AWARD NUMBER: W81XWH-21-1-0900

TITLE: Treat Implant Loosening of Percutaneous Osseointegrated Prosthetic Limbs with Intermittent Parathyroid Hormone

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CONTRACTING ORGANIZATION: Hospital for Special Surgery

REPORT DATE: OCTOBER 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2023	Annual	1 Sep 2022 – 31 Aug 2023

4. TITLE AND SUBTITLE

Treat Implant Loosening of Percutaneous Osseointegrated Prosthetic Limbs with Intermittent Parathyroid Hormone

5a. CONTRACT NUMBER
W81XWH-21-1-0900

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We aim to evaluate parathyroid hormone, an FDA-approved agent, as a candidate to prevent and reverse the failure of percutaneous osseointegrated prosthetic limbs (POPL) caused by implant loosening due to initial instability. We will also investigate the cellular origin and molecular mechanism of the formation of peri-implant fibrotic tissue. For this reporting period the major goals were to optimize the mouse model, to breed Lepr-creER mice, and to confirm that Lepr positive cells conditionally induced in this mouse line can mark the fibroblasts. We successfully bred these mice and confirmed that Lepr positive cells are the cellular origin of the fibrosis. We have tested various implants to improve the reproducibility of this mouse model. We demonstrated that mechanical loading is necessary for the formation of both bone and fibrosis in this model. To carry out the project as proposed we will need to develop an automated loading device and implants that can be attached to it.

15. SUBJECT TERMS
Implant loosening, Percutaneous Osseointegrated Prosthetic limbs, Mouse phenotype, Mouse breeding, Mouse line with gene reporter

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	6	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. Introduction

With this project, we aim to evaluate parathyroid hormone, an FDA-approved agent, as a candidate to prevent and reverse the failure of percutaneous osseointegrated prosthetic limbs (POPL) caused by implant loosening due to initial instability. We will also investigate the cellular origin and molecular mechanism of the formation of peri-implant fibrotic tissue. Successful completion of this project will lead to a clinical trial on using this agent as a therapy for fibrotic osseointegration failure to benefit veterans and civilians. The findings can also provide a new perspective for the research of fibrosis.

2. Keywords

Percutaneous osseointegrated prosthetic limbs, Implant loosening, Fibrosis, Parathyroid hormone (PTH), Leptin receptor (LEPR), C-X-C motif chemokine 12 (CXCL12)

3. Accomplishments

What were the major goals of this project?

In the last report, we described that the originally proposed Cxcl12-CreER mouse line had been found to only label a subset of the fibroblasts in our POPL model and in some of the mice induced for osseointegration failure, we did not see fibrosis. We therefore decided to additionally obtain a mouse line with a conditional reporter for LEPR+ cells and to optimize the model to create consistent fibrotic tissue. The goals were to: 1) breed Lepr-creER; Ai9 mice, 2) confirm whether the Ai9 reporter conditionally driven by tamoxifen injection will efficiently label the peri-implant fibroblasts in our model, and 3) test implants of various sizes and materials to optimize the reproducibility of this model.

What was accomplished under these goals?

According to the goals, we have done the following:

1) To breed Lepr-creER; Ai9 mice.

   Completed.

   The initial breeding was slow with low birth rate and high death rate.

2) To confirm whether the Ai9 reporter will label peri-implant fibroblasts.

   Completed.

   I. When we finally had enough Lepr-creER mice bred to the correct age, we conducted an experiment and found that in mice with fibrosis, it was marked by LeprCre-Ai9+ cells induced with tamoxifen one week before implantation.

   II. To confirm that the fibroblast origin was Lepr lineage cells, we had a group of mice given tamoxifen injection at age of 4 to 6 weeks and received implantation at age of 16 weeks. The fibrosis was marked with LeprCre-Ai9+ cells.

3) To test implants of various sizes and materials to optimize the reproducibility of this model.

   Completed.

   I. While waiting for the breeding of the above mouse line, we did several experiments to optimize the model with wild type mice. In the first experiment, we used implants made of bone cement (polymethyl methacrylate, PMMA) with a customized mold, with identical dimensions of our titanium implants. These implants should osseointegrate less well and induce more fibrotic tissue than titanium implants. However, they did not produce consistent fibrosis by two weeks post implantation. This made us speculated that the implants were too big to have enough micromotion and induce fibrosis.

   II. We next used implants with a stem diameter of 0.5 mm, 50% that of the original implants, to induce more micromotion and fibrosis. These implants fail to induce fibrosis in more mice

than the original implants by two weeks. In addition, some of these implants fell out of the amputated bone a few days after implantation.

III. Another possibility of the lack of consistent fibrosis as seen in our ossoeintegration failure model with an intraarticular tibial implant was that in this POPL model, the implant did not penetrate through the tibial growth plate, which might host the progenitors of fibroblasts. Therefore, we did an experiment in which the stem tip of the implant was inserted into the subchondral bone, penetrating the growth plate. This did not increase the percentage of mice that developed fibrosis.

IV. To have a bigger bone to provide enough space for overdrill and induce implant micromotion, we also performed amputations at distal femur or mid-shaft of femur. Although fibrosis was found in more mice by two weeks but in most of the mice fibrosis resolved and peri-implant bone decreased by four weeks. This make us hypothesize that mechanical loading is crucial to the induction and maintenance of the fibrosis.

V. We then conducted an experiment in which the implants were percutaneously loaded manually for three minutes every day starting on the first day after implantation until euthanasia. The loading was conducted under anesthesia with isoflurane inhalation and the mice tolerated it well. This induced fibrosis in 80% of mice by two weeks and the fibrosis lasted until four weeks.

VI. We did another experiment the same as in V but also gave daily injection of PTH or saline as control to each mouse until 4 weeks. MicroCT and histology showed that PTH prevented fibrosis in most of the mice.

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?
To have a reproducible model with consistent fibrosis we need to design a device to apply controlled loading to the POPL implant. We also need to design an implant can be attached to soft tissue well enough to not to fall out but loosely enough to have enough micromotion. These implants also need to be able to be attached to the loading device percutaneously. If these can be accomplished, we will breed Lepr-creER; $Pdgfa^{fl/fl}$, Ai9 mice to investigate the mechanism of osseointegration failure in POPL and the effect of PTH.

4. Impact

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
We have spent this reporting period to optimize the mouse POPL model. We have demonstrated that mechanical loading is crucial to both fibrosis and bone formation in this model. To develop an automated loading device and implants can be attached to it will be challenging. We probably will need to extend the project for at least one year.

Changes that had a significant impact on expenditures
Since we have not started the experiments approved by ACURO, we have only used the budget to cover personnel salary and general lab supply and antibodies for immunofluorescence used for the pilot experiments.

6. Products

Nothing to report.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	***Xu Yang***	***Matthew B. Greenblatt***	***Anastasia Oktarina***
Project Role	*PI*	*Co-PI*	*Post-doctoral Fellow*
ORCID ID	*0000-0001-8377-077X*	*0000-0001-9794-8532*	*0000-0003-0903-308X*
Nearest person month worked	3	1	6
Contribution to Project	*Dr. Yang oversaw all aspects of this project, including experimental design, animal surgeries, troubleshooting technical issues, communicating with consultants and collaborator.*	*Dr. Greenblatt supported Dr. Yang in the execution of this project, including "realtime" review of project data and technical troubleshooting, participating in project meetings and progress reporting.*	*Dr. Oktarina worked closely with Dr. Yang in animal surgeries, tissue collection and processing, and data collection and analysis.*

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?

Nothing to report.

8. Special Reporting Requirements

Nothing to report.

9. Appendices

None.