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
E-Mail: rhazel@aaortho.org

AAO Foundation Final Report Form
(a/o 5/31/2017)

Please prepare a report that addresses the following:

Type of Award,
Research Aid Award

Name(s) of Principal Investigator(s)
Xuanyu Lu

Title of Project
Characterization of Biomimetically Enhanced Bio-Oss for Bone Regeneration Applications

Period of AAOF Support
07-01-2017 to 06-30-2018

Amount of Funding
5,000

Summary/Abstract of Completed Project Results

Clefts of the lip and palate are the most prevalent congenital craniofacial birth defects. From an orthodontic perspective, the aim of bone grafting at the alveolar cleft is to provide continuity and stabilization of the maxillary arch and to permit tooth eruption and orthodontic tooth movement. Although autologous bone graft is the gold standard, it requires a secondary surgical site and the risks of pain, morbidity, infection and scarring at donor site. Tissue engineering approaches that are aimed at improving the functionality of existing clinical materials may provide clinicians with new alternatives.

For decade, Bio-Oss, the porous bone mineral substitute, has been widely and safely applied to dental bone grafting procedure. It is osteoconductive and functions primarily as a space maintainer. However, the clinical Bio-Oss® does not support cell attachment and is not osteoinductive. Biomimetic strategies that incorporate the native osteogenic extracellular matrix (ECM) within collagen-based materials have been developed to improve the osteoinductive nature of the biomaterials. This application will focus on utilizing this biomimetic strategy to integrate the osteoinductivity to a frequently used bone graft material Bio-Oss.

We hypothesize that: the biomimetically enhanced anorganic bone graft material will impart osteoinductivity by improving stem cell attachment, proliferation and osteogenic differentiation and, ultimately, facilitating new bone formation and remodeling.

The goal of this research project was to establish a stable 3D pro-osteogenic ECM coating

on Bio-Oss®, and investigate the improved osteoinductive capacity of the biomimetically enhanced Bio-Oss (BE Bio-Oss) by improving stem cell attachment, proliferation and osteogenic differentiation by a series of *in vitro* and *in vivo* tests.

Upon the SEM comparison, the particle size and surface morphologies of BE Bio-Oss demonstrated no difference compared to control but exhibited ECM fibers deposition. However, the HMSC proliferation and the expression of osteogenic marker genes, such as *Runx2, Bmp2, colI*, and *OCN* were increased significantly on BE-Bio-Oss. Applied the BE Bio-Oss and control Bio-Oss in the rat critical-sized calvarial bone defects, comparing the bone healing during 4-, 8- and 12-week periods, the μ-CT analysis showed that the bony content (BV/TV), bone structures and bone mineral density were different in BE-Bio-Oss group compare to the control. Under the histological analysis, the BE Bio-Oss demonstrated increased osteogenic cell infiltration and attachment on particle surface as well as enhanced particle remodeling and collagen deposition. Osteogenic markers proteins, DMP1, fibronectin, BMP2, TGFβ and osteocalcin, were strongly expressed in the experimental group compared to Bio-Oss controls. Nano-indentation was not applicable for this experiment due to the cortical bone was not consolidated at 12-weeks.

Together, our data indicate existing anorganic bone graft material, Bio-Oss, possess poor osteoinductive properites. The biomimetically enhanced Bio-Oss could promote better cell attachment, proliferation and osteogenic differentiation *in vitro*, and facilitate stem cell attachments, differentiation and mineralized tissue remodeling *in vivo*. Our results show a methodology to enhance existing anorganic clinical bone graft materials for improved osteoinductive ability.

Response to the following questions:

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

2. Were the results published?
   No publish yet, but the manuscript will be submitted to publication in the near future, and AAOF support will be acknowledged.

2. Have the results of this proposal been presented?
   Yes, this proposal has been presented at the Clinic and Research Day at University of Illinois at Chicago, and AAOF support was acknowledged. Also, it's plan to be presented at IADR/AADR 2019.

   Characterization of Biomimetically Enhanced Anorganic Graft Material for Bone Regenerative Application, *Xuanyu Lu, Chun-Chieh Huang, Praveen Gajendrareddy, Sriram Ranindran*, Clinic and Research Day, Chicago, IL, 3-2018

3. To what extent have you used, or how do you intend to use, AAOF funding to further your career?
   This RAA funding contributes significantly to my master thesis research. Without the financial support from AAOF, it's impossible to complete a series of animal experiments about this project.

   My career goal is to be an orthodontic scientist. This founding opportunity not only supported my interest in bone regeneration at basic science level, but also it encourages me to advance my knowledge in Craniofacial Orthodontics and conduct clinical research to evaluate the outcomes of the secondary alveolar graft in craniofacial anomalies patients.

After I finished my orthodontic residency, currently, I am a Craniofacial Orthodontic Fellow at Children's Hospital Los Angeles. Taking care of cleft lip and palate kids as well as seeking the optimized long-term bone graft outcomes will be my specialty in the future. I am very appreciated AAOF support my research and early career development.

Accounting for Project; i.e., any leftover funds, etc.

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