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Original Article

Alterations of FOXO1 protein expression during orthodontic tooth movement in rats

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Abstract: Objective: To study alternations in forkhead box, class O1 (FOXO1) protein expression in the periodontal tissue of rats during orthodontic tooth movement (OTM). Methods: A total of 60 Sprague-Dawley (SD) rats were randomly and evenly divided into six groups: an untreated control group and 5 groups treated with applied orthodontic force for 1, 3, 7, 10, and 14 days. The left maxillary first molar was used for experimental treatment, whereas the right side remained untreated as an internal control. OTM was established and tissue samples were collected. Hematoxylin-eosin (HE) staining was used to observe cell morphology; tartrate resistant acid phosphatase (TRAP) staining, to determine the number of osteoclasts on the alveolar pressure side; and immunohistochemical analysis, to analyze the expression of FOXO1. Results: The movement of the maxillary first molar initially increased with 1-3 days of orthodontic force, followed by a flattening between 3 and 7 days and then a continuous, rapid increase between 7 and 14 days. The number of osteoclasts initially increased, peaking at the 7th day after the start of orthodontic force, and then slowly decreased. The average optical density of FOXO1-positive immunostaining was significantly different in the pressure zone at 1, 3 and 7 days, and in the tension zone at 1, 3, 7, 10 and 14 days from that of the internal control (all $P < 0.05$). Conclusion: The alternations of FOXO1expression might participant in the periodontal tissue remodeling during the OTM.

Keywords: Sprague-Dawley rats, orthodontic tooth movement, forkhead box, class O1 protein, periodontal tissue, maxillary first molar, average optical density

Introduction

The number of patients seeking orthodontic treatment and correction for dentofacial deformities has increased; therefore, it is important to investigate the biological and molecular basis of orthodontic movement (OTM) [1]. OTM is a sequence of mechanical bone adaptation and occurs as a result of bone resorption and bone formation in alveolar bone as the bone mass and structure adapt to the mechanical environment [2]. As a result, OTM includes remodeling of alveolar bone and the periodontal ligament. Reducing the length of treatment for OTM might be beneficial for patients by reducing the long-term consequences of orthodontic treatment [3]. OTM requires alveolar bone resorption by osteoclasts at the compression side during tooth movement and bone formation on the alveolar wall (tension side) of the tooth [4, 5]. Because OTM is a complex process, it is important to monitor OTM non invasively by assessing the levels of growth factors, various enzymes, cytokines, and proteoglycans [6].

The forkhead box, class O (FOXO) proteins are characterized by the presence of a winged-helix DNA binding domain and play a key role in many biological processes, including apoptosis, gluconeogenesis, oxidative stress, and stress signaling [7]. Additionally, deregulation of FOXO1 has been shown to promote cell proliferation and tumorgenesis in endometrial, prostate and breast cancers, and FOXO1 has become a major target in preventing the development of cancers [8, 9]. Furthermore, FOXOs participate in redox balance and osteogenesis, and FOXO1 is the main regulator of osteogenesis. Downregulation of FOXO1 in osteoblasts results in decreased proliferation of osteoblasts and decreased bone formation [10]. Additionally, FOXO1 is essential for bone homeostasis and r osteocyte survival as it promotes the mainte-

nance and differentiation of early osteoblast progenitor but results in contradictory results of osteoclastgenesis [11].

Because FOXO1 plays a significant role in bone homeostasis, we aim to investigate the dynamic expression of FOXO1 in the periodontal tissues of rats during OTM. The results of this study may provide further clinical evidence on the mechanism of OTM.

Materials and methods

Study subjects

A total of 60 healthy male Sprague-Dawley (SD) rats (clean grade; 6-8 week-old; 210 ± 10 g) were provided by the Experimental Animal Center of China Medical University. The rats were randomly and evenly divided into six groups: an untreated control group and 5 treatment groups based on the duration of OTM application (1-, 3-, 7-, 10- or 14-day group). The OTM device was applied to the left maxillary first molar (experimental side) whereas the right left maxillary first molar remained untreated and served as an internal control. The rats were housed and bred under clean-level conditions (temperature of 18-25°C and a humidity of 40-60%) with access to food and water *ad libitum*. The rat's drinking water was subject to high pressure sterilization at 121°C for 30 min before drinking, and all food was replaced once per week. The experiments were carried out in strict accordance with the National Institutes of Health for the use of Laboratory Animals [12], and all procedures were approved by the Experimental Animal Center of China Medical University.

Generation of the animal model and postoperative animal feeding

The rats were anesthetized with 3 ml/kg 10% chloral hydrate (intraperitoneally). A high-speed dental turbine assembly with fine particles of silicon carbide burs was used to grind a fixing groove of approximately 0.5-1.0 mm on the maxillary incisor with the bolster on the bearing portion and the labial surface. An orthodontic stainless steel ligation wire (with a diameter of 0.25 mm) was used for fixing and a 0.3 mm Ni-Ticoil spring was ligated between the left maxillary first molar and the incisor of the rats. Achemically curing glass ionomer adhesive was used to reinforce the spring, and this process was repeated as necessary. The Ni-Ti coil spring was stretched to an application force of 50 g (detected by a dynamometer), which moved the left maxillary first molar to mesi occlusion. OTM was applied for 1, 3, 7, 10 and 14 days. After surgery, the rats were feda crushed mixture of food, water and sesame oil (stirred into a paste) to prevent pain with feeding and to keep the OTM device in place. The device was checked daily in case to monitor for damage or unintentional removal.

Measurement of tooth movement distance

Maxillary impressions and standard plaster models of the rats were accurately obtained before and after the application of OTM force. A caliper (with an accuracy of 0.02 mm) was used to measure the distance between the maxillary first molarmesio-lingual groove point and the third molar mesio-lingual groove point. The difference between the two measured values was the mesial-movement distance of the maxillary first molar. Each measurement was repeated three times by the same experimenter and the average measurement value was calculated.

Sample preparation

The rats in each group were sacrificed at predetermined time points (1, 3, 7, 10, and 14 days after the start of force application). The left and right maxillary first molars and the corresponding periodontal tissue blocks were obtained and fixed in 4% paraformaldehyde for 1 day. The samples were decalcified with 10% ethylene diaminetetraacetic acid (EDTA) at 4°C for 3 months, followed by dehydration with ethanol and paraffin embedding. Longitudinal slices (5 μm) in the mesiodistal direction were prepared from the upper left first molar and extended at 49°C. The slices were coated with poly-lysine, removed, and baked at 60°C for 2-3 hours. Hematoxylin-eosin (HE) staining tartrate resistant acid phosphatase (TRAP) staining and immunohistochemical staining were conducted on the slices (see below).

HE staining

The prepared slices were oven-dried and dewaxed in xylene (2 × 10 min) with rehydration using graded ethanol for 2 min each time. The slices were stained using hematoxylin (5 min),

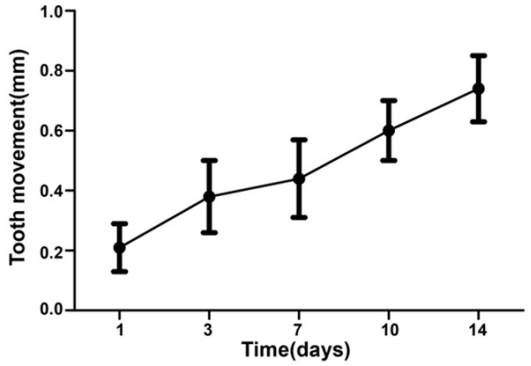

Figure 1. Tooth movement distance in rats during orthodontic tooth movement. The movement curve for the maxillary first molar showed an initial rise, followed by a flattening and then a continuous increase.

rinsed in tap water and differentiated by dipping in hydrochloric acid ethanol for 30 s. Next, the slices were soaked in tap water for 15 min or warm water at 50°C for 15 min and then put into an eosin solution for 2 min, dehydrated in graded ethanol, cleared with 2 changes of xylene (2 × 10 min), sealed with neutral resin, and observed and photographed under an optical microscope. Microscope showed the distribution of periodontal tissue cells in the mesi occlusion (pressure zone; 1/3 of the first molar root) and the distal occlusion (tension zone; the first molar root).

TRAP staining

Theslices were dewaxed and dehydrated, followed by staining with TRAP solution at 37°C for approximately 30 min. The slices were removed and rinsed with tap water and then restained with methyl green for approximately 5 min before being sealed by resin. Slides were randomly selected and observed under a high magnification lens to count the TRAP positive osteoclasts in alveolar bone.

Immunohistochemical staining

The samples were fixed in a 10% formalin solution, embedded with paraffin and continuously sectioned into 4 μm thick slices. The slices were dewaxed and dehydrated by alcohol. Hydrogen peroxide (3%) was used to block endogenous peroxidase before antigen retrieval. The slices were incubated with mouse anti-FOXO1 antibody (Abcam, UK) at 37°C for 1 h, followed by incubation with abiotin-labeled secondary antibody at 37°C for 30 min. Freshly prepared diaminobenzidine (DAB) developing solution was added for 1-2 min before the sluices were washed with phosphate buffered saline (PBS) buffer (3 × 2 min) and then re-stained with hematoxylin for 1 min, dehydrated, and mounted with neutral resin. Slices with known FOXO1 expression were used as a positive control, and no antibody slices were used as a negative control. FOXO1 expression was observed in randomly selected slices using a high-power microscope (400 ×). Image-Pro Plus image analysis software (Media Cybernetics, US) was used to obtain accurate pictures for analysis. The average optical densities of the positive staining areas in the tension zone and pressure zone of the periodontal tissue were measured in both the control and the experimental groups.

Statistical analysis

SPSS 21.0 statistical software was used for statistical processing. Measurement data were expressed as mean ± standard deviation and tested by t-test. Count data were expressed as percentages or ratios and tested by chi-square test. Paired t-test was used to compare the displacement distances of rats' teeth at varied timings, the number of TRAP-stained osteoclast at varied timings and the average *FOXO1*-positiveoptical density at the near-middle periodontium (pressure zone) and the far-middle periodontium (tension zone)of the case group at different stressing timings. *P* is two-tailed and $P < 0.05$ was considered statistically significant.

Results

Tooth movement distance

OTM consists of three stages: (1) the transient motion stage, (2) the slow stage and (3) the continuous movement stage. In this study, the movement curve for the maxillary first molar showed an initial rise, followed by a flattening and then a continuous increase. The tooth movement distances were 0.21 ± 0.08 mm in the 1-day group, 0.38 ± 0.14 mm in the 3-day group, 0.44 ± 0.16 mmin the7-day group, 0.60 ± 0.10 mm in the 10-day group and 0.74 ± 0.13 mmin the 14-day group. After orthodontic force was applied for 1-3 days, rapid tooth movement was observed, with significant differences in

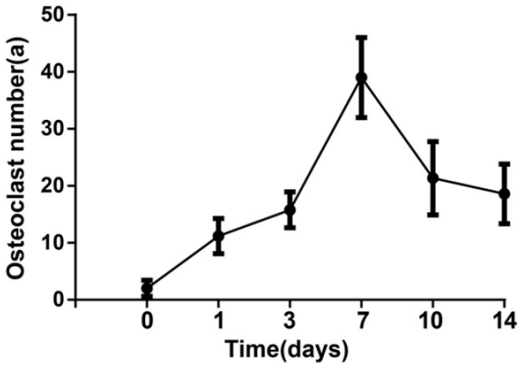

Figure 2. The number of osteoclasts following TRAP staining in the rat samples at the different time points. The total number of osteoclasts initially increased to a peak at the 7th day of orthodontic force application and then slowly decreased. Note: TRAP, tartrate resistant acid phosphatase.

tooth movement distance between the 1-day and 3-day groups ($P = 0.004$). Between 3 and 7 days of orthodontic force application, tooth movement slowed, and no significant differences were observed between the 3-day and 7-day groups ($P = 0.384$). However, between 7 and 14 days of orthodontic force application, tooth movement increased again, and significant differences were noted between the 7-day group and the 10-day group ($P = 0.015$) and between the 10-day group and the 14-day group ($P = 0.015$) (**Figure 1**).

Osteoclast counts after TRAP staining

Microscopy after TRAP staining revealed few or no osteoclasts in the control slices. Alveolar bone completely encompassed the tooth roots, showing a compact and organized structure. In addition, the periodontal ligament was intact and continuous, with no significant changes. The 1-day and 3-day groups showed TRAP-positive osteoclasts scattered throughout the pressure side of the alveolar bone. Using 7 days of force application, the periodontal ligament tissue in the pressure zone was compressed, the periodontal space was narrowed, and osteoclasts were observed on the edges of the resorption lacuna of the alveolar bone. In the 10-day and the 14-day groups, we observed ongoing resorption of periodontal bone in the pressure zone, and the surface of the lacuna appeared eroded; although TRAP positive osteoclasts were still observed, their expression was decreased. The osteoclast numbers were 11.202 ± 3.088, 15.778 ± 3.144, 39.013 ± 7.025, 21.336 ± 6.428 and 18.586 ± 5.219 in 1-, 3-, 7-, 10- and 14-day groups, respectively. After 1-7days of force application, the osteoclast numbers increased over time, and significant differences were found between the 1-day and 3-day groups ($P = 0.004$) and between the 3-day and 7-day groups ($P < 0.001$). Between 7 and 14days of force application, the osteoclast numbers gradually decreased, and significant differences were noted between the 7-day group and the 10-day group ($P < 0.001$), whereas no significant differences were observed between the 10-day group and the 14-day group ($P = 0.308$). The overall osteoclast number initially increased and then decreased, with a peak osteoclast number observed at 7days of force application (**Figure 2**).

HE staining results

For both the untreated control group and the internal control group, the periodontal fibers of the maxillary first molar were regularly arranged, and the alveolar bone had a smooth surface. The collagen fibers extended into the periodontal ligament from the cementum. The periodontal ligament consisted of collagen fibers and fibroblasts, and the fibroblasts were uniformly distributed between the main collagen fibers, which was inconsistent with the long axis of the main fibers and produced a wavy appearance. Blood vessels were wrapped around the loose connective tissue between the collagen fibers. Bundles of collagen fibers were attached to the alveolar bone. In addition, no obvious signs of osteoblasts and osteoclasts were found (**Figure 3A**, **3B**).

In the tension zone of the 1-day group, the periodontal gaps were slightly widened, the periodontal ligament fibers were relatively straight but loosely arranged, the number of fibroblasts had increased, and blood vessel dilation was visible. In the pressure zone, the periodontal gaps were narrowed, the collagen fibers were decreased in the periodontal ligament and a few individual osteoclasts located in the eroded lacuna were found on the surface of the alveolar bone. In the tension zone of the 3-day group, we observed ongoing widening of the periodontal gap, disarrangement of the periodontal ligament fibers and more vascular dilatation. In the pressure zone of the 3-day group, we observed continued narrowing of the peri-

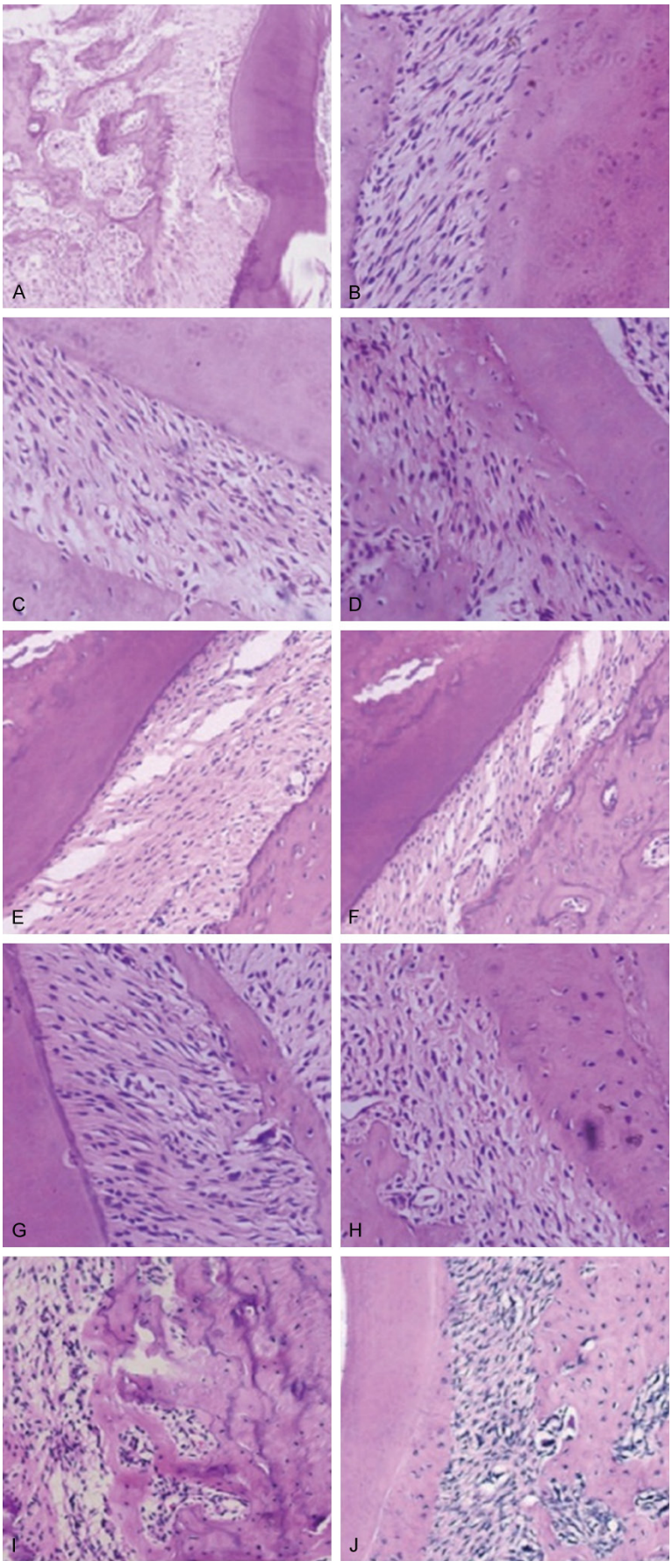

Figure 3. HE staining results (× 40) A: Untreated control group; B: Internal control group. C: The 1 day group (pressure zone); D: The 1-day group (tension zone); E: The 3-day group (pressure zone); F: The 3-day group (tension zone); G: The 7-day group (pressure zone); H: The 7-day group (tension zone); I: The 10-day (pressure zone); J: The 10-day group (tension zone). Note: HE, hematoxylin-eosin.

odontal gap, the appearance of the partial hyalinization zone and a few osteoclasts located in the eroded lacuna on the adjacent alveolar surface. The 7-day group showed disorganization and thickening of the periodontal ligament fibers, an increased number of dilated blood vessels, and the appearance of cubic osteoblasts on the alveolar surface and thickened new bone deposits in the tension zone. In addition, in the pressure zone of the 7-day group, the hyalinization zone was reduced, the periodontal gap was further narrowed, a large number of multi-core osteoclasts were seen in the lacuna, and there was significant alveolar bone absorption. In the 10-day and 14-day groups, the arrangement of the periodontal ligament fibers had gradually returned to normal in the tension zone, and in the pressure zone, the periodontal ligament was narrowed, osteoclasts were found in the eroded lacuna on the alveolar bone surface, periodontal tissue fibroblasts had gradually increased with time, the number of osteoclasts in the lacuna had decreased and a small number of osteoblasts were visible (**Figure 3C-J**).

Immunohistochemistry results

The periodontal ligament fibers of the periodontal tissue

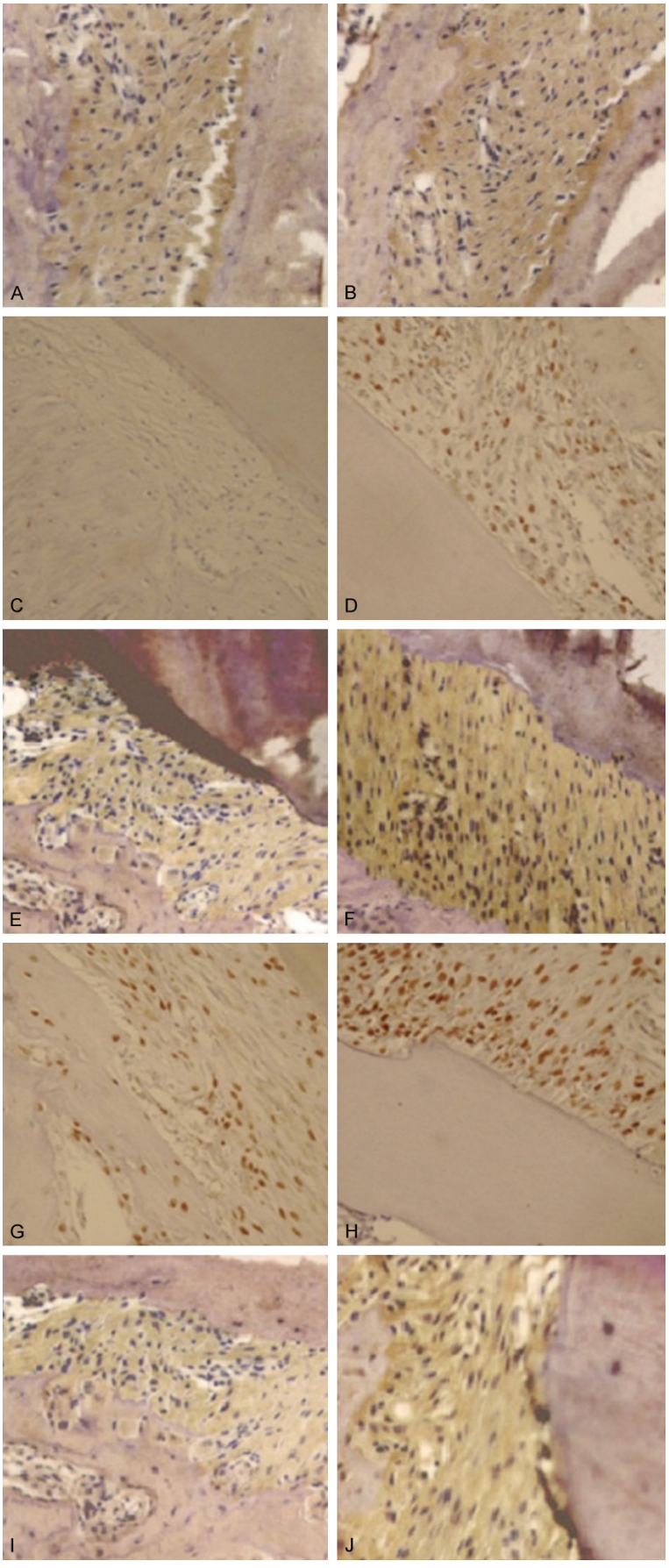

Figure 4. Immunohistochemical staining (× 200 magnification) A: Untreated control group; B: Internal control group; C: The 1-day group (pressure zone); D: The 1-day group (tension zone); E: The 3-day group (pressure zone); F: The 3-day group (tension zone); G: The 7-day group (pressure zone); H: The 7-day group (tension zone); I: The 10-day (pressure zone); J: The 10-day group (tension zone).

were regularly arranged, and the edges of the alveolar bone were continuous without significant absorption, showing evidence of new formation in both the untreated control group and the internal control group. In addition, nuclear staining for FOXO1 was found in the periodontal ligament fibroblasts, endothelial cells, alveolar bone cells and gingival epithelial cells in the normal periodontal tissue (as evidenced by brown staining). FOXO1-positive staining was also observed in the endochylema of a small number of fibroblasts and gingival epithelial cells. The gingival cells were negatively stained (**Figure 4A**, **4B**).

In the 1-day group, no significant changes were observed in the alveolar bone between the tension zone and the pressure zone. Proliferated fibroblasts with nuclear FOXO1 staining were observed. Although no significant difference was found between the 1-day and the internal control samples with respect to appearance, further analysis showed that the number of FOXO1 positive fibroblasts was significantly reduced. In the 3-day group, hyperplasia of the periodontal ligament fibers and enhanced FOXO1 expression fibers and enhanced in the tension zone; however, in the pressure zone, the number of FOXO1 positive cells was similar to that of the

Table 1. The average optical density of FOXO1 positive staining in the mesiocclusion periodontal tissue (pressure zone) between the experimental groups and the internal control group

Group	Pressure zone	Internal control	t	*P*
Untreated control	0.181 ± 0.019	0.178 ± 0.020	0.478	0.644
Experimental groups				
1-day group	0.140 ± 0.012	0.173 ± 0.021	4.423	0.002
3-day group	0.152 ± 0.009	0.171 ± 0.025	2.526	0.032
7-day group	0.157 ± 0.011	0.176 ± 0.019	2.973	0.016
10-day group	0.162 ± 0.018	0.172 ± 0.022	1.186	0.266
14-day group	0.169 ± 0.022	0.174 ± 0.024	0.320	0.756

Note: FOXO1, forkhead box protein O1.

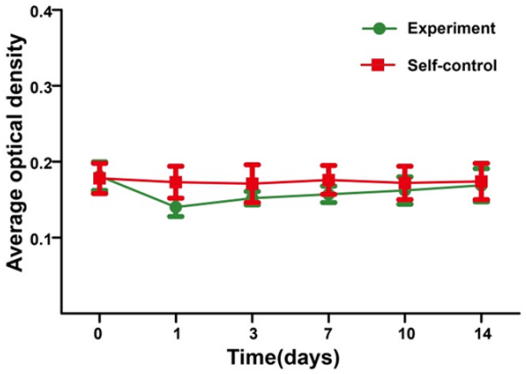

Figure 5. The average optical density of FOXO1 positive staining in the mesiocclusion periodontal tissue (pressure zone) between the experimental groups and the internal control group. A statistically significant difference was seen inFOXO1 positive staining in the pressure zone for the 1-, 3- and 7-day group compared with the internal control group (all $P < 0.05$). Note: FOXO1, forkhead box protein O1.

1-day group, with staining observed in only a small number of fibroblast nuclei. In the tension zone of the 7-day group, active new bone formation was noted, with newly generated osteoblasts visible on the surface. The level of FOXO1 expression was similar to that of the untreated control group. In the pressure zone, osteoclasts and bone resorption were noted throughout the lacuna, and FOXO1 expression was further increased. In the 10-day and 14-day groups, FOXO1 expression was significantly reduced in the tension zone compared with the levels observed in the 7-day group (**Figure 4C-J**).

Analysis results of FOXO1 expressionin periodontal tissue during tooth movement

FOXO1 expression in the pressure zone of the 1-day and 3-day groups decreased and then rebounded after 7-14 days of for-ce application. The average optical density of FOXO1 positive staining showed no statistically significant difference between the untreated control group and the internal control group ($P > 0.05$); However, the 1-, 3- and 7-day groups were significantly different from the internal control group (all $P < 0.05$). In addition, no statistically significant difference was found when comparing the 10-day and 14-day groups with the internal control group (both $P > 0.05$) (**Table 1** and **Figure 5**).

In the tension zone, the average optical density of FOXO1 positive staining showed no statistically significant difference between the untreated control group and the internal control group ($P > 0.05$). However, the 1-, 3-, 7-, 10- and 14-day groups were all significantly different from the internal control group (all $P < 0.05$) (**Table 2** and **Figure 6**).

In the pressure zone, FOXO1 expression in the periodontal tissue decreased significantly in the 1-day group before slowly increasing and returning to baseline levels. In the tension zone, FOXO1 expression in the periodontal tissue gradually increased to a peak in the 3-day group before slowly decreasing. The average optical density of the FOXO1 positive staining showed that there were statistically significant differences between the mesi occlusion (pressure zone) and the distal occlusion (tension zone) at 1, 3, 7, 10 and 14days (all $P < 0.05$) (**Table 3** and **Figure 7**).

Discussion

We investigated the dynamic expression of FOXO1 in the periodontal tissue of rats during OTM. Our results showed that the movement of the maxillary first molar was rapid after force was applied for 1-3 days. This level of movement slowed after 3-7 days and then increased again between 7 and 14 days. In OTM, alveolar bone remodeling may be induced and regulated by constant mechanical forces, and tooth movement is initiated and facilitated by compressive force-associated osteoclast formation and bone resorption [13]. The process of bone remodeling typically involves old bone resorption by osteoclasts and new bone formation by osteoblasts, and the total amount of skeletal tissue depends on the equilibrium between the

Table 2. The average optical density of FOXO1 positive staining in the distal occlusion periodontal tissue (tension zone) between the experimental groups and the internal control group

Group	Tension zone	Internal control	t	*P*
Uutreated control	0.181 ± 0.019	0.178 ± 0.020	0.478	0.644
Experimental groups				
1-day group	0.268 ± 0.018	0.173 ± 0.021	10.85	< 0.001
3-day group	0.324 ± 0.019	0.171 ± 0.025	20.90	< 0.001
7-day group	0.265 ± 0.015	0.176 ± 0.019	9.505	< 0.001
10-day group	0.236 ± 0.021	0.172 ± 0.022	7.980	< 0.001
14-day group	0.204 ± 0.022	0.174 ± 0.024	2.844	0.019

Note: FOXO1, forkhead box protein O1.

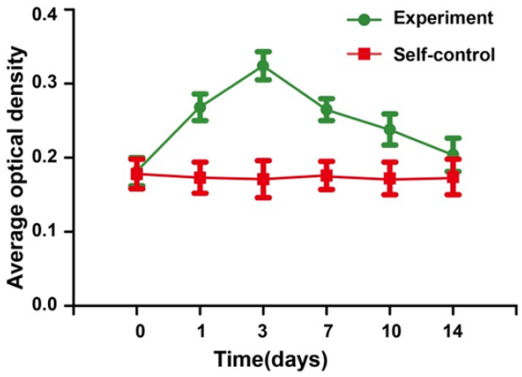

Figure 6. The average optical density of FOXO1 positive staining in the distal occlusion periodontal tissue (tension zone) between the experimental groups and the internal control group. A statistically significant difference was seen in FOXO1 positive staining in the tension zone, the average optical density of FOXO1 positive staining showed significant difference when comparing the 1-, 3-, 7-, 10-, and 14-day groups compared with the internal control group. Note: FOXO1, forkhead box protein O1.

rates of the two processes [14]. In our study, we found different speeds of tooth movement during the 14 days of orthodontic force application, which may be the result of different rates of bone formation and resorption.

Moreover, the number of osteoclasts increased between 1 and 7 days, peaked at 7 days and then gradually decreased between 7 and 14 days. Osteoclasts are multinucleated cells originating from hematopoietic mononuclear precursor cells of the monocyte/macrophage lineage that are responsible for bone resorption in bone metabolism [15]. Mechanical stress, such as orthodontic force, influences bone remodeling through repeated alveolar bone resorption by osteoclasts on the pressure side and new bone formation by osteoblasts on the tension side [16]. Therefore, the initial increase in osteoclasts indicated rapid bone resorption, and the subsequent decrease in osteoclasts suggested slowed bone resorption.

FOXO1 positive expression in the pressure zone initially decreased before increasing, whereas in the tension zone, FOXO1 expression initially increased and then decreased over time. In addition, the average optical density of FOXO1 protein immunostaining was significantly higher in the tension zone than in the pressure zone. OTM produces two different zones in the periodontal ligament: the pressure zone and the tension zone. On the pressure side, disturbed blood flow and cell death are followed by resorption of hyalinized tissue, whereas on the tension side, stretched periodontal ligament fibers are associated with increased blood flow [2]. Increased osteoclast activity may lead to bone resorption in the pressure zone, whereas increased osteoblast activity may lead to bone formation in the tension zone [17].

FOXO1 directly interacts with the Runx2 promoter, and FOXO1 silencing decreases the expression of Runx2 and impairs bone formation. In addition, Runx2 and FOXO1 interact with each other and cooperate in the transcriptional regulation of osteoblast markers [18]. The Runx2 runt-domain is normally expressed in osteoblastic mesenchymal cells, and the function of Runx2 is necessary for the differentiation and maturation of osteoblasts and bone formation [19]. Studies have shown that FOXO1 is upregulated during bone formation in response to stimulation by bone morphogenetic proteins and is an important transcription factor for promoting osteoblast differentiation by interacting with the RUNX2 promoter [20]. This suggests that FOXO1 is an early molecular regulator during osteoblastic mesenchymal cell differentiation. Moreover, FOXO1 has been shown to interact with ATF4, which may regulate amino acid important stress-dependent pathways to influence p53 signaling, thereby participating in osteoblast physiology and providing a direct mechanistic link between oxidative stress and the regulation of bone remodeling

Table 3. The average optical density of FOXO1 positive staining between the mesiocclusion (pressure zone) and the distal occlusion (tension zone) for the experimental groups

Group	Pressure zone	Tension zone	t	*P*
1-day	0.140 ± 0.012	0.268 ± 0.018	14.05	< 0.001
3-day	0.152 ± 0.009	0.324 ± 0.019	33.09	< 0.001
7-day	0.157 ± 0.011	0.265 ± 0.015	15.39	< 0.001
10-day	0.162 ± 0.018	0.236 ± 0.021	10.43	< 0.001
14-day	0.169 ± 0.022	0.204 ± 0.022	2.976	0.016

Note: FOXO1, forkhead box protein O1.

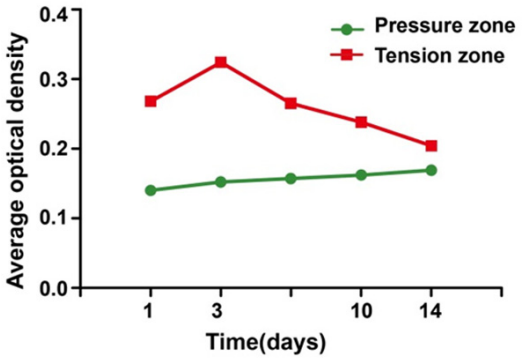

Figure 7. The average optical density of FOXO1 positive staining between the mesiocclusion (pressure zone) and the distal occlusion (tension zone) for the experimental groups. A statistically significant difference in FOXO1 positive staining was observed between the pressure zone and the tension zone at 1, 3, 7, 10, and 14 days (all $P < 0.05$). Note: FOXO1, forkhead box protein O1.

[21]. Furthermore, FOXO-dependent oxidative stress defense provides a mechanism to handle oxygen free radicals, which are constantly generated by osteoblast aerobic metabolism and are therefore essential for bone mass homeostasis, by activating free radical scavenging and apoptosis-related gene expression [22]. Moreover, FOXO proteins can restrain osteoclastogenesis and bone resorption by attenuating H_2O_2 accumulation, which is a critical control point for osteoclastogenesis and bone resorption. Furthermore, decreased H_2O_2 production in the mitochondria may represent a novel treatment target for diseases involving high bone resorption [23].

In conclusion, orthodontic force in OTM induced changes in FOXO1 expression in periodontal tissue leading to tissue and bone remodeling by regulating the balance of osteoblasts and osteoclasts. The results of our study may provide a new biomarker to monitor OTM and provide valuable insight into the mechanism of OTM.

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Disclosure of conflict of interest

None.

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