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

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CONTRACTING ORGANIZATION: Hospital for Special Surgery

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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 goal was to determine the role of CXCL12+/LEPR+ cells in and the therapeutic potential of iPTH for fibrotic osseointegration failure of a POPL implant. However, we were delayed by the initial difficulty in the breeding of Cxcl12-creER; Ai14 mice which required back-crossing the breeders with wildtype mice. We then did 5 experiments to demonstrated that the Cxcl12-creER; Ai14 mice, can only label some of the fibroblasts in our POPL model. We have started breeding mice of a recently developed Lepr-creER line. We have also noticed the lack of fibrosis phenotype in some mice. We are testing various implants to improve the reproducibility of this mouse model.

15. SUBJECT TERMS

Implant loosening, Percutaneous Osseointegrated Prosthetic limbs, Mouse phenotype, Mouse breeding, Mouse line with gene reporter

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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?

For this reporting period the major goal was to determine the role of CXCL12+/LEPR+ cells in and the therapeutic potential of iPTH for fibrotic osseointegration failure of a POPL implant.

What was accomplished under these goals?

In the original proposal Cxcl12-CreER mouse line was chosen to lineage trace LEPR+ cells because of the lack of Lepr-creER mouse line and the overlap of CXCL12+ and LEPR+ cells. Before we could finalize our IACUC proposal and submit it for ACURO approval, we needed to confirm that fibrosis phenotype and accumulation of CXCL12/LEPR+ cells in our mouse POPL model. We did the following:

1) We aimed to breed the Cxcl12-creER; iDTA; Ai14; Ocn-GFP mice. However, the initial breeding of Cxcl12-creER mice was slow due to low birth rate and high death rate. Since the Cxcl12-creER; Ai14 breeders were obtained from an outside investigator and it was not clear of which generations of this mouse line they were, we decided to back-cross them with wild type mice for three generations to get rid of potential unintentional mutations. After that the breeding was faster. However, it took more than 6 months to breed enough Cxcl12-creER; Ai14 mice for pilot experiments.
2) In the first experiment, we gave the mice two weeks of tamoxifen injection starting from one week before surgery. The Ai14 reporter driven by Cxcl12 only labeled a small percentage of the peri-implant fibroblasts in mice sacrificed at 2 weeks after surgeries.
3) One possibility of the lack of reporter signals in the peri-implant fibroblasts was that although these cells had derived from CXCL12+ lineage cells but they were not actively expressing *Cxcl12* genes when they were producing fibrosis. We therefore, did another experiment in which tamoxifen was given when the mice were 4 weeks old and surgeries were performed when they were 12, 16, or 20 weeks old. Again, the fibroblasts were not labeled efficiently by the reporter.
4) To determine whether the lack of reporter in peri-implant fibroblasts was due to the infidelity of this mouse line or the lack of accumulation of CXCL12+ cells in this model, we bred Cxcl12-creER; Ai14; Cxcl12-GFP mice for another experiment. We found that the Ai14+ cells only overlapped with some of the GFP+ cells, mostly in the area more proximal to the implant and closer to the growth plate. This indicated that the Cxcl12-creER; Ai14 reporter cannot label the peri-implant area in the POPL model.
5) We next used Lepr-cre; Ai9 mice for this POPL model. We confirmed that the reporter driven by *Lepr* labels most of the peri-implant fibroblasts.
6) Putting the above data together, we concluded that although Cxcl12-Cre; Ai14 mice have the advantage of temporally controlled labeling of Cxcl12-expressing cells, this line can only label a subset of the fibroblasts in our POPL model. We therefore found it advantageous to additionally obtain a mouse line with a conditional reporter for LEPR+ cells.

7) We found that an investigator in China reported a Lepr-creER mouse line after this project was funded by DOD. Although the process of mouse transfer was delayed by the pandemic of COVID19, we have recently received a pair of breeders of this mouse line.

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?
We are currently breeding Lepr-creER; Ai9 mice. We will confirm whether the Ai9 reporter conditionally driven by tamoxifen injection will efficiently label the peri-implant fibroblasts in our model. If so, we will implement the experiments as originally proposed. Otherwise, we will propose alternative experiments such as using wildtype mice to test the effect of PTH on peri-implant osseointegration and fibrosis, and targeting specific genes to block LEPR+ cells from converting to fibroblasts.

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
As aforementioned, we have spent this reporting period to demonstrate the Cxcl12-creER; Ai14 reporter could not efficiently label the peri-implant fibroblasts in our POPL model. We have obtained breeders of a recently developed Lepr-creER mouse line. We are currently breeding the mice and will confirm their efficiency in our model.
Another potential issue is that in some of the mice induced for osseointegration failure, we did not see fibrosis. One possibility is the smaller diameter of the mouse tibiae has restricted the instability of the implants. We are currently testing implants of various sizes and materials to optimize the reproducibility of this model.

Changes that had a significant impact on expenditures
Since we have not started the experiments approved by ACURO, we have delayed using the budget to cover the salary for postdoctoral fellow and have only charged the cost of some 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	2
Contribution to Project	*Dr. Yang oversaw all aspects of this project, including experimental*	*Dr. Greenblatt supported Dr. Yang in the execution of this project, including*	*Dr. Oktarina worked closely with Dr. Yang in animal surgeries,*

	design, animal surgeries, troubleshooting technical issues, communicating with consultants and collaborator.	*"realtime" review of project data and technical troubleshooting, participating in project meetings and progress reporting.*	*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
A report will be submitted by the Co-PI, Dr. Matthew Greenblatt.

9. Appendices
None.