AAO Foundation Award Final Report

Principal Investigator	James L. Borke, PhD
Co-Investigator	Weston Fortson, Jr., DMD
Secondary Investigators	Dr. James Whitehead (Orthodontic Resident)
Award Type	Biomedical Research Award
Project Title	Connexin 43 Expression in Orthodontic Tooth Movement
Project Year	1997
Institution	Medical College of Georgia School of Dentistry
Summary/Abstract	This project was completed in 1998. The objectives of this study were to define the spatial and temporal expression of connexin 43 in osteocytes associated with orthodontic tooth movement in rats. At that time we had little experience with orthodontic tooth movement in rats and this grant gave us the opportunity to gain some of that experience. The spring design selected for this study exerted too great a force and tooth movement was completed in the first few minutes of activation. We were not aware of this until several months into the project (see preliminary report attached). This caused some tissue necrosis and tooth evulsions and did not allow us to evaluate the temporal relationship of connexin expression with tooth movement. As designed, the results of this study were not publishable. However, much that was learned has been applied to parallel and subsequent studies which are listed below. *Published Manuscripts* Su M, **Borke JL**, Donahue HJ, Li Z, Warshawsky NM, Russell CM, Lewis JE. Expression of connexin 43 in rat mandibular bone and periodontal ligament (PDL) cells during experimental tooth movement. *Journal of Dental Research* 76(7): 1357-1366, 1997. Adamo CT, Mailhot JM, Smith AK, **Borke JL**. Connexin-43 expression in oral-derived human osteoblasts after transforming growth factor β and prostaglandin E_2 exposure. *Oral Implantology* 27: 25-31, 2001. *Published Abstracts* Zaki AE, **Borke JL**, Eisenmann DR and Medneiks MI. Differential expression of connexin-43 antigen in secretory and maturation ameloblasts. *Journal of Dental Research* 76(SI): 406, 1997. Zaki A, Eisenmann D, Mednieks M, Genutis S, Merheb N, **Borke J** and Hand A. Connexin 43 and actin distribution in rat secretory and maturation ameloblasts. *VIth International Conference on Tooth Morphogenesis and Differentiation*, Goteborg, Sweden,

June 11-15, 1997.

Adamo C, Mailhot J, **Borke J**. Increased connexin-43 expression in oral-derived human osteoblasts after TGF-β exposure. *Journal of Dental Research* 78: Abst. 2824, 458, 1999.

Orellana MF, **Borke JL**. Mechanotransduction in Orthodontic Tooth Movement. *The FASEB Journal* 15(4): Abst. 122.3, A68, 2001.

PRELIMINARY REPORT
Originally submitted December 30, 1997

December 30, 1997

Raymond George, D.M.D. - President
American Association of Orthodontists Foundation
401 N. Lindbergh
St. Louis, MO 63141

Dear Dr. George:

I am the recipient, along with Dr. Weston Fortson, of an American Association of Orthodontists Foundation Biomedical Research Award for our research project entitled, **Connexin 43 Expression in Orthodontic Tooth Movement**. In accordance with conditions of this award, I am writing to report on the progress we have made thus far with this project.

Dr. Fortson and I have recruited an orthodontics resident, Dr. James Whitehead, from our institution to work on this project for his Masters Degree thesis. Together we have manufactured and tested all of the springs prior to installation in the test animals.

After long discussions Dr. Fortson, Dr. Whitehead and I had decided that we could obtain additional information about direction and rate of tooth movement if we radiographed the mandibles of each animal over time with the springs engaged. It was our rational that we might be able to take measurements on the radiographs that would give us the rate and direction of movement in one or more axes prior to sacrifice of the animals, and thus more accurately assess the forces experienced by the cells we would later observe in the tissue sections processed for immunohistochemistry and *in situ* hybridization. We therefore tested several jig designs meant to reproducibly position the rat's head and the film, for multiple radiographs on the same animal. We placed films intra- and extraorally in multiple planes, and tried multiple and varied exposures. These studies were unsuccessful in that identification of reproducible landmarks and absolute repositioning of the rat heads in the anesthetized animals could not be

obtained.

We next proceeded to install springs in the rats and sacrifice them according to the schedule in the grant application. At the prescribed time points, the animals were perfused with formalin and the mandibles from each rat were excised, cleaned and fixed for the immunohistochemistry and *in situ* hybridization. Before decalcifying and processing the mandibles for these studies, we began radiographing each mandible for assessment of direction and rate of movement. In the excised mandible, reproducible positioning of film and mandible has been substantially easier. Comparisons of the side of each mandible subjected to force with the contralateral side of the same mandible, (without applied force), are being used for this determination.

At the present time we are finishing the radiographing of the excised mandibles. As each mandible is radiographed it is being decalcified, embedded in paraffin and sectioned for immunohistochemistry and *in situ* hybridization. We expect to finish all of the radiography, tissue processing and sectioning, by the end of January. I have a full-time technician who is processing and sectioning each tissue block as we finish the radiography and decalcification. Dr. Whitehead will continue making all of the measurements on the radiographs while the tissue processing is completed. In February my technician will begin labeling the tissue sections for immunohistochemistry and *in situ* hybridization with antibodies and mRNA probes specific for connexin 43. My technician is very proficient at these techniques and should have the labeled sections ready in the early part of March. Dr. Whitehead, Dr. Fortson, and myself will photograph and analyze these sections with image analysis software for studies of the distribution of connexin 43 and final quantitative histomorphometry. Correlation of this information with the radiographic data will be assessed for our final report.

Once again, Dr. Fortson and I would like to thank the members of the American Association of Orthodontists Foundation for their support. We are available anytime at your convenience to discuss this work further or to provide any additional information that you may require.