



## Role of Osteogenic Supplement in Reducing Orthodontic Relapse: Rabbit Model Study



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### Abstract

**O**RTHODONTIC relapse is a significant problem associated with orthodontic treatment. Therefore, increase bone density on the relapse side is important to decrease the relapse rate. This study attempted to evaluate the effect of local injection of osteogenic supplement on the rate of orthodontic relapse. Twenty-four male albino rabbits were used in this study. The animals were randomly divided into two groups (twelve rabbits in each group), positive control and osteogenic supplement groups. Each main group was subsequently divided into three subgroups (four rabbits in each). Modified orthodontic devices were cemented to the mandibular incisors. Orthodontic tooth movement was conducted for 2 weeks, followed by a retention phase of 3 weeks. Subsequently, the orthodontic appliances were removed, allowing the teeth to relapse over a period of 20 days. During this period, positive control and osteogenic supplement groups received local injection of phosphate buffer saline, and osteogenic supplement solutions, respectively. The relapse was estimated clinically after removal of orthodontic appliance. Histological analyses were carried out following the completion of the experiment. The osteogenic supplement group had a decreased relapse rate compared to the positive control group; but statistically not significant. The osteogenic supplement group showed a notable increase in the number of osteoblasts, larger areas of new bone formation, decreased in the number of osteoclasts, and narrower width of the periodontal ligament in most of the experimental periods. So, Local injection of an osteogenic supplement has been found to decrease orthodontic relapse in a rabbit model.

**Keywords:** Alveolar bone, Bone remodeling, Orthodontic relapse, osteogenic supplement.

### Introduction

Today, orthodontic treatments use a variety of fixed and removable appliances, occasionally in conjunction with supplementary techniques. They all employ and control forces acting on the teeth and surrounding structures, despite their different designs. The dentoalveolar system experiences the main changes as a result of these forces, which manifest as tooth movements [1]. Orthodontic tooth movement (OTM) is biologically dependent on the periodontal tissue's spatiotemporal remodeling process [2]. While an unfavourable orthodontic force does not produce a precise biological response and may cause harmful tissue reactions, a favourable

force aims to maximize cellular response and establish tissue stability [1].

The stability of orthodontic treatment outcomes is often unstable due to the need for gingival and periodontal tissues to reorganize after appliance removal. Additionally, the teeth may be in an intrinsically unstable position following treatment, and changes in growth can potentially affect the results of orthodontic treatment [3]. Therefore, a retention phase is important for all orthodontic tooth movements to uphold the teeth in their new position and prevent relapse [4]. Relapse is a condition that occurs after treatment, where teeth have a tendency to shift back towards their initial misalignment [5]. Relapse is considered a multifaceted problem with numerous possible causes. Several reasons contribute

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to this phenomenon, including the recovery of gingival and periodontal tissues, the growth of skeletal structures, and most significantly, the remodeling of alveolar bone and periodontal ligaments [6,7].

The standard procedure to stimulate osteogenic differentiation of mesenchymal stem cells in vitro is through culturing the cells in the presence of osteogenic supplement containing dexamethasone, ascorbic acid, and  $\beta$ -glycerophosphate [8].

Dexamethasone, a steroid, stimulates the transformation of mesenchymal stem cells into osteoblasts by stimulating the WNT/b-catenin signaling pathway. As a result, this process triggers the activation of Runx2 gene expression, which causes mesenchymal stem cells to undergo differentiation into immature osteoblasts [9].

Ascorbic acid is necessary for the biosynthesis of collagen as it acts as a cofactor for prolyl and lysyl hydroxylase enzymes, and also stimulates the expression of collagen genes [10]. It is also the predominant regulator of collagen type 1 secretion [9]. It was noted that ascorbic acid promotes cell growth, enhances collagen production, activates alkaline phosphatase activity, and triggers bone cell differentiation [11].

$\beta$ -Glycerophosphate is an inorganic phosphate that has been demonstrated to have a major impact on the process of mesenchymal stem cells differentiating into bone cells [12]. It also that is required for the formation of hydroxyapatite mineral [9], and controls the expression of genes such as osteopontin and bone morphogenic protein-2 [13].

To the best of our knowledge, there have been no previous studies carried out to evaluate the effect of osteogenic supplement on reducing of relapse after orthodontic tooth movement. Therefore, this study hypothesized that there is a positive effect of osteogenic supplement on reducing the orthodontic relapse following orthodontic tooth movement in animal model.

## **Material and Methods**

### *Ethical approval*

The protocol of study was approved by the research ethics committee of University of Mosul / College of Dentistry, REC referencec No. UoM.Dent/ DM.12/ 23.

The samples of the study consisted of 24 healthy male albino rabbits, with average weight 1450 g, age 6-12 months .The animals housed in a suitable big, clean and good ventilation metallic cage.The animals were divided randomly into two main groups, positive control and osteogenic supplement groups with 12 rabbits in each group. Each group was further divided into three subgroups (4 rabbits in

each) according to the day of euthanization as follow:

1. Positive control group day 0 (PCD0), Positive control group day 10 (PCD10), Positive control group day 20 (PCD20): Rabbits were received 200  $\mu$ l local injection of phosphate- buffered saline solution (PBS) every 3 days during retention period. The animals in (PCD0), (PCD10), and (PCD20) groups were euthanized on day 0, 10 and 20 respectively after removal of orthodontic appliance.
2. Osteogenic supplement group day 0 (OSD0), Osteogenic supplement group day 10 (OSD10), Osteogenic supplement group day 20 (OSD20): Rabbits were received local injection of OS (200  $\mu$ l) every 3 days [14], during retention period. The animals in (OSD0), (OSD10), and (OSD20) groups were euthanized on day 0, 10 and 20 respectively after removal of orthodontic appliance

### *Experimental design:*

The study design was based on previously published studies on orthodontic tooth movement and relapse in rabbits [15-17]:

Modified orthodontic appliance used in this study. It consists of two stainless steel orthodontic band for mandibular central incisors (LA 00) into which bracket with (0.022"  $\times$  0.030") slot dimension attached to it. Straight stainless steel wire (0.016"  $\times$  0.022") of about 2 cm length was inserted in the bracket's slot of the first orthodontic band and ligate to the bracket by using stainless steel ligature wire (0.010"). Then Nickel-titanium open coil spring (0.012"  $\times$  0.030") of 5 circles (about 5.5 mm length) was inserted along the stainless steel arch wire, then the other end of the wire was ligated to bracket's slot of the second orthodontic band by using stainless steel ligature wire. The arch wire was bended on both ends to avoid trauma to the rabbits. Finally the two bands ligated with each other with stainless steel ligature wire to compress the open coil spring from 5.5 mm to about 2.4 mm, in order to exert lateral reciprocal force approximately 50 grams to move the mandibular central incisors distally.

### *Insertion of Orthodontic Appliance*

Rabbits were anesthetized with an intramuscular injection of the mixture of 50 mg/kg body weight ketamine and 10 mg/kg body weight xylazine. Orthodontic separator was placed between mandibular incisors to open space for appliance, then, the modified orthodontic appliances was cemented to the teeth by using light curing flowable composite resin (Proflow, Korea). Then the orthodontic appliance allowed being active by removing the ligature wire that squeezed the open coil spring, as seen in Fig.(1).

The force was applied for 14 days. After that, the appliance was retained in position during the retention period (3 weeks). Adequate amount of flowable composite was added to the open coil spring that was served as a retainer during the retention period. After that, the modified orthodontic appliance was removed and allowed the teeth to relapse into their original position.

The degree of relapse was measured by employing an electronic digital vernier caliper with a precision of 0.01mm. Measurements were taken at the mesial tip of the two lower incisors at certain time intervals: 0, 2, 4, 7, 10, 13, 17, and 20 days following the removal of the orthodontic appliance. Subsequently, the animals were euthanized in accordance with the predetermined schedule and sent for histological studies.

#### *Drug administration*

During the retention period, OS and PC groups were received injections with osteogenic supplement (MSC osteogenic differentiation medium (Promocell, Germany) (100 ml C-28013,) and PBS (Himedia, India), respectively. The injection was administered locally into the submucosal area near and parallel to the mesial surface of the experimental left mandibular incisor through the attach gingiva (buccally, lingually and into the periodontal ligament space).

At the end of the experiment, the animals were euthanized using intravenous injection of an overdose of xylazine hydrochloride (200 mg/kg) [18], according to the time scheduled for each subgroup.

#### *Histological assessment*

The specimen placed in a solution of 10% neutral buffered formalin. Following fixation, the specimens underwent a series of procedures including decalcification, dehydration, clearing, infiltration, and embedding. The specimens were cut into slices with a thickness of 5 micrometres. The mandibular left incisor, along with its supporting components, was sectioned longitudinally in a labiolingual orientation. The specimens were stained using Hematoxylin & Eosin (H&E), which is a widely used histology stain [19-20].

#### *Histological Analysis*

The analysis was conducted in two regions: the upper half and the lower half. The delineation of these zones was achieved by tracing longitudinal line, along the symphysis region of the mandible. Then, two horizontal lines were drawn perpendicular to the vertical line. The first horizontal line was drawn at the alveolar crest level, and the other line was drawn at a distance of (9960 $\mu$ m) from the first horizontal line, which represent the straight part of the mandibular incisor's root. The distance between

the two horizontal lines was divided equally into two halves, the upper and lower halves. The target area was from the mesial aspect of the mandibular left central incisor's root, till the symphysis area.

#### Quantitative Evaluation of Histological Sections

All histological measurements were carried out by using light microscope with a color USB 2.0 digital image camera (Omax Toup view, 9 Megapixels, China) which was provided with image processing software. The software of camera was calibrated to all lenses of Microscope-Olympus-CX31 by aid of 0.01mm stage micrometer (ESM-11/Japan).

**Number of Osteoblasts:** On the alveolar wall next to the mesial side of the roots of the left mandibular incisors, osteoblasts were counted. Osteoblasts are cuboidal cells that have a plump shape and large nuclei. They can be observed on the surface of osteoid or bone [21], Fig.(2).

**New Bone surface Area:** New bone surface area (NBA) was measured in both upper and lower halves [22], Fig.(2).

**Numbers of Osteoclasts:** The number of osteoclasts on the alveolar wall close to the mesial surface of the left mandibular incisor roots was counted. These cells were big, stained with eosin, had many rounded nuclei, and were found in a resorption depression known as the Howships lacuna [21], Fig.(2).

**Periodontal ligament (PDL) width:** It is the distance between the mesial surface of the mandibular left central incisor's root and new bone surface at 2 regions (at the level of cervical and apical line), Fig.(2).

The data were analysed using IBM SPSS Statistics, Version 20 (IBM Corporation, USA). The data were checked for their normal distribution by using Shapiro-Wilk's test. Normal distribution of data was found for clinical and histological parameters, so that parametric statistical approach was used and included: Descriptive statistics, independent sample t-test was used to compare means within the same group and among both groups. Also, One-way ANOVA and Duncan's multiple range analysis were used to compare means within the same group. Differences at  $p < 0.05$  were considered statistically significant.

#### **Result**

##### *Clinical finding*

Throughout the entire experimental trial, none of the experimental animals exhibited any noticeable systemic disorders that would disrupt the activity, weight, or growth of the rabbits. Furthermore, there were no signs of gingival inflammation such as swelling, bleeding, or laceration noticed. This suggests that the solution used did not have any

negative effects during the whole 55-day experimental period.

The statistical analysis of the data by using independent sample t- test, ANOVA and Duncan for the amount of space gained for both groups at three experimental period showed that there was no significant differences in the amount of spaced gained for each group and between groups over the three experimental periods (0, 10, and 20 days) as illustrated in Tables (1,2).

*Mean of the amount of space gained, remaining space, relapse distance and percentage of relapse after removal of orthodontic appliances.*

During the relapse period, the greater rate of relapse was occurred in positive control group, and the relapse rate gradually reduces until the end of relapse period. Osteogenic supplement group had a relapse rate less than that of positive control group but statistically not significant. At the end of relapse period, the percentage of relapse was higher for the positive control group (89.75%), followed by osteogenic supplement group (72.32%) as demonstrated in Table (3).

Comparison of the amount of relapse distance for each day during relapse period between positive control and osteogenic supplement groups.

On days 2, 4, 7,10,13,17, and 20, although there was no significant differences in the relapse distance between positive control and osteogenic supplement groups, but the osteogenic supplement group had the lower rate of relapse as compared to control group. All these results were presented in Table (4)

#### *Histological analysis*

**Number of osteoblast:** On day 0, the osteoblast count was highest in both groups. There was a progressive decrease in the number of osteoblasts by day 10, reaching the lowest count on day 20. The OS group exhibited a significantly higher number of osteoblasts compared to the PC group throughout the relapse period in both halves, except for day 0 in the lower half where the OS group had a higher number of osteoblasts but the difference was not statistically significant. as seen in Table (5).

**New bone surface area:** The NBA reached its largest area in both groups on day 0. Over time, during the relapse period, there was a progressive decrease in the NBA until it reached its lowest value on day 20. The OS group exhibited greater NBA than the PC group throughout the relapse period, but the difference was not reach to the significant level, as seen in Table (6).

**Number of osteoclast:** The osteoclast population exhibited the lowest count on day 0, followed by a peak count on day 10, and subsequently experienced a decline on day 20. This image was observed in both groups. The OS group exhibited a significantly

reduced number of osteoclasts throughout the relapse period, compared to the PC group, in both the upper and lower halves. This difference was statistically significant, as shown in Table (7).

**Periodontal ligament width:** On day 0, the maximum width of the PDL was measured for both groups. Over the course of 10 days, the width gradually decreased, reaching its minimum on day 20. During the relapse period, the width of the PDL was consistently narrower in the OS group compared to the PC group, except on day 10 in both halves and day 0 in the lower half. On these specific days, the PDL width was narrower in the OS group, but the difference was not statistically significant, as seen in Table (8).

#### **Discussion**

In this study, white albino male rabbits were used as animal model because of their human-like biological structure [23] phylogenetic resemblance to humans [24], relatively docile, easy to and they are easily bred and maintained [25]. The male rabbits were chosen to ensure a hormonal balance for the study's results and to exclude any hormonal fluctuations caused by the estrous cycle in females, which could potentially impact bone metabolism and tooth movement [26-27].

The remodeling of the periodontal ligament and the adjacent alveolar bone is a crucial factor in the relapse process [7,28]. Relapse occurred in the two groups following appliance removal. However, a higher relapse rate was found immediately after appliance removal which may be due to that the tooth undergoes a rebound shift in the tooth socket as stated by Reitan, [29], then the relapse gradually decreased over the subsequent days. This trend is consistent with the findings in other studies [28,30]. The relapse rate decreased especially near the end of relapse period because the relapse force may be lower because of a decrease in PDL pressure as suggested by Franzen et al. [7]. Our findings clarified that greater distance was in relapse occurs within 7th days. This is consistent with the findings of Aoki et al. [31].

The relapse observed in the PC group can be related to a lack of fully mature and mineralized bone, which makes it unable to sustain the stress created by stretched transseptal fibers. This finding aligns with the study made by Hudson et al. [32] and Azami et al. [33].

In this study, the histological analysis revealed that the osteoblast count was maximum on day 0 during the relapse period in both groups. Subsequently, a larger area of new bone formation occurred. At day 0, where the mesial side considers as tension side at the end of orthodontic tooth movement, new bone is formed as a result of forces applied by braces during orthodontic treatment. On

the tension side, Osteoblasts are differentiated from their progenitor cells, that is, mesenchymal stem cells (MSCs). Mature osteoblasts lay down the osteoids and the mineralization processes follow [34]. The greater number of osteoblast in OS group as compare to PC group was due to the effect of OS on osteoprogenitor cells as reported by many authors. The study conducted by Urban et al. [35] revealed a beneficial impact of vitamin C on the proliferation of primary bovine osteoblasts in vitro. Additionally, on their study on ovariectomized Wistar, Choi et al., [36] demonstrated that vitamin C enhanced osteoblastogenesis and simultaneously suppressed osteoclastogenesis in vivo. Cells isolated from human alveolar bone are a good source of cells that can differentiate into the osteoblast lineage and that dexamethasone in the osteogenic medium increased the differentiation of osteoblast-like cells [37].  $\beta$ -glycerophosphate promoted the osteogenic differentiation of human bone marrow mesenchymal stem cells [38].

With time there was a gradual reduction in the number of osteoblast till reach the end of study period at day 20, because during the relapse, opposite tooth movement occurred, and the previous tension site become pressure site and alveolar bone resorption occurred according to the pressure-tension theory in orthodontics explains that when there is compression on the side of the periodontal ligament, it leads to the resorption of alveolar bone. Conversely, tension on the opposite side stimulates the production of new bone [39].

In this study, the highest number of osteoclasts was recorded on day 20 because during the relapse period, the previous tension side became the pressure side, resulting in an increase in the number of osteoclasts and associated bone resorption. The present study demonstrated a direct correlation between the number of osteoclasts and orthodontic relapse, underscoring the significance of bone resorption during the relapse period. Taking into account the total number of osteoclasts and the results of relapse, we found that the control group had high rates of relapse and osteoclast counts, while the OS group displayed the lesser relapse rate profile because of its impact on the bone cells and osteoprogenitor cells. Kanatani et al. [40] Research has shown that an increase in phosphate levels inhibits the process of osteoclast differentiation. Vitamin C has positive effects on bone health as it acts as an antioxidant, reducing inflammation and preventing the activation of RANKL and osteoclasts, which are responsible for bone loss [41].

While on day (20), the number of osteoclasts decreased, this may be due to decrease in the relapse force due to decrease in PDL pressure as the teeth

became near the end of relapse period, as concluded by Franzen et al. [7].

On day 0, just after removal of the orthodontic appliance, the mesial side was identified as the tension side. As a result, both experimental groups exhibited a wide periodontal ligament (PDL) width. Our findings align with the research conducted by Jeon et al. [42]. With the progression in the relapse, there was a reduction in the width of the PDL fibers on days (10) and reach its narrowest width at day (20), because the mesial side became compression side. This result come in agreement with studies carried out by Franzen et al.[7] and Aoki et al.[31], as both studies showed that with the application of orthodontic forces, the PDL width on the tension side widens, but toward the end of the relapse phase, it narrows. This suggests that tension of PDL fibres is involved in relapse.

OS group possessed narrower PDL when compared to CP group due to the anabolic effect of OS, as mesenchymal stem cells differentiation by OS lead to larger new bone formation as compared to PC group and subsequently narrower PDL width.

All researches use osteogenic media for osteogenic differentiation of mesenchymal stem cells from different origins into osteoblast cells, and all studies were carried out in vitro. To the best of our knowledge, there has been no previous studies use this media to differentiate the stem cells in vivo and enhance bone formation.

Since the number of animals is limited, so to achieve more accurate and reliable results, the number of animals should be increased. In addition, there was difficulty in sample selection as well as difficulty in determination the exact age of the animals.

## Conclusion

Although the present results showed insignificant differences in relapse percentages between control and experimental groups, the histological analysis proved that osteogenic supplement is still a potent osteogenic material which can be used with different parameters in future researches to evaluate its effectiveness.

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**TABLE 1.** Comparison of the amount of space gained for control positive and osteogenic supplement groups at three experimental periods.

Groups	Subgroups	Min*	Max*	Mean*	SD	P-value**	Duncan
CP	CPD0	3.33	3.81	3.580	0.198	0.403	A
	CPD10	2.96	3.60	3.258	0.340		A
	CPD20	2.95	3.88	3.345	0.420		A
OS	OSD0	3.29	3.80	3.503	0.216	0.524	A
	OSD10	2.95	3.70	3.350	0.308		A
	OSD20	3.27	3.79	3.558	0.241		A

\*: All measurements are in mm, \*\*: Significant difference at  $P < 0.05$  level.

**TABLE 2.** Comparison of the amount of space gained between control positive and osteogenic supplement groups at three experimental periods.

Subgroups	Mean* $\pm$ SD	T-value	Sig. **
CPD0	3.580 $\pm$ 0.198	0.529	0.616
OSD0	3.503 $\pm$ 0.216		
CPD10	3.258 $\pm$ 0.340	-0.403	0.701
OSD10	3.350 $\pm$ 0.308		
CPD20	3.345 $\pm$ 0.420	-0.878	0.414
OSD20	3.558 $\pm$ 0.241		

\*: All measurements are in mm, \*\*: Significant difference at  $P < 0.05$  level.

**TABLE 3.** The mean value for the amount of space gained, remaining space, relapse distance and percentage of relapse for control positive and osteogenic supplement groups.

Groups	Days	Gaining space*	Remaining space*	RD*	PR***
CP	0	3.345	0	0	0 %
	2		1.77	1.575	47.07 %
	4		1.51	1.835	54.86 %
	7		1.18	2.165	64.72 %
	10		0.925	2.42	72.35 %
	13		0.68	2.665	79.67 %
	17		0.498	2.848	85.14 %
	20		0.343	3.002	89.75 %
OS	0	3.558	0	0	0 %
	2		2.368	1.19	33.45 %
	4		2.065	1.493	41.96 %
	7		1.76	1.798	50.53 %
	10		1.465	2.093	58.83 %
	13		1.215	2.343	65.85 %
	17		1.025	2.533	71.19 %
	20		0.985	2.573	72.32 %

\*All measurement in mm, \*\*RD: Relapse distance, \*\*\* PR: Percentage of relapse.

**TABLE 4. Comparison the amount of relapse distance among the control positive and osteogenic supplement groups for each day.**

Groups	Mean* ±SD	t- Value	Sig.**
CPD0	0 ± 0	-	-
OSD0	0 ± 0	-	-
CPD2	1.575 ± 0.358	1.857	0.113
OSD2	1.190 ± 0.209		
CPD4	1.835 ± 0.382	1.673	0.145
OSD4	1.493 ± 0.147		
CPD7	2.165 ± 0.269	2.409	0.053
OSD7	1.798 ± 0.144		
CPD10	2.420 ± 0.319	1.600	0.161
OSD10	2.093 ± 0.256		
CPD13	2.665 ± 0.312	2.028	0.089
OSD13	2.343 ± 0.063		
CPD17	2.848 ± 0.318	1.814	0.120
OSD17	2.533 ± 0.139		
CPD20	3.003 ± 0.355	2.307	0.061
OSD20	2.573 ± 0.114		

\*All measurements are in mm, \*\*: Significant difference at P<0.05 level.

**TABLE 5. Comparison of osteoblast number among positive control and osteogenic supplement groups for three time intervals in two regions.**

Area	Time	Groups	Mean ± SD	T-value	Sig.*
Upper half	Day 0	PC	104.750 ± 9.323	-3.482	0.033
		OS	121.500 ± 2.380		
	Day 10	PC	76.250 ± 3.304	-5.5	0.002
		OS	93.750 ± 5.439		
	Day 20	PC	51.500 ± 13.478	-3.06	0.022
		OS	74 ± 5.888		
Lower half	Day 0	PC	109.250 ± 14.683	-1.647	0.151
		OS	121.750 ± 3.862		
	Day 10	PC	80.250 ± 3.862	-4.785	0.003
		OS	93.750 ± 4.113		
	Day 20	PC	56 ± 6.481	-3.758	0.009
		OS	71.750 ± 5.315		

\* Significant difference existed at the P < 0.05 level.

**TABLE 6. Comparison of new bone surface area among positive control and osteogenic supplement groups for three time intervals in two regions.**

Area	Time	Groups	Mean ± SD*	T-value	Sig.**
Upper half	Day 0	PC	121056 ± 47713.9	-1.012	0.369
		OS	150744.7 ± 17437.8		
	Day 10	PC	44260.7 ± 12245.2	-1.969	0.12
		OS	80716.3 ± 29632.5		
	Day 20	PC	33746 ± 7922.6	-1.669	0.171
		OS	60725 ± 26861.4		
Lower half	Day 0	PC	141634 ± 48444	-0.574	0.596
		OS	158693 ± 17286		
	Day 10	PC	63265 ± 12256.1	-1.636	0.177
		OS	96389 ± 32862.8		
	Day 20	PC	42677.3 ± 9281	-2.218	0.091
		OS	76641.3 ± 24848		

\* Significant difference existed at the P < 0.05 level.

**TABLE 7.** Comparison of osteoclast number among positive control and osteogenic supplement groups for three time intervals in two regions.

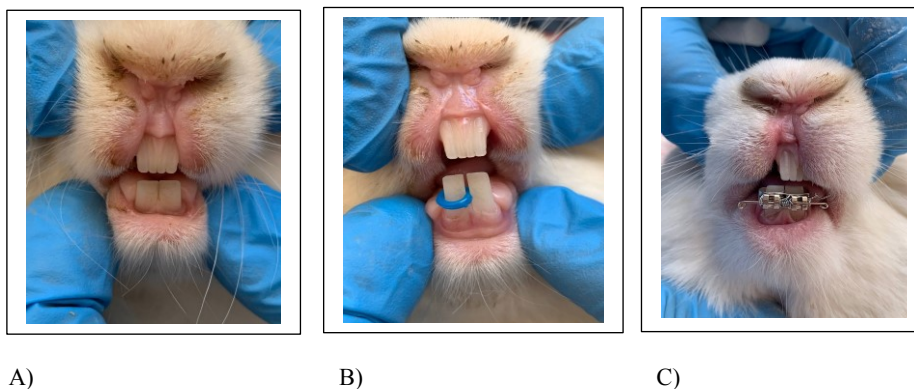
Area	Time	Groups	Mean $\pm$ SD	T-value	Sig.*
Upper half	Day 0	PC	8 $\pm$ 0.817	3.656	0.011
		OS	6.250 $\pm$ 0.500		
	Day 10	PC	19 $\pm$ 0.817	5.196	0.002
		OS	16 $\pm$ 0.817		
	Day 20	PC	11.750 $\pm$ 1.500	2.496	0.047
		OS	9.500 $\pm$ 1		
Lower half	Day 0	PC	9 $\pm$ 0.817	5	0.002
		OS	6.500 $\pm$ 0.577		
	Day 10	PC	19.750 $\pm$ 1.708	2.905	0.027
		OS	17 $\pm$ 0.817		
	Day 20	PC	12 $\pm$ 0.817	5	0.002
		OS	9.500 $\pm$ 0.577		

\* Significant difference existed at the  $P < 0.05$  level.

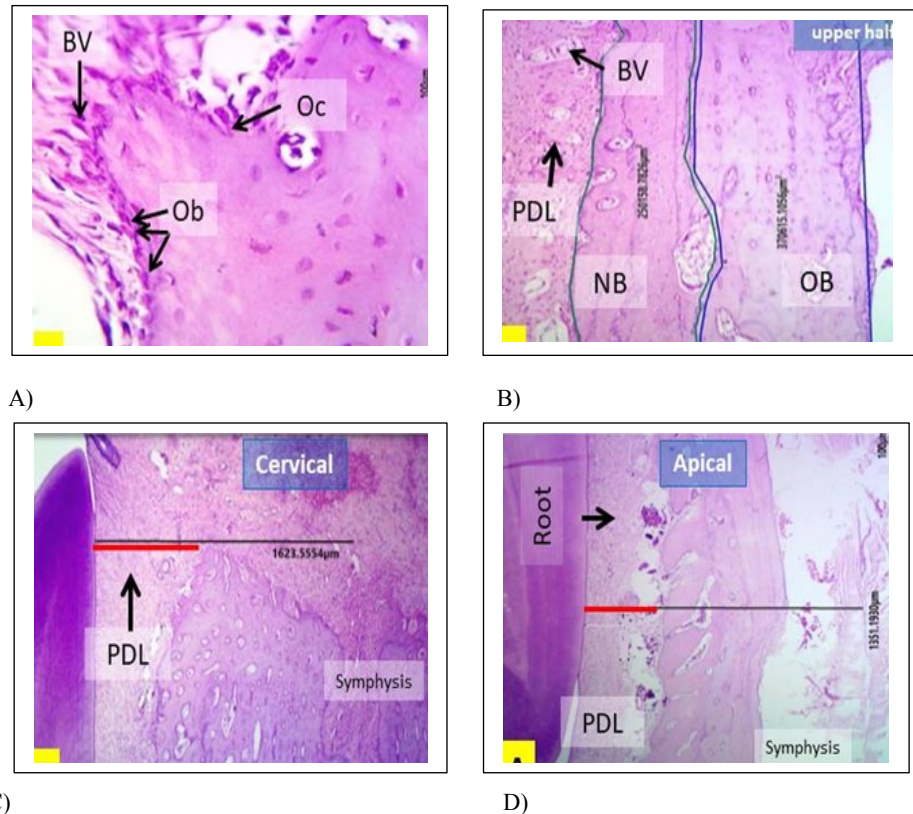
**TABLE 8.** Comparison of the periodontal ligament width among positive control and osteogenic supplement for three time intervals in two regions.

Area	Time	Groups	Mean $\pm$ SD*	T-value	Sig.**
Upper half	Day 0	PC	629 $\pm$ 12.832	3.889	0.008
		OS	581 $\pm$ 21.087		
	Day 10	PC	526.250 $\pm$ 31.224	2.078	0.083
		OS	490 $\pm$ 15.556		
	Day 20	PC	470.250 $\pm$ 27.536	4.095	0.006
		OS	378.250 $\pm$ 35.509		
Lower half	Day 0	PC	643.250 $\pm$ 19.670	1.7	0.14
		OS	602.250 $\pm$ 44.041		
	Day 10	PC	515 $\pm$ 32.506	1.799	0.122
		OS	482.250 $\pm$ 16.399		
	Day 20	PC	476 $\pm$ 19.950	3.961	0.007
		OS	399.750 $\pm$ 32.928		

\* Significant difference existed at the  $P < 0.05$  level.

**Fig. 1.** Steps of orthodontic appliance insertion. A) Pre orthodontic appliance insertion, B) Orthodontic separators between incisors, C) Orthodontic appliance in position.





**Fig. 2. Hematoxylin and eosin stained section photomicrograph of rabbit model. A: the number of osteoblasts (Ob), osteoclast (Oc), B: Surface area measurements of new bone formation (NB) C, D: measurements of (PDL) (red line) at cervical region and apical region respectively, Scale bar=100µm.**

## References

1. Graber, L.W., Vig, K.W., Huang, G.J. and Fleming, P. Orthodontics-: current principles and techniques. 7th Edition Elsevier Health Sciences. pp: 51. (2022).
2. Chen, Y. and Zhang, C., Role of noncoding RNAs in orthodontic tooth movement: New insights into periodontium remodeling. *Journal of Translational Medicine*, **21**(1), 101 (2023).
3. Proffit, W.R., Fields, H.W., Larson, B. and Sarver, D.M. Contemporary Orthodontics. 6<sup>th</sup> ed., Elsevier Health Sciences, Mosby pp. 579 (2019).
4. Gad, A., Abdallah, E., ElHarouni, N. and Soliman, S. Evaluation of the effect of systemic omega-3 polyunsaturated fatty acids on post-orthodontic relapse in a rabbit model. *Egyptian Orthodontic Journal*, **54**(12), 1–10 (2018).
5. Littlewood, S.J., Kandasamy, S. and Huang, G. Retention and relapse in clinical practice. *Aust. Dent. J.*, **62** (1), 51–57 (2017).
6. Melrose, C. and Millett, D.T. Toward a perspective on orthodontic retention? *Am. J. Orthod. Dentofacial Orthop.*, **113**, (5), 507-514 (1998).
7. Franzen, T.J., Brudvik, P. and Vandevska-Radunovic, V. Periodontal tissue reaction during orthodontic relapse in rat molars. *Eur. J. Orthod.*, **35**, 152- 159 (2013).
8. Ahmed, M.A., Exploring the impact of hypoxia mimetic agents on multipotent stem cell biology, Doctoral dissertation, Keele University (2018).
9. Langenbach, F. and Handschel, J. Effects of dexamethasone, ascorbic acid and  $\beta$ -glycerophosphate on the osteogenic differentiation of stem cells in vitro. *Stem Cell Res. Ther.*, **4** (5), 117 (2013).
10. Kishimoto, Y., Saito, N., Kurita, K., Shimokado, K., Maruyama, N. and Ishigami, A. Ascorbic acid enhances the expression of type 1 and type 4 collagen and SVCT2 in cultured human skin fibroblasts. *Biochem. Biophys. Res. Commun.*, **11**, 430 (2), 579-584 (2013).
11. Wang, C., Cao, X. and Zhang, Y. A novel bioactive osteogenesis scaffold delivers ascorbic acid,  $\beta$ -glycerophosphate, and dexamethasone in vivo to promote bone regeneration. *Oncotarget.*, **8** (19), 31612–31625 (2017).
12. Dey, D., Jingar, P., Agrawal, S., Shrivastava, V., Bhattacharya, A., Manhas, J., Garg, B., Ansari, M. T., Mridha, A. R., Sreenivas, V., Khurana, A. and Sen, S. Symphytum officinale augments osteogenesis in human bone marrow-derived mesenchymal stem cells in vitro as they differentiate into osteoblasts. *J. Ethnopharmacol.*, **10**, 248, 112329 (2020).
13. Tada, H., Nemoto, E., Foster, B.L., Somerman, M.J. and Shimauchi, H. Phosphate increases bone morphogenetic protein-2 expression through cAMP-

- dependent protein kinase and ERK1/2 pathways in human dental pulp cells. *Bone*, **48**(6), 1409–1416 (2011).
14. Sordi, M.B., Curtarelli, R.B., da Silva, I.T., Fongaro, G., Benfatti, C.A.M., de Souza Magini, R. and Cabral da Cruz, A.C., Effect of dexamethasone as osteogenic supplementation in in vitro osteogenic differentiation of stem cells from human exfoliated deciduous teeth. *J. Mater. Sci. Mater. Med.*, **32**(1), 1 (2021).
  15. Elkattan, A.E., Gheith, M., Fayed, M.S., Yazeed, M.A.E., Farrag, A.R.H. and Khalil, W.K.B. Effects of Different Parameters of Diode Laser on Acceleration of Orthodontic Tooth Movement and Its Effect on Relapse: An Experimental Animal Study. *J. Med. Sci.*, **7**(3), 412-420 (2019).
  16. Li, H., Li, Y., Zou, J., Yang, Y., Han, R. and Zhang, J. Sinomenine Inhibits Orthodontic Tooth Movement and Root Resorption in Rats and Enhances Osteogenic Differentiation of PDLSCs. *Drug Des. Devel. Ther.*, **16**, 2949-2965 (2022).
  17. Al-Fakhry, H.H. and Al-Sayagh, N.M. Effects of Injectable platelet rich fibrin (i-PRF) on reduction of relapse after orthodontic tooth movement: Rabbits model study. *J. Orthod. Sci.*, **11**, 10 (2022).
  18. Ozbek, M., Odabasi, M., Erdur, S. K., Senturk, F., Ozsutcu, M., Aras, C. and Eliacik, M. Determination of the retinal toxicity of intravitreal colistin in rabbit eyes. *Cutan. Ocul. Toxicol.*, **40**(4), 300–304 (2021).
  19. Kiernan, J.A. *Histological and Histochemical Methods. Theory and Practice.* 5th edition © Scion Publishing Ltd. pp: 12, 45 (2015).
  20. Suvana, S.K., Layton, C. and Bancroft, J.D. *Bancroft's theory and practice of histological techniques.* 8th edition. Elsevier. pp: 73-95 (2019).
  21. Alnajjar, H. A. A. M. and Al Groosh, D. H. The effects of calcitonin on post-orthodontic relapse in rats. *Clin. Exp. Dent. Res.*, **7**(3), 293–301(2021).
  22. Al Fakhry, H.H. Influence of local injection of platelet rich fibrin and osteoprotegerin on orthodontic relapse in a rabbit model. PhD. Dissertation. University of Mosul, College of Dentistry, Iraq (2022).
  23. Bozbiyik, C. and Kirbaş Doğan, G. Investigation of male genital system anatomy in the New Zealand rabbit (*Oryctolagus cuniculus* L.). *Anat. Histol. Embryol.*, **52**(3), 381-392 (2022).
  24. Andersen, M.L. and Winter, L.M.F. Animal models in biological and biomedical research—Experimental and ethical concerns. *An. Acad. Bras Cienc.*, **91** (suppl. 1), e20170238 (2019).
  25. Matsuhisa, F., Kitajima, S., Nishijima, K., Akiyoshi, T., Morimoto, M. and Fan, J. Transgenic Rabbit Models: Now and the Future. *Applied Sciences*, **10** (21), 7416 (2020).
  26. Syahputra, C. A., Abidin, T., & Harahap, N. Mast Cell Expression in Periodontal Ligaments Associated with Orthodontic Tooth Movement with the Use of Elastic Separator and Steel Ring in *Nesolagus netscheri*- In Vivo Study. *Journal of Evolution of Medical and Dental Sciences (JEMDS)*, **8**(52), 3989–3993 (2019).
  27. Alaa, S., Fouda, A.M., Grawish, M.E. and Abdelnaby, Y.L. The effect of submucosal injection of platelet-rich fibrin vs. Platelet-rich plasma on orthodontic tooth movement in rabbits; 28 days follow-up. *Int. Orthod.*, **21**(1), 100715 (2023).
  28. Franzen, T.J., Monjo, M., Rubert, M. and Vandevska-Radunovic, V. Expression of bone markers and micro-CT analysis of alveolar bone during orthodontic relapse. *Orthodontics & Craniofacial Research, Orthod. Craniofac. Res.*, **17**(4), 249-258 (2014).
  29. Reitan, K. Clinical and histologic observations on tooth movement during and after orthodontic treatment. *Am. J. Orthod.*, **53**(10), 721-724 (1967).
  30. Qi, J., Kitaura, H., Shen, W.R., Kishikawa, A., Ogawa, S., Ohori, F., Noguchi, T., Marahleh, A., Nara, Y. and Mizoguchi, I. Establishment of an orthodontic retention mouse model and the effect of anti-c-Fms antibody on orthodontic relapse. *PLoS ONE*, **14**(6), e0214260 (2019).
  31. Aoki, Y., Kako, S., Miyazawa, K., Tabuchi, M., Kimura, F., Kataoka, K., Kato, R., Sato, T. and Goto, S. Dynamics and observations of long-term orthodontic tooth movement and subsequent relapse in C57BL/6 mice. *Exp. Anim.*, **72**(1), 103–111 (2023).
  32. Hudson, J.B., Hatch, N., Hayami, T., Shin, J.M., Stolina, M., Kostenuik, P.J. and Kapila, S. Local delivery of recombinant osteoprotegerin enhances postorthodontic tooth stability. *Calcif. Tissue*, **90**(4), 330–342 (2012).
  33. Azami, N., Chen, P.-J., Mehta, S., Kalajzic, Z., Dutra, E.H., Nanda, R. and Yadav, S. Raloxifene administration enhances retention in an orthodontic relapse model. *Eur. J. Orthod.*, **42**(4), 371–377 (2020).
  34. Sprogar, S., Vaupotic, T., Cör, A., Drevensek, M. and Drevensek, G. The endothelin system mediates bone modeling in the late stage of orthodontic tooth movement in rats. *Bone*, **43**(4), 740–747 (2008).
  35. Urban, K., Höhling, H. J., Lüttenberg, B., Szuwart, T. and Plate, U. An in vitro study of osteoblast vitality influenced by the vitamins C and E. *Head Face Med.*, **8**, 25 (2012).
  36. Choi, H.K., Kim, G.J., Yoo, H.S., Song, D.H., Chung, K.H., Lee, K.J., Koo, Y.T. and An, J.H., Vitamin C Activates Osteoblastogenesis and Inhibits Osteoclastogenesis via Wnt/ $\beta$ -Catenin/ATF4 Signaling Pathways. *Nutrients*, **11**(3), 506 (2019).
  37. Tabassum, A. Effect of dexamethasone on the growth and differentiation of osteoblast-like cells derived from the human alveolar bone. *J. Taibah Univ. Med. Sci.*, **17**(4), 707e714 (2022).
  38. Yin, Z., Shen, J., Wang, Q., Wen, L., Qu, W. and Zhang, Y. miR-215-5p regulates osteoporosis development and osteogenic differentiation by targeting XIAP. *BMC Musculoskelet. Disord.*, **17**, 23(1):789 (2022).

39. Viazis, A.D. and Pagonis, T.C. Prediction of orthodontic treatment duration based on the alveolar bone formula. *J. Dent. Health Oral Disord. Ther.*, **14** (4), 142–149 (2023)
40. Kanatani, M, Sugimoto, T, Kano, J, Kanzawa, M, and Chihara, K. Effect of high phosphate concentration on osteoclast differentiation as well as bone-resorbing activity. *J. Cell Physiol.*, **196**(1),180-189 (2003).
41. Jain, S.K, McLean, W.E., Stevens, C.M. and Dhawan, R. The Positive Association of Plasma Levels of Vitamin C and Inverse Association of VCAM-1 and Total Adiponectin with Bone Mineral Density in Subjects with Diabetes. *Nutrients*, **14**(19), 893 (2022)
42. Jeon, H.H., Teixeira, H. and Tsai, A. Mechanistic Insight into Orthodontic Tooth Movement Based on Animal Studies: A Critical Review. *J. Clin. Med.*, **10** (8), 1733 (2021).

### دور المكملات العظمية في تقليل الانتكاسات التقويمية: دراسة نموذجية للأرنب

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#### الخلاصة

يعتبر الانتكاس التقويمي مشكلة كبيرة مرتبطة بعلاج تقويم الأسنان. ولذلك، فإن زيادة كثافة العظام في جانب الانتكاس أمر مهم لتقليل معدل الانتكاس. حاولت هذه الدراسة تقييم تأثير الحقن الموضعي للمكملات العظمية على معدل الانتكاس التقويمي. تم استخدام أربعة وعشرون أرنباً من الذكور البيضاء في هذه الدراسة. تم تقسيم الحيوانات عشوائياً إلى مجموعتين (اثني عشر أرنباً في كل مجموعة)، مجموعة السيطرة الإيجابية ومجموعة المكملات العظمية. ثم بعد ذلك تم تقسيم كل مجموعة رئيسية إلى ثلاث مجموعات فرعية (أربعة أرانب في كل منها). تم تثبيت أجهزة تقويم الأسنان المعدلة على قواطع الفك السفلي. ثم تمت عملية تحريك الأسنان التقويمية لمدة أسبوعين، تليها مرحلة التثبيت لمدة 3 أسابيع. بعد ذلك، تمت إزالة أجهزة تقويم الأسنان، مما سمح للأسنان بالانتكاس خلال فترة 20 يوماً. في أثناء هذه الفترة، تلقت مجموعات المراقبة الإيجابية ومجموعات المكملات العظمية حقنة محلية من محلول ملحي الفوسفات، ومحاليل المكملات العظمية، على التوالي. تم تقدير الانتكاس سريرياً بعد إزالة جهاز تقويم الأسنان. وأجريت التحليلات النسيجية بعد الانتهاء من التجربة. كان لدى مجموعة المكملات العظمية معدل انتكاس منخفض مقارنة بالمجموعة الضابطة الإيجابية. ولكن ليست ذات دلالة إحصائية. من الناحية النسيجية، أظهرت مجموعة المكملات العظمية زيادة ملحوظة في عدد الخلايا العظمية البانية، وزيادة مساحة العظم، وانخفاض في عدد الخلايا الهادمة للعظم، وعرض أضييق للرباط اللثوي في معظم الفترات التجريبية. لذا فإن الحقن الموضعي لمكمل عظمي المنشأ يقلل من انتكاس تقويم الأسنان في نموذج الأرنب.

**الكلمات المفتاحية:** العظم السنخي، إعادة تشكيل العظام، الانتكاس التقويمي، المكملات العظمية.