AAO Foundation Award Final Report

Principal Investigator	Zongyang Sun
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Award Type	Faculty Development Fellowship Award
Project Title	Characterize and Validate Pig Bone Marrow Stromal Cells
Project Year	July 1, 2010-June 30, 2011
Institution	The Ohio State University
Summary/Abstract (250 word maximum)	**Aim:** Autologous stem cells, such as bone marrow stromal cells (BMSC), may provide a better alternative to repair large craniofacial bone defects than autologous bone grafting. Clinical application of BMSCs relies on preclinical studies on large animals. The present project was aimed at establishing methodology to extract/expand pig bone marrow stromal cells (pBMSC) and characterizing their response to FGF2 and inflammatory cytokines. **Methods:** Bone marrow was aspirated from the tibia of 4-month-old pigs. Then pBMSCs were isolated and cultured following literature recommended protocols. Multipotency of cultured pBMSC was examined. Expansion efficiency was assessed by calculating cell doubling time. The effects of FGF2 and inflammatory cytokines (IL-1β and TNF-α) on pBMSCs were analyzed by varied methods. **Results:** Upon proper induction agents, pBMSCs were differentiated into osteogenic, adipogenic or chondrogenic lineages. Cell doubling time ranged between 1.5 and 3 days and about 200 million pBMSCs could be obtained within 3 weeks after a single bone marrow aspiration (~30ml). FGF2 enhanced osteogenic media in promoting pBMSC osteogenic differentiation. While treatment of 5 ng/mL FGF2 for 5 days most effectively induced pBMSC osteogenic commitment, higher concentration and longer time of FGF-2 treatment further stimulated osteogenic cells to mature. Short term (1-3 days) treatment of IL-1β and TNF-α tended to decrease pBMSC viability but had little effect on their osteogenic differentiation. **Conclusion:** This study established methodology to efficiently culture/expand pBMSC *in vitro* and identified an optimal FGF2 treatment to enhance pBMSC osteogenic differentiation, which will be used in our future *in vivo* preclinical studies.
Were the original, specific aims of the proposal realized?	Yes. The original specific aims have two major components, teaching and research.

	Teaching: ***S.A.1 To learn and practice skills of directing orthodontic case conference*** ***S.A.2 To develop didactic course materials on cellular and molecular mechanisms of craniofacial development, tooth eruption and orthodontic tooth movement*** During the last year, I have been co-directing the weekly orthodontic case conferences with a senior faculty member. This experience has improved my skill in delivering this type of teaching. I have also developed course materials in these areas for undergraduate and orthodontic graduate students. Research: ***S.A.3 To culture human bone marrow stromal cells (BMSCs) and characterize their response to mechanical loading*** ***S.A.4 To validate pig BMSc extraction and culturing techniques and compare pig and human BMSc for their osteogenic features and response to mechanical loading*** Since February 2011, we have conducted a series of studies to address these specific aims. Our initial work focused on human BMSCs using cell samples kindly donated to us by Dr. Pamela Robey (Director, Craniofacial and Skeletal Diseases Branch, NIDCR). These studies confirmed the stem-cell like features of human BMSCs and established protocols to culture this type of cells. In June 2010, we switched our focus to pig BMSCs. Through 5 sets of intensive experiments, we established and improved techniques and procedures to aspirate pig bone marrows and to isolate/culture pig BMSCs. We were able to confirm that the cultured pig BMSCs, similar to human BMSCs, possess multipotent differentiation capacity and can be quickly expanded *in vitro*. We did not examine their response to mechanical loading as we realize that *in vivo* loading during function or treatment (such as distraction osteogenesis) is hard to be duplicated in *in vitro* settings. Instead, as optimal osteogenic differentiation of BMSCs may be a desired feature for *in vivo* BMSC transplantation, and any transplanted cells will experience a short phase of inflammation challenge as a result of the surgery, we examined the effects of FGF2 and proinflammatory cytokine on pig BMSCs. We believe that these experiments are more relevant and useful for our *in vivo* studies.
Were the results published? If not, are there plans to publish? If not, why not?	Presently, the results have not been published in any full-size peer-reviewed papers yet, but we are preparing a manuscript based on the results. A peer-reviewed abstract has been accepted to publish at the 2011 Biomedical Engineering Society (BMES) meeting (detailed below).

Have the results of this proposal been presented? If so, when and where? If not, are there plans to do so? If not, why not?	Yes, the results have and will be presented in multiple meetings. 1. **Sun Z**, Tee BC, Gales MJ, Mallery SR, Fields HW. Pig Bone Marrow Stromal Cell Expansion and Response to FGF2 and Inflammatory Cytokines. OSU 2nd CCTS Annual meeting. May, 2011. Columbus, OH. 2. Tee BC, Mallery SR, Fields HW, **Sun Z**. Expand Pig BMSC in vitro and Use FGF2 to Enhance Osteogenic Differentiation. Biomedical Engineering Society Annual Meeting, October 2011. Hartford, CT 3. Gales MJ, Tee BC, **Sun Z.** Proinflammatory Cytokine Regulation of Bone Marrow Stromal Cells. OSU Denman Research Forum, May 2011, Columbus, OH.