Principal Investigator	Zongyang Sun, DDS, MS, MSD, PhD
Co-Investigator	
Secondary Investigators	
Award Type	AAOF Post-Doctoral Fellowship Award
Project Title	Regional Variation and Mechanoregulation of Pig Mandibular Periosteal Cells
Project Year	2008-2010
Institution	The Ohio State University
Summary/Abstract (250 words maximum)	**Objective:** Periosteal growth at human mandibular ramus is characterized by anterior resorption and posterior apposition, but the mechanism underlying this differential growth remains unknown. This study compared the expression profile of molecules involved in bone apposition/resorption at these regions of the ramal periosteum. **Methods:** Growing (3-4 month) pigs were used as a model. Periosteal growth at the mandibular ramus was assessed by vital staining and histological observation. Periosteal tissues were harvested and pulverized for direct RNA and protein extraction. Periosteal cells were extracted, expanded *in vitro* in osteogenic media, and subjected to a single dose of cyclic tensile strain (0.5Hz with 0, 5% or 10% magnitude). RNA and protein samples from periosteal tissues and cultured cells were examined by real-time RT-PCR and Western blot. **Results:** Histological observation confirmed that the pig mandibular ramus has the same anterior-resorption/posterior apposition pattern as in the human. Both *in vivo* tissue and *in vitro* cell samples demonstrated that the anterior region had stronger expression in RANKL/OPG (ratio) and BMP2, while less expression of OPG, than the posterior region. With the application of a single-dose cyclic tensile strain, cultured periosteal cells tended to change the expression profile of osteogenic markers, but not that of RANKL/OPG and BMP2. **Conclusion:** The unique regional variation of periosteal activity at the mandibular ramus is likely regulated by a differential expression in RANKL/OPG ratio (mainly through OPG) and BMP2, and a single-dose cyclic tensile strain *in vitro* is insufficient to reverse the osteoclastogenesis-promoting profile of anterior cells.
Were the original specific aims of the proposal realized?	All three specific aims proposed originally were realized. Detailed explanations are reported as follows. ***S.A.1 To establish and characterize periosteal osteoblast-like cell cultures from the anterior and posterior ramal surfaces of growing pigs.*** The procedures and protocols for harvesting and culturing of periosteal cells from the anterior and posterior regions were established. Osteogenic features of cultured cells were confirmed by alkaline phosphatase and alizarin red staining. All cells were passaged when reaching 80% confluence. Cells from passage 3-5 were used for subsequent experiments.

	S.A.2 To compare these two types of cells with regard to the expression of osteogenic differentiation markers (Runx 2, Smad5) and key regulators controlling the shift between bone formation and resorption (RANK, RANKL, and OPG). Molecular expression was examined by real-time RT-PCR and Western blot (for RANKL and OPG). All primer sets used in this study were designed based on the sequences from NCBI nucleotide database. The specificity of PCR products were confirmed by electrophoresis of PCR products. Housekeeping gene β-actin was used to normalize all PCR results by the comparative threshold cycle (C_T) method. Major findings: 1), the expression of osteogenic differentiation markers (Runx2 and Osteocalcin) were comparable between the anterior and posterior regions; 2) compared to posterior cells, anterior cells had significantly less OPG expression but significantly stronger BMP2 expression, while their expression of RANKL was comparable. ***S.A.3 To examine the effects of cyclic tension (cTENS) or compression (cCOMP) on the molecular regulation of osteogenesis in periosteal cells as compared to untreated controls.*** Cell loading (tension) experiments were performed using FX-4000 Tension Plus system. Cells were cultured in 6-well Bioflex culture plates coated with Collagen-Type I (Flexcell International, Hillsborough, NC). Upon confluence, the plates were stretched by mechanical strains of 0% (negative control), 5% or 10% at 0.5Hz for 2 hours, followed by using a FX-4000 Tension Plus system (Flexcell International). RNA and protein samples were subsequently extracted from strained cells and analyzed by real-time RT-PCR and Western blot. Major findings: 1) with the increase of strain magnitude, anterior cells tended to have upregulated expression of osteogenic differentiaon markers while posterior cells tended to downregulated expression of these markers; 2) this single dose of tensile strain did not decrease the expression of BMP2 and RANKL/OPG ratio in the anterior cells, suggesting their resorptive profile is not reversed. **In addition to the proposed specific aims, we also expanded the project to the following two areas:** 1. Confirmed that the pig has the same periosteal growth pattern at the mandibular ramus as in the human. This was achieved by vital staining using fluorescent bone labels (calcein and alizarin complexone) for mineralization followed by observing postmortem specimens containing the mandibular ramus. 2. Examined *in vivo* expression of the same molecules by directly assaying periosteal tissues from the two regions. This

	was achieved by pulverizing periosteum tissue (frozen with liquid nitrogen) followed by the extraction of RNA and proteins. Extracted samples were analyzed by RT-PCR and Western blot using the same methods as used for *in vitro* cell samples. **As originally predicted, this fellowship will be of great significance in three areas, all of which have also been fulfilled as detailed below.** "*First, a post-doctoral training is essential for becoming a full-fledged independent investigator. This post-doctoral experience will allow me a more equal footing with others when seeking peer reviewed research funding, such as from NIDCR.*" This AAOF support has certainly provided me an indispensable post-doctoral development towards an independent investigator. Part of the preliminary data generated by this project was used for an NIH/NIDCR R03 grant application and this application has been approved for funding earlier this year. *"Second, the planned research project will expand my area of expertise to molecular biology and signal transduction. I have not done in vitro studies before, and the opportunity to train with Dr. Agarwal will give me new skills. This, together with my extensive experience in studying bone growth at the tissue and cellular level, will greatly enhance my ability to investigate the relationship between mechanics and craniofacial growth."* This project has indeed expanded my research expertise into molecular biology and signal transduction. Through the experiments, I have become more knowledgeable and acquainted with the practical aspects of many techniques and methodology used to study cellular and molecular biology. This will significantly help my future research. *"Third, the planned research will start to unveil the mechanisms of an overlooked major craniofacial growth structure, the periosteum. The results of these studies will provide much needed clues to the underlying mechanisms of mandibular growth and remodeling, which will bring significant benefits to clinical orthodontics."* I believe that this project does provide a clearer understanding the differential periosteal growth at the mandibular ramus at the molecular level. The data proved from both *in vitro* and *in vivo* aspects that the periosteum is different between an appositional and a resorptive surface. The two molecules, OPG and BMP2, found to be critical for bone resorption/formation control at these regions, can potentially be used as biochemical supplements in orthodontic practice to change periosteal growth, an area that my lab will further investigate in the future.

Were the results published? If not, are there plans to publish? If not, why not?	Yes, the results have been submitted for publishing. Zongyang Sun, Boon Ching Tee. Molecular Variations Related to the Regional Differences in Periosteal Growth at the Mandibular Ramus. *Anat. Rec*., Under review.
Have the results of the proposal been presented? If so, when and where? If not, are there plans to do so? If not, why not?	Yes, the results have been presented. Sun, Z, Tee BC, Hueni S and Agarwal S. 2010. Location Variations of Mandibular Periosteum. 2010. AADR 38th Meeting, Washington, DC. Sun, Z. Molecular control of differential periosteal growth at the mandibular ramus. Invited Oral Presentation. 2010 International Conference on Craniofacial Research (Moyers Presymposium), University of Michigan, Ann Arbor, MI. Tee BC, Sun, Z, Molecular Variation of Mandibular Periosteum. 2010. Experimental Biology Meeting, Anaheim, CA.