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Effects of Different Parameters of Diode Laser on Acceleration of Orthodontic Tooth Movement and Its Effect on Relapse: An Experimental Animal Study

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Abstract

AIM: Recent studies have demonstrated that low-level laser therapy (LLLT) can accelerate orthodontic tooth movement. However, there is still controversy about the optimum parameters that can cause acceleration. The present study was designed to examine two different doses of LLLT in the acceleration of orthodontic tooth movement and their effect on relapse.

METHODS: An orthodontic appliance was designed to induce tooth movement on lower incisors of rabbits. The in-al-as diode laser was used to radiate different groups of rabbits according to a specific protocol. The amount of tooth movement was measured for 21 days, and then the orthodontic appliance was removed, and the relapse was measured till day 28.

RESULTS: The amount of tooth movement was significantly greater in the group with irradiation of low dose in comparison with the high dose group and the control group. The relapse was greatest in the group of low dose irradiation and least in the control group.

CONCLUSION: The findings suggest that LLLT with a low dose accelerate the orthodontic tooth movement while LLLT with a high dose was not able to have the same effect. LLLT had a reverse effect on relapse tendency; an increase in the relapse tendency was seen with low dose irradiation.

Introduction

The principle of orthodontics is to apply light pressure on the tooth for a long time, so that bone remodelling occurs and the tooth starts to move [1]. So, the orthodontic tooth movement normally takes a long time. This is usually non-desirable from the patient`s point of view and may also have adverse side-effects as the development of white spot lesions and root resorption. Moreover, the prolonged time of retention after the orthodontic treatment is another concern for the patient.

Previous methods for stimulation of Orthodontic tooth movement (OTM) have been investigated such as drug injections, electric stimulation and ultrasound application. One promising way to enhance tooth movements is the use of low-level laser therapy (LLLT). Many researches support the claim of increased osteoblastic activity following LLLT in vivo [2], [3], [4], [5] and in vitro [6], [7], [8], [9], [10], [11], [12], [13].

Other researchers suggest that bone resorption is the rate-limiting step in tooth movement. So, any procedure which potentiates osteoclastic activity can accelerate the rate of orthodontic tooth movement. Recent studies have indicated enhanced osteoclastic activity after low-level laser therapy in vivo and in vitro [14].

Low-level laser therapy (LLLT) has shown to affect many biological processes in the body and has many beneficial effects including effects on fibroblasts, chondral proliferation, collagen synthesis, nerve regeneration.

However, there is a great controversy in the literature concerning the effective parameters of (LLLT) that produce an acceleration of tooth movement [14] and there are many recommendations for further studies concerning the effective doses. Thus, the primary aim of the present study is to investigate the effect of different parameters of (LLLT) on orthodontic tooth movement.

A long period of retention is necessary to prevent early relapse. Although the reason for early relapse is not fully clear, bone regeneration after orthodontic treatment may affect the post-treatment relapse. It would be beneficial therefore to accelerate bone formation to prevent relapse to abbreviate the retention period [15]. This study will also investigate the effect of (LLLT) on the relapse of orthodontically moved teeth.

Material and Methods

The material and methods of this study were designed according to the ARRIVE guidelines for reporting In Vivo Experiments for animal research. All the checklist items were accurately followed.

Ethical statement

The ethical committee of the National Research Centre in Egypt has approved this research according to the protocol submitted in November 2015, and the approval had the reference number 15083.

Number of experimental and control groups

Forty-five rabbits were randomly divided into three groups according to the treatment modality: Group I, non- irradiation control group (n = 15); Group II, High dose irradiation group (n = 15); Group III, Low dose irradiation group (n = 15). The orthodontic appliance is fixed on the right and left lower incisors of each rabbit so the number of incisors in each group (n = 30).

Timeline of the experiment

The appliance was activated immediately before its fixation on the lower incisors of each rabbit, and a photograph (Fuji film, Finepix, 4X, 8.2 megapixels, Macro mode) is taken before the fixation of the appliance and immediately after the fixation. A scale is added to the photo so that the distance between the incisors can be measured accurately using computer software.

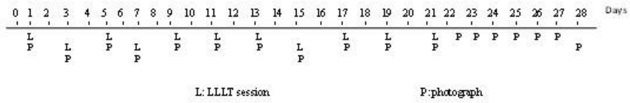

Figure 1: The timeline diagram

Then each rabbit was photographed at days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and the rabbits of group II and III undergone the laser sessions according to the specific protocol of each group at the same days while group I didn't receive any laser.

On day 21 all the appliances were removed, and the teeth were allowed to relapse, and they were photographed every day until day 28 which is the last day of the experiment.

The Orthodontic appliance

An orthodontic appliance was constructed for each rabbit which consisted of:

1. Two bands formed of stainless steel band material with 0.18-inch width and 0.005-inch thickness.

2. Omega loop constructed of stainless steel round orthodontic wire (0.012 inches) welded to the bands.

The bands were custom made on a lower incisor extracted from a rabbit so that all the bands had the same size. All the omega loops were standardised in size and shape and were welded at the middle of each band. (Height of omega loop is 0.4 mm approximately) (Figure 2).

Figure 2: The orthodontic appliance

The omega loops were placed on a grid and activated so that the range of movement was about

3mm. And the force was calibrated with a Stress and Tension gauge (1 pcs/set, Lotus Global Co., Ltd.) to 30 g so that it would be 15 g on each incisor [16], [17], [18]. The orthodontic appliance was activated once just before fixing it in the rabbit's mouth with no further activation during the experiment.

Animal sedation

Intramuscular injection of a mixture of Xylazine 20 mg\kg and Ketamine 50 mg\kg was used to anaesthetize each rabbit.

a) b) c)

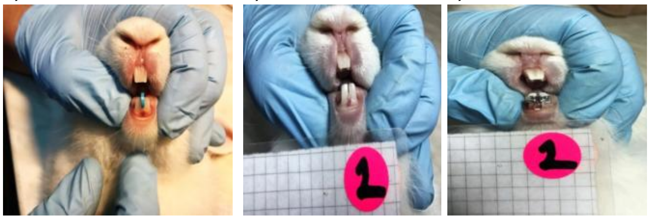

Figure 3: a) Separator between incisors to open space for appliance; b) After separation; c) Orthodontic appliance fixed

After anaesthetising each rabbit, the orthodontic appliance was fixed on the lower incisors of each rabbit using glass ionomer cement (Medicem; Promedica; Dental Material GmbH; Domagkstr.31; 24537 NeumÜnster; Germany) (Figures 3a, 3b and 3c).

Soft Laser application:

Group I (non-irradiation): Did not receive any laser throughout the experiment

Group II (High dose): Received laser with a high dose every other day from day 1 till day 21

Group III (Low dose): Received laser with a low dose every other day from day 1 till day 21

Figure 4: Laser machine Biolase Epic X

The soft laser was applied using Biolase (Epic X) (Figure 6) with active medium InGaAs (indium gallium arsenide) Semi-conductor diode using a probe with spot size area 0.002 cm2. The laser was applied distal to the distal incisal edge of the lower incisor, parallel to the long axis of the tooth and in contact with the distal periodontal pocket of the lower incisor [18] (Figure 4).

Figure 5: LLLT application distal to the right and left incisors

The laser irradiation was done on the lower right, and lower left incisor of each rabbit.

1. Wavelength: 940 ± 10 nm.

2. Power density: Group II (High dose): 250 W/cm^2; Group III (low dose): 250 W/cm^2.

3. Energy density: Group II (High dose): 5000 J/cm^2; Group III (Low dose): 2500 J/cm^2

4. Joules/session: Group II (high dose): 10 J; Group III (low dose): 5 J.

Total energy does: **Group II:** At day 3: 20 J; At day 7: 40 J; At day 15: 80 J; At day 21: 110 J; **Group III:** At day 3: 10 J; At day 7: 20 JAt day 15: 40 J; At day 21: 55 J.

5. Continuous wave

Experimental animals

The study included 45 white New Zealand rabbits. The rabbits used were males about 14 weeks and the average weight of 2 kg ± 100 gm. They were provided by the Animal House of the National Research Centre.

The orthodontic appliance was fixed on the lower incisors of each rabbit.

Housing and husbandry

All animals were housed in a 12-h light/dark environment at a constant temperature of 23 C and fed a standard pellet diet with tap water and libitum. The rabbits were kept in individualised cages, and special caretakers, as well as the veterinarian specialist of the animal house, is monitoring the animals around the clock, in regular shifts.

Sample size determination

Based on the assumption of normal distribution and equal variances of the amount of movement variable, assumed standard deviation of 1.2 and means equal 9.25 and 7.84 for the null and alternative hypothesis, Using ANOVA test, the required sample size is 12 rabbits in each group for a confidence level 95%, at alpha = 0.05 and power = 0.80[10]. To consider for drop- out, 15 rabbits per group was advised. So the total number of rabbits in the three groups was 45 rabbits.

Allocating animals to different groups

The animals were randomly divided using the online computer program Random sequence generator; random.org, into three groups. Groups I (non-irradiation control group), Group II (High dose), and Group.

(Low dose) Served as experimental groups containing 15 rabbits each. This randomisation was performed by a researcher who didn`t participate in the rest of the study.

In the first group, the orthodontic appliance was fixed on lower incisors for orthodontic tooth movement. In the second and third groups, the orthodontic appliance was fixed on the lower incisors, and laser application was made with different doses.

Blinding

Blinding of the operator was not possible due to the nature of the experiment, but blinding was done with the assessor doing the postoperative assessment.

Assessment of the rate of tooth movement

Each rabbit was photographed using a digital camera (Fuji film, Finepix, 4X, 8.2 megapixel, Macro mode) and a scale (Figure 6a), before the fixation of the appliance, immediately after fixation and at days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 22, 23, 24, 25, 26, 27 and 28.

a) b) c)

Figure 6: a) Photo was taken using a digital camera and a scale; Image converted into actual scale image; c) Lines showing the exact measurement of the space

The photographs were assessed using computer software (Corel Draw X6, Corel photopaint X6, Autocad) to exactly measure the distance moved by each tooth on these days (Figures 6b and 6c).

Statistical methods

Statistical analysis was done between groups comparing different variables using ANOVA test SPSS version 20. A p-value < 0.05 was accepted as statistically significant.

Results

Forty-five white New Zealand rabbits were included in this study. The study period lasted for twenty-eight days. During this period, the animals received veterinary care and were housed under the same environmental conditions. The animals lost some of their body weight during the first week then weight regain started during the second week and continued until the end of the experiment.

The rate of tooth movement

Days from zero to 3

The mean distances during the first 3 days were: (0.58 ± 0.39 mm, 0.62 ± 0.35 mm, 0.51 ± 0.28) for groups I, II, and III respectively.

The rate of tooth movement was highest in group II (high dose) than group I (no laser) then group III (low dose). Statistically, there was no significant difference (p > 0.05) between the three groups during the first 3 days (Figure 7).

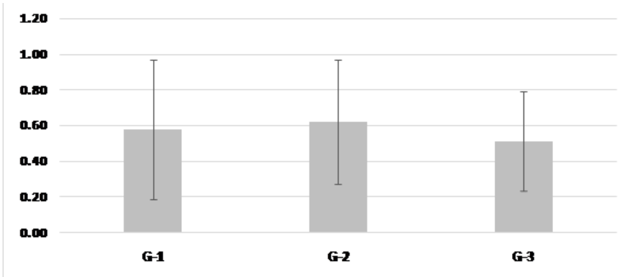

Figure 7: Mean (SD) of the distance from 0 to 3 days

Days from 3 to 7

The mean distances from day 3 to 7 were: (1.06 ± 0 mm, 1.02 ± 0.03 mm, 1.11 ± 0.11) for groups I, II and III respectively.

The rate of tooth movement was highest in group III then the group I than group II. Statistically, there was no significant difference (p>0.05) (Figure 8).

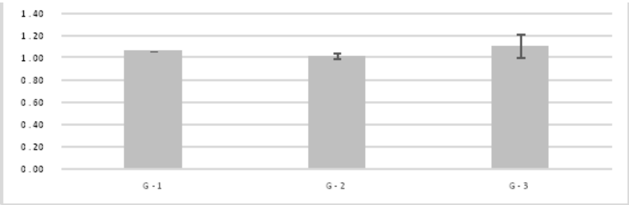

Figure 8: Mean (SD) of the distance from 3 to 7 days

Days from 7 to 15

The mean distances from day 7 to 15 were: (1.15 ± 0.07 mm, 1.09 ± 0.05 mm, 1.47 ± 0.13) for groups I, II and III respectively.

The rate of tooth movement was highest in group III then I then II. Statistically, there is no significant difference between group I and II (p1 > 0.05 (0.67). There is a significant difference between group II and III (p2 < 0.05 (0.001). There is a significant difference between group I and III p3 < 0.05 (0.002) (Figure 9).

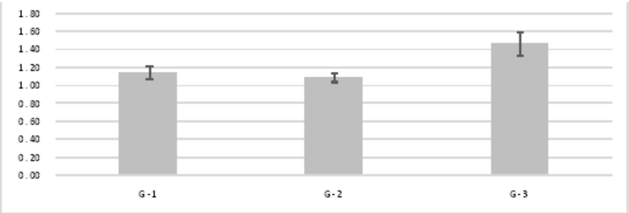

Figure 9: Mean (SD) of the distance from 7 to 15 days

Days from 15 to 21

The mean distances from day 15 to 21 were: (1.10 ± 0.05 mm, 1.11 ± 0.08 mm, 1.40 ± 0.07 mm) for groups I, II and III respectively.

The rate of tooth movement was highest in group III then II then I. Statistically, there is no significant difference between group I and II (p1 > 0.05 (0.93). There is a significant difference between groups II and III (p2 < 0.5(0.0004). There is a significant difference between groups I and III (p3 < 0.5 (0.0003) (Figure 10).

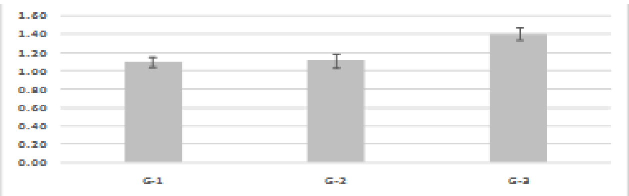

Figure 10: Mean (SD) of the distance from 15 to 21 days

Relapse: (After appliance removal)

Days from 21 to 28

The mean relapse distance from day 21 to 28 was: Group I: -0.41 mm, Group II: -0.6 mm, Group III: -0.74 mm.

Mean percentage relapse: Group I: 46%, Group II: 46.88%, Group III: 56% (Figure 11).

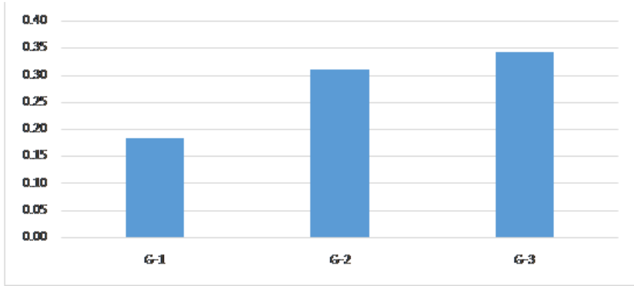

Figure 11: Coefficient of variation between the three groups during relapse

Discussion

It is beneficial to decrease the duration of orthodontic treatment as reflectively this can decrease the side effects as root resorption and white spots. Furthermore, it can increase the patient's compliance and satisfaction.

Orthodontic literature presents different methods to stimulate bone remodelling such as, drug injections like prostaglandin [19] and osteocalcin [20] that could be associated with pain and discomfort [21]. Electric stimulation [22], and ultrasound application are other methods [23] which need sophisticated apparatus and demands applications for the long term to achieve its therapeutic effects [24].

LLLT, a non-invasive and simple method has been recently investigated in literature for accelerating tooth movement and shows promising results [25]. There are only a few experimental studies concerning the optimal laser dose for biostimulation of orthodontic tooth movement.

The present study examined the biological response to LLLT on the speed of orthodontic tooth movement using two different dosages. Recently, a systemic review compared 11 studies of LLLT in animal models of OTM rates and concluded that different laser protocols could increase OTM rates [14], with total energy per tooth ranging from 0.5 J to 204.5 J and energy density ranging from 0.05 J/cm^2 to 4821.4 J/cm^2. In our study, two doses were examined which were the high dose given to group II (10 J per tooth and energy density 5000 J/cm^2), whereas the low dose given to group III was (5 J per tooth and 2500 J/cm^2).

Rabbits were used as the experimental animals in this study. Rabbits were chosen as they have lower central incisors of a good size so that the orthodontic appliance can be installed easily. Also, the structure of their oral tissues has close similarity to humans. Amongst various strains, New Zealand white strains of rabbits are commonly being used for research activities. These strains are less aggressive and have fewer health problems as compared with other breeds [26]. They are also genetically closer to humans than rodents.

The fixed orthodontic appliance used in this study was designed to produce a reciprocal force on the right and left lower first incisors, producing distal tipping movement of both incisors. This was by Altan 2012 [16] and Eldakroury 1998 [17].

The reciprocal force used on the lower incisors of each rabbit was 30 cN force so that it would be 15 cN on each incisor and this was by Altan 2012 [16] and Ren 2004 [18]. The split-mouth technique was not used in this study to avoid the systemic effect of phototherapy [27] that may give misleading results.

The activation of the orthodontic appliance was done once at the beginning of the study (just before the appliance fixation), and no further activation was done throughout the study period, and this meant a decrease in the amount of force throughout the study period.

Indium Aluminum Arsenide (In-Al-As) semiconductor diode laser (940 nm) was used in this study as currently, most commercially available lasers are characterized by a wavelength located in the near infrared band (790-1064 nm) very versatile - although most studied lasers are those that emit the visible red spectrum (635-685 nm) [14].

In a penetration test done by (Li-Fang Hsu 2018) [27] showed that at this longer wavelength 970 nm infrared light provides deep penetration, without being completely absorbed by 2 mm bone slice before reaching periodontal ligament and deeper bone tissue.

The results of the rate of tooth movement were compared among the three groups for the period of tooth movement and then the relapse period.

The tooth movement period lasted for 21 days then the orthodontic appliances were removed and the teeth allowed to relapse till day 28 which is the last day of the experiment.

Although there is a widespread acceptance for the use of LLLT in the clinical setting, there is still a lack of scientific evidence and insufficient guidelines in the use of the most effective parameters for different laser treatments. The laser biostimulation effect depends on laser units and doses that are continuously changed to reach the optimal treatment parameters in both in-vitro and in-vivo conditions [28]. Karu conducted a series of experiments that demonstrated that biologic stimulation followed dose dependency [29]. This was exhibited through bell-shaped curves where every laser wavelength produced a maximum stimulation at a specific dose. Doses greater and less than the optimal resulted in less stimulation. These bell-shaped curves are indicative of bio-stimulation's dose dependency. Application dose measures the amount of energy applied at any one given treatment and is measured in joules (J). To determine the application dose, or energy dose, delivered during one treatment session, the power output is multiplied by the time of application. Also, the treatment dose, or total energy dose, is an additive or accumulative value combining the energy delivered over the entire length of treatment. If a patient receives an application dose of 1 J every week for eight weeks, their total treatment dose would be 8 J [25].

The present findings suggest that low-level laser therapy with low dose significantly increased the rate of orthodontic tooth movement compared to the LLLT with a high dose and the control group that did not receive any laser therapy.

Twenty-one studies have been reported on how LLLT affects the rate of orthodontic tooth movement. Nine of them were clinical studies [30], [31], [32], [33], [34], [35], [36], [37], [38] and twelve were on experimental animals [15], [16], [25], [39], [40], [41], [21], [27], [42], [43], [44], [45].

However, making a direct comparison between studies is complicated by some factors. The varying experimental designs, varying laser parameters including laser wavelength, power output, mode of delivery, power density, energy density, the number of applications, the time separating each application, and the length of the experiment. In addition to the different animal models used as cats, rats, rabbits, dogs, and humans. So in our discussion, we will discuss our results in comparison with the twenty-one studies according to the total energy dose given in periods 3, 7, 15 and 22 days.

Experimental animal studies

Days from 0 to 3

Group II received 20 J total energy dose while group III received 10 J.

There was no significant difference between the 3 groups at this period, although the rate of tooth movement was highest in group II (High dose) than group I (control) then group III (low dose). These results agreed with Marquezan 2010 [42] and Rowan 2010 [25] and disagreed with Kawasaki and Shimizu 2000 [21], Duan 2012 [39], Yamaguchi 2010 [45] and Fujita 2008 [40] who found a significant increase in the rate of OTM. The reason for this disagreement may be because the total energy dose (20 J and 10 J) was still insufficient to cause biostimulation in comparison to these studies where the total energy dose was (162 J and 216 J) except for the study of Duan 2012 [39] who had 6.48 J total dose and still caused significant increase.

Days from 3 to 7

Group II has received total energy dose 40 J while group III 20 J.

Statistically, there was no significant difference in the rate of OTM between the three groups although it was highest in group III then I then II. These results agreed with Altan 2012 [16], Marquezan 2010 [42] and Rowan 2010 [25].

These results disagreed with Yamaguchi 2010 [45], Fujita 2008 [14] who found a significant increase in the rate of OTM (1.3 fold) at day 7 with total energy dose 432 J. And also disagreed with Duan 2012 [39] who found significant increase at day 7 with 6.46 J.

Days from 7 to 15

Group II has received total energy dose 80 J while group III has received 40 J.

Statistically, there is a significant increase in the rate of OTM III (low dose) and group II (High dose) and between group III and the control group, while there is no significant difference between group III and the control group although the rate was higher in the control group. These results agreed with Duan 2012 [39], Shirazi 2013 [44] and Kawasaki 2000 [21]. These results disagreed with Seifi 2007 [43], Rowan 2010 [25] and Li-Fang Hsu 2018 [27] who found no significant difference at the same period of the experiment.

Days from 15 to 21

Group II has received total energy dose 110 J while group III has received 55 J.

Statistically, there is a significant increase in the rate of OTM between group III and group II and the control group. There is no significant difference between group II and the control group. These results agreed with Goulart 2006 [41] and disagreed with Rowan 2010 [25].

These disagreements may be related to many factors, the different animal models used as rats, rabbits and dogs, the different study designs used, some tried to move molars, and others worked on upper incisors or lower incisors, the different designs of the orthodontic appliance used and the different amount of forces used. Moreover, the different laser parameters used as different wavelengths, energy densities, power densities, total energy dose, time of application and points of application, the duration of the experiment, the frequency of LLLT sessions and the different methods of measurements used.

Clinical Studies

Our results agreed with Gene 2012 [34] where the patient received at day 3: 4 J, at day 7: 6 J, at day 14: 8 J and at day 21: 10 J. And he found significant increase at days: 7, 14, 21, 28 and 35. Also agreed with Youssef 2008 [38] who found a significant increase at day 21 with total energy dose 32 J.

For the following studies, the study lasted for more than one month, and the measurements were done after 1 month. Our results agreed with Dosh-Mehta 2011 [33], Sousa 2011 [37], Cruz 2004 [31] and Dominguez 2013 [32] who all found a significant increase with total energy dose 320 J, 18 J, 8 J, 648 J per month respectively. And disagreed with Kansal 2014 [35], Altan 2014 [30] where all didn't find any significant change with total energy dose 69 J, 8.4 J and 30 J per month respectively.

The second part of our study aimed to investigate the effect of LLLT on the rate of relapse of orthodontically moved teeth. It started at day 21 of the experiment. The orthodontic appliance was removed at day 21, and the teeth were allowed to relapse till day 28 of the experiment. The amount of relapse was measured every day.

In group I the relapse was rapid at the first day after removal of the appliance, while in group II the relapse was rapid at the third day of appliance removal and in group III the relapse was rapid at day 2 and more rapid at day 3 of appliance removal.

At the end of the relapse period; by day 28 the mean percentage of relapse was measured as Group I (46%), Group II (46.88%) and Group III (56%). These results agreed with Kim 2010 [3] and disagreed with Franzen 2014 [46].

Relatively few studies have been carried out on the effect of LLLT on orthodontic relapse. Kim et al. studied the effects of LLLT on relapse and retention of rat molars and concluded that LLLT administered with retention facilitated collagen synthesis contributing to faster repair of damaged PDL tissue and better retention, while irradiation performed without retention in place would lead to an increased rate of relapse due to increased catabolic metabolism of collagen. So, LLLT appears to decrease orthodontic relapse but cannot inhibit it, so the use of retainers is mandatory after removal of the orthodontic appliance with the help of LLLT may decrease the period required for retention.

In conclusion, acceleration of orthodontic tooth movement using LLLT is dose-dependent, very low dose or very high dose will not cause acceleration**.** LLLT with the optimum dose can be used during retention to decrease the retention period, but a retainer should be used. Otherwise the relapse tendency would increase.

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