Infrared Light-Emitting Diode (LED) Effects on Orthodontic Tooth Movement

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The present study aimed to analyze the effect of LED phototherapy on the presence of hyalinization and root resorption during orthodontic tooth movement (OTM) in rats and to measure the amount of tooth movement. Eighty rats were allocated into two groups: LED and control (CON), where the LED rats were irradiated with infrared LED (850 nm, 30 mW) for 5 min during the first five days of OTM and where controls were not irradiated. Both groups were subdivided into four subgroups (n=10) according to the date of euthanasia (4, 7, 14 and 21 days). Five out of ten LED21 and five of ten CON21 rats were submitted to micro-computed tomography (µCT); µCT scans were taken on days 0, 7, 14 and 21. For histological study, maxillae were processed to light microscopy using Hematoxylin-Eosin (HE) and Tartrate-Resistant Acid Phosphatase (TRAP) histochemistry. The amount of tooth movement did not differ between LED and CON. Hyalinization was observed at the pressure areas in both groups, and it did not show a statistically significant difference between the groups. Root resorption was also observed in both groups after 7 days and it did not represent any differences between the two groups. LED phototherapy was not able to increase the amount of OTM. Similar characteristics of hyalinization and root resorption were observed in both groups.



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Introduction

Orthodontic tooth movement (OTM) results from an applied force to a tooth that is transmitted to the supporting periodontal tissues. OTM leads to coordinated biological responses that allow tooth displacement within the alveolus. The early biological reactions in the periodontal ligament after the application of force include vasodilation, exudation and migration of inflammatory cells. The inflammatory cells produce cytokines and signaling molecules, which are associated with the maintenance of pressure of the blood vessels, resulting in cell-free areas in the periodontal ligament that appears with a homogeneous staining pattern due to the alteration in the extracellular matrix in special collagen fibrils. As the histological appearance resembles that of hyaline cartilage, it is called "hyalinization"; alveolar bone resorption follows the removal of hyalinization areas (1). As bone resorption is necessary for allowing tooth movement in the direction of orthodontic force application, the amount of tooth movement is directly related to the rate of bone resorption (1). While active osteoclasts are responsible for resorption of alveolar bone at the pressure areas, odontoclasts could also resorb the adjacent cementum at the root surface (2), and the root resorption is an undesirable side effect during OTM.

There is a constant search for a safe mechanism to enhance the amount of OTM without inducing transitory

and permanent deleterious effects such as periodontal ligament hyalinization and root resorption. Phototherapy has been the topic of many studies investigating the effects of laser on OTM. It has been clinically demonstrated that low-level laser therapy (LLLT) reduces the pain occasioned by orthodontic force (3-5) and accelerates tooth movement (5,6). It has also been reported that laser coherent light (laser) and non-coherent light (LED) produce similar biological effects (7); however, infrared LEDs have not been well investigated. Previous clinical studies revealed that LED significantly increased the amount of tooth movement and reduced pain in patients (8-10). It reduces number of osteoclasts in the periodontal ligament (11) and accelerate tooth movement in rats (12).

The use of LED in in vivo experiments demonstrated an important photobiomodulatory effect on inflammation, including a decreased number of inflammatory cells, increased fibroblast proliferation, angiogenesis stimulation, granulation tissue formation and an increase of collagen synthesis (13–15).

Due to the scarce number of studies related to the amount of tooth movement, hyalinization, and root resorption during orthodontic tooth movement with non-coherent light, we aimed to analyze the effect of LED phototherapy on hyalinization in the periodontal ligament and on root resorption during OTM in rats. The null hypothesis was that infrared LED therapy would reduce hyalinization, avoid root resorption lacunae and accelerate tooth movement.

Material and Methods

Animals

Eighty 10-weeks-old Wistar rats (250-270 g) were randomly divided into eight groups of 10 rats according to infrared LED phototherapy (LED irradiation). The animals were submitted to general anesthesia using intramuscular injection of 12 mL/100 g body weight ketamine hydrochloride and 6 mL/100 g body weight xylazine hydrochloride mixed. In control groups, the animals were not irradiated. In infrared LED groups, the animals were exposed to infrared LED irradiation. In each LED subgroup, the animals were exposed to LED irradiation, and the time elapsed between the start of the OTM and euthanasia was 4, 7, 14, and 21 days (groups LED4, LED7, LED14, and LED21, respectively). The time elapsed between the start of the OTM and euthanasia for the control groups was 4, 7, 14, and 21 days (groups CON4, CON7, CON14, and CON21, respectively). The euthanasia was performed by decapitation after anesthesia.

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During the entire experiment, the animals were maintained on a vented shelf with controlled temperature (25 °C), 12/12 h light cycles and water and chow ad libitum. Principles of laboratory animal care (NIH publication 85-23, 1985) and national laws on animal use were observed. All the experiments using animals were approved by the Ethical Committee for Animal Research at the University of São Paulo (number of protocol: 028/2013).

Infrared LED Irradiation

A LED device displaying 850 nm wavelength emitting a power density of 30 mW/cm2 was used in the present

study. Irradiation was performed with continuous waves under a lower dose of general anesthesia (3 mL/100 g body weight ketamine hydrochloride and 1.5 mL/100 g body weight xylazine hydrochloride) to ensure the immobility of the animal. The left cheek that covered the upper molars was shaved before irradiations. The LED device was extrabuccally positioned close to the left cheek and the 2 cm² area was irradiated daily for 5 min.

Microcomputed Tomography

Five rats of the LED21 group and five of the CON21 group were submitted to in vivo μ CT SkyScan 1176[®] (SkyScan NV, Kontich, Belgium) (75 Kv,80 μ A, 18 μ m, 360°). The rats were anaesthetized to ensure that they remained still during scanning. μ CT scans were taken on day 0 (immediately after installing orthodontic appliances) and on days 7, 14 and 21.

Orthodontic Tooth Movement

Experimental OTM was performed using a closed-coil spring (Ø 1 mm, 7 mm) exerting 10 g force to mesially move the molar. The force magnitude was measured by a gauge; a stainless steel ligating wire (wire size: 0.008", Morelli®, SP, Brazil) was used to attach the coil-spring on the first maxillary molar (cervical) and incisors (cervical). The tying wire was analogous to a band encircling the crown of the maxillary left first molar while attaching to one end of the spring. The other end was attached to the maxillary incisor using a tying ligature wire; the teeth were covered by light-activated resin to improve coil spring retention (Fig. 1A).

Measurement of Tooth Movement

The amount of tooth movement was measured only

in the CON21 and LED21 groups. The determination of the amount of OTM (linear measurements) was performed on MIMICS software (version 19.0, Materialise, Leuven, Belgium), where the segmentation of the maxillary of the molar was acquired (Fig. 1B). A sagittal plane from occlusal to apical was constructed; this plane was perpendicular to the occlusal of the maxillary molars (Fig. 1C). Maxillary molars were constructed, and the 3D image of each rat was superimposed on different time points. For superimposition of the images, the marked points were the



Figure 1. A: The coil spring was calibrated to deliver 10 g of force and was attached to the upper central incisors and the first left upper molar; B: segmentation of the maxillary of the molar; C: A perpendicular plane from occlusal to apical was constructed.

anterior foramen of the maxilla, the mesial marginal crest of the left upper second molar, and the distal marginal crest of the left upper third molar. The spatial difference between the first molar and second molar determined the amount of movement of the tooth at different time points: T1 (7 days – 0 days), T2 (14 days – 7 days), and T3 (21 days – 14 days). All measurements were performed by the same blind and calibrated operator.

Histological Analysis

A histological examination was performed to verify the tissue hyalinization, while histochemistry was prepared for the identification of clastic cells. On days 4, 7, 14 and 21, the animals were anaesthetized and euthanized by decapitation. The maxillae were dissected out and fixed in the 0.1% glutaraldehyde + 4% formaldehyde, buffered in 0.1 M phosphate, pH=7.2, and left submersed overnight at 4 °C. The specimens were decalcified in 4.13% EDTA, pH=7.2, for 30 days, dehydrated in ethanol and embedded in paraffin. The paraffin blocks were oriented to cut the maxillae perpendicular to the occlusal plane of molars, producing 5-um-thick sections utilizing a microtome.

The blocks were stained with hematoxylin and eosin (HE) and analyzed in an optical microscope. Twenty adjacent slides of each animal were constructed, in which each slide contained three sections, covering a distance of 100 µm in the buccolingual direction. One section of each slide showing the biggest mesial surface length of the distal palatal root of the first molar was selected for evaluation. For measurements of hyalinization, light micrographs were digitalized in an Olympus BX60 light microscope equipped



Figure 2. The extension of hyalinization was calculated using ImageJ software. A line was drawn from the highest point (H) to the lowest point of the hyalinized necrosis area (L).

with an Olympus DP72 CCD camera (Olympus Corp., Tokyo, Japan) with a 10' objective using CellF software (Olympus Soft Imaging Solution GmbH, Munster, Germany). The extension of hyalinization and root resorption area were calculated using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). A straight line was drawn throughout the hyalinized area, from the highest point to the lowest point of the hyalinized necrosis area; the length in micrometers for each slide was predicted by the software (Fig. 2), every measurement was repeated three times, followed by the average calculation for the hyalinization in the periodontal ligament. Likewise, the area of root resorption lacunae was calculated by the software, utilizing the values obtained from three slides of each animal (Fig. 3).

Additional sections of all groups were incubated for Tartrate-resistant acid phosphatase (TRAP) histochemistry. The sections were incubated for 2 h in Burstone's complete medium at 37 °C (Sigma Chemical, St. Louis, MO, USA), rinsed in tap water and counterstained with Harris hematoxylin. Coverslips were assembled with Entellan® (Merck, Germany) and the three slides analyzed with a 10' objective lens in an Olympus BX60 light microscope. The number of clastic cells was recorded as acidophilic multinucleated gigantic cells on the surfaces of the root or alveolar bone and in the periodontal ligament of the pressure area.

Statistical Analyses

The data are expressed as the means \pm SD for each parameter in all groups. Comparisons between groups using the nonparametric Mann-Whitney test were performed for the amount of tooth movement, root resorption area, and extent of hyalinization. A p value less than 0.05 was considered statistically significant.



Figure 3. The area of root resorption lacunae was calculated using Image J software. A Line was drawn bypassing the root resorption lacunae.

Results

Effects of LED Phototherapy on the Amount of Tooth Movement

The values obtained with the measurements of the distance between the first and second molars for the time points T1 and T2 demonstrated that the control group (CON) presented a greater distance compared to the LED group, as shown in Table 1. For the time points T1, T2 and T3, no statistically significant difference (p>0.05) was observed in the groups.

Effects of Hyalinization in the Periodontal Ligament and Root Resorption

The data are presented according the different days (4, 7, 14, and 21 days). Figure 4, represents the histological analysis and TRAP histochemistry from the different

treated groups.

Day 4

Areas of hyalinization were observed in the periodontal ligament of specimens from both groups (Fig. 4A, C). Although the average for the extension of hyalinization was higher in the control group, there was no statistically

Table 1. Distribution of the mean and standard deviation (SD) of the amount of tooth movement for CON group versus LED group

Time interval	GCON (n=5) Mean (SD) mm	GLED (n=5) Mean (SD) mm	pa
0-7 days	3.87 (3.72)	3.06 (2.08)	0.873
0-14 days	5.65 (3.39)	3.80 (3.84)	0.285
0-21 days	4.65 (4.68)	4.18 (4.70)	0.714

^aMann-Whitney (0.05).



Figure 4. Light micrographs showing in the pressure areas; A, B: Control specimens at 4 days; C, D: LED group. The mesial portion of the periodontal ligament shows hyalinization areas (asterisk), whereas few TRAP positive cells (arrows) can be seen at the alveolar septum in both groups. In control specimens at 7 days (E, F), the periodontal ligament adjacent to the mesial portion of the distal-buccal root shows hyalinization (asterisk). In the LED group at 7 days (G, H), numerous osteoclasts (arrows) are found at the alveolar bone (AB) surrounding the distal-buccal roots. At 14 days, an area of hyalinization (asterisk) is seen in the control group (I, J). The LED specimens (K, L) show numerous osteoclasts (arrows) adjacent to root resorption areas. At 21 days, both the control (M, N) and LED (O, P) groups show resorption surfaces (heads arrow) but osteoclasts are absent. D, root dentin. [HE - stained slide, original magnification 100' (A, C, E, G, I, K, M, O); TRAP – stained slide, original magnifications 200' (B, D, F, H, J, L, N, P)].

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significant difference between groups (p=0.58) (Table 2). TRAP histochemistry revealed the presence of osteoclasts in the control group (Fig. 4B), despite the fact that they were more evident in the LED group (Fig. 4D). Root resorption was not detected in the control and LED groups (Fig. 4A, C).

Day 7

Light microscopy demonstrated areas of hyalinization in the periodontal ligament of specimens from both groups (Fig. 4E). The average extent of hyalinization was higher in the control group. However, there was no statistically significant difference between groups (p=0.93) (Table 2). TRAP histochemistry revealed the presence of numerous osteoclasts (Fig. 4F), which were more evident in the LED group (Fig. 4H). In both groups, the TRAP-positive osteoclasts were observed adjacent to the root surface.

Day 14

The hyalinization areas at this time point were more evident in the control group (Fig. 4A, E, I). TRAP histochemistry revealed the presence of few osteoclasts in the control group (Fig. 4J). In both groups, most osteoclasts appeared adjacent to the root surfaces (Fig. 4L). Resorption lacunae were observed on the root surfaces analyzed (p=0.85) (Fig. 4 J, L) (Table 3).

Day 21

The periodontal ligament of specimens from both groups appeared free from hyalinization areas (Fig. 4M, O). TRAP histochemistry did not reveal the presence of osteoclasts in both groups (Fig. 4N, P). Resorption lacunae were observed on the root surfaces analyzed in both group (p=0.76) (Fig. 4M, P) (Table 3).

Discussion

In the current study, we describe the use of infrared phototherapy on hyalinization and root resorption during experimental OTM and the amount of tooth movement. The measurements of the distance between first and second molars showed no significant difference between the control group and the LED group at T1, T2, and T3.

Table 2. Distribution of the mean and standard deviation (SD) of hyalinization for CON versus LED groups

Time interval	GCON Mean (SD) μm	GLED Mean (SD) μm	$\mathbf{p}^{\mathbf{a}}$
4 days	101.44 (114.46)	71.77 (106.14)	0.5784
7 days	71.54 (128.57)	53.57 (118.39)	0.9341
14 days	67.61 (135.64)	13.28 (37.57)	0.4667
21 days	-	-	-

^aMann-Whitney (0.05).

The closed-coil stainless steel coil spring was used to move the upper first molar. The maxillary incisors were chosen for anchoring. To the best of our knowledge, there are no data on the mechanical properties of stainless steel coil springs during induced tooth movement in rats, such as force decay. As the roots of upper first molars present different cross-sectional areas and shapes, the 10 g mesial initial force applied had a higher impact on the periodontal ligament of the distal palatal root. Similar force has been used previously in laser studies and induced tooth movement (16-18). Due to the small intraoral space in rats, we performed the irradiation on the facial skin area (adjacent to the upper molars) to accommodate the probe of the LED instrument (12-19).

The inflammatory process can be observed in the periodontal ligament some hours after orthodontic force application in the molar. Consequently, the clastic cells begin the resorption of alveolar bone. After 24 h, phagocyte cells remove the pre-surface layer of cement. After 3 days, the mineralized cementum is removed by clastic cells (20-21). In this study, we report that the infrared LED was applied on days 0 to 4 in order to control the inflammatory process during the initial phase of the OTM.

There were no significant differences between the CON and LED groups in relation to the amount of OTM. Recently, μ CT has been employed to evaluate the amount of tooth movement, bone architecture, and alveolar bone density (17,22). One study evaluated the effect of LED on OTM (11) using an electronic digital caliper on dental cast models of rat maxillae, where they demonstrated differences between the experimental and control groups.

In the present study, µCT was chosen due to the possibility of high accuracy (micrometers) and there was no significant difference between the LED and CON groups. Although the resolution chosen (18 µm) did not allow for the analysis of bone architecture and alveolar bone density, the rebuilt 3D images through CTan® software made it possible to measure the distance between molars and evaluate the rate of OTM. Considering the amount of movement in different periods, no acceleration of OTM was detected under the LED effect. The acceleration of

Table 3. Distribution of root resorption mean and standard deviation (SD) for CON versus LED groups

Time interval	GCON Mean (SD) μm	GLED Mean (SD) μm	$\mathbf{p}^{\mathbf{a}}$
4 days	-	-	-
7 days	295.07 (457.87)	134.21 (270.10)	0.485
14 days	1472.62 (3027.69)	3574.85 (6373.11)	0.854
21 days	2521.44 (4042.08)	824.02 (936.21)	0.759

^aMann-Whitney (0.05).

OTM has been reported in other studies, which employed the following approaches: lasers and evaluation of the amount of tooth movement by plaster model scans (16); lasers and evaluation of the amount of tooth movement by microcomputed tomography (17); lasers and evaluation of the amount of tooth movement by a digital caliper (18); and LED and evaluation of the amount of tooth movement in dental cast models by a digital caliper (12).

Unfortunately, tooth movement did not show significant differences between the CON and LED groups regarding the extension of hyalinization areas in the periodontal ligament at the mesial surface of the distal palatal root of the first molar. Some authors observed that the removal of necrotic tissue from the periodontal ligament, under laser influence, might accelerate tooth movement (23). On the other hand, it could have allowed the remodeling of periodontal tissues, resulting in faster tooth movement (18,24). However, LED had no effect on hyalinization, and this can be explained due to the applied dose or to the distance between the equipment and the target tissue.

The LED utilized in the present study did not inhibit root resorption during the OTM. A few resorbed areas were observed on the root surface of first molars in both groups at the pressure areas on day 7. Although previous studies showed that orthodontic force-induced root resorption could be significantly decreased by LED irradiation (9,11,12), other authors considered that the inhibitory effect on resorption activity was difficult to predict. These studies were unable to show differences in the amount of resorption between the laser and control groups (24,25). The difference in results between studies may be directly related to the Arndt-Schutz law, which means that the biological tissue response is dose-dependent. In both groups of our study, osteoclasts were absent on day 21. The absence of osteoclasts in the CON and LED groups on day 21 may have occurred due to the inactivation of the stainless steel coil spring at that time point. In addition, since rat incisors erupt continuously, the change of applied force angle may have gradually reduced the force intensity.

In conclusion, the results obtained in the present study did not show the effectiveness of infrared LED therapy on amount of tooth movement, hyalinization, and root resorption, with the applied dose. According this study, the null hypothesis was rejected. Further studies using different application protocols are needed to evaluate the benefits of infrared phototherapy to reduce hyalinization, root resorption areas, and accelerate tooth movement.

Resumo

O presente estudo tem como objetivo analisar o efeito da fototerapia LED na presença da hialinização e reabsorção radicular durante o movimento dentário ortodôntico (MDO) em ratos, e a mensuração da quantidade de movimento dentário. Oitenta ratos foram alocados em dois grupos: LED e Controle (CON), os ratos foram irradiados com um LED infravermelho (850nm, 30mW) por 5 minutos durante os cinco primeiros dias da MDO; e o grupo controle não foi irradiado. Ambos os grupos foram subdivididos em 4 subgrupos (n=10) de acordo com a data da eutanásia (4, 7, 14 e 21 dias). Cinco dos dez ratos LED21 e cinco dos dez ratos CON21foram submetidos a microtomografia computadorizada (µCT); As µCT foram realizadas nos dias 0, 7, 14 e 21. Para o estudo histológico, as maxilas foram processadas para microscopia de luz, usando hematoxilina-eosina (HE) e Fosfatase ácido Tartrate-Resistente (TRAP) para histoquímica. A quantidade de movimento dentário não diferiu entre o LED e o CON. A hialinização foi observada nas áreas de pressão em ambos os grupos e não mostrou diferença estatisticamente significante. Reabsorção radicular também foi observada em ambos os grupos depois de 7 dias e não houve diferença entre os grupos. A fototerapia LED não aumentou a guantidade de MDO. Características similares de hialinização e reabsorção radicular foram observadas em ambos os grupos

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