AAO Foundation Award Final Report

Principal Investigator	Zongyang Sun
Co-Investigator	Kelly Kennedy
Secondary Investigators	
Award Type	Faculty Development Fellowship Award
Project Title	Use autologous bone marrow stromal cells to enhance pig mandibular distraction osteogenesis
Project Year	July 1, 2011-June 30, 2013
Institution	The Ohio State University
Summary/Abstract (250 word maximum)	**Background:** Bone regeneration through distraction osteogenesis (DO) is promising but remarkably slow. This pilot project investigated a scaffold-based approach for cell delivery to the distraction site in a porcine mandibular DO model. **Methods:** Eleven adolescent domestic pigs were used. The in-vitro study established methodologies to: aspirate bone marrow from the tibia; isolate, characterize and expand bone marrow-derived mesenchymal stem cells (BM-MSCs); enhance BM-MSC osteogenic using FGF-2; and confirm cell integration with a gelatin-based Gelfoam scaffold. The in-vivo study transplanted autologous stem cells into the mandibular distraction sites using Gelfoam scaffolds; completed a standard DO-course and assessed bone regeneration by macroscopic, radiographic and histological methods. Repeated-measure ANOVAs and t-tests were used for statistical analyses. **Results:** From aspirated bone marrow, multi-potent, heterogeneous BM-MSCs without contamination of hematopoietic stem cells were obtained. FGF-2 significantly enhanced pig BM-MSC osteogenic differentiation and proliferation, with 5 ng/ml determined as the optimal dosage. Pig BM-MSCs integrated readily with Gelfoam and maintained viability and proliferative ability. After integration with Gelfoam scaffolds, 2.4-5.8 x10^7 autologous BM-MSCs (undifferentiated or differentiated) were transplanted to each experimental DO site. Among 8 evaluable DO sites included in the final analyses, the experimental DO sites demonstrated less interfragmentary mobility, more advanced gap obliteration, higher mineral content and faster mineral apposition than the control sites, all indicative of enhanced bone regeneration. **Conclusion:** It is technically feasible and biologically sound to deliver autologous BM-MSCs to the distraction site immediately after osteotomy using a Gelfoam scaffold to enhance mandibular DO.
Were the original, specific aims of the proposal realized?	Yes. The original specific aims have two major components, experiments and grant application. <u>Experiments:</u> ***S.A.1 To optimize the method to integrate pig BM-MSCs with a scaffold appropriate for the distraction osteogenesis site.*** ***S.A.2 To assess bone-regeneration capacity of autologous BM-MSCs (carried by Gelfoam) in a pig mandibular distraction***

	osteogenesis model. Over the award period, our lab has successfully addressed these specific aims. We have conducted a series of *in vitro* studies to characterize pig BM-MSC integration with Gelfoam scaffold. These studies confirmed that the integrated BM-MSCs, either undifferentiated or osteogenic-differentiated, were able to maintain their viability and proliferation. We also optimized the loading efficiency and duration according to the cell type. Subsequently, we conducted the *in vivo* mandibular DO studies on 6 pigs with blank control (no Gelfoam), Gelfoam-only control, and experimental sites transplanted with either undifferentiated or osteogenic-differentiated autologous BM-MSCs carried by Gelfoam scaffolds. Our results demonstrated that a scaffold-based cell-delivery approach is feasible and promising to stimulate mandibular DO site bone regeneration. <u>Grant application:</u> ***S.A.3 To prepare an NIDCR R01 grant proposal to investigate mandibular bone regeneration using combined cell and mechanical approaches.*** We have submitted an R01 grant to the NIH/NIDCR in June, 2013 based on the preliminary data generated by this project.
Were the results published? If not, are there plans to publish? If not, why not?	Sun Z, Tee BC, Kennedy KS, Kennedy PM, Kim D-G, Mallery SR, Fields HW. Scaffold-based delivery of autologous mesenchymal stem cells for mandibular distraction osteogenesis: preliminary studies in a porcine model. *PLoS One.* 2013; In press. Additionally, results of this project have been published in several abstracts: 1. Sun Z, Tee BC, Kennedy KS, Kim DG, Low E. Optimizing gelatin-based sponges as stem-cell carriers for mandibular distraction osteogenesis. *J Dent Res.* 2012;91(Spec Iss A):53. 2. Kennedy PM, Tee BC, Kennedy KS, Mallery S, Fields HW, Sun Z. Enhance mandibular distraction osteogenesis using a tissue engineering approach. *J Oral Maxillofac Surg.* 2012; 70(9):Supp. 2, e88-e89. 3. 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. 2011. 206:116.
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 been presented at several meetings. 1. Poster presentation: Sun Z, Tee BC, Kennedy KS, Kennedy P., Kim DG, Mallery SR, Fields HW. Enhance Mandibular Distraction Osteogenesis Using Autologous Stem Cells in Gelatin-based Scaffolds. 3rd OSU Annual CCTS Scientific Meeting, May 2012, Columbus, OH. [CCTS poster award winner] 2. Oral presentation: Sun Z, Tee BC, Kennedy KS, Kim DG, Low E.

	Optimizing gelatin-based sponges as stem-cell carriers for mandibular distraction osteogenesis. American Association of Dental Research 2012. Tampa, FL. 3. Poster presentation: Kennedy P, Tee BC, Kennedy KS, Mallery SR, Fields HW, Sun Z. Enhanced Mandibular Distraction Osteogenesis Using a Tissue Engineering Approach. American Association of Oral and Maxillofacial Surgeons Annual Meeting 2012. San Diego, CA. 4. Poster presentation: Tee BC, Mallery SR, Fields HW, Sun Z. Expand Pig BMSC in vitro and Use FGF2 to Enhance Osteogenic Differentiation. Biomedical Engineering Society meeting 2011. Hartford, CT.