Principal Investigator	Nan Hatch, DMD PhD
Co-Investigator	
Secondary Investigators	
Award Type	AAOF Post-Doctoral Fellowship Award
Project Title	Nan E. Hatch Post-Doctoral Fellowship Award
Project Year	2007-2009
Institution	University of Michigan
Summary/Abstract (250 words maximum)	The research aspect of this post-doctoral fellowship involved two projects. **Aim 1. Investigate the role of cellular pyrophosphate elaboration in FGF stimulated changes in mineralization.** Hypothesis: FGF effects mineralization via induction of the pyrophosphate generating enzyme, PC-1, in osteoblastic cells. Pyrophosphate and phosphate are critical mediators of bone mineralization. Pyrophosphate is an established inhibitor of hydroxyapatite deposition and crystal growth yet, when hydrolyzed into phosphate, it becomes a substrate for hydroxyapatite deposition. Altered expression of pyrophosphate/phosphate elaborating factors downstream of FGF signaling could provide a potential mechanism for the changes in mineralization seen in FGF2 treated and FGF2 transgenic mice, as well as for the aberrant mineralization phenotype of the FGFR associated craniosynostosis syndromes. PC-1 is the primary enzymatic generator of pyrophosphate in osteoblastic cells therefore the induction of PC-1 by FGF2 may be a primary mechanism by which FGF2 effects mineralization. Studies in this aim will 1) confirm that FGF2 treatment of calvarial osteoblastic cells increases pyrophosphate elaboration, 2) investigate the mechanism by which FGF signaling regulates expression of PC-1, 3) confirm that FGF2 induces expression of PC-1 *in vivo*, and 4) elucidate the role of PC-1 activity in FGF2 effects upon mineralization *in vitro* and *in vivo*. **Aim 2. Evaluate the ability of FGF2 gene therapy to stimulate bone regeneration in mice.** Hypothesis: Adenoviral expression of FGF2 will promote pre-osteoblastic cell proliferation and elaboration of factors for generation of osteoid at bone repair sites so as to enhance overall bone regeneration at these sites. FGF2 stimulates both the proliferation and differentiation of osteoblast precursor cells. While FGF2 itself appears to have an immediately inhibitory effect upon mineralization, it has been shown to have an overall bone anabolic effect in both FGF2 treated mice and osteoblastic cells in culture, if the cells or mouse is allowed to

	recover for a period of time prior to bone formation or mineralization assays. Additionally, treatment with recombinant FGF2 protein has been shown to significantly enhance repair of bony fractures *in vivo*. Unfortunately, use of recombinant proteins is limited both by expense of production and potential for rapid degradation *in vivo*. These results suggest that the use of gene therapy to express FGF2 will significantly contribute to bone repair. Development of a well-regulated adenoviral FGF expression system could ultimately lead to a less costly, highly practical and efficient method for stimulating bone regeneration via gene therapy in clinical settings. Additionally, adenoviral expression of FGF2 in mice will enable a systematic investigation of the mechanism by which FGF signaling alters bone mineralization and cranial suture fusion *in vivo*. Studies in this aim will optimize and evaluate the biologic activity of an FGF2 adenovirus by 1) inserting an FGF4 secretion signal sequence into the amino terminus of FGF2 cDNA for construction of an FGF2/FGF4 signal sequence adenovirus with higher FGF2 secretion levels, 2) confirming efficient secretion of FGF2 from transduced cells, 3) measuring changes in proliferation and differentiation of osteoblastic cells upon transduction with FGF2 in culture, and 4) investigating the bone formation effects of adenoviral FGF2 expression in ectopic (subcutaneous) and orthotopic (calvarial bone defect) sites *in vivo*.
Were the original specific aims of the proposal realized?	The goals of this proposal are in the process of being met. Substantial data has been generated and presented at both national and international conferences. Manuscripts have been submitted for publication. Some have been accepted and some are still under review. Perhaps most significantly, an R01 research application has been written and submitted to the National Institutes of Health (NIH). This application is under review for the 2nd of three possible revisions. Unfortunately, NIH funding is currently near an all time low, due to economic constraints. Competition for funding is high and includes competition with PhD scientists who have commonly completed 2-3 post-doctoral fellowships prior to submitting to NIH for funding. I am optimistic though, that given adequate time I will be able to successfully compete for NIH funding and achieve independence as a biomedical researcher with an orthodontic perspective. During this fellowship period I have also developed didactic curriculum on the biology of orthodontic tooth movement, orthopedic growth modification and orthodontic associated root resorption. I am teaching this course for the first time this fall. Feedback thus far has been very positive. Additionally, I am an active member on two Orthodontic Resident Master of Science Thesis Committees. One of these projects is an investigation of biologic mediators for enhanced orthodontic retention. I am currently submitting a proposal to Amgen (independent biomedical company) for collaborative work and access to recombinant biologic mediators for enhancement of orthodontic tooth movement, retention and anchorage. While translation of this work into clinical practice will take time, we should be able to determine the clinical utility of these recombinant

	proteins through a series of orthodontic resident projects. These mediators are also actively being pursued by others for the treatment of osteopenia and osteoporosis, which should ultimately help for translation into the clinic.
Were the results published? If not, are there plans to publish? If not, why not?	**Hatch** N, Roca, H and Franceschi RT (2008). *FGF2 stimulated expression of the pyrophosphate generating enzyme, PC-1, is mediated by Runx2 in pre-osteoblast cells*. J Bone Min Res, in review. **Hatch**, N, Franceschi RT (2007*).* Osteoblast Differentiation Stage Specific Expression of the Pyrophosphate Generating Enzyme, PC-1*.* Cells Tiss Organs, 10:53-8. **Hatch** N. (2007). *Potential role of PC-1 Expression and Pyrophosphate Elaboration in the Molecular Etiology of the FGFR Associated Craniosynostosis Syndromes*. Orthod Craniofac Res, 10: 53-8.
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?	**Hatch**, N (2008). *Cellular mechanisms of premature cranial suture fusion.* Oral presentation and published abstract, Fourth Biennial Conference on Orthodontic Advances in Science & Technology, Monterey, CA. **Hatch**, N and Franceschi RT (2008). *FGF2 Induced Expression of the Pyrophosphate Generating Enzyme, PC-1, is Mediated by Runx2 and Msx2.* Oral presentation and published abstract, 38th International Sun Valley Workshop On Skeletal Tissue Biology, Sun Valley, ID. **Hatch**, N (2008). *FGF Stimulated Pre-osteoblastic Expression of the Pyrophosphate Generating Enzyme, PC-1, is regulated by Msx2 and Twist.* Oral Presentation and published abstract. Annual Meeting of the International Association of Dental Research, Toronto, Canada. **Hatch**, N (2008). *Current Concepts in Craniofacial Skeletal Development.* Oral presentation, Annual Session of the American Association of Orthodontists, Denver, CO. **Hatch** N, Roca H and Franceschi RT (2007). *Runx2 Mediates FGF2 Stimulated Expression of PC-1 in Osteoblastic Cells. P*oster presentation and published abstract. Annual meeting of the International Association of Dental Research. New Orleans, LA. **Hatch** N and Franceschi RT (2007). *Runx2 Mediates FGF2 Stimulated Expression of the Pyrophosphate Generating Enzyme, PC-1, in Osteoblastic Cells.* Oral Presentation and published abstract, 9th International Conference on the Chemistry and Biology of Mineralized Tissues, Austin, TX.
Awards	2008 Harold M. Frost Young Investigator Award International Sun Valley Workshop on Skeletal Tissue Biology American Society for Bone and Mineral Research

	2007 American Association of Dental Research Hatton Award 1st Place, Post-doctoral Category 2007 International Association of Dental Research Hatton Award 1st Place, Senior Basic Science Category