A micro-CT study of microstructure change of alveolar bone during orthodontic tooth movement under different force magnitudes in rats

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Abstract. The dynamic changes of the microstructure of alveolar bone during orthodontic tooth movement in rats was explored by employing micro-computed tomography (micro-CT) system and to provide theoretical reference for clinical orthodontic treatment. Ten rats were selected randomly as control among 70 adult female Wistar rats, and the other 60 rats were divided into 25-g and 75-g groups of equal number. Orthodontic appliance with force of 25 g and 75 g was installed to perform the molar mesial movement. Microstructural parameters for trabecular bone mesial to the distobuccal root were evaluated at different time points using micro-CT system. Moreover, distance for mesial movement of the molar were measured. Microstructural parameters for trabecular bone of two groups showed no significant changes from day 0 to day 3 (P>0.05); from day 3 to day 7, bone mineral density (BMD), bone volume/total volume (BV/TV) and trabecular thickness (Tb.Th) decreased significantly (P<0.05), whereas trabecular separation (Tb.Sp) and structure model index (SMI) increased significantly (P<0.05); from day 7 to day 14, in 25-g group, BMD, BV/TV and Tb.Th increased significantly (P<0.05), while Tb.Sp and SMI decreased significantly (P<0.05). Correspondingly, in 75-g group, changes of parameters did not carry any statistical significance (P>0.05). Furthermore, the 75-g group showed larger distance than 25-g group only at day 14 (P<0.05). In conclusion, in order to maintain the health of periodontal tissues, adequate time for repair and recovery is needed to ensure reasonable remolding of alveolar bone and healthy movement of the orthodontic tooth.

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Introduction

During the process of orthodontic treatment, tooth movement is closely related to alveolar bone remodeling, which consists of bone resorption in direction of tooth movement and bone formation on the opposite side. The use of an optimal force system is important for an adequate biological response in the periodontal system. Heavy forces could overcompress the periodontal ligament and induce hyalinization, which could impede resorption of the alveolar bone surface (1,2).

A previous study on the periodontal tissues, and especially on the microstructure of the trabecular bone used to adopt specimens destructive methods of histological techniques which was capable of the two-dimensional microstructure, but was hard to obtain and describe the parameters of the alveolar bone internal microstructure (3). Micro-computed tomography (micro-CT) is a new imaging examination and analysis technique of high resolution and non-intrusive has developed fast in recent years, which can provide three-dimensional qualitative as well as quantitative data of the tested specimens, to better understand and study the remolding of alveolar bone microstructure (4,5).

The purpose of this study was to evaluate the dynamic changes of the microstructure of alveolar bone during orthodontic tooth movement under different force magnitudes in rats employing micro-CT system, and to explore the pattern of changes of the trabecular bone microstructure in the alveolar bone so as to provide theoretical reference for clinical orthodontic treatment.

Materials and methods

Grouping of the laboratory animals. Seventy 11-week-old adult female Wistar rats (approximate weight, 220-260 g) were used for this study. A total of 10 rats were selected randomly as control, and the remaining 60 rats were divided into 25-g and 75-g groups of equal number. The rats were raised under standard conditions: Room temperature at 25±1°C, relative humidity at 55±5%, and 12-h light and dark cycles. All the experimental protocols followed were approved by the Ethics

Committee of Harbin Medical University, and the experiments were carried out under the control of the University's Guidelines for Animal Experimentation.

Installing orthodontic appliance. A total of 30 rats of 25-g group and 30 rats of 75-g group were anesthetized by intraperitoneal injection of 10% chloral hydrate, a cervical groove was prepared on the incisors using a round burr with a dental low-speed handpiece (NSK Ltd., Tokyo, Japan). The maxillary left first molar was connected with both maxillary central incisors with nickel-titanium coil spring 0.008 inch in diameter (Shinye Odontology Materials Corp. Co., Hangzhou, China) using a 0.2 mm ligature wire (Changsha Tiantian Dental Equipment Co., Ltd., Changsha, China) to perform the mesial movement (Fig. 1). The ligature wire was then secured with bond adhesive (Transbond; 3M Unitek, Monrovia, CA, USA) on the incisors. Forces of 25 and 75 g were applied separately to observe the reactions of the alveolar bone. Spring retention was checked daily to ensure the stability of the applied force. The incisors, molar, and spring were cleaned and irrigated with tap water as needed to prevent potential trauma and irritation to the gingival and periodontal tissues.

Experimental samples. A total of 10 rats as control without installing orthodontic appliance were euthanized by perfusion though heart with 4% paraformaldehyde on day 0, and every 10 rats in each group were euthanized by the same way on days 3, 7 and 14, respectively. Then the maxillae were dissected and stored in 4% paraformaldehyde.

Micro-CT scanning. A total of 70 samples were scanned with a micro-CT scanner (μCT 35; Scanco Medical AG, Bassersdorf, Switzerland) with a 7 μm voxel size using the following parameters: 114 mA, 70 kVp, exposure time of 300 msec, and a horizontal scan angle. The scanning procedure lasted ~1 h per sample and generated ~1,000 two-dimensional images with a resolution of 1,024x1,024 pixels.

Evaluating the microstructural parameters. The region of interest (ROI) of the alveolar bone was selected according to previously described method which showed the integral microstructure of alveolar bone (6). A 210x210x210 μm cube of trabecular bone mesial to the cervial third of the distobuccal root of the maxillary left first molar was selected separately for analysis. The distance between the cube and the root was 100 μm (Fig. 2).

Three-dimensional microstructure of the alveolar bone was analyzed using the software affiliated to the micro-CT (Scanco® software ver. 3.7; Scanco Medical AG®, Brüttisellen, Switzerland). We evaluated the bone mineral density (BMD, mg cm^{-3}); bone volume/total volume (BV/TV, %); trabecular thickness (Tb.Th, mm); trabecular number (Tb.N, mm^{-1}); and trabecular separation (Tb.Sp, mm), microstructure model index (SMI), showing whether the trabecular bone is microstructured as rod-like or plate-like. Theoretical value for a complete rod-like microstructure is 3, while theoretical value for an ideal plate-like microstructure is 0. The stress that the tabular microstructure can bear is larger than that of clave microstructure (6,7).

Figure 1. The orthodontic appliance is installed to the maxillary left first molar and the incisors.
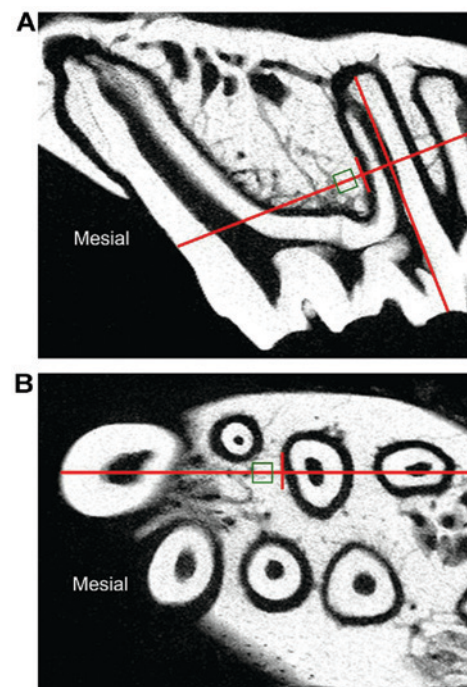

Figure 2. The region of interest. A cube of trabecular bone (210x210x210 μm) mesial to the cervical third of the distobuccal root of the maxillary left first molar (100 μm) was selected for analysis in micro-computed tomography images: (A) Sagittal surface passing the centers of mesial root and distobuccal root; (B) horizontal surface perpendicular to the cervical third of the distobuccal root.

Measuring the distance for mesial movement. Distance for mesial movement of the molar were measured between the nearest two landmark points (a,b) in the crowns of the first molar (M1) and second molar (M2) which were indentified on the longitudinal section through maximal diameter of the distobuccal root and mesial root of the first molar (Fig. 3).

Statistical analysis. The data were analyzed by using SPSS, version 21.0 (SPSS Inc., Chicago, IL, USA). Parameters of 25-g and 75-g groups on days 0, 3, 7 and 14 were compared using Student-Newman-Keuls of ANOVA. P<0.05 was considered to indicate a statistically significant difference.
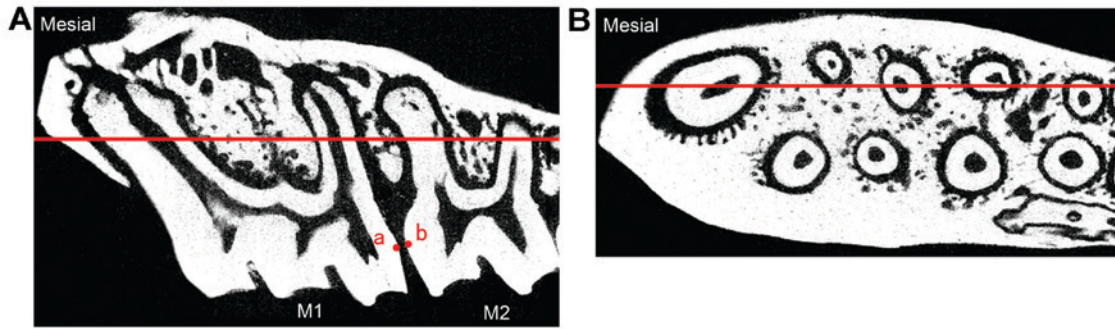
Figure 3. Landmark point locations and methods for the measurement of molar mesial movement in micro-computed tomography images. Landmark points a,b are indicated. M1, first molar; M2, second molar. (A) Sagittal view; (B) horizontal view.
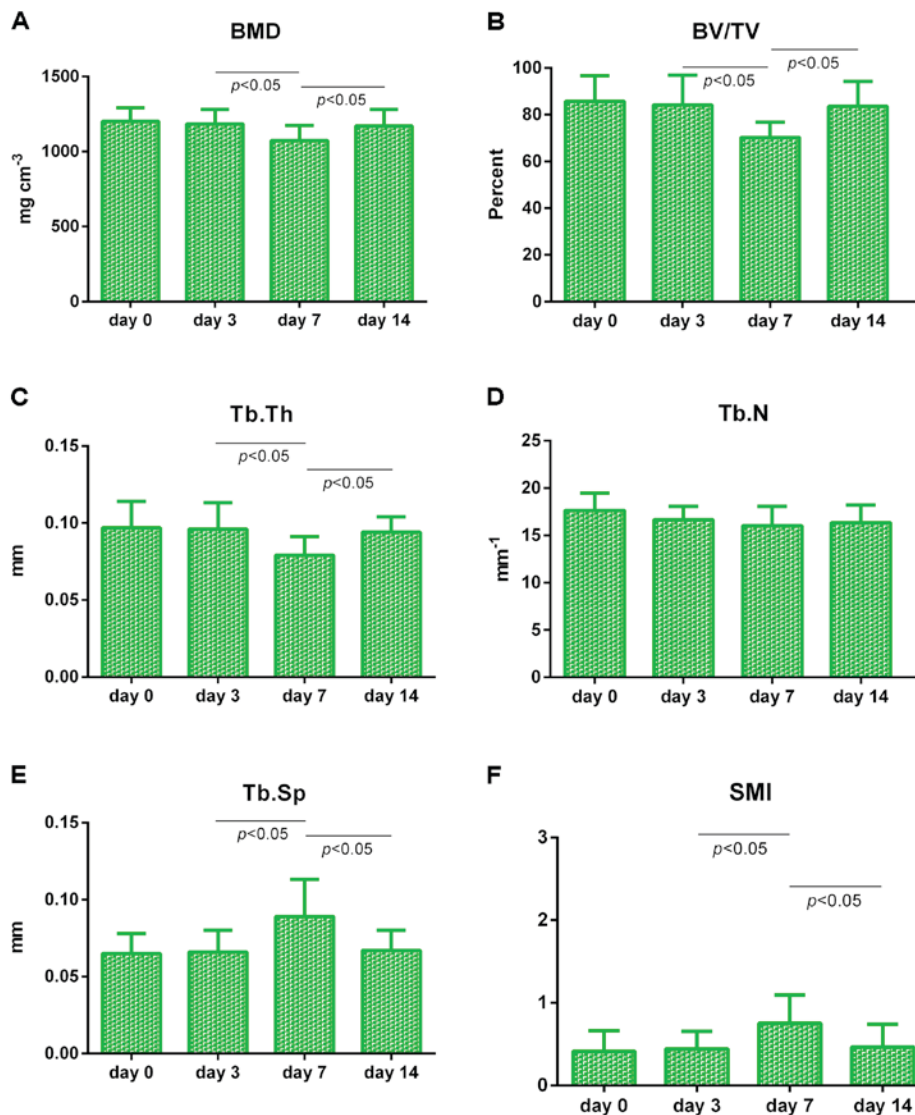

Figure 4. Microstructural parameters for trabecular bone mesial to the distobuccal root of the maxillary left first molar at different time points in 25-g group after installing orthodontic appliance. (A) Bone mineral density (BMD); (B) bone volume fraction (BV/TV); (C) trabecular thickness (Tb.Th); (D) trabecular number (Tb.N); (E) trabecular separation (Tb.Sp); (F) structure model index (SMI). Error bars indicate standard deviations.

Results

Microstructural parameters for trabecular bone mesial to the distobuccal root of the maxillary left first molar of 25-g group at different time-points are summarized in Fig. 4. From day 0 to day 3, the parameters did not display any significant changes ($P>0.05$); from day 3 to day 7, BMD, BV/TV and Tb.Th significantly decreased ($P<0.05$), whereas Tb.Sp and SMI significantly increased ($P<0.05$); while from day 7 to day 14, BMD, BV/TV and Tb.Th increased significantly ($P<0.05$),
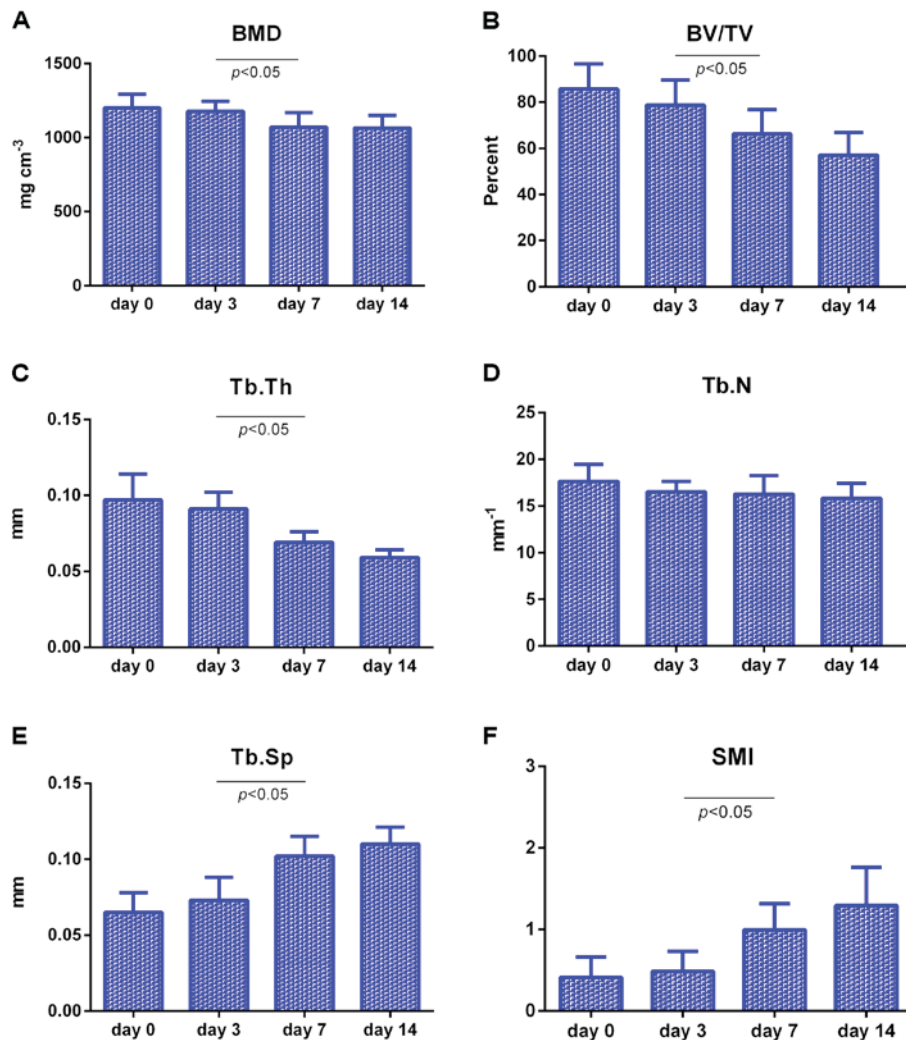
Figure 5. Microstructural parameters for trabecular bone mesial to the distobuccal root of the maxillary left first molar at different time points in 75-g group after installing orthodontic appliance. (A) Bone mineral density (BMD); (B) bone volume fraction (BV/TV); (C) trabecular thickness (Tb.Th); (D) trabecular number (Tb.N); (E) trabecular separation (Tb.Sp); (F) structure model index (SMI). Error bars indicate standard deviations.

while Tb.Sp and SMI decreased significantly (P<0.05). Tb.N did not have any clear change during the whole process of the experiment (P>0.05).

Microstructural parameters for trabecular bone mesial to the distobuccal root of the maxillary left first molar of 75-g group at different time points are summarized in Fig. 5. Statistical results of parameters on day 0 to day 7 are consistent with those of 25-g group, while from day 7 to day 14, changes of parameters did not carry any statistical significance (P>0.05), Tb.N did not have any significant change during the whole process of the experiment (P>0.05).

Microstructural parameters for trabecular bone mesial to the distobuccal root of the maxillary left first molar between 25-g and 75-g groups at different scanning time points are summarized in Fig. 6. From day 0 to day 7, the change trends of microstructural parameters of two groups were similar, but from day 7 to day 14, the change in trends between two groups showed significant difference (P<0.05).

Distance for mesial movement of the molar at different scanning time points between 25-g and 75-g groups are summarized in Fig. 7. The 75-g rats showed larger distance than 25-g only at day 14 (P<0.05).

Discussion

Previous studies frequently employed methods such as histological analysis to explore microstructure of the alveolar bone, which was sophisticated in operation and hard to obtain and describe the internal parameters of the specimens (3-5). The method of histological sections was also disadvantageous in limited number of slices and in turn deviation and substantial loss of information (3). In contrast, micro-CT is easy to operate with a high resolution of μm that is capable of accurate qualitative as well as quantitative analysis of the specimens. It allows improved study of the bone remolding and later histological or electron microscopic observation of the specimens, whenever needed, after having completed scanning (4,5).

The model of mesial movement of the maxillary left first molar by means of orthodontic extrusion was first proposed by Waldo and Rothblatt (8). Based on literature review, Ren *et al* (9) held the view that one orthodontic force loading cycle should be 14 days. Repair and reconstruction of alveolar bone would be dominant after 14 days. Since the molar volume of human is 50 times than that of the rat, orthodontic extrusion
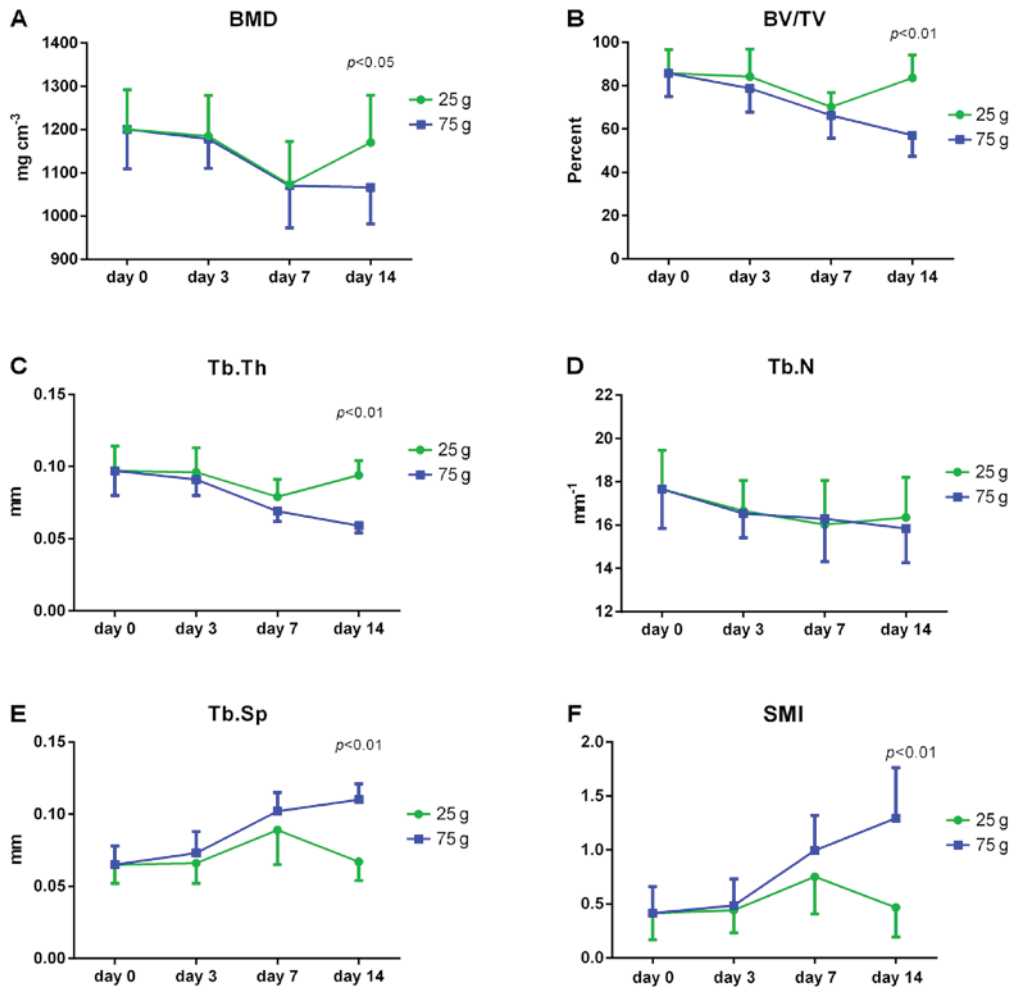
Figure 6. Microstructural parameters for trabecular bone mesial to the distobuccal root of the maxillary left first molar at different time points between 25-g and 75-g groups after installing orthodontic appliance. (A) Bone mineral density (BMD); (B) bone volume fraction (BV/TV); (C) trabecular thickness (Tb.Th); (D) trabecular number (Tb.N); (E) trabecular separation (Tb.Sp); (F) structure model index (SMI). Error bars indicate standard deviations.
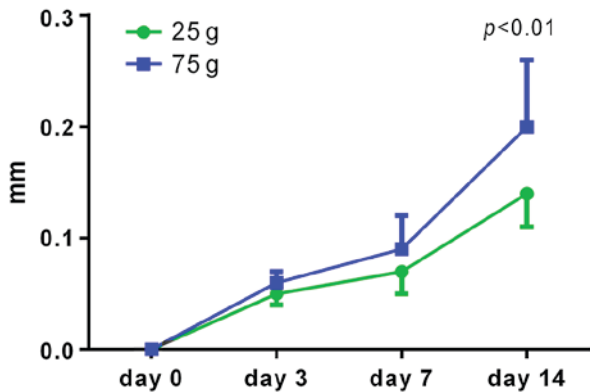

Figure 7. Distance for mesial movement of the maxillary left first molar at different time points between 25-g and 75-g groups after installing orthodontic appliance. Error bars indicate standard deviations.

of 20 g was considered as an ideal value for orthodontic molar tooth movement mesially, and 75 g belonged to heavy force. Taking the factor of the basic scale value of 25 g on the dynamometer used in the experiment, for more accurate measurement, this study selected 25 and 75 g orthodontic extrusion as the orthodontic force loading.

Based on the previous study on the three-dimensional finite elements of the rat molar, under the application of mesial force loading, the mesial side of the disbuccal root was subject to the largest compression (10). During the process of orthodontic tooth movement, alveolar bone on the compression side would pose the main resistance to the tooth movement, so if the alveolar bone on the compression side could always maintain a reasonable and stable rate of resorption and reconstruction throughout the orthodontic treatment, then the tooth would be able to move into the ideal position healthily and effectively. This study chose the alveolar bone mesial to the cervial third of the distobuccal root of maxillary first molar as its interest observation region, which is the location of the largest compression and of typical significance in terms of bone resorption and remodeling. Some studies using micro-CT system observed the changes of the microstructure of alveolar bone during orthodontic movement *in vivo* (11,12). However, they did not choose the location of the largest compression as interest observation region, so the results of the experiments were affected inevitably. Moreover, our study which used 70 rats tried to avoid the result error caused by sample difference as far as possible.

Currently, there exists controversy from histological experimental studies concerning the time points of alveolar

bone reconstruction during orthodontic tooth movement. Kohno *et al* (13) showed that hyaline change in the alveolar bone appeared on day 7, and bone resorption and reconstruction took place on day 14 after the force loading. However, Tomizuka *et al* (14) indicated that bone resorption happened on day 10 after force loading through histological observation.

The results showed that on day 0 to day 3 after the application of the force loading, the parameters of the two groups did not have any evident change (P>0.05), indicating that resorption did not occur in the alveolar bone. From day 3 to day 7, BMD, BV/TV and Tb.Th significantly decreased (P<0.05), while Tb.Sp and SMI significantly increased (P<0.05), indicating that resorption occurred in the alveolar bone. The difference was that from day 7 to day 14, in 25-g group, BMD, BV/TV and Tb.Th increased significantly (P<0.05), while Tb.Sp and SMI decreased significantly (P<0.05), indicating that alveolar bone was in the phase of repair and reconstruction.

Correspondingly, in 75-g group, changes of various parameters did not carry any statistical significance (P>0.05), indicating that alveolar bone was still in the phase of resorption. The comparison of microstructural parameters for trabecular bone between 25-g and 75-g groups at different scanning time points also supported above conclusion.

Obviously, on day 14, for the rats of 25-g group that were already in the repair and reconstruction, it was feasible to continue applying force loading; however, for the rats of 75-g group that were still in the phase of resorption, it was disputable to continue applying any force loading. Thus, further studies are still needed.

Previous studies showed that orthodontic tooth movement included three phases: The first phase is initial rapid tooth movement after the force loading; the second phase is delayed tooth movement; the third phase is linear tooth movement (2,15-17). In our study, the tooth movement of both groups conformed with the description above. Among them, from day 0 to day 3 belonged to phase 1; from day 3 to day 7 was in phase 2; and from day 7 to day 14 belonged to phase 3. However, 75-g group showed larger distance than 25-g group only at day 14 (P<0.05). Apparently, this kind of accelerated tooth movement at the expense of excessive alveolar bone was not ideal and healthy pattern.

The aforementioned findings may provide reference to orthodontic practitioners that, in order to maintain the health of periodontal tissues, low force should be advocated, and it is not suggestive to frequently apply orthodontic force loading. Periodontal tissues should be allowed adequate time for repair and recovery so as to ensure reasonable reconstruction of alveolar bone and healthy movement of the orthodontic tooth.

In conclusion, these findings indicated that on day 14, the rats of 25-g group that were in repair and reconstruction phase were feasible to apply continuous force load, however, it was disputable to continue applying any force loading for the rats of 75-g group.

In addition, the movement distance in 75-g group was significantly larger only at day 14, compared with 25-g group. Apparently, the fast tooth movement at the expense of excessive alveolar bone resorption was not ideal.

Taken together, frequently applying orthodontic force loading during orthodontic treatment was demonstrated to be unhelpful. In order to maintain the health of periodontal tissues, adequate time for repair and recovery is needed to ensure reasonable remolding of alveolar bone and healthy movement of the orthodontic tooth.

Acknowledgements

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