



Original Article

Comparison of the efficiency of alveolar decortication and low level laser therapy on orthodontic tooth movement and alveolar metabolism in rats



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Abstract *Background/purpose:* Reducing orthodontic treatment duration has many advantages for both clinicians and patients. This study was designed to compare the effects of alveolar decortication and low level laser therapy methods on tooth movement rate and alveolar bone metabolism.

Materials and methods: A total of 42 Wistar albino rats were divided into three main groups as: Alveolar decortication (AD), low level laser therapy (LLLT) and only orthodontic force (F). The groups were evaluated at 7 and 14 day time points. Tooth movement rates were calculated by measuring the space between the contact points of the first and second molars. Comparisons regarding the alveolar bone metabolism were accomplished by evaluating osteoclast counts and RANKL - OPG expressions.

Results: The rate of tooth movement, at all time points, was significantly higher for the AD group than the other groups and was significantly higher in the LLLT group than the F group. At both time points, the RANKL and OPG expression in the AD group was significantly higher than the other groups and these parameters in the LLLT group was significantly higher than the F group. The osteoclast count values in the AD and LLLT groups were significantly higher than the F group and there were no significant differences between these two groups at all time points.

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Conclusion: This study shows that, to be more effective at AD, both AD and LLLT therapy significantly increases the level of tooth movement in the early period through their stimulating effects on the alveolar bone metabolism.

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Introduction

The level of alveolar bone remodeling rate is one of the most important factors determining the duration of orthodontic treatment.^{1–3} A shorter treatment time comes with many advantages, therefore many approaches have been proposed in order to reduce treatment time by accelerating tooth movement.^{4–6} Alveolar decortication (AD) and low-level laser therapy (LLLT) are among the popular methods, receiving emphasis by the researchers. AD method relies on the induction of the regional acceleratory phenomenon (RAP) by surgically injuring the cortical bone. Upon triggering RAP, bone metabolism is accelerated and density of regional bone tissue is decreased.^{7,8} Studies on this subject have shown that these events are the contributing factors for tooth movement and acceleration of these events lead to an increase in tooth movement rate.^{9,10} It is thought that LLLT increases the number and the activity of osteoclasts, and promotes the formation of mineralized bone. As a result bone metabolism is increased, leading to an acceleration in tooth movement.^{11,12}

Studies up to date have mostly investigated the biostimulant effects of these methods, while studies comparing these methods are limited. The aim of the present study is to compare the effects of AD and LLLT methods on orthodontic tooth movement rate and alveolar bone metabolism.

Materials and methods

Study design

This study has been approved by Istanbul University Experimental Animals Ethics Committee (2012/72). In this study, a total of 42, 10–12 week old Wistar albino rats weighing 250 ± 20 gr were used. Specimens were divided into three main groups of 14 and each group was evaluated in two subgroups of 7 based on 7- and 14- day time points. Tooth movement was induced by Ni-Ti coil springs of 10 g force (GAC international, Bohemia, NY, USA) positioned between the first molars and the incisors (Fig. 1). Appliance conditions, force magnitudes and body weights were checked 2 times a week. All procedures were performed under general anesthesia with ketamine HCl 100 mg/kg (50 mg/ml Ketalar; Parke-Davis, Morris Plains, NJ, USA) and Xylazine (Alfazyne 2% Injectable Solution; Ege Vet, Izmir, Turkey) injections. At the end of the experimental period, the animals were euthanized with sodium thiopental injection (Pentothal sodium flakon; Abbott, Istanbul, Turkey).

Group 1 (F) underwent only orthodontic force application. *In Group 2 (AD)*, AD was performed at the buccal and palatal aspects of left first molar following elevation of full thickness flaps. A total of 6 micro-osteoperforations were performed, three on each side of the alveolar bone, by a low speed hand piece and a No.1/4 round bur under water irrigation (Fig. 1). *In Group 3 (LLLT)*, LLLT irradiation was performed with an 830-nm wavelength and 100 mW power output Ga-Al-As Diode laser device (Doris CTL-1106MX; CTL, Warsaw, Poland), using 2-mm diameter application probe (CTL). Irradiation was applied to a total of nine spots: three spots (60 s per spot) in each of the mesial, vestibular and palatal aspects of the alveolar bone of the left first molar (Fig. 1). In a total of nine-minute irradiance episode, 54.0 J energy/day was delivered per episode. Laser irradiations were performed every day during the experimental period.

Preparation of histological specimens and measurements

The level of tooth movement was evaluated by measuring the space between the contact points of the first and second molars using an interproximal reduction gauge set (DentsplySirona, York, PA, USA). Following the euthanasia, maxillary bones were removed and prepared for routine histology. After preparation, multiple 3-micron thick transversal sections were cut and the sections containing the largest views of the mesiobuccal root were examined after staining with hematoxylin and eosin (H&E). The sections selected for immunohistochemical examination were stained with primary antibodies of receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) (Biorbyt Ltd., Cambridge, United Kingdom), and the number of positively-stained cells in each section were counted at 400x magnification. All sections were examined under a light microscope (Olympus BX60, Tokyo, Japan) and an imaging analysis system (Olympus analysis FIVE Soft, Olympus Soft Imaging Solutions GmbH, Münster, Germany).

Osteoclast counts were performed in the coronal, middle and apical regions of the distal and mesial parts of the mesiobuccal root of the first molar teeth at 100x magnification, by scanning the entire root surface in a fixed view field of 2.27 mm^2 (Fig. 2). The presence of hyalinization areas in this field was also defined. Hyalinization areas were identified as, homogeneous cell free zones in the periodontal ligament. For statistical evaluation samples presenting hyalinization areas were rated as 1, and samples without hyalinization areas were rated as 0.

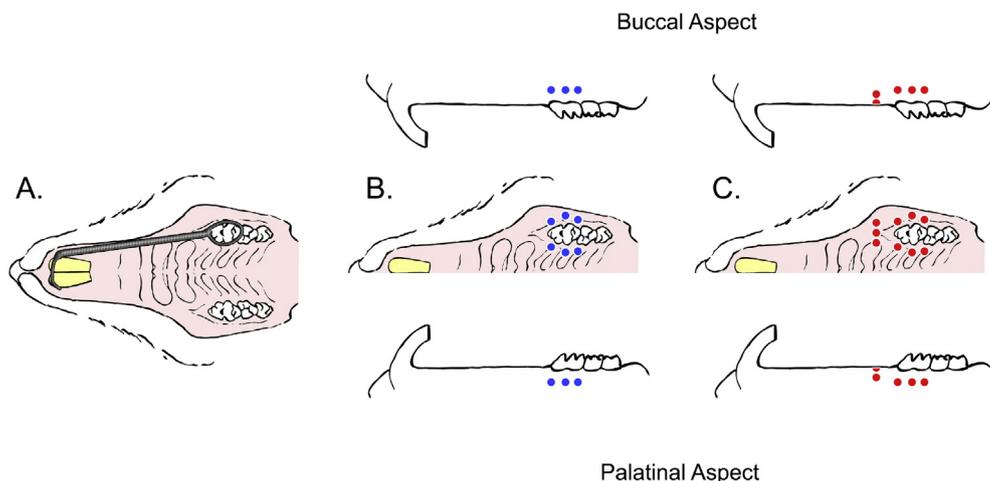


Figure 1 Illustration of the molar mesialization mechanic (A) locations of the alveolar decortication cuts (B) and locations of the laser irradiation points.

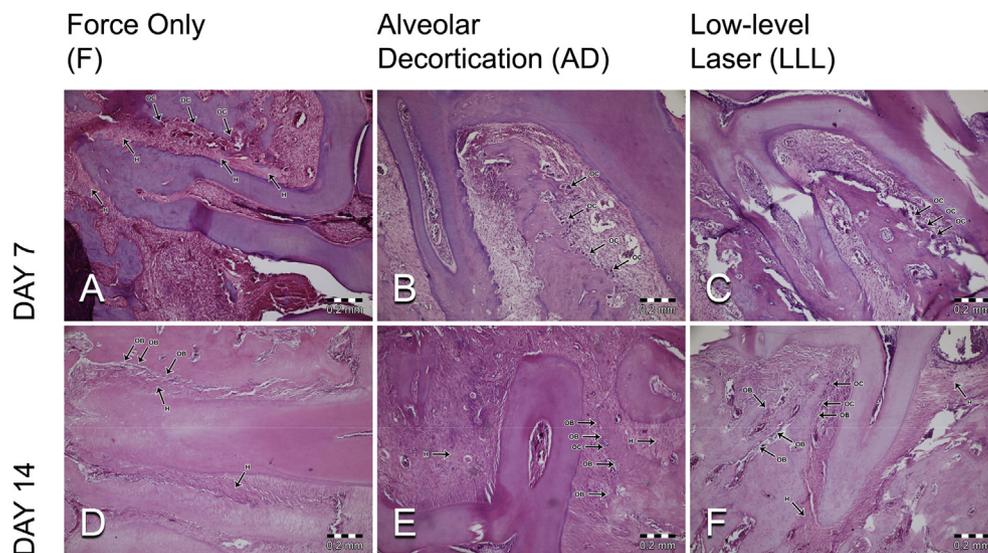


Figure 2 Representative photomicrographs of the sections stained with hematoxylin and eosin. A, D: Force only group on day 7–14; B, E: Alveolar decortication group on day 7–14; C, F: Low-level laser irradiation group on day 7–14. Arrows indicate: OC osteoclast, OB osteoblast, H hyalinization. (Original magnification 100 \times).

Statistical analysis

SPSS Statistics 22 (IBM SPSS, Turkey) software was used for the analysis of the study data, and data was presented as mean values. The amount of tooth movement was measured three times at different time points, with the average values taken for analysis. A Mann-Whitney U-test was used to compare parameters between two groups, and a Kruskal Wallis test was used to compare parameters between more than two groups. A Mann-Whitney U-test was also applied to determine the group that contributed to the difference. The level of statistical significance was set at $p < 0.05$.

Results

Amount of orthodontic tooth movement

The rate of tooth movement at day 7 was significantly higher in the AD group than in the LLLT ($p = 0.004$) and F ($p = 0.001$) groups ($p < 0.01$) (Fig. 3). When the LLLT and F groups were compared, tooth movement rate was found to be significantly higher in the LLLT group ($p = 0.003$; $p < 0.01$). On day 14, tooth movement rate was significantly higher in the AD group than in the LLLT ($p = 0.001$) and F ($p = 0.001$) groups ($p < 0.01$). When the LLLT and F groups were compared, the rate of tooth movement in the LLLT

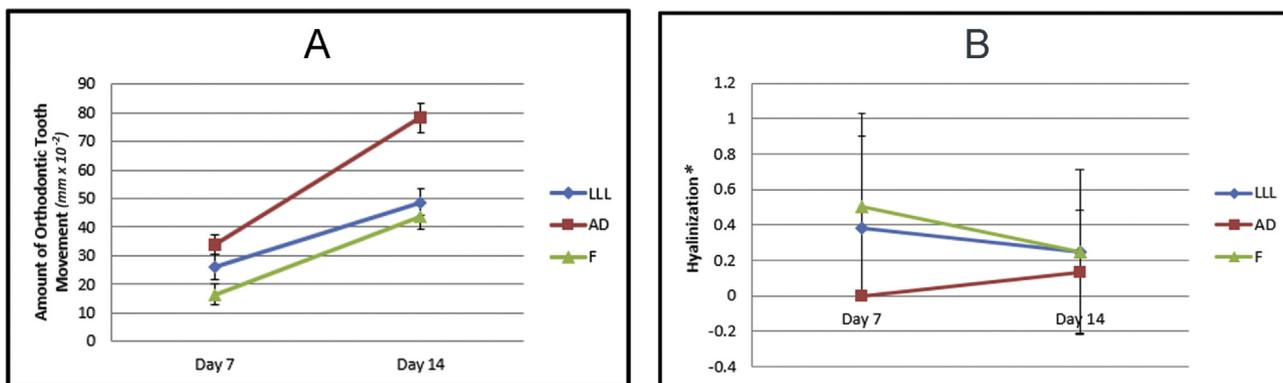


Figure 3 Graphic presentation of the comparison of the amount orthodontic tooth movement (A) and hyalinization (B) of the study groups on days 7 and 14. * Samples with any hyalinization zone was rated as 1 and samples without any hyalinization zone was rated as 0.

group was significantly higher than in the F group, although the difference was reduced when compared to the measurements on day 7 ($p = 0.048$; $p < 0.05$).

Histological evaluation

Osteoclast count

On day 7, the osteoclast count in the F group was significantly lower than in the LLLT ($p = 0.003$) and AD ($p = 0.001$) groups ($p < 0.01$) (Fig. 4). There was no

significant difference between the LLLT and AD groups ($p > 0.05$). Similarly, on day 14 the count in the F group was significantly lower than in the LLLT ($p = 0.048$) and AD ($p = 0.007$) groups ($p < 0.05$; $p < 0.01$) and there was no significant difference between the LLLT and AD groups ($p > 0.05$).

Hyalinization

There was no statistically significant difference between the groups, in the level of hyalinization, on days 7 and 14 ($p > 0.05$) (Fig. 3).

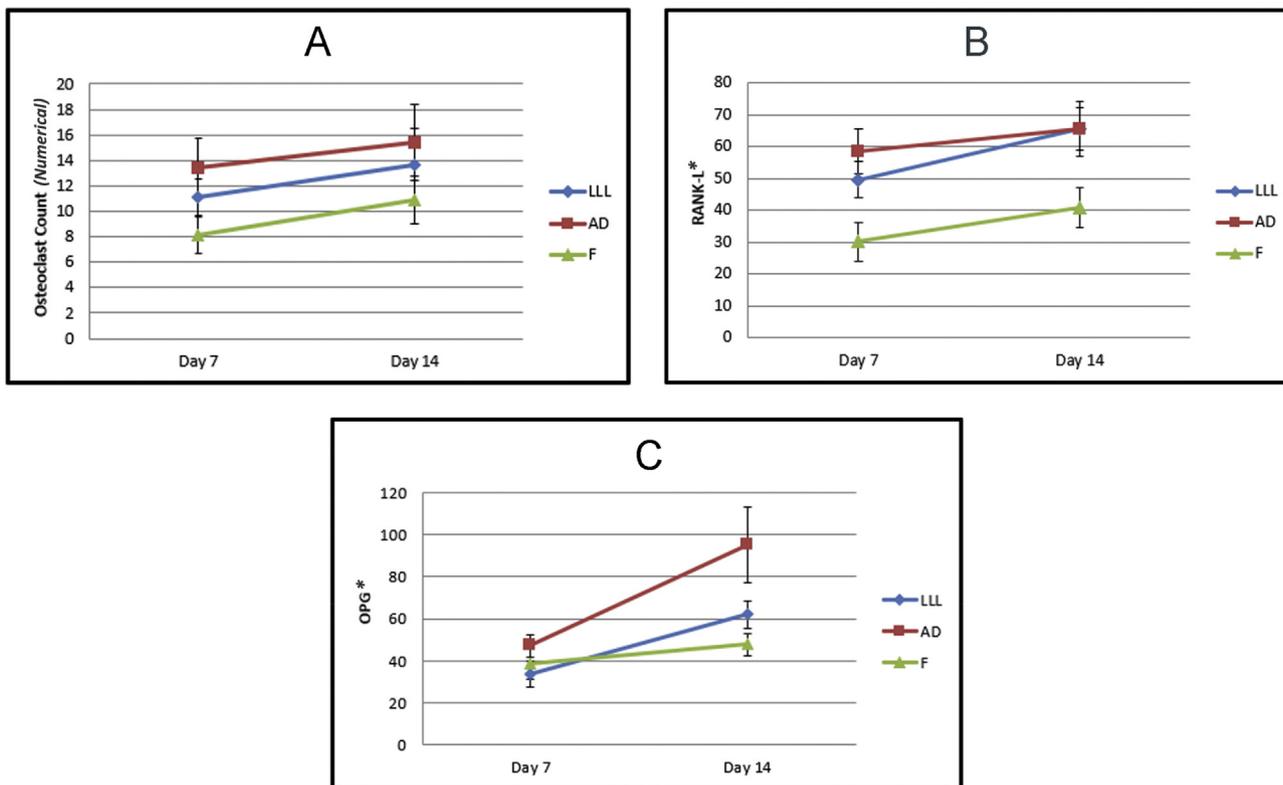


Figure 4 Graphic presentation of the comparison of osteoclast count numbers (A) RANKL expression levels (B) and OPG expression levels (C) on days 7 and 14. * Number of positively-stained cells.

Immunohistochemical evaluation

Receptor activator of nuclear factor kappa-B ligand (RANKL)

The RANKL expression in the AD group on day 7 was significantly higher than in the F ($p = 0.001$) and LLLT ($p = 0.030$) groups ($p < 0.05$) (Fig. 4). When the LLLT and F groups were compared, expression in the LLLT group was significantly higher than in the F group ($p = 0.001$; $p < 0.05$). On day 14, the RANKL expression in the F group was significantly lower than in the LLLT and AD groups ($p = 0.001$; $p < 0.05$) and no significant difference was found between the LLLT and AD groups ($p > 0.05$).

Osteoprotegerin (OPG)

The OPG expression in the AD group on day 7 and 14 was significantly higher than in the F ($p = 0.031$) and LLLT ($p = 0.001$) groups ($p < 0.05$) and there was no statistically significant difference between the F and LLLT groups ($p > 0.05$) (Fig. 4). Similar to day 7, on day 14, the expression in the AD group was significantly higher than in the F and LLLT groups ($p = 0.001$; $p < 0.05$) and there was no significant difference between the F and LLLT groups ($p > 0.05$).

Discussion

Orthodontic tooth movement basically occurs in three phases.^{13,14} In the initial phase, the tooth under displacing forces moves in the direction of the force to the limits of the periodontal space. And thus, the compression and tension of the periodontal membrane triggers regional inflammation. The subsequent phase is the lag phase, during which hyalinization caused by ischemia as a result of compression of the periodontal membrane brings about a pause in tooth movement. In the third phase, the tooth movement starts accelerating following the removal of hyalinized necrotic tissue and this movement turns into a nearly steady amount of displacement in time.

In a study by Cuoghi et al., the periodontal ligament thickness of the mesiobuccal root of the first molar tooth in rats was measured as 0.171 mm.¹⁵ In the present study, the mean tooth movement in the F group on day 7 was found to be 0.164 mm, and when this result is compared with that of Cuoghi et al., it can be concluded that tooth movement in the F group on day 7 was limited to the initial movement within the alveolar socket. This finding shows that, in the F group, the initial tooth movement was followed by the lag phase in the first seven days. However, the level of tooth movement on day 7 was 2.1-fold greater in the AD group and 1.6-fold greater in the LLLT group than in the F group. These findings suggest that the lag phase was by passed or shorter in duration in the AD and LLLT groups. In this study no hyalinization area was observed in the AD group on day 7. However, although statistically not significant, hyalinization areas were observed in both LLLT and F groups to be lesser in the LLLT group. These findings are also supported with recent studies suggesting that tooth movement rate increases with the application of AD by significant reduction of regional bone density, increase in bone metabolism and elimination or reduction of hyalinization.^{2,7,10} Also

regarding LLLT, some studies show that, LLLT enhances alveolar bone metabolism especially in favor of catabolic activity which leads to a decrease in bone mineral density. It is advocated that by this response the resistance of the alveolar bone at the compression site is reduced leading to a decrease in hyalinization areas and root resorption.^{16–18}

In this study, the level of tooth movement on day 14 was 1.8 fold higher in the AD group than in the F group and 1.1-fold higher in the LLLT group than in the F group. These ratios for day 7 were 2.1 and 1.6 respectively. The decline in tooth movement ratios between AD and LLLT groups and the F group, within time, can be attributed to the acceleration of tooth movement in the group F following day 7 (the 3. phase of tooth movement).

When the AD and LLLT groups were compared, the level of tooth movement in the AD group on day 7 was 1.3-fold higher than the level of tooth movement in the LLLT group and the difference increased to 1.6-fold on day 14. These findings suggest that AD is significantly more efficient in accelerating tooth movement in all phases of tooth movement when compared to LLLT. Based on these results it can be concluded that the efficacy of AD in terms of accelerating tooth movement is sustained for a longer period than the LLLT. However, as a limitation of the study, the evaluation of a 14-day period is quite limited for obtaining adequate information on the efficacy of both methods in the long-term.

In the present study, the osteoclast count and RANKL and OPG expressions were evaluated to compare the effects of the AD and LLLT methods on bone metabolism. Recent studies show that both methods have positive effects on the differentiation and maturation of the osteoclasts.^{2,8,12,16,18,19} Similarly in this study, the osteoclast count was significantly higher in both time periods in the AD and LLLT groups when compared to the F group. Although all groups showed the same pattern of change, the differences between the AD and LLLT groups and the F group were highest in the first seven days. This finding suggests that bone catabolism have triggered in an earlier period in the AD and LLLT groups than in the F group. Although statistically not significant, the osteoclast count was found to be higher in the AD group on days 7 and 14 than that in the LLLT group. This finding may suggest that AD is more influential on catabolic activity when compared to LLLT. In accordance with this result, RANKL expression, which induces osteoclast differentiation and activation, was significantly higher in the AD group on day 7, when compared to the other groups. The statistically significant difference in RANKL expression between the AD and LLLT groups disappeared on day 14, but its expression in both groups was still significantly higher than that in the F group. Similar to the osteoclast count, RANKL expression decreased in the AD and LLLT groups between days 7 and 14, and these findings suggest that both methods, particularly the AD method, stimulate catabolic activity, and that the efficacy of these methods declines over time.

OPG, which antagonizes the effects of RANKL, plays a role in reducing osteoclastic activity by inhibiting osteoclast differentiation.^{20,21} Accordingly, continued osteoblastic activity becomes more prominent and regulates bone metabolism. In the present study, OPG expression was found to be significantly higher in the AD group on days 7

and 14 when compared to the expression levels in the other groups, while there was no significant difference between LLLT and F groups. When osteoclast count, RANKL and OPG expressions were evaluated as a whole, both methods were found to accelerate bone metabolism in the early period, and both methods increase the level of tooth movement per unit time. In paired comparisons, AD was found to be more efficient in increasing tooth movement rate and bone metabolism, than LLLT.

One of the main limitations of this study was that the subjects employed were rats and surely their bone metabolism are not identical to humans. However, the use of different methods on the same type of subject has allowed us to compare the investigated parameters. Another limitation of the present study was the limited experimental period. Daily laser irradiations under general anesthesia substantially increase the likelihood of animal death, and so the experimental period in this study was limited to 14 days in order to minimize the number of animals. This study evaluated the efficacy of the investigated methods on the initial phases of tooth movement, while their efficacy in the long term has not been evaluated. Studies are needed to analyze the long-term effects of these methods.

In this study it is found that alveolar decortication and low-level laser application significantly increased the level of tooth movement in the early period through their effects on the bone metabolism. The efficacy of AD and LLLT applications decreased during the experimental period. The comparisons made within the scope of this study show that AD is a more effective method than LLLT application in accelerating tooth movement and alveolar bone metabolism. Although laser applications at frequent intervals and surgical interventions are not preferred methods in the course of orthodontic treatment, they may be preferred in conditions if shortening the treatment period is desired. Accordingly, we believe that the results of the present study, comparing the effects and efficacy of both methods, will be beneficial in the selection of the most appropriate method for a specific patient.

Conflicts of interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jds.2019.08.004>.

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