Low-level laser therapy stimulates bone metabolism and inhibits root resorption during tooth movement in a rodent model

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This study evaluated the biological effects of low-level laser therapy (LLLT) on bone remodeling, tooth displacement and root resorption, occurred during the orthodontic tooth movement. Upper first molars of a total of sixty-eight male rats were subjected to orthodontic tooth movement and euthanized on days 3, 6, 9, 14 and 21 days and divided as negative control, control and LLLT group. Tooth displacement and histomorphometric analysis were performed in all animals; scanning electron microscopy analysis was done on days 3, 6 and 9, as well as the immunohistochemistry analysis of RANKL/OPG and TRAP markers. Volumetric changes in alveolar bone were analyzed using MicroCT images on days 14 and 21. LLLT influenced bone resorption by increasing the number of TRAP-positive osteoclasts and the RANKL expression at the compression side. This resulted in less alveolar bone and hyalinization areas on days 6, 9 and 14. LLLT also induced less bone volume and density, facilitating significant acceleration of tooth movement and potential reduction in root resorption besides stimulating bone formation at the tension side by enhancing OPG expression, increasing trabecular thickness and bone volume on day 21. Taken together, our results indicate that LLLT can stimulate bone remodeling reducing root resorption in a rat model.

LLLT improves tooth movement via bone formation and bone resorption in a rat model.
1. Introduction

Bone is a mineralized tissue with specific mechanical and metabolic functions that constitutes the skeleton. This tissue has the ability to adapt to its environment according to functional demand in a way that its morphology can be transformed. The physiological bone turnover that occurs after puberty, known as bone-remodeling process, is a lifelong process involving tissue renewal [1]. Bones are continuously being remodeled through repeated cycles of destruction and rebuilding, as a consequence of physiological stimulus and mechanical stress. This remodeling most likely serves as a repair function, especially in bones subjected to heavy loading [2]. A delicate balance between the osteoblast and the osteoclast cells, the two major bone cells involved in bone turnover, maintains bone integrity and function.

Orthodontic tooth movement is a process that involves physiologic responses induced by externally applied forces. In this process, orthodontic force causes a minor reversible-injury to the tooth-supporting tissues, followed by physiologic adaptation of alveolar bone in response to this mechanical strain, and subsequently the tooth movement occurs [3]. Therefore, the orthodontic tooth movement can be used as a model for studying the bone metabolism and understanding the complex cellular and biochemical remodeling processes.

The controlled force applied on a tooth causes inflammatory reactions in the periodontal tissues, releasing the inflammatory mediators and triggering the biological processes associated with alveolar bone resorption and apposition [4].

In order to facilitate bone remodeling and promote acceleration of orthodontic tooth movement, the effect of various drug administration and surgical trauma have been studied; and they includes the use of prostaglandins [5], vitamin D [6], prostacyclin and thromboxane A2 [7], osteocalcin [8], kappa factor B [9], external electrical stimuli [10], hormones like tirinoxina [11], and various form of surgical protocols as in corticotomias [12, 13].

Since orthodontic tooth movement requires controlled inflammatory process, various studies have investigated the effect of low-level laser therapy (LLLT) on pain relief [14], inflammation control [15], and modulation of bone resorption and apposition during orthodontic tooth movement [16]. In fact, the utilization of LLLT for accelerating the orthodontic tooth movement can be considered. It is a safe and relatively non-invasive technique, with minimal discomfort and without the risk of drug interactions and side effects [17].

Literature reports that LLLT therapy can accelerate tooth movement by increasing number of osteoclasts in the compression site and subsequent bone resorption, but promoting bone formation and cellular proliferation on the tension site [15, 16]. However, little is known regarding the role of LLLT on metabolic pathway during the bone remodeling and root resorption. Although there are some reports have been published on the effect of LLLT on tooth movement, and most of the reports have found an acceleration on tooth movement compared to the control group, there is a lack of studies on the effect of LLLT correlating tooth movement metabolism at molecular and cellular level either in the periodontal ligament and alveolar bone occurred in the initial phase of tooth movement. Also, there are no studies that associate molecular and cellular metabolism to bone remodeling and root resorption process at tissue level, mainly showing the microstructural change on trabecular bone and root surface.

In this study we evaluated the influence of low-level laser therapy on bone metabolism, tooth movement and root resorption by the following analysis at days 0, 3, 6, 9, 14 and 21: histomorphometry of alveolar bone, root resorption areas and periodontal ligament (PDL); immunohistochemistry of RANKL, OPG and TRAP markers related to osteoclastogenesis; volumetric bone structure analysis by μCT and root resorption analysis by scanning electron microscopy and μCT. Our hypothesis is that LLLT could enhance bone remodeling by stimulating metabolic changes and cellular differentiation, and LLLT also could promote periodontal healing and minimize root resorption.

2. Materials and methods

This research was conducted in accordance with the ethical principles of animal experimentation and the Brazilian norms for the practical, educational, and scientific uses of vivisection. The Animal Research and Ethic Committee of IPEN/CNEN-SP evaluated and approved this study (Animal Care and Use Committee IPEN-CNEN/SP #100/12).

Sixty-eight male Wistar rats (10 weeks, body mass 200–250 g) were used for the experiments. The animals were housed under normal laboratory conditions, and fed with the standard powdered food and water ad libitum. The food was checked and changed every day and a standard 12 hours’ light and dark cycle was maintained.

Animals were placed under general anesthesia with xylazine (30 mg/kg) and ketamine (70 mg/kg). Each animal was immobilized with an open mouth on a table made especially for this purpose. A tapered mini-screw (1.5 diameter × 3 mm total length, Pedlab, Belo Horizonte, MG, Brazil) was inserted behind upper incisors and a NiTi coil spring (RMO, Denver, CO, USA) was used to create a 50 g force to move the left upper first molars (Fig-
This force was chosen based on previous studies that have indicated that 50 g force on rat’s molar promoted tooth movement and root resorption [18–20].

The animals were divided in negative, control and low-level laser groups. Thirty animals received a unilateral appliance (left side) and were euthanized for histomorphometric and immunohistochemistry analysis on days 3, 6, and 9 (n = 5 per group for day 3, 6, and 9). During the tooth movement, fifteen of them received LLLT (experimental group) on the left side with the appliance and the other fifteen animals experienced tooth movement without LLLT (control group). Right side was used as negative control with no active tooth movements in all thirty animals. Additional eighteen rats received the same unilateral appliance and left upper molars were extracted and submitted to scanning electron microscopy (SEM) analysis (n = 3 for both control and LLLT groups on days 3, 6, and 9). For MicroCT analysis, twenty animals received split-mouth designed appliance: left side was irradiated during the tooth movement and right side was used as control. Third molars were used as a negative control. The MicroCT animals were euthanized on days 14 and 21 (n = 5 per group). No space was observed between the second and third molars, indicating lack of mesial movement of the second molar during the experiment and, therefore, allowing right upper third molars of the both groups to be used as negative control group (no intervention) at the MicroCT analysis (n = 10).

The laser irradiation was performed using a GaAlAs diode laser (λ = 810 nm, 100 mW output power, spot of 0.02 cm², Therapy XT, DMC, São Carlos, SP, Brazil). The labial and palatal sides were punctually irradiated for 15 s every other day. Energy per point was 1.5 J resulting in a fluence of 75 J/cm² [21, 22]. The contra-lateral side, that was not irradiated, received a metal barrier to avoid scattered light or indirect irradiation. The LLLTs groups received laser irradiation every 48 h, until the end of the experimental period.

Tooth movement was evaluated clinically in rat’s mouth by measuring the space between the first molar (on mesial aspect) and the center of miniscrew with metal compass and with an electronic digital caliper (Mitutoyo Co., Miyazaki, Japan) as displayed in Figure 1.

### 2.1 Histomorphometric analysis of alveolar bone, periodontal ligament and root surface

Rat euthanasia was performed on days 3, 6, and 9, with an overdose of anesthesia and appliances were removed. Maxillas containing LLLT, control and negative control sites were dissected and prepared for histological analysis. The specimens were dehydrated in a series of alcohol baths beginning with 50% and progressing to 100%. Thereafter, the samples were embedded in paraffin, and 4 µm sections were prepared, stained with haematoxylin and eosin and photographs were captured in an optical microscope. The software ImageJ (National Institute of Health, USA) was used to quantify the amount of alveolar bone area delimited by standardized rectangles (200 µm width × 1400 µm length) at the tension and compression sides. Similar methodology was used to measure the area of hyalinization tissue in the periodontal ligament at the compression side for all groups. Also, outline of each root resorption lacunae was drawn following the internal contour of the lacunae and the external margin was estimated by the continuation of the root resorption surface. After drawing, the areas were measured and the sum was used to calculate the total area of root resorption in each sample. Posteriorly, data were classified according to their location: mesial and distal root, both at the tension and compression side.

### 2.2 Immunohistochemistry analysis (IHC)

Additional slices were prepared for immunohistochemistry analysis on days 3, 6 and 9, and they were incubated with polyclonal anti-RANKL, anti-OPG e anti-TRAP (Goat anti-RANKL, goat anti-OPG, goat anti-TRAP polyclonal, Santa Cruz Biotechnology, California, USA). Working dilution was 1:100 for all antibodies, left for overnight incubation at room temperature. Secondary antibody, biotylated anti-goat produced in rabbits, was used (Biotinylated Link Universal, DAKO). The immunohistochemistry reaction with a streptavidin/biotin system (LSAB +Sys-HRP – DAKO, California, USA) and final color reactions were performed by counter-staining.
with Mayer-hematoxylin. A potential marker for bone resorption is the evaluation of tartrate-resistant acid phosphatase activity (TRAP). This enzyme is secreted by osteoclasts during the bone resorption, and it enables the identification of active osteoclasts [23, 24]. The number of TRAP positive cells was counted at the tension and compression sides.

### 2.3 MicroCT analysis

For μCT, rats were euthanized on days 14 and 21. The samples were scanned using Skyscan 1172 (Bruker MicroCT, Kontich, Belgium) and associated software (Version 1.5.23) at a resolution of 5 μm using an X-ray and source potential of 70 kV, amperage of 142 μA, power of 10 W through a 180° of rotation around the vertical axis and a rotation step of at 0.4°. Raw image data were reconstructed using nRecon software Version 1.5.23 and were analyzed using CT Analyser software (Version 1.15.4.0, Bruker MicroCT). Two rectangular volumes (200 μm width × 400 μm thickness × 1400 μm height) of the alveolar bone next to the mesial and distal roots were analyzed. Bone mineral density (BMD), bone volume and total volume fraction (BV/TV), trabecular thickness (TbTh), trabecular number (TbN) and separation (TbSp) were measured at the tension and compression sites (Figure 2).

Root resorption lacunae were evaluated using ex-vivo microcomputerized tomography. After 3D images of the upper first molars were reconstructed, the compression and tension sides of the mesial and distal roots were analyzed using CT Analyser software. Cross-sectional views of the areas with root resorption lacunae were generated [25]. All root resorption craters were drawn by outlining the boundary of the lacunae individually (region of interest) in all cross-sectional MicroCT images (200 slices per root), and posteriorly the volume of each lacunae was calculated. Total volumetric root resorption value in a particular region was the sum of all root resorption volume lacunae in that area, and then this value was assigned to its particular location: the tension or compression sites of the mesial and distal roots (Figure 2).

### 2.4 Scanning electron microscopy (SEM) evaluation of the roots

For this assay, eighteen upper first molars from laser and control groups and their surrounding bone were cut as a block on days 3, 6 and 9 (n = 3 per group per day). The alveolar bone was then removed gently in order to avoid any root surface damage. All molars were washed in 1% sodium hypochlorite to eliminate periodontal ligament residues [26], left to air dry for 1 day, and placed on the equipment stub. Scanning electron microscopy was performed using a TM 3000 tabletop microscope (Hitashi, Krefeld, Germany) with accelerating voltage of 15 kV at low magnification (60 ×) and higher magnification scanning (300 ×). Two areas at the lateral root surfaces from the tension and compression sides, of each root (distal and mesial) in all groups were scanned and photographed (total sample size based on number of area was 12). A qualitative assessment of root resorption of the experimental teeth was performed using a modification of the method proposed by Han et al. [27]. The severity of root resorption was classified by score as follows:

- 1 = no resorption
- 2 = less than 1/3 of root length at root resorption lacunae at the cementum level
- 3 = more than 1/3 of root length at root resorption lacunae at the cementum level
- 4 = less than 1/3 of root length at root resorption lacunae at the dentin level
- 5 = more than 1/3 of root length at root resorption lacunae at the dentin level

![Figure 2 MicroCT images showing the rectangles delimiting the volume of alveolar bone (dot arrows) evaluated in this study. AB: alveolar bone, PDL: periodontal ligament (A). Root resorption region of interest highlighted in red and draw in all slices for volumetric measurement of lacunae individually and posteriorly calculation of total lacunae (B).](image-url)
Root resorption was considered extensive when RR lacunae found more than 1/3 of the total root length and considered deep when reached the dentin level. Figure 3 represents the experimental design of the study.

2.5 Statistical analysis

Values are given as means, and error bars are standard deviations. Shapiro-Wilk test was employed to investigate normal distribution. Analysis of variance (two-way ANOVA) and Bonferroni test for post hoc were used to compare all measurements. Statistical significance was found at $p < 0.05$.

3. Results

Body mass of the animals increased during the experiment, and no lost of miniscrews or any parts of the orthodontic appliance were observed in any of the animals, allowing for a constant force loading on molars throughout the experiment.

3.1 Effect of LLLT on tooth movement

Figure 4 indicates that the tooth movement has the same asymptotic behavior for LLLT and control groups, i.e., the movement exponentially increases over time until it plateaus, as it approaches a certain value. However, LLLT group demonstrated greater tooth movement throughout the experimental period compared with the control group. Tooth movement was significantly greater in LLLT group in all studied periods (3, 6, 9, 14 and 21 days). These results indicated that LLLT was able to increase the distance of tooth movements by 46% at day 3, 31% at day 6, 42% at day 9, 44% at day 14 and 43% at day 21 (Figure 4). The acceleration of tooth movements was pronounced during the first six days, and no difference in the rate of tooth movement was found after sixth day for both groups.

3.2 Effect of LLLT on alveolar bone and periodontal ligament

The histomorphometric analysis of HE stained slices (Figure 5) showed no significant differences on alveolar bone area after 3 days comparing the two groups. However, less areas of the alveolar bone in
the compression side were observed in the LLLT group on 6 (p < 0.01), 9 (p < 0.01) and 14 (p < 0.05) days. Also, statistically significant differences were found in alveolar bone area in the tension side between LLLT and control groups on days 14 and 21, where the LLLT group showed greater bone area. Interestingly, on days 6 and 9, LLLT group showed less bone area than control group. Moreover, alveolar bone area was found to be significantly reduced in all experimental days compared to the negative control group (no intervention). On days 14 and 21, both groups showed higher amount of alveolar bone compared to days 3, 6 and 9 in tension and compression sides.

The hyalinization areas in the LLLT group were significantly less than that of the control group on days 3, 6 and 9 at the compression side. LLLT was able to progressively reduce hyalinization areas on 25% on day 3, 57% on day 6 and 70% on day 9 (Figure 6).

3.3 Immunohistochemical analysis

The results of this study indicated significant differences for TRAP-positive cell counts at the compression side between control and LLLT groups on day 9. Moreover, there was a tendency to decrease the number of TRAP-positive cell over time for control group (Figures 7 and 8).

Increase in immunoreactivity evidenced by positive RANKL in the periodontal ligament and alveolar bone was observed in the compression side, and this expression of osteoclastic activity for LLLT group, measured by the presence of RANKL, was significantly higher than that of the control group. For the control group, RANKL expression decreased over time; while for the irradiated group, it has increased. There also was a significant reduction in OPG expression for LLLT group than control group in the compression side. On the other hand, OPG expression was higher than the expression of RANKL in the tension side. There was a progressive increase in OPG expression in the LLLT group from day 3 on at the tension side compared to the control group which showed progressive reduction of OPG expression (Figure 9).

3.4 MicroCT analysis

The evaluation of the trabecular bone volume in the compression side showed that bone mineral density (BMD) was significantly lower in the LLLT group compared to control after day 14 (p < 0.01). LLLT group also showed lower percentage of bone volume (BV/TV) at day 14 (p < 0.01). However, there was no significant difference in BMD and BV/TV between the groups at day 21. Also, the laser irradiation group showed significantly lower trabecular thickness compared to the control group in the compression side at day 21 (p < 0.001), but no significant difference was observed at day 14. Increased trabecular separation was observed in LLLT group at day 14 (p < 0.001) but no significant difference was observed at day 21. A greater osteoclastic activity in the compression side for the LLLT group at day 14 was evident (Figure 10).

On the tension side, BMD was greater for the LLLT group at day 14 (p < 0.05) but no significant difference was observed at day 21. BV/TV ratio was
significantly higher for LLLT group compared at day 14 ($p < 0.05$) and day 21 ($p < 0.01$). LLLT group showed larger trabecular thickness and less separation at 21 days ($p < 0.001$). Irradiation with low-level laser at the tension side of alveolar bone increased BMD, BV/TV and TbTh, but decreased TbSp during the tooth movement (Figure 11).

### 3.5 Effect of LLLT on root resorption

Semiquantitative analysis of the images obtained by scanning electron microscopy showed that LLLT group had lower score of root resorption, i.e., the root resorption lacunae found were less extensive and with less depth, when compared to the control group. Statistically significant differences were obtained between the two groups on day 9 ($p < 0.05$). The control group had a higher number of roots classified with a higher score, which represented more than 1/3 of the root length with resorption craters at the level of cementum and dentin (Figures 12 and 13).

The results of the measurement of the areas of root resorption obtained from histological slides showed statistically significant reductions in the areas of root resorption ($p < 0.05$) for days 14 and...
21 in the tension side of the distal root and days 6, 9 and 14 on the tension side of the mesial root. The compression side, there was significant reduction in the laser group at day 21 in the mesial root. In the distal root statistically significant reductions were observed on days 9, 14 and 21 (Figure 14).

The results of total areas of root resorption lacunae showed statistically significant reductions in the areas of root resorption ($p < 0.05$) on days 6, 9, 14 and 21 at the tension side of the mesial and distal roots. There was no significant change over time in the LLLT group with an increase on day 21. Control group showed a high increase of root resorption lacunae over time, while LLLT remained stable.

Figure 10 Mean values ±SD of the bone mineral density (BMD), bone volume and total volume ratio (BV/TV), trabecular thickness (TbTh) and trabecular separation (TbSp) at the compression side. ** $p < 0.01$ and *** $p < 0.001$ ($n = 6$). NC – negative control.

Figure 11 Mean values ±SD of the bone mineral density (BMD), bone volume and total volume ratio (BV/TV), trabecular thickness (TbTh) and trabecular separation (TbSp) at the tension side. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ ($n = 6$). NC – negative control.

Figure 12 Mean values ±SD of the root resorption on days 3, 6 and 9. * $p < 0.05$. 
The volume of root resorption lacunae measured by MicroCT images showed a significant reduction of the volume (in mm³) in the LLLT groups at tension side of the mesial roots on day 21 and distal roots on day 14. At the compression side, a significant reduction in root resorption lacunae on the mesial root on day 21 and on distal root at days 14 and 21. It was also observed that between the two studied roots, distal root presented larger resorption lacunae in average. Comparing sides, compression side, as expected, showed higher volumes of root resorption in both groups (Figure 15).

4. Discussion

It has been well documented that bone tissue is sensitive to its mechanical environment [28–30], for example, cyclic stretch appears to be involved in regulation of osteoblastic differentiation, but the exact mechanisms remain elusive [31, 32]. Induced orthodontic tooth movement consists in apply physiological forces over tooth to create strains, categorized as compressive and tensile stress, in the tooth-supporting tissues (periodontal ligament) [3]. These controlled forces initiate an inflammatory event at compression site causing constriction at the periodontal ligament’s microvasculature and consequently, resulting in a local hyalinization, compensatory hyperemia in the adjacent periodontal ligament and pulpal vessels [33].

The tissues around the compressed area start to release a numerous chemo-attractants, such as interleukin, prostaglandin [34] and also the RANK-RANKL-OPG system, that draw osteoclast cells locally. These cells produce resorption of the periodontal ligament, nearby alveolar bone and in some cases cementum layer from the root.

In orthodontic induced movement, generally low tension areas have been characterized as being primarily osteogenic, usually without a significant inflammatory component [35]. On the other hand, high tensile strains act as pro-inflammatory stimuli and increase the expression of inflammatory cytokines [36].

This study used induced orthodontic tooth movement in rats as a model to evaluate the effect of LLLT on bone metabolism and bone turnover. A controlled force was applied to the first molar using...
a NiTi coin-spring in order to create a compression area on adjacent bone in the force direction and a tension area at the opposite site [13, 14, 19, 33]. Also the control group was the contra-lateral side, avoiding or at least reducing the differences among individual biologic response.

The model using the same animal to evaluate the effect of LLLT by having experimental and control sides can be controversial. The presence of systemic effect as consequence of laser irradiation cannot be ignored, and it may cause unwanted bias. Evidence of systemic effect of LLLT have been shown by Rodrigo et al. and Coelho et al. [35, 36] on wound and bone healing, nervous system, and burns. However, other studies [37, 38] used the same animal as the control group and did not experience any evidence of this effect. In those studies, irradiated groups showed statistically significant differences when compared to the untreated control side, which agrees with this study. In fact, we observed statistically significant differences between the groups for the most assays, suggesting that even if the systemic effect was present, this effect was not enough to promote a significant influence on the counter-part side used as a control.

Several studies on the amount of tooth movement utilizing animal [22, 39, 40] and humans [21, 41] models reported positive acceleration of bone turnover and consequently tooth displacement after laser irradiation. Although this process of bone metabolic modulation induced by LLLT could have beneficial effect on tooth movement, it can also promote root resorption, since it is based in the same cellular activation [4, 42, 43]. Our study hypothesized that the LLLT could improve bone turnover and, consequently, tooth displacement, but also control or even decrease the process of root resorption.

Our results demonstrated an improvement on tooth displacement after laser irradiation, revealing a faster tooth movement (around 40%) compared to the control group. This finding is slightly higher than those reported in Refs. [39, 42]. This factor could be explained by the different forces applied and distinct laser parameters reported by literature such as fluence, exposure time and fluence rate, as well as total number of irradiations.

Regarding the applied force, our study used 50 g of force, which may be considered excessive for rat’s molar but enough to induce root resorption and acute inflammation. If lighter force was applied, such as 10 g force, it would probably have facilitated tooth movement but on the other side no root resorption would have been found, not allowing the hypothesis of this study which was the influence of LLLT on tooth displacement and root resorption process to be tested. Also, clinically it is very hard to control the amount of force delivered by the orthodontic appliance, so it is likely that heavy forces reach the periodontal ligament and alveolar bone in some regions of the dental root.

Thus, LLLT may have acted as a powerful adjunct in enhancing bone-remodeling process immediately after the first laser irradiation. After 9 days, the LLLT/control asymptotes leveled off at 42%. We assume that LLLT may have more impact in tooth movement at the initial irradiation and, under the conditions used in this study, gradually lost its effect. Probably, light parameters should be adapted to the target cells [44]. Since orthodontic tooth movement and root resorption are dependent of the inflammatory process [40, 45], this study explored the mechanisms by which LLLT modulating the inflammation.

Yamaguchi et al. [46] showed that under compressive force the cells in PDL could increase the
production of RANKL inducing osteoclastogenesis. Ozawa et al. [47] demonstrated that laser irradiation stimulates cell proliferation and differentiation of bone-forming cells (osteoblast-like cells), resulting in an increase in the number of differentiated osteoblasts and subsequent bone formation. Laser irradiation also promote differentiation of osteoclasts on the pressure side during the induced movement [39]. These effects increase the bone turnover rate and enhance tooth movements. In addition, Aihara et al. [48] applied LLLT in osteoclast precursor cells in vitro and showed an increase of multinucleated cells. Their study suggested that LLLT facilitates the differentiation and activation of osteoclasts, and it was observed that areas of resorption were more abundant in the LLLT groups compared to the control. In this study, LLLT increased RANKL production and TRAP expression on days 6 and 9 at the compression sites demonstrating the induction of osteoclastic activity, which resulted in lower bone area values (see Figures 7 and 9) indicating amplified bone turnover [42, 48].

High expression of RANKL is also associated with severe root resorption cases [49]. One of the hypothesis for root resorption during orthodontic treatment is that excessive force limits blood flow to the compressed PDL space and deprive oxygen, leading to ischemic necrosis and formation of hyalinized areas along the PDL space, death of cementoblasts and PDL cells, and exposure of root surfaces to osteoclastic activity [43, 50].

In contrast to the above theory, the increase of RANKL and TRAP expression at the compression sites for LLLT group on days 6 and 9, validated by the decrease of bone area on days 6, 9 and 14, could be an indication of reduced load at the tooth/ligament/bone interface in this study. This hypothesis is also confirmed by the lower values of BMD and BV/TV ratio which indicates that bone metabolism was enhanced in favor of osteoclastic activity, reducing bone density in the compression site and facilitated tooth displacement. Moreover, modulating lower/shorter inflammation process by LLLT could result in less root resorption, since cementoblast (cell layer covering and protecting root dentine) is not subjected to a high level of stress for a longer period of time. After 3 days of compressive load (day 3) on PDL, it was possible to see hyalinized area in both groups, but significantly less for the LLLT group. However, TRAP and RANKL expression show no significant differences between two groups on day 3. Subsequently, on days 6 until 14, the histomorphometric analysis showed that there was decrease in bone areas, and IHC values showed significant increase in RANKL and TRAP expression, resulting in lower BMD and BV/TV ratio. In addition, trabeculae were thinner and more spaced for LLLT group (see Figure 10). On the other hand, PDL complex in the control group had been subjected to greater compression forces, since the boney wall was denser due to less osteoclastic activity. A longer period of PDL excessive compression and higher load caused by surrounding denser bone resulted in a larger hyalinized area (see Figure 6) and more root resorption (see Figure 12) for control group.

Another advantage of LLLT on bone remodeling is that new bone formed after laser irradiation is better quality than non-irradiated tissue [39, 51, 52]. In this study, alveolar bone on the tension side after 14 and 21 days presented higher values of BMD, BV/TV ratio and trabecular thickness for the LLLT group, corroborating with the previous studies. More alveolar bone area was also noted by the histomorphometric analysis on days 14 and 21 for this group. Interestingly, the LLLT group showed less bone area on days 6 and 9 compared to control group at the tension side. This may be explained by the faster tooth displacement of the LLLT group triggered by enhanced osteoclastic activity at the compression side, causing initial low bone area at the tension side. As the subsequent osteoblastic activity at the tension side becomes escalated by LLLT, the bone density improves beyond that of the control group.

This study showed that low-level laser therapy may affect tooth movement by increasing osteoclast activity TRAP-positive at the alveolar bone mediated by the increase of RANKL expression and reduction of OPG expression since day 3, maintaining this process on days 6 and 9. This high metabolism on the process of bone resorption induced less formation of hyalinized areas (areas of cell and tissue necrosis) at the compression side at days 3, 6 and 9. We hypothesize that LLLT acted on mitochondria, increasing levels of reactive oxygen species (ROS) and nitric oxide (NO). It has been described that NO plays an important role during tooth movement influencing capillary vascularization, increasing microvascular permeability and monocytes, therefore increasing the number of osteoclasts [53, 54]. The enhancement of the tissue oxygenation could lead to activation of cell signaling pathways, inducing transcription factors such as nuclear factor kappa B (NF-κB) [55]. NF-κB is essential for RANKL-induced osteoclast formation [56]. In fact, our results demonstrated an increase on RANKL expression, promoting osteoclast differentiation and activation, increasing bone resorption at the compression side, thus providing rapidly force dissipation at the periodontal ligament reducing area of necrosis (hyalinization).

Formation of hyalinization area at the periodontal ligament precedes root resorption during tooth movement. According to Brezniak and Wasserstein [4], during the removal of hyalinized tissue formation induced during tooth movement by clasts cells
and macrophages, root resorption may occur if cementoblast layer at root surface was damaged. From our results, it seems that LLLT reduced root resorption since we noticed less amount of area and volume of root resorption by reducing formation of hyalinization area.

The present study evaluated, histomorphometrically and by MicroCT images, the effect of the LLLT during the process of root resorption and showed that there was a reduction in the total area of root resorption lacunae on both roots mesial and distal at tension and compression sides. Significant reduction was seen at the tension side after day 6 on both roots and at the compression side on day 21 on the mesial root and after day 9 on the distal root. Histomorphometric results was consistent with the data obtained by SEM. On the other hand, we used MicroCT to quantify root resorption volumes since this approach provides high accuracy of measurements when high resolution is used during image acquisition. We observed a significant reduction in total volume of root resorption lacunae in the mesial root on day 21 for both tension and compression side and also in the distal root, at the tension side on day 14 and especially at the compression side on days 14 and 21.

The literature is scarce relating the effect of LLLT in minimizing the root resorption process. Seifi et al. demonstrated a reduction in the areas of resorption in the group irradiated with low-level laser during tooth movement after induction of bone loss followed by synthetic bone graft placement [57], while Han et al. could not observe a reduction of root resorption lacunae during the tooth movement in dogs after corticotomy using LLLT [58]. Probably different light parameters explain these contradictory results.

Summarizing, this study reports a treatment protocol that was able to biomodulate both bone resorption and root resorption processes at the same time, by stimulating bone resorption through osteoclast activation and inhibiting root resorption by preserving the cementoblast layer and therefore avoiding root damage even in the presence of high bone resorptive activity.

5. Conclusion

Our study suggests that LLLT can accelerate induced tooth movement by increasing the activity of osteoclastic cells and thereby stimulating the process of bone resorption at the compression side, which consequently led to reduction in root resorption lacunae. Furthermore, LLLT can have a positive effect on bone formation at the tension side. We hope that this work provides better comprehension of the biological mechanism involving tooth movement and root resorption to encourage clinical studies in this area.

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Conflict of interest None of the authors have any conflicts of interest.

Author biographies Please see Supporting Information online.

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