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TITLE: Laser Application on Orthopaedic Bone Repair

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT The goal of this project is to improve the treatment of bone defect by leveraging a rat cortical bone allograft model and Er:YAG laser currently used in the dental field. To achieve this goal we will test the hypothesis that: 1) segmental bone defects treated with Er:YAG laser irradiation will form significantly more and stronger cortical bone allograft incorporation; and 2) segmental bone defects treated with cortical bone allograft pre-conditioned by Er:YAG laser will form significantly more and stronger cortical bone allograft incorporation. The project is currently in progress and definitive results await further analysis. However, post-operative observations and body weight gain indicated there were no complications such as allergic reactions, abscesses or infections. Body weight gain in the laser-treated animals was similar to that in non-laser treated animals during the healing period. These results showed a successful feasibility of the Er:YAG laser system for the cortical bone allograft model.					
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INTRODUCTION

It has been reported that more than 30,000 American service members have been wounded in the wars in Iraq and Afghanistan during the last six years. The majority of service members who are wounded in action sustain musculoskeletal injuries such as the orthopedic-related trauma involving the upper and lower extremities present with a significant bone defect. Bone allografts have become an accepted technology to replace bone loss. However, major complications reported for grafting procedures are infection, bone graft fracture, non-union at the graft-host interface, and, rarely, massive allograft resorption. Recent advances in technology have led to a development of novel approaches for use of lasers in hard tissue surgeries in the dental field. The laser that show the most promise for hard tissue surgery is the erbium:YAG (Er:YAG) laser. Recent reports indicates Er:YAG laser provides; (1) advantageous bone surface for bone tissue repair; (2) bactericidal effect; (3) applications for both soft and hard tissue. However, it has not been shown whether Er:YAG laser has a positive effect on orthopedic bone repair. The goal of this project is to improve the treatment of bone defect by leveraging a rat cortical bone allograft model and Er:YAG laser currently used in the dental field. We hypothesis that: 1) allograft bed treated with Er:YAG laser irradiation will lead to more and stronger cortical bone allograft incorporation; and 2) segmental bone defects treated with cortical bone allograft pre-conditioned by Er:YAG laser will lead to more and stronger cortical bone allograft incorporation. The project is currently in progress and definitive results await further analysis. However, post-operative observations and body weight gain indicated there were no complications such as allergic reactions, abscesses or infections. Body weight gain in the laser-treated animals was similar to that in non-laser treated animals during the healing period. These results showed a successful feasibility of the Er:YAG laser system for the cortical bone allograft model. Samples are currently under investigation for micro CT analysis and mechanical testing.

BODY

The following is a summary of the work completed to the present time based on the project's accepted Statement of Work. Comments by PI follow below.

Statement of Work

Task 1: Determine the effect of Er:YAG laser on allograft bed.

1a. Preparation for the animal study - completed

Obtain animal use approvals and order the supplies.

1b. Preparation of the allografts - completed

Obtain cortical bone grafts from the donor rats (n=26; 2 grafts/animal) prior to the surgery. Allografts will be sterilized with 70% ethanol and then fresh frozen at -80°C until use.

1c. Perform surgery – harvest after 4 & 8 week post-surgery - in progress

Perform surgery; create segmental defect (n=78) and utilize cortical bone allograft grafts (n=52) prepared above (1b). Some animals (group 2 & 7) will undergo laser irradiation during the surgery. Four and 8 weeks post-surgery, for mechanical testing, the dissected bones will be wrapped in saline soaked gauze and frozen at -20°C. For undecalcified histology, the dissected bones will be fixed in 10% neutral buffered formalin.

1d. Analyze the samples - in progress

Analyze the samples by μ CT, mechanical test and histology.

Task 2: Determine the effect of Er:YAG laser on allograft.

2a. Preparation for the animal study - completed

Obtain animal use approvals and order the supplies.

2b. Preparation of the allografts - completed

Obtain cortical bone grafts from the donor rats (n=26; 2 grafts/animal) prior to the surgery. Allografts will be sterilized with 70% ethanol and then fresh frozen at -80°C until use. Some allografts (for group 3, 4, 8, and 9) will undergo laser irradiation prior to the surgery.

2c. Perform surgery - harvest after 4 & 8 week post-surgery - **in progress**

Perform surgery; create segmental defect (n=52) and utilize cortical bone allograft grafts (n=52) prepared above (2b). Some animals (group 4 & 9) will undergo laser irradiation during the surgery. Four and 8 weeks post-surgery, for mechanical testing, the dissected bones will be wrapped in saline soaked gauze and frozen at -20°C. For undecalcified histology, the dissected bones will be fixed in 10% neutral buffered formalin.

2d. Analyze the samples - **in progress**

Analyze the samples by μ CT, mechanical test and histology.

Comments:

The project is currently in progress and definitive results await further analysis. However, post-operative observations and body weight gain indicated there were no complications such as allergic reactions, abscesses or infections. Body weight gain in the laser-treated animals was similar to that in non-laser treated animals during the healing period. These results showed a successful feasibility of the Er:YAG laser system for the cortical bone allograft model. Contact X ray images obtained during the healing period indicate similar or larger callus in laser-treated groups compared to non-treated groups. However, these qualitative observations are done in two-dimensional, projected image while subsequent micro CT analysis will provide better understanding in three-dimensions. Moreover, mechanical testing is the primary endpoint for the project which will be performed after non-destructive-X ray analysis. Initiation of animal surgery and subsequent sample analysis were delayed because completion of the MTA to obtain the laser device and training personnel for using the laser device took longer than anticipated. Nevertheless, we anticipate completing the project on schedule.

KEY RESEARCH ACCOMPLISHMENTS

- IACUC approved by the USAMRMC Animal Care and Use Review Office
- MTA concluded with HOYA Photonics, Inc.

REPORTABLE OUTCOMES

The investigators are currently awaiting results of ongoing analysis from the project. Following personnel received research training based on animal procedures in this research project which includes introduction and training for Er:YAG laser.

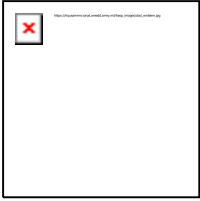
- Vbenosawemwinghayeh Orhue, M.D. – Postdoctoral fellow
- David F. GomezGil, D.D.S., M.S. – Ph.D. candidate
- Siddhesh R. Angle, Ph.D. – Ph.D. candidate
- David G. Karwo, B.S. – Research assistant
- Julie E. Brown, B.S. – Research assistant

CONCLUSION

- MTA issue slightly delayed progress, but the project is in progress to meet the completion by the end of the project period.
- Preliminary results indicate no adverse effect or complication by use of Er:YAG laser in cortical allograft model.

APPENDICIES

1. Memorandum for the Record – Approval of Animal Protocol (8/6/2010)
2. Memorandum for the Record – Completed MTA (12/16/2010)



**DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MD 21702-5012**

August 06, 2010

Director, Office of Research Protections
Animal Care and Use Review Office

Subject: Review of USAMRMC Proposal Number OR090783, entitled, "Laser Application on Orthopaedic Bone Repair"

Principal Investigator Kotaro Sena
Rush University Medical Center
Chicago, IL

Dear Dr. Sena:

Reference: (a) DOD Directive 3216.1, "The Use of Laboratory Animals in DOD Programs"
(b) US Army Regulation 40-33, "The Care and Use of Laboratory Animals in DOD Programs"
(c) Animal Welfare Regulations (CFR Title 9, Chapter 1, Subchapter A, Parts 1-3)

In accordance with the above references, protocol OR090783 entitled, "Laser Application on Orthopaedic Bone Repair," IACUC protocol number 10-061 is approved by the USAMRMC Animal Care and Use Review Office (ACURO) for the use of rats and will remain so until its modification, expiration or cancellation. This protocol was approved by the Rush University Medical Center IACUC.

When updates or changes occur, documentation of the following actions or events must be forwarded immediately to ACURO:

- IACUC-approved modifications, suspensions, and triennial reviews of the protocol (All amendments or modifications to previously authorized animal studies must be reviewed and approved by the ACURO prior to initiation.)
- USDA annual program/facility inspection reports
- Reports to OLAW involving this protocol regarding
 - a. any serious or continuing noncompliance with the PHS Policy;
 - b. any serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals; or
 - c. any suspension of this activity by the IACUC
- USDA or OLAW regulatory noncompliance evaluations of the animal facility or program
- AAALAC, International status change (gain or loss of accreditation only)

Throughout the life of the award, the awardee is required to submit animal usage data for inclusion in the DOD Annual Report on Animal Use. Please ensure that the following animal usage information is maintained for submission:

- Species used (must be approved by this office)
- Number of each species used
- USDA Pain Category for all animals used

For further assistance, please contact the Director, Animal Care and Use Review Office at (301) 619-2283, FAX (301) 619-4165, or via e-mail: acuro@amedd.army.mil.

NOTE: Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer or Grant Officer can authorize expenditure of funds. It is recommended that you contact the appropriate Contract Specialist or Contracting Officer regarding the expenditure of funds for your project.

Sincerely,

For 

Alec Hail, DVM, DACLAM
Colonel, US Army
Director, Animal Care and Use
Review Office

Copies Furnished:

Mr. Joshua McKean, US Army Medical Research Acquisition Activity (USAMRAA)
Dr. Miriam Darnell, Congressionally Directed Medical Research Program (CDMRP)
Dr. Thomas M. Schmid, Rush University Medical Center
Mrs. Jennifer L. Garcia, Rush University Medical Center

MATERIAL TRANSFER AGREEMENT - EQUIPMENT

This Material Transfer Agreement – Equipment is made and becomes effective this 16TH day of DECEMBER 2010 (the "Effective Date") by and between,

Rush University Medical Center, a not-for-profit corporation with its principal place of business at 1653 West Congress Parkway Chicago, IL 60612 (hereinafter referred to as "Recipient") via its employee Kotaro Sena, DDS (hereinafter referred to as "Recipient Scientist") who is not a party to this agreement and **HOYA ConBio, Inc.**, a company organised and existing under the laws of the State of California, with its registered offices at 47733 Fremont Boulevard, Fremont CA, 94538 USA (hereinafter referred to as "Supplier").

RECITALS

- WHEREAS Supplier has developed and produced certain Equipment (as defined below) and owns all proprietary and intellectual property rights in and to and/or has the right to use and otherwise dispose of such Equipment.
- WHEREAS the Recipient wishes to obtain the Equipment for the sole purpose of conducting the Research Project (as defined below).
- WHEREAS Supplier agrees to provide the Recipient with the Equipment according to the following terms and conditions.

Now therefore, the parties have agreed as follows:

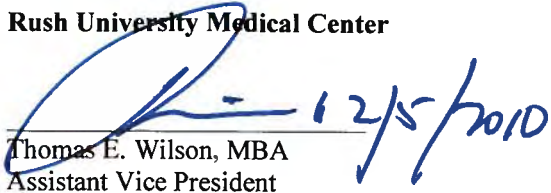
1. Supplier agrees to transfer to Recipient the following Equipment : **VersaWave Er:YAG laser system and accessories** ("Equipment").
2. The Equipment shall be used by Recipient in connection with the project described as follows: Laboratory Animal Study which will investigate the use of the VersaWave in orthopedic bone repair. The VersaWave Er:YAG Laser System is commercially cleared by the FDA for use in hard, soft, and osseous tissue dental procedures. The objective of the Study is to investigate the effects of Er:YAG laser in orthopedic bone repair as defined in greater detail in the Project Narrative attached in "Exhibit A". ("Research Project").
3. Recipient agrees that the Equipment shall only be used for the Research Purpose by the Recipient Scientist or any person under its control and/or supervision, in the Recipient Scientist's laboratory.
4. Recipient agrees to comply with all federal, state and local laws, regulations and rules applicable to the Research Project, the handling, storage, use and otherwise disposal of the Equipments. The Recipient agrees to notify the consulting laboratory, contractor, or grantee that the study must be conducted in compliance with 21 CFR Part 58, Good Laboratory Practices for Nonclinical Laboratory Studies but only to the extent that such third party actually follows 21 CFR Part 58.
5. The Equipment remains the property of Supplier. Recipient therefore agrees to retain control over the Equipment, and further agrees to retain disclosure, access and transfer of the Equipment only to people who have a "need to know" to conduct the Research Project and who are under the Recipient Scientist's supervision. Recipient shall promptly inform Supplier in writing of the completion of the Research Project and of the results thereof. In addition, Recipient shall make available information as to the progress of the Study as requested by Supplier, and will provide reports as agreed between Supplier and the Recipient Scientist. The Supplier may use the data at their discretion including the submission of the data for review by the Food and Drug Administration, Ethics Committees and/or other domestic and/or international regulatory bodies in support of marketing efforts.
6. The Recipient and Supplier agree that, as a condition for Supplier to provide the Equipment hereunder and for Recipient to use said Equipment in Recipient's research plan, both Supplier and Recipient shall not attempt to reverse engineer, disassemble, or otherwise perform analyses directed or intended at learning the methodology, components, formulae, processes or other information pertaining to the make-up or production of the Equipment, Recipient's assays or methods, unless expressly authorised in

writing. If so authorised, either party shall furnish copies of any such analyses to the other and shall make no use thereof except as agreed in writing. If no further agreement is reached by the parties as to the Equipment, Recipient or Supplier shall at Supplier's or Recipient's request either furnish all copies of such analyses to one another or destroy all copies thereof.

7. Recipient shall inform Supplier in writing of the existence of any Developments. In this Agreement, "Developments" shall mean any modifications or improvements made to the Equipment by Recipient. Developments shall specifically exclude modifications or improvements made to Recipient's assays or other use of the Equipment including but not limited to any progeny, extracts, replications, summaries, or derivatives thereof, and any discoveries, inventions or developments made by Recipient using or incorporating all or part of the Equipment or based on the Confidential Information. Recipient shall provide Supplier with a fully paid, non-exclusive license to Recipient's rights in the Developments with the right to sublicense to affiliates and a six (6) month option to take an exclusive license to the Developments on reasonable commercial terms. If Supplier elects to exercise that option, Recipient and Supplier shall negotiate in good faith for an additional six (6) months towards a license. Should Recipient and Supplier be unable to reach terms in such a license, the parties may mutually elect to extend the negotiation period for one (1) additional six (6) month period or may choose to end negotiations, in which case, Recipient shall have no further obligation to Supplier other than the non-exclusive license provide in this Section 8.
8. Any intended publication or otherwise public disclosure relating to all or part of the results of the Research Project and/or the Development and/or Confidential Information by the Recipient, whether oral or written, shall be subject to Supplier's prior review of the content of such publication or disclosure. Supplier shall have the right to place a thirty (30) day hold on submission of any publication in order to determine if a patentable Development or other proprietary confidential information is contained therein and may request an additional thirty (30) day hold on submission in order to file a patent application and/or revise or remove the confidential information. In case of a publication or otherwise public disclosure, Recipient shall acknowledge Supplier's contribution of the Equipment unless otherwise agreed in writing.
9. **THE EQUIPMENT IS PROVIDED "AS IS" TO RECIPIENT WITHOUT ANY WARRANTIES WHATSOEVER, WHETHER EXPRESS OR IMPLIED, INCLUDING ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE NOR ANY WARRANTY AS TO THE PROPERTIES, PERFORMANCES, SAFETY, NOVELTY OR SUITABILITY OF THE EQUIPMENT.** Recipient agrees to accept liability for all damages, expenses and losses arising out of or as a result of Recipient's use, storage, and otherwise disposal of the Equipment and/or Confidential Information for any purpose.
10. Nothing in this Agreement shall or may be construed as granting Recipient any intellectual property right, licence, title or interest in and to the Equipment and the Confidential Information for any use other than the Research Project nor may it be construed to grant Supplier any intellectual property right, license, title of interest in and to Recipient's assays and Developments specifically excluded in Section 7 herein.
11. This Agreement and the confidentiality and restriction of use obligations contained herein shall remain in effect for a period of five (5) years from the Effective Date.

IN WITNESS WHEREOF, the parties have cause this Agreement to be executed in duplicate by their duly authorised representatives, as of the Effective Date.

Rush University Medical Center


Thomas E. Wilson, MBA
Assistant Vice President

MAL - OK DATE NEXT pg

Supplier: HOYA ConBio, Inc.

By: 
Name: Timothy S. Gehlmann
Title: President and CEO

12/15/10

Date : _____

Date : _____

READ AND ACKNOWLEDGED BY:

Recipient Scientist: Kotaro Sena, DDS

By : _____

Name : Kotaro Sena, DDS

12/8/2010

Exhibit "A"

Project Narrative