Dynamic Evaluation of Orthodontically-Induced Tooth Movement, Root Resorption, and Alveolar Bone Remodeling in Rats by in Vivo Micro-Computed Tomography

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Background: The aim of this study was to dynamically evaluate tooth movement, root resorption, and remodeling of alveolar bone using different forces to cause tooth movement in rats.

Material/Methods: 12-week-old male Sprague-Dawley rats were selected. Nickel-titanium (Ni-Ti) coil springs (20 g, 50 g, and 100 g forces) were placed for mesial movement of the left first maxillary molar teeth. Tooth movement, root resorption, and microarchitectural parameters of the trabecular bone were evaluated by in vivo micro-CT. Histological examination was used to observe the root resorption, alveolar bone remodeling, and changes in osteoclasts from day 0 to day 14.

Results: The tooth movement distance increased significantly over the initial 3 days in the 3 groups. The 20 g force group showed more tooth movement than in the 50 and 100 g force groups after 14 days (P<0.05). From days 7 to 10, root resorption lacunae appeared in the 3 groups and then stabilized, and the 100 g force group produced more lacunar resorption than in the other 2 groups (P<0.05). Compared to day 0, the trabecular thickness and bone volume fraction on the pressure side gradually decreased from day 7 to day 14. The structure model index increased significantly from day 3 to day 14. Histological examination showed remarkable root resorption craters and osteoclasts positive for tartrate-resistant acid phosphatase in the root resorption lacunae in the 50 g and 100 g groups from day 7 to day 14.

Conclusions: A 100 g heavy force can be used to establish a root resorption model in rats.

MeSH Keywords: Root Resorption • Tooth Movement • X-Ray Microtomography

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Background

Orthodontic treatment involves an inflammatory reaction in periodontal tissue with the application of orthodontic force. Many factors affect the tooth movement, and some adverse effects may occur during this process [1]. Root resorption is one of these adverse effects and has been reported in approximately 80–90% of treated adolescents, with up to 12–17% of these cases showing severe apical resorption of more than 4 mm [2–4]. Severe root resorption can cause pulp necrosis and even tooth mobility, causing great pain to patients. Orthodontically-induced root resorption is related to factors such as age, dental vulnerability, force magnitude and duration, tooth movement direction, and orthodontic appliance type [5–9].

Histological staining, X-ray tomography, and scanning electron microscopy have previously been used to detect tooth movement and root resorption in 2 dimensions [10,11]. However, these methods require the sacrifice of experimental animals and cannot be used to in dynamic observation. Cone-beam computed tomography (CBCT) is widely used in clinical practice, but the accuracy is not sufficient to detect the details of the roots [12]. Another method employed is micro-computed tomography (micro-CT), which is an imaging technology that has a high resolution at 1 micron per pixel and can be used to view the alterations in root resorption and tooth movement in 3 dimensions in vivo. Previous studies have used micro-CT to view the progression and repair of root resorption [13–16]. However, there has been little research on or use of in vivo micro-CT to dynamically evaluate tooth movement, root resorption, and alveolar bone remodeling that occur after different forces caused tooth movement in rats.

In clinical practice, elastics or springs are used to move the molar and incisor to close the extraction space. During tooth movement, talveolar bone remodeling occurs, which on the pressure edge consists of bone resorption and on the tension edge consists of bone formation. To assure adequate biological response in the tooth movement process, it is important to use an optimal force system. Heavy forces can induce alveolar bone hylalization, which affects the tooth movement. In previous studies, a model of rat tooth movement was established to simulate human tooth movement and to observe the changes of movement distance and root resorption. Rat molars are multirooted teeth with an anatomical structure and physiological function similar to that of human molars. In rats, bone metabolism homeostasis is preserved with an equilibrium between alveolar bone resorption and regeneration, and the relevant genes in rats and humans are homeotic. Thus, rats are used to establish models of tooth movement and root resorption. Previous studies have used multiple force magnitudes in such tooth movement and root resorption models without a reference.

In the present study, a rat tooth movement model was established using different force magnitudes to detect dynamic variations in the tooth movement distance, root resorption lacunar volume, and alveolar bone microstructure during tooth movement using micro-CT in vivo. Tartrate-resistant acid phosphatase (TRAP) staining and histology were also used to view alterations in the periodontal tissue and osteoclast number. The present study aimed to dynamically evaluate the tooth movement, root resorption, and alveolar bone microstructure elicited by different force magnitudes to provide a reference force magnitude for rat tooth movement and root resorption models and theoretical reference for clinical orthodontic treatment.

Material and Methods

Experimental Animals

Sprague-Dawley rats (12-week-old males, n=90) (Animal Experimental Center of Chongqing Medical University, Chongqing, China) weighing 225±25 g were used as experimental animals and allowed to acclimate for 1 week before the experiment. The animals were randomly divided into 20 g, 50 g, and 100 g groups, with 30 rats in each group. All the animals were in an environment with 55±5% humidity and 25±5°C temperature and fed a general diet with unrestricted access to water under a light-dark cycle of 12 h. All animals were handled in accordance with the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH, 80-23). All Experiments were conducted as approved by the Ethics Committee of the Stomatology Hospital of Chongqing Medical University, Chongqing, China (permit no. CQHS-REC-2015-02).

Establishing the tooth movement model

The tooth movement model was modified based on previous models [17,18] (Figure 1). All rats were anaesthetized using a 10% solution of chloral hydrate (0.03 ml/kg) with intraperitoneal injection. After anaesthetization, the nickel-titanium (Ni-Ti) coil spring (ø: 0.12 mm, Ormco Corporation, Glendora, CA, USA), with forces of 20, 50, and 100 g were placed for mesial movement to the left first maxillary molar teeth. After surgery, the animal’s vital signs were observed carefully until recovery from anaesthesia. Previous studies have found that non-steroidal anti-inflammatory drugs affect the tooth movement [19,20]; therefore, neither analgesics nor antibiotics were used in this experiment.

In vivo micro-CT scanning

First, the animals were anaesthetized as previously described [16]. The nickel-titanium coil spring was removed.
at each time point to avoid influencing the micro-CT images. All samples were scanned dynamically on days 0, 3, 7, 10, and 14 by micro-CT (SCANCO Medical Co., Zurich, Switzerland). The micro-CT scanning conditions: the voltage was 70 kVp and 114 mA, integration time was 350 ms, slice thickness was 0.01 mm, and image voxel size was 7.0 μm. The average scanning time of each animal per each time point was about 45 min, and approximately 600 images were produced per session.

The nickel-titanium coil spring was reattached for continuous force after scanning.

Tooth movement measurement

The data obtained from micro-CT scanning were converted into DICOM files, then imported into Mimics software (version 10.01) to reconstruct first and second molars. The method used to measure tooth movement has been previously reported [16]. The tooth movement distance was calculated for days 3, 7, 10, and 14. The measurement procedure was repeated 3 times by the same researcher, who then calculated the mean value.

Root resorption lacunar volume measurement

Mimics software (version 10.01) was used to import the scanned data and to segment the mesial root of the maxillary left first molar. The lacunar volume of root resorption was measured using a convex hull algorithm [21,22] on days 3, 7, 10, and 14. To calculate the root resorption lacunar volume, the root volume on day 0 was subtracted from each subsequent time point. Data for each time point were measured 3 times by the same researcher to obtain the mean value.

Trabecular bone microarchitectural parameters measurement

Trabecular bone cubes (700×700×700 μm), which were 200 μm away from the mesial side of the apical third of the distal

Figure 1. Tooth movement model. Tooth movement model: the Ni-Ti spring was ligated between left first maxillary molar and incisor with 20 g, 50 g, and 100 g of force delivered.

Figure 2. A selected trabecular bone cube. The selected trabecular bone cube: The cubes (700×700×700 μm) of trabecular bone on the mesial side of the apical third of the distal buccal root of the maxillary first molar was selected for analysis. (A) Sagittal, (B) Horizontal.
buccal root of the maxillary first molar, were selected for analysis (Figure 2). The following trabecular bone microstructural parameters were measured by the affiliated program of the micro-CT (μCT V6.1): trabecular thickness (Tb.Th), trabecular number (Tb.N), the bone volume fraction (BV/TV), trabecular separation (Tb.Sp), and structure model index (SMI).

**Histology**

After the animals were sacrificed, the left maxillae, comprising both the first and second molars, were first dissected and then fixed for 48 h with 4% paraformaldehyde. Samples were then decalcified for 6 weeks using 10% EDTA, embedded in paraffin, and cut to 4-μm thickness sections after the parasagittal sections of the mesio-distal part of the tooth. Haematoxylin and eosin (H-E) staining was carried out to view root resorption and alveolar bone remodeling. TRAP with wine-red staining was used to detect osteoclasts, achieved with an acid phosphatase leukocyte kit (Sigma, St. Louis, MO, USA).

**Statistical analysis**

Repeated-measures ANOVA was performed on all data using SPSS V20.0 (Chicago, IL), where a P value of < 0.05 was taken to be statistically significant.

**Results**

No serious weight loss, mucosal infection, or other adverse effects were observed in any animals.

### Tooth movement distance

On day 3, the tooth movement distance in all groups was significant compared to day 0 (0.04 mm, 0.045 mm, and 0.076 mm in the 20, 50, and 100 g groups, respectively), with the 100 g group showing the most remarkable change. From days 3 to 10, the movement distance changed slowly in all groups. From days 10 to 14, the 20 g force group acquired significantly greater movement distance than in the 50 g force group and the 100 g force group (P<0.05) (Table 1, Figure 3A).

### Root resorption lacunar volume

From days 0 to 3, the root resorption volume increased slightly in all groups. From days 7 to 10, obvious root resorption appeared in the 3 groups and then stabilized. After 14 days, the volume of resorption in the 100 g force group was significantly greater than that in both the 20 g and 50 g force groups (P<0.05), but there was no observed difference among the 20 g and 50 g force groups (P>0.05) (Table 2, Figure 3B, 3C).

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**Table 1.** Tooth movement by different magnitudes of force (mm), (±s).

| Time (days) | 20 g | 50 g | 100 g |
|------------|------|------|-------|
| 0 days     | 0    | 0    | 0     |
| 3 days     | 0.06±0.008 | 0.045±0.017 | 0.076±0.009* |
| 7 days     | 0.128±0.034 | 0.056±0.011* | 0.090±0.004* |
| 10 days    | 0.181±0.039 | 0.059±0.011* | 0.110±0.008** |
| 14 days    | 0.237±0.045 | 0.079±0.027* | 0.134±0.014** |

* Significant difference compared with 20 g group (P<0.05); ** Significant difference compared with 50 g group (P<0.05).

**Table 2.** Root resorption crater volume by different magnitudes of force (×10^7 μm^3), (±s).

| Time (days) | 20 g | 50 g | 100 g |
|------------|------|------|-------|
| 0 days     | 0    | 0    | 0     |
| 3 days     | 0.368±0.1582 | 0.473±0.2104 | 0.579±0.2611 |
| 7 days     | 0.994±0.2137 | 1.370±0.3410 | 2.089±0.4112** |
| 10 days    | 1.776±0.2997 | 2.121±0.4626 | 2.926±0.3894** |
| 14 days    | 1.891±0.2342 | 2.373±0.4190 | 3.389±0.6266** |

* Significant difference compared with 20 g group (P<0.05); ** Significant difference compared with 50 g group (P<0.05).
Microarchitectural parameters of the trabecular bone

Compared with day 0, on days 3 and 7 the BV/TV decreased significantly in the 100 g group and on days 7 and 10 in the 20 and 50 g force groups. The SMI significantly increased from days 3 to 14 in the 50 g and 100 g groups and from days 10 to 14 in the 20 g group. From day 3 to 14, the Tb.N increased slowly in each group, but there was no statistically significant difference. From days 3 to day 7, the Tb.Th reduced significantly in all groups and then remained the same. The Tb.Sp reduced from days 3 to 7 and then increased from days 7 to 10, but no statistically significant alterations were detected. There were

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**Figure 3.** Tooth movement and root resorption crater volume by different magnitudes of force. Tooth movement distance and root resorption crater volume by different magnitudes of force. (A) Tooth movement distance in 20 g, 50 g and 100 g groups from day 0 to day 14; (B) Root resorption crater volume in 20 g, 50 g, and 100 g groups from day 0 to day 14; (C) The 3D reconstructed root resorption lacuna in 20 g, 50 g, and 100 g groups from day 0 to day 14. (* Significant difference compared with 20 g group, P<0.05; # Significant difference compared with 50 g group, P<0.05).
Figure 4. Alveolar trabecular bone microstructural properties by different magnitudes of force. Alveolar trabecular bone microstructural properties by different magnitudes of force. (A) Bone volume fraction; (B) Structure model index; (C) Trabecular number; (D) Trabecular thickness; (E) Trabecular separation; (F) The 3D reconstructed alveolar trabecular bone in 20 g, 50 g, and 100 g groups from day 0 to day 14. (* Significant difference compared with day 0 in 20 g group, P<0.05; # Significant difference compared with day 0 in 50 g group, P<0.05; @ Significant difference compared with day 0 in 100 g group, P<0.05).
no statistically significant alterations in the trabecular bone microarchitectural parameters among the 3 groups (Figure 4).

**Histological examination**

H-E staining showed no resorption of the mesial root on the surface of the left first molar or on the pressure side on the alveolar bone on day 3 in any group, but the periodontal ligament was compressed. From days 7 to 14, resorption lacunae on the alveolar bone on the pressure side were detected in the 3 groups, with remarkable root resorption craters in both the 50 and 100 g force groups (Figure 5A–5C). From days 10 to 14, slight root resorption appeared in the 20 g group.

TRAP-positive osteoclasts showed wine-red staining in the cytoplasm. From days 0 to 3, osteoclasts appeared along the edge of the alveolar bone in all groups. The number of osteoclasts increased in the root resorption lacunae from days 7 to 10 in the 50 and 100 g force groups (Figure 5D–5F) and then decreased by day 14.

**Discussion**

CBCT is used in orthodontic clinics, and many studies have compared the accuracy of CBCT vs. micro-CT. They found micro-CT has high accuracy to detect the detail of tooth and roots [23,24]; therefore, it is widely used in oral experiments, and many
studies have used micro-CT to investigate the 3D microstructure of alveolar and root resorption. Ru et al. [15] established a tooth movement model and then evaluated changes in root resorption lacunae and alveolar microstructure using micro-CT in vivo; they found obvious root and alveolar resorption after 7 days of force loading. Gonzales et al. [25] used in vivo micro-CT to investigate rat models and found that the tooth movement pattern showed mesial incline and intrusion and distal extrusion. As micro-CT can be used to analyze the microstructure of periodontal tissue without causing tissue damage, in vivo micro-CT was applied in this study.

Clinical observations have shown that orthodontic tooth movement can be divided into 3 phases: rapid tooth movement during the initial phase of force loading; delay, with no obvious tooth movement; and another period of rapid tooth movement [26]. Gonzales et al. [27] established a rat tooth movement model and applied forces of different magnitudes to rat molars. The results showed slow tooth movement from days 1 to 3, a delay from days 3 to 10, and faster tooth movement to day 28 with the application of 25, 50, and 100 g of force; additionally, in the 25 g group the tooth movement distance was double that of the other groups. Our results show significant tooth movement distances in all of the groups, with the most remarkable change on day 3 of the 100 g force group. From days 3 to 10, the movement distance changed slowly in all groups. The tooth movement after 7 days in the 20 g force group was significantly larger than that of the 50 g and 100 g force groups. These findings are in accordance with previous studies and were confirmed by histological examination. H-E staining showed that the periodontal ligament was compressed on day 3, and on the alveolar bone on the pressure side, resorption lacunae were detected in the 3 groups from day 7 to 14. Osteoclasts that were TRAP-positive emerged along the alveolar bone edge on day 3 in all of the groups, and then increased in the alveolar resorption craters.

The in vivo micro-CT examination showed that from day 0 to 3, the root resorption volumes were increased slightly in all groups, but H-E staining showed that the root surface was smooth from day 0 to 3. This difference between the micro-CT and H-E staining results may be ascribed to the precision difference. From day 7 to 10, micro-CT examination showed obvious root resorption in all groups, followed by stabilization. Remarkable root resorption craters were revealed using H-E staining in both the 50 and 100 g force groups from day 7 to 14. TRAP staining validated the results, as TRAP-positive osteoclasts increased in the root resorption lacunae in the 50 g and 100 g groups from day 7 to 10 and then decreased on day 14. These variations are also consistent with the findings of Ru et al. [15].

The result of orthodontic force magnitude on the resorption of roots is controversial. We found in the 100 g group that the resorption volume was significantly larger than both the 20 and 50 g force groups after 7 days, but with no significant difference among the 20 and 50 groups. These results are not in accordance with the previous study. They used common micro-CT to measure root resorption during tooth movement and found that both light and heavy forces could cause root resorption, without a difference between the 2 groups. This difference between the results may be due to the examination method, as we used in vivo micro-CT to obtain dynamic results. Previous studies have shown that while the tooth movement distance does not increase with increasing orthodontic force, the root resorption increases; additionally, greater root resorption appears with the use of heavier orthodontic forces [14]. Darendeliler et al. [28] loaded a force of 25 g or 225 g to a premolar for 28 days and then extracted the tooth in the clinic. After 28 days, the detected root resorption was more obvious in the heavy (100 g) than in the light (20 g) force group. These are the same results as our study – the root resorption lacunar volume increased significantly from day 7 to 10; thus, we can intervene during this period to prevent root resorption in future experiments.

The microstructure of trabecular bone is an important index for determining bone strength. By micro-CT observation, Ide [29] found that the 3D shape of the trabeculae in normal mandibular alveolar bone was a plate-like structure. However, in edentulism, the height of the alveolar bone decreased rapidly, the volume and width of the trabeculae decreased, the trabeculae were arranged irregularly, and the trabecular shape changed from plate-like to rod-like. In this study, a third of the proximal alveolar bone in the first molars distal buccal root was observed. Under the action of traction, the BV/TV on the pressure side gradually decreased, the trabecular shape narrowed, and the trabecular number decreased. The Tb.Sp was initially compressed and then widened because of the continuous absorption of bone mass. The SMI increased significantly from day 3 to 14, and the trabecular bone changed from plate-shaped to rod-shaped. The microarchitectural parameters of the trabecular bone indicated, in response to force, the alveolar bone on the pressure side was absorbed. The trends were the same in all 3 groups, and the results are similar to those of previous studies [30]. A model of rat tooth movement was established using in vivo micro-CT to observe the microstructural parameters of alveolar bone in the model of rat tooth movement. Significantly decreased BV/TV, Tb.Sp, Tb.Th, and SMI, values were detected on the pressure side.
Conclusions

Root resorption and tooth movement can both be induced by different orthodontic forces. With increasing orthodontic force, the tooth movement distance decreases and the root resorption volume increases. Therefore, future experiments may choose a light force of approximately 20 g to initiate a rat tooth movement model and a heavy (100 g) force to establish a model of root resorption in rats. As the root resorption lacunar volume increased significantly from day 7 to 10, we can intervene during this period to prevent root resorption in future experiments. To avoid root resorption, an excessively heavy force should not be used to move teeth in clinical orthodontic treatment.

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