Original Article

Three-dimensional morphology of root and alveolar trabecular bone during tooth movement using micro-computed tomography

Li Zhuang[a]; Yuxing Bai[b]; Xianying Meng[c]

ABSTRACT

Objective: To investigate the effects of force magnitude on three-dimensional alveolar trabecular bone structure and root resorption.

Materials and Methods: Twenty-two 11-week-old Sprague Dawley rats were randomly assigned to two groups that received a mesially directed orthodontic force to the upper right first molars at different magnitudes of force, 30 g or 100 g, for 2 weeks. The contralateral molars served as controls. The teeth and alveolar bone around the teeth were dissected from the sacrificed animals and were scanned with micro-computed tomography (CT). Structural properties of the trabecular bone and resorption crater volume on the mesial roots of the maxillary first molars were analyzed.

Results: The bone volume fraction of the 30 g group and the 100 g group increased significantly in both groups, and trabecular separation of the 100 g group decreased significantly compared with controls ($P < .05$). The total root resorption volume in all experimental groups and the resorption volume of the lower distal surface in the 100 g group increased significantly compared with controls ($P < .01$). The volume of the upper mesial root surface in the 30 g group increased significantly compared with the 100 g group and controls ($P < .05$).

Conclusion: The alveolar trabecular bone was denser after orthodontic force was applied for 14 days. The effects of 30 g and 100 g orthodontic forces on root resorption were different at the upper mesial and lower distal surfaces of the mesial roots of maxillary first molars. (*Angle Orthod.* 2011;81:420–425.)

KEY WORDS: Orthodontic; Trabecular bone; Root resorption; Micro-computed tomography; Morphology

INTRODUCTION

Mechanical stress may affect bone mass and architecture. The microarchitecture of trabecular bone has been shown to play an important role in mechanical bone strength.[1,2] The effects of bone loss are more prevalent in trabecular bone owing to its relatively high surface area. Also, the greater bone mineral content of cortical bone can conceal small changes in the trabecular bone. In addition, trabecular geometry is a main factor underlying bone quality. Some research[3–6] has focused on bone mass or bone mineral density during orthodontic treatment. However, few studies have examined three-dimensional changes in the microstructure of alveolar trabecular bone during tooth movement.

External apical root resorption (EARR) is a common side effect of orthodontic treatment that results in permanent loss of tooth structure in the root apex. The frequency of severe EARR during orthodontic treatment is reported to be 5%–18%,[7] and progressive EARR can lead to a compromised crown-to-root ratio and compromised tooth functioning.[8] However, the causes and mechanisms underlying EARR remain poorly characterized; this increases the risk associated with some orthodontic treatments.

The relationship between root resorption and orthodontic force magnitude is very important to consider and allows orthodontists to manage treatment risk; although several studies have investigated this relationship,[9–15] no clear consensus has been reached

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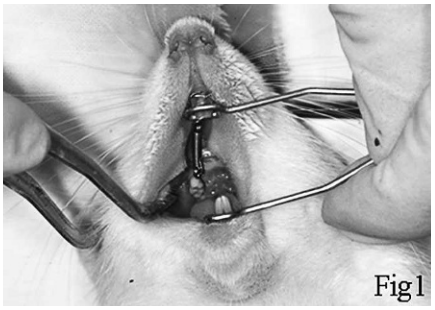

Figure 1. The NiTi coil was ligated to each of the rat's maxillary right first molars with a ligature, and the contralateral molars served as controls.

because of study differences, such as methods and samples. We propose that it is necessary to study the relationship between root resorption and orthodontic force using a new method.

Micro-computed tomography (micro-CT) is designed to image and quantify bone with high resolution. Micro-CT can be used to study trabecular architecture and the mechanical properties of bone by assessing specific parameters in three dimensions.[2] In addition, high resolution allows the method to detect tiny resorption craters before EARR is diagnosed.

The aim of the present study was to use micro-CT to observe three-dimensional changes at the root surface and in trabecular bone around the roots of teeth under different orthodontic forces, in an attempt to enhance understanding of the processes associated with tooth movement.

MATERIALS AND METHODS

Eleven-week-old male Sprague Dawley rats ($n = 22$) were allowed 1 week to acclimatize to the laboratory environment. The rats were then randomly assigned to two groups ($n = 11$ in each). A split-mouth design was used. A nickel-titanium (NiTi) coil spring (IMD, Shanghai, China) was ligated to each rat's maxillary right first molar and incisors with a ligature, and the contralateral molars without an orthodontic appliance served as controls (Figure 1). The spring was calibrated to produce a continuous force of 100 g in one group and 30 g in the other group. A cervical groove was prepared on the incisors and secured with bond adhesive (3M Unitek, Monrovia, Calif). During the experimental period, the rats were fed a powder diet and water ad libitum. After 2 weeks, the rats were sacrificed by an overdose of carbon dioxide (CO_2), and the maxillae were dissected and stored in 10% formalin. This research was approved by the institutional Animal Experimentation Ethics Committee (Ethics Approval Number 0001478).

Samples were placed on the stage of a SkyScan1172 (SkyScan, Kontich, Belgium) micro-CT with the mesial root in an upright position. The scanning procedure lasted approximately 15 minutes per sample. During the cone-beam acquisition step of the technique, the samples were rotated 180 degrees and were scanned with a resolution of 5 μm per pixel. After the samples were scanned, the raw data underwent further reconstruction to provide axial cross-sections using NRecon provided by the SkyScan1172. Roughly 40 minutes was necessary to reconstruct a sample, and 900 cross-sections were collected per sample. The 16-bit bitmapped picture files had a resolution of 1024×1024 pixels.

Three-dimensional microarchitecture information for the trabecular bone included the following measures. First, the bone volume fraction was calculated as the ratio of bone volume (BV) to total volume (TV), with TV being the volume of the whole sample examined and with BV calculated using tetrahedrons corresponding to the enclosed volume of the triangulated surface. Second, the mean trabecular thickness was determined from the local thickness at each voxel representing bone. Also, trabecular number was calculated by taking the inverse of the mean distance between the middle axes of the structure, and trabecular separation was calculated by applying the technique used for the direct thickness calculation to the nonbone portions of the three-dimensional image.[16] Last, the structure model index (SMI) was determined, which varied from zero–3 for ideal plate and rod structures.

We selected a 300 μm × 300 μm × 300 μm cube of trabecular bone distal to the apical third of the mesial root of the maxillary right first molar for analysis (Figure 2). The distance between the cube and the root was 500 μm, as determined by preliminary experiments to ensure that the cubes were in the trabecular bone. Quantitative analysis was carried out using CT Analyser software (SkyScan, Kontich, Belgium).

The teeth were subsequently extracted from the images using software, and the bone was removed. Rat maxillary first molars have five roots. We analyzed the mesial roots. Custom programming of software, based on a convex hull algorithm similar to the one used by Harris,[9] was used to analyze the volume of the root resorption craters on the root surface. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 11.5 (SPSS Inc., Chicago, Ill). Univariate analysis of variance (ANOVA) was used to compare the differences between groups.

RESULTS

The bone volume fraction in the 30 g and 100 g groups was significantly greater than the values obtained for controls ($P < .05$; Figure 3); the trabecular separation of the 100 g group was significantly

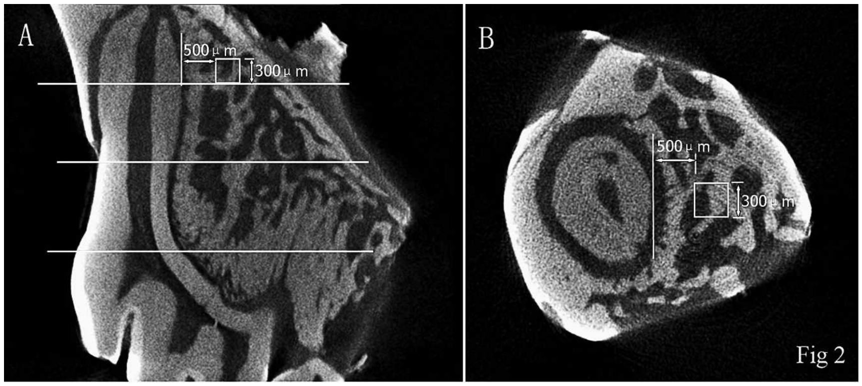

Figure 2. A 300 μm × 300 μm × 300 μm cube distal to the apical third of the mesial root of the maxillary right first molar was extracted from the trabecular bone for analysis. (A) Sagittal view. (B) Horizontal view.

less than that found in controls ($P < .05$; Figure 4). However, no significant difference was found between the 30 g and 100 g groups. Differences among the other indices (SMI, trabecular thickness, and trabecular number) were not significant ($P > .05$; Table 1). Three-dimensional images of the selected bone cube were subsequently reconstructed, and trabecular bone in the experimental groups was clearly compacted (Figure 5).

The mesial roots of the maxillary first molars were divided into four surfaces (upper mesial, upper distal, lower mesial, and lower distal; Figure 6). Crater volumes at the root surfaces were compared between groups (Table 2). Total root resorption volumes in all experimental groups and the resorption volume of the lower distal surface in the 100 g group were significantly greater than the volumes measured in controls ($P < .01$) (Figures 7 and 8). No significant difference was found between the 30 g and 100 g groups. The volume of the upper mesial root surface in the 30 g group was significantly greater than that in the 100 g group and in controls ($P < .05$), but no significant difference was noted between the 100 g group and controls (Figure 9). Three-dimensional images of the mesial roots were also reconstructed (Figure 10).

DISCUSSION

The internal structure of bone constantly adapts to its functional environment via both modeling and remodeling. Masticatory muscle function and occlusal force applied directly by the teeth can influence alveolar trabecular bone.[17,18] In the present study, we found that the bone fraction increased significantly after orthodontic force was applied for 2 weeks, and trabecular separation decreased significantly with higher orthodontic force (100 g). These results suggest that the microarchitecture of the alveolar trabecular bone became denser, so that it could adapt to greater mechanical stress—similar to the results of previous studies.[17,18]

Orthodontic forces can transmit through the periodontal ligament to the supporting alveolar bone, leading to bone changes.[19] Previous studies[3,4] indicated that bone fraction is reduced during tooth movement, which is not consistent with results of the

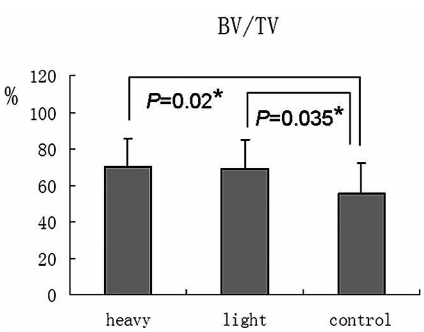

Figure 3. Bone volume (BV)/total volume (TV) values among the experimental and control groups. The BV/TV values of the 100 g force (heavy) and 30 g force groups (light) were not significantly different, but both were significantly higher than control values. Data represent the mean ± standard deviation.

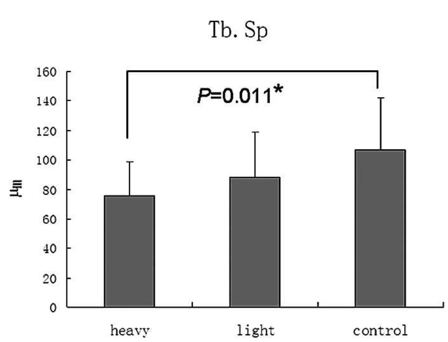

Figure 4. Trabecular separation (Tb.Sp) in the experimental and control groups. Tb.Sp in the 100 g force group (heavy) was significantly less than that of controls, but differences between the 100 g force group and the 30 g force group (light) and between the 30 g force group and controls were not significant. Data represent the mean ± standard deviation.

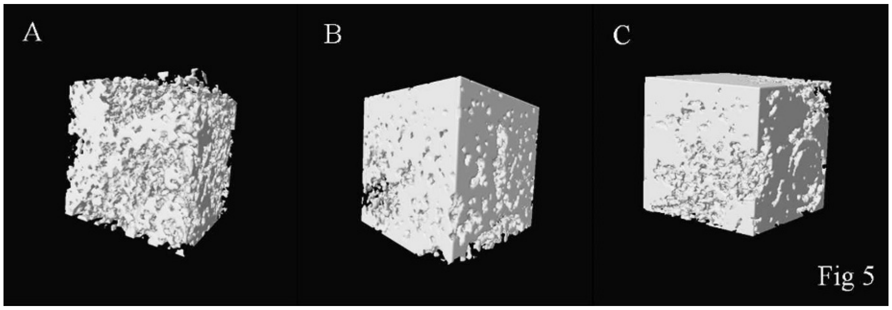

Figure 5. Three-dimensional micro-CT image of the alveolar trabecular bone. (A) Control group. (B) 30 g force group. (C) 100 g force group.

present study. The reason for this difference may be that two-dimensional histomorphometric indices were observed in earlier studies, whereas the three-dimensional microstructure of alveolar bone was evaluated in the present study.

Trabecular bone is generally accepted to be regulated by estrogen[20]; thus, male rats were selected for the present study to avoid this effect. Small differences in some indices between experimental and control groups may have been due to the short loading period. Additional studies are needed to investigate changes in alveolar bone after longer loading time.

Many factors may be related to root resorption, one of which is orthodontic force. However, the relationship between force magnitude and root resorption remains unknown. Many studies have demonstrated that increased orthodontic force magnitude may lead to an increased incidence of root resorption, which then results in an increased incidence of EARR,[9–12] but other studies have reported that increased force magnitude does not lead to increased root resorption.[13,14]

Radiographs or histologic methods were used in most of the previously mentioned studies. Quantitative analysis of root resorption craters using radiographs or histologic analysis has been shown to be inaccurate, difficult to reproduce, and insensitive.[12] Two-dimensional studies are limited to the measurement of only small amounts of root apex loss.[21,22] The micro-CT system used in the present study is a relatively new approach for studying root resorption and allows the reconstruction of teeth in three dimensions. With the help of a convex hull algorithm, we were able to quantify the volume of root resorption craters.

We found that the volume of root resorption craters in the experimental groups was significantly greater than the volume measured in the control group, indicating that orthodontic force can lead to root resorption; this is consistent with findings of previous studies reporting significant root resorption when greater than 25 g of force was applied to roots for 14 days.[15] Also, the amount of force usually used to move rat molars mesially is 50–60 g[23–25]; this is why we selected 30 g and 100 g of force. These experimental conditions produced obvious root resorption and allowed us to investigate the effects of different magnitude forces. However, our results were different from those reported in some studies using micro-CT[9,26,27] because the forces used on human premolars in these studies were 25 g and 225 g. The other studies found that heavy force produced significantly greater root resorption than was produced by light

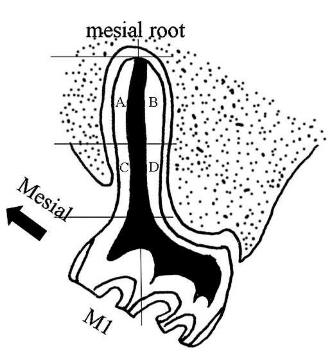

Figure 6. The mesial root was divided into four surfaces: A is the lower mesial surface; B the lower distal surface; C the upper mesial surface; and D the upper distal surface.

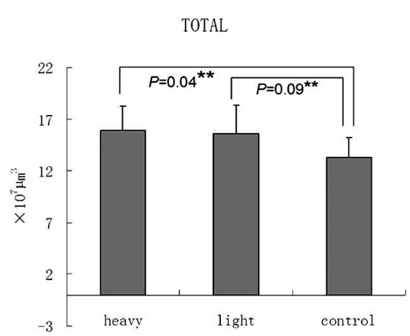

Figure 7. Total crater volume in the three groups. The volume was significantly greater in both the 100 g force (heavy) group and the 30 g force (light) group compared with control values, but the difference between the 100 g force group and the 30 g force group was not significant. Data represent the mean ± standard deviation. $^{**}P < .01$.

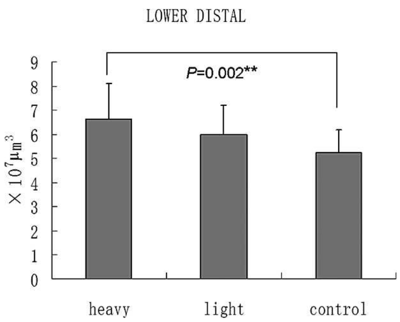

Figure 8. Crater volume on the lower distal surface. The volume was significantly greater in the 100 g force group (heavy) than in the control group. Differences between the 100 g force group and the 30 g force group (light), and between the 30 g force group and controls, were not significant. Data represent the mean ± standard deviation. $^{**}P < .01$.

force. This difference may be due to the force magnitude range.

Although the difference in root resorption volume between 30 g and 100 g force groups in the present study was not significant, root resorption was not the same between experimental groups. In the upper mesial surface of the mesial root, resorption was more likely to occur when 30 g of force was applied, whereas the lower distal surface was more involved when a 100 g force was applied. This difference may be due to the distribution of stress and the anatomic characteristics of the roots, but the exact reasons remain unknown and require further study. The conclusion drawn from this result is that the effects of 30 g and 100 g of orthodontic force on root resorption were different at the upper mesial and lower distal surfaces of the mesial roots of maxillary first molars.

CONCLUSIONS

- Orthodontic force resulted in increased alveolar trabecular bone density.
- The difference in root resorption between 30 g and 100 g of force was not significant.

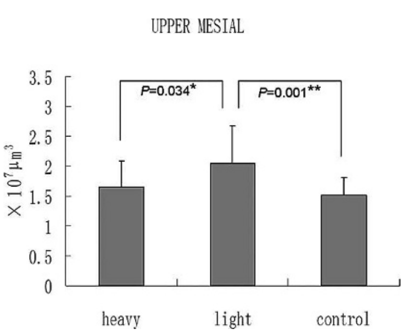

Figure 9. Crater volumes on the upper mesial surface. The volume of the 30 g force group (light) was significantly greater than that of the 100 g force group (heavy) and the control group. Data represent the mean ± standard deviation. $^{*}P < .05$. $^{**}P < .01$.

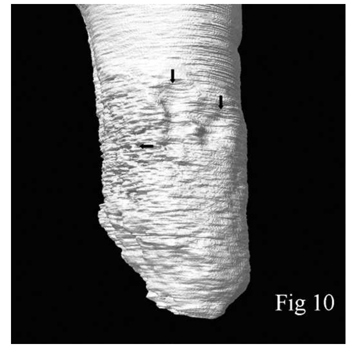

Figure 10. Three-dimensional micro-CT image of the root. Root lacunae (arrows) are visible.

- The effects of 30 g and 100 g of orthodontic force on root resorption were different at the upper mesial and lower distal surfaces of the mesial roots of maxillary first molars.

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