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PRINCIPAL INVESTIGATOR: Brad M. Isaacson, Ph.D.

CONTRACTING ORGANIZATION: Henry M. Jackson Foundation for Advancement of Military Medicine
Bethesda, MD 20817

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Establishing the Mineral Apposition Rate of Heterotopic Ossification for Prevention of Recurrence.

Brad M. Isaacson, Ph.D.; Paul F. Pasquina M.D.
Roy D. Bloebaum Ph.D.; Kyle Potter M.D.
E-Mail: brad.isaacson@utah.edu

Henry M. Jackson Foundation for Advancement of Military Medicine
Bethesda, MD 20817

U.S. Army Medical Research and Materiel Command
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In Year 1 of this grant the team established the necessary infrastructure and received full IRB approval. The investigators recruited 40% of their intended study population and demonstrated the ability to flurochrome label ectopic bone samples. Histological data is still being analyzed however; the first four patients of our study have mineral apposition rates (MAR) that are approximately 2x as rapid as non-pathological human bone (1.92 +/- 0.09 microns per day vs. 1.0 micron per day). Additionally, localized regions of bone demonstrated high metabolic growth, with MAR approaching 3.5 microns per day. Scanning electron microscopy has provided images of the varying stages of bone mineralization and highly vascular channels in HO. Salary support for the investigators has resulted in 2 peer-reviewed publications (1 accepted, 1 submitted) and grant W81XWH-12-2-0017 was acknowledged.
Table of contents

1. Introduction .......................................................... 4
2. Body........................................................................ 4
3. Key Research Accomplishments ............................................. 6
4. Reportable Outcomes .......................................................... 7
5. Conclusions ..................................................................... 7
6. References ....................................................................... 7
7. Appendix A ..................................................................... 8
1. Introduction
Investigating ways to alleviate complications with heterotopic ossification (HO) has high clinical significance for our wounded warriors. As noted in the original submission, approximately 63% of service members who sustain amputations as a result of improvised explosive device (IED)-trauma, experience problems with this ectopic bone formation (1). HO may require surgical revision and delay rehabilitation regimens for those seeking to return to active duty or reintegrating to the community. Therefore, this grant focuses on using novel histological techniques to determine the rate of HO growth and markers such as osteoblast/osteoclast indices and mineral apposition rate (MAR) to improve surgical planning. The primary aim of this research initiative is to correlate HO growth with recurrence and thereby provide histological evidence to support clinical recommendations as to when the bony mass should be excised.

2. Body
2.1 Institutional Review Board Approval
In order to meet our grant deliverables in a timely manner, the investigators received full IRB approval for this study during Q1 and actively engaged the orthopedic surgeons at Walter Reed National Military Medical Center (WRNMMC). We explained the intent of the study and how the work collected herein may impact the future clinical care for those requiring HO removal.

2.2 Patient Recruitment
The investigators have been proactive and given PowerPoint presentations, prepared posters for recruitment outside of the rehabilitation locations and regularly attended the doctor’s clinics during pre-operative counseling. Because of this level involvement, the investigators have recruited 16 patients in year 1 (40% of the intended study sample size in 8 month). Patients have been administered tetracycline and symptomatic HO has been removed in 14 of these patients thus far.

2.3 HO Characterization
Fourteen wounded warriors recruited in our study have had their surgeries to remove the ectopic bone in their residual limbs. Two patients have also been scheduled for surgical intervention at WRNMMC in the upcoming weeks. A complete breakdown of the histological timeline for the fourteen participants who have had surgical intervention is as followed:

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Goss Photography (Fig 1)</th>
<th>Radiographs</th>
<th>Dehydrated</th>
<th>PMMA Embedment</th>
<th>Section</th>
<th>Micro-Radiograph (Fig 1)</th>
<th>SEM (Fig 2)</th>
<th>Ground to 70µm</th>
<th>MAR (Fig 3)</th>
<th>Counting Labels</th>
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Table 1: Breakdown of each patient’s histology schedule since resection.
Figure 1: Gross photography and micro/macro radiography from one of the bone samples removed from the wounded warriors included in this study. Note that there is a combination of cortical and trabecular bone (as demonstrated in the microradiograph).

Figure 2: SEM images from three regions within one patient’s excised bone samples. These images demonstrate the continued growth and development of this bone even at surgical resection (as indicated by the gray regions of the bone which are still remodeling). Also, this bone is highly vascular and will likely correlate
with the increase MAR rates (vascular networks shown by large pores within the bone sections – see top right image).

Figure 3: Tetracycline labels from patient 1’s resected bone samples (as indicated by arrows). These images were used for quantitative measurement to determine MAR. Note: this is the first time ectopic bone has been seen with flurochrome labeling.

2.4 Data Analyses
Correlation between SEM, MAR, patient demographical information, preoperative serum alkaline phosphatase levels and bone scan activity with the rate and size of HO recurrence have not yet been evaluated in this study. Complete histological analysis of each patient’s bone specimens requires 3-4 months; therefore, once more patients have had full microscopic analysis, data will be compared using parametric and non-parametric statistics. However, the team has provided a brief synopsis in the Reportable Outcomes section below.

2.5 Peer-Reviewed Publications
The investigators have had 1 manuscript accepted in the *Journal of Prosthetics and Orthotics* and submitted another to the *Journal of Spinal Cord Medicine* since the inception of this grant. Since W81XWH-12-2-0017 supported the PI’s salary, this grant is listed in the acknowledgments section (See Appendix A).

2.6 Literature Review
To ensure that no key information is omitted from future publications, the PI has focused a great deal of time reading a diverse collection of HO literature. An extensive list of articles has been collected and is being reviewed on a daily basis.

3. Key Research Accomplishments
* Established the infrastructure needed to conduct research
* Received full IRB approval from WRNMMC
* Achieved 40% patient recruitment within 8 months
* Photographed excise bone samples and performed contact microradiographs (Fig 1)
* Obtained MAR measurements and SEM images of HO (Fig 2)
* Demonstrated the ability to flurochrome label ectopic bone (Fig 3)
* Wrote 2 manuscripts (1 accepted, 1 submitted) (See Appendix A)
4. Reportable Outcomes
* MAR data is still being analyzed for each patient’s resected HO mass. However, to date, the first four patients have an average bone growth that is approximately 2x as rapid as non-pathological human bone (1.92 +/- 0.09 microns per day vs. 1.0 micron per day). Additionally, localized regions of bone demonstrated high metabolic growth, with MAR approaching 3.5 microns per day. These values are quite high considering that many patients are proscribed anti-inflammatories such as Celebrex prior to surgery.
  * Since patients 1-4 have not yet made it 6 months post-operatively, histological data cannot yet be assessed with pre and post-operative AP levels, demographical information or recurrence. This is planned in Year 2 of the study.

* SEM images demonstrated the continued growth and development of HO even at time of surgical resection. Ectopic bone is highly vascular and may correlate with the increased MAR rates (Fig 2).

5. Conclusions
Work conducted during Year 1 of this grant has resulted in our team being the first group to flurochome labeling HO bone. We have recruited 14 patients (40% of our intended patient sample size in 8 months time) and data collected to date of MAR measurements has indicated that ectopic bone may grow 2-3x faster than that of non-pathological “normal” human cortical bone. Additionally, salary support for the investigators has resulted in 2 peer-reviewed publications.

6. References
Appendix A: Publications

1. “Relationship Between Heterotopic Ossification Volume and Clinical Screening Tools in Combat-Injured Transfemoral Amputees.” (Accepted for Publication in the Journal of Prosthetics and Orthotics.)

Abstract: Heterotopic ossification (HO) often causes symptoms requiring surgical resection and may delay rehabilitation regimes for wounded service members. Clinical screening tools for assessing HO have included serum alkaline phosphatase (AP), nuclear scintigraphic activity and patient pin scores. However, no studies to date have investigated the relationship of these clinical predictors with HO incidence and volume. Ten servicemen with transfemoral amputations were included in this retrospective study. Volumetric measurement of HO was calculated using thresholding software and computed tomography cans were performed 12.6 +/- 6.8 months after injury. Subject AP levels, white blood cell (WBC) counts and pain scores were assessed to determine if these factors were predictors of ectopic bone volumes. The mean volume of HO was 44.73 +/- 39.35 cm³. Statistical analysis demonstrated that the volume of HO and serum AP levels was significantly correlated (p=0.002). However, average pain scores were not significant predictor of HO volume (p=0.212). Infections developed in nine of the ten subjects and WBC counts and HO volumes were significant correlated (p=0.028). The magnitude of serum AP levels and WBC counts may be effective factors for predicting the expected volume of ectopic bone in combat-injured service members with transfemoral amputations.


Abstract: This paper seeks to describe how novel technologies such as the computer assisted research environment (CAREN) may improve physical and cognitive rehabilitation for wounded warfighters. Design: This paper focuses on the CAREN system as a means to assist service members who have sustained improvised explosive device (IED) injuries during Operation Enduring Freedom (OEF), Operation Iraqi Freedom (OIF) and Operation New Dawn (OND). The complex nature of warfighter injuries present unique rehabilitation challenges that demand new tools for quick return to active duty or the civilian community. Findings: The CAREN system provides a safe, interactive environment for the user while providing kinematic and kinetic data capture to improve rehabilitation regimens. A detailed literature review concludes virtual reality-based gait training programs have demonstrated the ability to directly influence physiological and biomechanical performance. Originality / Value: This paper provides an overview of the CAREN system and describes how this has been used as a rehabilitation aid for wounded warriors.