

Evaluation of two protocols for low-level laser application in patients submitted to orthodontic treatment

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Introduction: Different low-level laser (LLL) irradiation protocols have been tested to accelerate orthodontic tooth movement (OTM). Nevertheless, divergent results have been obtained. It was suggested that the stimulatory action of low level laser irradiation occurs during the proliferation and differentiation stages of bone cellular precursors, but not during later stages. **Objective:** The purpose of this study was to determine the effect of two protocols of LLL irradiation on experimental tooth movement: One with daily irradiations and another with irradiations during the early stages. **Methods:** Thirty-six rats were divided into control groups (CG1, CG2, CG3) and irradiated groups (IrG1, IrG2, IrG3) according to the presence of: experimental tooth movement, laser irradiation, type of laser irradiation protocol and date of euthanasia (3th or 8th day of experiment). At the end of experimental periods, a quantitative evaluation of the amount of OTM was made and the reactions of the periodontium were analyzed by describing cellular and tissue reactions and by counting blood vessels. **Results:** The amount of OTM revealed no significant differences between groups in the same experimental period ($p < 0.05$). Qualitative analysis revealed the strongest resorption activity in irradiated groups after seven days, especially when using the daily irradiation protocol. There was a higher number of blood vessels in irradiated animals than in animals without orthodontic devices and without laser irradiation ($p < 0.05$). **Conclusion:** Moreover, angiogenesis was verified in some of the irradiated groups. The irradiation protocols tested were not able to accelerate OTM and root resorption was observed while they were applied.

Keywords: Lasers. Low-level laser therapy. Tooth movement. Angiogenesis inducing agents.

Introdução: diferentes protocolos de irradiação por laser de baixa potência (LBP) têm sido testados para potencializar o movimento ortodôntico; entretanto, há resultados divergentes. Foi sugerido que seu efeito bioestimulador ocorre nas fases de proliferação e diferenciação celular, não agindo em estágios tardios. **Objetivo:** avaliar o efeito de dois protocolos de irradiação do LBP na movimentação ortodôntica: um com irradiações diárias e outro em que irradiações foram realizadas apenas nos períodos iniciais. **Métodos:** trinta e seis ratos Wistar foram divididos em grupos controles (GC1, GC2 e GC3) e irradiados (G1r1, G1r2 e G1r3), de acordo com a presença de dispositivo ortodôntico, a presença de irradiação, o tipo de protocolo de irradiação e a data de eutanásia (3º ou 8º dia de experimento). Ao final dos períodos experimentais, foram realizadas mensurações da movimentação dentária, análise qualitativa das reações celulares e teciduais do periodonto e contagem de vasos sanguíneos no ligamento periodontal. **Resultados:** a quantidade de movimentação não diferiu entre os grupos num mesmo tempo experimental ($p < 0,05$). A análise qualitativa revelou maior atividade absorviva nos grupos irradiados ao final de 7 dias, especialmente quando as irradiações foram diárias. Nos grupos irradiados diariamente, a contagem de vasos foi aumentada em relação aos animais isentos de dispositivo ortodôntico e de aplicações de LBP ($p < 0,05$). **Conclusão:** apesar de verificada angiogênese em certos grupos irradiados, os protocolos de irradiação testados não foram capazes de acelerar a movimentação dentária, e foi possível verificarem-se absorções radiculares. **Palavras-chave:** Lasers. Terapia a laser de baixa intensidade. Movimentação dentária. Agentes indutores da angiogênese.

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Submitted: July 13, 2009 - **Revised and accepted:** April 27, 2010

» The author reports no commercial, proprietary or financial interest in the products or companies described in this article.

How to cite this article: Marquezan M, Bolognese AM, Araújo MTS. Evaluation of two protocols for low-level laser application in patients submitted to orthodontic treatment. *Dental Press J Orthod.* 2013 Jan-Feb;18(1):33.e1-9.

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INTRODUCTION

In orthodontics, low-level laser (LLL) has been used to relieve pain associated with tooth movement,^{23,26} accelerate bone regeneration during rapid maxillary expansion,²¹ as well as faster orthodontic tooth movement (OTM).^{2,4,9} The latter is the main focus of studies on laser therapy in orthodontics.

Methodological variations in studies evaluating the relationship between irradiation by LLL and the rate of OTM have generated conflicting results. Positive results can be observed, with increase in movement rate,^{2,4,11} no effect, when experimental and control groups did not differ,¹³ and inhibitory effect in the irradiated groups.²²

Although most of the protocols used for applying LLL during OTM are punctual, there are variations in the wavelengths, doses and the number of irradiation sessions. Some authors have used daily irradiations of LLL,^{4,11,15} while others have set different intervals of laser application.^{2,13,22} Considering that the effect of laser is dose-dependent,^{6,10} greater attention should be given to the irradiation protocols.

It has been suggested that the biostimulatory effect of LLL occurs during cellular proliferation and differentiation, and does not act in late stages.¹⁸ Furthermore, studies that observed an increase in OTM with daily laser irradiation have demonstrated that the rate of OTM rises between the second and third days, keeping a constant difference between groups after this period.^{4,11} Thus, it is necessary to test protocols for LLL application which include irradiation only in the initial stage of OTM.

The aim of this study was to investigate the effect of two LLL irradiation protocols on OTM, one with a daily irradiation, and another in which the irradiation was given only in the earlier period. A macroscopic quantitative analysis of OTM was performed, followed by a microscopic qualitative analysis of cel-

lular and tissue reactions of the periodontium and quantification of the number of blood vessels in the periodontal ligament (PDL).

MATERIAL AND METHODS

For the present *in vivo* experimental study, 36 adult male Wistar rats, 12 weeks old, with a mean weight of 250 g were used. The rats were divided into 2 groups, Irradiated Group (IrG) and Control Group (CG), and then subdivided into 6 groups according the presence of experimental tooth movement, number of laser irradiation sessions and euthanasia day (Table 1). The sample size calculation was made at a level of significance of 5% and power test of 80%. The rats were housed in cages inside a room with a 12 hours light/dark cycle and provided with powdered food to avoid damage to the orthodontic appliance and filtered water *ad libitum*. All procedures were carried out under general anesthesia using intraperitoneal injection of a mixed solution of ketamine hydrochloride and xylazine hydrochloride. This protocol was reviewed and approved by the Ethics Commission on Animal Use in Scientific Research at the Heath Center of the “Universidade Federal do Rio de Janeiro”.

The orthodontic device was composed of a 7 mm nickel titanium closed coil spring (Dental Morelli Ltda, Sorocaba/SP) linked to the maxillary left first molar by a stainless steel ligature wire (Dental Morelli Ltda, Sorocaba/SP), stretched to achieve 40 cN and then tied to the maxillary incisors with a second ligature wire. The orthodontic appliance was set with the rats sedated, administering intraperitoneal injection of the combination of ketamine (1.33 ml / kg) and xylazine (0.67 ml / kg). Composite resin (Transbond XT, 3M Unitek, Monrovia, California, USA) was used to cover the incisors to ensure maximum retention of the ligature and to improve the macroscopic analysis of OTM through the

| Group | Subgroup | OTM | Number of irradiation sessions | Euthanasia | Number of animals |
|----------------------------|----------|-----|--------------------------------|------------|-------------------|
| CG (Control Groups) | CG1 | No | Zero | 1st day | 6 |
| | CG2 | Yes | Zero | 3rd day | 6 |
| | CG3 | Yes | Zero | 8th day | 6 |
| IrG (Irradiated Groups) | IrG1 | Yes | 2 | 3rd day | 6 |
| | IrG2 | Yes | 2 | 8th day | 6 |
| | IrG3 | Yes | 7 | 8th day | 6 |

Table 1 - Group division of animals according OTM, number of irradiation sessions and euthanasia day.

landmark created (Fig 1). Finally, the mandibular incisors were cut to avoid damage to the orthodontic appliance by occlusal trauma.

A continuous wave gallium-aluminum-arsenide (Ga-Al-As) diode laser (Thera Lase, DMC Equipamentos, São Carlos/SP) with a wavelength of 830 nm, power output of 100 mW and spot area of nearly 0.003 cm² was used for this research. A fluency of 6000 J/cm² was applied.^{4,11} Irradiation was performed for 3 minutes at each of the three elected points (mesial, buccal and palatal) around the moved tooth, corresponding to a total energy of 54 J. The number of irradiation sessions was performed according to the group division (Table 1).

The distance between the mesial surface of the first molar and the landmark created in composite resin was measured by a single blind and calibrated examiner (ICC = 0.996), using an orthodontic caliper (Odin, Orthopli, Philadelphia, Pennsylvania, USA) immediately after the coil spring was placed, and before euthanasia (Fig 2). The subtraction was considered the amount of OTM. The negative control group, CG1, was not included in this analysis because no OTM was performed.

The animals were euthanized by decapitation and maxillary bones were dissected. The left maxillary molars and their associated periodontium and supporting bone of each rat were fixed in 10% neutral buffered formalin, decalcified in Morse Solution and placed in paraffin blocks. Cross sections 5 µm thick were obtained from the cervical third of the root, and were hematoxylin and eosin stained (HE).

Qualitative analysis of cellular and tissue reactions of the periodontium of maxillary left first molar was performed on Nikon Eclipse E600 microscope at 40X, 100X and 400X magnifications. Quantitative analysis of the number of blood vessels in the PDL was also performed to verify the presence or absence of angiogenesis during OTM and the LLL irradiation. Photomicrographs at 400X magnification were obtained in four areas of the periodontal ligament of the mesial root (mesial, buccal, distal and palatal) and blood vessel counts were performed visually in the software Image Pro Plus 4.5 (Media Cybernetics, Silver Spring, Maryland, USA). The ICC for counting was 0.841 showing good reliability.

The amount of OTM (recorded in millimeters) and the absolute number of vessels in each specimen were statistically analyzed using the Statistical Package for the Social Sciences (version 16, SPSS Inc., Chicago, Illinois, USA). The verification of normality and homogeneity was performed using the Shapiro-Wilk and Levene tests, respectively, at a significance level of 0.05. Having checked the normal distribution and homogeneity of the variables, analysis of variance (ANOVA) and Tukey's multiple comparisons were applied to detect differences between groups.

RESULTS

Quantification of OTM

Macroscopic analysis revealed no significant difference between irradiated and control groups in the evaluated periods (3rd and 8th days) ($p < 0.05$) (Table 2).

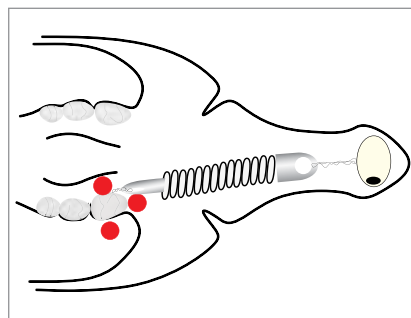


Figure 1 - Schematic illustration of the LLL irradiation (red circles) on the first upper left molar.



Figure 2 - Measurement of tooth movement: Measure of the distance between the mesial surface of the first upper left molar and the reference point on the resin covering the upper incisors, with orthodontic caliper.

Table 2 - Mean and standard deviation (S.D.) for OTM (mm).

| Group | Mean (S.D.) |
|-------|--------------------------|
| CG2 | 0.39 ± 0.04 ^a |
| CG3 | 1.28 ± 0.10 ^b |
| IrG1 | 0.40 ± 0.05 ^a |
| IrG2 | 1.04 ± 0.06 ^b |
| IrG3 | 1.25 ± 0.11 ^b |

Different letters indicate statistical difference at $\alpha = 0.05\%$ (ANOVA/ Tukey).

Table 3 - Qualitative analysis of cellular and tissue reactions in the periodontium.

| | Root surface | PDL | Alveolar bone |
|------|--|--|---|
| CG1 | Thin layer of acellular cementum, surrounded by a thick layer of cellular cementum; Organized cementoblast layer. | Uniform thickness; Fibers inserted perpendicularly to the alveolar bone; Presence of blood vessels around the ligament. | Osteogenesis in the mesial region due to eruptive process; Small-sized marrow spaces filled with hematopoietic marrow and blood vessels. |
| CG2 | Disorganized cementoblast layer on the pressure side. | On the pressure side, the fibers were compressed and the ligament thickness was reduced. On the tension side, the thickness was increased and fibers were extended; Increased number of vessels; Active osteoclasts in the pressure and tension areas; Mixed inflammatory infiltrate. | Howship lacunae evident; Small-sized marrow spaces filled with connective tissue, fatty marrow, blood vessels, mixed inflammatory infiltrate and active osteoclasts. |
| CG3 | Disorganized cementoblast layer on the pressure side. | Increased thickness due to resorptive activity; Reduced number of vessels; Active osteoclasts sparse; Chronic inflammatory infiltrate. | Resorption on pressure side and thin osteoid layer on the tension side; Larger marrow spaces filled with connective tissue, fatty marrow, blood vessels, chronic inflammatory infiltrate, active osteoblasts and few osteoclasts. |
| IrG1 | Disorganized cementoblast layer on the pressure side. | On the pressure side, the fibers were compressed and the ligament thickness was reduced. On the tension side, the thickness was increased and fibers were extended; Increased number of vessels; Active osteoclasts in the pressure and tension areas; Mixed inflammatory infiltrate. | Howship lacunae evident; Small-sized marrow spaces filled with connective tissue, fatty marrow, blood vessels, mixed inflammatory infiltrate and active osteoclasts. |
| IrG2 | Disorganized cementoblast layer on the pressure side. Root resorption. | Increased thickness due to resorptive activity; Reduced number of vessels; Active osteoclasts sparse; Chronic inflammatory infiltrate. | Resorption on pressure side and thin osteoid layer on the tension side; Very large marrow spaces filled with connective tissue, fatty marrow, blood vessels, chronic inflammatory infiltrate active osteoblasts and few osteoclasts. |
| IrG3 | Disorganized cementoblast layer on the pressure side. Root resorption. | Alveolus increased in size; Increased number of vessels; Large number of active osteoclasts; Chronic inflammatory infiltrate. | Absence of osteoid on the tension side; Great resorption of septa-root and very large marrow spaces filled with connective tissue, fatty marrow, chronic inflammatory infiltrate, many vessels and active osteoclasts. |

Qualitative analysis of cellular and tissue reactions of the periodontium

Analysis of cellular and tissue reactions are described below and summarized in Table 3.

CG1 without laser applications and without orthodontic appliance

These physiologically normal animals were considered standard for comparison. In the mesial root, the presence of a thin layer of acellular cementum was observed, surrounded by a thin layer of cellular cementum in the buccal and mesial regions. There was a cementoblast layer covering it and some cementoid areas. The PDL presented a relatively uniform thickness and its fiber bundles were organized and surrounded by fibroblasts. The distal segment showed parallel fiber beams

inserted perpendicularly in the cementum and alveolar bone. On the mesial surface, this parallelism was not so clear. Blood vessels of different sizes were observed throughout the extension of the PDL. In the mesial portion of alveolar bone, a line of bone apposition was observed, indicating recent osteogenesis. The bone apposition was probably due to the normal eruptive process, which occurs in a distal direction. The marrow spaces were small in extent (Fig 3) and filled with hematopoietic tissue and blood vessels.

CG2 with orthodontic appliance, without LLL irradiation and euthanasia at 3rd day

OTM changed the organization of cementoblasts, especially in the pressure zone. In these areas, a reduction in the PDL space was observed, and fiber bundles

were compressed and diffuse. In tension areas the fibers were stretched. There were blood vessels along the entire length of the ligament. These vessels were more numerous when compared to the group without orthodontic appliance (CG1). Active osteoclasts were observed in both pressure and tension sides, indicating the initial enlargement of the alveoli. Mixed inflammatory infiltrate was also observed, with mononuclear and polymorphonuclear inflammatory cells. In bone tissue, an irregular contour was observed, due to the presence of Howship lacunae. In the tension area, there were no

areas of bone formation. The marrow spaces were still normal in size and were filled with connective tissue, fatty marrow, blood vessels, mixed inflammatory infiltrate and active osteoclasts (Fig 3).

CG3 with orthodontic appliance, without LLL irradiation and euthanasia at 8th day

The characteristics of cement were the same as in CG2. The ligament thickness was increased due to resorption. Active osteoclasts were scarce in pressure and tension sides, and in the marrow spaces.

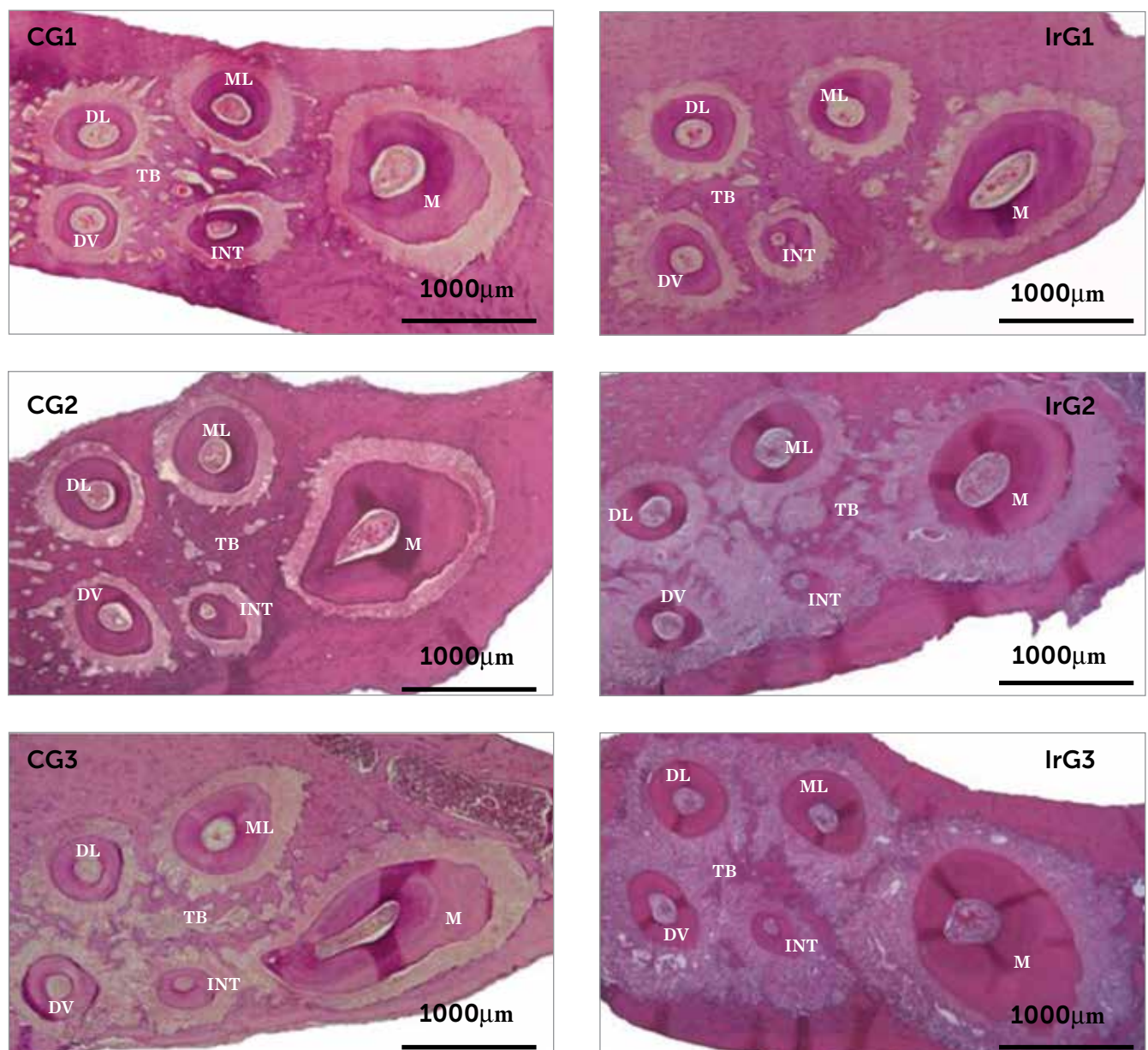


Figure 3 - Photomicrograph of supporting tissues and first upper molars undergone orthodontic movement in the different groups. M = mesial root; ML = mesiolingual root; DL = distolingual root; INT = intermediate root; DV = distovestibular root; TB = trabecular bone (HE, 40X).

PDL vascularization remained well distributed, with little reduction in the number of blood vessels. Lymphoplasmacytic inflammatory infiltrate (chronic) was observed. In the alveolar bone, the compression side presented irregularities due to the resorptive process, while on the tension side narrow osteoid areas were observed. Marrow spaces were greater in extent than in CG2, filled with connective tissue, fatty marrow, blood vessels, chronic inflammatory infiltrate, active osteoblasts and few osteoclasts (Fig 3).

IrG1 with orthodontic device, two LLL applications and euthanasia at 3rd day

The characteristics were similar to the CG2 (Fig 3).

IrG2 with orthodontic device, two LLL applications and euthanasia at 8th day

Bigger differences were observed when teeth were moved in a period of seven days. Differences in the characteristics of cement and marrow spaces were evident when compared with group CG3. The cement showed areas of resorption (Fig 4), and the marrow spaces were larger, with great reduction of the septa-root (Fig 3).

IrG3 with orthodontic device, seven LLL applications and euthanasia at 8th day

Root resorptions were also observed in this group (Fig 4) and further enlargement of the periodontal space was observed when compared with group IrG2 (Fig 3). The number of blood vessels was higher than in CG3.

Mononuclear inflammatory cells were abundant (chronic inflammatory infiltrate). The septa-root showed significant reduction, even disappearing in some animals. In the space between the roots, connective tissue, fat marrow, a high number of blood vessels, chronic inflammatory infiltrate and active osteoclasts in the remaining bone were observed.

In all of the groups that received an orthodontic appliance on the first molar, there were similar reactions in the periodontium of the second molar, but in lower extension. It was possible to see areas of pressure and tension in the PDL of the second molar as well as changes in the cells and size of marrow spaces.

Blood vessels counting

Means and standard deviations for each group are shown in Table 4. Statistically significant difference was found ($p < 0.05$) only among CG1 and groups CG2, IrG1 and IrG3, in which the number of vessels was higher (Fig 5).

DISCUSSION

Two different LLL irradiation protocols were tested during OTM in this study: One with daily irradiations and another in which the irradiation occurred only on the first two days of OTM. The macroscopic evaluation of OTM showed no significant difference between groups on the evaluated days (3rd and 8th). This means both tested protocols were unable to increase the amount of OTM ($p < 0.05$).

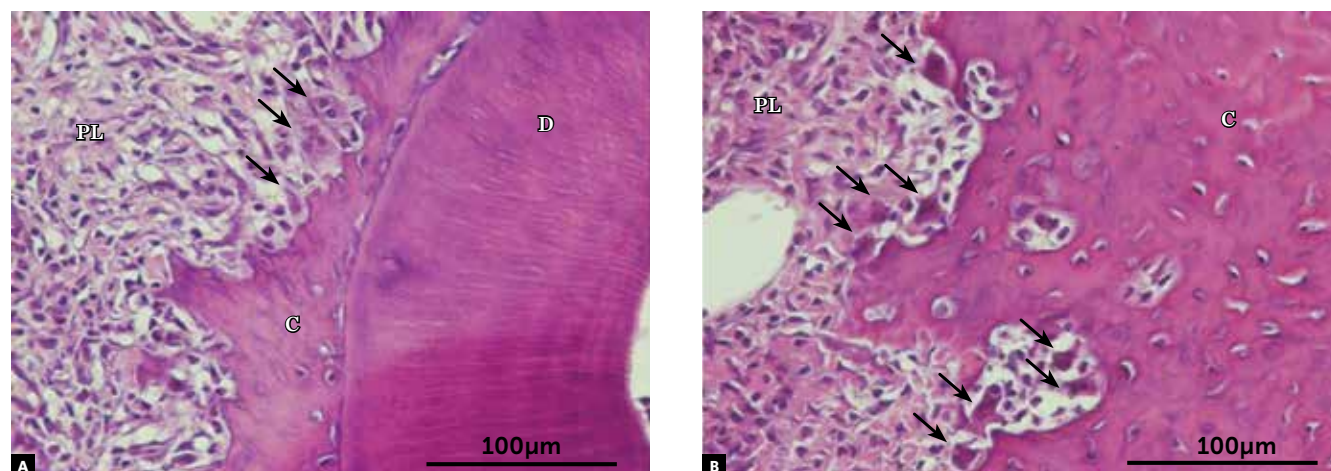


Figure 4 - Photomicrograph of mesial root resorption areas of first molars of mice belonging to IrG2 (A) and IrG3 (B). D = dentin; C = cement; PL = periodontal ligament; black arrows = osteoclasts; (HE, 400X).

This result is in agreement with the findings of Limpanichkul et al,¹³ in which the irradiated group and control group did not differ in the speed of OTM. Another study demonstrated the inhibitory effect of LLL, in other words, the amount of OTM decreased.²² On the other hand, others have demonstrated the effectiveness of LLL.^{2,4,11} It is believed that LLL is dose-dependent and it can speed up or slow down biological processes depending on the fluency applied and the irradiation protocol.^{1,6,9,10} In addition, the mentioned studies used different subjects: Some of them used animals (rats or rabbits), and others used humans. Considering the body height and functional differences of these organisms, the interpretation of fluency as high or low may vary.

Table 4 - Mean of induced tooth movement (mm) and standard deviation (S.D.).

| Group | Mean \pm S.D. |
|-------|--------------------------------|
| CG1 | 22.00 \pm 3.16 ^a |
| CG2 | 37.40 \pm 9.91 ^b |
| CG3 | 30.40 \pm 5.41 ^{ab} |
| IrG1 | 37.00 \pm 6.89 ^b |
| IrG2 | 27.66 \pm 3.78 ^{ab} |
| IrG3 | 38.00 \pm 5.19 ^b |

Different letters indicate statistical difference at $\alpha = 0,05\%$ (ANOVA/Tukey).

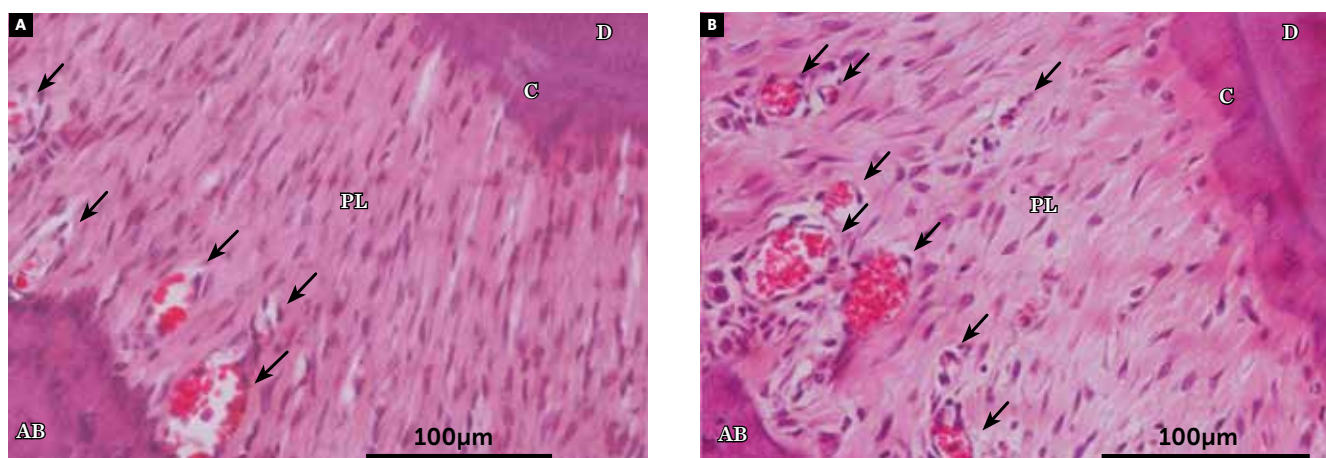


Figure 5 - Photomicrograph of periodontal ligament of rats' mesial root belonging to CG1 (A) and IrG1 (B). Black arrows indicate blood vessels; D = dentin; C = cement; PL = periodontal ligament; AB = alveolar bone; (HE, 400X).

Two of the above-mentioned researches used the same animal model and the same fluency as used in this study and obtained a positive result: The movement rate increased when LLL was applied,^{4,11} disagreeing with the results of the present study. This may be explained by the different measurement method. They measured the distance between the occlusal surface of first and second molars in digital dental casts under magnification. In the present study, an orthodontic caliper was used directly in the animal's oral cavity, taking the measurement from the mesial surface of first molar up to the incisors, as described by Drevenšek et al.³ Although Kawasaki, Shimizu¹¹ and Fujita et al⁴ used an indirect method for measurement associated

with magnification, which may have made it more sensitive, the choice of the second molar as a reference point does not seem adequate since this tooth position is influenced by the first molar movement through the action of transeptal fibers.^{8,28}

Another factor that differed between the studies of Kawasaki, Shimizu¹¹ and Fujita et al⁴ and this research was the force applied. Both used 10 cN while in this study 40 cN was used.^{7,12,14,15,24,25} This factor, however, does not seem to explain the difference in the results obtained, because Gonzales et al⁵ tested the strengths of 10, 25, 50 and 100 cN for moving rat molars and observed that over a period of 14 days, the forces of 10, 25 and 50 cN did not differ in the amount of OTM.

Moreover, the force used in this study was able to generate bone remodeling without evidence of hyalinization, suggesting that it was appropriate.

Qualitative analysis of cellular and tissue reactions have been little explored in scientific articles. The importance of quantifying data and submitting them to statistical analysis is known, however, the description of phenomena makes it easier for the reader to understand, and may arouse her/his curiosity to conduct further analysis and to quantify other data. In this research, interesting phenomena were observed. Root resorptions were evident during the eighth day of OTM in the irradiated groups (IrG2 and IrG3), while in the other groups it was not observed. Another interesting aspect was the effect on the alveolar bone tissue, specifically as regards the marrow spaces. Groups submitted to seven days of OTM and irradiated by laser, in particular those in which the irradiations were daily (IrG3), presented an increased resorptive activity, showing extremely large marrow spaces, including in the second molar region, as a consequence of the action of transseptal fibers.²⁸

Root resorptions were observed after seven days in the mesial roots of first molars undergoing OTM and irradiated by LLL. This finding disagrees with the study conducted by Mendes,¹⁵ in which root resorption was considered similar between irradiated and unirradiated groups. The literature provides no information on the relationship between LLL and changes in the marrow spaces.

In this research, the aim was to quantify the blood vessels of the PDL, since and angiogenic effect of LLL on wound repair has previously been reported.^{20,29} Angiogenesis is also beneficial to OTM by allowing greater movement of oxygen, nutrients and chemical mediators of inflammation, and by facilitating the infiltration of repair cells.³⁰ It also promotes the arrival of osteoclasts, cells that arise from monocytes in the bone marrow and are transported through the bloodstream.¹⁶

Groups CG2 and IrG1, submitted to two days of OTM, differed from CG1, confirming that there is an increase in the vascularity during the early periods of OTM.^{17,20,28} These two groups, however, presented

similar number of vessels, showing that LLL was unable to produce further increase in angiogenesis in the earlier period of OTM.

On the eighth day of OTM, vascularization tended to return to normal, with no statistically significant difference between CG3, IrG2 and the other groups. Exception was found in IrG3, showing that daily applications of laser were able to continue increasing the number of vessels. This result is in agreement with Mendes,¹⁵ who observed an increase in the vascularization of the periodontium during the 8th day of OTM when rats were irradiated daily.

Among the irradiated groups, only group IrG2 did not differ from CG1. This finding confirms the conclusion of Saito and Shimizu.²¹ These authors suggested that while applications of LLL during the initial stages of bone regeneration trigger stimulatory effects, applications in the late periods seem to have the function of maintaining such an effect. The discontinuation of applications appears to reduce the stimulus, as observed in this study.

CONCLUSION

Quantitative analysis demonstrated that OTM did not differ between control and irradiated groups in the equivalent experimental period, demonstrating that both LLL irradiation protocols tested were unable to increase the rate of OTM.

Qualitative analysis showed greater periodontal absorptive activity, in both root and bone tissues, in irradiated groups on the eighth day of OTM (IrG2 and IrG3), especially when irradiations were carried out daily (IrG3).

The number of blood vessels in the PDL was increased by OTM and daily LLL irradiation. IrG1 and IrG3 presented a higher number of blood vessels in comparison with CG1 (device-free animals and not irradiated), showing that irradiations in later periods are necessary to maintain the stimulatory effect of LLL.

Despite the increased number of blood vessels found in certain groups, the tested LLL irradiation protocols were unable to accelerate OTM and caused root resorption as a side effect.

REFERENCES

- Coombe AR, Ho C-TG, Darendeliler MA, Hunter N, Philips JR, Chapple CC, et al. The effects of low laser irradiation on osteoblastic cells. *Clin Orthod Res.* 2001;4(1):3-14.
- 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(2):117-20.
- Drevensek M, Sprogar S, Boras I, Drevensek G. Effects of endothelin antagonist tezosentan on orthodontic tooth movement in rats. *Am J Orthod Dentofacial Orthop.* 2006;129(4):555-8.
- 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(3):143-55.
- Gonzales C, Hotokezaka H, Yoshimatsu M, Yozgatian Jh, Darendeliler Ma, Yoshida N. Force magnitude and duration effects on amount of tooth movement and root resorption in the rat molar. *Angle Orthod.* 2008;78(3):502-9.
- Hawkins DH, Abrahamse H. The role of laser fluence in cell viability, proliferation, and membrane integrity of wounded human skin fibroblasts following Helium-Neon laser irradiation. *Lasers Surg Med.* 2006;38(1):74-83.
- Hayashi H, Konoo T, Yamaguchi K. Intermittent 8-hour activation in orthodontic molar movement. *Am J Orthod Dentofacial Orthop.* 2004;125(3):302-9.
- Heller IJ, Nanda R. Affect of metabolic alteration of periodontal fibers on orthodontic tooth movement. An experimental study. *Am J Orthod.* 1979;75(3):239-58.
- Hourelid NN, Abrahamse H. Laser light influences cellular viability and proliferation in diabetic-wounded fibroblast cells in a dose- and wavelength-dependent manner. *Lasers Med Sci.* 2008;23:11-8.
- Karu T, Pyatibrat LV, Ryabykh TP. Nonmonotonic behavior of the dose dependence of the radiation effect on cells in vitro exposed to pulsed laser radiation at $\lambda = 820$ nm. *Lasers Surg Med.* 1997;21(5):485-92.
- Kawasaki K, Shimizu N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. *Lasers Surg Med.* 2000;26(3):282-91.
- King GJ, Archer L, Zhou D. Later orthodontic appliance reactivation stimulates immediate appearance of osteoclasts and linear tooth movement. *Am J Orthod Dentofacial Orthop.* 1998;114(6):692-7.
- 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(1):38-43.
- Madan MS, Liu ZJ, Gu GM, King GJ. Effects of human relaxin on orthodontic tooth movement and periodontal ligaments in rats. *Am J Orthod Dentofacial Orthop.* 2007;131(1):8.e1-10.
- Mendes OF. Influência do laser de baixa potência na movimentação ortodôntica: estudo experimental em ratos [dissertação]. Rio de Janeiro (RJ): Universidade Federal do Rio de Janeiro; 2005.
- Mundy GR. Bone remodeling and its disorders. 2nd ed. London: Ed. Martin Dunitz; 1999.
- Murrel EF, Yen EHK, Johnson RB. Vascular changes in the periodontal ligament after removal of orthodontic forces. *Am J Orthod Dentofacial Orthop.* 1996;110(3):280-6.
- Ozawa Y, Shimizu N, Kariya G, Abiko Y. Low-energy laser irradiation stimulates bone module formation at early stages of cell culture in rat calvarial cells. *Bone.* 1998;22(4):347-54.
- Rygh P, Bowling K, Hovlandsdal L, Williams S. Activation of the vascular system: a main mediator of periodontal fiber remodeling in orthodontic tooth movement. *Am J Orthod.* 1986;89(6):453-68.
- Rocha JCT. Terapia laser, cicatrização tecidual e angiogênese. *Rev Bras Promoção Saúde.* 2004;17(1):44-8.
- Saito S, Shimizu N. Stimulatory effects of low-power laser irradiation on bone regeneration in midpalatal suture during expansion in the rat. *Am J Orthod Dentofacial Orthop.* 1997;111(5):525-32.
- 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(4):261-4.
- Shimizu N, Yamaguchi M, Goseki T, Shibata Y, Takiguchi H, Iwasawa T, et al. Inhibition of prostaglandin E2 and interleukin 1- β production by low-power laser irradiation in stretched human periodontal ligament cells. *J Dent Res.* 1995;74(7):1382-8.
- Stuani AS. Aspectos histológicos do periodonto no lado de pressão de dentes, quando submetidos à força ortodôntica [dissertação]. Rio de Janeiro (RJ): Universidade Federal do Rio de Janeiro; 2003.
- Taweechaisupapong S, Srisuk N, Nimitpornsuko C, Vatrapphoudes T, Rattanayatikul C, Godfrey K. Evening primrose oil effects on osteoclasts during tooth movement. *Angle Orthod.* 2005;75(3):356-61.
- Turhani D, Scheriau M, Benesch T, Jonke E, Bantleon HP. Pain relief by single low-level laser irradiation in orthodontic patients undergoing fixed appliance therapy. *Am J Orthod.* 2006;130(3):371-6.
- Vandevska-Radunovic V, Kristiansen Ab, Heyeraas Kj, Kvinnsland S. Changes in blood circulation in teeth and supporting tissues incident to experimental tooth movement. *Eur J Orthod.* 1994;16(5):361-9.
- Verna C, Zaffe D, Siciliani G. Histomorphometric study of bone reactions during orthodontic tooth movement in rats. *Bone.* 1999;24(4):371-9.
- Walsh LJ. The current status of low level laser therapy in dentistry. Part 1. Soft tissue applications. *Aust Dent J.* 1997;42(4):247-54.
- Watson JC, Redmann JG, Meyers MO, Alperin-Lea RC, Gebhardt BM, Delcarpio JB, et al. Breast cancer increases initiation of angiogenesis without accelerating neovessel growth rate. *Surgery.* 1997;122(2):508-14.