401 N. Lindbergh Blvd.
St. Louis, MO 63141
Tel.: 314.993.1700, #546
Toll Free: 800.424.2841, #546
Fax: 800.708.1364
Cell: 314.283.1983
Send via email to: jbode@aaortho.org and cyoung@aaortho.org

AAO Foundation Final Report Form
(a/o 6/30/2020)

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?*

Please prepare a report that addresses the following:

Type of Award: **Research Aid Award**

Name(s) of Principal Investigator(s): **Jia Liu**

Institution: **University of Connecticut**

Title of Project: **Dynamic Transcriptome Changes During Orthodontic Tooth Movement in Rats**

Period of AAOF Support: **07-01-19 to 12-31-20**

Amount of Funding: **5,000**

Summary/Abstract

Objectives:

The biology behind orthodontic tooth movement (OTM) is extremely complex yet very crucial to be understood by clinicians. A thorough knowledge of the biological responses involved in OTM would provide clinicians with the ability to better determine prognoses and the occurrence of orthodontically induced inflammatory root resorption, as well as elucidate the etiology of post-treatment relapse. Additionally, understanding the dynamics of remodeling pathways will also help clinicians to design more effective appliances which could target specific cells for a

controlled and safe acceleration of tooth movement. The aim of this study is to determine global dynamic changes in gene expression using RNA sequencing approach to assess the biological effects of OTM on the PDL and alveolar bone in a rat model.

Materials and Methods:

Wistar rats with body weight of 400-450g were used for experiments. 8 to 10 grams of protraction force was applied to the maxillary first molar using a nickel titanium coil spring.
Three hours, 1, 3, 7, and 14 days after the placement of the appliance, rats were sacrificed at each time point, respectively. PDL and the alveolus around the left maxillary first molar were excised on compression side. The samples were immediately frozen in liquid nitrogen for subsequent RNA extraction. Total RNA samples were prepared for mRNA-Sequencing using the Illumina TruSeq Stranded mRNA Sample Preparation kit. Sample libraries were prepared for Illumina sequencing by denaturing and diluting the libraries. RNA-Seq reads were aligned to the rat genomes using the STAR Aligner v2.7.0b with version 6.0 (July 2014) of the rat genome (http://www.ensembl.org). The gene expression profiles were quantified using read counts from the feature counts. DESeq2 was performed for determining differential expression genes (DEG) and threshold with adjusted P-value < 0.05 and absolute values of log2 (fold change) > 1. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were enriched by R package (v 3.5.3, https://www.r-project.org) to better understand the functions of the DEGs.
Results: Our data showed three processes, hypoxia, inflammation, and bone remodeling, are major mechanisms behind OTM. Hypoxia induces Hif1a/Arntl2 expression and inhibits Hif1an expression, which in turn leads to the induction of AP-1 heterodimers and Cebpb, promoting expression of a range of genes, especially cytokines (Il11 and Il6), chemokines (Cxcl1, Cxcl2 and Cxcl3) and MMPs. The activation of these pathways leads to various downstream activation including osteoclast differentiation, MMPs expression, and their substrates collagens turnover. The counteractive genes of MMPs, and cytokines/chemokines were also increased in the expression during OTM, since signaling pathways in biology system always have feedback mechanisms to balance the biological reaction cascades. **Conclusion:** By performing RNA-sequencing analysis on all the time points, we identified genes related to hypoxia, inflammatory cascade, MMPs activity and osteoclast differentiation have been significantly and dynamically changed during OTM.

Detailed results and inferences:

1. If the work has been published, please attach a pdf of manuscript OR
2. Describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis and interpretation of results should be included.

Respond to the following questions:

1. Were the original, specific aims of the proposal realized? **Yes**
2. Were the results published? **Manuscript is under preparation**
    a. If so, cite reference/s for publication/s including titles, dates, author or co-authors, journal, issue and page numbers **N/A**
    b. Was AAOF support acknowledged? **It will be included in the manuscript**

    c. If not, are there plans to publish? If not, why not? **Yes**
3. Have the results of this proposal been presented? **No**
    a. If so, list titles, author or co-authors of these presentation/s, year and locations
    b. Was AAOF support acknowledged?
    c. If not, are there plans to do so? If not, why not? **Yes**
4. To what extent have you used, or how do you intend to use, AAOF funding to further your career? **The AAOF RAA is essential to this project by assisting in the funding and support, especially in conducting the costly RNA sequencing studies.**

Accounting for Project; i.e., any leftover funds, etc.
The leftover funds were returned to AAOF.