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Comparison of horizontal and vertical osteoperforation on biological response and tooth movement in rabbits

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Abstract

Objective: The aims of this study were to compare the amount of tooth movement after multiple-horizontal and single-vertical microosteoperforation (MOP), and to evaluate the histological changes after orthodontic force application in rabbits.

Materials and Methods: Mandibles of 24 white rabbits have received two experimental interventions: a multiple-horizontal (MH) and a single-vertical (SV) MOP. Defect volume of MOP between two groups was controlled similarly. A hundred cN force was applied via coil spring between the incisor teeth to the first premolars. The amount of tooth movement was measured. Differences in amount of tooth movement and bone variables according to the three time points and the two groups were evaluated using repeated measures ANOVA.

Results: The first premolar showed 1.47 mm of mesial movement in the MH group and 1.84 mm in the SV group, which showed significant difference at week 3 ($P < 0.05$). There was no significant difference in bone volume and bone fraction between the groups. TRAP-positive cell count was significantly greater at week 3 in both the SV and MH groups compared to week 1.

Conclusions: The amount of tooth movement showed significant difference between the 1st and 3rd weeks for the SV and MH MOP groups, but not between the two groups. Therefore, SV MOP could be considered an effective tool for enhancing tooth movement, especially for molar distalization, uprighting, and protraction to an edentulous area.

Keywords: Corticotomy; Tooth movement; Bone biology

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INTRODUCTION

With increased demand for orthodontic treatment in adults, long treatment time is often a main factor that influences patient satisfaction. As acceleration of tooth movement has become the main concern of clinicians, many studies have been conducted on accelerated tooth movement.[1–3]

Surgical acceleration of tooth movement can be explained by the regional acceleratory phenomenon (RAP), in which injury to bone creates a temporary localized bone demineralization-remineralization. Tooth movement is expedited as the dense cortical bone resistance is removed.[4,5]

Among these modalities, corticotomy is the most effective method because it is a technique that induces RAP and stimulates tooth movement on the buccal side.[6,7] Less invasive techniques such as piezocision, corticision, and microosteoperforation (MOP) without flap elevation have been reported.[8–13]

Wilcko et al.[14] applied selective decortication lines and made cuts as deep as 0.5 mm around the target teeth; and Baloul et al.[15] demonstrated that this surgical intervention accelerated the rate of tooth movement during the earlier stages of treatment. Dutra et al.[16] reported that alveolar decortications resulted in enhanced bone remodeling at the region of tooth movement. In addition, some studies have demonstrated that MOP significantly shortens the duration of orthodontic treatment.[8,17] Recently, two randomized controlled studies were conducted to assess the effect of MOP on tooth movement.[18,19] Sivarajan et al[18] showed a statistical increase in tooth movement but not a clinically significant one. Meanwhile, Aboalnaga et al[19] reported no significant effect of MOP on canine retraction. Moreover, Fu et al[20] reported the lack of evidence in a systematic review that a single application of MOP may result in accelerated tooth movement.

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On the other hand, a vertical osteotomy of the alveolar extraction socket was reported to accelerate canine distalization into the first premolar extraction space. Liou and Huang[21] created vertical grooves inside the extraction socket. They discovered that the canines showed 6.5 mm of distal movement in 3 weeks with no significant complications. Yu et al.[22] evaluated the histologic effects and the rate of orthodontic tooth movement after vertical osteoperforation in mandibles of rabbits. They found that such a procedure produced greater tooth movement. Also, McBride et al.[23] undermined the interseptal bone by creating vertical grooves in dogs. They concluded that less dense and less mature bone was induced by surgical insults. However, no study has compared the histologic effect of the vertical osteotomy to that of the MOP or compared the resultant amount of tooth movement.

Therefore, the aim of this study was to compare the rate of tooth movement after multiple horizontal (MH) and single vertical (SV) osteoperforation, and to evaluate the histological changes in response to orthodontic force application following the surgical procedure in rabbits. The null hypothesis is that there are no significant differences between MH and SV MOP as far as their biologic effect and the resultant tooth movement are concerned.

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MATERIALS AND METHODS

The sample comprised mandibular halves of 24 male New Zealand white rabbits (mean age, 14 weeks) weighing 1.7 to 3.1 kg. For 1 week before the experiments, animals were acclimatized in individual cages under controlled temperature (22.0 ± 2.0°C), 12-hour light/dark periods, and had free access to water and a commercial diet. Institutional Animal Care and Use Committee of Uijeongbu St. Mary's Hospital, Catholic University of Korea (Approval Number UJA2018-04) has approved the animal protocol used in this study.

Experimental and orthodontic procedures

All rabbits underwent two experimental procedures: SV osteoperforation on the right mandible and MH osteoperforations on the left. Surgical procedures were performed by a single operator in a region 1 mm mesial to the mandibular first premolar (P1) under anesthesia administrated intramuscularly (35.0 mg/kg Ketamine and 5.0 mg/kg Rompun). This region was selected because it is an edentulous region that resembles the atrophied alveolar ridge in humans.

In MH osteoperforations sites, six horizontal osteoperforations (1.0 mm wide and 1.5 mm deep) were performed by a fissure bur 1.0 mm in diameter (MCTBIO, Yongin, Korea), at 600 rpm with saline coolant. Three osteoperforations were performed on the buccal side and 3 on the lingual. In the SV osteoperforation sites, only one vertical osteoperforation (1.4 mm in width and 4.6 mm in depth) was made at the crest of the ridge by a fissure bur 1.4 mm in diameter, 600 rpm with saline coolant (MCTBIO, Yongin, Korea). Depths of the osteoperforations were controlled via markers on the burs. The bone defect volume was intended to be similar in the horizontal and vertical groups. Nickel-titanium closed coil springs (Ultimate Wireforms Inc., Bristol, CT, USA) were connected between P1s and

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anterior teeth to deliver 100 cN of traction force in Figure 1.

Rabbits were divided randomly into 3 groups according to sacrifice time: one, two, and three weeks after the experimental procedure. At each timepoint, eight rabbits were sacrificed with an overdose of potassium chloride under a double dose of ketamin.

Micro-computed tomography (Micro-CT) images

Micro-CT images of the 24 mandibles were acquired after sacrifice for morphometric analysis at 130 kV and 60 µA using a lab scanner (SkyScan1173; Bruker-CT, Kontich, Belgium). Images were reconstructed by the scanner software. The assessment of bone volume and percentage of bone volume was performed by CT-Analyser 1.14.4.1 (Bruker-CT). The region of interest extended for 3 mm mesially starting from the mesial surface of P1 root. Vertically, it extended coronal to the plane passing through P1 root apex parallel to the occlusal plane.

Tooth movement:

On micro-CT images, tooth movement was measured by a single investigator from the center of the distal marginal ridge of P1 to the center of mesial marginal ridge of the second premolar (P2) (Figure 2). The distance between those 2 points before traction was assumed to be zero as in an intact contact point between the 2 premolars. Several weeks later, the sample was remeasured to assess the intraobserver reliability. Intraclass correlation coefficient (ICC) was calculated and the ICC values ranged between 0.997 and 1.00.

Tissue preparation:

Specimens extended from P2 till 8 mm mesial to P1 and were fixed for 24 hours in 4% paraformaldehyde at 4°C, then decalcified in 10% Ethylene Diamine Tetraacetic acid by BioWave Pro (CPELCO, CA, USA), embedded in paraffin, and then finally sectioned into 4-

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μm-thick mesiodistal slices and stained with hematoxylin and eosin (H&E). The regions of interest were mesial to P1 and between P1and P2.

TRAP-positive cell count

To confirm the presence of osteoclasts, Tartrate-resistant acidic phosphatase (TRAP) staining has been applied following a previously described protocol.[24] Digital slide images from 8 mm mesial of the P1 were obtained by PANNORAMIC 250 Flash III (3DHISTECH, Budapest, Hungary). TRAP-positive cells were counted by CaseViewer 2.0 (3DHISTECH, Budapest, Hungary).

Statistical analysis

Statistical analysis was conducted by IBM SPSS Statistics, version 20.0 (IBM Corp., Armonk, NY). Shapiro-Wilk test was applied to affirm normal distribution. Differences in tooth movement and bone variables between the two groups and between the three timepoints were evaluated using repeated-measures ANOVA. Significance value was 0.05.

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RESULTS

Amount of tooth movement:

The amount of tooth movement revealed a significant difference according to time points (p = 0.001). The MH group showed 0.57±0.20 mm at week 1, 0.89±0.46 mm at week 2 and 1.47±0.56 mm at week 3. The SV groups showed 0.57±0.29 mm at week 1, 0.98±0.46 mm at week 2 and 1.84±1.15 mm at week 3. No significant difference was reported between the MH and SV groups. There was no significant difference in tooth movement between weeks 1 and 2 (P = 0.322) but a significant difference between weeks 2 and 3 (P = 0.025), and between weeks 1 and 3 (P < 0.001) in Figure 3 and Table I.

Micro-CT bone evaluation

There was no significant effect on either group based on the bone variables or bone volume based on the time point effect. The bone fraction was significantly smaller at week 3 than at weeks 1 and 2 (P = 0.007 and 0.004, respectively) (Figure 4). Meanwhile, trabecular separation was significantly greater at week 3 than at weeks 1 and 2 (P <0.001). The trabecular number was greater at week 1 than that at week 3 (P = 0.027), while trabecular thickness was significantly different between weeks 2 and 3 (P = 0.014) in Table II.

TRAP-positive cell count

There was no significant difference in TRAP-positive cell count between the MH and SV groups. However, TRAP-positive cell count was significantly increased between week 1 and week 3 (P = 0.002) as in Figures 5 and 6, and Table III.

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Microscopic examination of undecalcified specimens

Microscopic examination of undecalcified specimens showed widened space of periodontal ligament (PDL) and an increased number of osteoclast-like cells, especially at week 3. Moreover, no significant root resorption was noticed in either group (Figure 7).

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DISCUSSION

Alveolar corticotomy-assisted rapid tooth movement is a relatively new category for adult orthodontic treatment. Recently, several studies have been conducted on MOP which does not require flap elevation.[21-23]

Kim et al.[25] reported that there was no significant differences in both the amount of tooth movement and the TRAP-positive osteoclasts between corticotomy and flapless MOP groups. Therefore, in our study, to increase the region affected by RAP, we adjusted the vertical and horizontal osteoperforations. A single large injury was created vertically and multiple small injuries were created horizontally, and the amount of bone removed in both methods was intended to be the same.

MOP was performed in a region 1 mm mesial to P1. Cramer et al.[26] reported that this intervention which was 3 mm away from the teeth to be moved was not effective in accelerating tooth movement. Also, it had no apparent differences in mineralization of bone, osteoblasts and osteoclasts. In addition, van Gemert et al.[27] demonstrated that the principal effects of MOP were limited to 1.5 mm extension.

In our rabbit study, tooth movement increased over the time, but without a significant difference between the MH and SV groups (1.5 mm and 1.8 mm, respectively at the 3rd week). Previous studies showed 2.3 mm of tooth movement at the 4th week after horizontal MOP,[25] and 1.6 mm at 3rd week after a vertical osteoperforation.[22] Chen et al.[28] showed a 0.9 mm tooth movement 2 weeks after corticotomy compared to just 0.8 mm for the control group, in rabbits. In our study, both groups showed 0.9 mm tooth movement in the second week.

Liou and Huang[21] made vertical grooves inside the extraction socket for rapid tooth movement. They showed that canines were distracted 6.5 mm without significant distal

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tipping and other complications during 3 weeks. On the contrary, another study claimed three MOPs was not effective for canine retraction.[29]

Regarding micro-CT analysis, there was no significant difference in bone volume and bone fraction between the MH and SV groups in our study, but bone fraction showed a significant decrease at week 3. McBride et al.[23] found that vertical grooves made in the extraction socket yielded a decreased density of bone material than in the control. But it showed no difference in bone volume.

In our study, trabecular number, thickness, and separation revealed no significant differences between the MH and SV groups. The trabecular number decreased and trabecular separation increased at week 3. But McBride et al.[23] showed increased numbers of osteoclasts and greater bone surface areas on the insult side in histologic evaluations. This might be due to a difference in the number and volume of MOPs and without the flap elevation in our study that was performed in the other study.

Regarding root resorption, Tsai et al.[9] demonstrated that MOP group resulted in a decreased root resorption compared to control. Murphy et al.[30] found no significant effect of corticision on root resorption. Patterson et al.[32] demonstrated that piezocision applied to initiate RAP may increase root resorption when used in conjunction with orthodontic forces. Our study showed no significant root resorption in either the horizontal or vertical MOP group. The disagreement among the studies might be due to the different protocols used to conduct the studies and the different methods of recording root resorption. Further investigations on the effect of surgically-induced RAP on root resorption might be warranted.

Regarding TRAP-positive cells, Kim et al.[25] reported no significant difference in TRAP-positive cell counts between the two corticotomy groups and a horizontal MOP group. Yu et al.[22] reported an increased number of TRAP-positive cells after vertical osteoperforation. Chen et al.[28] reported gradual rises in the osteoclast count at week 1 and

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week 2 after decortication compared to the control group. Likewise, Zou et al.[31] have found greater number of osteoclasts and stronger staining of the osteogenic marker in the corticotomy group on weeks 1, 2, and 4, compared to control. In our study, there was a significant increase in the TRAP-positive cell count between week 1 and week 3. However, there was no significant difference in the number of osteoclasts between the MH and SV groups. This similarity of histologic findings might explain the absence of differences in the amount of tooth movement between the 2 groups.

The lack of a control group for comparison purposes might be a drawback of this study, so future studies including a control group as a split mouth design are recommended. Also, it might have been better if the MH and SV procedures were randomized between the right and left sides. Further randomized controlled studies on different osteoperforation techniques such as MH and SV osteoperforation might be meaningful as well to evaluate the effects of these treatment modalities. Also, future studies might focus on the pain and discomfort of the patients due to vertical and horizontal osteoperforation.

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CONCLUSIONS

- Each of the MH and SV MOP techniques was able to generate a RAP, since the TRAP-positive osteoclast count was significantly greater at week 3 compared to week 1 in both MOP groups.

- The effectiveness of the RAP generated by both techniques was similar since the amount of tooth movement, the bone variables and the TRAP-positive cell counts showed no significant differences between the 2 groups over the 3 time points.

This suggests that a single, vertical MOP might be an effective tool for enhancing tooth movement, especially with molar distalization, uprighting, and protraction in an edentulous area.

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FIGURE LEGENDS

Figure 1. A, 3D Micro-CT reconstructed scan of left side horizontal micro-osteoperforation (MOP)s and right single vertical MOP; B, A force of 100 cN was applied by connecting the anterior teeth to the first premolars with NiTi closed coil springs; C, Design of six MH MOPs and one SV MOP group.

Figure 2. Tooth movement distance (A, Occlusal section; B, Sagittal section; White Arrow, the amount of tooth movement)

Figure 3. Amount of tooth movement distance in 1, 2, 3 weeks (mm).

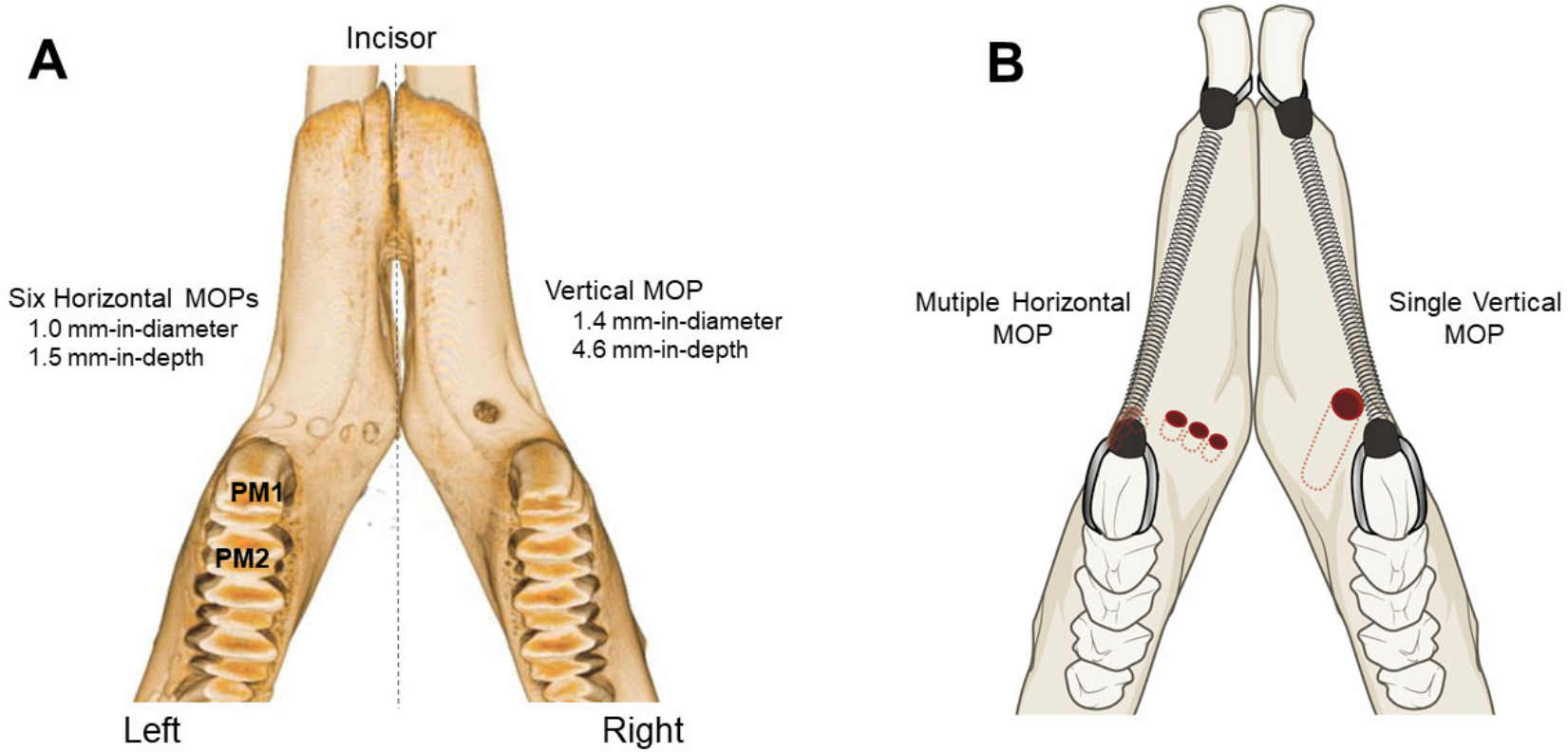
Figure 4. Micro-CT evaluation (A, Bone volume; B, Bone fraction)

Figure 5. TRAP-positive cell count: comparison between groups and timepoints.

Figure 6. Microphotograph of periodontal tissues stained with tartrate-resistant acidic phosphatase (TRAP) at ×200 magnification. A, B and C, Multiple horizontal osteoperforation at week 1, 2 and 3 respectively; D, E and F Single vertical osteoperforation at week 1, 2 and 3 respectively. TRAP-positive cells are detectable along the resorbed alveolar bone on the compression side.

Figure 7. Microphotograph of an H&E stained buccolingual section at the mesial periodontium of the first premolar at ×40 magnification. A, B and C Multiple horizontal (MH) osteoperforation at week 1, 2 and 3 respectively; D, E and F Single vertical (SV) osteoperforation at week 1, 2 and 3 respectively. Note the widened periodontal ligament (PDL) space in C and F. ALV, Alveolar bone.

Figure 1. Study design (A, Occlusal view; B, Schematic drawing of occlusal view; C, sagittal view)



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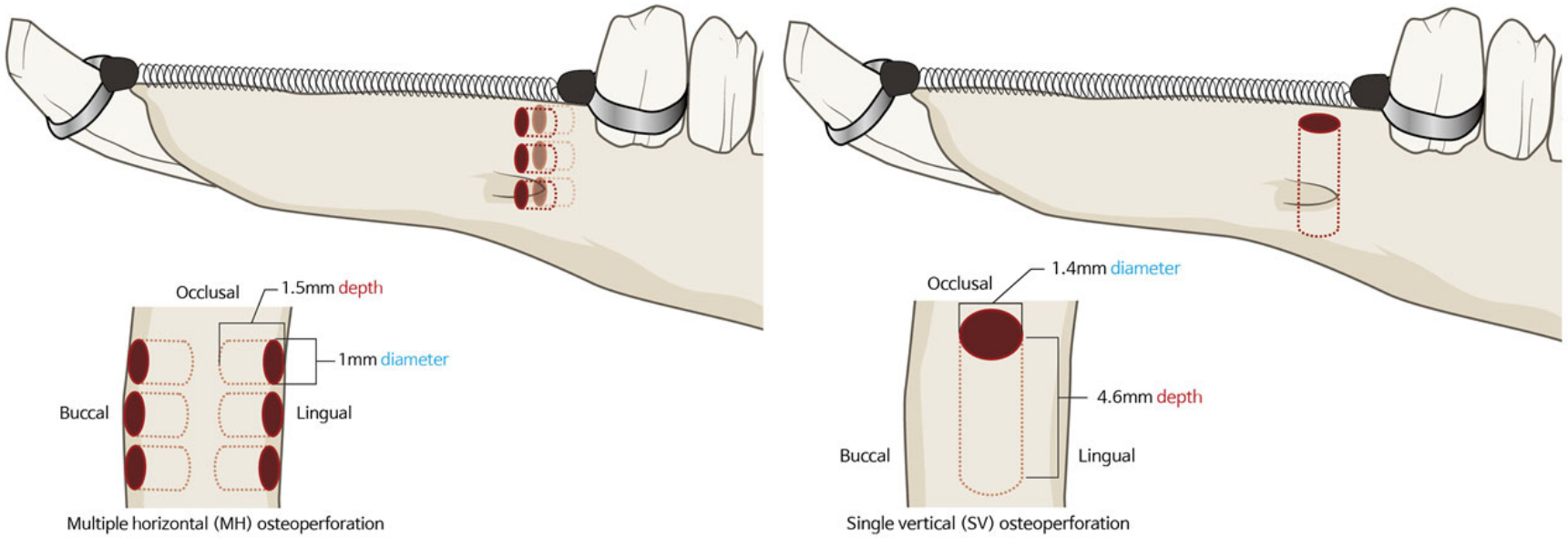
Occlusal
1.5mm depth
1mm diameter
Buccal
Lingual
Multiple horizontal (MH) osteoperforation
Occlusal
1.4mm diameter
4.6mm depth
Buccal
Lingual
Single vertical (SV) osteoperforation

Figure 2. The measurement of tooth movement
(A, Occlusal section; B, Sagittal section; White Arrow, the amount of tooth movement)

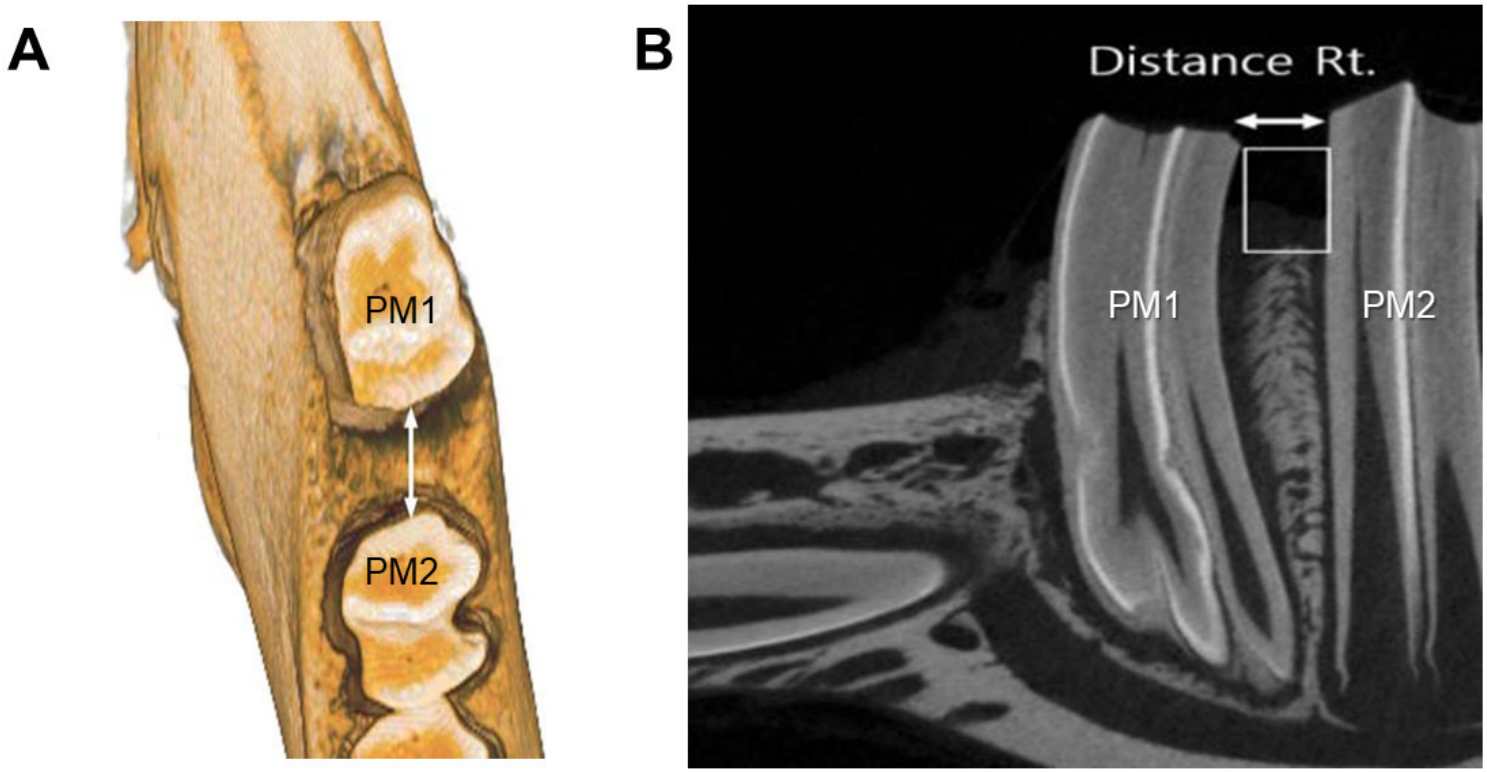

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Figure 3. Amount of tooth movement distance in 1, 2, 3 weeks (mm)

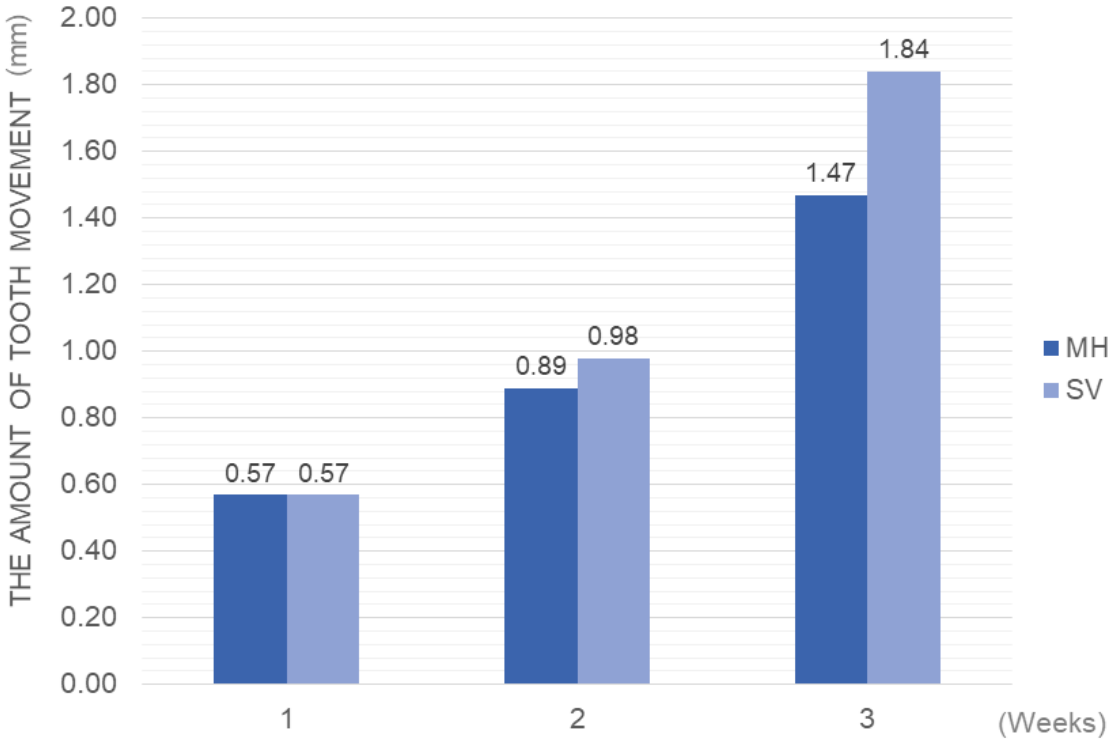

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Figure 4. Micro-CT evaluation (A, Bone volume; B, Bone fraction)

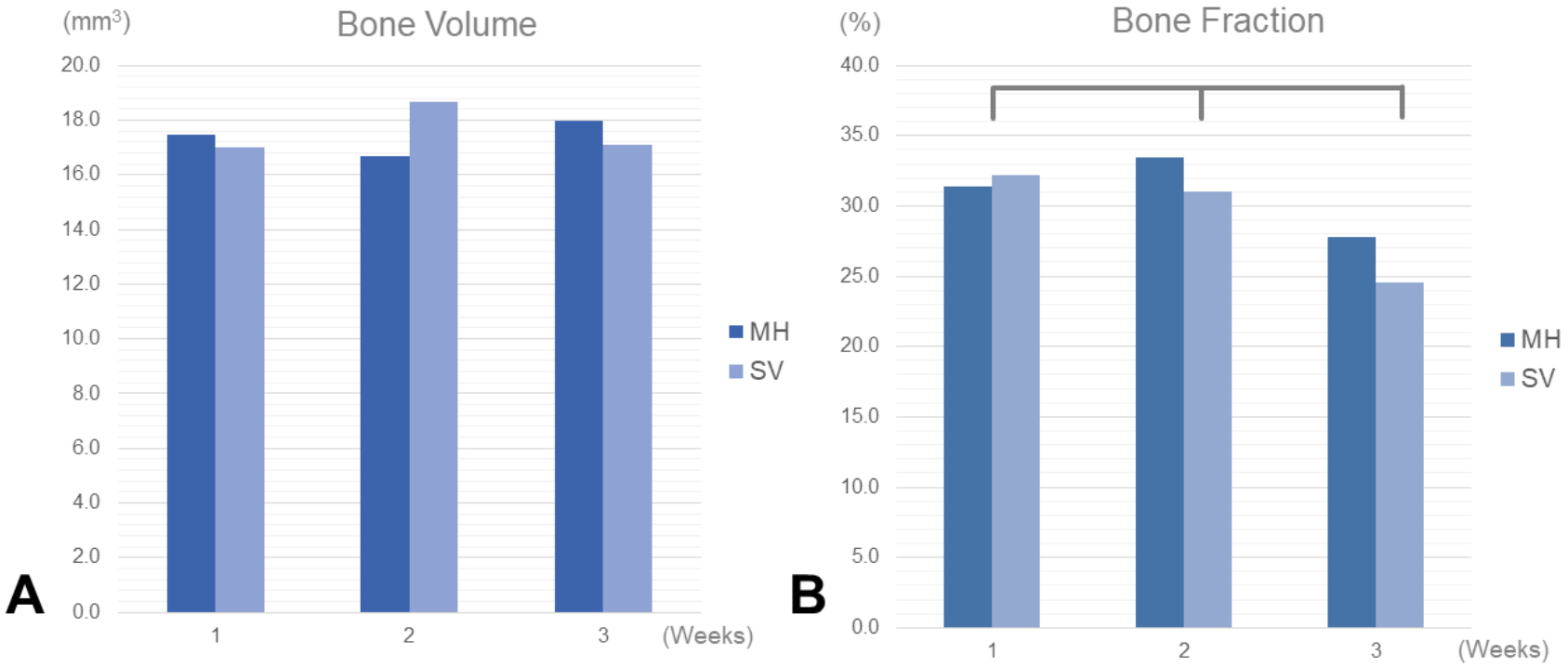

Figure 5. Comparison of tartrate-resistant acidic phosphatase (TRAP) - positive osteoclast counts.

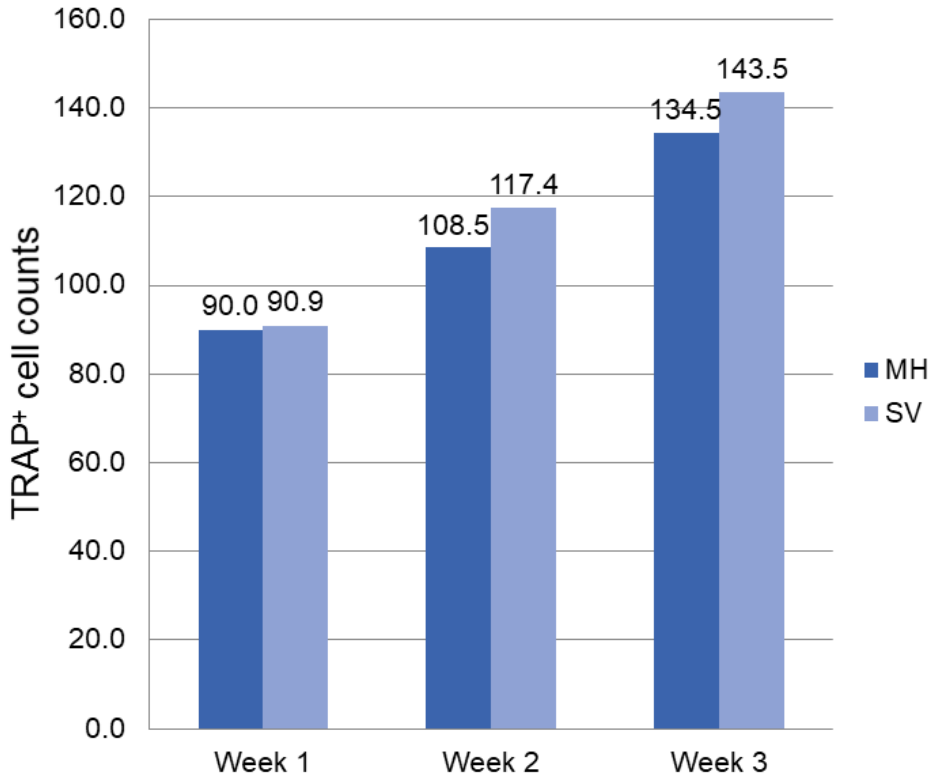

Figure 6. Microphotograph of periodontal tissues with tartrate-resistant acidic phosphatase (TRAP) staining (×200).

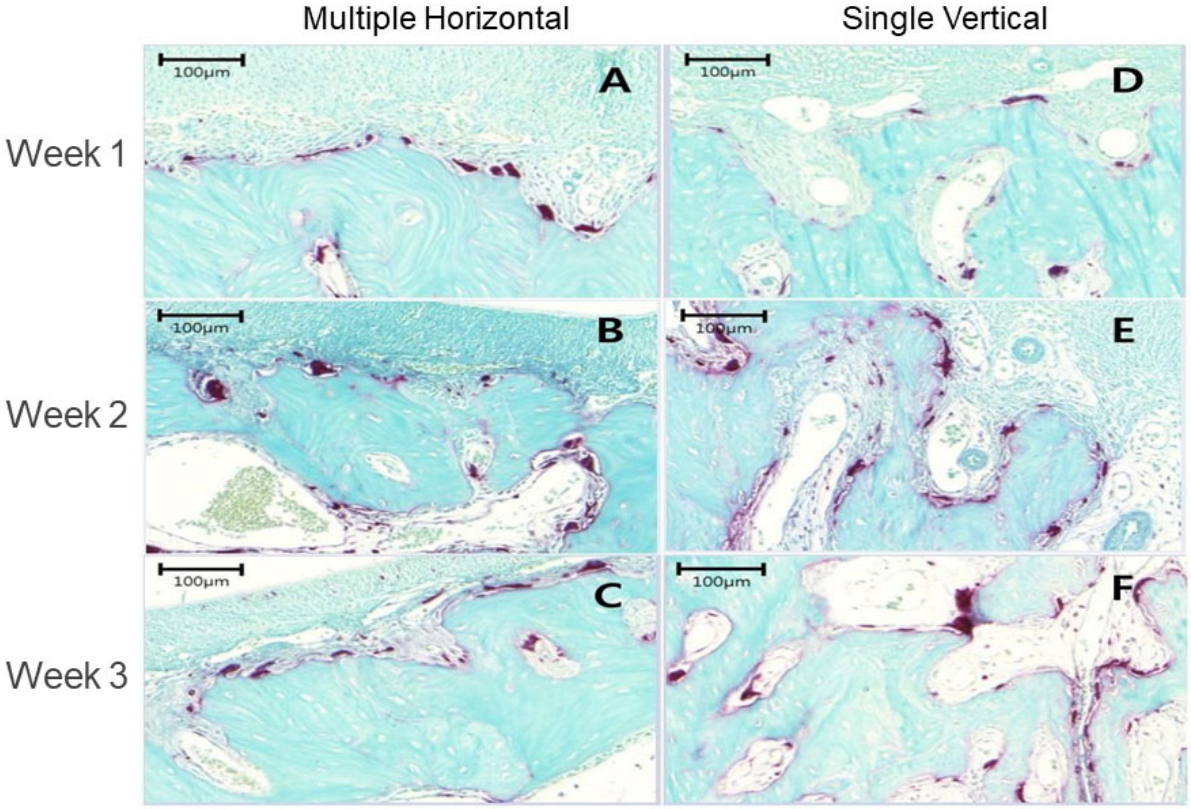

Figure 7. Microphotograph of buccolingual section of the mesial periodontium of the first premolar with H&E staining (×40) of decalcified specimens.

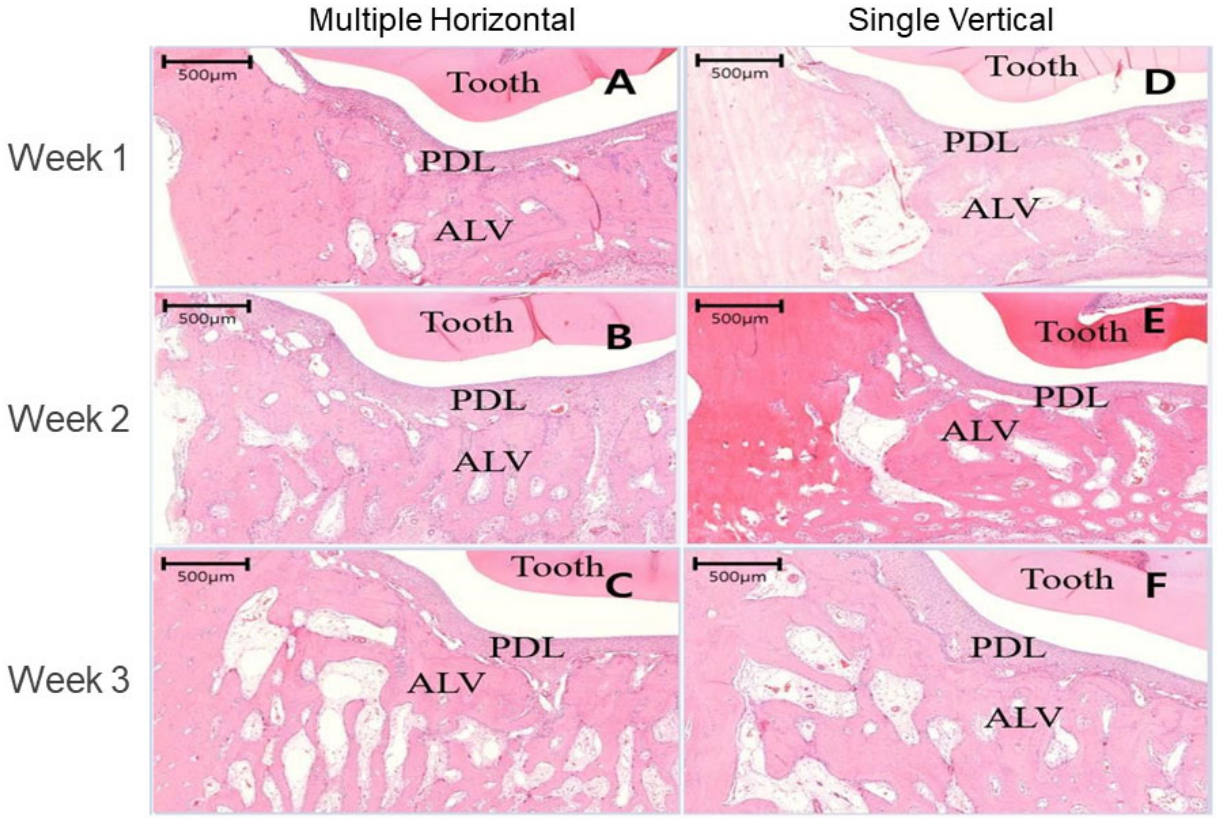

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Table I. Amount of tooth movement distance in 1, 2, 3 weeks (mm)

	Week 1	Week 2	Week 3	P-value*	P-value†		
	Mean±SD	Mean±SD	Mean±SD		1 vs 2	2 vs 3	1 vs 3
Multiple horizontal (N=8)	0.57±0.20	0.89±0.46	1.47±0.56	0.001	0.322	0.025	< 0.001
Single vertical (N=8)	0.57±0.29	0.98±0.46	1.84±1.15				
P-value‡		0.166					

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Table II. Micro-computed tomography bone evaluation

	Week						P value		
	1		2		3				
	MH Mean±SD	SV Mean±SD	MH Mean±SD	SV Mean±SD	MH Mean±SD	SV Mean±SD	Between groups	Between timepoints	Interaction
Bone volume (mm^3)	17.49±4.61	17.02±2.77	16.69±3.02	18.66±3.05	17.97±3.02	17.10±2.58	0.695	0.96	0.082
Bone fraction (%)	31.41±4.65	32.20±5.26	33.45±4.75	31.02±4.77	27.79±4.73	24.52±3.97	0.246	0.002	0.458
Trabecular number (n/mm)	1.31±0.17	1.26±0.19	1.21±0.14	1.13±0.20	1.19±0.10	1.06±0.18	0.114	0.026	0.787
Trabecular thickness (mm)	0.24±0.04	0.26±0.04	0.28±0.02	0.28±0.03	0.23±0.03	0.23±0.02	0.408	0.016	0.419
Trabecular separation (mm)	0.43±0.08	0.39±0.04	0.45±0.05	0.45±0.04	0.59±0.07	0.72±0.24	0.308	<0.001	0.081

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Table III. Evaluation of tartrate-resistant acidic phosphatase positive (TRAP) osteoclasts.

	Week			P value			
	1	2	3	1 vs 2	2 vs 3	1 vs 3	Between time points
Multiple horizontal (N=8)	90.0 ± 18.3	108.5 ± 29.6	134.5 ± 27.8	0.340	0.132	0.006	0.01
Single vertical (N=8)	90.9 ± 27.6	117.4 ± 36.9	143.5 ± 9.2	0.149	0.156	0.002	<0.001
P value between 2 groups	0.900	0.282	0.408				

(paired t-test: comparison between groups at each time point independently. ANOVA: comparison between time points. Multiple Comparisons: Tukey test)