Local orthodontic force initiates widespread remodelling of the maxillary alveolar bone

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Objectives: To clarify the effects of a local orthodontic force on alveolar bone by analysing bone remodelling in different regions of the maxilla during orthodontic tooth movement (OTM).
Methods: An OTM model was established in rats. Histological changes in the maxilla were analysed using TRAP staining, IHC staining for CTSK and haematoxylin and eosin (H and E) staining. The root bifurcation region of the alveolar bone of the first (M1), second (M2) and third (M3) molars were selected as the regions of interest (ROIs), which were further divided into a cervical and an apical level. Sequential fluorochrome labelling was performed to analyse bone deposition rates.
Results: The maxillary left first molars were moved mesially. TRAP staining and IHC staining for CTSK showed orthodontic force increased osteoclast numbers in all six ROIs at both the cervical and apical levels. H and E staining indicated elevated osteoblast numbers in the OTM group in all induced regions. Sequential fluorochrome labelling exhibited increased bone deposition rates around M1, M2 and M3 in the OTM group.
Conclusions: An orthodontic force applied to the first molar could initiate widespread remodelling of the maxillary alveolar bone, which was not restricted to the tension and pressure sites. This may revise the orthodontic biomechanical theory and provide new insights for clinical work.
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Introduction

Biomechanical alveolar bone remodelling induced by an orthodontic force lays the physiological foundation for orthodontic treatment.1,2 Previous studies have revealed the effects of orthodontic stress in the periodontal ligament (PDL)3,4 or on the alveolar bone surrounding a moving tooth.5,6 Most of the previous studies have focused on local alveolar bone remodelling associated with the loaded tooth during OTM, indicating bone resorption at the compression site and deposition at the tension site.7,9 However, the bone around the tooth roots is only a small percentage of the total alveolar bone and whether all of the alveolar bone responds to the local orthodontic force is still not clearly clarified.

According to Wolff’s law, established in 1892, mechanical loading is crucial to maintaining the internal architecture and the external form of bone in living beings.10 Bone is able to remodel itself under mechanical stress, while loading reduction could break the balance of remodelling and induce bone metabolic diseases such as osteoporosis.11,12 Astronauts, without skeletal loading, exhibit more than a 10% decrease in bone mineral density and suffer a higher risk of osteoporosis.13,14 In vivo studies have also suggested that skeletal unloading in mice, by tail suspension,
induced disrupted bone metabolism, marked bone loss, and disorganised bone architecture.\textsuperscript{15} Furthermore, the biological reaction of bone under a mechanical stimulus may not be localised.\textsuperscript{16} In 1990, Tanne et al. demonstrated that remodelling of an entire long bone was initiated under mechanical stress.\textsuperscript{17} Generally, biomechanical bone remodelling is a tightly systematic physiological process involving synchronised co-ordination of different cell types.\textsuperscript{18} It indicates that alveolar bone remodelling induced by an orthodontic force might not be locally restricted.

The present study hypothesised that alveolar bone may react to an orthodontic force in its entirety and not be localised to the compression and tension sites. Hence, the histological changes in different regions of the maxillary alveolar bone during OTM in rats were investigated and analysed. The activities of osteoblasts and osteoclasts were evaluated to detect the catabolic and anabolic metabolism of the maxillary alveolar bone. This study aims to provide new understanding and insights regarding the orthodontic biological process and, further, update biomechanical theory, as well as offer guidance for clinical practice.

**Materials and methods**

**Animals**

The study was conducted on 27 non-pregnant, female, Sprague-Dawley (SD) rats (eight weeks old, and weighing 180–200 g). Standard laboratory environmental conditions related to room temperature (22–26°C) and humidity (50–55%) were provided for all animals. Standardised laboratory pellet food and water were available, \textit{ad libitum}. All of the experimental protocols were approved by the ethics committee of the Shanghai Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine.

**Orthodontic force application**

The OTM model was constructed as previously described.\textsuperscript{19,20} After anaesthesia, an orthodontic force was delivered by closed-coil springs (0.25 mm wire size, 0.76 mm diameter; Innovative Material and Devices, Shanghai, CHN) to the maxillary left first molar and measured with the dynamometer (Cathaysian Dentistry, Zhejiang, CHN) to a force of 50 g. The other end of the spring was ligated to the central incisor by a 0.2 mm steel ligature wire supported by self-curing restorative resin (3M ESPE, MN, USA) for reinforcement (Figure 1A). The orthodontic appliance was checked every day and an experimental rat would be excluded if dislodgement occurred. The animals were euthanased at four different time points after the application of OTM (day 0, 1, 3, 7), comprising six rats in each group. The maxillae were harvested for histological examinations. The OTM distance was measured between the midpoints of the mesial marginal ridge of M2 and the distal marginal ridge of M1 by an electronic calliper (Figure 1B).

![Figure 1](image-url)
**Histology and immunohistochemistry**

Histological examinations were carried out as previously reported.\textsuperscript{21,22} The samples of the maxillary bones were fixed in paraformaldehyde (4\%) for 48 hours and demineralised using EDTA (10\%) for five weeks. Following processing, 4 \(\mu\)m consecutive paraffin sections were obtained in the transverse plane. These were divided into two areas incorporating the cervical (section A) and apical level (section B) (Figure 1C). The alveolar bone of M1, M2 and M3 in sections A and B were defined as regions of interest (ROIs) (Figure 1D). To analyse the histologic change in the maxillae, tartrate-resistant acid phosphatase (TRAP) staining using an acid phosphatase leukocyte kit (Sigma, St Louis, MO, USA)\textsuperscript{23} and haematoxylin and eosin staining were applied.

Immunohistochemical (IHC) staining was conducted as previously described.\textsuperscript{22,24} After deparaffinisation and rehydration, prepared sections were blocked with 3\% \(\mathrm{H}_2\mathrm{O}_2\) and underwent antigen retrieval using 0.2\% protease K. The slices were pretreated with a blocking reagent (M.O.M. Kit, Vector Laboratories Inc., CA, USA) to inhibit nonspecific binding, and subsequently incubated with mouse anti-cathepsin K antibody (CTSK, dilution 1:250, CA, USA). Following incubation, horseradish peroxidase–conjugated antibody (HRP, DAKO, Glostrup, Denmark) was applied. The diaminobenzidine substrate (DAB, DAKO) was used for staining prior to counterstaining in haematoxylin.

To analyse the histological characteristics, light microscopy (Olympus Corporation, BX-51, Tokyo, JPN) incorporating a digital camera was utilised to examine and photograph the sections. Osteoblasts (identified by H and E staining) and osteoclasts (identified by TRAP-positive \([\text{TRAP}^+]\) staining and cathepsin K-positive \([\text{CTSK}^+]\) staining) were counted in the ROIs.

**Sequential fluorochrome labelling and histomorphometric analysis**

The sequential fluorochrome labelling was performed according to a previous method.\textsuperscript{22} Three rats were injected intraperitoneally with calcein (CA) (Sigma) (20 mg/kg.bw) on day 5 and alizarin red (AL) (40 mg/kg.bw) (Sigma) on day 9 after the commencement of OTM. The animals were euthanised on day 12 and the maxillae were harvested. Processing by dehydration and embedding in polymethyl methacrylate were performed. The specimens were subsequently sectioned in the transverse plane into 200 \(\mu\)m (thickness) sections using a hard sectioning microtome (Leica, Wetzlar, Germany). Examination was undertaken via laser scanning confocal microscopy (Leica). The commonly-used parameters to detect bone formation rates, the mineral apposition rates (MAR) and bone formation rates / bone surface (BFR/BS) were calculated.\textsuperscript{25}

**Statistical analysis**

Statistical analyses were conducted to estimate significant differences (\(p < 0.05\)) between the OTM side and the control side in each group. All of the data were shown as mean ± SD and the paired-samples \(t\)-test was applied using SPSS, version 24.0 (SPSS Inc).

**Results**

**Local orthodontic force induces catabolic metabolism of the maxillary alveolar bone**

The OTM models were constructed using the spring apparatus (Figure 1A-B) and routinely checked daily. The success of the OTM model was confirmed by the increase in the distance between M1 and M2 (Figure 1B). To study the effects of a local orthodontic force on the total alveolar bone, six regions of alveolar bone were observed, and identified as a cervical level (section A) and an apical level (section B) of M1, M2, and M3 (Figure 1C-D).

Bone remodelling included bone resorption by osteoclasts and bone formation by osteoblasts. Changes in bone resorption during OTM were determined by TRAP staining and IHC staining of CTSK. The orthodontic force was directly applied to M1 and, therefore, the initial analysis was the alveolar bone of M1. As shown (wure 2A-G, 2V), there were more TRAP\(^+\) multinuclear osteoclasts in the cervical level of the alveolar bone on day 1, 3, and 7 after the initiation of the OTM, when compared with the corresponding control group and the day 0 group (\(p < 0.05\)). The apical region of the alveolar bone of M1 during OTM also contained more TRAP\(^+\) osteoclasts (Figure 2W, \(p < 0.05\)). Similarly, IHC staining of CTSK indicated an increased number of osteoclasts in the cervical and apical level of the alveolar bone.
of M1 during OTM (Figure 3A–G, \( p < 0.05 \)). Furthermore, changes in the ROIs of M2 and M3, on which the orthodontic force was not loaded directly, were analysed. The results demonstrated that in the ROIs of M2 and M3, the number of osteoclasts in the OTM group also increased when compared with the corresponding control group and the day 0 group at both the cervical and apical levels (Figure 2 and 3, \( p < 0.05 \)). This data indicated that the local orthodontic force acting on M1 promoted catabolic metabolism of the maxillary alveolar bone.

**Local orthodontic force elevates anabolic metabolism of the maxillary alveolar bone**

Bone quantity and quality is maintained by a balance in catabolic and anabolic metabolism. The effects of local orthodontic forces on alveolar bone and its anabolic metabolism during OTM were studied. As shown by H and E staining (Figure 4G, N, U), new alveolar bone was formed on day 7 when compared with the control group. Osteoblast numbers were elevated in six ROIs on day 3 and 7 during OTM (\( p < 0.05 \)). This reaction was observed in the alveolar bone at both the cervical and apical levels (Figure 4V-W).
Furthermore, sequential fluorochrome labelling analysis determined bone deposition rates of alveolar bone during OTM. It appeared that the mineral apposition rates of the OTM group of M1, M2 and M3 increased in comparison with the corresponding control group (Figure 5, \( p < 0.05 \)). These results demonstrated that local orthodontic forces activated anabolic metabolism of the maxillary alveolar bone.

### Discussion

Based on the classic pressure-tension hypothesis, orthodontic force-induced tooth movement is considered an alveolar bone remodelling process, involving bone resorption on the pressure side and deposition on the tension side.\(^8,9,26\) However, the biological reaction of bone under a mechanical stimulus appeared not to be localised. The conventionally studied alveolar bone adjacent to the roots\(^6,27\) during OTM only occupies a small proportion of the alveolar bone, while biological responses of the entire alveolar bone under local orthodontic force are seldom reported. In the present study, the widespread tissue reaction of the alveolar bone was highlighted. It was found that an orthodontic force acting on M1 not only initiated partial bone remodelling...
surrounding the loaded tooth (M1) but, additionally, triggered remodelling of the entire maxillary bone in rats. It may become a supplemental development of the orthodontic biomechanical theory. The results suggested that orthodontists should attach more clinical importance to the biomechanical effects on the entire alveolar bone.

Bone remodelling involved bone resorption by osteoclasts and bone deposition by osteoblasts. Osteoclast precursor cells function as pioneers during the early stages of OTM. Subsequently, differentiation and polarisation of osteoclasts occurs, releasing hydrogen ions and proteolytic enzymes that dissolve minerals and degrade the organic matrix of bone. As a widely used marker of osteoclast development, cathepsin K is a major extracellular bone-degrading enzyme predominantly expressed by osteoclasts. In the present study, IHC staining for CTSK revealed osteoclastic activity. Both TRAP and CTSK osteoclasts were upregulated on day 1, 3, and 7 after OTM in the M1 zones, as well as exhibiting moderate increases in M2 and M3 zones, at both the cervical and apical levels. The data indicated that osteoclastic activity was enhanced throughout the entire maxillary alveolar bone, which meant that the local orthodontic force induced entire catabolic
metabolism of the surrounding alveolar bone. It has been reported that the osteoclasts could be recruited and migrate to the adjacent alveolar bone around the roots on the pressure site in the early phase of OTM.\textsuperscript{28-30} It was demonstrated that the activation of osteoclasts was not localised but widespread, which implied a total adaption to a local mechanical stimulus.

The process of new bone formation ensures the maintenance of adequate bone mass during tooth movement.\textsuperscript{31,32} According to the H and E analysis, osteoblast numbers were elevated on day 3 and 7 after the initiation of OTM in all of the M1, M2, and M3 zones, at both the cervical and apical levels. New bone formation was observed in all regions following OTM. In addition, bone deposition rates were increased in six ROIs, compared with a non-OTM control group, which further demonstrated an enhanced osteoblast activity in the maxilla. The results indicated that the local orthodontic force also elevated anabolic metabolism of the surrounding maxillary alveolar bone. The widespread adaptive activity of osteogenesis contributes to maintaining balance during osteoclastogenesis, which helps to maintain bone mass and architecture of the total alveolar bone. Therefore, bone integrity and security are assured during orthodontic tooth movement. The present study indicates and supports the adaptive capacity of the entire alveolar bone when subjected to local mechanical loading.

The CT results by Verna et al.\textsuperscript{33} support the premise that the whole maxilla responds to a local partial mechanical load. The enhanced catabolic and

![Figure 5](image.png)

Figure 5. Sequential fluorochrome labeling analysis indicated that the local orthodontic force increased the entire bone deposition rates. The MAR and BFR/BS of the alveolar bone in M1, M2 and M3 zones were all elevated during OTM. (*p < 0.05, N = 3)
anabolic metabolism of the entire alveolar bone may have been caused primarily by the regional acceleratory phenomenon (RAP), which functions as an “SOS” system in the body. This phenomenon is generally a response to a mechanical stimulus or other directed perturbation.\textsuperscript{16,34} The RAP suggests that basic multicellular units (BMUs) are substantially activated, thereby accelerating bone re-organisation and enlarging modelling spaces.\textsuperscript{35} During orthodontic tooth movement, the maxillary tissue reaction with elevated osteoblastic and osteoclastic activity under local loading revealed the instant adaption to a new mechanical environment, which may also be considered a form of RAP. Correspondingly, the bone remodelling process is initiated and every BMU of the entire alveolar bone becomes activated, followed by accelerated bone resorption and deposition, which is not exclusively limited to the load-bearing alveolar bone. However, whether the present results are applicable to humans is still uncertain, remains the limitation of the study, and provides the stimulus for further clinical research.

Conclusions

The present research demonstrated that a local orthodontic force was not restricted to the partial tension and pressure sites but generated remodelling of the entire alveolar bone. According to the data, the activities of osteoblasts and osteoclasts were both elevated, which indicated that catabolic and anabolic metabolism of maxillary alveolar bone were activated under the locally applied force. The results emphasise the integrity and balance of bone remodelling during orthodontic tooth movement, and provide evidence for the orthodontic biomechanical theory. The present study therefore offers novel insights for the clinical work of orthodontists in highlighting the widespread impact of an orthodontic force, although further human clinical research will be required in the future.

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Conflict of interest

The authors report no professional or financial conflict of interest in relation to this study.

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