401 N. Lindbergh Blvd.
St. Louis, MO 63141
Tel.: 314.993.1700, #546
Toll Free: 800.424.2841, #546
Fax: 800.708.1364
Send via email to: jbode@aaortho.org and cyoung@aaortho.org

AAO Foundation Final Report Form
(a/o 1/3/2018)

In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Type of Award: Research Aid Award

Name(s) of Principal Investigator(s): Joy Chang

Title of Project: Effects of Sustained Release of RANKL on Orthodontic Tooth Movement

Period of AAOF Support (e.g. 07-01-17 to 06-30-18): 07/01/2017-06/30/2018

Amount of Funding: $5,000

Summary/Abstract

Objectives: Orthodontic tooth movement (OTM) requires 18-36 months of treatment. Current methods to accelerate treatment are either ineffective or transient. OTM is dependent on bone modeling, and RANKL is essential in osteoclast activation. We hypothesize a sustained dose of RANKL through localized delivery will increase osteoclast formation and increase OTM.

Methods: Polylactic-acid-co-glycolic acid (PLGA) is used to prepare microparticles using double emulsion. 40µg/mL RANKL is adsorbed on microparticles at 37°C for 1 hour, then embedded in 10% hydroxyethylcellulose (HEC) gel. MPs are characterized for morphology and release kinetics in PBS at physiologic conditions (pH = 7.4). RANKL activity is tested on mice osteoclast precursor cells (RAW 264.7). The effects on OTM is determined using 15-week old male Wistar rats for 14 days. In the experimental group, the formulation is injected palatal to the left maxillary first molar, and OTM is accomplished with a NiTi coil spring applying 10g of force. Control groups will involve only the application of NiTi spring, with and without placebo formulation. The outcome measure includes the distance of tooth movement, bone volume fraction, and tissue density determined with microCT. The amount of osteoclast activity will also be determined using tartrate-resistant acid phosophatase (TRAP) staining.

Results: PLGA microspheres display a spherical appearance 250-425 µm in diameter with a dimpled, porous surface morphology. RANKL release kinetics were determined at physiologic

conditions, where the RANKL-PLGA microspheres embedded in HEC gel released approximately 14% of total RANKL in the first hour, followed by steady release achieving 82% total release in 28 days. The RANKL formulation successfully allowed differentiation of RAW 264.7 cells into osteoclasts-like cells, while addition of the blank MP-HEC formulation did not contribute to any differentiation of the cells, suggesting maintenance of RANKL bioactivity. MicroCT analysis of tooth movement shows the amount and rate of orthodontic tooth movement in all of the groups over the 14-day period. OTM only control, OTM + blank formulation, and OTM + RANKL-MP-HEC formulation had 0.24±0.05 mm, 0.32±0.1 mm, and 0.55±0.2 mm tooth movement, respectively, in 14 days. Statistical analysis shows significant difference ($p<0.001$) between the RANKL-MP-HEC group with the OTM only and the OTM + blank formulation group but did not reveal a statistical difference between the OTM + blank formulation and the OTM only group. The bone volume fraction and bone tissue density were significantly less in the OTM + RANKL formulation in comparison with the OTM only and OTM + blank formulation controls. The bone volume fraction was approximately 55% in the OTM only group, 47% in the OTM + blank formulation group, and 27% in the OTM + RANKL formulation group. The difference between OTM only and the OTM + blank formulation control was not significant, but was significant with the OTM+RANKL group. Similarly, for tissue density, the OTM only group had 1120 mg/ccm HA, the OTM + blank formulation had 1040 mg/ccm HA, and the OTM + RANKL formulation had 950 mg/ccm HA, with the only significant difference between the OTM + RANKL formulation with the other two groups. Histological analysis and quantification of osteoclast formation was obtained with TRAP staining for all 3 groups. The OTM + RANKL formulation had a significant increase ($*p < 0.001$) of approximately twice the amount of TRAP activity in comparison with the OTM only and OTM + blank formulation groups. Determination of distant effects and systemic effects of the RANKL formulation was assessed through analysis of the contralateral (right) maxilla for the animals which underwent OTM only in comparison with the OTM + RANKL formulation administration. No significant difference was found in bone volume fraction and TRAP activity on the contralateral maxilla between both groups, suggesting that the effects of the RANKL formulation was localized to the region of tooth movement.

Conclusions: The duration of orthodontic treatment is often the cause of many adverse effects, and there is currently a lack of an effective method which would continuously accelerate OTM without systemic effects. In this study, we developed a novel RANKL-loaded formulation which could control the release of RANKL to biologically and effectively accelerate OTM. Our study shows promising results in producing a novel, targeted therapeutic to sustain the effects of accelerated OTM. Further studies looking at the long-term effects and possibility of adverse, long-term systemic effects are needed to bring this therapeutic from bench to chairside.

Response to the following questions:

1. Were the original, specific aims of the proposal realized?
   Yes

2. Were the results published?
   a. If so, cite reference/s for publication/s including titles, dates, author or co-authors, journal, issue and page numbers
      Not yet.

   b. Was AAOF support acknowledged?
      Once it is published, yes, AAOF support will be recognized.

c. If not, are there plans to publish? If not, why not?
Yes, we plan to publish these results in the next few months.

3. Have the results of this proposal been presented?
    a. If so, list titles, author or co-authors of these presentation/s, year and locations
        - Chang J, Nanda R, Kumbar S, Yadav S. Effects of Sustained Release of RANKL on Osteoclast Formation. 2017, AADR, San Francisco
        - Chang J, Nanda R, Kumbar S, Yadav S. Effects of Sustained Release of RANKL on Osteoclast Formation. 2017, WIOC, Kobe, Japan
        - Chang J, Chen P, Nanda R, Kumbar S, Yadav S. Effects of Sustained Release of RANKL on Orthodontic Tooth Movement. 2018, AADR, Ft. Lauderdale
        - Chang J, Chen P, Nanda R, Kumbar S, Yadav S. Effects of Sustained Release of RANKL on Orthodontic Tooth Movement. 2018, AAO, Washington D.C.
    b. Was AAOF support acknowledged?
    Yes.
    c. If not, are there plans to do so? If not, why not?

4. To what extent have you used, or how do you intend to use, AAOF funding to further your career?

    AAOF funding has been instrumental for my career development as a clinician-scientist. Without this funding, my Master's Research would not be possible, especially with the costly materials as well as the high cost of the animal study. My Master's Thesis is a critical stepping stone for my career, as it allows me to develop and hone my research interests in accelerating tooth movement. I intend to use this project to develop further research studies for my career as a scientist. I am forever grateful for the opportunity that AAOF funding has given me in developing and completing my project, which I hope would benefit orthodontic research.

Please return to AAOF via email attachment to
aaof@aaortho.org