View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Biblioteca Digital da Produção Intelectual da Universidade de São Paulo (BDPI/USP)

Universidade de São Paulo

Biblioteca Digital da Produção Intelectual - BDPI

Departamento de Odontopediatria , Ortodontia e Saúde Coletiva - Artigos e Materiais de Revistas Científicas - HRAC FOB/BAO

2013-11

Influence of low-level laser on bone remodeling during induced tooth movement in rats

Angle Orthodontist, Appleton, v. 83, n. 6, p. 1015-1021, Nov. 2013.
http://www.producao.usp.br/handle/BDPI/46305

Downloaded from: Biblioteca Digital da Produção Intelectual - BDPI, Universidade de São Paulo

Original Article

Influence of low-level laser on bone remodeling during induced tooth movement in rats

Eliziane Cossetin[a]; Guilherme Janson[b]; Maria Goretti F. de Carvalho[c]; Rejane A. de Carvalho[d]; José Fernando Castanha Henriques[e]; Daniela Garib[f]

ABSTRACT

Objective: To analyze the effect of low-level laser on bone remodeling during induced tooth movement in rats.

Materials and Methods: A diode laser (808 nm, 100 mW, 54 J on an area of 0.0028 cm^2) was used. The application was continuous, punctual, and with contact. Forty-two 70-day-old Wistar rats had the maxillary left first molar moved using a force level of 25 g. In two experimental subgroups the movement was performed over 7 days and in three subgroups the movement occurred over 14 days. In the 7-day movement subgroups, one subgroup received laser irradiation on day 1 only; the other subgroup received laser irradiation on days 1, 3, and 5. In the 14-day movement subgroups, one subgroup received laser irradiation on day 1 only; the second on days 1, 3, and 5; and the third on days 1, 3, 5, 7, 9, 11, and 13. The control group was also divided into two subgroups, and movement occurred over two different periods of treatment (7 days and 14 days) without laser application; these were used as controls for the respective experimental subgroups. Inter-subgroup comparison was performed with Kruskal-Wallis, followed by Mann-Whitney and analysis of variance, followed by Tukey tests within the 7- and 14-day subgroups.

Results: The subgroup with three laser applications showed significantly greater osteoclastic activity and bone resorption than the other subgroups in the 7-day movement subgroups.

Conclusions: Low-level laser application significantly increased the osteoclastic but not the osteoblastic activity during the initial phases of tooth movement. In addition, the osteoclastic activity was dose-dependent. (*Angle Orthod.* 2013;83:1015–1021.)

KEY WORDS: Experimental animal model; Laser therapy; Orthodontic movement

INTRODUCTION

Low-level laser (LLL) has demonstrated analgesic, anti-inflammatory, and biostimulatory effects.[1] Among all methods studied to accelerate induced dental movement and consequently decrease orthodontic treatment time, low-level laser is minimally invasive, extremely simple, safe, and fast to apply.[2] In spite of these advantages, studies on low-level laser have shown contradictory findings. Although some studies showed an increase in osteoclastic activity or tooth movement with low-level laser,[3–5] others found no differences between irradiated and nonirradiated groups,[6,7] and some concluded that the speed of tooth movement decreased in lased compared with non-lased samples.[8]

Because of the aforementioned divergent results, the aim of this study was to analyze the influence of low-level laser application on osteoclastic and osteoblastic activities and on degree of bone neoformation during induced tooth movement in Wistar rats.

[a] Graduate student, Department of Orthodontics, Bauru Dental School, University of São Paulo, Bauru, São Paulo, Brazil.

[b] Professor, Department of Orthodontics, Bauru Dental School, University of São Paulo, Bauru, São Paulo, Brazil.

[c] Professor, College of Medicine, Potiguar University, Natal, Rio Grande do Norte, Brazil.

[d] Professor, Department of Operatory Dentistry, Potiguar University, Natal, Rio Grande do Norte, Brazil.

[e] Professor, Department of Orthodontics, Bauru Dental School, University of São Paulo, Bauru, São Paulo, Brazil.

[f] Associate Professor, Department of Orthodontics, Bauru Dental School, University of São Paulo, Bauru, São Paulo, Brazil.

Corresponding author: Dr Eliziane Cossetin, Department of Orthodontics, Bauru Dental School, University of São Paulo, Alameda Octávio Pinheiro Brisolla 9-75, Bauru - SP - 17012-901, Brazil
(e-mail: elizianecv@gmail.com)

Accepted: March 2013. Submitted: October 2012.
Published Online: May 14, 2013
© 2013 by The EH Angle Education and Research Foundation, Inc.

Table 1. Specimen Distribution According to Group, Number of Laser Applications, and Time of Humane Killing

Subgroup[a]	Laser Frequency	No. of Laser Administrations	Day Killed
C7d	No administration	0	Day 7
I1ap7d	Day 1 only	1	Day 7
I3ap7d	Days 1, 3, and 5	3	Day 7
C14d	No administration	0	Day 14
I1ap14d	Day 1 only	1	Day 14
I3ap14d	Days 1, 3, and 5	3	Day 14
I7ap14d	Days 1, 3, 5, 7, 9, 11, and 13	7	Day 14

[a] C7d indicates control subgroup, 7-day tooth movement; I1ap7d, experimental subgroup that received one laser application over 7 days; I3ap7d, experimental subgroup that received three laser applications over 7 days; C14d, control subgroup, 14-day tooth movement; I1ap14d, experimental subgroup that received one laser application over 14 days; I3ap14d, experimental subgroup that received three laser applications over 14 days; I7ap14d, experimental subgroup that received seven laser applications over 14 days.

MATERIALS AND METHODS

Permission to conduct this study was granted by the Ethics Committee in Animal Experimentation of Potiguar University (RN – Brazil). Forty-two 70-day-old female Wistar rats weighing 170–190 g were used for this experiment. During the experimental period, the animals remained inside appropriate cages at a constant temperature ranging between 23°C and 25°C, in a 12-hour light/dark environment and provided with food and water ad libitum.

The animals were divided into two groups: the experimental, or irradiated group (I), which had 30 rats, and the control group (C), which had 12 rats. The experimental group was divided into five subgroups containing six rats each, according to laser irradiation frequency and duration of treatment (Table 1). In two experimental subgroups, movement was induced over 7 days, and in the remaining three subgroups, movement was induced over 14 days. In the two 7-day movement subgroups, one received laser irradiation on day 1 only, and the other was irradiated on days 1, 3, and 5. In the three 14-day movement subgroups, one received laser irradiation on day 1 only; the one on days 1, 3, and 5; and one on days 1, 3, 5, 7, 9, 11, and 13. The control group was also divided into two subgroups of six rats each. In these subgroups movement was also induced for two different periods of time (7 days and 14 days) but these rats received no laser application (Table 1). All procedures were carried out under general anesthesia, with 0.3 mL/100 g body weight intramuscular injection of tiletamine chlorhydrate 125 mg/zolazepam chloridrate 125 g (Zoletil 50, Virbac, São Paulo, Brazil).

A modified model described by Heller and Nanda[9] was used to move the maxillary left first molar in both groups. Tooth movement was performed by means of nickel titanium closed coil springs (Morelli, Sorocaba, Brazil) using both maxillary central incisors as anchorage. The closed coil spring characteristics were standardized at 0.25 mm of wire diameter, 0.76 mm internal diameter, and 7 mm total length. The coil was fixed to the teeth with a 0.25-mm stainless steel wire ligature. To calibrate the force magnitude, the spring was fixed to the first molar above the proximal contact point. The closed coil spring was stretched until a force of 25 g was achieved before fixation around both maxillary incisors. The teeth were covered by photocured resin around the ligature wire to improve coil spring retention (Figure 1).

Gallium-aluminum-arsenide laser (Whitening Laser II – DMC, São Carlos, SP, Brazil) was used to generate low-level laser irradiation. The wavelength was 808 nm (infrared laser), and a continuous emission regimen was used. The output power was set to 100 mW, the optic fiber diameter corresponded to 0.6 mm, and the energy density was 642 J/cm²/point (Table 2). Dosimetry was obtained by the following formula:

$$(J/cm^2) = \left(\frac{P(W) \times T(s)}{A(cm^2)}\right),$$

considering area (A) as $\pi \times R^2$ (radius of the optic fiber active point). Following the protocol used by Kawasaki and Shimizu,[3] irradiation was applied in three points by the punctual method with 3 minutes of contact for each point, totaling 9 minutes. The application points were the buccal, palatal, and mesiocervical aspects of the first left maxillary molar. Laser was applied 1, 3, or 7 times in each animal during the experimental period,

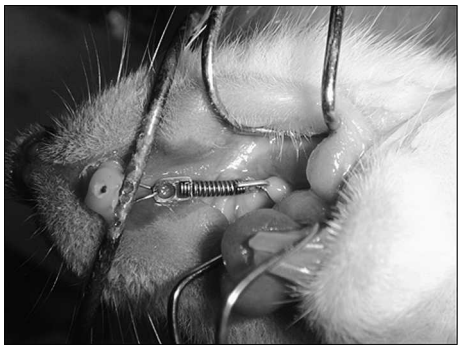

Figure 1. Appliance used to move the maxillary left first molar.

Table 2. Phototherapy Parameters

Phototherapy Parameters	Values
Energy density	1926 J/cm^2
Energy	54 J
Output power	100 mW
Wavelength	808 nm
Color	Invisible
Emition regimen	Continuous
Optic fiber diameter	0.6 mm
Distance of application	In contact/punctual
Time	3 min/3 points of application (9 min total)

with 48-hour intervals, according to the subgroup (Table 1).

The animals were humanely killed in a carbonic gas chamber 7 or 14 days after force application. Their heads were submerged in 10% formaldehyde solution for 48 hours. After fixation, the samples were decalcified using 7.5% nitric acid for 5 days. The left maxillary hemi-arches were then divided and embedded in paraffin, sectioned with a rotary microtome of 4 μm in thickness, perpendicular to the occlusal plane of the first molar up to the radicular pulp level. Finally, the samples were stained with hematoxylin and eosin.

Histologic evaluation was performed using a binocular microscope (CX31, Olympus, Tokyo, Japan). The blades were photographed using a digital camera (Olympus) connected to a computer. Two examiners who were blinded to the study groups performed the readings.

The analyzed area corresponded to the inter-root region, especially the distal aspect of the mesial root, the mesial aspect of the distal root, and under the furcation. For interpretation, the same parameters were performed for histologic graduation in the experimental and control groups. The most evident manifestation of the cellular events in each specimen was recorded. The presence of active osteoblastic and osteoclastic cells and the amount of alveolar bone in the inter-root region were analyzed. Osteoblastic activities were interpreted by counting the young cells that had a cuboid shape in bone surfaces and that presented basophilic cytoplasm and polarized nucleus, arranged in palisade, in two fields of large magnification; these were classified as low (1 to 10 cells), moderate (11 to 25 cells), or intense (more than 25 active cells) (Figure 2). The osteoclasts were considered when their outline was irregular, filling the Howship's lacuna, or near bone; the activity was registered as low (maximum of three osteoclasts per region), moderate (four to six cells), and intense (more than six, cells) (Figure 2). These analyses were performed in three inter-root regions (distal root, mesial root, and furcation).

A 10× magnification ocular lens placed on the right ocular with a micron graduated, 1 mm long ruler

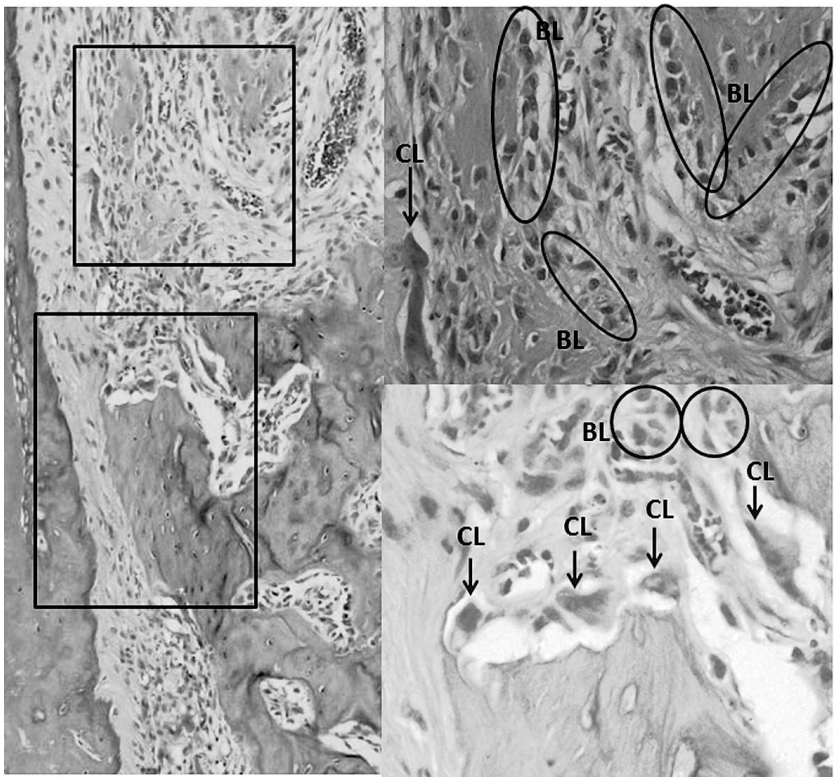

Figure 2. Photomicrography for histologic evaluation. Osteoblasts (BL in circle) and osteoclasts (CL, arrows). Hematoxylin and eosin 400×.

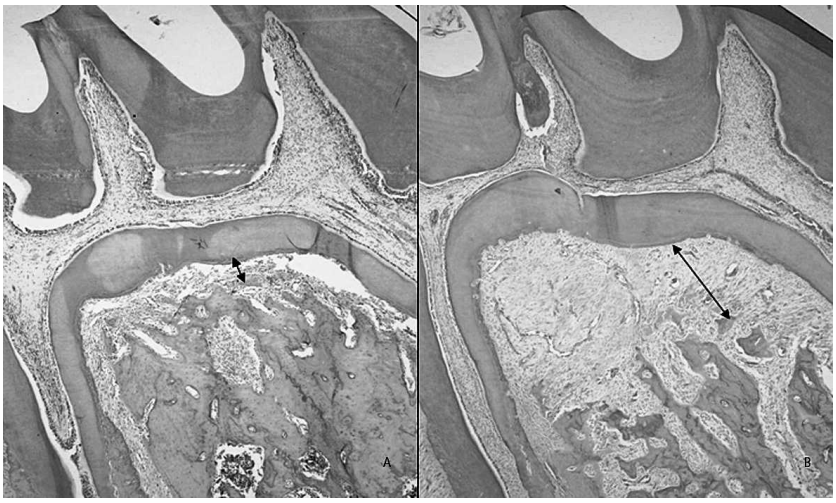

Figure 3. Photomicrography of the distance between the furcation region and alveolar bone (arrow). (A) Specimen of the subgroup C7ap; hematoxylin and eosin (HE) 40×. (B) Specimen of the experimental subgroup that received three laser applications over 7 days (I3ap7d); HE 40×.

(1/0.01 mm of graduation) was used to measure the distance from the furcation wall to the nearest vertical alveolar bone present. The smallest measurement in each blade was recorded, and the greater the distance, the greater the bone loss (Figure 3).

Statistical Analyses

Inter-subgroup comparisons of the magnitude of osteoclastic and osteoblastic cellular activity were performed with Kruskal-Wallis tests, followed by Mann-Whitney tests, for the animals in which movement was induced for 7 and for 14 days and their respective control subgroups. Analysis of variance and then Tukey tests were performed to compare the distances between the furcation and the inter-root alveolar bone within the 7- and 14-day movement subgroups and their respective control subgroups. Results were considered statistically significant at $P < .05$.

RESULTS

Among the 7-day tooth movement subgroups, the experimental subgroup that received three laser applications (I3ap7d) had a significantly greater osteoclastic cellular activity than the control group (C7d) and the experimental subgroup that received one laser application (I1ap7d) (Table 3). There was no inter-subgroup difference among the 14-day tooth movement subgroups (Table 4).

Among the 7-day tooth movement subgroups, the experimental subgroup with three laser applications (I3ap7d) had a significantly greater amount of bone loss than the other groups (Figure 3; Table 5). There was no inter-subgroup difference among the 14-day tooth movement subgroups (Table 6).

DISCUSSION

Decalcification with 7.5% nitric acid in the determined time and concentration was adequate to prevent structural cellular changes.[10] Cell marking was not necessary because it is perfectly possible for an experienced examiner to count them by observing their characteristics as described in the methodology.[11]

The dosimetry used in this study was similar to the one proposed by Kawasaki and Shimizu,[3] but the frequency of applications and force magnitude were distinct. Kawasaki and Shimizu used a daily frequency of laser application, whereas in this research the irradiations consisted of one, three, and seven applications within a 48-hour interval (Table 1). Twenty-five grams of force were applied instead of 10 g[3] and this

Table 3. Cellular Activity in the 7-Day Tooth Movement Subgroups (Kruskal-Wallis Followed by Mann-Whitney Tests)[a]

	C7d (N = 6)			I1ap7d (N = 6)			I3ap7d (N = 6)			
	Median	25%	75%	Median	25%	75%	Median	25%	75%	*P
Osteoclasts	1.5[y]	1.0	2.0	1.0	1.0[y]	2.0	2.0[z]	2.0	3.0	.0057
Osteoblasts	2.0	1.0	2.0	2.0	1.0	2.0	1.0	1.0	1.0	.253

[a] C7d indicates control subgroup, 7-day tooth movement; I1ap7d, experimental subgroup that received one laser application over 7 days; I3ap7d, experimental subgroup that received three laser applications over 7 days.

* Statistically significant at $P < .05$. Different letters represent statistically significant differences.

Table 4. Cellular Activity in the 14-Day Tooth Movement Subgroups (Kruskal Wallis Test)[a]

	C14d (N = 5)			I1ap14d (N = 5)			I3ap14d (N = 5)			I7ap14d (N = 5)			
	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	*P*
Osteoclasts	1.0	0	1.0	1.0	0	1.0	0	0	0.1	1.0	1.0	2.0	.429
Osteoblasts	1.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0	.824

[a] C14d indicates control subgroup, 14-day tooth movement; I1ap14d, experimental subgroup that received one laser application over 14 days; I3ap14d, experimental subgroup that received three laser applications over 14 days; I7ap14d, experimental subgroup that received seven laser applications over 14 days.

difference may have exacerbated the laser stimuli. Twenty-five grams of force for moving rat teeth is the amount often found in the literature.[12] Therefore, the additional laser stimuli may be responsible for the differences between bone resorption and bone formation in our results.

Tissue changes were greater on the mesial aspect of the distal root than on the mesial aspect of the mesial root. This finding was previously reported.[13] Therefore, it was decided to analyze the region under the furcation of the mesial aspect of the distal root (compression) and of the distal aspect of the mesial root (tension), instead of the both aspects of the same root as other authors had done,[3] enabling simultaneous visualization of the bone remodeling events (Figure 3).

Our results showed that osteoclastic activity was influenced by laser phototherapy, demonstrating greater stimulation with the increase of application frequency, in the 7-day experimental subgroups (Tables 3 and 4). The osteoclasts appeared in greater amounts in the 7-day period and decreased after 14 days of force application. These findings suggest that laser is capable of activating the pre-osteoclasts from the periodontal ligament to become mature but does not induce bone marrow cells to differentiate into new pre-osteoclasts fast enough. Fujita and colleagues[14] also found stimulation only in the early stages. It seems that when the pre-osteoclast cells present in the ligament come to an end, the laser effect in the process of bone resorption is inexpressive. In this way, laser should ideally be recommended only at the initial period of force application, as demonstrated in our findings and according to the literature.[15] It may be reasonable to assume that the effect of laser in stimulating the osteoclasts is dependent on the number of existing pre-osteoclasts, once the laser increases the speed in which these cells are activated, as has also been previously demonstrated.[3]

Osteoblasts were not significantly influenced by low-intensity laser in the dosage used in this study (Tables 3 and 4). The 7-day experimental subgroup that received three laser applications showed the numerically smallest osteoblastic activity. There was a similar response in the 14-day experimental subgroups. It has been previously demonstrated that laser does not have a significant effect on osteoblastic proliferation or activation, and is only beneficial to maintain cellular viability.[16] On the other hand, a positive result of laser osteoblastic stimulation was found in other studies.[17] These facts suggest that there is a limit of stimulation for osteoclastic and osteoblastic cells, or even an ideal dosage for each cell type that cannot be surpassed to achieve a stimulatory response, as has already been demonstrated in the literature.[18]

The result in this study was bone loss with consequent increase of the connective tissue area between the furcation wall and the inter-root alveolar bone during the experiment, which was more intense in the experimental subgroup that received three laser applications over 7 days (I3ap7d; Table 5). The increase of connective tissue area means that bone resorption was larger than bone apposition, or bone apposition was not fast enough to balance bone remodeling with the applied dosage in the first 7 days of movement (Figure 3). Therefore, considering that bone remodeling for tooth movement depends on the synchronized activity of both cells, it is not practical that different ideal dosages are necessary to stimulate each cell type. The explanation for no significant

Table 5. Bone Remodeling Represented by the Distance Between Furcation (F) and Alveolar Bone (AB) in the 7-Day Subgroups (ANOVA Followed by Tukey Tests)[a]

	C7d (N = 6)		I1ap7d (N = 6)		I3ap7d (N = 6)		
	Mean	SD	Mean	SD	Mean	SD	**P*
F – AB (μm)	55.5[y]	21.23	71[y]	15.47	161[z]	16	.000

[a] C7d indicates control subgroup, 7-day tooth movement; I1ap7d, experimental subgroup that received one laser application over 7 days; I3ap7d, experimental subgroup that received three laser applications over 7 days.

* Statistically significant at $P < .05$. Different letters represent statistically significant differences.

Table 6. Bone Remodeling Represented by the Distance Between Furcation (F) and Alveolar Bone (AB) in the 14-Day Subgroups (ANOVA)[a]

	C14d (N = 5)		I1ap14d (N = 5)		I3ap14d (N = 5)		I7ap14d (N = 5)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*
F – AB (μm)	222	77.96	287	27.84	225.4	94.50	310.4	92.48	.222

[a] C14d indicates control subgroup, 14-day tooth movement; I1ap14d, experimental subgroup that received one laser application over 14 days; I3ap14d, experimental subgroup that received three laser applications over 14 days; I7ap14d, experimental subgroup that received seven laser applications over 14 days.

difference among the 14-day subgroups (Table 6) is based on the early laser-stimulating effect already mentioned. After the seventh day, the laser activated the osteoclasts less intensively, allowing time for the non-irradiated group to achieve a bone resorption degree similar to that of the irradiated group. Considering that the number of osteoblasts during the 7- and 14-day periods of movement is relatively the same according to the literature[19] and that the osteoclasts are stimulated only at the first period of experiment in irradiated group, a statistically significant difference is present only among the 7-day subgroups.

The literature is not clear about the differences between different cell responses and the ideal dosage for each cell type. In any event, if there were a laser radiation modulation band for each cellular type, it would be impractical to stimulate processes that involve many different and synchronized tissues, such as tooth movement. After an accelerated movement by laser stimulation, relapse would be facilitated and greater anchorage reinforcement or greater retention time would be necessary. If this hypothesis is confirmed, the correct indication for laser phototherapy would be regeneration of tissues with similar metabolic pattern.

Studies involving epithelial, connective,[20] and bone[21] regeneration, or even dental implant osteointegration,[22] showed positive results in the irradiated groups. Conversely, studies on the effects of laser on tooth movement are divergent and inconclusive.[3–8] In rats, low-level laser irradiation facilitated turnover of connective tissues during tooth movement and was dependent on dosage and frequency of laser application.[23] The dosage used in this study seemed to be stimulatory for the connective tissue cells, including osteoclasts, but inhibitory or less stimulatory for osteoblasts, consequent to the lack of synchrony in bone remodeling. Furthermore, in respect to tooth movement, synchrony between cellular events would be more important than the speed in which they occur.[24] In other words, the ideal situation would be to increase the speed of the events, maintaining the physiological tissue organization. Considering these aspects, it would be interesting to test other laser dosages in tooth-movement stimulation.

CONCLUSIONS

- Osteoclastic activity was greatest in the 3-day laser irradiation administration subgroup, which received laser irradiation on days 1, 3, and 5 in the first 7 days of movement. Therefore, osteoclastic activity was dose-dependent.
- Osteoblastic activity was not influenced by laser irradiation.

REFERENCES

1. Tortamano A, Lenzi DC, Haddad AC, Bottino MC, Dominguez GC, Vigorito JW. Low-level laser therapy for pain caused by placement of the first orthodontic archwire: a randomized clinical trial. *Am J Orthod Dentofacial Orthop*. 2009;136:662–667.
2. Cruz DR, Kohara EK, Ribeiro MS, Wetter NU. Effects of low-intensity laser therapy on the orthodontic movement velocity of human teeth: a preliminary study. *Lasers Surg Med*. 2004; 35:117–120.
3. Kawasaki K, Shimizu N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. *Lasers Surg Med*. 2000;26:282–291.
4. Youssef M, Ashkar S, Hamade E, Gutknecht N, Lampert F, Mir M. The effect of low-level laser therapy during orthodontic movement: a preliminary study. *Lasers Med Sci*. 2008;23:27–33.
5. Yoshida T, Yamaguchi M, Utsunomiya T, et al. Low-energy laser irradiation accelerates the velocity of tooth movement via stimulation of the alveolar bone remodeling. *Orthod Craniofac Res*. 2009;12:289–298.
6. Limpanichkul W, Godfrey K, Srisuk N, Rattanayatikul C. Effects of low-level laser therapy on the rate of orthodontic tooth movement. *Orthod Craniofac Res*. 2006;9:38–43.
7. Kim SJ, Moon SU, Kang SG, Park YG. Effects of low-level laser therapy after corticision on tooth movement and paradental remodeling. *Lasers Surg Med*. 2009;41:524–533.
8. Seifi M, Shafeei HA, Daneshdoost S, Mir M. Effects of two types of low-level laser wave lengths (850 and 630 nm) on the orthodontic tooth movements in rabbits. *Lasers Med Sci*. 2007;22:261–264.
9. Heller IJ, Nanda R. Effect of metabolic alteration of periodontal fibers on orthodontic tooth movement. An experimental study. *Am J Orthod*. 1979;75:239–258.
10. Mawhinney WH, Richardson E, Malcolm AJ. Control of rapid nitric acid decalcification. *J Clin Pathol*. 1984;37:1409–1413.
11. Fonseca PD, de Lima FM, Higashi DT, et al. Effects of light emitting diode (LED) therapy at 940 nm on inflammatory root resorption in rats. *Lasers Med Sci*. 2013;28:49–55.
12. Verna C, Hartig LE, Kalia S, Melsen B. Influence of steroid drugs on orthodontically induced root resorption. *Orthod Craniofac Res*. 2006;9:57–62.

13. Ashizawa Y, Sahara N. Quantitative evaluation of newly formed bone in the alveolar wall surrounding the root during the initial stage of experimental tooth movement in the rat. *Arch Oral Biol*. 1998;43:473–484.
14. Fujita S, Yamaguchi M, Utsunomiya T, Yamamoto H, Kasai K. Low-energy laser stimulates tooth movement velocity via expression of RANK and RANKL. *Orthod Craniofac Res*. 2008;11:143–155.
15. Sousa MVS, Scanavini MA, Sannomiya EK, Velasco LG, Angelieri F. Influence of low level laser on the speed of orthodontic movement. *Photomed Laser Surg*. 2011;29: 191–196.
16. Coombe AR, Ho CT, Darendeliler MA, et al. The effects of low level laser irradiation on osteoblastic cells. *Clin Orthod Res*. 2001;4:3–14.
17. Ueda Y, Shimizu N. Pulse irradiation of low-power laser stimulates bone nodule formation. *J Oral Sci*. 2001;43:55–60.
18. Karu T. Photobiology of low-power laser effects. *Health Phys*. 1989;56:691–704.
19. Pavlin D, Dove SB, Zadro R, Gluhak-Heinrich J. Mechanical loading stimulates differentiation of periodontal osteoblasts in a mouse osteoinduction model: effect on type I collagen and alkaline phosphatase genes. *Calcif Tissue Int*. 2000;67: 163–172.
20. Rezende SB, Ribeiro MS, Nunez SC, Garcia VG, Maldonado EP. Effects of a single near-infrared laser treatment on cutaneous wound healing: biometrical and histologic study in rats. *J Photochem Photobiol B*. 2007; 87:145–153.
21. Weber JB, Pinheiro AL, de Oliveira MG, Oliveira FA, Ramalho LM. Laser therapy improves healing of bone defects submitted to autologous bone graft. *Photomed Laser Surg*. 2006;24:38–44.
22. Dortbudak O, Haas R, Mailath-Pokorny G. Effect of low-power laser irradiation on bony implant sites. *Clin Oral Implants Res*. 2002;13:288–292.
23. Kim YD, Kim SS, Kim SJ, Kwon DW, Jeon ES, Son WS. Low-level laser irradiation facilitates fibronectin and collagen type I turnover during tooth movement in rats. *Lasers Med Sci*. 2010;25:25–31.
24. Thilander B, Rygh P, Reitan K. Tissue reaction in orthodontics. In: Graber TM, Vanarsdall RL, eds. *Orthodontics Current Principles and Techniques*. St Louis, Mo: Mosby; 2000:128–39.