0.078) in the percentage of damaged area at 14 days post injury (Figure 2e). No significant difference was found between LIV and control mice for collagen deposition or angiogenesis (Figure 3a-b). With this most recent data reaffirming the trends we saw from cohort 1, we are reasonably confident that LIV at 0.2 g and 90 Hz leads to improved muscle regeneration following laceration injury as seen by the increased normal area and increased fiber area, although more needs to be tested in regards to the mechanisms and cell types involved as well as other vibration parameters that may be optimal.

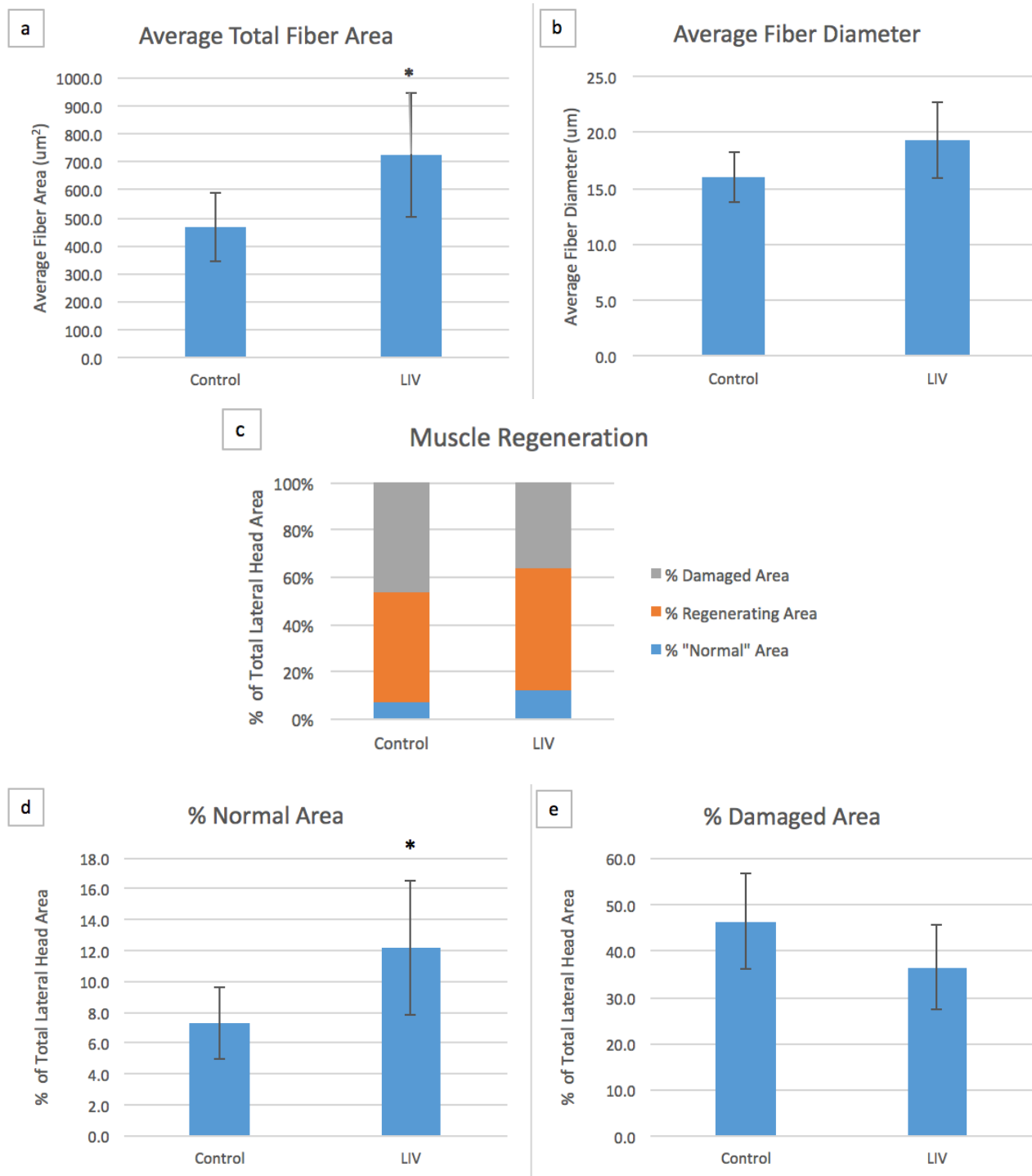


Figure 2. Effect of low-intensity vibration (LIV) at 0.2 g and 90 Hz on muscle healing following laceration injury. Mouse gastrocnemius muscles subjected to laceration injury and then mice either subjected to 30 minute bouts of whole-body LIV at 0.2 g at 90 Hz, 5 d/wk or handled identically without LIV treatment for controls. Fourteen days after injury, muscles were harvested and healing was assessed in cryosections stained with hematoxylin and eosin. * p-value < 0.05 vs control. Data represent mean ± SD, n=7-8 per group.

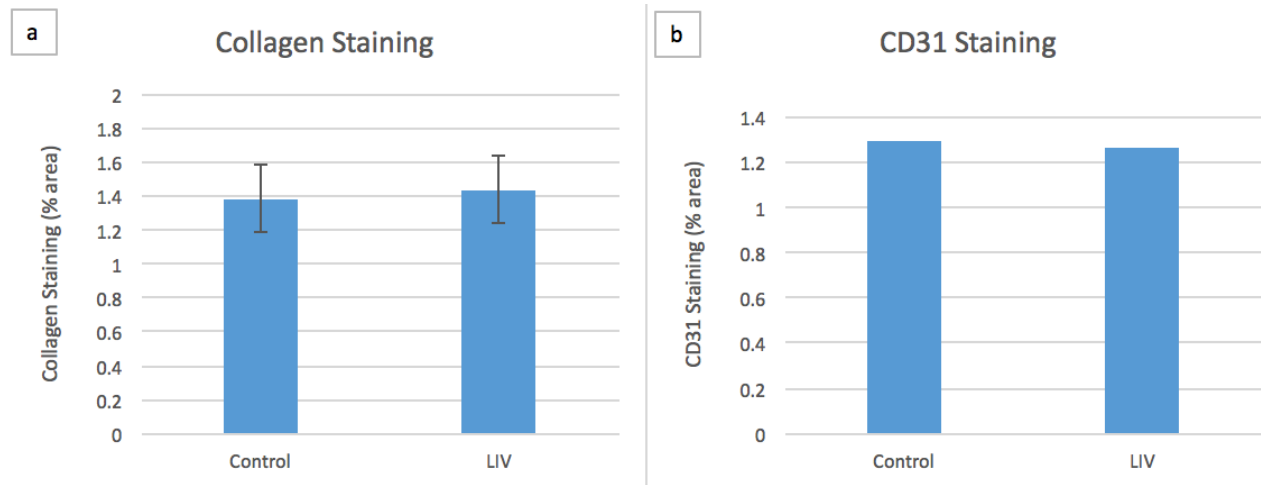


Figure 3. Effect of low-intensity vibration (LIV) at 0.2 g and 90 Hz on muscle healing following laceration injury. Mouse gastrocnemius muscles subjected to laceration injury and then mice either subjected to 30 minute bouts of whole-body LIV at 0.2 g at 90 Hz, 5 d/wk or handled identically without LIV treatment for controls. Fourteen days after injury, muscles were harvested and healing was assessed in cryosections stained with (a) Masson's Trichrome, or (b) CD31 (angiogenesis). Data represent mean \pm SD, n=7-8 per group.

As seen in some of the above histological data, the current model of injury being used to investigate Specific Aim 1 has some inherent variability which makes obtaining consistent results challenging. In an effort to deal with this, we are developing a similar model of injury that can be used with MRI analysis. MRI analysis will allow us to analyze inflammation, necrosis, and fibrosis non-invasively in a serial manner over the time course of healing and may allow more consistent analysis. However, our previous laceration model of injury does not generate a large enough volume injury to be consistently detected by MRI. Thus, rather than using a scalpel to make a thin laceration through the lateral gastrocnemius muscle, we are testing the use of a 3 mm biopsy punch to create an injury with a larger volume that is more easily detectable with MRI. We also expect this model of injury to be even more representative of traumatic muscle injury induced perhaps by a projectile than the current model. Representative MRI images have been included using the original model of laceration (Figure 4). Experiments using the biopsy punch model are in progress.

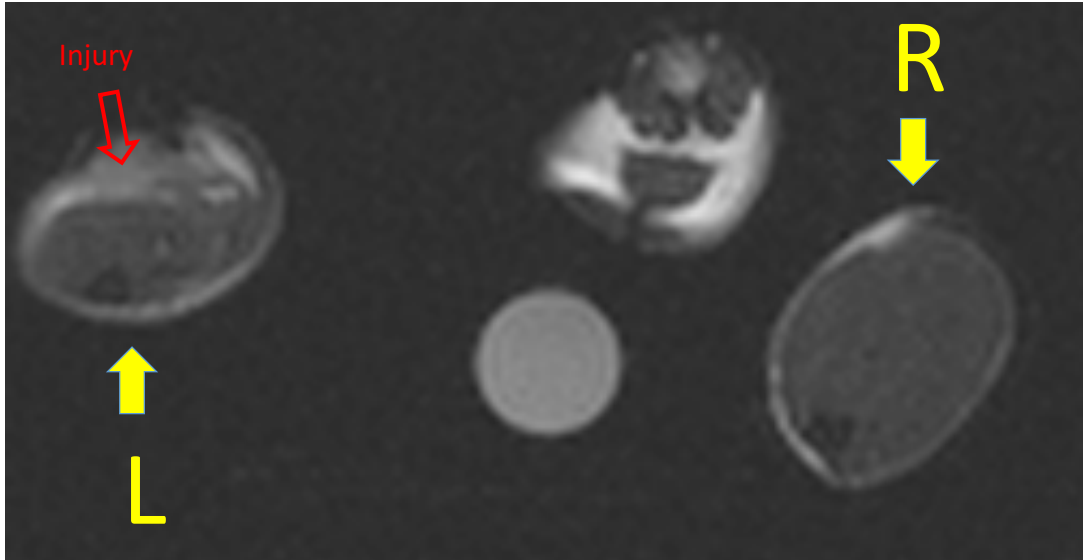


Figure 4. Magnetic Resonance Imaging of muscle laceration injury with T2 contrast and gadolinium enhancement. Mouse gastrocnemius muscles were subjected to laceration injury and scanned using MRI 2 days post-injury. In this image, the left (L) gastrocnemius was injured while the right (R) gastrocnemius was left uninjured. Using T2 contrast, areas of bright contrast represent inflammation (indicated by red arrow) and edema within the muscle while darker areas show normal muscle tissue. Area and volume measurements can be taken using these images in a serial manner over various time points.

The manufacturing of the device for local delivery of LIV, as outlined in Subtask 2, has been completed. The equipment has been set up and calibrated. Thus, experiments for Specific Aim 1, Major Task 1, Subtasks 3 and 4 that apply LIV signals locally are in the planning stages.

Specific Aim 2

In the Statement of Work for this project, the goal of Specific Aim 2 is to determine the role of bone marrow-derived cells (BMDC) in LIV-induced improvements in muscle healing. Experiments were performed in which mouse gastrocnemius muscles were subjected to laceration injury and then mice were subjected to daily bouts of whole-body LIV at 0.2 g and 90 Hz or handled identically without LIV treatment for controls. Fourteen days after injury, muscles were harvested and healing was assessed. Macrophage accumulation showed a trend towards increasing with LIV, however, high variability prevented this trend from becoming significant. These trends alongside the in vitro data suggest that macrophages may play a role in the response to LIV.

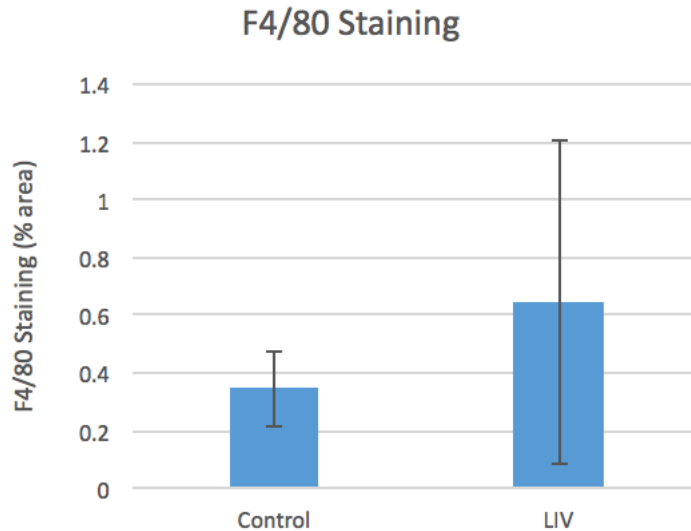


Figure 5. Effect of low-intensity vibration (LIV) at 0.2 g and 90 Hz on muscle healing following laceration injury. Mouse gastrocnemius muscles subjected to laceration injury and then mice either subjected to 30 minute bouts of whole-body LIV at 0.2 g at 90 Hz, 5 d/wk or handled identically without LIV treatment for controls. Fourteen days after injury, muscles were harvested and healing was assessed in cryosections stained with F4/80 (macrophages). Data represent mean \pm SD, n=7-8 per group.

Specific Aim 3

In the Statement of Work for this project, the goal of Specific Aim 3 is to identify specific cells that detect and transduce the LIV signal. As reported in the previous annual report, *in vitro* LIV experiments were performed in murine macrophages (J774.1) to evaluate the effect of varying LIV signals on cell viability, proliferation, and phenotype. The student performing this work graduated and left the project and it took Dr. Judex some time to find a replacement. Aaron Damato was hired and is currently being trained on the methods required for work on Specific Aim 3. Mr. Corbiere was also trained on cell culture work and will be planning experiments to perform at UIC pertaining to Specific Aim 3 using C2C12 cells.

In the past year, we published data indicating macrophages are responsive to LIV, which downregulates expression of pro-inflammatory markers and upregulates expression of pro-healing markers (Specific Aim 3, Major Task 1, Subtask 1 and Major Task 2, Subtask 1) in the *Journal of Biomechanics*[1]. An abstract of these data was also submitted for presentation in the American Society for Bone and Mineral Research annual meeting and was published in the *Journal of Bone and Mineral Research* [2].

References

1. Pongkitwitoon, S., et al., *Low-intensity vibrations accelerate proliferation and alter macrophage phenotype in vitro*. *Journal of Biomechanics*, 2016. **49**(5): p. 793-796.
2. Pongkitwitoon, S., et al., *Low Intensity Vibrations Alter Macrophage Phenotype*. *Journal of Bone and Mineral Research*, 2015. **30**: Supplement 1

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

Nothing to report

How were the results disseminated to communities of interest?

1. Ms. Pongkitwitoon presented data at the 2015 American Society for Bone and Mineral Research Annual Meeting on October 12, 2015 which showed that cultured macrophages are responsive to LIV by downregulating expression of pro-inflammatory markers and upregulating expression of pro-healing markers (Specific Aim 3, Major Task 1, Subtask 1 and Major Task 2, Subtask 1).
2. Mr. Corbiere gave a presentation at the Clinical and Rehabilitative Medicine Research Program (CRM RP) In-Progress Review Conference on February 5, 2016.

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 a new model of laceration injury using a biopsy punch and MRI. MRI will be used to assess inflammation, necrosis, and fibrosis.
2. 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.
3. 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).
4. 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).
5. 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

1. Dr. Weinheimer-Haus left the project in September 2015 and Thomas Corbiere was hired in her place to continue the work that she began as of January 2016.

Changes that had a significant impact on expenditures

1. The amount expended in quarters 1 and 2 were below projections because of departure of Dr. Weinheimer-Haus and the training of Thomas Corbiere on the in vivo experiments required for Specific Aim 1.
2. The amount expended in quarter 3 was below projections because Dr. Judex had not yet hired a new graduate student and, thus, increased his effort to 1.56 summer months to facilitate the training of Aaron Damato for work on Specific Aim 3.

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 written using the data indicating macrophages are responsive to LIV, which downregulates expression of pro-inflammatory markers and upregulates expression of pro-healing markers (Specific Aim 3, Major Task 1, Subtask 1 and Major Task 2, Subtask 1) from the experiments performed in the previous grant year and was published in the Journal of Biomechanics[1]. An abstract was also submitted for presentation to the American Society for Bone and Mineral Research annual meeting. This abstract was published in the Journal of Bone and Mineral Research [2].

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:	Thomas Corbiere
Project Role:	PhD Student
Researcher Identifier (e.g. ORCID ID):	0000-0001-5408-0024
Nearest person month worked:	12 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.