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ORIGINAL ARTICLE

Effects of the GaAlAs diode laser (780 nm) on the periodontal tissues during orthodontic tooth movement in diabetes rats: histomorphological and immunohistochemical analysis

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Abstract The purposes of the present study are to assess the effects of the GaAlAs diode laser on the periodontal tissues and to investigate its action on the alveolar bone remodeling process during orthodontic tooth movement in normoglycemic and diabetic rats. Sixty adult male Wistar rats were divided into four groups of 15 rats: normoglycemic (N), diabetic (D), laser-normoglycemic (LN), and laser-diabetic (LD) rats. Diabetes mellitus was induced by a single intravenous injection of 40 mg/kg monohydrated alloxan. The orthodontically moved tooth underwent a force magnitude of 20 cN. The laser irradiation with a continuous emission of a 780-nm wavelength, an output power of 20 mW, and a fiber probe with a spot size of 0.04 cm in diameter and an area of 0.00126 cm^2 were used. Moreover, an energy density of 640 J/cm^2 was applied in an exposition time of 40 s. Histomorphological and immunohistochemical analysis was performed. The photobiomodulation (PBM) strongly stimulated the periodontal tissue response, establishing mainly the balance between the bone formation and resorption. Intense inflammatory cell infiltration and extensive loss of bone tissue were mainly found in the D group from 14 days. The number of osteopontin-positive osteocytes was significantly greater in the LN group, followed by the LD, especially at 7 and 14 days, whereas osteoprotegerin-positive osteoblasts were significantly higher in the LN and LD groups than in the N and D groups, respectively, in all periods. The PBM strongly stimulated the alveolar bone remodeling and favored the continuous reorganization of the soft periodontal tissues, leading to the maintenance and integrity of the periodontal microstructure under orthodontic force, especially in uncontrolled diabetic rats.

Keywords Type 2 diabetes · Periodontitis · Orthodontics · Photobiomodulation · Low-level laser therapy · OPN · OPG · RANKL

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Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by deficiency of insulin secretion or action, leading to chronic hyperglycemia and disturbances of carbohydrate, fat, and protein metabolism. The major complications of diabetes include microangiopathy, retinopathy, nephropathy, neuropathy, cardiovascular disease, susceptibility to infections, delayed tissue repair, and severe periodontal disease [1–7]. Considering the bone metabolic disorder in diabetes, imbalance between the alveolar bone formation and resorption processes is found due to decreased osteoblast activities and/or enhanced apoptosis of osteoblastic cells [4].

Several diabetic individuals have searched for orthodontic treatment, which has encouraged orthodontists to acquire a basic knowledge about the systemic complications of DM and its impact on the oral cavity.

However, if diabetes is uncontrolled or poorly controlled, severe degradation of periodontal tissues may occur, becoming a contraindication for orthodontic treatment until the metabolic disorder is compensated [1]. As an alternative support treatment to obtain an appropriated tooth movement and bone remodelation, the photobiomodulation (PBM), also known as low-level laser therapy (LLLT), has been largely used to promote therapeutic and biostimulating effects, such as analgesia, anti-inflammatory action, angiogenesis, and mitogenesis [7–11].

Taking previous studies into consideration, the purpose of this study was to assess the effects of the GaAlAs diode laser (780 nm) on the periodontal tissues and to investigate, in particular, its action on the alveolar bone remodeling process during orthodontic tooth movement in normoglycemic and diabetic rats through the histomorphological and immunohistochemical analysis using antibodies for OPN (osteopontin), OPG (osteoprotegerin), and RANKL (receptor activator of nuclear factor kappa B ligand) to assess the intensity of alveolar bone formation and resorption.

Material and methods

Animals

Sixty healthy adult male Wistar rats, with an average body weight of 200–250 g, were selected. These animals were randomly selected and divided into four groups of 15 rats: normoglycemic (N), laser-normoglycemic (LN), diabetic (D), and laser-diabetic (LD) rats. Subsequently, they were placed under natural lighting in ambient temperature conditions, fed a standard commercial diet, and given water ad libitum. This research was approved by the Animal Experimentation Ethics Committee of the Institute of Science and Technology-UNESP (protocol No. 11/2011-PA/Ethical in Research Committee). This work is in accordance with guidelines approved by the Council of the American Psychological Society (2010) for the use of animal experiments. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Alloxan-induced diabetes model

Initially, the animals were anesthetized with ketamine (5 mg/kg; Dopalen®; Agribrands, São Paulo, Brazil) and xylazine (10 mg/kg; Anasedan®; Agribrands, São Paulo, Brazil) solution, via intraperitoneal route, prior to inducing diabetes and applying the orthodontic apparatus. After, they received a single intraperitoneal injection of 40 mg/kg monohydrated alloxan (Sigma-Aldrich, St. Louis, MO, USA), dissolved in 5 mL of NaCl (0.15 mol/L), through the penile vein. Hyperglycemia was indicated when the blood glucose level rose above 200 mg/dL. The blood glucose level was assessed at 7 days after alloxan injection and before euthanasia of the animals to confirm the diagnosis of diabetes.

Installation of orthodontic appliance

The orthodontic appliance was installed 7 days after diabetes was confirmed. The anesthetized rats were placed in the dorsal decubitus position, with the four limbs affixed to a surgical table. Additionally, two adapted mouth openers were developed, with stainless steel wire of round section 0.8 mm in diameter, to allow the full visualization of intrabuccal structures and to inhibit any forward movement of the head. After these procedures, an adapted orthodontic appliance, composed of a 4.0-mm NiTi closed coil spring (Morelli® Campinas, São Paulo, Brazil), was attached to the animals. This spring was installed around the right mandibular first molar and stretched until the right mandibular central incisor above the level of the gingiva, applying a force magnitude of 20 cN (Fig. 1). This apparatus was fixed over the mandibular central incisor surface with light-cured composite resin (Quick Cure, Reliance Orthodontic Products, Itasca, USA).

Photobiomodulation

A GaAlAs diode laser (Twin Flex II, MMOptics, São Paulo, Brazil) with a continuous emission of a 780 nm wavelength, an output power of 20 mW, and a fiber probe with a spot size of 0.04 cm in diameter and an area of 0.00126 cm^2 were used. Without movement, the tip was held perpendicular and in contact with the alveolar mucosa. This procedure was done by a single operator. The root surface of the right mandibular first molar was irradiated at one point, located in the middle third of the root, on alternating days during each studied

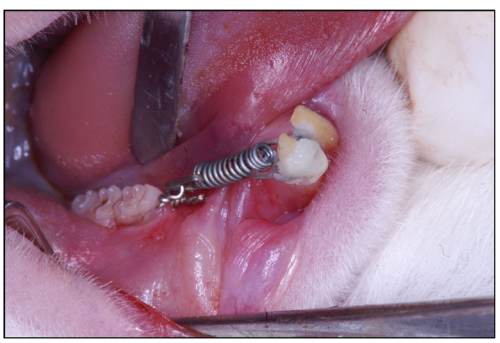

Fig. 1 Orthodontic appliance, composed of a 4.0-mm NiTi closed coil spring, installed between the right mandibular first molar and mandibular central incisor

period until the euthanasia of the animals. For this study, the energy density of 640 J/cm^2 was applied in an exposition time of 40 s to the LN and LD groups. The irradiations were performed on alternate days at a dose of 640 J/cm^2. The 7-day groups received a total of three doses, the 14-day groups received a total of six doses, and the 21-day groups received a total of ten doses, totaling 1920, 3840, and 6400 J/cm^2, respectively.

Histology

Five anesthetized animals in each group were euthanized at time intervals of 7, 14, and 21 days after the installation of the orthodontic appliance and the PBM application. The hemimandible that contained the orthodontically moved tooth was fixed in a 4% buffered paraformaldehyde solution (Sigma-Aldrich Chemical, St. Louis, MO, USA) for 48 h, decalcified in a 10% EDTA aqueous solution (Sigma-Aldrich Chemical, St. Louis, MO, USA) at room temperature and constant blend, then processed, and embedded in paraffin. Histological sections were cut to a thickness of 3 μm for histomorphological (hematoxylin–eosin staining) and immunohistochemical analysis.

Immunohistochemistry

For immunohistochemical analysis, slices were placed on silane-coated glass slides. The amplification labeled streptavidin-biotin (LSAB) method was used for the following rat monoclonal antibodies: anti-OPN (osteopontin, code: LF-175, National Institute of Dental and Craniofacial Research (NIDCR)) to evaluate the intensity of primary bone tissue formation and anti-OPG (osteoprotegerin, code: scN-20/SC-8468, Santa Cruz Biotechnology, CA, USA) and anti-RANKL (receptor activator of nuclear factor kappa B ligand, code: scN-19/SC-7628, Santa Cruz Biotechnology, CA, USA) to assess the inhibition-stimulation degrees of osteoclast activity. The alveolar bone formation and resorption during orthodontic tooth movement may be measured. The antigenic retrieval was performed in a 0.1 M Tris buffer and in citrate at pH 6.0 for anti-OPN and anti-OPG antibodies, respectively, using a microwave, while for the anti-RANKL, pepsin at pH 1.8 was used during 10 min at 60 °C and 50 min at 37 °C. The negative controls were obtained through histological sections with no primary antibodies for anti-OPN, anti-OPG, and anti-RANKL. The area of the right mandibular first molar was delimited using a drawing tool in the 4.7.2 AxioVision program (Carl Zeiss Vision Imaging Systems, Oberkochen, Germany). Images were taken using a ×40/0.65 objective lens (ACHROPLAN, Carl Zeiss) and an ocular ×10 (W-PI, Carl Zeiss) in a light microscope (Axioskop 40; Carl Zeiss) with a coupled digital camera (AxioCam MRc5, Carl Zeiss). The immunoreactive cell counting was accomplished in five random histological fields for each antibody, which are indicated in Fig. 2. Subsequently, a mean value was calculated.

Statistical analysis

The histomorphometric results for immunohistochemistry, expressed as the mean ± standard deviation (SD), were submitted to analysis of variance (one-way ANOVA) and the Tukey test using GraphPad InStat software version 5.0 for Windows 7 (GraphPad Software, San Diego, CA, USA). The level of significance was set at $P < 0.05$.

Results

Histomorphological analysis

The analyzed areas were gingival epithelium (oral, sulcus, and junctional epitheliums), lamina propria (connective tissue), periodontal ligament (PDL), and alveolar bone, especially in the furcation and interdental regions; additionally, the presence of tooth root resorption or the lack thereof was investigated. These structures were examined after the PBM in the normoglycemic and diabetic groups.

7 days

N group Discrete bone loss was observed at the interdental region; on the other hand, newly formed bone trabeculae were evidenced at the furcation region. The PDL displayed well-cellularized and well-vascularized connective tissue and mild mononuclear inflammatory cell infiltration. The gingival

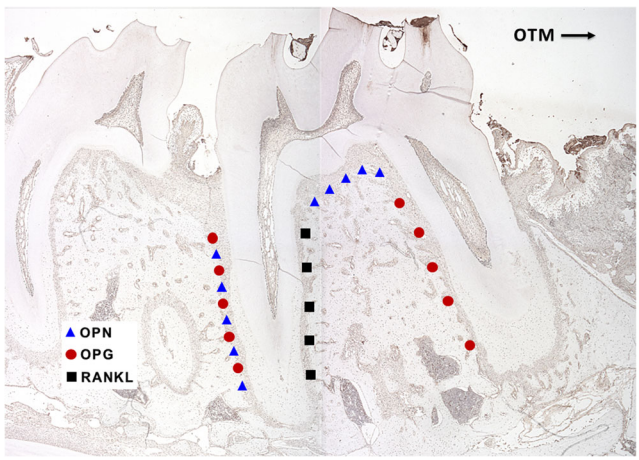

Fig. 2 Diagram showing the five histological fields for count of immune-positive cells for OPN (*triangles*), mesial surface of the interdental septum and mandibular first molar furcation, OPG (*circles*), mesial surface of the interradicular and interdental septa, and RANKL (*squares*), distal surface of the interdental septum. Direction of the orthodontic tooth movement (*OTM* ➔) (original magnification ×25)

tissue showed preserved; however, mild mononuclear inflammatory cell infiltration was evidenced.

LN group The alveolar bone formation was continuous at the interdental and furcation regions. The morphological features of the PDL were similar to the N group. The gingival tissue exhibited mild mononuclear inflammatory cell infiltration and congested blood vessels, especially in the subepithelial region. The biological width of the periodontium was better preserved in relation to the N group.

D group Moderate alveolar bone loss was evidenced at the interdental and furcation regions, which was replaced by granulation tissue. The gingival surface was covered by a serofibrinous pseudomembrane and the lamina propria showed a highly cellular connective tissue, numerous congested blood vessels, and intense mononuclear inflammatory cell infiltration.

LD group The interdental region was similar to the D group; however, there was discrete alveolar bone loss at the furcation region. The biological width of the periodontium was more preserved in some specimens when compared with the D group (Fig. 3a).

14 days

N group Conspicuous alveolar bone resorption triggered formation of periodontal pockets at the interdental region, as well as newly formed bone trabeculae and granulation tissue were evidenced at the furcation region in some specimens. Loss of collagen fibers in the PDL was observed; however, the periapical and oblique fibers showed still preserved. The gingival tissue was covered by serofibrinous pseudomembrane, which was supported by a granulation tissue. Intense inflammatory cell infiltration and numerous congested blood vessels were also noted.

LN group The alveolar bone structure presented normal aspects in most specimens at the interdental and furcation regions. The gingival tissue exhibited discrete mononuclear inflammatory cell infiltration, especially in the subepithelial region.

D group Intense alveolar bone loss was observed in most specimens at the interdental and furcation regions. The gingival tissue was covered by hyperplasic epithelium, whereas the connective tissue displayed intense cellularity, numerous blood vessels, and intensive mononuclear inflammatory cell infiltration.

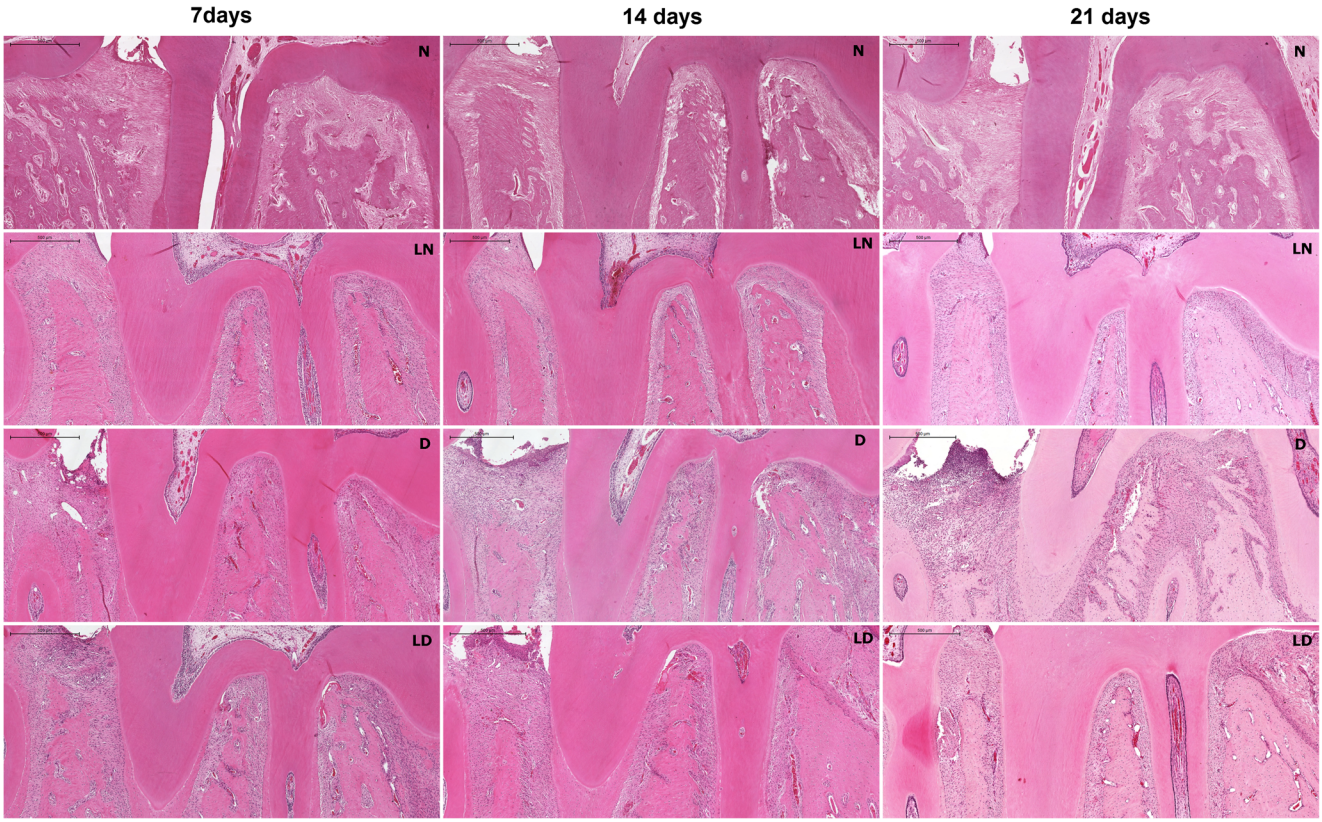

Fig. 3 Seven, 14, and 21 days after orthodontic tooth movement. Photomicrographs showing right mandibular first molar furcation and interdental region (between the mandibular first and second molars): N, LN, D, and LD groups: hematoxylin–eosin

LD group Moderate alveolar bone loss was evidenced at the furcation region; on the other hand, the interdental region showed intense. The gingival tissue was similar to the histological findings of the D group; however, moderate mononuclear inflammatory cell infiltration was evidenced (Fig. 3b).

21 days

N group Periodontal pockets were evidenced at the interdental region, as well as irregular new bone trabeculae and granulation tissue were found at the furcation region in most specimens. The gingival tissue was covered by stratified squamous epithelium and serofibrinous pseudomembrane; additionally, moderate inflammatory cell infiltration and numerous blood vessels were found. The periapical and oblique fibers of the PDL were preserved.

LN group The alveolar bone microarchitecture was maintained at the interdental and furcation regions. The gingival tissue showed epithelialized, as well as discrete mononuclear inflammatory cell infiltration and numerous blood vessels were found. The biological width of the periodontium was preserved in most specimens.

D group Extensive alveolar bone loss and intense polymorphonuclear and mononuclear inflammatory cell infiltration were found at the interdental and furcation regions, in some specimens. The gingival tissue was covered by serofibrinous pseudomembrane and composed by granulation tissue.

LD group The wall of the interdental alveolar bone exhibited thin thickness and discrete loss of height, whereas the bone microstructure was kept at the furcation region. The gingival tissue showed preserved epithelium and rare mononuclear inflammatory cell infiltration in most specimens (Fig. 3c).

Finally, tooth root resorption of the right mandibular first molar was strongly evidenced in the diabetic group, in particular, at 21 days.

Immunohistochemical analysis

The amount of immune-positive osteocytes for OPN was significantly higher in the LN group when compared with the N, D, and LD groups at 7 days ($P < 0.001$) and 14 days ($P < 0.05$, $P < 0.001$, and $P < 0.05$). Additionally, OPN immunoreactivity was stronger in the LD group than in the D group at 14 days ($P < 0.001$) and 21 days ($P < 0.01$); however, no statistical significance was found in relation to the N group in both the periods. Furthermore, there was a statistical significance between the N and D groups at 14 days ($P < 0.001$) and 21 days ($P < 0.05$). The number of immune-positive osteoblasts for OPG was higher in the LN and LD groups ($P < 0.0001$) when compared with the N and D groups at 7 days, respectively. The LN and LD groups showed significant difference in relation to the D group at 14 days ($P < 0.01$ and $P < 0.05$) and 21 days ($P < 0.01$); however, no statistical significance between the N, LN, and LD groups was found at 14 days. There was statistical significance between the N ($P < 0.01$) and D groups at 14 days. Although the number of immune-positive osteoclasts for RANKL has been variable, no statistical significance was found among the studied groups and periods (Fig. 4 and Table 1).

Discussion

It is largely accepted that the rapid progress of periodontal disease in diabetes occurs due to the function and reduction of polymorphonuclear leukocyte chemotaxis leading to an increase in infection susceptibility, collagen synthesis and maturation reduction, increased collagenase activity, and the formation of advanced glycation end products (AGEs) that bind to the receptor for advanced glycation end products (RAGEs) in macrophages and monocytes [3, 6, 9]. Researchers have described that the effects of AGE accumulation increase tissue oxidant stress, alter the endothelial cell functions, elevate the activity of matrix metalloproteinases leading to the production of free radicals, and promote vascular dysfunction and cellular death [9, 12]. These factors may directly affect the migration and activity of inflammatory cells, impairing the mechanisms of defense against microorganisms and delaying the periodontal tissue repair processes [2]. Based on these assumptions, we hypothesize that AGE accumulation in the periodontium of the diabetic rats could be present especially in longer observation periods; however, the measurement of markers for anti-AGEs and RAGEs is necessary to validate this speculation.

Some studies have shown that the low resistance of bone structure in diabetes is related to alterations of proteoglycan synthesis and decreased osteoblastic activity causing loss of bone matrix production and, subsequently, delayed primary bone formation. This may promote an imbalance between osteoid tissue formation and bone mineral homeostasis, leading to osteopenia or osteoporosis [13]. Our histomorphological findings showed that the intense osteoblastic and osteoclastic activities were especially located in the tension and pressure sides, respectively, in the PDL. This phenomenon resulted in bone formation and resorption during the orthodontic tooth movement in the studied periods and groups; however, the alveolar bone remodeling varied from intense bone loss to bone homeostasis. According to literature, the orthodontic mechanical stress generates two different forces in the PDL: compression and tension. In the compression site, the force that is generated by the root against the alveolar bone induces bone resorption. In the tension site, PDL fibers are strained and bone tissue is formed [14, 15]. It is known that under normal physiological

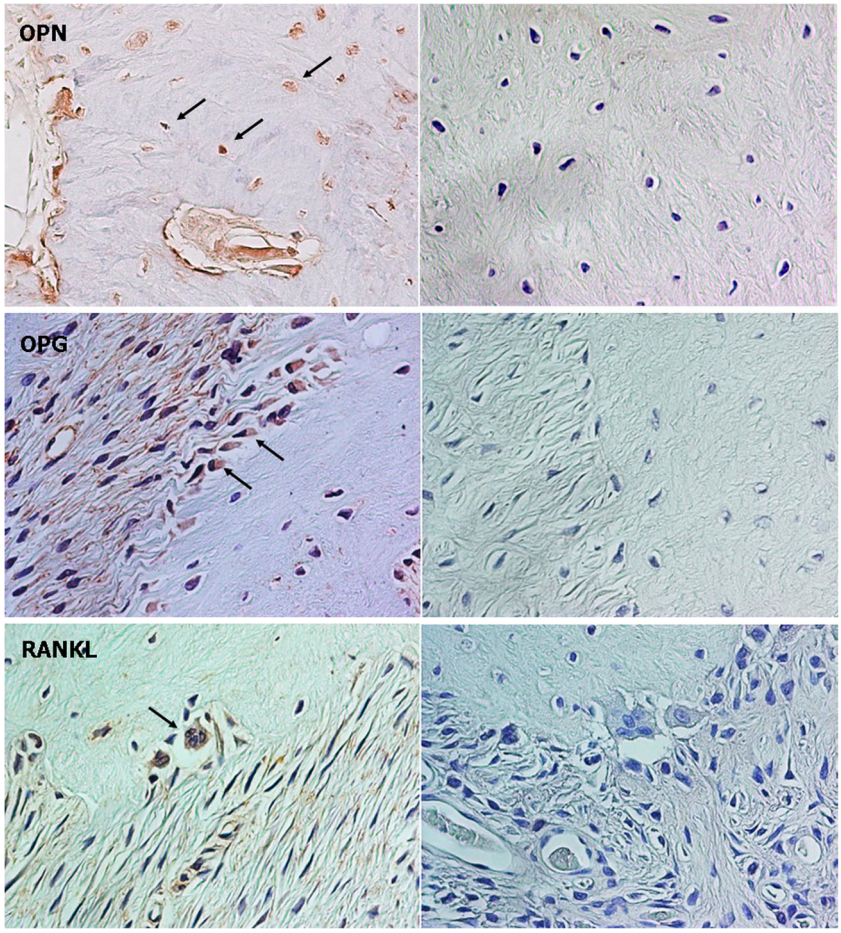

Fig. 4 Representative immunohistochemical reactivity (*arrows*) for OPN (immune-positive osteocytes), OPG (immune-positive osteoblasts), and RANKL (immune-positive osteoclasts) at 21 days and their respective negative controls (immunohistochemical staining, original magnification ×400)

conditions, the differentiation of mature osteoclasts takes place within the bone marrow and that only very few mature osteoclasts migrate to the PDL at the compression side during orthodontic tooth movement [14].

It is important to note that the laser irradiation stimulated alveolar bone turnover during the orthodontic tooth movement, as well as favored the preservation of periodontal structures, especially in the irradiated hyperglycemic rats (LD

Table 1 Amount of immunoreactive cells for primary antibodies anti-OPN, anti-OPG, and anti-RANKL (mean ± SD)

Periods of observations (days)	Antibodies	N Mean ± SD	LN Mean ± SD	D Mean ± SD	LD Mean ± SD
7	OPN	17.63 ± 1.53	24.20 ± 2.86 aABb***	17.23 ± 0.51	17.70 ± 0.87
	OPG	8.40 ± 0.26	10.93 ± 0.05 aA***	8.33 ± 0.20	11.70 ± 0.34 bB***
	RANKL	1.30 ± 0.34	0.66 ± 0.20	0.80 ± 0.36	1.10 ± 0.45
14	OPN	19.70 ± 0.43 AB***	23.43 ± 0.70 aAb*, aB***	13.37 ± 0.47	19.57 ± 0.32 bB***
	OPG	12.33 ± 0.20 AB**	12.43 ± 0.15 aB**	10.47 ± 0.40	12.13 ± 0.86 bB*
	RANKL	0.83 ± 0.15	0.76 ± 0.41	0.73 ± 0.60	1.20 ± 0.17
21	OPN	13.53 ± 0.65 AB*	13.62 ± 1.33	9.82 ± 1.17	14.72 ± 0.82 bB**
	OPG	9.93 ± 0.45	11.03 ± 0.47 aB**	9.16 ± 0.49	11.23 ± 0.11 bB**
	RANKL	0.80 ± 0.17	0.43 ± 0.30	0.73 ± 0.35	0.56 ± 0.46

The letters "AaBb" indicate the statistical significance in relation to the experimental groups; where a group of these letters is found, the result for the group represented by the first letter is statistically significant in relation to the others (*A*, N group; *a*, LN group; *B*, D group; *b*, LD group)

SD standard deviation, *N* normoglycemic rats, *LN* laser-normoglycemic rats, *D* diabetic rats, *LD* laser-diabetic rats

Statistical significance was considered at the level $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$

group). In contrast, intense inflammatory cell infiltration, alveolar bone resorption, and destruction of PDL fibers were found, mainly in the D group from 14 days, while tooth root resorption was strongly proven at 21 days. Most likely, these findings were intensified by the effects of the orthodontic device on periodontal tissue, which was installed 7 days after the diagnosis of diabetes. Previous studies have described that the newly formed alveolar bone in diabetes does not present the same structural features when compared with normoglycemic animals during orthodontic tooth movement [3, 7], agreeing with our findings.

Mechanical loading associated with actions of hormones and cytokines may change the bone architecture through a spectrum of molecules, which are involved in stress-dependent regulation of bone formation, affecting the stimulation of bone remodeling [16, 17]. Among them, the OPN protein is a major noncollagenous bone matrix protein, which is distributed throughout the entire mineralized matrix. It is an important indicator for assessing primary bone formation under mechanical stress in expansion force-induced osteogenesis models [16, 18]. Researchers report that expression of OPN in osteocytes of the alveolar bone was greatly increased, particularly in the area that received the tension force, becoming an important triggering factor that leads to bone remodeling caused by orthodontic mechanical stress [16–18]. In our study, the use of anti-OPN identified immunoreactive osteocytes on the mesial surface of the interdental septum, especially in the LN and LD groups at 7 and 14 days, showing intense activity of alveolar bone formation.

The RANK/RANKL/OPG system is essential for skeletal homeostasis, and has helped in concepts of bone metabolism, resulting in a better understanding of the pathogenesis of bone disorders. RANKL/RANK signaling pathway regulates osteoclast formation, activation in physiological bone remodeling, and a variety of pathological (osteoporotic) conditions. OPG protein, produced by osteoblasts and undifferentiated cells, protects the bone tissue from excessive osteoclastic resorption through binding to the RANKL, preventing it from binding to RANK [19]. Therefore, this system reflects osteoclast differentiation and function, which determine skeletal bone mass and integrity [11]. This may lead to the development of new therapeutic strategies for stimulating the bone repair process.

Concerning the immunohistochemical results, we evidenced that the irradiated rats (LN and LD groups) presented intense primary bone tissue formation when compared with the non-irradiated rats (N and D groups) due to a great amount of OPN-positive osteocytes, especially at 14 days. Additionally, we found intense bone resorption-formation activities, intense vascularization, and well-organized collagen fibers on the PDL, therefore, better preservation of the alveolar bone microstructure. This confirms the efficiency of the GaAlAs laser irradiation on the periodontal tissues. In contrast, the amount of OPN-positive osteocytes in the LN and LD groups decreased at 21 days. This finding could be justified due to the biomechanical loss of orthodontic springs, reducing the magnitude of the force on the periodontal tissues at 21 days. However, other hypotheses more likely could be the inhibitory effects of the periodontal tissue responses to the PBM, which were generated by high irradiances.

The literature reports that the laser PBM can generate a biphasic response on several tissues, displaying patterns of stimulation or inhibition. In relation to these biomodulating properties, the stimulatory effects are applied to enhance the cellular and molecular response in tissue repair, while the inhibitory effects are used to attenuate the inflammation and to reduce pain and swelling caused by injuries, degenerative disease, or autoimmune diseases. In practice, if irradiance is lower than the physiological threshold value for a given target, it does not produce beneficial effects even when irradiation duration is extended. Moreover, photoinhibitory deleterious effects may occur at higher irradiances, inducing hyperthermia. This provides further evidence of the Arndt-Schulz law which states that there is only a narrow window where you can activate a cellular response using specific sets of parameters [20, 21]. Thus, we consider very likely that the switch from a stimulatory to inhibitory response of the periodontal tissues under orthodontic force has occurred due to high levels of irradiances used in this experiment, in particular, at 21 days.

According to previous studies of Schindl et al. [22], low doses of laser irradiation promote the formation of a transmembrane photoelectrochemical proton gradient in mitochondria leading to liberation of intracellular Ca^{2+}, which stimulate various biological processes including RNA and DNA synthesis, mitosis, and protein secretion. Elevated doses result in excessive liberation of intracellular Ca^{2+} causing hyperactivity of calcium-ATPase pumps and exhaust ATP pool in the cells, thereby inhibiting the cell metabolism.

It is important to emphasize that the hyperglycemic rats showed more accentuated periodontitis in comparison to the normoglycemic rats, especially in the 21-day period. We hypothesize that the appearance of diabetes complications could also be associated with two more factors: orthodontic forces on the periodontal tissues and psychological stress caused during the various handlings of the animal for administration of general anesthesia and PBM applications. Therefore, as a compensatory mechanism, the PBM may act largely in the bioregulation or normalization of cellular functions, enhancing the cellular metabolism and, consequently, stimulating the repair process of the periodontal tissues. According to Braga et al. [4], severe periodontitis occurs due to the high levels of pro-inflammatory mediators and chemokines, leading to an increased number of osteoclasts and, consequently, intense bone resorption, as well as a greater range of orthodontic tooth movement in experimentally induced diabetes.

Additionally, there was a statistical significance ($P < 0.01$) in the number of OPG-positive osteoblasts in the N group

when compared with the D group at 14 days. Furthermore, we evidenced that the LD group displayed an increased significant difference in the number of OPG-positive osteoblasts in relative to the D group on 7 days ($P < 0.0001$), 14 days ($P < 0.05$), and 21 days ($P < 0.01$). The convergence of these findings demonstrates the effectiveness of the biostimulating effects of the GaAlAs laser irradiation on the periodontal tissues, submitted to the mechanical loading, resulting in the preservation of the periodontal structures.

Although no statistical significance in the amount of RANKL-positive osteoclasts in all the groups and periods was found, we noted that the number of immunoreactive osteoclasts was inversely proportional to the number of immunoreactive osteoblasts, expressing a regulatory activity of the OPG protein. This fact confirms the pathophysiological dynamics of the bone remodeling process on the periodontium during orthodontic tooth movement in diabetic rats treated with PBM. According to investigations of Amorim et al., the diabetes may trigger an imbalance of the RANK/RANKL/OPG system, causing intense alveolar bone resorption and, consequently, exacerbation of the periodontitis. This explains our findings that diabetes, without the use of laser therapy, promoted large alveolar bone loss resulting in severe periodontal disease.

On the basis of this study, we suggest that the GaAlAs diode laser could be used in diabetic individuals, as a support treatment in orthodontics. Moreover, we can emphasize that the indication of the PBM must be carefully studied, since it depends exclusively on the general and oral health status of patients with diabetes. Given the above, it is extremely important that dentists have knowledge and understanding about the systemic and oral manifestations of diabetes mellitus and the biological properties of the PBM on the periodontal tissues during orthodontic tooth movement.

Conclusions

Based on the results, we can to conclude that the GaAlAs diode laser irradiation enhanced the biological responses of the periodontal tissue during the orthodontic tooth movement. Therefore, the PBM strongly stimulated the alveolar bone remodeling and favored the continuous reorganization of the soft periodontal tissues, leading to the maintenance and integrity of the periodontal microstructure under orthodontic force, especially in uncontrolled diabetic rats.

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Compliance with ethical standards This work is in accordance with guidelines approved by the Council of the American Psychological Society (2010) for the use of animal experiments and is in accordance with the Animal Welfare Act (7 U.S.C. §2131 et.seq.). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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

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Human and animal rights and informed consent This research was approved by the Animal Experimentation Ethics Committee of the Institute of Science and Technology-UNESP (protocol No. 11/2011-PA/Ethical in Research Committee).

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