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ORIGINAL ARTICLE

Effects of corticopuncture (CP) and low-level laser therapy (LLLT) on the rate of tooth movement and root resorption in rats using micro-CT evaluation

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Abstract
The aim of this study was to compare the rate of tooth displacement, quantity of root resorption, and alveolar bone changes in five groups: corticopuncture (CP), low-level laser therapy (LLLT), CP combined with LLLT (CP + LLLT), control (C), and negative control (NC). A total of 60 half-maxilla from 30 male Wistar rats (10 weeks old) were divided randomly into five groups: three (CP, LLLT, and CP + LLLT) test groups with different stimulation for accelerated-tooth-movement (ATM), one control (C) group, and one negative control (NC) group with no tooth movement. Nickel-titanium coil springs with 50 g of force were tied from the upper left and right first molars to micro-implants placed behind the maxillary incisors. For the CP and CP + LLLT groups, two perforations in the palate and one mesially to the molars were performed. For the LLLT and CP + LLLT groups, GaAlAs diode laser was applied every other day for 14 days (810 nm, 100 mW, 15 s). The tooth displacements were measured directly from the rat's mouth and indirectly from microcomputer (micro-CT) tomographic images. Bone responses at the tension and compression sites and root resorption were analyzed from micro-CT images. The resulting alveolar bone responses were evaluated by measuring bone mineral density (BMD), bone volume fraction (BV/TV), and trabecular thickness (TbTh). Root resorption crater volumes were measured on both compression and tension sides of mesial and distal buccal roots. The tooth displacement in the CP + LLLT group was the greatest when measured clinically, followed by the CP, LLLT, and control groups (C and NC), respectively (p <0.05). The tooth movements measured from micro-CT images showed statistically higher displacement in the CP and CP + LLLT groups compared to the LLLT and control groups. The BMD, BV/TV, and TbTh values were lower at the compression side and higher at the tension side for all three test groups compared to the control group. The root resorption crater volume of the distal buccal root was higher in the control group, followed by CP, LLLT, and CP + LLLT, mostly at the compression site. Combining corticopuncture and low-level laser therapy (CP + LLLT) produced more tooth displacement and less root resorption at the compression side. The combined technique also promoted higher alveolar bone formation at the tension side.

Keywords Low-level laser therapy · Tooth movement · Orthodontics · Corticopuncture

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Introduction

Orthodontic tooth movement is a result of bone remodeling where bone resorption and deposition occurs in the compressed and stretched sides of the periodontal ligament, respectively [1]. Several methods have been proposed to increase the speed of tooth movement: low-level laser irradiation [2, 3], vibration [4], pulsed electromagnetic fields [5], pharmacologic agents [6], and various surgical approaches, such as corticotomy [7, 8], osteotomy [9], and dental distraction [10].

Since the introduction of corticotomy [11] and the concept of regional acceleratory phenomena (RAP) [7, 12], less invasive surgical techniques have been proposed: corticision [13], piezopuncture [14], micro-osteoperforation [15, 16], and corticopuncture. All surgical approaches produced acceleration in tooth movement by increasing bone turnover through a phenomenon called RAP (regional acceleratory phenomenon). RAP occurs when a physical bone trauma is performed by surgical intervention and is usually limited to the cortical portion of the alveolar bone. This process initiates and potentiates the normal healing process, increasing the turnover of alveolar bone and finally promoting faster tooth movement.

Another promising method to speed the tooth movement is by applying low-level laser therapy (LLLT). The biomodulator, analgesic, and anti-inflammatory effects of LLLT have been used in orthodontics to reduce post-adjustment pain [17], to treat traumatic ulcers in the oral mucosa [18], and to accelerate tooth movement induced by stimulating bone-resorption and bone-formation [19–23]. More recently, the effect of LLLT in the root resorption process has been studied and shown that LLLT not only accelerate tooth movement but may decrease the area and volume of root resorption craters that may occur and represents an undesired side effect of orthodontic treatments [24]. However, only a few studies have explored the relationship between RAP and LLLT. Kim et al. (2009) studied the tooth movement of 24 premolars from 12 beagle dogs and compared 4 experimental groups: control, corticision, LLLT, and corticision and LLLT. Corticision and LLLT increased tooth movement significantly compared to the control but the authors found an inhibitory effect when the two procedures were combined. They suggested that LLLT may have influenced the healing of alveolar defect after corticision instead of allowing the RAP to promote bone resorption but pointed out that LLLT is dependent on optimal dose for stimulatory effects [25].

Also, the literature is scarce relating the effect of RAP and LLLT in minimizing the root resorption process [20, 26, 27]. Since bone resorption and root resorption processes appear to occur in a similar manner by metabolic and cell pathway [28, 29], the application of LLLT and corticopuncture technique may have a concomitant effect on root resorption. The objective of this study was to compare the effect of corticopuncture (CP), low-level laser therapy (LLLT), and the combined technique (CP + LLLT) on the surrounding bone, root surface, and rate of tooth movement after 14 and 21 days of force application in rats.

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).

Thirty male Wistar rats (10 weeks, body weight 300–350 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-h light-and-dark cycle was maintained.

The animals were divided in groups so that the left side of the maxilla received treatment (CP, LLLT, or CP + LLLT). Right sides were randomly distributed in the control group ($n = 10$) or negative control ($n = 5$) for each experimental period (i.e., 14 or 21 days) as shows Fig. 1:

Induction of tooth movement

Animals were placed under general anesthesia with xylazine (30 mg/kg) and ketamine (70 mg/kg). Each animal was immobilized with open mouth on a table specifically designed for this experiment. A tapered miniscrew (1.5 × 3 mm, Peclab, Belo Horizonte, MG, Brazil) was inserted 1 mm distal to the upper incisors, and NiTi coil springs (RMO, USA) were used to create a 50 g force to the right and left upper first molars in mesial direction using a split-mouth design (Fig. 1). This force was chosen based on previous studies that indicated that 50 g force on rat's molar promotes tooth movement and root resorption [29–31]. The magnitude of each spring was measured three times using a force gauge (Zeusan, Brazil).

Low-level laser therapy

The laser irradiation was performed using a gallium-aluminum-arsenide (GaAlAs) diode laser ($\lambda = 810$ nm, 100 mW output power, 600 μm fiber diameter involved in a metal tube that diverges the laser beam at a spot of 0.02 cm^2 – Therapy XT – DMC, Brazil). The power of the laser beam was measured before each irradiation using a laser power meter (LM-01, Coherent, USA). The parameters used for irradiation were: punctual irradiation for 15 s at the labial and palatal sides on days 0, 2, 4, 6, 8, 10, and 12. Energy per point was 1.5 J resulting in a fluence of 75 J/cm^2 per side (Fig. 2) [32, 33]. The contra-lateral side was not irradiated and received a metal barrier to avoid scattered light or indirect irradiation.

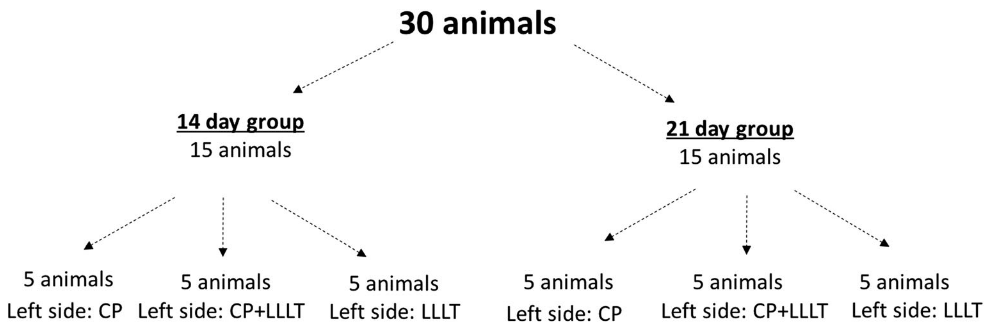

Fig. 1 Flowchart of the sample distribution used in this study

Corticopuncture technique

Corticopuncture was performed on the mesial and palatal aspects of the maxillary left first molar (target tooth). Micro-implant with 1.5 mm diameter was used to produce three perforations, two with 0.7 mm depth in the palatal bone and one with 5 mm depth mesial to the first molar using a manual driver (Fig. 3a, b).

Tooth movement distance measurement

Tooth movement was evaluated clinically in rat's mouth by measuring the distance between the cervical area at the mesial aspect of the first molar and the center of the miniscrew, with metal compass, when the rats were under anesthesia. Subsequently, this distance was transferred to a paper card and measured with an electronic digital caliper (Mitutoyo Co., Miyazaki, Japan). By subtracting the distance (in millimeters) measured on day 0 from day 14, molar movement was calculated. All measurements were performed three times by the same investigator to eliminate bias, and the average value was calculated. Subsequently, the distances between points at the occlusal level of first and second molars on micro-CT images were also measured.

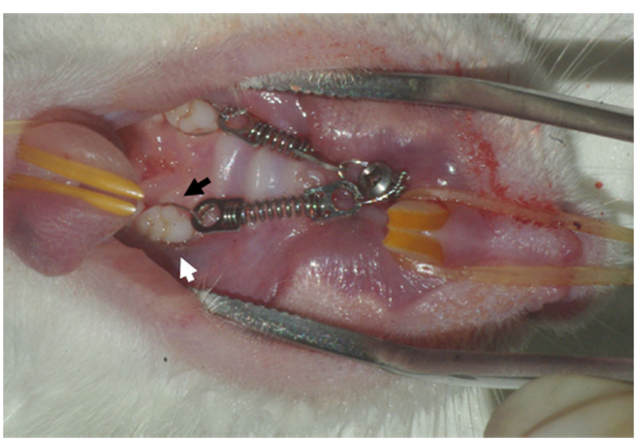

Fig. 2 View of the orthodontic appliance in a split-mouth design. Tooth was irradiated in palatal (black arrow) and labial (white arrow) sides using an infrared laser emitting at 810 nm, $p = 100$ mW. The energy was 1.5 J/side corresponding to a dose of 75 J/cm^2/side

Micro-CT scan and analysis

All the animals were euthanized on the 14th and 21st day of the study in a CO_2 chamber. Maxilla from each rat was dissected, sectioned in hemi-arches, fixed in 4% paraformaldehyde, and stored at room temperature in alcohol solution. Half of the animals from each group was euthanized on day 14 and the other half was euthanized on day 21, i.e., $n = 5$/group except for the control group ($n = 10$).

These samples were scanned using Skyscan 1172 (Bruker MicroCT, Kontich, Belgium) and associated software (Version 1.5.23) at a resolution of 5 μm, an X-ray and source potential of 70 kV, amperage of 142 μA, power of 10 W through a 180^o of rotation around the vertical axis, and a rotation step of at 0.4^o. Raw image data were reconstructed using the nRecon software Version 1.5.23, and they were analyzed using the CT Analyser software (version 1.15.4.0, Bruker MicroCT) [34].

From these 3D images, two rectangular volumes (200 μm width × 400 μm thickness × 1400 μm height) of the alveolar bone next to the mesial and distal buccal roots were analyzed. Bone mineral density (BMD), bone volume and total volume fraction (BV/TV), and trabecular thickness (TbTh) and separation (TbSp) were measured at the tension and compression sites (Fig. 4b, c).

Root resorption craters (RRC) were evaluated using ex vivo microcomputerized tomography on day 21. After 3D images of the upper first molars were reconstructed, the compression and tension sides of the mesial and distal buccal roots were analyzed using the CT Analyser software. Cross-sectional views of the areas with root resorption lacunae were generated [35]. All root resorption craters were drawn by outlining the boundary of the lacunae individually (region of interest) in all cross-sectional micro-CT images (200 slices per root), and the volume of each RRC was calculated. Total volumetric root resorption value in a particular region was the sum of all root resorption volume craters in that area, and this total value was assigned to the particular location: the tension and compression sites of the mesial and distal buccal roots (Fig. 4a). All samples were randomly named and allocated during the analysis to secure blindness of the experiment.

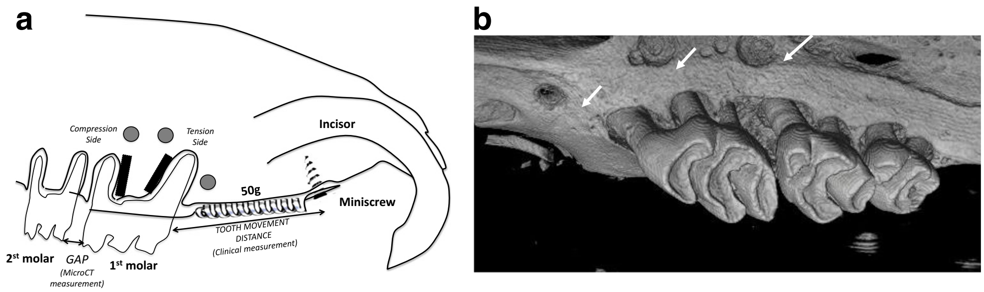

Fig. 3 **a** Representative drawing of the appliance used to facilitate tooth movement, and the schematic representation of trabecular bone volume at the tension and compression sides analyzed by micro-CT images (black rectangles). **b** Micro-CT 3D image of a sample that received corticopunctures around the upper first molar: one at the mesial and two at the palatal sides

Histology analysis

Maxillas from all groups 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 hematoxylin 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 at the tension and compression sides. A descriptive analysis of bone quality and root resorption craters was performed, and the number of osteoclasts was counted at the tension and compression sides.

Statistical analysis

Measurements were performed three times in distinct periods. Smirnov-Kolmogrov test was employed to investigate for normal distribution. A parametric statistical analysis of variance (two-way ANOVA) and Bonferroni test for post hoc were used to compare all the measurements. All tests were performed using Graphpad Prism 5 (GraphPad Software, San Diego, USA). The significance level was set at $p < 0.05$.

Results

All animals experienced initial weight loss, about 5%, during the first week in average, but the initial weight returned by the end of experiment. Appliance breakage or micro-implant loss did not occur during the study.

Tooth movement in distance

On the 14th and 21st day, tooth movement measured by electronic digital caliper was significantly greater in the combined corticopuncture and laser group (CP + LLLT or Cort+LLLT) compared to the CP group and LLLT group ($p < 0.05$). The corticopuncture group (CP or Cort) also showed a great amount of tooth displacement, followed by the laser group (LLLT). All three methods, CP + LLLT, CP, and LLLT groups, produced significantly greater tooth movements compared to control by 1.61-fold, 1.43-fold, and 1.3-fold and 1.78-fold, 1.4-fold, and 1.23-fold on days 14 and 21, respectively. Although CP group showed an increase in tooth displacement compared to LLLT, it did not show statistically significant difference at day 21 ($p > 0.05$). Measurements were also performed using micro-CT 3D images and were in agreement with the clinical findings (Fig. 5).

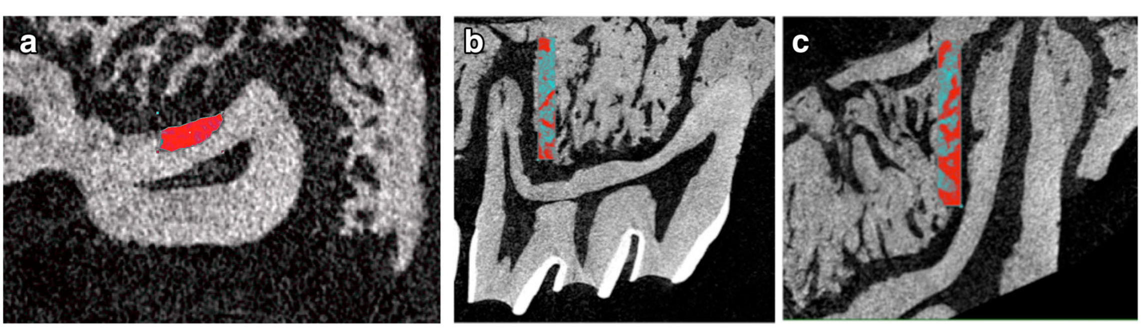

Fig. 4 **a** Root resorption: region of interest was highlighted and draw in all slices for volumetric measurement of RRC individually and used for the calculation of total RRC. **b** Alveolar bone region of interest highlighted at the compression side for bone evaluation. **c** Alveolar bone region of interest highlighted at the tension sides for bone evaluation

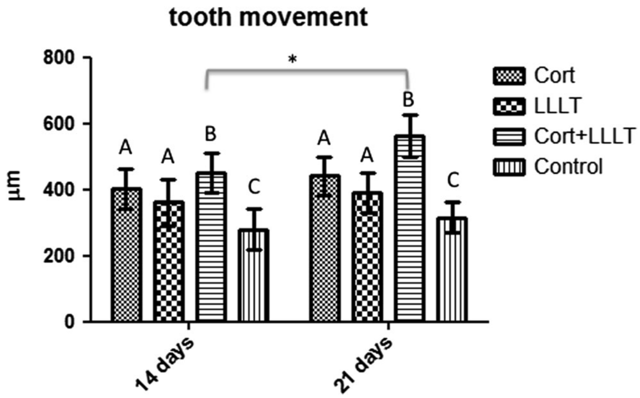

Fig. 5 Means ± standard deviation values of tooth movement evaluated by clinical measurement and micro-CT 3D images. Different capital letters indicate differences between groups at each time and *indicate $p < 0.05$

Volumetric analysis of trabecular bone: compression side

Bone mineral density (BMD) of the trabecular bone at the compression side of the distal buccal root was significantly lower in all three treatment groups (CP + LLLT, CP, and LLLT) compared to both control groups (C and NC) ($p < 0.05$) on day 14. Between treatment groups, BMD was significantly lower in CP + LLLT than in LLLT ($p < 0.05$), but the difference was not significant between CP + LLLT and CP groups on day 14. On day 21, no difference was found between all groups. All treatment groups showed a significant decrease in BMD compared to the negative control group (no movement) on day 14, maintaining it until day 21 (Fig. 6a). The control group did not show difference from the negative control group on day 14, but showed a statistically significant decrease on day 21.

Regarding bone volume to total volume ratio (BV/TV), the CP + LLLT group showed the lowest value, followed by CP and LLLT showing significant difference compared to the control group on day 14. Among treatment groups, significant difference was found between CP + LLLT and CP and LLLT groups. On day 21, CP groups showed the lowest BV/TV ratio compared to all other groups. Over time, both lasers groups (LLLT and CP + LLLT) showed a significant increase on BV/TV ratio compared to other groups. BT/TV was not significantly different between control group on days14 and 21(Fig. 6b).

All treatment groups showed significantly less trabecular thickness (TbTh) compared to the control group. However, no significant differences were found between three treatment groups on day 14. On day 21, laser groups (LLLT and CP + LLLT) showed higher trabecular thickness compared to CP and control groups. Also, over time, laser groups showed significant increase in trabecular thickness compared to CP and control groups. The control group showed less trabecular thickness on day 21 compared to day 14 and the NC group (Fig. 6c).

Trabecular separation (TbSp) presented significantly larger separation for all treatment groups compared to control on day 14. On day 21, only CP+LLLT group showed larger trabecular separation than control. No difference was observed for all groups between day 14 and 21. (Fig. 6d).

Volumetric analysis of trabecular bone: tension side

At the side tension side of the mesial root, among treatment groups, BMD was the highest for CP + LLLT, followed by LLLT and CP, and no difference was found between CP and LLLT. All treatment groups had significantly higher BMD than C on days 14 and 21. (Fig. 7a). Between treatment groups, CP showed less bone density throughout time. Over time, control and CP groups showed a statistically significant decrease in BMD while LLLT groups showed no differences compared to the negative control group.

BV/TV measurements between three treatment groups were not significantly different on day 14. However, laser groups had significantly higher values than CP and control groups on day 21. Over time, the control group showed less BV/TV ratio on day 14 and an increase on day 21. All treatment groups showed increase on BV/TV over time (Fig. 7b).

CP + LLLT showed greater TbTh compared to LLLT and CP on day 14. Both laser groups presented greater TbTh than CP and control groups ($p < 0.05$) on day 21. Trabecular thickness increased over time in all treatment groups. The control group showed a slight reduction on day 21 (Fig. 7c).

The control group showed less trabecular separation at the tension side compared to all treatment groups on day 14 but on day 21, the control group showed greater and the LLLT group showed significantly less trabecular separation compared to other groups.

Volumetric analysis of root resorption crater

The mesial root surface showed less volume of RCC than control groups but the differences were not significant among all the groups. However, on the distal buccal root surface, all test groups had significantly less RRC total volume than the control group. Among the test groups, no significant difference was found between CP and LLLT, but RRC total volume was significantly lesser for CP + LLLT than CP ($p < 0.05$).

Total RRC volume measurement of the mesial roots had no significant differences between CP and CP + LLLT and control groups. Only the LLLT group showed significant reduction on RRC compared to control. For distal buccal roots, all intervention groups showed significant decrease of RRC total volume compared to the control group, as CP + LLLT showing the least volume of RRC, followed by LLLT and CP (Fig. 8).

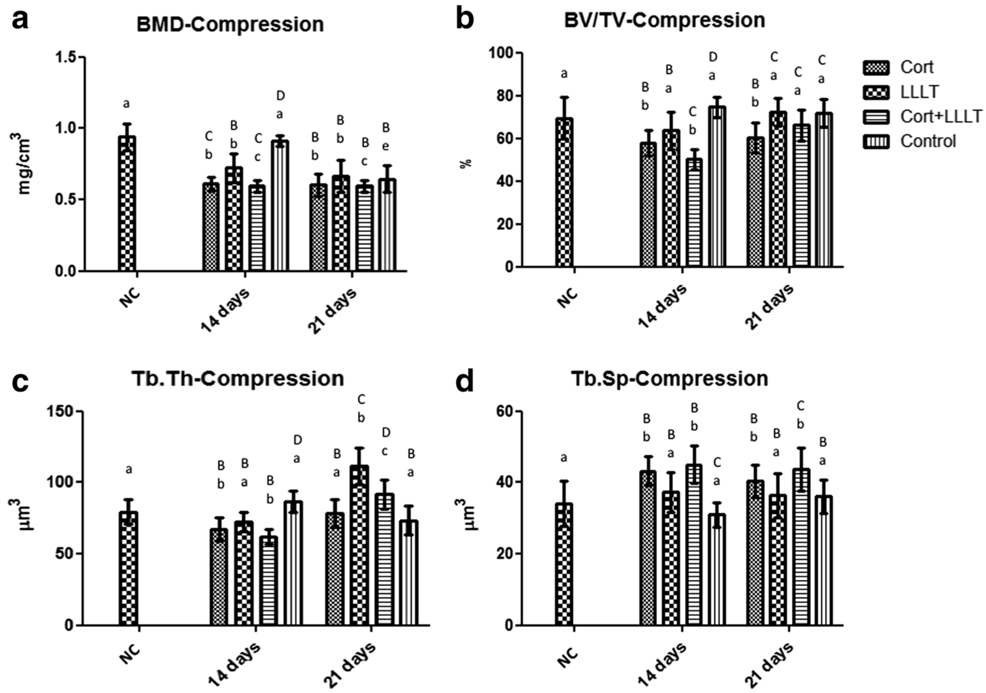

Fig. 6 **a** Bone mineral density (BMD). **b** bone volume to total volume ratio (BV/TV). **c** trabecular thickness (TbTh). **d** trabecular separation (TbSp) at the compression side. Error bar indicates standard deviation. Different capital letters indicate differences between groups at each time and lower case letters represent differences for each group over time ($p < 0.05$)

Histology findings

The reorganization of collagen fibers in the PDL and re-establishment of the PDL width with normal vascularization occurred at the compression side by day 21, and the signs of hyalinized tissue were not observed for all groups. However, the control group had extensive areas of root resorption with the presence of abundant alveolar bone near PDL areas. The irradiated groups (LLLT and CP + LLLT) exhibited less extensive RRC as indicated in Fig. 9 (white brackets). The CP groups (CP and CP + LLLT) had less alveolar bone near PDL with wider PDL spaces at the compression side (*).

All groups exhibited more elongated and parallel collagen fibers at the tension side. The CP + LLLT group showed neoformation of alveolar bone with more area of bone near PDL (white rectangle). LLLT and CP groups had similar amount of alveolar bone area, whereas the control group had less alveolar bone formation.

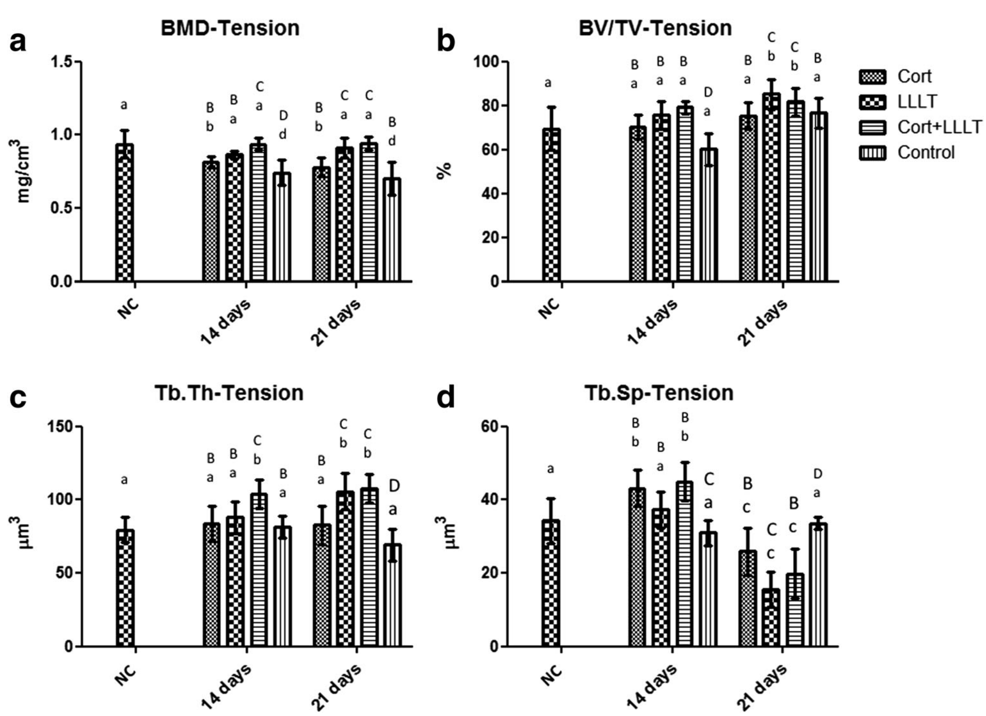

Fig. 7 **a** Bone mineral density (BMD). **b** bone volume and total volume ratio (BV/TV). **c** trabecular thickness (Tb.Th). **d** trabecular separation (Tb.Sp) at the tension side. Error bar indicates standard deviation. Different capital letters indicate differences between groups at each time and lower case letters represent differences for each group over time ($p < 0.05$)

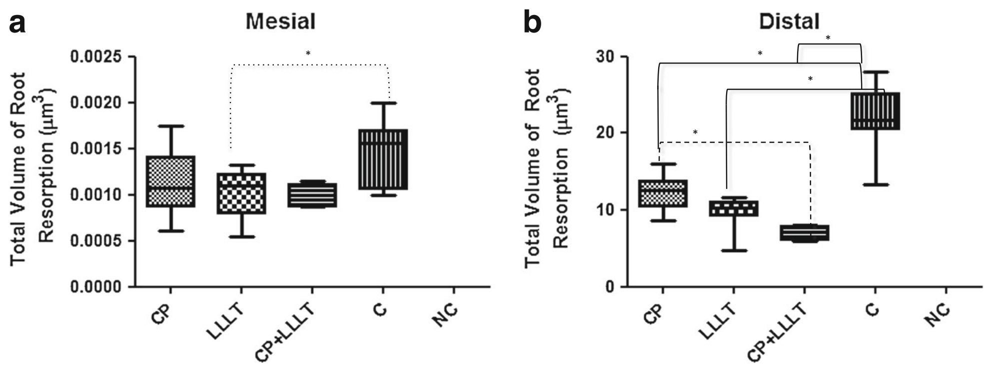

Fig. 8 Total volume of root resorption craters (RRC) found on the mesial (**a**) and distal (**b**) roots surfaces. Error bar indicates standard deviation. *indicate $p < 0.05$

Discussion

Nowadays clinicians face a new challenge concerning the duration of orthodontic treatment due to patient's demand for speedy process without compromising the results and comforts, while reducing the risk of decay, gingival inflammation, bone deterioration, and root resorption. Some attempts [13, 15, 36–39] have been made to establish the best approach to achieve the above objectives without extensive surgical and costly procedures. In this study, tooth displacement, bone remodeling, and root resorption were evaluated in CP, LLLT, and CP + LLLT groups on days 14 and 21 after tooth induction.

Taddei et al. [30] compared different protocols for tooth movement induced in rats by varying the amount of applied force. Based on that study, 50 g of applied force was specifically chosen to promote bone remodeling and to ensure root resorption for the current experimental model. If lighter force was applied, such as 10 g force, it would have facilitated tooth movement but root resorption may not have been found.

Since bone remodeling dictates the rate of tooth movement, applying corticotomy in the alveolar bone or low-level laser irradiation can effectively increase the speed of tooth movements by modulating bone metabolism. CP and LLLT were effective when they were applied independently; however, this effect was heightened when CP was combined with

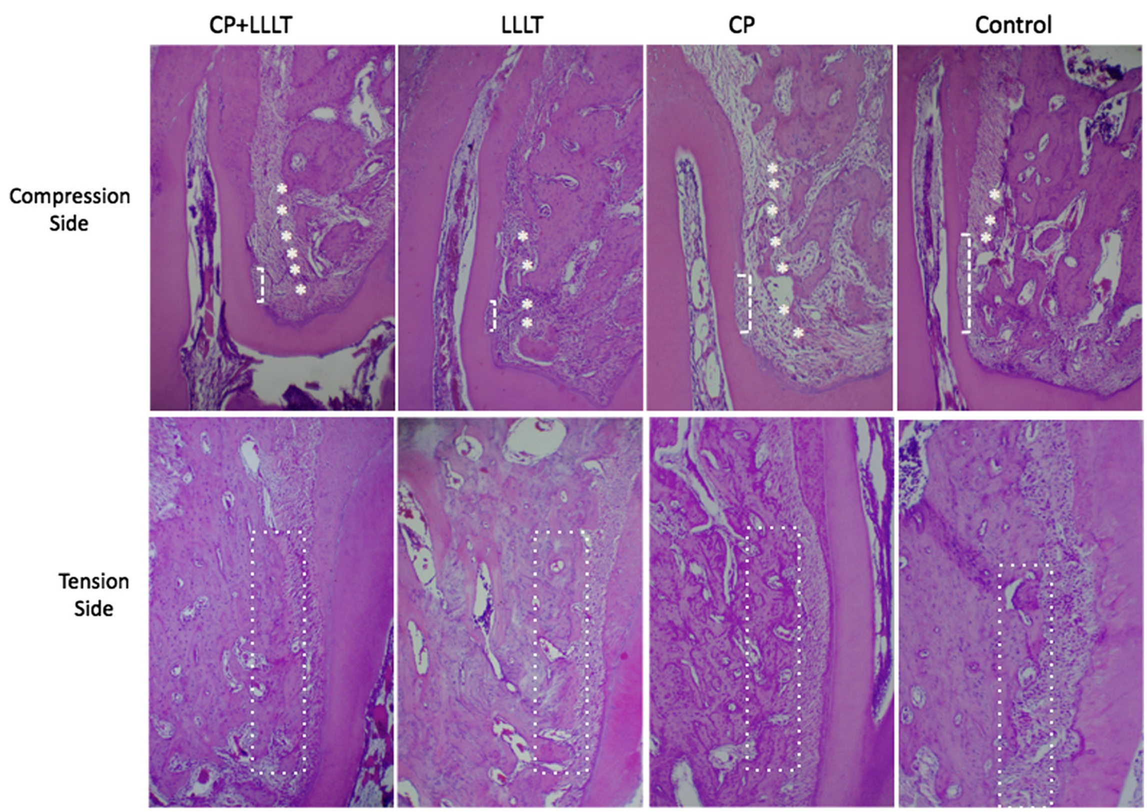

Fig. 9 Photomicrographs of rat's molars in the compression and tension sides of all studied groups. In the compression side, root resorption lacunaes (white brackets) and bone resorption (*) are observed. In the tension side, more bone formation can be seen for the CP + LLLT group near the PDL (white rectangle) (HE stain, original magnification ×40)

LLLT (CP + LLLT group), indicating the existence of synergy between the two stimulations. When both techniques were combined, it resulted in a faster tooth movement on days 14 and 21, 12.5% more than CP, 23.8% more than LLLT, and 61% more than control on day 14, and 27.1% more than CP, 44.7% more than LLLT, and 78% more than control on day 21. Although procedures such as corticision [13], micro-osteoperforation [16, 40], and corticopuncture may clinically present most predictive results, low-level laser therapy is a non-invasive method [20, 39, 41, 42]; therefore, combining both methods as proposed in this study may be a valuable option. In fact, the increase in speed of tooth movement by 60% (LLLT) and 78% (CP + LLLT) observed in our study could envisage benefits in clinical practice.

One of the aims of this study was to evaluate the differences in bone remodeling after 14 and 21 days of tooth movement with three different methods to promote ATM. BMD for all treatment groups was significantly lower than control groups in the compression side. When CP and LLLT were combined (CP + LLLT), BMD became significantly lower than that of the LLLT group but not compared to the CP group. The cumulative nature of heightened catabolic activity may be responsible for the increased rate of tooth movement in the CP + LLLT group, with CP having more impact in catabolic activity. The results of bone volume ratio (BV/TV), trabecular thickness (TbTh), and trabecular space (TbSp) and the presence of less alveolar bone area in the CP + LLLT group found in the histological analysis support the abovementioned finding. Although studies have shown that LLLT stimulated tooth movement and osteoclasts formation [21, 24, 43, 44], it seems that CP was more effective than LLLT in promoting catabolic activity in the compression side; however, their impacts were cumulative together. The bone quality was not significantly different between the control and negative control groups, indicating that compressive orthodontic force alone either does not boost the catabolic activities as significantly as CP, LLLT, and CP + LLLT groups or requires longer time to achieve the same level of catabolic activities and subsequently increasing the treatment duration.

The bone density measurements (BMD and BV/TV) in the tension side were higher for the three test groups than the control, indicating stimulation of anabolic activity. Unlike the findings from the compression side, LLLT had higher impact than CP on the tension side based on BMD, BV/TV, and TbTh on day 21 although the difference was not statistically significant between the two groups on day 14. However, CP + LLLT yielded significantly higher BMD than CP and thicker TbTh than both individual groups. It seems that LLLT played a more significant role in increasing the anabolic activity during the new bone formation than CP; however, combining CP with LLLT had a cumulative effect. In fact, histological analysis also showed larger area of alveolar bone in the CP + LLLT group, suggesting bone neoformation. Bone density (BMD, BV/TV) for the control dropped significantly on day 14 in comparison to the negative control group with no tooth movement, indicating that new bone formation in unstimulated orthodontic treatment is slow. CP and LLLT both increased anabolic activity, and the quality of newly formed bone improved in CP + LLLT in the 14 and 21 days compared to the negative control group.

The total volume measurements of RRC for distal buccal root surface illustrated similar results as in anabolic activity. All the three treatment groups successfully reduced the RRC formation. CP and LLLT did not show significant differences with each other in reducing RRC during the tooth movement. When they were combined (CP + LLLT), the RRC measurement was significantly lower than CP. This may indicate that LLLT may have played a slightly larger role in preventing root resorption than CP. The mesial root has a much larger surface area than the distal buccal root, and this may explain why the distal buccal root had more resorption. The effect of LLLT on root resorption has been elucidated in a few studies [24, 26, 45, 46] that showed less volume of root resorption in agreement with our findings.

It has been reported that higher level of inflammatory markers related to recruitment and activation of osteoclasts, causing subsequent accelerated tooth movement in patients who were subjected to micro-osteoperforations [15]. More recently, Cheung and collaborators used mini-implant-facilitated micro-osteoperforation (MOP) associated to microcomputed tomography volumetric analysis on all five roots of the maxillary first molars and no statistically significant differences between root volumes of the control and the MOP sides were observed [16]. Regarding association of LLLT and CP, authors have showed rapid tooth movement in dogs without any associated root resorption [25] and less hyalinization areas as well as faster removal of this hyalinization tissue in cats [13]. Our results, in a rat model, are in agreement with those studies, as we found a reduction of root resorption crater volume for the two groups treated with corticopuncture (CP and CP + LLLT), for both mesial and distal buccal roots in the compression side compared to the control. This may be due to the heightened bone resorption process that occurred in the CP and CP + LLLT groups, providing rapidly force dissipation at the PDL, preserving the cementoblast layer, and therefore avoiding root damage even in the presence of high bone resorptive activity. The LLLT group also showed the similar result.

Treatment dosage is one of the most important clinical parameters when applying LLLT. Finding the correct protocol for a particular condition is not an easy task [40]. In this study, we used the parameters reported by Suzuki et al. [24] who used total energy of 1.5 J, and showed acceleration of tooth movement at 14 days. Other parameters must be taken into account, such as wavelength, irradiance, contact or non-contact application, exposure time, type of tissue, physiological conditions, and optical properties of the tissue. In this study, the wavelength of 810 nm allowed a deeper tissue penetration compared to lasers with red emission, the wavelength was unchanged in both groups, and the fluence rate and fluence were kept similar in all animals. The laser beam was positioned carefully to ensure a uniform direct contact in order to reduce the energy loss by the reflection. The use of the same animal to compare an experimental and control group is a controversial subject with laser studies. The presence of bias due to systemic effect of LLLT cannot be discharged [47]. However, Shirazi et al. [48] compared three groups: one control group received unilateral orthodontic appliance design and the laser-irradiated group received split-mouth design, with orthodontic appliance on both sides and laser irradiation on one side only. The results showed that LLLT affected tooth movement on the side that was irradiated but no difference was found between the unilateral control group and the non-irradiated contra-lateral side group. In our study, we assumed that the systemic effect was not enough to significantly influence the control side, as in accordance with the study by Garcez. [33].

Although procedure such as corticision, micro-osteoperforation, and corticopuncture may clinically present most predictive results, LLLT is a non-invasive and promising method. Thus, combining both methods as proposed in this study may be a valuable alternative to other more invasive procedures. The use of considerably less aggressive and relatively benign procedures than performing extensive corticotomy can aid in clinical application of these new approaches. The synergic effects of LLLT when combined with CP may even reduce the number of corticopuncture sites and sessions to achieve the same bone response. Further studies are necessary to elucidate the kinetics of tooth movement using corticopuncture and LLLT as well as to establish the optimal clinical protocol and LLLT dosage for human tissues.

Conclusion

In this study, the results demonstrated that corticopuncture and low-level laser therapy could independently accelerate tooth movement and reduce the volume of root resorption craters by enhancing the bone remodeling process, causing greater bone resorption at the compression side and stimulating bone formation at the tension side compared to the control group. Corticopuncture seemed to have more impact in catabolic activity in the compression site, and laser therapy had more impact in anabolic activity in the tension site. CP seems to have more impact on tooth movement and LLLT on reducing RRC volume. Combining the two techniques showed cumulative effects in both accounts, and this novel therapeutic approach may serve to improve the clinical orthodontic outcome.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval 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).

Informed consent No informed consent was needed in this study.

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