Testing a new micro-osteoperforation modality on the rate of orthodontic tooth movement in a rabbit model

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ABSTRACT

Objectives. The current study objective was to test and compare the impact of a new micro-osteoperforation (MOP) modality with 2 other modalities on the rate of tooth movement (RTM) in rabbits.

Methods. The sample comprises 45 adult male albino rabbits aged (24 weeks) and divided equally into 3 groups regarding MOP modalities; a single vertical MOP (1V-MOP), a single vertical MOP + 2 horizontal MOPs (1V+2H-MOP), as the new MOP design; and 3 horizontal MOPs (3H-MOP). The first right mandibular premolar (RMP1) received MOP(s) + orthodontic force (50g) by traction spring. The contralateral tooth on left (LMP1), as positive (+ve) control,

received only (50g) orthodontic force by another spring. Both springs were fixed by a mini-screw between the 2 lower incisors. Evaluations of the clinical RTM and histopathology with H and E stain of the compressed PDL at the cervical, middle, and apical thirds of MP1 root were performed for comparing after (1, 2, and 3 weeks) of OTM respectively.

Results. The RTM ($P<0.001$) and number of osteoclasts, osteoblasts, blood vessels and PDL width ($P<0.001$ to $P=0.039$) significantly increased in the MOP groups; the 1V+2H-MOP was in between the higher 1V-MOP and the lower 3H-MOP.

Conclusion: All MOP modalities could efficiently accelerate RTM and increase the activity of the compressed PDL, but with a variant rate. The new 1V+2H-MOP modality can be chosen as the best modality for faster RTM, because it showed moderate RTM after the 1V-MOP.

Keywords: Bone remodeling, micro-osteo-perforation, regional acceleratory phenomenon, tooth movement rate

INTRODUCTION

Overall extended orthodontic intervention may result in many unwanted side effects [1,2] like; root resorption (RR) [3], high risk of caries, decreased patient compliance [4], and discomfort [5]. Thus, multiple attempts were conducted for the aim of facilitating OTM (orthodontic tooth movement) and hence, eliminating most of the OTM complications [6,7]. Most general methods used for facilitating OTM involve surgical interventions such as the most efficient, but also the most

invasive technique, corticotomy [8], followed by the less invasive methods, the corticision [9] and piezocision [10]. Moreover, another surgical technique categorized by its minimally induced trauma, known as MOPs or micro-osteo-perforations has emerged as a way for causing also faster OTM via stimulating the remodeling of alveolar bone [11-13]. The MOPs are based on the regional acceleratory phenomenon (RAP) that can be triggered by selective decortications of the alveolar bone [14]. Thus, the most suitable alternative to all other surgical procedures is MOPs owing to their least invasive nature [15].

Alikahni *et al*. [16] show that MOPs could result in about (2.3 times) faster OTM than the traditional orthodontic techniques. Other studies indicate that MOPs could result in a significantly shorter OT duration [7,11,15,17], a clinically non-significant increase in TM [18]. In contrast, a number of studies found no significant effect of MOPs on TM [19,20], unless the procedure is repeated [21].

However, no studies have evaluated and compared a mixture of the features of MOPs categorized by performing vertical and horizontal holes at the same site adjacent to the moved root. This study aims to test and compare a new MOP modality with another 2 previously tested MOP modalities on clinically measured RTM and also to histo-pathologically compare the influence of the 3 MOP modalities with regard to the number of osteoclasts, osteoblasts, and blood vessels, and also PDL width and organization of the compression mesial side of mandibular 1st premolar (MP1) 3 root regions; cervical, middle and apical thirds-exposed MOPs through rabbit experimental OTM after (1, 2, and 2 weeks) of TM.

MATERIALS AND METHODS

The Study Design and Protocol

This study was approved by the Research Ethics Committee of the College of Dentistry, University of Mosul (UoM. Dent. 23/2). It is an experimental split mouth study design that employed a rabbit model. The right mandibular first premolar (RMP1) was designed as the experimental part, and the left side (LMP1) was designed as the control part.

The Sample

The mandibular halves were obtained from 45 healthy adult male albino rabbits with mean age (24 weeks old) weighing (1.5 to 2.2 kg). For 1 week before the experiment, the animals were housed and acclimatized in individual metallic cages under a controlled temperature (22.0 ± 2.0°C) simulating the natural environment as much as possible. They had also free access to a commercial diet and water [17].

Animal Grouping

The rabbits were randomly divided into 3 main groups of 15 rabbits each on the basis of the MOP modality which in turn further subdivided according to the euthanize time into 1, 2, and 3 weeks after the experimental procedure.

Experimental and Orthodontic Procedures

Regarding the MOPs, 3 modalities were undertaken at the buccal cortical and at the alveolar crest of bone of the experimental side mesial to RMP1. The mesial of the RMP1 was chosen because this region is an edentulous area resembling the atrophied alveolar ridge in humans as described by Kim *et al*. [17,22]. The 1st MOP modality, the 1V-MOP, was performed with 1 vertical MOP (1.4 mm

diameter, 4.5 mm depth) at alveolar crest, 1mm mesial to RMP1. The 2nd MOP modality, the 1V+2H-MOP, was a new proposed MOP intervention consisting of 2 horizontal MOP holes: one buccally and the other lingually (1.4mm diameter, 1.5mm depth) 5mm away from alveolar crest, 1mm mesial to RMP1 plus 1 vertical MOP (1.4mm diameter, 1.5mm depth) at alveolar crest. The 3rd MOP modality, the 3H-MOP, was performed with 3 horizontal MOPs (1.4mm diameter, 1.5mm depth, 2mm apart) 1mm away from alveolar crest, 1mm mesial to RMP1. The 3 MOP modalities were shown in (Figure 1).

By a single operator, the MOP procedures were accomplished under general anesthesia (GA) administrated I.M. (35mg/kg of ketamine & 5mg/kg of xylazine) [17]. All MOPs were performed with a self-drilling mini-screw (diameter 1.4mm and length 6mm, OSTEONIC, South Korea) and their depths were determined via the use of a rubber stopper added to the mini-screw [23], as shown in (Figure 2A); the bone defect volume was intended to be similar in all experimental groups for a standardized work [19]. Before drilling with the mini-screw, the soft tissue thickness should be assessed [1]. The same mini-screw (1.4mm × 6mm) (OSTEONIC- South Korea) that was used for the creation of the MOPs, was then inserted into bones between the two mandibular central incisors by the use of a manual screw driver (GSSEM / 11-244- South Korea), (Figure 2B).

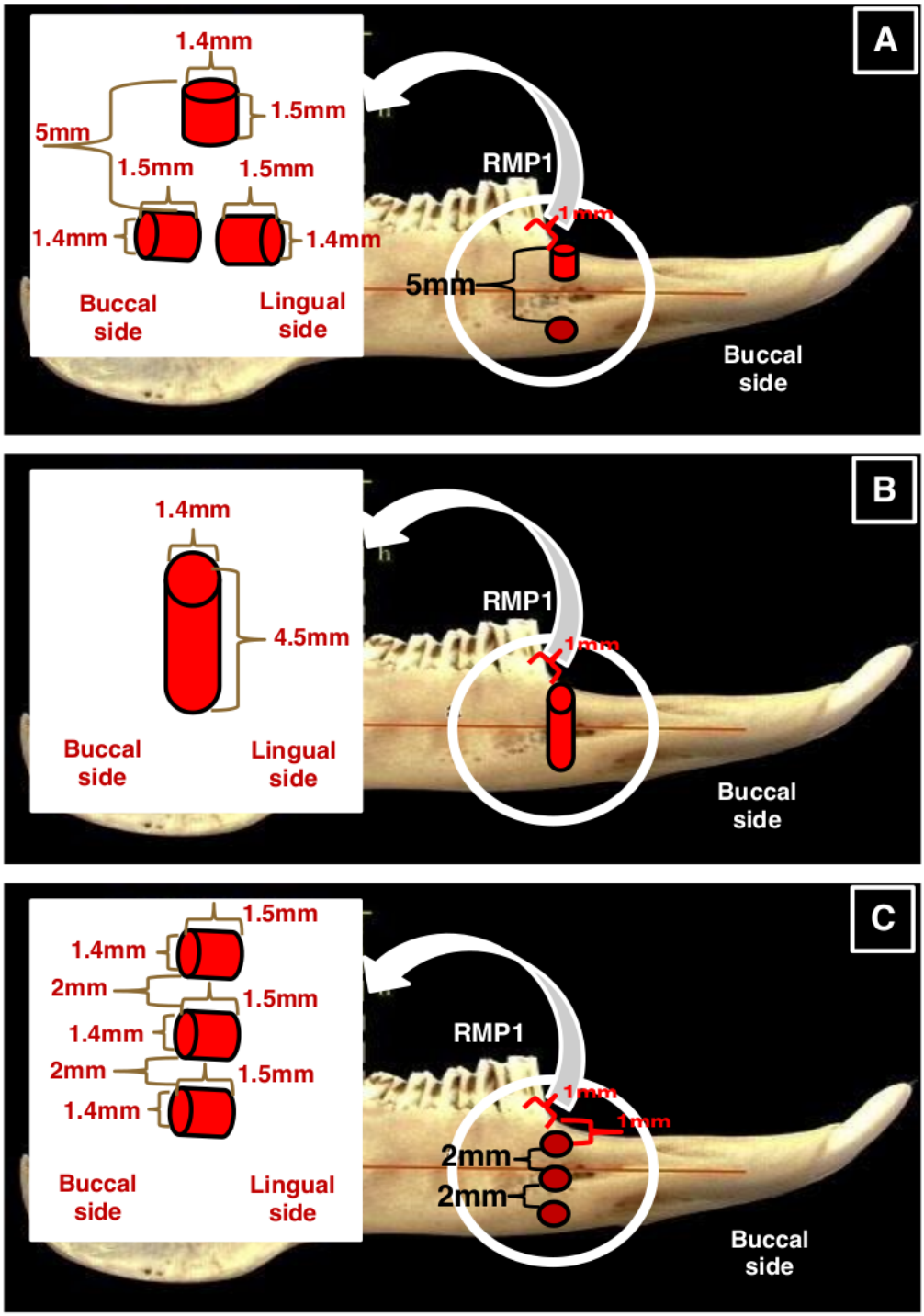

FIGURE 1. Rabbit mandibular right side showing: (A) The new modality of vertical and horizontal MOPs (1V+2H-MOP) (B) Vertical modality of MOP (1V-MOP) (C) Horizontal modality of MOP (3H-MOP)

The traction force was assumed via a nickel-titanium (NiTi) closed-coil springs (0.9mm diameter, 0.22mm lumen size, 1m total length, Zugfeder / 758-205- Dentaurum, Germany) connected between the mini-screw and the MP1 at both sides of mandible to deliver (50g) of force [15], measured by a tension gauge (dynamometer) (Hahnkolf / Stuttgart-Germany) for standardization (Figure 2C & D). At the end of each time period of (1, 2, and 3 weeks), the rabbits were euthanized with an overdose of (potassium- chloride) and a double dose of (ketamine) [17].

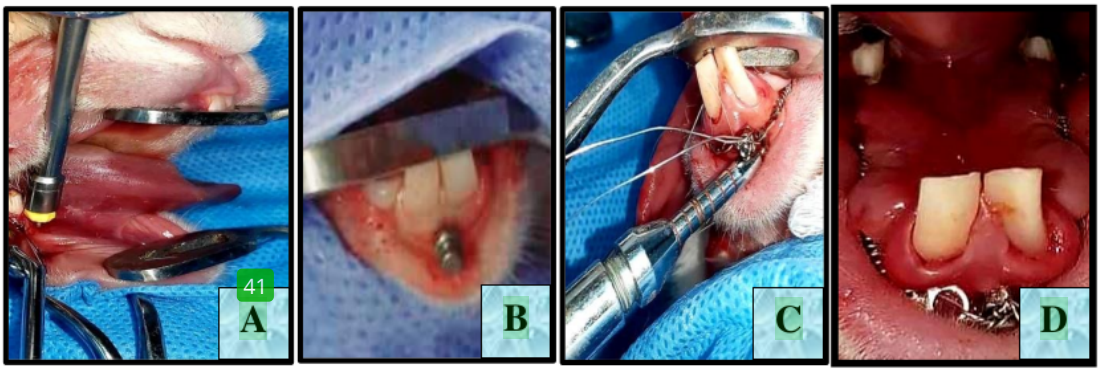

FIGURE 2. (A) Creating vertical MOP with a stopper added mini-screw (B) The self-drilling mini-screw positioned between the mandibular central incisors (C) to measuring the amount of the traction force (50g) by the tension gauge at the left side of the mandible (D) Close front-superior view of the bilateral orthodontic appliance placement.

Clinical Measurement

Clinical measurements were used to assess the rate of tooth movement (RTM) (distance) which was done before the experiment (T1) = 0, then every 2 and 3 days and at the end of every time period according to the sub-groups (1, 2 and 3 weeks) after insertion of orthodontic appliances and spring traction (T2) by using a digital vernier caliper (with 0.01mm accuracy) (Mefine-China) from the center of the distal surface of the MP1 to the center of the mesial surface of the MP2 at both sides of the mandible. All measurements were repeated 3 times by

the same investigator and the average was taken to ensure consistency [22]. Tooth movement distance or RTM, (in mm), was calculated by subtracting the measured distance at T1 (before intervention) from the measured distance at T2 (after intervention) [15].

Histological Staining

Sections of the mesial side of the cervical, middle, and apical thirds of the MP1 root in each specimen (MP2-10mm mesial of MP1) were obtained. The histological staining was accomplished in accordance with many studies [15,24, 25].

The specimens were sectioned into 5-µm-thick sections (mesio-distal slices) so that they were perpendicular to the MP1's occlusal plane. The sections of the mesial side of the cervical, middle, and apical thirds of the MP1 root were then stained with H & E (hematoxylin and eosin) for evaluating the whole tissue morphology. These sections were examined under a light microscope (LM) (OPTIKA, Italy), and the captured images with the digital camera (NIS-Elements AR 3.1, OPTIKA, Italy) were under 2 magnifications (100x and 40x).

Statistical Analysis

All statistical analyses were performed with the Statistical Package for the Social Sciences software (SPSS 22.0, Chicago, IL). As the data of the present study were non-parametric, thus, the median and interquartile range (IQR) were used for analyzing the data statistically, and then for comparing among groups, the Kruskal-Wallis H test was used, while for comparing among periods, the Friedman test was used. The statistical significance level was set at $P \leq 0.05$.

Both intra-class and inter-class calibrations were assumed in the current study. Intra-class calibration was examined by individual researchers who

evaluated 10 samples of rabbits, 2-times and with a minimum interval of 2 weeks. Inter-class calibration examined the same rabbit samples evaluated by researchers (A.M., M.G.). Random errors were measured by the Dahlberg equation, while systemic errors were estimated by the paired t test. For the intra-class calibration, random errors were (0.061) and systemic errors were (0.81), while for the inter-class calibration, random errors were (0.090) and systemic errors were (0.83); both calibrations showed statistically non-significant results.

RESULTS

Throughout the whole experiment, all NiTi closed coil springs stayed intact. The compression mesial moving side of the MP1 at both sides was evaluated in all groups; and all the MP1s were properly moved in the correct direction.

Rate of Tooth Movement

The results of the Shapiro-Wilks statistical test confirmed the distributing normality for the RTM. ANOVA and Duncan tests showed that the RTM on the surgical MOP groups was greater than the +ve control groups and there was a significant difference in the RTM among all groups in every time period ($P<0.001$), and also among different week (wk) periods for every experimental group ($P<0.001$). The RTM increased significantly over time in all groups.

For 1V-MOP groups, RTM was 0.78 ± 0.005, 1.24 ± 0.01, 2.24 ± 0.02. For 1V+2H-MOP groups, RTM was 0.70 ± 0.005, 1.12 ± 0.02, 2 ± 0.02. For 3H-MOP groups, RTM was 0.61 ± 0.003, 1.02 ± 0.03, 1.76 ± 0.03. And for +ve control groups, RTM was 0.40 ± 0.02, 0.75 ± 0.009, 1.24 ± 0.01, after 1, 2, and 3 weeks respectively. Thus, the 1V-MOP group showed the greater RTM, and the 3H-MOP showed as the smallest RTM among the MOP groups. Moreover, the greatest RTM was the 3wk / 1V-MOP (2.24 ± 0.02) and the smallest RTM was the 1wk / +ve control (0.40 ± 0.02), all measured in (mm), (Table 1) & (Figure 3).

The measurements of the RTM that were taken every 2 and 3 days were shown in (Figure 4) for all experimental groups.

TABLE 1. Clinical measurements of the rate of tooth movement (RTM) in (mm) of all experimental groups after 1, 2 and 3 weeks of OTM or OTM+MOP

Groups	1V-MOP	1V+2H-MOP	3H-MOP	+ve Control	*P*-Value
1 week	0.78 ± 0.005 A c	0.70 ± 0.005 B c	0.61 ± 0.003 C c	0.40 ± 0.02 D c	<0.001
2 weeks	1.24 ± 0.01 A b	1.12 ± 0.02 B b	1.02 ± 0.03 C b	0.75 ± 0.009 D b	<0.001
3 weeks	2.24 ± 0.02 A a	2 ± 0.02 B a	1.76 ± 0.03 C a	1.24 ± 0.01 D a	<0.001
***P*-Value**	<0.001	<0.001	<0.001	<0.001	

Data expressed as median and interquartile range (IQR) (Number (N) = 5 animals).

Different capital letters among material groups in rows mean there is significant difference at $p \leq 0.05$.

Different small letters among periods in columns groups mean there is significant difference at $p \leq 0.05$.

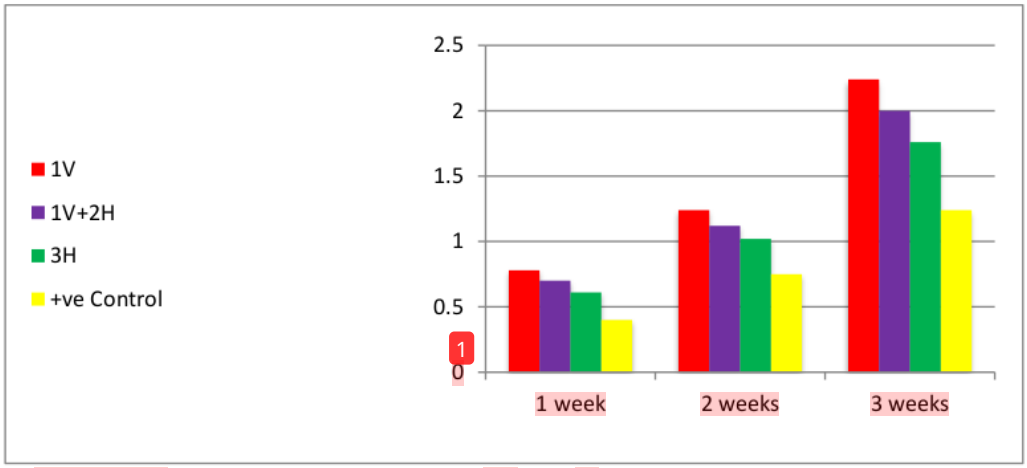

FIGURE 3. Clinical measurements of the rate of tooth movement (RTM) in (mm) of all experimental groups (1V-MOP, 1V+2H-MOP, 3H-MOP, and +ve control) after 1, 2 and 3 weeks of OTM or OTM+MOP.

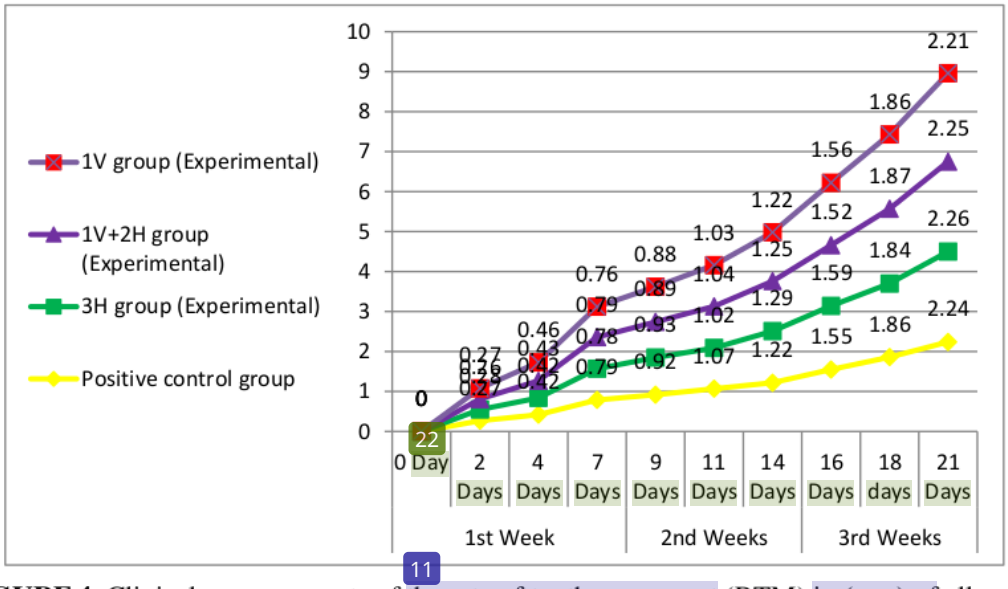

FIGURE 4. Clinical measurements of the rate of tooth movement (RTM) in (mm) of all experimental groups (1V-MOP, 1V+2H-MOP, 3H-MOP, and +ve control) after follow-up periods of days during OTM or OTM+MOP

Histological Analysis

The histological results of the current study demonstrated that all experimental MOP groups revealed within the mesial side (compression) of the MP1 root were significantly wide PDL and high number of osteoclasts (active cells, including also osteoclast-like cells or called cementoclasts), osteoblasts, and blood vessels, as compared to the +ve control group. At the compressed region of MP1 root in all groups, there was an alveolar osteopenia in addition to the multi-nucleated cells seen on the margin of the alveolar bone near the pressed PDL. Also, a significant difference was found among all groups ranged between ($P<0.001$) and ($P=0.039$). The width and number also increased significantly over time in all groups. The 1V-MOP group always showed the widest/more irregular

PDL and greatest number, and the 3H-MOP showed the narrowest / less irregular PDL and smallest number among the MOP groups at every time period.

Moreover, a significant difference of PDL width (P<0.001) and (P=0.002) and of osteoclasts (P<0.001) and (P=0.038), osteoblasts (P<0.001) and (P=0.039) & blood vessels number (P<0.001) and (P=0.029) were also present between the middle and apical 1/3, and cervical and apical 1/3, while the results were non-significant between the middle and cervical 1/3. The middle 1/3 had the greatest width and number, and the apical 1/3 had the smallest width and number in all groups, (Tables 2-5) and (Figures 5-10).

TABLE 2. The mean numbers of *Osteoclasts* (100X field) in the apical, middle, and cervical 1/3 regions after 1, 2 and 3 weeks of OTM or OTM+MOP

Periods	Groups / Regions	1V-MOP	1V+2H-MOP	3H-MOP	+ve Control	*P*-Value
1 week	Apical	7.88± 0.55 A f	5.1± 0.98 B f	4.38± 0.41 C f	3.38± 0.2 D f	<0.001
	Middle	11±0.93 A e	9.3±0.68 B e	6.98±0.41 C e	6.38±0.41 D e	0.002
	cervical	10.61±0.2 A e	9 ±0.2 B e	6.61± 0.2 C e	6±0.25 D e	0.039
2 weeks	Apical	15.61±0.2 A d	12.6±0.25 B d	12±0.8 C d	8.38± 0.2 D d	<0.001

	Middle	21.9± 0.25 A c	17± 0.2 B c	13.38± 0.41 C c	10.38± 0.2 D c	<0.001
	cervical	20.61±0.2 A c	16.38± 0.2 B c	13.18± 0.2 C c	10±0.25 D c	0.004
3 weeks	**Apical**	27.38±0.8 A b	22.61±0.55 B b	19.61±0.55 C b	16.38±0.2 D b	<0.001
	Middle	38.38±0.41 A a	33.61±0.41 B a	28.38±0.41 C a	23.38±0.41 D a	<0.001
	cervical	36.61± 0.2 A a	32.61±0.2 B a	27±0.25 C a	22±0.25 D a	<0.001
***P*-Value**		<0.001	<0.001	<0.001	0.038	

Data expressed as median and interquartile range (IQR) (Number (N) = 5 animals).

Different capital letters mean there is significant difference among groups at $p \leq 0.05$.

Different small letters mean there is a significant difference among periods at $p \leq 0.05$.

TABLE 3. The mean numbers of *Osteoblasts* (100X field) in the apical, middle, and cervical 1/3 regions after 1, 2 and 3 weeks of OTM or OTM+MOP

Periods	**Groups / Regions**	**1V-MOP**	**1V+2H-MOP**	**3H-MOP**	**+ve Control**	***P*-Value**
1 week	**Apical**	17.38± 0.55 A f	14.6± 0.98 B f	12.38± 0.41 C f	9±0.25 D f	<0.001
	Middle	22±0.93 A e	19±0.68 B e	18.38±0.41 C e	12.61±0.2 D e	0.002
	cervical	21.61±0.2 A e	18.38±0.2 B e	17.61± 0.2 C e	11±0.25 D e	0.039
2	**Apical**	28.61±0.2	24±0.25	22.6±0.8	15.38± 0.32	<0.001

weeks		A d	B d	C d	D d	
	Middle	35± 0.25 A c	33± 0.2 B c	28.38± 0.41 C c	18.38± 0.32 D c	<0.001
	cervical	33.61±0.2 A c	32.38± 0.2 B c	27.38± 0.2 C c	18±0.25 D c	0.004
3 weeks	**Apical**	43.38±0.8 A b	39.61±0.55 B b	36.61±0.55 C b	22±0.31 D b	<0.001
	Middle	57.38±0.41 A a	47.61±0.41 B a	41.38±0.41 C a	28.2±0.2 D a	<0.001
	cervical	54.61± 0.2 A a	46.61±0.2 B a	40±0.25 C a	27±0.31 D a	<0.001
***P*-Value**		<0.001	<0.001	<0.001	0.039	

Data expressed as median and interquartile range (IQR) (Number (N) = 5 animals).

Different capital letters mean there is significant difference among groups at $p \leq 0.05$.

Different small letters mean there is a significant difference among periods at $p \leq 0.05$.

TABLE 4. The mean numbers of *Blood Vessels* (100X field) in the apical, middle, and cervical 1/3 regions after 1, 2 and 3 weeks OTM or OTM+MOP

Periods	**Groups / Regions**	**1V-MOP**	**1V+2H-MOP**	**3H-MOP**	**+ve Control**	***P*-Value**
1 week	**Apical**	15± 0.51 A f	11± 0.93 B f	8.27± 0.4 C f	6.61±0.41 D d	<0.001
	Middle	18±0.25 A e	15.61±0.8 B e	13.61±0.61 C e	11.61±0.66 D c	<0.001
	cervical	17±0.25 A e	14.38±0.8 B e	12.38± 0.32 C e	10.38±0.41 D c	<0.001
2 weeks	**Apical**	21.38±0.32 A d	17.38±0.41 B d	18.38±0.66 C d	16.61± 0.41 D c	0.029

	Middle	26.61 ± 0.8 A c	23.61 ± 0.41 B c	21.61± 0.41 C c	19.61± 0.66 D b	0.027
	cervical	25.61±0.2 A c	22.61± 0.2 B c	21± 0.44 C c	17.38±0.41 D b	<0.001
3 weeks	**Apical**	31.61±0.8 A b	28.61±0.8 B b	25.61±0.55 C b	19.61±0.41 D b	0.022
	Middle	38±0.25 A a	33±0.25 B a	28±0.25 C a	23.61±0.66 D a	0.028
	cervical	37± 0.25 A a	32±0.25 B a	27±0.25 C a	22.38±0.41 D a	0.030
***P*-Value**		<0.001	<0.001	<0.001	0.029	

Data expressed as median and interquartile range (IQR) (Number (N) = 5 animals).

Different capital letters mean there is significant difference among groups at $p \leq 0.05$.

Different small letters mean there is a significant difference among periods at $p \leq 0.05$.

TABLE 5. The *thickness of PDL* in (micrometer μm) in the apical, middle, and cervical 1/3 regions after 1, 2, and 3 weeks of OTM or OTM+MOP

Periods	**Groups / Regions**	**1V-MOP**	**1V+2H-MOP**	**3H-MOP**	**+ve Control**	***P*-Value**
1 week	**Apical**	220.6± 11.8 A e	205 ± 15.9 B f	193.2± 16.5 C f	152±14.6 D d	0.033
	Middle	270 ±5.1 A d	220.4 ±5.2 B e	208.4±6.4 C e	184.6±16.4 D c	<0.001
	cervical	255.6±2.6 A d	215.8±16.5 B e	201.2±1.6 C e	181.± 14.8 D c	<0.001
2 weeks	**Apical**	309.6 ±4.5 A d	251 ±7.4 B d	224.8±10.3 C d	173.2±14.6 D c	<0.001
	Middle	330±14.2	292± 15.1	258.4± 4.10	224.6±16.4	<0.001

		A c	B c	C c	D b	
	cervical	318.7±2.7 A c	281.4±6.9 B c	249.4±9.7 C c	221.± 14.8 D b	<0.001
3 weeks	**Apical**	364.2±7.2 A b	323.2±8.3 B b	288.4±15.3 C b	231±14.6 D b	<0.001
	Middle	398.4±9.6 A a	357±9.2 B a	322.8±8.3 C a	264.6±16.4 D a	<0.001
	cervical	385±34.6 A a	343.8±31 B a	310.8±24.8 C a	259.± 14.8 D a	<0.001
***P*-Value**		<0.001	<0.001	<0.001	0.002	

Data expressed as median and interquartile range (IQR) (Number (N) = 5 animals).

Different capital letters mean there is significant difference among groups at $p \leq 0.05$.

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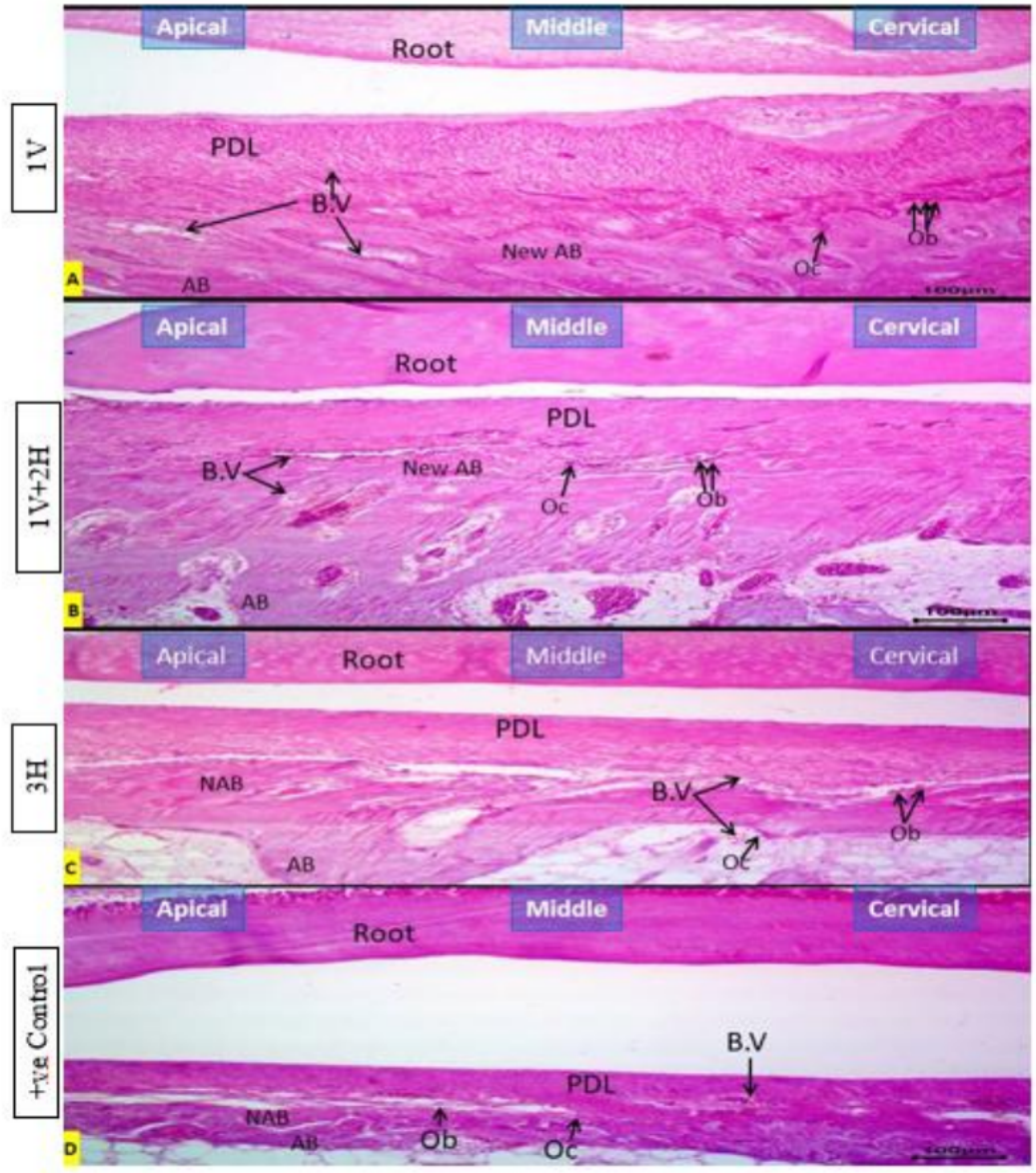

FIGURE 5. Histological section of rabbit MP1 tooth root after removal of orthodontic appliance at **1 week** from the [A]: 1V group, [B]: 1V+2H group, [C]: 3H group and [D]: +ve control group; showing high surface area of the new alveolar bone formation NAB with highly numbers of osteoblasts Ob, osteoclast OC, and blood vessels in the 1V group then 1V+2H group then 3H group and then the +ve control group. Alveolar bone AB. H&E stain, (A, B, C, D: 40X), Scale bar=100μm.

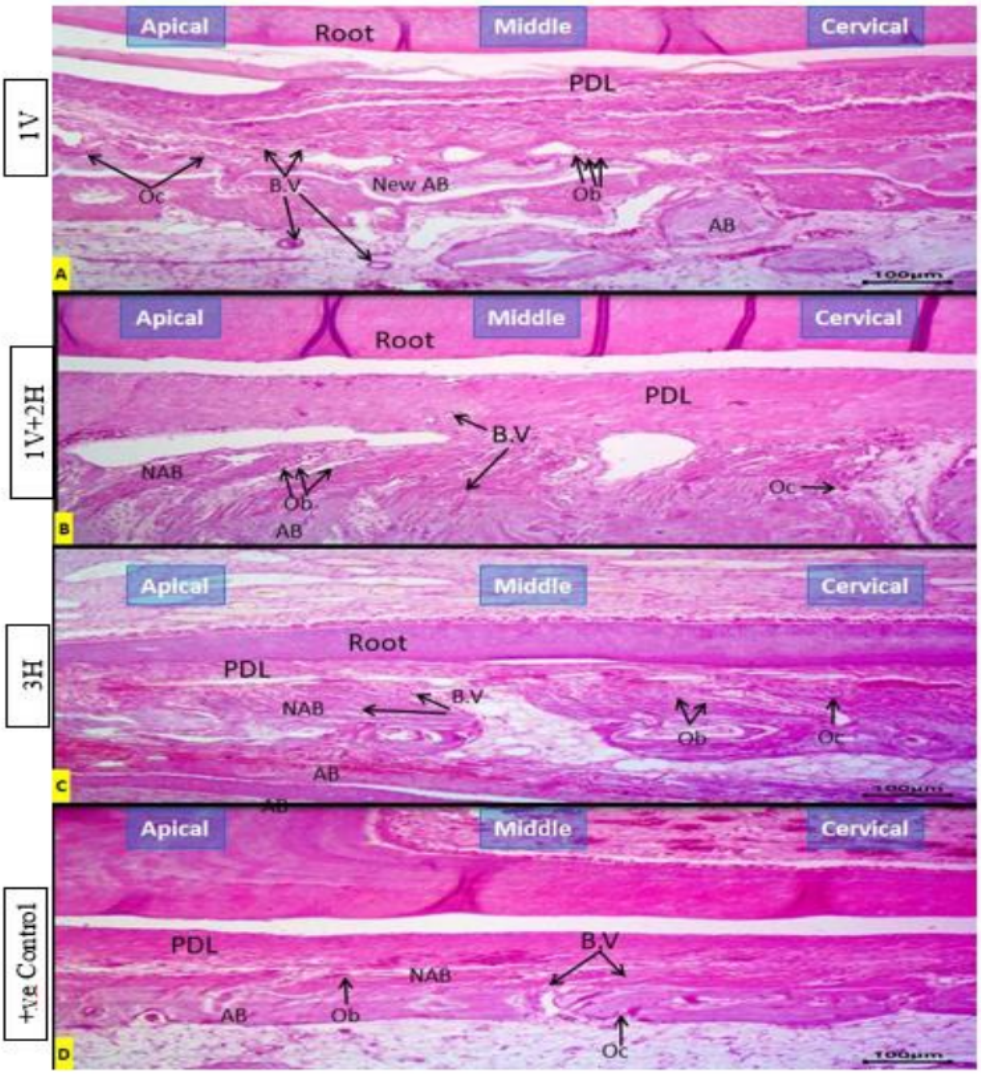

FIGURE 6. Histological section of rabbit MP1 tooth root after removal of orthodontic appliance at **2 weeks** from the [A]: 1V group, [B]: 1V+2H group, [C]: 3H group and [D] +ve control group showing; high surface area of the new alveolar bone formation NAB with highly numbers of osteoblasts Ob, osteoclast OC, and blood vessels in the 1V group then 1V+2H group then 3H group and then the +ve control group. Alveolar bone AB. H&E stain, (A, B, C, D: 40X), Scale bar=100µm.

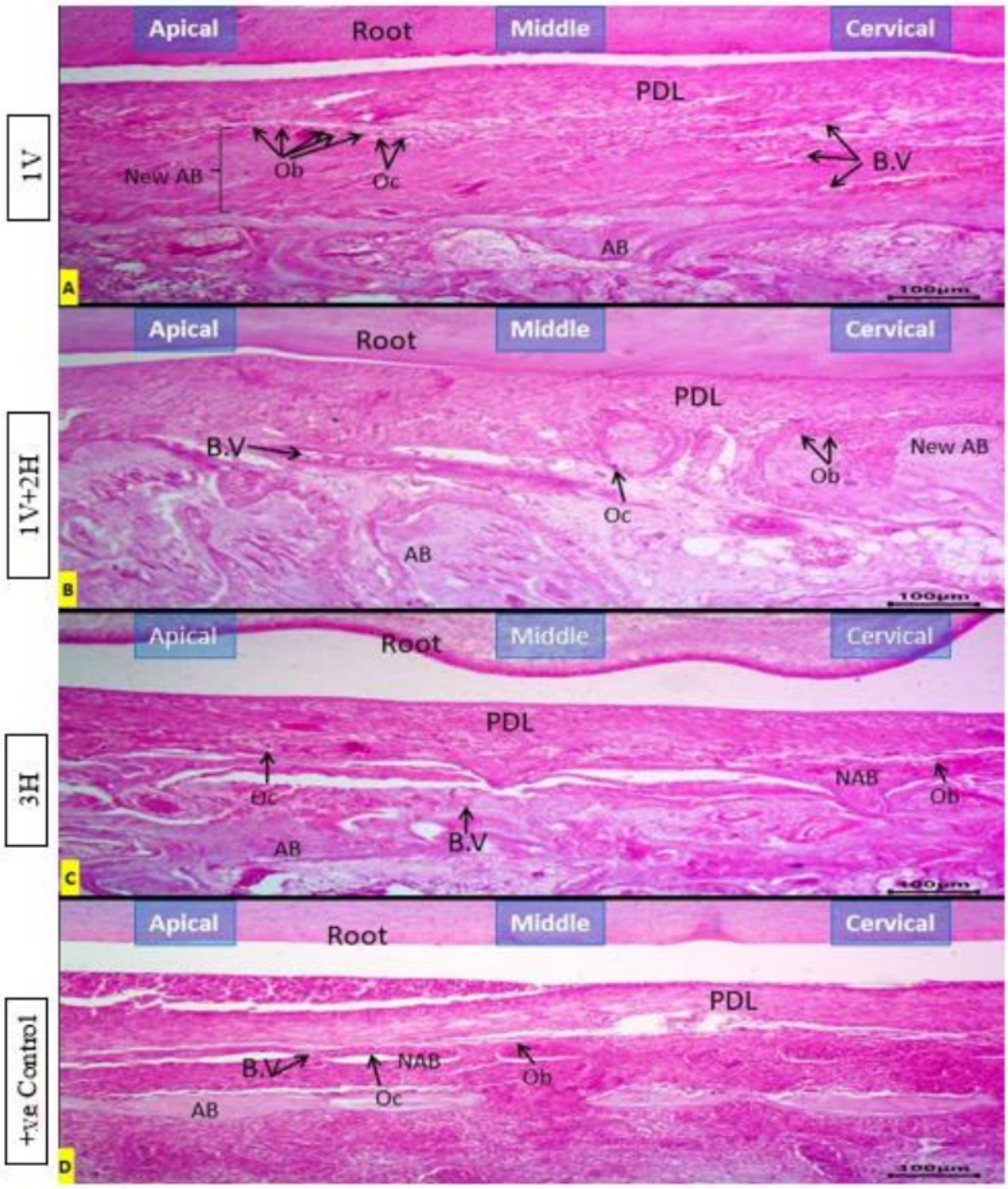

FIGURE 7. Histological section of rabbit MP1 tooth root after removal of orthodontic appliance at **3 weeks** from the [A]: 1V group, [B]: 1V+2H group, [C]: 3H group and [D] +ve control group showing; high surface area of the new alveolar bone formation NAB with highly numbers of osteoblasts Ob, osteoclast OC, and blood vessels in the 1V group then 1V+2H group then 3H group and then the +ve control group. Alveolar bone AB. H&E stain, (A, B, C, D: 40X), Scale bar=100µm.

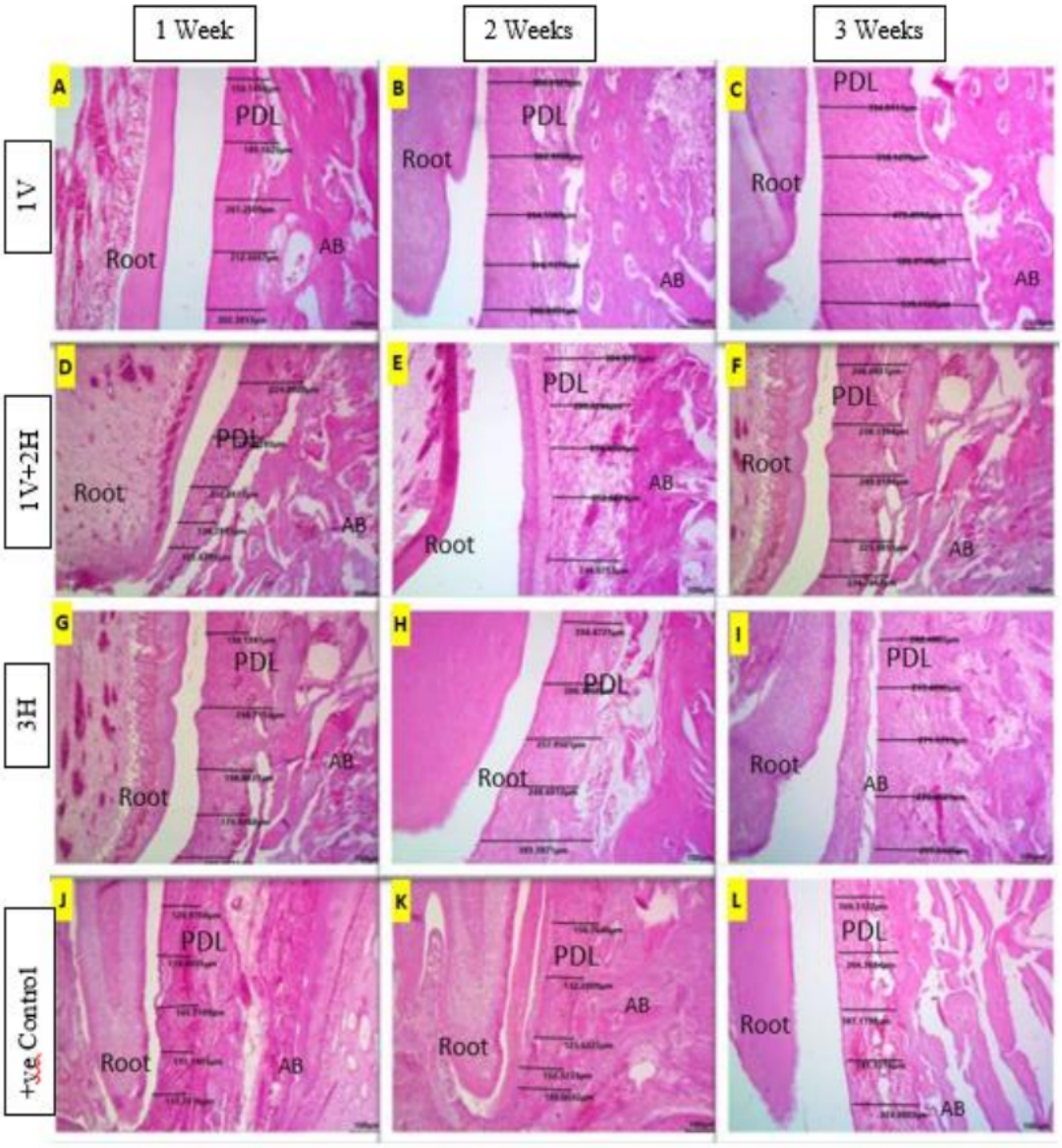

FIGURE 8. Histological section of the **apical** region of rabbit MP1 tooth root after removal of orthodontic appliance at 1, 2 and 3 weeks from the [A, B, C]: 1V group, [D, E, F]: 1V+2H group, [G, H, I]: 3H group and [J, K, L]: +ve control group showing; the root, measurements of the PDL width with highly disorganized, highest PDL width in the 1V group; then disorganized, wide PDL in the 1V+2H group; then slightly disorganized, less PDL width in the 3H group; and lastly the least disorganized, least PDL width in the +ve control group. Alveolar bone AB. H&E stain, (A, B, C, D, E, F, G, H, I, J, K, L: 100X), Scale bar=100µm.

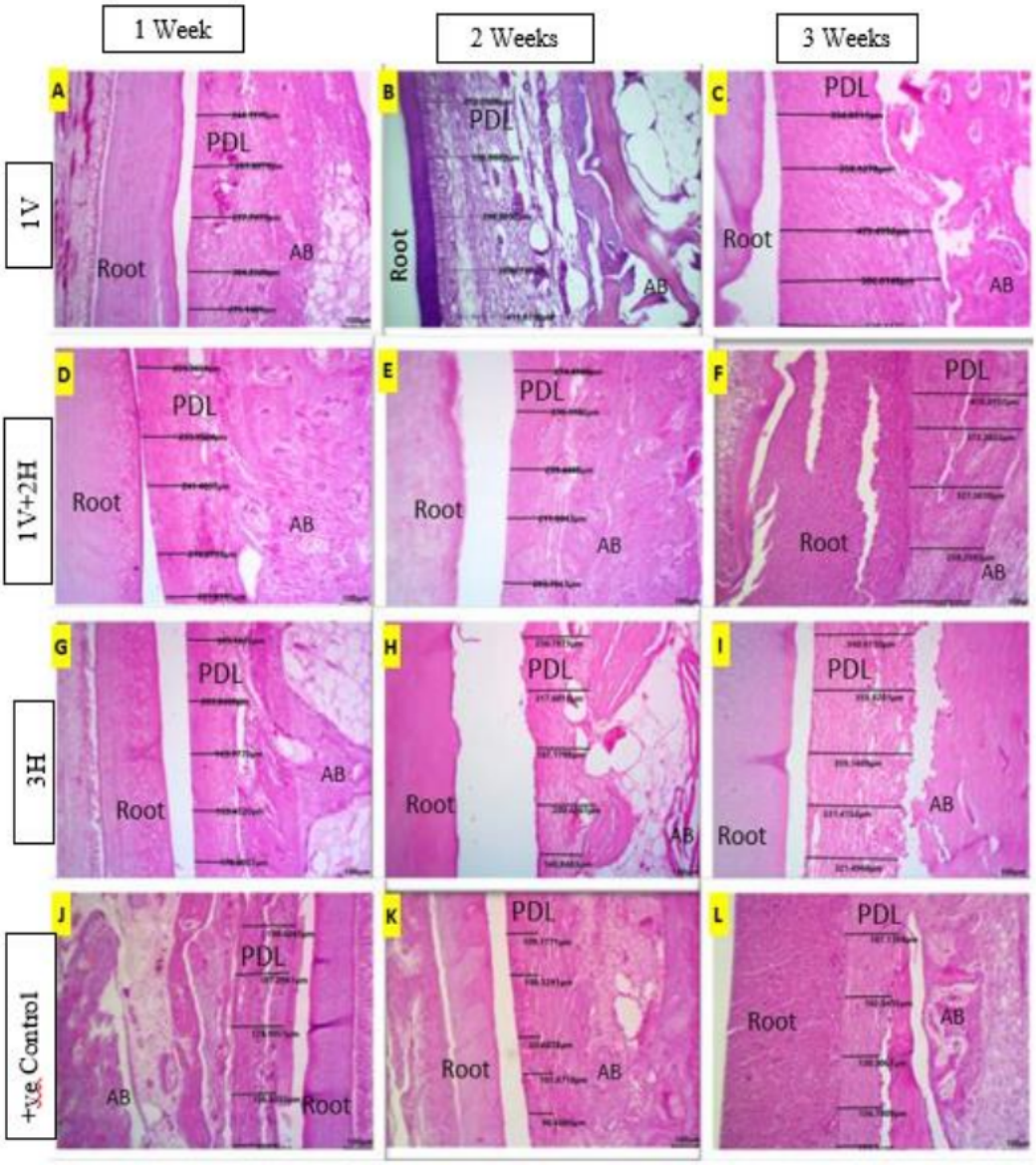

FIGURE 9. Histological section of the **middle** region of rabbit MP1 tooth root after removal of orthodontic appliance at 1, 2 and 3 weeks from the [A, B, C]: 1V group, [D, E, F]: 1V+2H group, [G, H, I]: 3H group and [J, K, L]: +ve control group showing; the root, measurements of the PDL width with highly disorganized, highest PDL width in the 1V group; then disorganized, wide PDL in the 1V+2H group; then slightly disorganized, less PDL width in the 3H group; and lastly the least disorganized, least PDL width in the +ve control group.. Alveolar bone AB. H&E stain, (A, B, C, D, E, F, G, H, I, J, K, L: 100X), Scale bar=100µm.

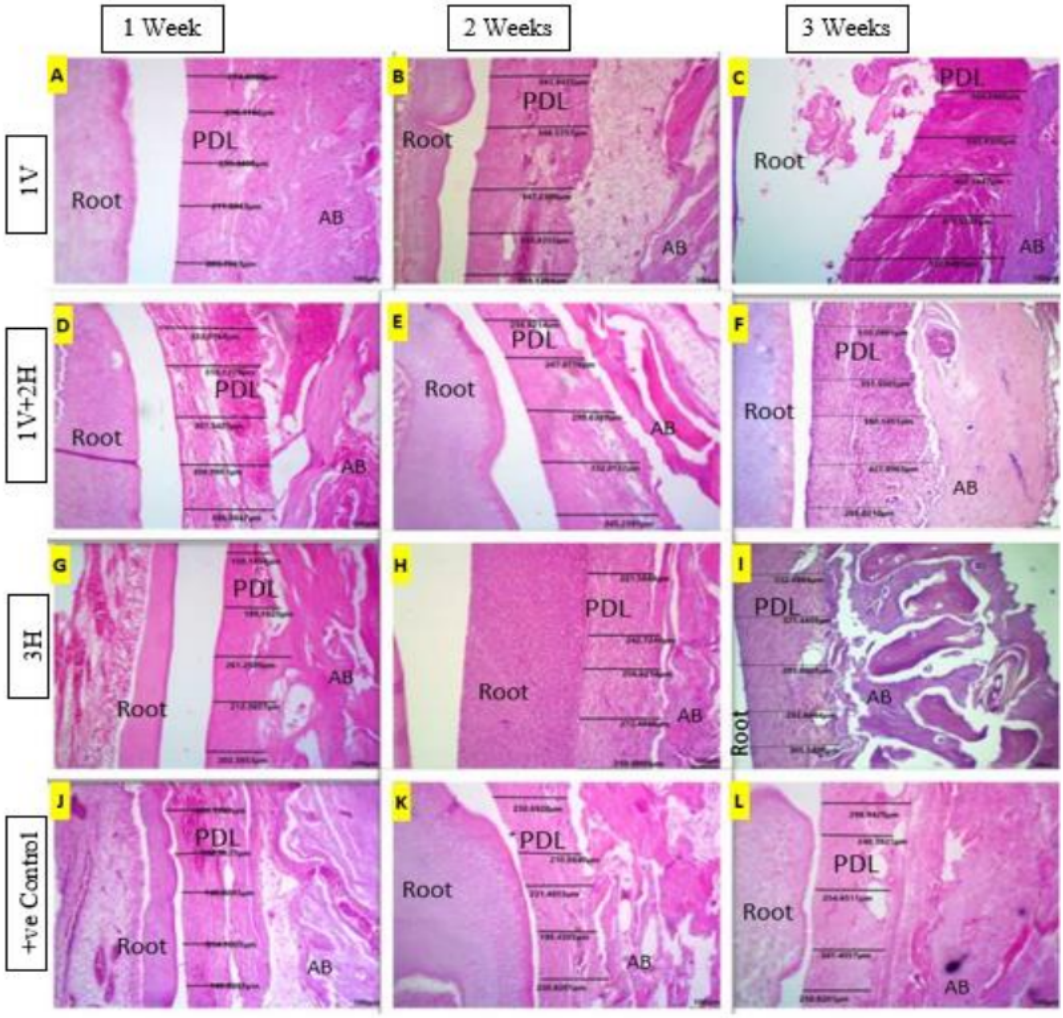

FIGURE 10. Histological section of the **cervical** region of rabbit MP1 tooth root after removal of orthodontic appliance at 1, 2 and 3 weeks from the [A, B, C]: 1V group, [D, E, F]: 1V+2H group, [G, H, I]: 3H group and [J, K, L]: +ve control group showing; the root, measurements of the PDL width with highly disorganized, highest PDL width in the 1V group; then disorganized, wide PDL in the 1V+2H group; then slightly disorganized, less PDL width in the 3H group; and lastly the least disorganized, least PDL width in the +ve control group. Alveolar bone AB. H&E stain, (A, B, C, D, E, F, G, H, I, J, K, L: 100X), Scale bar=100µm.

DISCUSSION

In the current study, the MOPs are modified and designed from many previous studies. Numerous studies designed MOPs of either (0.25mm) or (0.5mm) diameter in a rat model [11,26]. For albino white rabbits, Kim *et al*. [17,22] designed MOPs of 2 diameters, (1mm) and (1.4mm), Kim also [17] designed MOP depths of (1.5mm) and (4.6mm), Huang *et al*. [15] designed (1mm) diameter for MOPs. Uribe *et al*. [27] suggested that if the corticotomy injury was limited to a depth of only a (1mm), the RAP response may not be triggered. Based on these facts, the suggested dimensions of MOPs in the current study were (1.4mm) as the diameter and (1.5mm or 4.5mm) as the depths of MOPs in a rabbit model. The location of MOPs and the choice of MP1 of rabbits to deliver the traction force were also in agreement with the studies of Kim *et al*. [17,22] and Huang *et al*. [15]. The design of orthodontic appliance was also in congruence with Huang *et al*. [15]. The one mini-screw that was inserted between the 2 lower incisors of rabbits, offers an absolute, successful anchorage [28], to continuously and efficiently tract the MP1s by the NiTi closed coil springs at each side of the mandible. Moreover, the rabbit's lower incisors are very small with inadequate retention [15]. Huang *et al*. [15] and Nugraha *et al*. [29] suggested a (50g) of force for an effective traction of MP1 in rabbits. Sugimori *et al*. [11] and Zhong *et al*. [30] used the same force in rats for (2 weeks).

The clinical findings of the RTM (in mm) showed that all experimental MOP groups were significantly higher than the controls and a significant difference was found among MOP groups and the RTM increased significantly over time. Recently, many studies have examined the use of MOP; it is the 2nd most surgical method after corticotomy that was studied for the aim of accelerating OTM due to its least invasive nature without elevating a flap [3,11-13,17,22]. The reason

behind such acceleration and the significance of the clinical results between MOP and control groups may be due to the micro-trauma induced by MOPs which can promote the inflammation of the alveolar bone, leading to high cellular activity, high rate of bone turnover, low bone density [31], high biological changes and an increase of certain biomarkers (chemokines/cytokines), which in turn supports the differentiation of osteoclasts and thus facilitate OTM, but without any adverse effect on pulp vitality [13]. The RTM could be increased over time [17]. Sugimori *et al*. [11] agree that the RAP induced by MOP are similar to that observed after bone grafting, osteotomy, arthrodesis and fracture, thus causing rapid stimulation & mobilization of the aggregated precursor cells at the injury area and faster bone remodeling and so rapid healing of the wound. However, MOP action range still exists as a wondering matter, and in need of further investigations [32].

The current study may come in agreement with numerous findings in animal studies [7,11,15,17,22]. They found significant rise in the RTM at the MOP groups after various time periods; 1, 2, and 3 weeks in rabbit, rat, and mouse models. Moreover, many human studies may agree with the current study's significant results [3,6,16,33]. Other studies show higher RTM, but with non-significant outcomes [18]. However, multiple studies show no significant influence of MOPs at all [19,20,34], without repeating the procedure [21]. The variability between the studies may be attributed to the biologic individual factors [35], geometrical variations between both sides of the jaw [36], sample size [37], variations in the design of the study, animal model, amount of bone removed when creating the MOP, amount of traction force such MOP itself, number of MOPs [34], MOP position according to the tooth root [22], repeatability of the procedure [21], injury depth [9], the protocol while creating MOPs [1], and inadequate follow-up period [19].

The current study reveals the presence of significant outcomes among the MOP groups. The 1V-MOP shows the greatest RTM and the 3H-MOP shows the smallest RTM. Bakr *et al*. [1] and Wagh *et al*. [3] concentrated on the effect of the surgical insult; they suggested that a minimal insult might not be able to induce the inflammatory response for triggering the RAP or the expression of different cytokines. Other surgical factors, in addition to the injury depth, are the MOP design / direction, and the number of MOPs may be attributed also to the evident difference in the RTM [34]. The deep large vertical injury of the 1V-MOP group at the alveolar bone crest could efficiently expand the RAP region and eliminate the cortical bone resistance at the alveolar bone crest which may agree with many studies [38, 39]. They suggested that the injury magnitude and amount can efficiently determine the RTM, which are proportionally related to the RTM. According to previous studies, the RAP can only be stimulated if the cortical injury depth is more than (1mm) [27] or (3-7mm) [40]. Moreover, the large injury created with this design can resemble an area of extracted tooth resulting in statistically significant faster TM [16]. McBride *et al*. [41] created numerous vertical grooves in dogs and noticed low bone density resulting from these traumatized regions. Besides, removing bone resistance at the cervical region can make the tooth move much faster, which may agree with the conclusions of Aboalnaga *et al*. [19].

This can also explain that the 1V+2H-MOP group had the 2nd highest RTM after the 1V-MOP group, as the 1V hole was created with the same manner but with less depth than the 1V-MOP; but the 2H-MOPs were made in a manner that every hole opposes the other and both were at the same level from the alveolar crest (5mm below). Thus, they created nearly a continuous horizontal hole of depth of about (3mm), which agrees with Alikhani [40]. Also, the lingual

horizontal hole created near the apex of RMP1 root in addition to the buccal horizontal hole, may add an advantage for removing part of the obstacle against TM created by the lingual cortical bone unlike that observed in the 3H-MOP group, which had the lowest RTM among the 3 experimental MOP groups. According to Bakr *et al*. [1]; the more the resistance of the harder tissues is reduced, the more the OTM during corticotomy procedures is increased. In addition, Yang *et al*. [42] demonstrated that creating only mesial cuts on the labial side of the dentoalveolar region can just exert a minor effect, unlike when making distal cuts also near the canine root, which might be more beneficial during corticotomy-assisted faster canine retraction. Alikhani [40] concluded that 3MOPs made distally to canines can enormously increase the RTM by (2.3 times). Meanwhile, Kim *et al*. [22] examined the histologic effects and RTM after multiple horizontal MOPs in rabbits' mandible and found greater OTM with such intervention. However, Alkebsi *et al*. [34] and Aboalnaga *et al*. [19] claimed that creating 3H-MOPs was not effective for retracting a canine tooth. They explained this by the presence of a less surgical insult via MOPs, which could not be enough to stimulate the inflammatory response for triggering cytokine expression and the RAP.

The findings in the current study came in agreement with Kim *et al*. [17]. Kim [17] examined and compared 1V-MOP (1.4mm diameter and 4.6mm depth) with 6H-MOPs (1mm diameter and 1.5mm depth), 1mm mesially to RMP1 in rabbits at 1, 2, and 3 weeks, and claims an increase in RTM over time, the 1V-MOP (1.84mm RTM) is higher than 6H-MOPs (1.47mm RTM), but disagrees with the current study about the significance. Kim reported a non-significance at week 3 and both experimental groups (1V-MOP and 6H-MOPs) show the same

RTM (0.9mm) at week 2. The absence of the control group for comparing the results in Kim's study may be related to this drawback.

In the current study, the measurements of the RTM of the groups that were taken every 2 and 3 days were done in order to demonstrate the changes of TM in a short period among groups. This will help for a better quantification of the results and diminishes the time effect. In the present study, TM increased over time nearly with the same rhythm among the groups. This may be due to the fact that rabbits have an open apex of the roots (elodont) [43]; it is usually highly vascularized and undergoes continuous remodeling, and thus can elongate the inflammatory response, particularly with the MOP groups. Besides, such criteria of the roots may be responsible for the inexistent of the 2nd phase (lag phase; necrotic tissue removal) of OTM, shown by the continuous TM in all groups over the 3 time periods. In addition, the light force used in the current study, could make this skip of the lag phase [44,45]. Kim *et al*. [17] suggested a significant increase of the TRAP +ve cell between weeks 3 and 1, and the highest increase of osteoclast−like cells was at (week 3) caused by (SV-MOP) and (MH-MOPs) in rabbits, the (SV-MOP) was higher than (MH-MOPs) in bone resorption cells and RTM, which agrees with the current study. Furthermore, according to Raghav *et al*. [31]; after the 1st (3 weeks) of MOP in humans, there was a persistent increased activity of osteoclasts due to the increased biomarkers activity which may be responsible for the faster OTM shown after (4 weeks). Moreover, Patterson *et al*. [46] showed a slight increase in the RTM via RAP effect in the 1st few days after corticotomy-assisted orthodontics, then peaked at (1-2 months) and lasted for the 1st (3-4 months) post-surgery. After this, it declined to the pre-surgery state in humans, which may come in agreement with the current study in rabbits. Moreover, Sugimori *et al*. [11] depicted a continuous increase in the RTM

after (7, 10 and 14 days) of orthodontic intervention, higher in MOP group than control in a rat model. Besides, Ratanasereeprasert *et al*. [47] demonstrated that the PDL during OTM could show a high response at (day 1), declines at (day 3) due to the lag phase which was omitted in rabbits. It elevates gradually from (day 7) by the osteoclast differentiation till (day 28) in humans; while in mice, the PDL response (more stable and slower) showed an upregulation from (day 7) to (day 14), which may agree with the current study.

The histological feature of the current study showed that the MOP groups were significantly higher in the osteoclasts, osteoblasts, and blood vessels number than the controls. According to various animal studies [7,11,29] MOP technique could cause heavy bone remodeling and decreased bone density/volume at the compressed PDL due to the significant increase in the osteoclastic activity (area and number) (12.9–55%) after MOPs in mice, rats, and rabbits, respectively. Kim *et al*. [17] showed that the highest osteoclast–like cells number was particularly depicted at (week 3) in rabbits. Moreover, Nugraha *et al*. [29] depicted a rise in the osteoblastic activity (via osteo-pontin expression) in response to the increase in bone resorption by the high osteoclastic activity and a time-dependent manner, (+ve) correlation between alkaline phosphatase (ALP)-osteoblastic activity levels and the RTM at the compression side after (1, 2 and 4 weeks) of OTM in rabbits. The generated hypoxia at the compression side may efficiently elevate the osteogenic differentiation as concluded by Nugraha *et al*. [29]. Furthermore, the homeostatic mechanism triggered by MOP may explain this elevation in the osteoblasts number at the compression side in order to maintain structural integrity [31]. Huang *et al*. [15] suggested the presence of significantly higher osteoblasts activity at the MOP side when compared to the control side after (2 weeks) in rabbits. Besides, Erdenebat *et al*. [7] concluded that the Wnt / β-catenin

pathway was highly expressed and significantly activated at the PDL compression site in the crater area during OTM, and it is also strongly expressed and promoted in the MOP groups than in controls. Zhong *et al*. [30] found the presence of a concomitant relationship between tissue vascularity and bone formation / remodeling. Increased vascularization could speed up bone synthesis (bone neogenesis). This may explain the elevation of both osteoblasts (which were already elevated in response to elevated osteoclasts) and blood vessels number in the same manner at the groups with the progress of time in the current study.

The PDL width can reflect the activity of the bone remodeling cells; the width increased with the increase in the number of the remodeling cells, which could come in agreement with the study of Kim *et al*. [17] on rabbits. Kim *et al*. [17] upon their comparison between the SV-MOP and MH-MOP studied groups at 1, 2 and 3 weeks showing that both of them depicted an increase in the PDL width over time, but SV-MOP was higher than MH-MOP, which agrees with the current study. The current study may come also in agreement with Rizk *et al*. [36]; who concluded that and because of the inherently deep connection between PDL and alveolar bone, the PDL could also undergo remodeling, thus its thickness & volume would be increased significantly over time during OTM after (5 weeks) in a mouse model. Rizk *et al*. [36] demonstrated also that the reason for such widening of the volume and thickness of PDL after its deformation at the compression side, might be due to its inability to compensate its extension at the other side (tension force region), particularly OTM of longer duration, and because the PDL not compensated, its whole system would be deformed, remodeled, and thus widened. The visco-elastic nature of PDL could explain such huge deformation in a time-dependent manner during OTM [48]. According to this and because the MOP groups showed the higher PDL remodeling and width,

they may exhibit the most disfigured and disordered feature of PDL collagen fibers when compared with the controls, graduating from the most irregular fibers at the 1V-MOP groups to the less irregular fibers at the 3H-MOP groups among the MOP groups.

Although there were different designs and locations of the MOPs in the current study, the middle 1/3 of the compression region at the MP1 root always showed the highest numbers of osteoclasts, osteoblasts, and blood vessels, and also the widest PDL with the most irregular region among the groups, followed by the cervical 1/3 and then the apical 1/3. Moga *et al*. [49] concluded that the stress induced via OTM could be distributed un-uniformly inside the PDL shown by the analysis of the finite element. Moreover, this might also be due to the fact that the center of resistance (C_{Res}) of every single-rooted tooth is located approximately at the middle of the root [50]. According to this, this area may exhibit a great resistance against the mechanical or stress-induced inflammatory response, or changes stimulated by OTM, in addition to the effect of the continuous erupting phenomenon (elodont) shown at rabbit teeth [43]. Thus, the more resistant middle 1/3 may always be under a continuous stress, which can be translated by the great bone remodeling and turnover at this area noticed histologically via the increase in the number of osteoclasts exacerbated by MOPs. Numerous previous studies [15, 30], focused on reducing this resistance in order to get faster TM. Huang *et al*. [15] made MOPs near this region and the cervical 1/3 of roots to facilitate OTM in a rabbit model, while Zhong *et al*. [30] applied photo-bio-modulation (PBM) therapy for (2 weeks) at the middle 1/3 of roots to make them move faster in a rat model.

In the current study, the cervical 1/3 showed the 2nd highest numbers and PDL width after the middle 1/3. According to Yang *et al*. [42] and Aboalnaga *et*

al. [19]; the location of the maximum stress was almost distributed on the tooth cervix. Aboalnaga *et al*. [19] emphasized also that the cusp tip of the canine tooth had the capability to move in a larger distance more than the root apex in both the MOP and (+ve) control sides, which means that the remodeling cells were higher at the cervical 1/3 than the apical 1/3, which agrees with the current study. Moreover, Li *et al*. [51] noticed that the stress of PDL was concentrated at the cervical 1/3 of the compression region of upper 1st molar roots in rats after (1 week) of OTM. Besides, the continuous orthodontic traction force applied near the cervical region of the tooth, and thus this close pressure may induce alveolar bone remodeling that was increased over time. Regarding the apical 1/3; the high vascularization and remodeling property of the apical 1/3, can make this region less influenced by the OTM stresses and less resorbed [52]. The unique property of the apical 1/3 of rabbit root could be a disadvantageous in another way, it may mask the outcomes of the study and therefore it could be unpredictable. Huang *et al*. [15] examined the effect of MOPs at the middle 1/3 of MP1 root region in rabbits. They excluded the cervical and apical 1/3. The current study showed that the highest bone cells and blood vessels number, and PDL width in all groups was present at the middle 1/3, which may be introduced as a more dependable region for the study, and this agrees with Huang *et al*. [15].

Verna *et al*. [53] introduced a finite element study, which shows the influence of surgical interventions on OTM. They claimed that the intervening of any surgery may affect the type and amount of TM. Thus, the formed transitory osteopenia via the injury for the aim of facilitating OTM could make the center of rotation (C_{Ro}) of the moved teeth to be shifted more apically; thus, the corticotomized teeth may undergo larger TM, especially for the uncontrolled tipping. This is why the MOP groups in the current study showed the highest

results. At the same time, this can add extra-stress induced changes at the supporting tissues of the tooth, particularly the middle and cervical 1/3 regions (middle is more because of C_{Res}) of PDL in the MOP groups, which may explain why the widening of these regions is more than the apical 1/3 in the current study which also increased over time. In addition, the latter (apical) of the open apex [43]; is highly vascularized and so less affected by the stress-induced inflammatory changes. Furthermore, the current study also agrees with Mena Laura *et al*. who [54] showed that the PDL thickness can be changed asymmetrically along the whole root that might occur due to TM at the 2nd phase of OTM.

LIMITATIONS

The present study provided a new and extended methodology which could introduce a comprehensive insight into the effect of different MOP modalities on the RTM. However, the following limitations and research shortcomings were observed:

1. The current study investigated only a single application of MOP. Repeated and frequent MOP performance at different intervals was not studied because of the limited and somewhat small lower jaw of the rabbit.
2. Rabbit teeth are categorized by the continuous eruption and the apex patency; thus, the outcomes of the apical 1/3 region of the root might not be so accurate due to the continuous remodeling at this region.

CONCLUSION

Within the limitations of the current study, the following conclusions were depicted:

1. Micro-osteoperforation (MOP) of different modalities can significantly increase the RTM when evaluated clinically.
2. The MOP may exacerbate the activity of the compressed PDL, thus increasing its width and the number of bone remodeling cells, the osteoclasts and osteoblasts, in addition to blood vessels number, particularly at the middle and cervical 1/3 regions of the root.
3. Moderate RTM was observed in the new 1V+2H-MOP modality in comparison to the higher RTM in the 1V-MOP and the lesser RTM in the 3H-MOP. Thus, the new 1V+2H-MOP modality might be chosen as the best modality for faster TM after the 1V-MOP.

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