How does the amount of surgical insult affect bone around moving teeth?

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Introduction: The purpose of this study was to determine how the amount of surgical insult affects the quantity and maturity of dentoalveolar bone around teeth that have been orthodontically moved. Methods: A split-mouth design with 8 foxhound dogs was used to evaluate bone surrounding maxillary second premolars that were protracted for 15 days and retained for 7 weeks. The maxillary first premolars were extracted, and the interseptal bone was removed to within 1 mm of the second premolars; on the insult (lesser surgical insult) side, buccal and lingual vertical grooves were made in the extraction socket to undermine the mesial root of the second premolar; the insult+ (greater surgical insult) side was flapped and had modified corticotomies extending to, but not through, the lingual cortex 1 mm distal to the distal root, and 3 to 5 mm apical to both roots. Microcomputed tomography analyses were used to evaluate the material density, bone volume fraction, and trabecular characteristics of surrounding bone. Hematoxylin and eosin sections were used to determine osteoclast numbers, bone surface areas, and bone volumes. Results: After 7 weeks of consolidation, there was significantly ($P<0.05$) less bone on the insult+ side; it was less dense and less mature than the bone on the insult side. Relative to the control bone, bone on the insult+ side was significantly less dense but showed no differences in bone volume. Preliminary histologic evaluations indicated increased numbers of osteoclasts and greater bone surface areas on the insult+ side than the insult side, but no differences in bone volume. Conclusions: Increased surgical insults produce less dense and less mature bone but have no effect on bone volume at 9 weeks after surgery. (Am J Orthod Dentofacial Orthop 2014;145:S92-9)

Studies evaluating orthodontic treatment duration indicate that the average patient requires 23.5 to 28.6 months to complete treatment. Altering the biology of bone to enhance tooth movement is appealing because it could dramatically improve treatment efficiency. In addition to fewer appointments, more efficient treatment might prevent the patient from experiencing negative sequelae secondary to orthodontic treatment, such as external apical root resorption and enamel decalcification. Conventional tooth movement is a biologic rate-limiting process that depends on the ability of the periodontal ligament to respond to the strains induced by orthodontic forces and mediate the repair of bone. Under normal conditions, cells of the periodontal ligament secrete osteoprotegerin to inhibit osteoclast formation and maintain the integrity of the periodontal ligament. Upon force application, the bone becomes strained, cell membranes disrupt, and a biochemical cascade of events occurs, stimulating osteoclastogenesis and bone formation. As the bone adapts to the strain by simultaneous resorption and formation, tooth movement occurs. Based on the clinical research, force levels of 60 to 300 g produce average tooth movement rates of approximately 1 mm per month in humans. Tooth movements can be accelerated by altering bone biology. After a noxious stimulus (ie, bone injury or operation), acceleration of most ongoing normal hard-tissue and soft-tissue processes occurs, including increased bone metabolism, turnover, and modeling. This complex reaction, known as the regional acceleratory phenomenon, initiates the healing process of the injured tissue, resulting in a temporary decrease in regional bone density and an increase in remodeling. The use of corticotomies to produce the regional...
acceleratory phenomenon and facilitate orthodontic treatment dates back to the late 1950s, but corticotomy procedures were not used clinically to enhance treatment outcomes until the late 1980s and early 1990s. Wilcko et al popularized this approach to accelerate orthodontic tooth movement rates. Since then, corticotomies have been used to facilitate the intrusion, distalization, and extrusion of teeth. Experimental studies have demonstrated positive effects of corticotomies on tooth movements. Cho et al reported 2 to 4 times greater experimental than control tooth movements in beagle dogs. Others have shown greater experimental tooth movements as early as the first week but not after the fourth week. Longitudinal models have demonstrated that experimental tooth movements in the mandibles of dogs peak after the third week, when they were moving 85% faster than the control teeth. These studies indicate that there is a relatively small window of opportunity to take advantage of the regional acceleratory phenomenon.

Importantly, the effects of the regional acceleratory phenomenon on tooth movements are not all or nothing. Sanjideh et al demonstrated that an additional corticotomy procedure, initiated 4 weeks after the first surgical insult, produced significantly greater tooth movements than a single corticotomy. Increased amounts of injury around multirooted teeth also cause increased tooth movements, with the maxillary second premolars protracted 1.8 and 2.9 mm on the limited and extensive surgical insult sides, respectively.

Although increased surgical insults are related to increased tooth movements, it remains unknown how different amounts of surgical insult affect the bone around treated teeth. The purpose of this study was to evaluate the dentoalveolar bone subjected to heavy orthodontic forces and differing amounts of surgical insult. The null hypothesis was that there are no differences in the quantity and maturity of dentoalveolar bone between 2 treatments producing differing amounts of surgical insult.

**MATERIAL AND METHODS**

Eight skeletally mature (1-2 years of age) male foxhound dogs, weighing 25 to 30 kg, were used for this study. The surgical and orthodontic procedures were fully described by Cohen et al, who evaluated their tooth movements. Before the surgeries, the maxillary right and left first premolars were extracted. The extraction sites were extended distally to within 1 mm of the second premolars (to simulate the width of human interseptal bone). The maxillary quadrants were randomly assigned to 1 of 2 groups, insult or insult+, signifying the level of insult to the alveolar bone surrounding the second premolar.

On the insult side, the investigators undermined the interseptal bone mesial to the second premolars by making vertical grooves in the extraction socket along the buccal and lingual sides, extending the cuts obliquely toward the base of the interseptal bone to weaken its resistance and stimulate the regional acceleratory phenomenon (Fig 1). On the insult+ side, a full-thickness gingival flap was raised, and the buccal plate between the second premolar and the canine was removed, along with any possible interference to translation of the tooth and bony segment. A vertical osteotomy extending to, but not through, the lingual cortex was performed 1 mm distal to the second premolar. The vertical osteotomy and extraction sites were then connected by a horizontal corticotomy 1 to 2 mm deep and 3 to 5 mm apical to the second premolar (Fig 1).

Immediately after surgery, custom orthodontic appliances, consisting of bands soldered to a hyrax screw (opened to allow protraction of the maxillary second premolar upon activation), were cemented in place. Protraction of the maxillary second premolars was initiated at a rate of .5 mm per day (2 closing turns of the hyrax screw) for 15 days. After protraction, the second premolars were retained by the appliance over a 7-week consolidation period, after which the experimental animals were sacrificed. The maxillas were then sectioned and removed from the skulls and stored in 70% ethanol.

Insult, insult+, and control bone specimens were harvested from each dog. Seven regions of interest were evaluated on the insult and insult+ sides, including the (1) mesial trabecular, (2) interradicular trabecular, (3) distal trabecular, (4) buccal cortical, and (5) lingual cortical bones, as well as the cortical (bundle) bone along the (6) mesial and (7) distal root surfaces of the mesial root (Fig 2). An eighth region of interest, located in the interradicular trabecular bone of the maxillary third premolar, served as the untreated control bone.

Bone quantity was based on the material density (the amount of bone mass per unit of volume, minus any voids) and the percent of bone volume (ratio of the number of bone voxels to the total number of voxels in a specific region of interest). Bone maturity was measured based on trabecular number, trabecular thickness, and trabecular separation.

The heights of the specimens from the most occlusal aspect of the experimental crown to within 5 mm of bone apical to the root apices were approximately 30 mm. The specimens were trimmed to fit in 30-mm-diameter tubes, using foam to stabilize their positions; 70% ethanol was poured over the specimens, and the tubes were covered with Parafilm (Pechiney Plastic
Packaging, Chicago, Ill) to prevent dehydration. The specimens were then scanned using a Micro-CT 35 (Scanco Medical, Brütisellen, Switzerland) at a medium resolution of 30 μm. Three-dimensional reconstructions, 50 slices thick occlusoapically, were obtained from the 8 areas of interest of each specimen. Vertically, the bone analyzed was located approximately midway between the crests of the alveolar bone and the apices of the mesial and distal roots. At this level, segments of bone from the buccal and lingual cortices, bone mesial to the mesial root and distal to the distal root, and mesial and distal bundle bone were outlined. Fifty-slice-thick segments of interradicular control bone were also outlined at the same level. The threshold boundaries for the scans were set between 250 and 1000 Hounsfield units for all specimens.

The histologic specimens were obtained by removing with a trephine bur (Ace Surgical, Brockton, Mass) 3-mm-diameter samples of interradicular bone, taken midway between the crests of the alveolar bone and

**Fig 1.** Surgical procedure diagrams with corresponding photos: A, insult group diagram (buccal view); B, sample insult group photo (occlusal view); C, insult+ group diagram (buccal view); D, sample insult+ group photo (buccal view).

**Fig 2.** Diagrams showing regions of interest: A, regions 1 (mesial), 2 (interradicular), 3 (distal), 6 (mesial radicular), and 7 (distal radicular) of the insult and insult+ groups, and 8 (interradicular) of the control group (buccal view); B, regions 1 (mesial), 2 (interradicular), 3 (distal), 4 (buccal), and 5 (lingual) of the insult and insult+ groups (occlusal view).
the mesial and distal root apices, from 6 specimens on the insult + side, along with their respective controls (total of 12 samples). The tissues were fixed in 10% buffered formalin and washed thoroughly with tap water. They were then decalcified in 0.5 mol per liter of EDTA (ethylenediamine tetraacetate; tetrasodium salt) using the PELCO BioWave (Redding, Calif). Radiographs were used to monitor the decalcification process. After decalcification, the specimens were dehydrated, cleared, infiltrated with paraffin and an automatic tissue processor (VIP model 2000; Miles Scientific, Fergus Falls, Minn), and embedded in paraffin blocks. The blocks were hardened on a cold plate and sectioned at 6 μm with a microtome. Five sections were chosen per block and mounted on 1 × 3-in coated glass slides, dried on a warming tray at 42 °C for 2 hours, and then dried overnight in an oven at 54 °C.

One slide per block was randomly selected to be stained using standard hematoxylin and eosin procedures. The number of osteoclasts on each slide was manually counted 3 times under 20-times magnification with a microscope (Zeiss, Thornwood, NY) and then averaged. Osteo software (version 11.2.6; Bioquant, Nashville, Tenn) was used to segment matrix bone tissue by adjusting threshold values; the bone was traced, and the bone surface area was calculated. When analyzing surface areas, the cut margins resulting from retrieval of the specimens with the trephine bur were segmented and excluded from the evaluation.

Statistical analysis

SPSS statistical software (version 18; IBM SPSS, Chicago, Ill) was used to describe the results and compare the groups. The nonparametric Wilcoxon signed rank test was used for all comparisons. Statistical significance was based on a P value of ≤0.05.

RESULTS

The 3-dimensional reconstructions obtained from the scans were all 1.5 mm thick occlusoapically and ranged in width from 1 to 3 mm, depending on the distances between the tooth roots and/or the surgical cuts. The amount of surgical insult had a direct effect on the quantity and maturity of bone. Bone volume fraction and bone density were consistently less on the insult + than on the insult sides. The insult + side had significantly less material density in the distal, buccal, and lingual regions than did the insult side (Table I). The insult side also showed a significantly greater bone volume fraction than did the insult + side in the buccal and mesial radicular regions.

The insult side also showed significantly (P = 0.036) denser bone than did the insult + side in the interradicular region. The interradicular bone of the insult and insult + sides showed significantly (P = 0.012) less material density than did the control bone (Table II). There were no statistically significant group differences in bone volume fraction of interradicular bone between the treatment groups and the controls.

The distal bone of the insult + side showed significantly more trabeculae, significantly thinner trabeculae, and significantly less trabecular separation than did the insult side (Table III). Compared with the controls (Fig 3, Table II), the interradicular bone of the insult + group showed significantly greater numbers of trabeculae (P = 0.017), significantly thinner trabeculae (P = 0.042), and significantly less trabecular separation (P = 0.012).

The experimental sections (Fig 4, A–D) showed mature lamellar bone with concentric lamellae radiating from central osteons. Osteocytes were found in the calcified tissues, and osteoblasts lined the endosteal surfaces. There also were mature multinucleated osteoclasts with ruffled borders lining the periosteal surfaces and in the Howship’s lacunae. The trabecular spaces contained multiple spindle-shaped fibroblasts. The control bone (Fig 4, E–H) showed mature lamellar bone containing osteocytes. Although osteoblasts and osteoclasts were found lining the periosteal surfaces, there were fewer compared with the experimental group.

Quantitative analyses showed no difference in the size of interradicular bone areas between the insult + and control groups. The insult + group showed significantly more osteoclasts (P = 0.028) than did the control group (Table IV). The differences in osteoclasts per unit of surface (P = 0.028) in the interradicular bone of the insult + and control groups were also statistically significant.

<table>
<thead>
<tr>
<th>Bone index</th>
<th>Insult+</th>
<th>Insult</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material density (mg HA/cm³)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesial</td>
<td>847.2</td>
<td>30.7</td>
<td>866.4</td>
</tr>
<tr>
<td>Distal</td>
<td>795.7</td>
<td>32.6</td>
<td>867.6</td>
</tr>
<tr>
<td>Buccal</td>
<td>857.3</td>
<td>23.3</td>
<td>898.4</td>
</tr>
<tr>
<td>Lingual</td>
<td>881.5</td>
<td>19.6</td>
<td>905</td>
</tr>
<tr>
<td>Mesial radicular</td>
<td>835.8</td>
<td>35.6</td>
<td>866.1</td>
</tr>
<tr>
<td>Distal radicular</td>
<td>816.4</td>
<td>46.4</td>
<td>859</td>
</tr>
<tr>
<td>Bone volume fraction (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mesial</td>
<td>50.3</td>
<td>19</td>
<td>56.3</td>
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<tr>
<td>Distal</td>
<td>48.8</td>
<td>17</td>
<td>52.5</td>
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<tr>
<td>Buccal</td>
<td>70.2</td>
<td>8</td>
<td>90.1</td>
</tr>
<tr>
<td>Lingual</td>
<td>86.2</td>
<td>9</td>
<td>88.3</td>
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<tr>
<td>Mesial radicular</td>
<td>65.2</td>
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<tr>
<td>Distal radicular</td>
<td>57.1</td>
<td>23</td>
<td>73.9</td>
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mg HA/cm³, Milligrams of hydroxylapatite per cubic centimeter.
DISCUSSION

Greater surgical insults resulted in less dense bone. The insult + side showed less material density than did the insult side in all regions. Cattermole et al,30 who used dual-energy x-ray absorptiometry to measure bone density, found less dense bone in the fractured than in the contralateral unfractured areas. Whereas others have shown decreased density associated with the regional acceleratory phenomenon, this is the first time that decreased material density with increased surgical insults has been demonstrated.31-34 The differences identified in the interradicular and distal regions were probably due to the extension of the surgical insult apical to the roots, and distal to the distal root on the insult + side. The buccal bone on the insult + side might be expected to be less dense because of the removal of the buccal plate and disruption of the blood supply from the flap surgery. Additionally, the lingual bone on the insult + side might have been less dense because of increased strain secondary to increased tooth movement.

Greater surgical insults also produced greater decreases in the bone volume fraction, which measures the relative amount of bone. Although the amount of bone has not been previously related to the extent of the insult, insults have been shown to reduce the amounts of bone.35-37 The fact that bone density was affected to a greater extent than the bone volume fraction can be explained by the normal healing process. After 7 weeks of consolidation, it can be assumed that the blood supply had been reestablished, new bone had formed, and bone was becoming more mature. The density of the bone remains low until the newly formed bone has remodeled into mature lamellar bone.

The amount of mesial radicular bone on the insult + side was less than on the insult side. Since this is a type of cortical bone, with less blood supply than trabecular bone, the increased strain in cortical bone secondary to increased tooth movement could have delayed the angiogenic response necessary for bone formation.38 The other regions displayed similar differences in bone volume fractions, but the differences were smaller.

The magnitude of the surgical insult also affected bone maturity. The bone on the insult + side showed greater trabecular numbers, less trabecular thickness, and less trabecular separation than did the bone on the insult side. This pattern indicates newly formed woven bone composed of greater amounts of thin and densely packed collagen fibrils. The differences were greatest in the distal region, probably because it was the site of the greatest difference in the surgical insult. Previous microcomputed tomography comparisons of regenerate bone after distraction osteogenesis found significant increases in trabecular numbers and significant decreases in trabecular thickness compared with the controls after 6 weeks of consolidation.39,40 Although a decrease in bone maturity is expected after injury, this study is the first to show that greater injury to bone results in decreased bone maturity.

After 7 weeks of consolidation, bone that had been surgically insulted showed less material density than did the control bone. The interradicular bones on the insult + and insult sides were 9.9% and 7.9% less dense, respectively, than the control bone, but only the insult + side showed statistically significant differences. Zapata

### Table II. Material densities, bone volume fractions, trabecular numbers, trabecular thickness, and trabecular separation in the interradicular region of the maxillary second premolars

<table>
<thead>
<tr>
<th>Bone index</th>
<th>Insult+ Mean</th>
<th>SD</th>
<th>Insult Mean</th>
<th>SD</th>
<th>Control Mean</th>
<th>SD</th>
<th>Insult+ vs insult</th>
<th>Insult+ vs control</th>
<th>Insult vs control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material density (mg HA/cm³)</td>
<td>837.5</td>
<td>19.9</td>
<td>856.1</td>
<td>16.3</td>
<td>929.1</td>
<td>30.8</td>
<td>0.036</td>
<td>0.012</td>
<td>0.012</td>
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<tr>
<td>Bone volume fraction (%)</td>
<td>36.1</td>
<td>7</td>
<td>35.1</td>
<td>11</td>
<td>34.7</td>
<td>13</td>
<td>0.779</td>
<td>0.779</td>
<td>0.779</td>
</tr>
<tr>
<td>Trabecular number (n/mm)</td>
<td>2.1</td>
<td>0.3</td>
<td>1.8</td>
<td>0.3</td>
<td>1.6</td>
<td>0.2</td>
<td>0.093</td>
<td>0.017</td>
<td>0.263</td>
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<tr>
<td>Trabecular thickness (mm)</td>
<td>0.2</td>
<td>0</td>
<td>0.3</td>
<td>0.1</td>
<td>0.3</td>
<td>0.1</td>
<td>0.111</td>
<td>0.042</td>
<td>0.203</td>
</tr>
<tr>
<td>Trabecular separation (mm)</td>
<td>0.5</td>
<td>0.1</td>
<td>0.6</td>
<td>0.1</td>
<td>0.7</td>
<td>0.1</td>
<td>0.123</td>
<td>0.012</td>
<td>0.176</td>
</tr>
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*mg HA/cm³*, Milligrams of hydroxylapatite per cubic centimeter.

### Table III. Trabecular number, trabecular thickness, and trabecular separation mesial and distal to the maxillary second premolars

<table>
<thead>
<tr>
<th>Bone index</th>
<th>Insult+ Mean</th>
<th>SD</th>
<th>Insult Mean</th>
<th>SD</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trabecular number (n/mm)</td>
<td>2.5</td>
<td>0.5</td>
<td>2.6</td>
<td>0.6</td>
<td>0.779</td>
</tr>
<tr>
<td>Distal</td>
<td>3.7</td>
<td>0.8</td>
<td>2.4</td>
<td>0.4</td>
<td>0.017</td>
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<tr>
<td>Trabecular thickness (mm)</td>
<td>0.3</td>
<td>0.1</td>
<td>0.3</td>
<td>0.1</td>
<td>0.401</td>
</tr>
<tr>
<td>Distal</td>
<td>0.2</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>0.012</td>
</tr>
<tr>
<td>Trabecular separation (mm)</td>
<td>0.4</td>
<td>0.1</td>
<td>0.4</td>
<td>0.1</td>
<td>0.799</td>
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<tr>
<td>Distal</td>
<td>0.3</td>
<td>0.1</td>
<td>0.5</td>
<td>0.1</td>
<td>0.012</td>
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</tbody>
</table>

April 2014 ● Vol 145 ● Issue 4 ● Supplement 1 American Journal of Orthodontics and Dentofacial Orthopedics
et al,\textsuperscript{41} who evaluated regenerate bone density after distraction osteogenesis and 6 weeks of consolidation in foxhound mandibles, found that the bone was 11.6\% less dense compared with the controls. Similar studies evaluating regenerate bone densities have reported 22.3\%\textsuperscript{42} less dense bone after 4 weeks of consolidation, and no differences after 12 weeks.\textsuperscript{43}

Surgically insulted bone was also less mature than control bone. Bone generated by distraction osteogenesis in dogs has been shown to be fully mineralized after 8 weeks of consolidation, but the regenerate remained distinguishable from host bone because of incomplete remodeling.\textsuperscript{44} After distraction osteogenesis in dogs, approximately 24 weeks of consolidation is needed before new bone resembles control bone.\textsuperscript{45} The insult group showed the same pattern of maturity as the insult+ group, but the changes relative to the controls were not significant. This study is unique in showing that increased surgical insults result in decreased overall bone maturity, and that the decrease in maturity matches the level of the insult.

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Surgical insults increased the rates of remodeling at 9 weeks postsurgery. Compared with the controls, both bone surface area and osteoclastic activity increased in the insult+ group. Since the insult group was not evaluated and only 1 slice per block was counted, these findings remain preliminary. Increases in bone surface area are related to increased metabolic activity\textsuperscript{56} and

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Comparisons of the measures of bone maturity of the insult+, insult, and control groups for the interradicular regions: A, trabecular numbers; B, trabecular thickness; C, trabecular separation.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Sample photomicrographs showing differences in osteoclastic activity between the insult+ (top row) and their respective controls (bottom row): A-D, photomicrographs of the insult+ group showing selected osteoclasts (*), osteoblasts (+), and osteocytes (o); E-H, photomicrographs of the respective controls showing absence of osteoclasts.}
\end{figure}
CONCLUSIONS

Based on a split-mouth design comparing bone surrounding maxillary second premolars that had and had not been surgically insulted, the following preliminary conclusions can be drawn.

1. With greater surgical insults, there is less bone, less dense bone, and less mature bone around teeth than with a lesser surgical insult.

2. Surgical insults produced bone that was less dense than the control bone.

3. Surgical insults produced bone with increased numbers of osteoclasts and greater bone surface areas.

REFERENCES


