RESEARCH REPORTS
Biological

E.J. Toro[1], J. Zuo[1], A. Guiterrez[1], R.L. La Rosa[1], A.J. Gawron[1], V. Bradaschia-Correa[2], V. Arana-Chavez[2], C. Dolce[1], M.F. Rivera[3], L. Kesavalu[3], I. Bhattacharyya[4], J.K. Neubert[1], and L.S. Holliday[1,5]*

[1]Department of Orthodontics, University of Florida College of Dentistry, Gainesville, FL 32610, USA; [2]Laboratory of Oral Biology, Department of Dental Materials, School of Dentistry, University of São Paulo, 05508-900 São Paulo SP, Brazil; [3]Department of Periodontology, University of Florida College of Dentistry, Gainesville, FL 32610, USA; [4]Department of Oral & Maxillofacial Diagnostic Sciences, University of Florida College of Dentistry, Gainesville, FL 32610, USA; and [5]Department of Anatomy and Cell Biology, University of Florida College of Medicine, Gainesville, FL 32610, USA; *corresponding author, sholliday@dental.ufl.edu

J Dent Res DOI: 10.1177/0022034513501876

Bis-enoxacin Inhibits Bone Resorption and Orthodontic Tooth Movement

APPENDIX

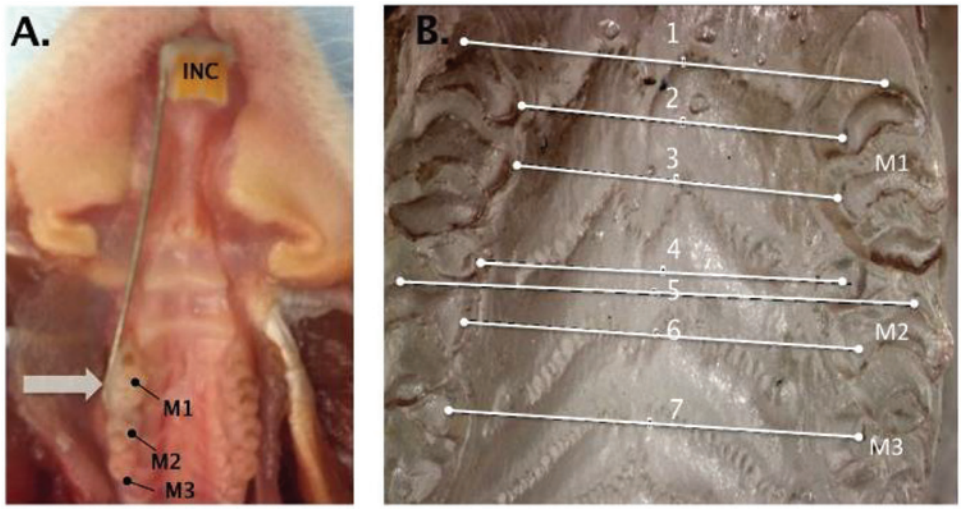
MATERIALS & METHODS

Orthodontic Tooth Movement

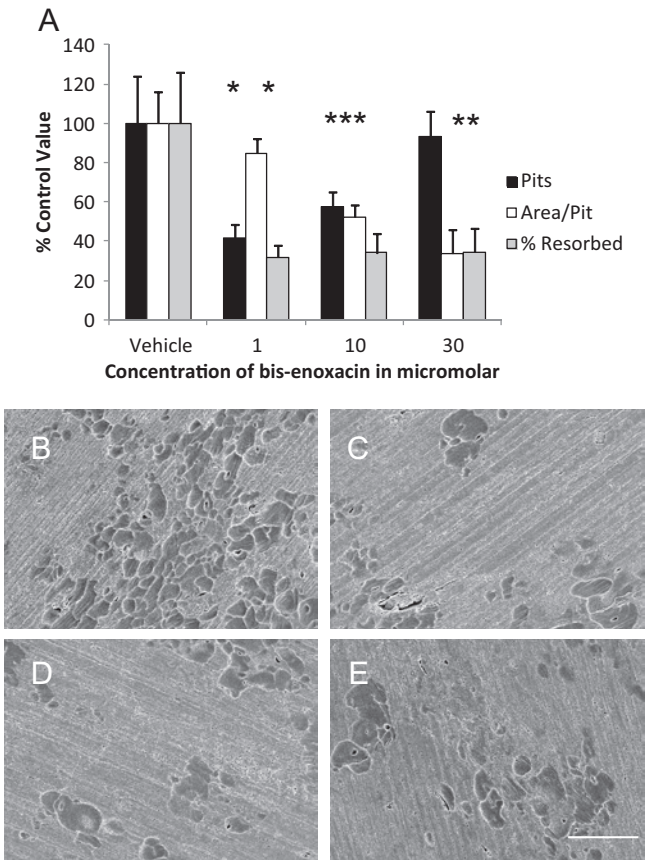
Custom plastic trays were filled with light body impression material (Aquasil Ultra XLV, Dentsply Caulk, Milford, DE, USA), and impressions were made for 5 min. The general integrity of the impression was checked under magnification to ensure that the maxillary teeth were captured, and animals were re-impressed as needed. The impressions were allowed to off-gas for at least 24 hrs and were then prepared for model fabrication. The custom tray was trimmed to allow the tray/impression to be placed into a 2.5-cm-diameter PVC tube that was partially filled with warm paraffin. Epoxy resin (EpoFix Resin, Struers, Cleveland, OH, USA) was poured into the PVC ring of the tray to fill the impression, with large bubbles removed by means of a sharp explorer tip. Small bubbles were released and removed under vacuum. These impressions and models were obtained 1 wk prior (baseline) to appliance bonding, and then at weekly intervals post-appliance application until the end of the study. A 0.014" high tensile stainless steel Australian wire (A.J. Wilcock/Webster and Horsfall Ltd, West Midlands, UK) appliance was custom-fitted to the baseline epoxy model for each animal such that a 90° bend was placed at the mesiobuccal line angle of the right incisor (Appendix Fig. 1). The facial part of the wire wrapped around the mesiobuccal portion of the left incisor, and the distal arm of the wire extended back to the distal portion of the first molar. The amount of activation for each wire was measured (30 g, Pesola Micro-Line 20031 scale; Kapuskasing, ON, Canada) and recorded prior to bonding. This wire was chosen because it provided a light, continuous force (<13 cN) when activated.

The maxillary teeth were isolated, and retention grooves were placed on the gingivodistal portion of both maxillary incisors and interproximally near the gingiva by means of a flame-tipped diamond bur. The incisors and maxillary first molar were etched with 35% phosphoric acid (Ultra Etch, Ultradent, South Jordan, UT, USA) and bond enhancer (Ortho Solo Bonding, Ormco, Orange, CA, USA), and then the wire was bonded with flowable composite (Henry Schein, Melville, NY, USA) at the gingival margin of the incisors (*INC*) such that the distal end lay passively against the palatal surface of the maxillary first molar (*M1*). The appliance was activated by bonding to the buccal surface of the maxillary molar, producing a force that tipped the tooth in the palatal direction. Animals were monitored for signs of distress, including failure to groom, with excessive red porphyryn around the eyes and abnormal resting postures.

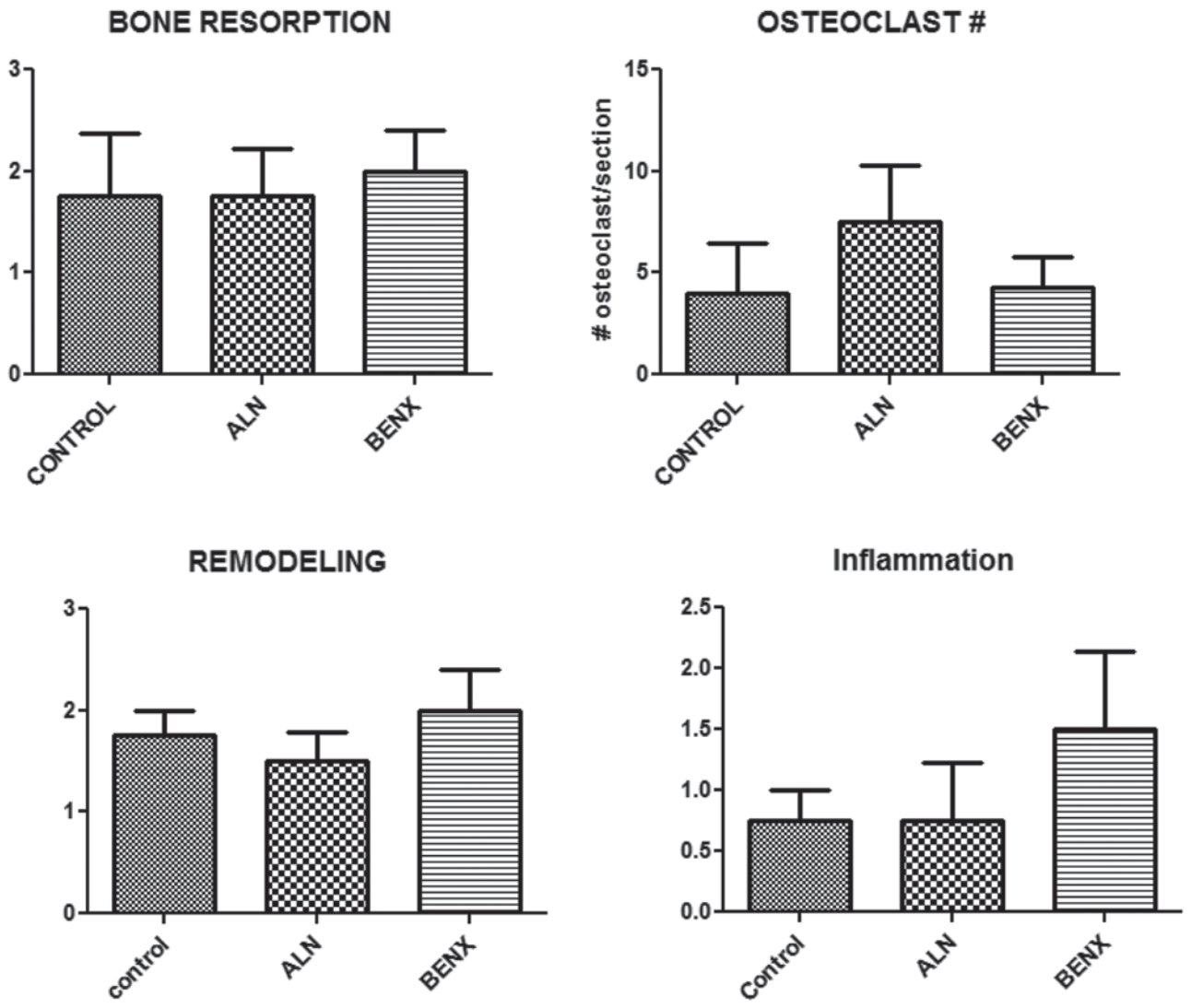
To determine the amount of orthodontic tooth movement, we imaged the epoxy models for each animal at every time-point (Zeiss AxioCam MR microscope, 10x magnification; Carl Zeiss, Göttingen, Germany). In total, 7 points were measured between each right and left molar tooth with Image J software (NIH, Bethesda, MD, USA) to determine the amount of palatal movement. The value for each respective set of points was subtracted from the pre-activation baseline value to determine the relative movement. Points 1 to 3 were averaged for assessing 1st molar movement, points 4 to 6 for 2nd molar movement, and point 7 for 3rd molar movement. Orthodontic tooth movement (OTM) was considered the movement of the first molar, while the second and third molars served as non-moving controls.



Appendix Figure 1. New rat model for orthodontic tooth movement studies. **(A)** Image of rat with the mandible removed to demonstrate the set-up of the orthodontic apparatus. M1, M2, and M3 indicate molars, and the white arrow shows the direction of force. **(B)** Digital image of impression showing locations where movement was measured. The distances indicated by lines 1 to 3 were averaged, and these data are presented in Fig. 4B (main article).



Appendix Figure 2. BE inihibts bone resorption by mouse marrow osteoclasts. **(A)** Examination of the effects of BE on bone resorption. The total area analyzed *per* image was 745,000 μm^2. Mean control values were 13.6% resorption, 15 pits, and 5,713 μm^2/pit. Asterisks indicate $p < .05$. **(B-E)** Representative scanning electron micrographs for (A), (B) (vehicle), (C) 1 μM BE, (D) 10 μM BE, and (E) (30 μM BE). The scale bar in (E) equals 50 μm in all panels.



Appendix Figure 3. Analysis of histological sections from tooth and alveolar bone from rats treated with vehicle, alendronate, or enoxacin and subjected to orthodontic force for 28 days. Bone resorption, remodeling, and inflammation were rated by an oral pathologist on a 0 to 3 scale. Osteoclasts were counted in sections with approximately equal areas of alveolar bone. Note that osteoclasts were abundant under each condition, and evidence of bone resorption and bone remodeling was evident despite the inhibition of orthodontic tooth movement by alendronate and BE.