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PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
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INFLUÊNCIA DA LLLT (GaAlAs) COM DIFERENTES ENERGIAS NA MOVIMENTAÇÃO ORTODÔNTICA EM RATOS

INFLUENCE OF THE LLLT (GaAlAs) WITH DIFERENT ENERGIES ON THE ORTHODONTIC MOVEMENT IN RATS

Prof. Dr. João Batista Blessmann Weber

Orientador

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Tese apresentada como requisito para obtenção do Título de Doutor pelo Programa de Pós-Graduação da Faculdade de Odontologia da Pontifícia Universidade Católica do Rio Grande do Sul. Área de Concentração, Cirurgia e Traumatologia Bucomaxilo Facial.

SIMONE TORRI

Orientador: Prof Dr João Batista Blessmann Weber

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EPÍGRAFE

“Se fui capaz de ver mais longe, é porque me apoiei em ombros de gigantes.”

Isaac Newton

DEDICATÓRIA

“Aos meus pais, Ivo e Dinacyr, que me ensinaram que o conhecimento é o bem mais valioso do ser humano e que para conquistá-lo, o caminho nem sempre é o mais fácil, mas, com certeza, é um dos mais gratificantes.”

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RESUMO

RESUMO

Introdução: Um dos grandes desafios da ortodontia nos dias atuais é a diminuição do tempo de tratamento. Estudos tem demonstrado que o *laser* pode ser um auxiliar na movimentação ortodôntica, influenciando na reparação óssea e na analgesia. O objetivo desse trabalho foi analisar a influência da *Low-Level Laser Therapy* (LLLT) com diferentes energias na quantidade da movimentação ortodôntica em ratos.

Métodos: Vinte e cinco ratos machos *Wistar* foram divididos aleatoriamente em cinco grupos de acordo com a energia de *laser* aplicada. Uma força de 10 gramas foi aplicada ao primeiro molar superior esquerdo. Nele foi aplicado *laser*, 830nm, com diferentes energias durante a movimentação ortodôntica (12J, 15J, 18J, 21J por ponto) em 3 pontos. A quantidade de movimentação dentária foi mensurada durante o experimento e calceína foi injetada nas amostras para marcar e possibilitar a mensuração da área do osso neoformado.

Resultados: Com relação à quantidade de movimentação, não houve diferença estatisticamente significante entre o grupo controle e os grupos LLLT ($P<0,01$). Histologicamente, houve aumento significativo da área de osso neoformado nos grupos LLLT com energias de 12, 15 e 18J ($P<0,05$).

Conclusões: Esses achados sugerem que a LLLT nas energias e protocolos aplicados nesse estudo não interferem na quantidade da movimentação dentária ortodôntica, apesar de estimular a neoformação óssea com a aplicação de determinadas energias.

Palavras chave: Ortodontia, Movimento dentário, Laserterapia, Rato *Wistar*

SUMMARY

SUMMARY

Introduction: Prolonged treatment times are one of the greatest challenges of orthodontic practice. Research has shown that laser therapy can be used as an adjunct to orthodontic movement, with effects on bone repair and analgesia. This study sought to assess the influence of low-level laser therapy (LLLT), using different energy settings, on orthodontic tooth movement in rats.

Methods: Twenty-five male Wistar rats were randomly allocated across four different energy setting groups and one control group. A 10-g load was applied to the left maxillary first molar. During orthodontic movement, 830-nm laser radiation was administered to three spots at different energy settings depending on group allocation (12J, 15J, 18J, or 21J per site). Orthodontic movement was measured throughout the experiment and calcein dye was injected into the specimens for measurement of the area of neoformed bone.

Results: There were no significant quantitative differences in orthodontic movement between the control and LLLT groups ($P<0.01$). On histological examination, LLLT groups 12J, 15J, and 18J exhibited a significant increase in area of neoformed bone ($P<0.05$).

Conclusions: At the energy settings and protocols used in this study, LLLT does not appear to influence the rate of orthodontic movement, although different energy settings encourage bone neoformation.

Keywords: Orthodontics; Tooth Movement; LLLT; Rats, Wistar

LISTA DE SIGLAS E ABREVIAÇÕES

GaAlAs - Arseneto de Gálio e Alumínio

DNA – Deoxyribonucleic acid (ácido desoxirribonucleico)

LED - *Light Emitting Diode* (diodo emissor de luz)

LLLT - Low Level Laser Therapy

RANK - receptor ativador do fator nuclear- κB

RANKL - ligante de RANK

LISTA DE SÍMBOLOS

Ø - diâmetro (ponteira do *laser*)

cm - centímetro

cm^2 - centímetro quadrado

E – energia

eVo – elétron-volt

g - grama

Hz - hertz

J - Joule

J/cm^2 - Joules por centímetro quadrado

J/cm^2/point - Joules por centímetro quadrado por ponto

J/cm^2/session - Joule por centímetro quadrado por sessão

mg/Kg - miligramas por quilo

min – minuto

mJ - miliJoule

mm – milímetro

mW - miliWatt

nm - nanômetro

P - potência

s - segundo

W - Watt

W/cm^2 - Watt por centímetro quadrado

SUMÁRIO

INTRODUÇÃO

1 INTRODUÇÃO

A palavra *LASER* significa *Light Amplification by Stimulated Emission of Radiation*, ou seja, luz amplificada por emissão estimulada de radiação (BRUGNERA JÚNIOR; PINHEIRO, 1998).

Os *lasers* podem ser classificados em alta ou baixa potência. O *laser* de baixa potência, também conhecido como *laser* de baixa intensidade ou LLLT (*low level laser therapy*), apresenta propriedades que produzem efeito biológico em nível celular (BRUGNERA JÚNIOR; PINHEIRO, 1998; GENOVESE, 2000; MELLO; MELLO; MELLO, 2001) e constitui uma alternativa terapêutica para a modulação do processo inflamatório (VIEGAS et al, 2005).

Segundo Gutknecht & Eduardo (GUTKNECHT; EDUARDO, 2004), os principais efeitos biológicos associados à LLLT são crescimento celular estimulado, regeneração celular, efeito anti-inflamatório (redução da capacidade dos linfócitos de reagir a estímulos antigênicos), redução de edema, revascularização (aceleração na regeneração de vasos linfáticos e veias), redução na formação de tecido fibroso (retarda a fibrose tissular após injúrias no tecido), maior atividade tissular (mudanças no conteúdo de prostaglandina, maior conteúdo de enzimas específicas e aumento da formação de produtos celulares) e, função nervosa estimulada (aumento na amplitude dos potenciais de ação).

Os equipamentos de *laser* utilizados para tratamento médico-odontológico emitem radiações que estão situadas na faixa das radiações visível, infravermelha e ultravioleta, todas não ionizantes (ALMEIDA-LOPES, 2004), com fótons de energia menores que 2,0 elétron-volt, portanto, inferior à energia da ligação das moléculas biológicas e do DNA, de maneira a não promover quebras das ligações químicas e não induzir mutação e carcinogênese (BRUGNERA; GENOVESE; VILLA, 1991).

O *laser* infravermelho, mais penetrante, é o comprimento de onda de eleição para reparos neurais e também quando se busca tecidos mais profundos (DAVIDOVITCH et al, 1980), como por exemplo, o tecido ósseo.

O tratamento ortodôntico é baseado no princípio do movimento dentário resultante de uma aplicação de força prolongada em um dente promovendo a criação de regiões de tensão e pressão no ligamento periodontal. Esse processo é caracterizado por inflamação nas estruturas adjacentes ao elemento dentário. Tais alterações nos tecidos periodontais causam remodelação óssea que são fundamentais para o movimento dentário ortodôntico (KAWASAKI; SHIMIZU, 2000).

A utilização do *laser* na Odontologia, mais especificamente na ortodontia, vem sendo observada a mais de uma década e continua crescendo. Estudos em animais e em seres humanos tem demonstrado que o *laser* pode ser um auxiliar na movimentação ortodôntica, influenciando na reparação óssea e na analgesia (LIM; LEW; TAY, 1995; SAITO; SHIMIZU, 1997; KAWASAKI; SHIMIZU, 2000; CRUZ et al, 2004; GOULART et al, 2006; LIMPANICHKUL et al, 2006; TURHANI et al, 2006; SOUZA et al, 2011).

Algumas pesquisas vem sendo realizadas sobre a influência da LLLT na quantidade da movimentação ortodôntica. Porém, apresentam diferenças na aplicação do *laser* com relação ao comprimento de onda, a potência, a dosagem e o tempo de aplicação, produzindo, desta forma, resultados divergentes (SAITO; SHIMIZU, 1997; CRUZ et al, 2004; GOULART et al, 2006; LIMPANICHKUL et al, 2006; TURHANI et al, 2006; SEIFI et al, 2007; FUGITA et al, 2008; YOUSSEF et al, 2008).

A presente tese consiste em dois artigos científicos que investigam os efeitos da LLLT sobre a movimentação ortodôntica. No primeiro, é feita uma revisão de literatura sobre o tema, enquanto o segundo artigo apresenta o experimento desenvolvido em modelo animal que teve como objetivo avaliar a ação da aplicação de diferentes densidades de energia da LLLT, com comprimento de onda de 830nm, na movimentação dentária ortodôntica em ratos. O experimento teve como variável dependente, a modulação do movimento ortodôntico do primeiro molar superior esquerdo dos ratos e como variável independente, a aplicação da LLLT infravermelho em diferentes densidades de energia.

ARTIGO 1

2 ARTIGO 1

O artigo “**Influence of LLLT on the rate of orthodontic movement: a literature review**” foi formatado, submetido e aceito de acordo com as normas do periódico *Photomedicine and Laser Surgery* (Anexos A, B e C).

Influence of LLLT on the rate of orthodontic movement: a literature review

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Running title: LLLT and orthodontic movement

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Abstract

Objective: To review low level laser therapy (LLLT) protocols that have been used to date and indicate which parameters appear to be most effective to guide future research.

Background data: Studies assessing the influence of LLLT on the rate of orthodontic tooth movement have produced controversial results as a result of methodological differences.

Methods: The MEDLINE database (1975-2012) and the Cochrane library (subject 8) were reviewed. Clinical studies and animal experiments written in English and focusing on the effects of LLLT on the rate of orthodontic movement were browsed. Article selection was conducted by one reviewer and checked by second investigator.

Results: A total of 109 articles were identified, of which 14 were selected for detailed analysis. Diode laser was used in all studies with different energies, frequencies, and doses. In animal studies, the most common and effective energy input was 54J per session daily; in humans, 2J per session on the first days of each month, with 72-96-hour intervals. Orthodontic force also influenced orthodontic movement. A force of 10 grams seems to be indicated for moving molars in rats, vs. 150 grams for canines in humans.

Conclusions: Most authors report positive effects of the use of LLLT on speed increase of orthodontic tooth movement when compared with control or placebo groups. Diode laser, especially gallium aluminum arsenide, used continuously and in direct contact with the irradiated areas, were the most frequent protocols. Further studies are warranted to determine the best protocols with regard to energy, dose, and intervention schedule.

Introduction

Laser has been used in dentistry for over a decade now and the phenomenon continues to grow. Orthodontic treatment is based on the principle of tooth movement resulting from the application of prolonged forces on a tooth, creating areas of tension and pressure in the periodontal ligament. The process is characterized by acute followed by chronic inflammation, once again followed by acute inflammation (after reactivation of orthodontic forces). These changes to periodontal tissues cause bone remodeling, essential for the promotion of orthodontic tooth movement.[1]

Studies conducted in animal models and human beings have shown that low-level laser therapy (LLLT) can improve orthodontic tooth movement by influencing bone repair and analgesia.[2-9] Specifically, some studies have been designed to assess the influence of LLLT on the rate of orthodontic tooth movement. However, differences in laser application protocols, such as type of laser used, wavelength, output power, dose, and treatment time, have produced controversial results. [2, 3, 5, 7, 9-12]

To the authors' knowledge, no literature review has been conducted to investigate the influence of LLLT on the rate of orthodontic movement. Therefore, the objective of the present article was to review the literature for LLLT protocols that have been used to date and indicate which parameters appear to be most effective to guide future research.

Materials and Methods

A computerized literature review was performed using the MEDLINE database (1975-2012) and the Cochrane library (subject 8). The following keywords were used: orthodontic, movement, laser, and LLLT.

The following selection criteria were taken into consideration: articles written in English, disclosing the wavelength employed, clearly describing LLLT application protocols, measuring the rate or speed of orthodontic movement, including control and/or placebo groups. Specifically for clinical studies, patients should not present any systemic disease, should not have taken any medication likely to influence orthodontic movement, and should have permanent dentition; animal studies should describe adequate animal maintenance conditions. Figure 1 shows the article selection process.

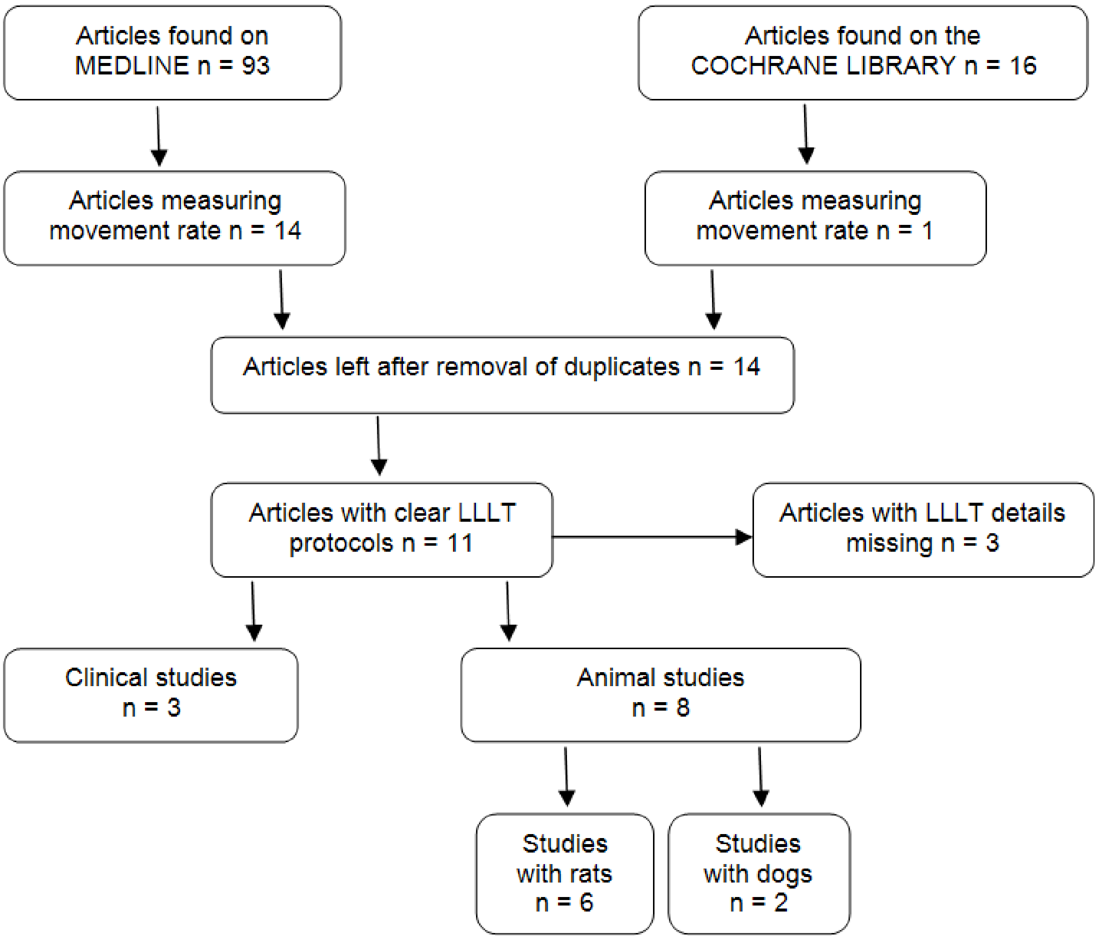

FIG. 1 Article selection flowchart.

Clinical studies and animal experiments studying the effects of LLLT on the rate of orthodontic tooth movement were browsed. The selection of articles was performed by one reviewer and checked by a second investigator. The titles and abstracts of potentially relevant articles were analyzed before full-text analysis.

Results

The computerized literature review yielded a total of 109 articles. The abstracts of these articles were read and screened. One article was found to be a duplicate, resulting in a total of 14 papers selected for a more detailed analysis, with full-text reading.

Of the 14 papers read in full,[2-6, 8, 10, 12-18] three were excluded either for not reporting all information necessary for study reproduction or for containing inconsistencies.[11-13] As a result, the present literature review included a total of 11 articles, namely three clinical studies and eight animal studies.

Because of the biological differences between animals and humans, and also because of the impossibility to affirm that the doses applied to animal models are appropriate for humans, the results of the present review are divided in two major sections, one devoted to the analysis of animal studies and the other to human studies.

Following the separate analysis of these two groups (animals and humans), the data found for both types of studies will be compared and discussed.

Animal studies

Tables 1 and 2 summarize the results of the animal studies assessed.

Sample characteristics

Of the eight studies assessing animal models, six used male Wistar rats aged 6 to 12 weeks,[6, 10, 14, 16-18] and the other two used dogs.[3, 15]

The tooth chosen for orthodontic movement in rats was the maxillary first molar, except for the study of Altan et al.,[18] in which maxillary incisors were used. In the studies with dogs, maxillary first molars[15] and second premolars[3] were selected for treatment.

Orthodontic movement

Nickel-titanium closed coil springs were used in most experiments for orthodontic movement. In only one study, a steel wire pendulum appliance was used to move the maxillary incisors of rats.[18]

Kawasaki and Shimizu,[6] Fujita et al.,[10] and Yoshida et al.[17] applied a force of 10 grams on the maxillary molars of rats. Gama et al.,[14] Marquezan et al.,[16] and Altan et al.,[18] in turn, applied higher forces, of 20 to 40.78 grams. In the studies conducted with dogs, higher forces were used, namely of 85 and 150 grams.

Laser type and wavelength

All animal experiments included in the review used diode laser, most often gallium aluminum arsenide (GaAlAs).[3, 6, 10, 15-18] Infrared was the wavelength most frequently used, ranging from 780 to 830 nanometers.

Laser application power

Laser output power ranged from 40mW to 100mW. Five of the studies used an output power of 100mW.[6, 10, 16-18] Among the authors working with rat models, only Gama et al.[14] reported an output power different from 100mW, namely 40mW. Goulart et al.[3] and Kim et al.,[15] both working with dogs, used 70mW and 76.3mW, respectively.

Application protocol, irradiation points, and energy input

Kawasaki and Shimizu,[6] Fujita et al.,[10] and Marquezan et al.[16] applied laser to three distinct points (mesial, buccal, and palatal) around the tooth subjected to orthodontic movement. Yoshida et al.[17] used four laser application points (mesial, distal, buccal, and palatal). All four studies used a total energy input per session of 54J, and an energy density per session of 18000J/cm². Gama et al.[14] used LLLT in three points, one extraoral (buccal surface). Total energy per session was 0.6J, and energy density per session, 20J/cm².

Incisors were the teeth selected for analysis by Altan et al.[18] LLLT was applied to five distinct points: two distobuccal, two distopalatal, and one distal point. Total energy per session was 54J in group II and 15J in group III, with energy densities per session of 1717.2J/cm² and 477J/cm², respectively.

With dogs, Goulart et al.[3] used only a palatal point for irradiation, at a total energy input of 0.21J per session and an energy density of 5.25J/cm² per session. Kim et al.,[15] in turn, applied laser to eight different points, four buccal and four palatal. Total energy density per session was 333.6J/cm². All studies applied laser continuously, in direct contact with the points irradiated, except Kim et al.,[15] who used pulsed laser.

Laser intervention schedule

Of the eight animal studies selected, five used daily laser applications,[6, 10, 16-18] but not necessarily throughout the study period.[17, 18] One study applied laser every 48 hours,[14] another every 72 hours,[15] and one study applied laser every 7 days only.[3]

Influence of LLLT on orthodontic movement

The results of our animal models show that application of LLLT during orthodontic treatment increases the rate of tooth movement when compared with non-irradiated control groups.[3, 6, 10, 15, 17]

Altan et al.[18] did not observe statistically significant differences with regard to the rate of orthodontic tooth movement between control and study groups, but they reported that LLLT accelerated the bone remodeling process, stimulating osteoblast and osteoclast cell proliferation and their functions. Those authors also suggested that their non-significant results may be due to the small size of their sample.

Gama et al.[14] and Marquezan et al.[16] also failed to find significant results associated with LLLT.

Clinical studies

Table 3 describes the results found in the three clinical studies reviewed.

Sample characteristics

The samples of the clinical studies selected for review included both male and female patients, aged from 10 to 22 years, and requiring orthodontic treatment with extraction of first premolars. Sousa et al.[8] irradiated maxillary and mandibular canines; the other two groups of authors irradiated maxillary canines only.

Orthodontic movement

Cruz et al.[2] and Limpanichkul et al.[5] used straight-wire brackets with Roth prescription and continuous arch wires. Sousa et al.[8], however, used Andrews prescription and segmented arch wires, all with 0.22 X 0.25 slots.

Cruz et al.[2] used a modified Nance holding arch cemented to the second premolars and a transpalatal bar attached to the first premolars for anchorage during retraction of the upper canine, which was tied to the stainless steel rectangular arch wire (0.17 X 0.25) with a 0.10 stainless steel ligature wire.

Limpanichkul et al.[5] used for anchorage a 3-mm vertical loop with stops mesial to first premolar tubes tied to the hook of the device, and the upper incisors tied together to the 0.45mm stainless steel arch wire, which served as a guide for the retraction of the upper canines. Retracted canine teeth were bracketed with a self-ligating bracket to standardize the effects of friction during movement.

Souza et al.[8] did not describe the anchorage system used, only the segmented arch wire from the first molar to the canine, with a 0.016 stainless steel wire used as a guide for retraction.

In all three studies, nickel-titanium closed coil springs were used for the retraction of canines, with a force of 150 g for canine retraction.

Laser type and wavelength

All clinical studies used GaAlAs diode laser with an infrared wavelength ranging from 780 to 860nm.[2, 5, 8]

Laser application power

Cruz et al.[2] and Sousa et al.[8] used a laser application output power of 20mW, compared to 100mW in Limpanichkul et al.[5]

Application protocol, irradiation points, and energy input

The three clinical studies applied laser continuously, in direct contact with the areas to be irradiated. Cruz et al.[2] and Sousa et al.[8] used the same points of irradiation and the same energy input at each point and session. The authors used five buccal points and five palatal or lingual points. Energy and energy density per session were 2J and 50J/cm², respectively. Limpanichkul et al.,[5] used three buccal, three palatal, and two distal points in relation to the irradiated canine.

Laser intervention schedule

Laser application frequencies were different in each study. Cruz et al.,[2] for example, irradiated teeth on days 0, 3, 7, and 14 in the first month, and on days 33, 37, and 44 in the second month, always with the same intervals. Springs were reactivated on days 0 and 30 in the control and irradiated experimental groups after the measurement of distances.

Limpanichkul et al.[5] used daily applications from the first to the third day of the study. At the end of the first month, laser applications were performed daily once again, as well as in the end of the second and third months. In that study, the authors reactivated springs once a month.

Finally, Sousa et al.[8] adopted a similar protocol to that of Cruz et al.,[2] with irradiation sessions on days 0, 3, and 7, and in the beginning of the second and third months, always maintaining the same intervals. Canine retraction springs were reactivated at the beginning of each month.

Influence of LLLT on orthodontic movement

Cruz et al.[2] and Sousa et al.[8] observed positive results, i.e., a higher rate of orthodontic tooth movement in the irradiated group when compared with the placebo group, at a statistically significant difference. Conversely, Limpanichkul et al.[5] did not find any effect of LLLT on the rate of orthodontic tooth movement.

TABLE 1. STUDIES ASSESSING THE EFFECT OF LLLT ON THE RATE OF ORTHODONTIC MOVEMENT IN RATS

Paper	*Laser type*	*Sample*	*Method*	*Results*
Kawasaki and Shimizu[6]	Ga-Al-As 830nm Continuous, direct contact at each point 100mW Ø 0.6mm 0.0028cm² (tip) 6000J/cm²/point 18000J/cm²/session 234000J/cm²/13 days 18J/point 54J/session 702J/13 days	24 male Wistar rats 6 weeks old 180 g 12 molars irradiated	Control group LLLT group: left maxillary first molar. Three points at the gingiva: mesial, buccal, and palatal. Time/point = 3 min Time/session = 9 min Once a day. Total of 13 days. Force of 10g. Measurement in plaster models. Distance between the top of the mesiobuccal cusp of the first and second maxillary left molars.	LLLT stimulates tooth movement, accelerates bone remodeling by increasing the number of osteoclasts and stimulating cell proliferation in the periodontal ligament and mineralization of the newly formed bone. A higher rate of movement on days 2, 4, and 12.
Fujita et al.[10]	GaAlAs 810nm Continuous, direct contact at each point Ø 0.6mm 0.0028cm² (tip) 18J/point 54J/session 432J/8 days 6000J/cm²/point	75 male Wistar rats 6 weeks old 180g 50 first molars irradiated	3 groups with 25 animals each Control group LLLT group 1 mesial, 1 mesiobuccal, and 1 mesiopalatal point Time/point = 3 min Time/session = 9 min Once a day. Total of 8 days. LED group	LLLT accelerates orthodontic tooth movement via induction of RANK/RANKL. The expression of RANK was detected in osteoclast precursor cells at an early stage (days 2 and 3) in the irradiated group. These findings suggest that LLLT accelerates bone remodeling, shortening the duration of orthodontic treatment.

	18000J/cm^2/session 144000J/cm^2/ 8 days LLLT group 810nm 100mW LED group 850nm 75mW		1 mesial, 1 mesiobuccal, and 1 mesiopalatal point Tempo/point = 4 min Once a day. Total of 8 days. Force of 10g. Measurement in models. Distance between the central fossa of the right maxillary first molar and the mesial surface of the right maxillary second molar.	
Yoshida et al.[17]	GaAlAs 810nm Continuous, direct contact at each point 100mW Ø 0.6mm 0.0028cm^2 (tip) 4 intraoral points 4500J/cm^2/point 18000J/cm^2/session 162000J/cm^2/9 days 13.5J/point 54J/session 486J/9 days	60 male Wistar rats 6 weeks old 180g 30 first molars irradiated	2 groups Control group LLLT group 1 mesial, 1 buccal, 1 palatal, and 1 distal point Time/point = 2 min 15 s Time/session = 9 min Once daily from day 0 to 6 One session on day 13 One session on day 20 Force of 10g. Measurement on tomographic images. Distance between the point of contact on the right maxillary first and second molars.	This LLLT protocol accelerates orthodontic tooth movement, stimulating bone remodeling. The rate of tooth movement was significantly higher in the LLLT group on days 3 (1.4-fold), 7 (1.19-fold), 14 (1.26-fold), and 21 (1.34-fold). Bone density in the LLLT group was higher on days 7 (1.08-fold), 14 (1.09-fold), and 21 (1.14-fold).
Gama et	Diode	30 male Wistar rats	2 groups (random division):	In this protocol, the use of LLLT did not

al.[14]	790nm Continuous, direct contact at each point 40mW Ø 2mm= 0.03cm² (tip) 2 intraoral points 0.135J/point 4.5J/cm²/point 9J/cm²/session Extraoral point 0.33J/point 11J/cm²/point/session 0.6J/session 20J/cm²/session	3 months old 250 to 300g 15 irradiated molars	Group I – orthodontic treatment (control) Group II – orthodontic treatment + LLLT 1 mesial, 1 distal, and 1 buccal point (extraoral application) 48-hour intervals between applications Total of 19 days. Force of 40g. Intraoral clinical measurement. Distance between the mesial surface of the first molar and a perforation made in the resin of incisors.	significantly interfere with orthodontic tooth movement. A lower rate of movement was observed in the LLLT group up to day 7 when compared to the control group.
Marquezan et al.[16]	GaAlAs 830nm Continuous, direct contact at each point 100W 0.0028cm² (tip) 6000J/cm²/point 18000J/cm²/session 18J/point 54J/session	36 male Wistar rats 12 weeks old 250g 18 irradiated molars	2 groups (random division): Control group: CG1 - no orthodontic treatment, death day 0 CG2 - orthodontic treatment, death day 2 CG3 - orthodontic treatment, death day 7 LLLT group: IrG1 - orthodontic treatment + 2 laser, death 2 (108J)	The two protocols did not have significant effects on the rate of orthodontic tooth movement when compared with the control group. Laser applications at late stages can have a role in maintaining the stimulatory effect of LLLT. The absence of laser can decrease stimulus. Daily laser application caused an increase in the number of osteoclasts after 7 days, but inhibited the expression

			IrG2 - orthodontic treatment + 2 laser, death day 7 (108J) IrG3 - orthodontic treatment + 7 laser, death day 7 (378J) Laser: 1 mesial, 1 buccal, and 1 palatal point Time/point = 3 min Total of 7 days. Force of 40.78g. Intraoral clinical measurement. Distance between the mesial surface of the first molar and a perforation made in the resin of incisors.	of immature collagen on the tension side.
Altan et al.[18]	GaAlAs 820nm Continuous, direct contact at each point 100W Ø 2mm= 0.03cm² (tip) Group II 10.8J/point 54J/session 343.9J/cm²/point 1717.2 J/cm²/session	38 male Wistar rats 10 weeks old 175g 22 irradiated incisors	4 groups (random division): Group I – orthodontic treatment Group II – orthodontic treatment + laser Time/point = 108s Group III – orthodontic treatment + laser Time/point = 30s Group IV – control Laser: 5 points on the right incisor: 2 distobuccal, 1 distal, and 2 distopalatal point Days 0, 1, 2 Total of 9 days.	No statistically significant differences were observed between the groups in the rate of orthodontic movement, even though group II (54J) showed a higher rate of movement. During orthodontic tooth movement, LLLT accelerates the bone remodeling process by stimulating osteoblast and osteoclast cell proliferation and their functions.

Group III 3J/point 15J/session 95.5J/cm²/point 477J/cm²/session	Force of 20g. Spring between maxillary incisors. Intraoral clinical measurement. Distance between the incisors and the level of the gingival papilla.

LLLT = low-level laser therapy.

TABLE 2. STUDIES ASSESSING THE EFFECT OF LLLT ON THE RATE OF ORTHODONTIC MOVEMENT IN DOGS

Paper	*Laser type*	*Sample*	*Method*	*Results*
Goulart et al.[3]	Ga-As-Al 780nm Continuous, direct contact at each point 70mW 0.04cm^2 LLLT group 5.25J/cm^2/point/session 0.21J/point/session LED group 35J/cm^2/point/session 1.4J/point/session	18 adult dogs (male and female) 4650-9600g Maxillary third molars extracted 18 first molars irradiated	2 groups with 9 first molars each: placebo group contralateral LED application Time/point = 20s LLLT group second premolar 1 point in the middle third of distal root time/point = 3s application every 7 days. Total of 9 weeks. Force of 85g. Intraoral clinical measurement. Distance between perforations made on molars and premolars.	Irradiation at 5J/cm^2 stimulates orthodontic tooth movement in the early stage (0 to 21 days). Lower doses are indicated for anti-inflammatory effects. Higher doses are indicated for anchorage results with increased bone formation.
Kim et al.[15]	GaAlAs 808nm Pulsed, no direct contact with each point 763mW Ø 0.4mm (fiber) Ø 1.75mm (focal spot) 10 Hz 75mJ per pulse	12 Beagles 12 maxillary second premolars irradiated	4 groups with 6 dogs each: Grupo A – orthodontic treatment (control) Grupo B – orthodontic treatment + corticotomy Grupo C – orthodontic treatment + LLLT Grupo D – orthodontic treatment + corticotomy + LLLT Laser: 4 buccal points and 4 palatal points Time: 20 s each point (9s of laser	Orthodontic tooth movement increased with LLLT in this protocol. The use of LLLT had late effects (from 5th to 8th week). Effect of LLLT on healthy alveolar bone differs from application on injured bone.

	41.7J/cm^2/ point 333.6J/cm^2/session	application) Every 3 days. Total of 8 weeks. Force of 150g. Measurement in models. Distance between cervicodistal point on the second premolar and third molar.

LLLT = low-level laser therapy.

TABLE 3. STUDIES ASSESSING THE EFFECT OF LLLT ON THE RATE OF ORTHODONTIC MOVEMENT IN HUMANS

Paper	*Laser type*	*Sample*	*Method*	*Results*
Cruz et al.[2]	Ga-Al-As 780nm Continuous, direct contact at each point 20mW 0.04cm^2 (tip) 5J/cm^2/point 50J/cm^2/session 200J/cm^2/month 0.2 J/point 2J/session 8J/month	11 patients (male and female) 12 to 18 years old Maxillary first premolars extracted 11 maxillary canines irradiated	Placebo group: contralateral canine LLLT group: irradiated maxillary canine 5 buccal and 5 palatal points Time/point = 10s Time/session = 100s Days 0, 3, 7, 14, 33, 37, and 44 (no reactivation) Total of 60 days. Force of 150g. Intraoral clinical measurement. Distance from the distal bracket slot of the canine to the mesial slot of the first molar.	LLLT application accelerates orthodontic tooth movement.
Limpanichkul et al.[5]	Ga-Al-As 860nm Continuous, direct contact at each point 100mW 0.09cm^2 (tip) 25J/cm^2/point 204J/cm^2/session 612J/cm^2/month	12 young adults (male and female) Mean age of 22.11 years Maxillary first premolars extracted 12 maxillary canines	Placebo group: contralateral canine LLLT group: irradiated maxillary canine 3 buccal, 3 palatal, and 2 distal points. Time/point = 23s Time/session = 184s Days 0, 1, 2, 28, 29, 30, 58, 59, 60, 88, 89, and 90. Total of 90 days.	LLLT applied with these parameters do not affect the speed of orthodontic tooth movement. Possible error due to small sample. An energy input of 25J/cm^2/session is probably too low to cause stimulatory or inhibitory effects.

	2.3J/point 18.4J/session 55.2J/month	irradiated	Force of 150g Measurement in models. Distance from the most mesial point of each retracted canine to the incisive papilla.	
Sousa et al.[8]	Ga-Al-As 780nm Continuous, direct contact at each point 20mW 0.04cm^2 (tip) 5J/cm^2/point 50J/cm^2/session 150J/cm^2/month 0.2J/point 2J/session 6J/month	10 patients (male and female) 10.5 to 22.2 years old Maxillary and/or mandibular first premolars extracted 13 maxillary and mandibular canines irradiated	Placebo group: contralateral canine LLLT group: canine randomly selected 5 buccal and 5 palatal/lingual points. Time/point = 10s Time/session = 100s Days 0, 3, 7, 30, 33, 37, 60, 63, and 67 (after reactivation) Total of 90 days. Force of 150g. Measurement in models. Distance from the distal bracket slot of the canine to the mesial slot of the first molar.	Statistically significant differences were observed between the two groups. The irradiated group showed an almost double increase in the rate of orthodontic tooth movement when compared with the placebo group.

LLLT = low-level laser therapy.

Discussion

Animal studies

Most of the animal studies included in this review found that LLLT increases the rate of orthodontic tooth movement, stimulating bone remodeling by increasing the number of osteoclast and osteoblast cells and reinforcing their functions.[3, 6, 10, 15, 17, 18]

With regard to the type of laser employed, the use of diode laser predominated, especially GaAlAs, as did infrared wavelengths. Infrared laser is known to penetrate biological tissues more deeply than red lasers, stimulating deeper tissues such as bone tissue, heavily implicated in orthodontic tooth movement. Fujita et al.[10] found a higher number of multinucleated osteoclast cells in the irradiated group, as well as an increased expression of RANK in osteoclast precursor cells at early stages.[10]

Continuous laser emission, in direct contact with irradiated tissues and limited to each point was the most frequent and effective method for producing positive effects on orthodontic tooth movement.[3, 6, 10, 17] When laser is applied directly to an irradiation point and in direct contact with tissue, the chances of energy absorption by the irradiated tissue increase, avoiding laser reflection. The only study reporting the use of pulsed laser, not in direct contact with tissues,[15] found significant results later in the course of LLLT when compared with the other papers.[6, 10, 15, 17]

Among the animal studies that reported positive results, three used rats and applied an energy of 54J per session distributed over different points around the orthodontically moved tooth, on a daily basis, at a total dose of 18000J/cm² per session.[6, 10, 17] Goulart et al.[3] and Kim et al.[15] used an energy input of 0.21J and 75mJ per pulse in dogs, with doses of 35J/cm² and 333,6J/cm² per session, respectively. Not only did these two latter studies use different energy inputs, doses, and application frequencies, they were also applied differently, as previously mentioned. Even though the number of studies conducted with dogs is too small to allow comparisons, we hypothesize that different energy inputs and doses may be most adequate to different animals to produce an increase in the rate of tooth movement.

The studies conducted by Gama et al.,[14] Marquezan et al.,[16] and Altan et al.[18] failed to observe increased tooth movement associated with LLLT. Those authors used older Wistar rats, aged 70 to 120 days, and also employed higher forces, at least double when compared with those used in the studies reporting positive associations.

Marquezan et al.[16] did not find statistically significant differences between the irradiated and control groups with regard to the rate of orthodontic tooth movement. Those authors used the same parameters described in studies with positive results, used the same teeth (maxillary molars), used GaAlAs laser applied at 54J and 18000J/cm² per session, continuously, in direct contact with the irradiation point. The only difference was the age of rats, which was double the age of rats from other studies,[6, 10, 17] and the orthodontic force employed, four times higher. The age of animal models can be an important variable, as a result of the effects of aging on periodontal tissues, which determine different responses to forces when compared with young tissues (e.g. injury and consequently a decreased rate of orthodontic movement). According to the histological findings described in the studies, the daily use of LLLT caused an increase in the number of osteoclasts after 7 days, but inhibited the expression of immature collagen on the tension side.

Altan et al. also applied the laser continuously, in direct contact with the irradiation point, using energy inputs of 54J and 15J and doses of 1717.2J/cm² and 477J/cm² per session. However, those authors failed to observe a statistically significant effect of LLLT on tooth movement. This finding may be due to their small sample size or to the fact that incisors were the teeth selected for orthodontic treatment in their study rather than molars, as in Kawasaki and Shimizu,[6] Fujita et al.,[10] and Yoshida et al.[17] Another possible explanation for the non-significant results observed is the lower total dose used per session by the latter authors, namely 18000J/cm², vs. only 1717.2J/cm² in Altan et al.[18] Despite these differences, Altan et al.[18] observed a trend toward an increased rate of orthodontic tooth movement in the group irradiated with 54J per session when compared with the one irradiated with 15J. Histologically, an accelerated proliferation of osteoclast cells was observed, corroborating the idea that LLLT interferes with bone remodeling during orthodontic tooth movement.

With the use of an older sample (12 weeks) and a higher orthodontic force, Gama et al.[14] showed that LLLT application may decrease induced tooth movement in comparison with controls when specific energy inputs and doses are applied. Another difference in that study was the use of an extraoral irradiation point. Even though the authors try to address the loss of energy in the course of penetration until reaching the desired tissue (by increasing the energy applied), it remains to be known how much energy was actually absorbed.

Clinical studies

All three clinical studies included in the review used GaAlAs diode laser with infrared wavelengths (as also observed for animal studies), applied continuously and in direct contact with irradiation points.[2, 5, 8] Sample size was very similar across the studies, including both male and female patients; however, this aspect is worthy of further consideration to determine whether the sample size is actually reliable. Also with respect to study samples, patient age varied greatly including different age groups, such as adolescents and adults, which may directly affect the results, since skeletal age and bone maturity are determining factors in orthodontic tooth movement rates. The teeth chosen for orthodontic retraction and LLLT were the maxillary canine in the studies by Cruz et al.[2] and Limpanichkul,[5] vs. the maxillary and mandibular canines in Sousa et al.[8]

The type of orthodontic mechanics used in the three studies varied with respect to bracket prescription, continuous or segmented arch wire for retraction, and reactivation of orthodontic force.[2,10,14] All these factors are of great importance to orthodontic tooth movement and may directly interfere with the results of the experiment.

Regarding laser application, Cruz et al.[2] and Sousa et al.[8] used 2J of energy at a dose of 50J/cm² per session and found statistically significant effects of LLLT during orthodontic tooth movement. Laser intervention schedule was a major difference between those two studies: Cruz et al.[2] used LLLT on days 0, 3, 7, 14, 33, 37, and 44, whereas Sousa et al.[8] skipped day 14, included day 30, and repeated the same application sequence adopted in the first month. Moreover, Sousa et al.[8] extended applications up to 67 days, whereas Cruz et al.[2] terminated the experiment on day 44. These findings suggest that even a lower number of applications at a lower intervention schedule may produce positive effects on the rate of tooth movement.

Limpanichkul et al.[5] used 18.4J at a dose of 204J/cm² per session, applied daily during the first 3 days and again in the last 3 days of the first, second, and third months of treatment. Results were negative, showing no influence of these LLLT parameters on the rate of orthodontic tooth movement. The authors hypothesize that their sample was too small and that the dose of 25J/cm² was too low to produce any stimulatory or inhibitory effect. If we compare it with the other two studies reporting positive effects, we can observe that sample size is adequate and that the source of a possible failure may lie in the dose used per session and intervention schedule of laser application. Perhaps, in humans, higher doses cause a decrease or even no effect on the speed of orthodontic movement, while lower doses increase the speed of orthodontic movement, unlike what occurs in animals.

Despite the small number of studies, failures in patient selection and differences in the type of orthodontic mechanics employed, we can learn from these mistakes and not repeat them in the future, thereby producing more reliable results.

Conclusions and Summary

In this review of the literature, we observed that most authors report positive effects of the use of LLLT on speed increase of orthodontic tooth movement when compared with control or placebo groups. GaAlAs diode laser, applied continuously, in direct contact with irradiation points, seems to be the most frequently indicated to produce such effects. Also, the energies and doses that produced the desired effect were different for animals and humans, leading us to believe that these parameters are different between these two groups. Further studies are warranted to determine the best protocols with regard to energy, dose, and intervention schedule. Sample standardization as to size and patient age, as well as to the type of orthodontic mechanics used, should be rigorously studied, especially in clinical trials, so that the results of such studies can be compared and validated.

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Author Disclosure Statement

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ARTIGO 2

3 ARTIGO 2

O artigo "**Influence of the LLLT with diferents energies on the rate of orthodontic movement in rats**" foi formatado e submetido de acordo com as normas do periódico *American Journal of Orthodontics and Dentofacial Orthopedics* (Anexos D e E).

INFLUENCE OF LOW-LEVEL LASER THERAPY (GaAlAs) AT DIFFERENT ENERGY SETTINGS ON ORTHODONTIC TOOTH MOVEMENT IN RATS

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Abstract

Introduction: Prolonged treatment times are one of the greatest challenges of orthodontic practice. Research has shown that laser therapy can be used as an adjunct to orthodontic movement, with effects on bone repair and analgesia. This study sought to assess the influence of low-level laser therapy (LLLT), using different energy settings, on orthodontic tooth movement in rats.

Methods: Twenty-five male Wistar rats were randomly allocated across four different energy setting groups and one control group. A 10-g load was applied to the left maxillary first molar. During orthodontic movement, 830-nm laser radiation was administered to three spots at different energy settings depending on group allocation (12J, 15J, 18J, or 21J per site). Orthodontic movement was measured throughout the experiment and calcein dye was injected into the specimens for measurement of the area of neoformed bone.

Results: There were no significant quantitative differences in orthodontic movement between the control and LLLT groups (P<0.01). On histological examination, LLLT groups 12J, 15J, and 18J exhibited a significant increase in area of neoformed bone (P<0.05).

Conclusions: At the energy settings and protocols used in this study, LLLT does not appear to influence the rate of orthodontic movement, although different energy settings encourage bone neoformation.

Keywords: **Orthodontics; Movement; Laser Therapy, Low Level; Rats, Wistar**

Introduction and literature review

One of the major challenges of current orthodontic practice is prolonged treatment time, which is reported by patients as one of the greatest disadvantages of orthodontic therapy.[1,2]

As is widely known, orthodontic treatment is based on the principle of tooth displacement resulting from the prolonged application of mechanical force on the tooth, which exerts localized tension and pressure on the periodontal ligament and, consequently, induces an inflammatory process. These changes lead to bone remodeling, which plays an essential role in orthodontic tooth movement.[3]

Laser therapy has been employed in dentistry (and particularly in orthodontic practice) for over 10 years, and its use is becoming increasingly widespread. Animal and human studies have shown that laser therapy can aid orthodontic movement by influencing bone repair and analgesia.[4-11]

Some research has focused on the influence of low-level laser therapy (LLLT) on the rate of orthodontic movement. However, these studies have been heterogeneous in terms of laser wavelength, power, dosage, and treatment duration, and have thus produced divergent findings.[5,7-10,12-14] Within this context, the objective of this article was to assess the influence of LLLT at different energy settings in the rate of orthodontic tooth movement in rats.

Material and Methods

Animals

Twenty-five male Wistar rats, age 6 weeks, were obtained from the animal rearing facilities of Universidade Federal de Pelotas, Pelotas, state of Rio Grande do Sul, Brazil. The animals were kept in properly labeled plastic cages, which were stored on ventilated cage racks (Alesco, Monte Mor, SP, Brazil), under a constant temperature of 22±1°C and a 12-hour light-dark cycle, at Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS). Water and chow were provided *ad libitum*. This experiment was approved by the PUCRS School of Dentistry Research Ethics Committee with protocol no. 0014/10 and by the PUCRS Animal Research Ethics Committee (CEUA/PUCRS) with protocol no. 10/00182.

Experimental tooth displacement

The initial sample was randomly subdivided into five groups of five animals each, according to exposure to LLLT following application of orthodontic force.

Group 1 was considered the control group, as the animals were not exposed to laser radiation. Animals in groups 2, 3, 4, and 5 received LLLT at spot energy levels of 12, 15, 18, and 21J respectively.

All procedures were performed under general anesthesia, which was induced by intraperitoneal injection of ketamine, 5% (100 mg/kg body weight) and xylazine, 2% (10 mg/kg body weight).

This study employed Kawasaki and Shimizu[1] model of orthodontic tooth movement. One end of a 7-mm closed-coil nickel–titanium (NiTi) alloy spring (wire diameter .10mm, internal coil diameter.30mm, Dental Morelli Ltda, Sorocaba, SP, Brazil) was attached to the left maxillary first molar with the aid of .10mm-thick stainless steel orthodontic wire (Dental Morelli, Sorocaba, SP, Brazil) and the other end was tied to the maxillary incisors. A 1/4 diamond bur was used to fashion a groove in the cervical zone of the maxillary incisors, immediately above the interdental papilla, to prevent displacement of the wire attachment (Figure 1). An orthodontic force of 10g was applied to achieve tooth movement.[6,12,15]

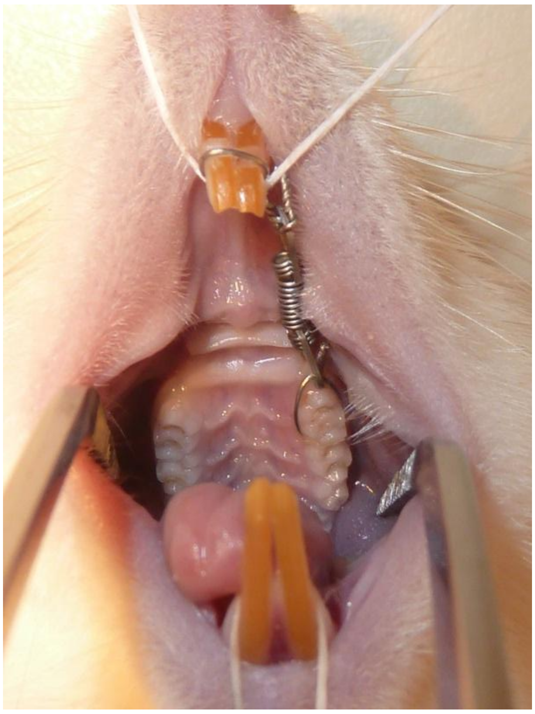

Figure 1. Orthodontic appliance. Closed-coil NiTi spring attached to first molar and maxillary incisors.

Laser therapy protocol

The laser used in this experiment was a gallium aluminium arsenide (GaAlAs) unit (Thera Lase, DMC Equipamentos Ltda, São Carlos, SP, Brazil), with a wavelength of 830nm, power 90mW, tip diameter 0.6mm, and cross-sectional area 0.0028cm^2. Laser radiation was applied in a spot fashion, in continuous mode and in direct contact with the oral mucosa. Three intraoral sites were chosen for application: mesial aspect of palatal surface, mesial aspect of buccal surface, and mesial aspect of left maxillary first molar (Figure 2). Irradiation was performed every 48 hours until experiment day 6 (Figure 3).

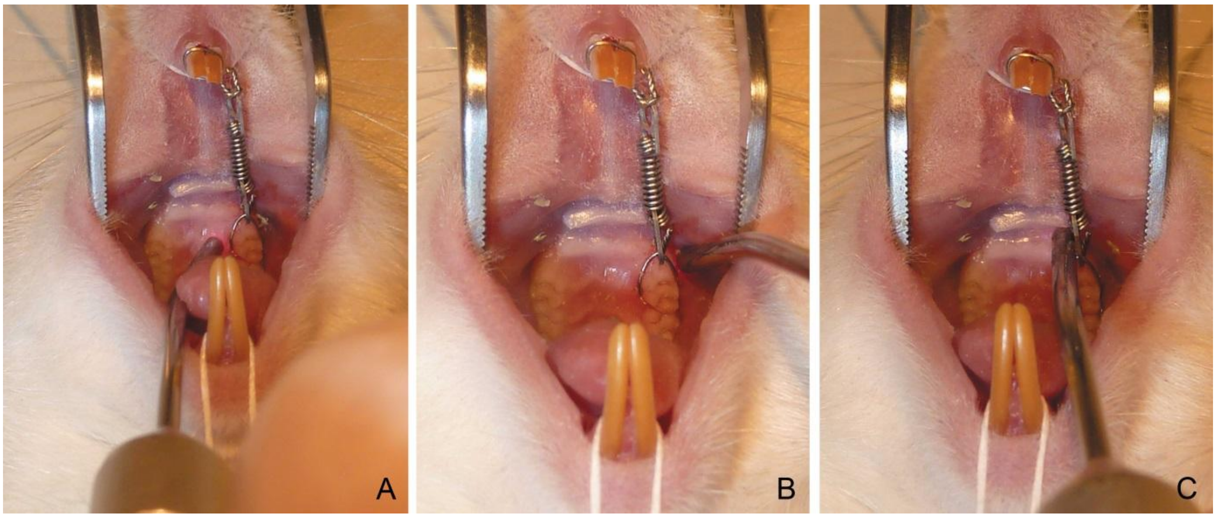

Figure 2. Laser application sites: mesial aspect of palatal surface, mesial aspect of buccal surface, and mesial aspect of left maxillary first molar.

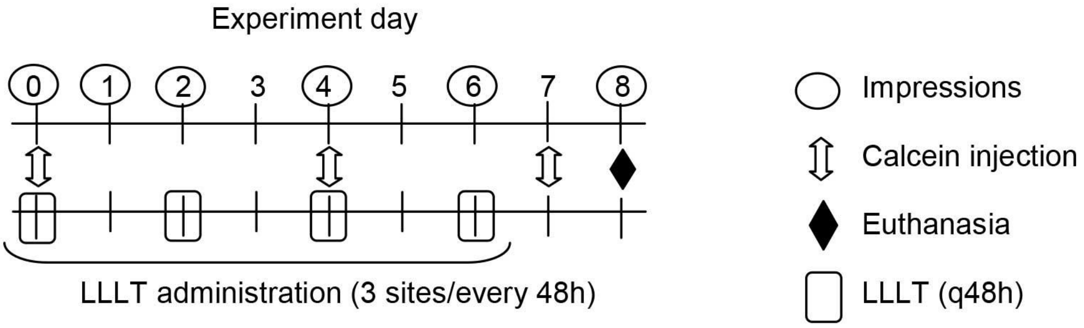

Figure 3. Experiment timeline.

Measurement of orthodontic tooth movement

To measure the extent of tooth movement achieved by application of orthodontic force, dental impressions were obtained using pourable, addition-cured silicone impression material (Express®, 3M ESPE, Minnesota, USA), with the aid of a cartridge dispenser with mixing tips and intraoral tips, before (day 0) and after application of orthodontic force (days 1, 2, 4, 6, and 8).

Type IV dental stone plaster casts were obtained and examined under a surgical microscope (MC-M31, DF Vasconcellos, Brazil) at x10 magnification, with a 200mm focal length lens. Images were captured with a digital video camera (Moticam 2000, Motic) coupled to the microscope.

Images were analyzed in the ImageJ software suite (National Institutes of Health, Bethesda, MD, USA) for measurement of the distance of left maxillary first molar movement at different time points in each animal. The landmarks used for measurement were the centers of the distal fossae of the left first and second molars (Figure 4).

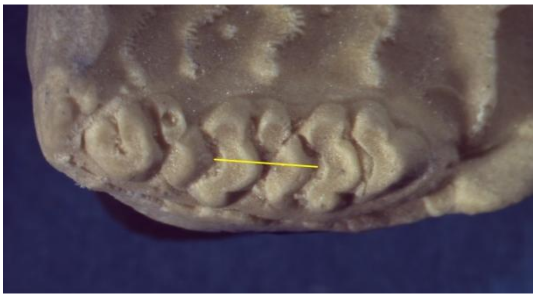

Figure 4. Measurement of orthodontic movement on plaster casts.

Bone staining

For bone staining, the fluorescent dye calcein (Sigma Aldrich, Japan) was injected subcutaneously at a dose of 8mg/Kg on days 0, 4, and 7 (Figure 3). On day 8, the rats were euthanized with isoflurane (Cristália, Porto Alegre, RS, Brazil) in an appropriate induction chamber. The maxilla of each animal was removed, fixed in 10% formalin, skeletonized, and dehydrated in a graded ethanol series (70–95%) and acetone A.R. (Merck, Darmstadt, Germany). The bones were then embedded in epoxy resin (Electron Microscopy Sciences, Pennsylvania, USA) according to manufacturer instructions.

The embedded samples were ground down to 1.5mm below and parallel to the occlusal plane of the molars, and 250 to 500-grain wet sandpaper was used to achieve a final slide thickness of 0.5mm.

The area chosen for measurement of bone neoformation on the side of application of orthodontic force was determined by drawing a line from the center of the mesiobuccal root of the left maxillary first molar to the center of its mesio-palatal root. Another line was drawn perpendicularly to the former so as to divide the mesiobuccal root into four segments. The site chosen for measurement of neoformed bone area was the root segment facing the distobuccal root of the same tooth.[12]

Slides were examined with the aid of confocal microscope (LSM 5 Exceiter, Zeiss, Germany), under x10 magnification, with images obtained using a 488nm laser. A camera coupled to the microscope was used to acquire micrographs, which were manipulated in the ZEN 2008 software suite, exported to TIFF format, and analyzed in the ImageJ software. Fluorescent areas on the bone surface were measured and the area of neoformed bone on the stress side was calculated (Figure 5).

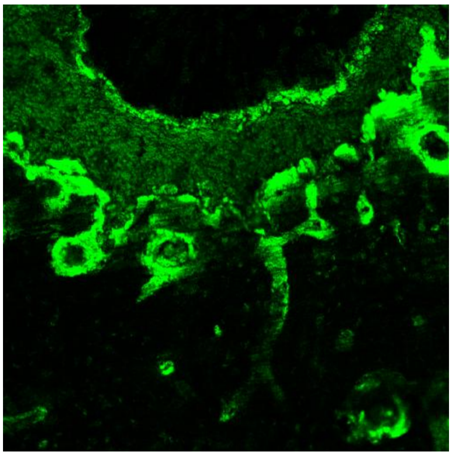

Figure 5. Confocal micrograph showing calcein-stained neoformed bone.

Results

To determine experimental error, samples were measured twice, with an interval of at least 1 month between measurements. These measurements were then compared by means of intraclass correlation coefficients, which showed excellent agreement between the two (p<0.001) (Tables I and II).

Table I. Intraclass correlation coefficients for measurements obtained from plaster models.

Intraclass Correlation Coefficient							
	Intraclass Correlation[a]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.946[b]	.926	.960	36,027	149.0	149	.000
Average Measures	.972[c]	.962	.980	36,027	149.0	149	.000

Two-way mixed effects model where people effects are random and measures effects are fixed.

[a] Type A intraclass correlation coefficients using an absolute agreement definition.

[b] The estimator is the same, whether the interaction effect is present or not.

[c] This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

Table II. Intraclass correlation coefficients for measurements of bone staining.

Intraclass Correlation Coefficient							
	Intraclass Correlation[c]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.998[b]	.996	1172,785	.999	24.0	24	.000
Average Measures	.999[c]	1.000	1172,785			24	.000

[a] Type A intraclass correlation coefficients using an absolute agreement definition.

[b] The estimator is the same, whether the interaction effect is present or not.

[c] This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

Measurements obtained from the plaster casts were analyzed by means of repeated-measures analysis of variance (ANOVA) followed by Tukey’s multiple comparison test, at a significance level of 0.05 (Tables III and IV). There were no statistically significant differences between the LLLT groups (Groups 2, 3, 4, and 5) and the control animals (Group 1), and no significant interaction of time and group. Regardless of group allocation, mean tooth displacement distances increased significantly with the passage of time, except between time points 1 and 2, where the difference was not significant.

Table III. Analysis of variance (ANOVA) with Tukey’s post-hoc multiple comparisons test.

Time	Group					Overall
(days)	A1	A2	A3	A4	A5	
0	1.96±0.11	2.06±0.08	1.99±0.05	1.98±0.07	2.06±0.1	2.01^{e}±0.09
1	2.21±0.04	2.37±0.16	2.23±0.07	2.18±0.11	2.29±0.06	2.26^{d}±0.11
2	2.36±0.04	2.42±0.08	2.31±0.13	2.28±0.14	2.39±0.13	2.35^{d}±0.12
4	2.44±0.15	2.49±0.11	2.52±0.12	2.44±0.08	2.49±0.10	2.47^{c}±0.11
6	2.71±0.22	2.58±0.06	2.56±0.14	2.52±0.13	2.66±0.18	$2.60^{b\pm}$0.16
8	2.93±0.19	2.86±0.06	2.70±0.35	2.79±0.05	2.87±0.14	2.83^{a}±0.19
Total	2.44^{AB}±0.35	2.47^{B}±0.26	2.38^{AB}±0.29	2.36^{A}±0.28	2.46^{AB}±0.29	2.42±0.29

Different superscript uppercase letters and different superscript lowercase letters denote statistically significant differences in means (repeated measures ANOVA with Tukey’s multiple comparisons, P<0.05).

Table IV. Repeated-measures analysis of variance (ANOVA).

Cause of variation	Degrees of freedom	F	P
Group	4	3.29	0.031
Time	5	119.23	<0.001
Group*Time	20	0.84	0.661

The nonparametric Kruskal–Wallis test, followed by the multiple comparisons test, was used for analysis of data on area of bone neoformation (Table V). The control group has significantly lower stained bone area values than groups 2, 3, and 4 at the 0.05 level. Group 5 did not differ from the control or other LLLT groups.

Table V. Kruskal–Wallis test with multiple comparisons.

Group	Median (interquartile range)	Mean rank
1	2968.67 (2030.63-5989.62)	4.20 ^{B}
2	9739.31 (5455.05-17381.74)	14.40 ^{A}
3	18061.05 (9200.69-25710.95)	19.80 ^{A}
4	10326.71 (6433.78-15814.35)	15.00 ^{A}
5	8189.60 (4903.11-10821.52)	11.60 ^{AB}

Different superscript letters denote significant differences in mean ranks (nonparametric Kruskal–Wallis test with multiple comparisons, $P<0.05$).

Discussion

GaAlAs infrared laser was chosen as it has the greatest ability to penetrate biological tissue and can act on bone and periodontal tissue alike; furthermore, no other laser type has been used as widely in studies of the influence of LLLT on orthodontic tooth movement.[6,10,12,15-19]

The energy settings employed in the present study were based on prior studies conducted in rats.[6,12,15-19] A spot energy of 18J (54J per session) was used in three previous studies. Two of these reported a positive response (increased distance of orthodontic tooth movement) in LLLT-treated animals as compared with the control group,[6,12] whereas the other study found no significant between-group differences.[16] Yoshida et al. used an energy setting of 13.5J per spot (54J per session) and found that LLLT increases the rate of orthodontic tooth movement.[15] Altan et al. also used a total energy of 54J per session, but distributed it across five irradiation sites (10.8J each), and found no significant between-group differences.[17] Gama et al. found no statistically significant results with a spot dose of 0.135J (intraoral)/0.33J (extraoral) and a total dose of 0.6J/session.[18] The 12, 15, 18, and

21J energy settings chosen for the present study cover the 13.5–18J range in which previous studies have obtained significant improvements in the rate of orthodontic movement after LLLT as compared with non-irradiated controls, with an additional margin to account for potential variation.

Standardization of LLLT protocols is essential to enable comparison of future studies and achievement of reliable results.

In the present study, LLLT was administered every 48 hours, although most previous research used daily exposure.[6,12,15-17] There is evidence in the literature that LLLT exerts positive effects on bone neoformation when administered in this schedule.[20-24]

According to Kawasaki and Shimizu,[6] Fujita et al.,[12] Yoshida et al,.[15] Goulart et al.,[10] and Marquezan et al.,[16] most orthodontic tooth movements occurs during the first few days of orthodontic force application and LLLT administration. Therefore, the experimental period of this study had a duration of 8 days to cover the period of greatest tooth displacement.

Our findings did not demonstrate statistically significant quantitative differences in orthodontic tooth movement between the control group and any of the LLLT groups. Similar findings have been reported by other authors[16-18], which suggests that, when administered using these protocols, LLLT does not interfere with the rate of orthodontic movement.

On histological examination, specimens from animals in the 12J, 15J, and 18J LLLT groups showed a significant increase in the area of neoformed bone as compared with animals in the control group, corroborating previous evidence that LLLT stimulates bone neoformation.[6,15,17] Only in group 5 (21J) was the difference in stained bone area not significant. This suggests that the high level of energy applied may have delayed the bone neoformation process.

Altan et al.[17] used energy settings of 54J and 15J per session and failed to observe any difference in distance of orthodontic movement between the LLLT and control groups, but LLLT did speed the bone remodeling process by stimulating osteoblast and osteoclast proliferation and activity. As in the present study, there was no quantitative difference in orthodontic tooth movement between the LLLT and control groups, but histological changes did occur that suggest LLLT may increase bone neoformation. This, in turn, may be a major determining factor of recurrence of tooth displacement.

In 1997, Saito and Shimizu found that repeated application of LLLT over the active area during the early period of orthodontic movement stimulates bone regeneration.

Although several studies have used this experimental model[6,12,15-18], we found that it may not be optimal for this specific assessment, as the incisors used as fastening elements were also displaced by the applied force, which may bias results. Furthermore, as LLLT has systemic effects[25-27], laser administration in the experiment groups may also have facilitated displacement of the incisors rather than of the molars alone, as we originally intended. Gama et al.[18] and Marquezan et al.[16] modified the model by adding resin to the incisors, which may have improved anchorage, but would not have eliminated the systemic influence of LLLT.

Therefore, this variable must be eliminated if more reliable results are to be obtained. Use of mini implants is a potential solution, but the procedure would have to be adapted to rats, or a different animal model used instead.

Although laser therapy protocols have yet to be completely defined, several clinical studies have employed LLLT in teeth subjected to orthodontic movement to assess whether the rate of said movement can be increased by laser irradiation.[7,8,11] Results have been divergent, although there is a general trend toward an increased rate of orthodontic movement with a certain LLLT protocol.[7,8,11]

However, the samples of these studies have been very heterogeneous in terms of participant age, which may have a direct influence on results, as skeletal age and bone maturity are determinants of the extent of orthodontic tooth movement. The type of orthodontic appliance employed has also varied widely among studies in terms of bracket placement, use of a continuous archwire versus wire segments, and appliance reactivation.[7,8,11]

Conclusions

At the energy settings and protocols used in this study, LLLT did not interfere with the rate of orthodontic tooth movement, although certain energy settings encouraged bone neoformation, which may help reduce recurrence. Further research is required to ascertain the effects of laser therapy on orthodontic tooth movement.

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DISCUSSÃO GERAL

4 DISCUSSÃO GERAL

Um dos grandes desafios da ortodontia nos dias atuais é a diminuição do tempo de tratamento, que os pacientes relatam como sendo uma das principais desvantagens desse tipo de tratamento (LEW, 1991; SKIDMORE et al, 2006).

Alguns estudos vem sendo realizados sobre a influência da LLLT na quantidade da movimentação ortodôntica. Porém, apresentam diferenças na aplicação do *laser* com relação ao comprimento de onda, a potência, a dosagem e o tempo de aplicação, produzindo, desta forma, resultados divergentes (SAITO; SHIMIZU, 1997; CRUZ et al, 2004; GOULART et al, 2006; LIMPANICHKUL et al, 2006; TURHANI et al, 2006; SEIFI et al, 2007; FUGITA et al, 2008; YOUSSEF et al, 2008).

Optou-se pela pesquisa em ratos por não haver um consenso sobre os benefícios e protocolos da utilização da LLLT em pacientes sob tratamento ortodôntico e por haver na literatura um modelo experimental de movimentação dentária, bastante utilizado, em ratos (KAWASAKI; SHIMIZU, 2000; FUGITA et al, 2008; YOSHIDA et al, 2009; GAMA et al, 2010; MARQUEZAN; BOLOGNESE; ARAÚJO, 2010).

A maioria dos artigos em animais, sobre a aplicação da LLLT na movimentação ortodôntica, reporta que há um aumento da quantidade de movimentação dentária, estimulando a remodelação óssea através do incremento do número de osteoclastos e osteoblastos e suas funções (KAWASAKI; SHIMIZU, 2000; GOULART et al, 2006; FUGITA et al, 2008; KIM et al, 2009; YOSHIDA et al, 2009; ALTAN et al, 2012).

O *laser* de diodo GaAlAs foi o escolhido para a utilização nessa pesquisa por ser o mais aplicado em estudos sobre o efeito da LLLT na ortodontia, assim como o comprimento de onda de 830nm, que encontra-se na faixa do infravermelho (KAWASAKI; SHIMIZU, 2000; GOULART et al, 2006; FUGITA et al, 2008; KIM et al, 2009; YOSHIDA et al, 2009; GAMA et al, 2010; MARQUEZAN; BOLOGNESE; ARAUJO, 2010; ALTAN et al, 2012). Sabe-se que o *laser* infravermelho tem maior penetrabilidade em tecidos biológicos quando comparado com o vermelho, estimulando tecidos encontrados mais profundamente como é o caso do tecido ósseo, intimamente relacionado com a movimentação dentária ortodôntica. Fugita et al encontraram no grupo irradiado um maior número de osteoclastos multinucleados assim como a expressão do RANK em células precursoras de osteoclastos em estágio mais inicial (FUGITA et al, 2008).

As aplicações da LLLT foram realizadas de forma contínua, em contato com o tecido irradiado e de forma pontual, por ser o método mais utilizado e eficaz para produção de resultados positivos (KAWASAKI; SHIMIZU, 2000; GOULART et al, 2006; FUGITA et al, 2008; YOSHIDA et al, 2009) . Quando o *laser* é aplicado em um único ponto e não em uma região, e em contato direto com o tecido, há maior chance de absorção dessa energia evitando a reflexão do *laser*. Kim et al aplicaram o *laser* de forma pulsátil e sem contato e encontraram resultados mais tardios da LLLT quando comparado com outros estudos (KAWASAKI; SHIMIZU, 2000; FUGITA et al, 2008; KIM et al, 2009; YOSHIDA et al, 2009).

As energias de *laser* aplicadas nessa pesquisa foram determinadas observando estudos prévios em ratos (KAWASAKI; SHIMIZU, 2000; FUGITA et al, 2008; YOSHIDA et al, 2009; GAMA et al, 2010; MARQUEZAN; BOLOGNESE; ARAÚJO, 2010; ALTAN et al, 2012). A energia de 18J por ponto, 54J por sessão, foi utilizada em três estudos, dois deles apresentaram resposta positiva para o aumento na quantidade de movimentação quando comparado com o grupo controle (KAWASAKI; SHIMIZU, 2000; FUGITA et al, 2008), o outro estudo mostrou não haver diferença estatisticamente significativa entre os grupos avaliados (MARQUEZAN; BOLOGNESE; ARAÚJO, 2010). Yoshida et al aplicaram energia de 13,5J por ponto e energia total de 54J por sessão, e obtiveram resultados indicando que o uso da LLLT acelera a velocidade da movimentação ortodôntica (YOSHIDA et al, 2009). Altan et al também aplicaram energia de 54J por sessão, mas distribuída em cinco pontos com 10,8J cada, observaram que não houve diferença estatística entre os grupos (ALTAN et al, 2012). Já Gama et al não encontraram resultados estatisticamente significativos utilizando energia de 0,135J por ponto intra oral e 0,33J no ponto extra bucal com energia total por sessão de 0,6J (GAMA et al, 2010). As energias de 12, 15, 18 e 21 Joules selecionadas para aplicação nesse estudo, cobrem o intervalo de 13,5 à 18J que estudos obtiveram resultados significativos para o aumento na quantidade de movimentação ortodôntica com a aplicação da LLLT comparado com o grupo controle, e deixa uma margem para avaliar possíveis variações.

Os estudos conduzidos por Gama et al, Marquezan et al e Altan et al não mostram aumento na quantidade de movimento dentário quando a LLLT é aplicada. Esses autores utilizaram ratos Wistar com idade mais elevada entre 70 e 120 dias e utilizaram forças maiores, no mínimo o dobro, que as utilizadas nos estudos que apresentaram respostas positivas (GAMA et al, MARQUEZAN; BOLOGNESE; ARAÚJO, 2010; ALTAN et al, 2012).

Marquezan et al não acharam diferença estatisticamente significante entre o grupo irradiado e o controle quanto a quantidade de movimentação dentária.

Utilizaram os mesmos parâmetros que os estudos com resultados positivos, *laser* GaAlAs com aplicação de 54J e 18000J/cm² por sessão de forma contínua, em contato e pontual. As únicas diferenças foram as idades dos ratos, o dobro da idade dos utilizados nos estudos com resultados positivos (KAWASAKI; SHIMIZU, 2000; FUGITA et al, 2008; YOSHIDA et al, 2009), e a força, quatro vezes maior. A idade dos espécimes pode ser uma variável importante visto que o tecido periodontal envelhece com o tempo e pode responder de forma diferente de um tecido jovem e as forças utilizadas podem causar danos a esse tecido, e consequente diminuição da movimentação ortodôntica. Os achados histológicos desse estudo mostraram que a aplicação da LLLT diariamente promoveu o aumento no número de osteoclastos após sete dias, mas inibiu a expressão de colágeno imaturo no lado de tensão.

Altan et al também aplicaram o *laser* de forma contínua, em contato e pontual, utilizando uma energia de 54J e 15J e doses de 1717,2J/cm² e 477J/cm² por sessão. Entretanto, os autores não observaram aumento estatisticamente significante na quantidade da movimentação dentária. Esse achado pode ser devido a uma amostra pequena, ou ao fato de que os dentes movimentados foram os incisivos ao invés dos molares utilizados por Kawasaki e Shimizu, Fugita et al e Yoshida et al.Outra possível explicação para a obtenção dos resultados não significantes é o fato da dose total da sessão ser inferior a aplicada por esses últimos autores, que utilizaram dose de 18000J/cm² por sessão enquanto Altan et al utilizaram apenas 1717,2J/cm². Apesar dessas diferenças, Altan et al encontraram uma tendência de aumento da quantidade de movimentação no grupo irradiado com 54J por sessão comparado com o de 15J. Histologicamente encontraram uma aceleração na proliferação celular de osteoclastos reforçando a ideia de que a LLLT realmente interfere na remodelação óssea durante o movimento dentário ortodôntico.

Com o uso de amostra mais velha (12 semanas) e força ortodôntica maior, Gama et al demonstraram que a aplicação da LLLT pode diminuir a movimentação dentária induzida quando comparada com grupo controle, se aplicadas energias e doses específicas. Outra variável desse estudo é a utilização de um ponto extra bucal para aplicação da LLLT. Apesar dos autores considerarem a perda de energia até a chegada ao tecido desejado e aumentarem a energia aplicada, não temos como saber o quanto de energia foi absorvida efetivamente.

A padronização dos protocolos de aplicação da LLLT é fundamental para que estudos possam ser comparados e resultados fidedignos possam ser obtidos.

A aplicação da LLLT a cada 48 horas foi estabelecida nesse estudo, apesar da maioria dos estudos prévios utilizarem aplicação diária (KAWASAKI; SHIMIZU, 2000; FUGITA et al, 2008; YOSHIDA et al, 2009, MARQUEZAN; BOLOGNESE; ARAÚJO, 2010; ALTAN et al, 2012). Na literatura há evidências que suportam que nesse intervalo de tempo há efeitos positivos da LLLT na neoformação óssea (BLAYA, 2005; VIEGAS et al, 2005; PINHEIRO; GERBI, 2006; WEBER et al, 2006; DREYER et al, 2011).

Estudos anteriores evidenciam que a maior movimentação ocorre durante os primeiros dias da aplicação da força e da LLLT (KAWASAKI; SHIMIZU, 2000; GOULART et al, 2006; FUGITA et al, 2008; YOSHIDA et al, 2009; MARQUEZAN; BOLOGNESE; ARAÚJO, 2010). Dessa forma, esse estudo teve duração de 9 dias para avaliar o efeito da LLLT durante o período de maior movimentação dentária.

Nesse experimento padronizamos o animal (rato), a idade do mesmo (6 semanas), a forma de movimentação dentária, a força dessa movimentação (10g/força), o tipo e o comprimento de onda do *laser* (GaAlAs, 830 nm), a forma de aplicação (contínua, pontual e em contato), a frequência dessa aplicação (cada

48horas) e os pontos de aplicação da LLLT (3 pontos intrabucais). A única variável independente foi a quantidade de energia aplicada, e suas doses, em cada grupo experimental.

Os resultados obtidos não demonstraram diferença estatisticamente significante do grupo controle aos demais grupos com aplicação da LLLT na quantidade da movimentação ortodôntica. O mesmo resultado foi obtido por outros autores (GAMA et al, 2010; MARQUEZAN; BOLOGNESE; ARAÚJO, 2010; ALTAN et al, 2012) indicando que a LLLT aplicada nesses protocolos não interfere na velocidade da movimentação dentária.

Histologicamente observamos que nos grupos com energias de 12, 15 e 18J houve aumento na área de neoformação óssea estatisticamente significante comparado com o grupo controle, corroborando evidências anteriores que a aplicação da LLLT estimula essa ação (KAWASAKI; SHIMIZU, 2000; YOSHIDA et al, 2009; ALTAN et al, 2012). Apenas no Grupo 5, com aplicação de energia de 21J, a diferença da área marcada não foi significativa. Esse achado pode ser devido à energia elevada aplicada que pode ter retardado o processo de neoformação óssea.

Altan et al aplicaram 54J e 15J de energia por sessão e não obtiveram diferença na quantidade de movimento entre os grupos em que foram aplicadas a LLLT e o controle, mas observaram que a LLLT acelera o processo de remodelação óssea estimulando a proliferação celular de osteoblastos e osteoclastos e suas funções. Assim como o presente estudo, não houve diferença nas quantidades de movimentações entre os grupos da LLLT e o controle, mas histologicamente houve alterações que indicam que a LLLT pode aumentar a neoformação óssea o que pode ser um fator importante quando consideramos a recidiva do movimento ortodôntico e o período de contenção.

Saito e Shimizu, em 1997, realizaram um estudo em que observaram que o estímulo da regeneração óssea ocorre quando a LLLT é feita sobre a área ativa no período inicial do movimento, por repetidas vezes.

Apesar de vários estudos já utilizarem esse modelo experimental (KAWASAKI; SHIMIZU, 2000; FUGITA et al, 2008; YOSHIDA et al, 2009; GAMA et al, 2010; MARQUEZAN; BOLOGNESE; ARAÚJO, 2010; ALTAN et al; 2012), pudemos observar durante o experimento que o modelo pode não ser o ideal para essa avaliação pois os incisivos utilizados como ancoragem também se movimentam devido a aplicação da força ortodôntica, o que pode alterar os resultados. Ainda, como a LLLT possui efeito sistêmico (BRAVERMAN et al; 1989; ROCHKIND et al, 1989; RODRIGO, 2007), é possível que a aplicação do *laser* nos grupos experimentais também possa ter favorecido a movimentação dos incisivos e não somente a dos molares como era a intenção. Gama et al e Marquezan et al modificaram o modelo adicionando resina aos incisivos o que pode ter aumentado a ancoragem mas não eliminou o fator sistêmico.

Dessa forma, há a necessidade de removermos essa variável para obtermos resultados mais fidedignos. A utilização de mini-implantes pode ser a solução para essa questão, mas há a necessidade de se adaptar tal procedimento para ratos ou mudar o animal experimental.

Apesar do protocolo de irradiação a *laser* não estar completamente definido, estudos clínicos tem sido realizados com a aplicação do LLLT em dentes movimentados ortodonticamente para avaliar se há um aumento na velocidade dessa movimentação (CRUZ et al, 2004; LIMPANICHKUL et al, 2006; SOUZA et al, 2011). Seus resultados são divergentes apesar de mostrarem uma tendência para o aumento da velocidade ortodôntica quando a LLLT é aplicada em determinado protocolo (CRUZ et al, 2004; LIMPANICHKUL et al, 2006; SOUZA et al, 2011). Porém, as amostras utilizadas nesses estudos variam muito com relação à idade dos participantes, o que pode influenciar diretamente os resultados já que a idade esquelética e a maturação óssea são fatores determinantes na quantidade da movimentação ortodôntica. O tipo de mecânica ortodôntica empregada também varia muito entre os estudos com relação à prescrição dos braquetes, a utilização de fios contínuos ou segmentados para a realização do movimento e a reativação dos forças ortodônticas (CRUZ et al, 2004; LIMPANICHKUL et al, 2006, SOUZA et al, 2011).

Com relação à aplicação da LLLT, Cruz et al e Souza et al, utilizaram 2J de energia com dose de 50J/cm² por sessão e apresentaram diferença estatisticamente significante, sugerindo que a aplicação da LLLT durante a ortodontia aumenta a quantidade de movimentação dentária. A diferença entre os dois estudos foi a frequência na aplicação do *laser*. Cruz et al aplicaram LLLT nos dias 0,3,7,14,33,37 e 44, enquanto Souza et al não aplicou nos dias 14 substituindo pelo dia 30 e repetindo a mesma sequencia de dias de aplicação do primeiro mês. Souza et al também estenderam as aplicações para 67 dias enquanto Cruz et al terminou o experimento no dia 44. Esses achados sugerem que as aplicações, mesmo em menor número, e diminuindo a frequência, também podem produzir aumento da quantidade de movimentação dentária.

Limpanichkul et al aplicaram 18,4J e dose de 204J/cm² por sessão com frequência diária nos 3 primeiros dias e se repetindo nos últimos três dias do primeiro, segundo e terceiro meses. Seus resultados foram negativos, demonstrando que, com esses parâmetros e nessa frequência, a aplicação da LLLT não produz influência alguma na quantidade de movimentação ortodôntica. Os autores sustentam a hipótese de que a amostra tenha sido muito pequena e que a dose de 25J/cm² por ponto seja muito baixa para produzir efeito estimulatório ou inibitório. Se compararmos com os estudos de Cruz et al e Sousa et al, que reportaram efeitos positivos, observamos que o tamanho da amostra está adequado e que a possível falha esteja na dose utilizada por sessão e a frequência das aplicações. Talvez, em humanos, doses altas de *laser* não causem efeitos sobre a movimentação ortodôntica, ou até mesmo possam causar a diminuição dessa velocidade, enquanto doses mais baixas produzam aumento da quantidade de movimentação dentária, ao contrário do que ocorre com animais.

Apesar do pequeno número de estudos, falhas na seleção de pacientes e diferenças nas técnicas ortodônticas empregadas, pode-se aprender com esses erros e não repeti-los no futuro, produzindo assim resultados mais fidedignos.

CONCLUSÃO

CONCLUSÃO

No presente estudo observamos que a aplicação da LLLT nesse determinado protocolo e nas energias empregadas não interfere na quantidade da movimentação ortodôntica, apesar de estimular a neoformação óssea com a aplicação de determinadas energias o que pode ser favorável para diminuição de recidivas e períodos de contenção. Novos estudos devem ser realizados para determinar o efeito do *laser* na movimentação ortodôntica.

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ANEXOS

ANEXO A

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4. Figures. Digital images should be in TIF or EPS format, CMYK or grayscale, at least 5 inches wide and at least 300 pixels per inch (118 pixels per cm). Do not embed images in a word processing program. If published, images could be reduced to 1 column width (about 3 inches), so authors should ensure that figures will remain legible at that scale. For best results, avoid screening, shading, and colored backgrounds; use the simplest patterns available to indicate differences in charts. If a figure has been previously published, the legend (included in the manuscript proper) must give full credit to the original source, and written permission from the original publisher must be included. Be sure you have mentioned each figure, in order, in the text.
5. Tables. Tables should be self-explanatory and should supplement, not duplicate, the text. Number them with Roman numerals, in the order they are mentioned in the text. Provide a brief title for each. If a table has been previously published, include a footnote in the table giving full credit to the original source and include written permission for its use from the copyright holder. Submit tables as text-based files (Word or Excel, for example) and not as graphic elements.
6. Model release and permission forms. Photographs of identifiable persons must be accompanied by a release signed by the person or both living parents or the guardian of minors. Illustrations or tables that have appeared in copyrighted material must be accompanied by written permission for their use from the copyright owner

and original author, and the legend must properly credit the source. Permission also must be obtained to use modified tables or figures.

7. Copyright release. In accordance with the Copyright Act of 1976, which became effective February 1, 1978, all manuscripts must be accompanied by the following written statement, signed by all authors:

"The undersigned author(s) transfers all copyright ownership of the manuscript [insert title of article here] to the American Association of Orthodontists in the event the work is published. The undersigned author(s) warrants that the article is original, does not infringe upon any copyright or other proprietary right of any third party, is not under consideration by another journal, has not been previously published, and includes any product that may derive from the published journal, whether print or electronic media. I (we) sign for and accept responsibility for releasing this material." Scan the printed copyright release and submit it via EES.

8. Use the International College of Medical Journal Editors Form for the Disclosure of Conflict of Interest (ICMJE Conflict of Interest Form). If the manuscript is accepted, the disclosed information will be published with the article. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of interest. Guest editorials, Letters, and Review articles may be rejected if a conflict of interest exists.

9. Institutional Review Board approval. For those articles that report on the results of experiments of treatments where patients or animals have been used as the sample, Institutional Review Board (IRB) approval is mandatory. No experimental studies will be sent out for review without an IRB approval accompanying the manuscript submission.

10. Systematic Reviews and Meta-Analyses must be accompanied by the current PRISMA checklist and flow diagram (go to Video on CONSORT and PRISMA). For complete instructions, see our Guidelines for Systematic Reviews and Meta-Analyses.

11. Randomized Clinical Trials must be accompanied by the current CONSORT statement, checklist, and flow diagram (go to Video on CONSORT and PRISMA). For complete instructions, see our Guidelines for Randomized Clinical Trials.

Other Articles

Follow the guidelines above, with the following exceptions, and submit via EES.

Case Reports will be evaluated for completeness and quality of records, quality of treatment, uniqueness of the case, and quality of the manuscript. A high quality manuscript must include the following sections: introduction; diagnosis; etiology; treatment objectives, treatment alternatives, treatment progress, and treatment results; and discussion. The submitted figures must include extraoral and intraoral photographs and dental casts, panoramic radiographs, cephalometric radiographs, and tracings from both pretreatment and posttreatment, and progress or retention figures as appropriate. Complete Case Report Guidelines can be downloaded from Case Report Guidelines.

Techno Bytes items report on emerging technological developments and products for use by orthodontists.

Miscellaneous Submissions

Letters to the Editor and their responses appear in the Readers' Forum section and are encouraged to stimulate healthy discourse between authors and our readers. Letters to the Editor must refer to an article that was published within the previous six (6) months and must be less than 500 words including references. Send letters or questions directly to the editor, via e-mail: vgkokich@u.washington.edu. Submit a signed copyright release with the letter.

Brief, substantiated commentary on subjects of interest to the orthodontic profession is published occasionally as a Special Article. Submit Guest Editorials and Special Articles via the Web site.

Books and monographs (domestic and foreign) will be reviewed, depending upon their interest and value to subscribers. Send books to the Editor in Chief, Dr. Vincent G. Kokich, Department of Orthodontics, University of Washington D-569, HSC Box 357446, Seattle,WA98195-7446. They will not be returned.

Checklist for authors

____Title page, including full name, academic degrees, and institutional affiliation and position of each author, and author to whom correspondence and reprint requests are to be sent, including address, business and home phone numbers, fax numbers, and e-mail address
____Abstract
____Article proper, including references and figure legends
____Figures, in TIF or EPS format
____Tables
____Copyright release statement, signed by all authors
____Photographic consent statement(s)
____ICMJE Conflict of interest statement
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ANEXO E

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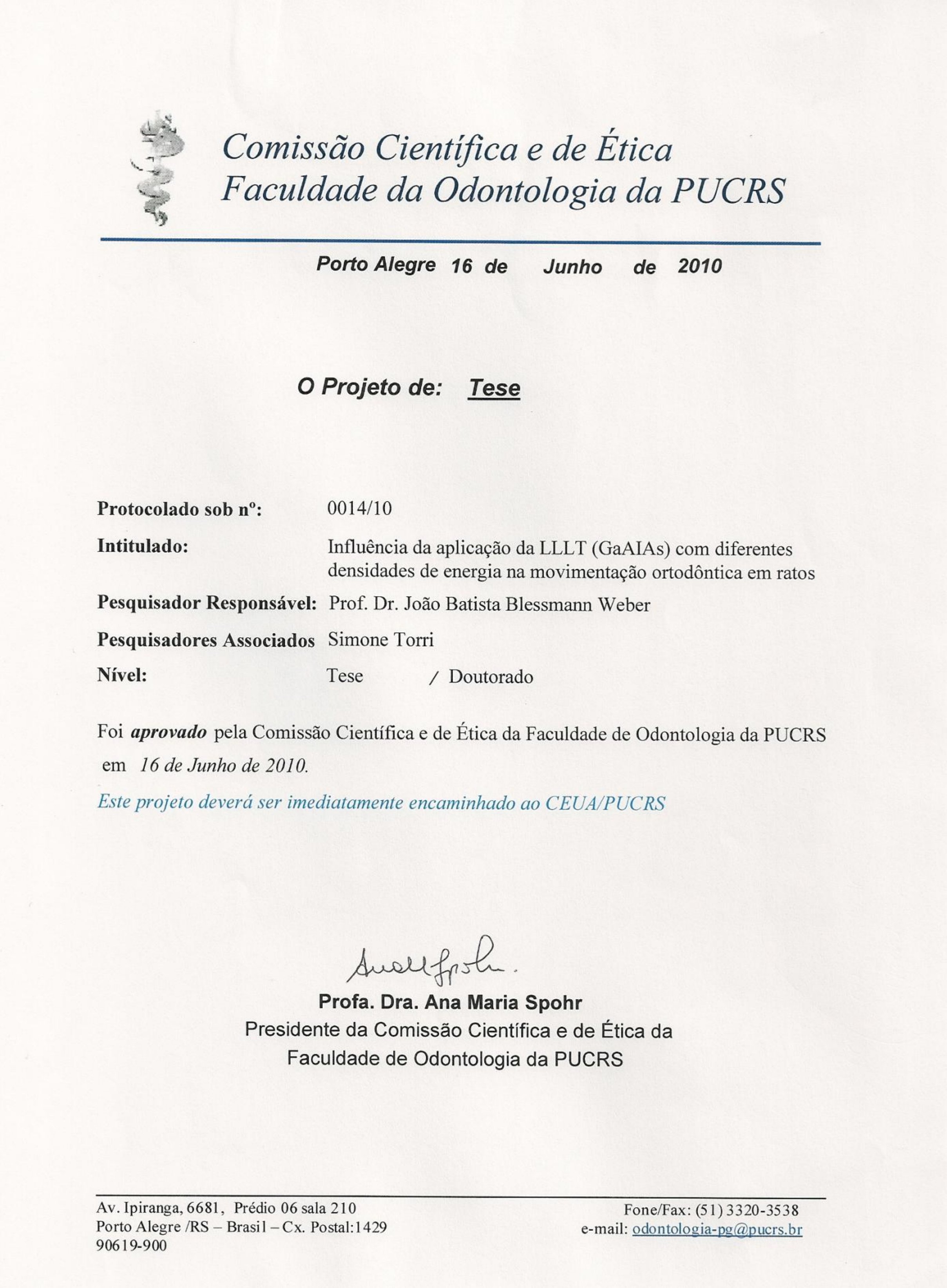
Author's Decision

Thank you for approving "INFLUENCE OF LOW-LEVEL LASER THERAPY (GaAlAs) AT DIFFERENT ENERGY SETTINGS ON ORTHODONTIC TOOTH MOVEMENT IN RATS". An email has been sent to you confirming that the journal has received this submission. Your Co-Author(s) may also receive this email, depending on the journal policy.

ees.elsevier.com/ajodo/default.asp 1/1

ANEXO F



Comissão Científica e de Ética
Faculdade da Odontologia da PUCRS

Porto Alegre 16 de Junho de 2010

O Projeto de: **Tese**

Protocolado sob nº:	0014/10
Intitulado:	Influência da aplicação da LLLT (GaAIAs) com diferentes densidades de energia na movimentação ortodôntica em ratos
Pesquisador Responsável:	Prof. Dr. João Batista Blessmann Weber
Pesquisadores Associados	Simone Torri
Nível:	Tese / Doutorado

Foi ***aprovado*** pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS em *16 de Junho de 2010.*

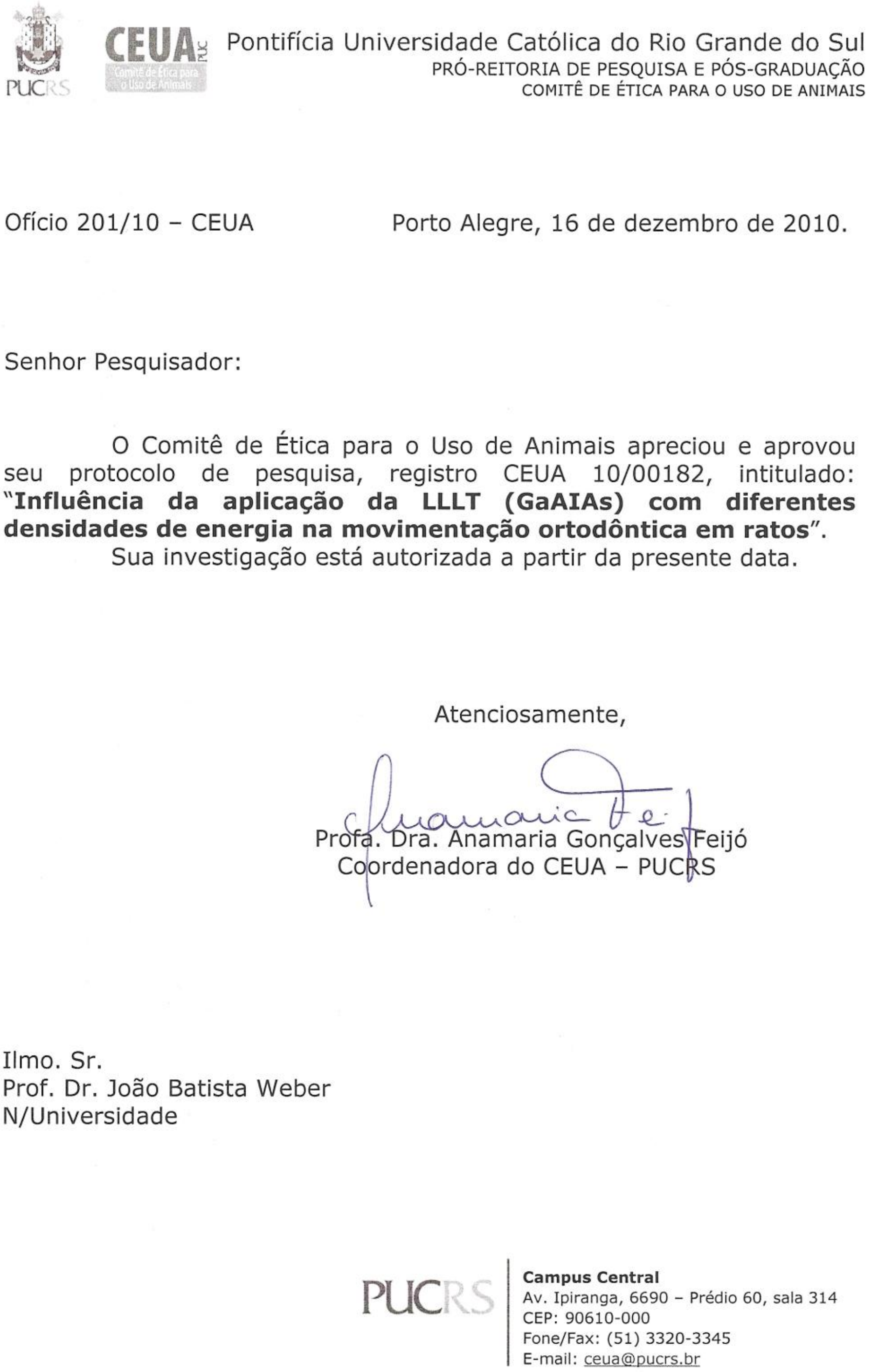
Este projeto deverá ser imediatamente encaminhado ao CEUA/PUCRS

Profa. Dra. Ana Maria Spohr
Presidente da Comissão Científica e de Ética da
Faculdade de Odontologia da PUCRS

Av. Ipiranga, 6681, Prédio 06 sala 210
Porto Alegre /RS – Brasil – Cx. Postal:1429
90619-900

Fone/Fax: (51) 3320-3538
e-mail: odontologia-pg@pucrs.br

ANEXO G



PUCRS
CEUA PUC
Comitê de Ética para o Uso de Animais
Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA PARA O USO DE ANIMAIS

Ofício 201/10 – CEUA Porto Alegre, 16 de dezembro de 2010.

Senhor Pesquisador:

O Comitê de Ética para o Uso de Animais apreciou e aprovou seu protocolo de pesquisa, registro CEUA 10/00182, intitulado: "**Influência da aplicação da LLLT (GaAIAs) com diferentes densidades de energia na movimentação ortodôntica em ratos**".

Sua investigação está autorizada a partir da presente data.

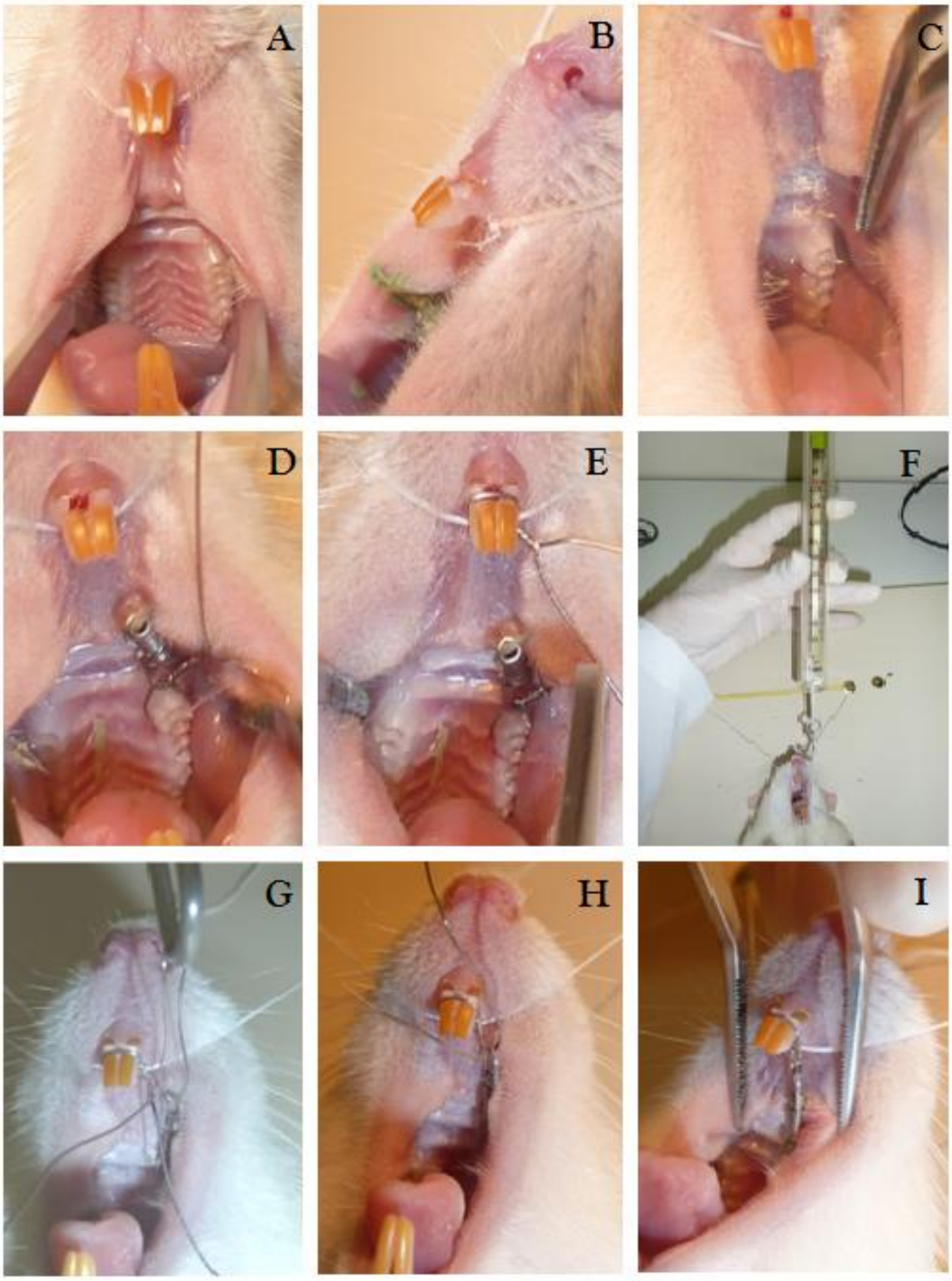
Atenciosamente,

Profa. Dra. Anamaria Gonçalves Feijó
Coordenadora do CEUA – PUCRS

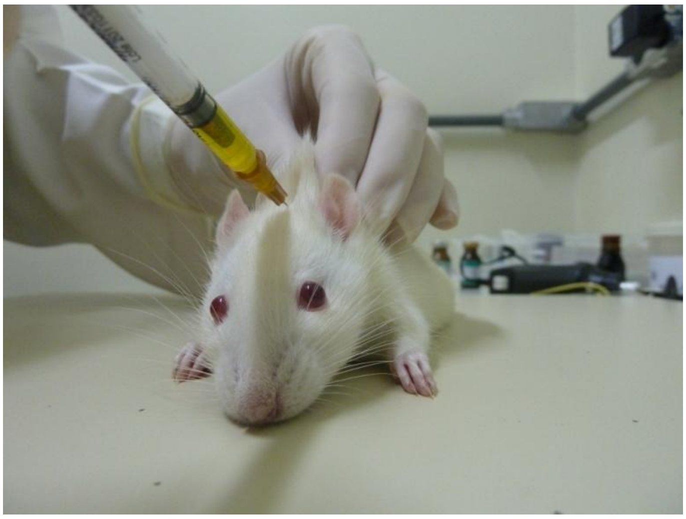
Ilmo. Sr.
Prof. Dr. João Batista Weber
N/Universidade

PUCRS
Campus Central
Av. Ipiranga, 6690 – Prédio 60, sala 314
CEP: 90610-000
Fone/Fax: (51) 3320-3345
E-mail: ceua@pucrs.br

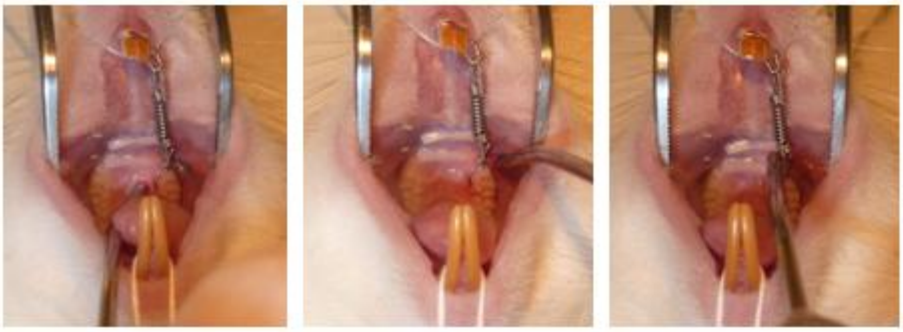
APENDICES



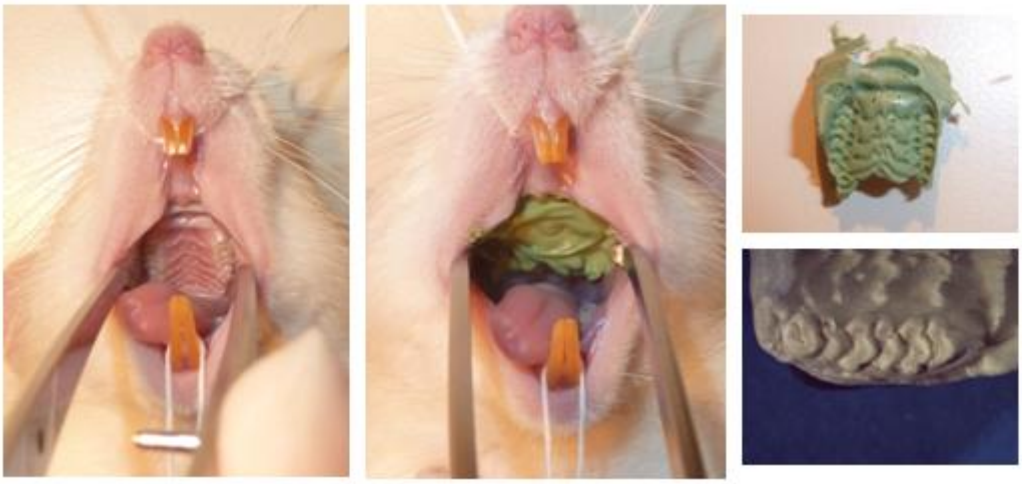
Montagem do aparelho ortodôntico. (A) Cavidade bucal do rato; (B) Canaleta nos incisivos superiores confeccionada com broca diamantada para retenção do amarrio; (C) Passagem do fio de amarrio entre o segundo e terceiro molares superiores; (D) Mola fechada de NiTi amarrada ao primeiro molar superior; (E) Amarrio posicionado nos incisivos superiores; (F) Ativação da mola em 10g; (G) Fio de amarrio dos incisivos marcado na posição de 10g; (H) mola posicionada na marcação para força de 10g; (I) Mola amarrada aos incisivos e finalização da montagem do aparelho ortodôntico.



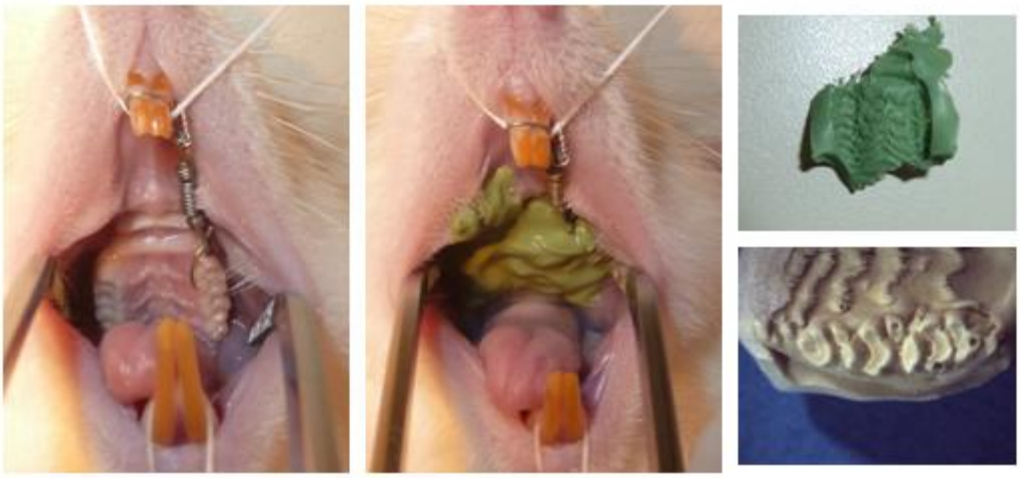
Injeção subcutânea do corante Calceína com o animal anestesiado.



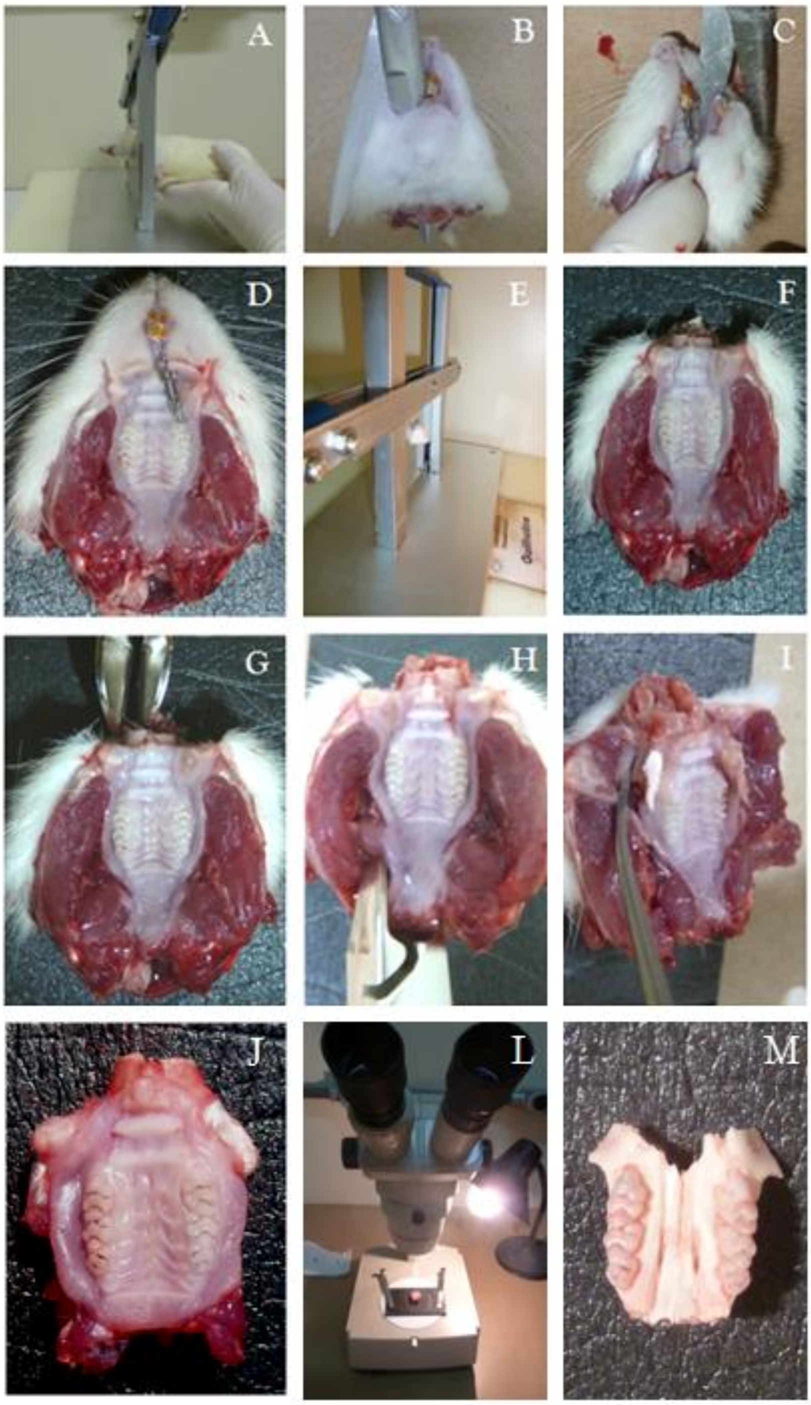
Pontos de aplicação da LLLT. Ponteira do *laser* perpendicular e em contato com a mucosa.



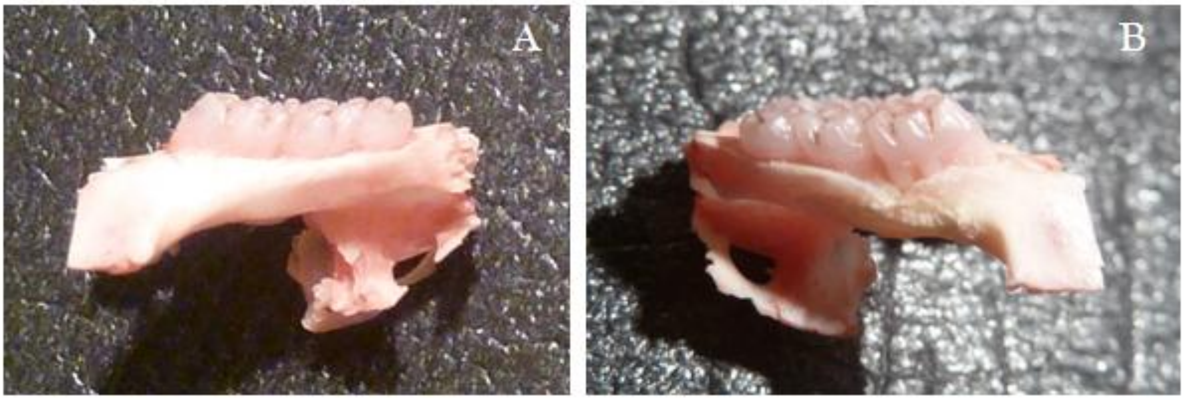
Moldagem antes da instalação do aparelho ortodôntico. A pasta fluida da silicona de adição foi aplicada diretamente sobre a maxila com o auxílio de pontas misturadoras e intra-bucais.



Moldagem após a instalação do aparelho ortodôntico. A pasta fluida da silicona de adição foi aplicada diretamente sobre a maxila com o auxílio de pontas misturadoras e intra-bucais, com o cuidado de não colocar o material de moldagem sobre a mola e assim diminuir o risco do deslocamento do aparelho ortodôntico.



Sequência para remoção da maxila após a eutanásia do animal experimental. (A) Remoção da cabeça com auxílio da guilhotina; (B, C) Remoção da mandíbula com tesoura; (D) Após a remoção da mandíbula e com o aparelho ortodôntico; (E) Remoção da parte anterior dos incisivos com auxílio da guilhotina; (F) Após remoção dos incisivos; (G) Início separação da maxila da base do crânio com o auxílio de um alicate de corte; (H) Separação das estruturas de união na parte posterior da maxila; (I) Corte realizado pelo alicate mostrando a separação da maxila da base do crânio; (J) Maxila solta com tecido mole; (L) Remoção do tecido mole do osso da maxila com o auxílio de um microscópio; (M) Osso da maxila com os dentes. Estrutura pronta para fixação em formol 10%.



Elementos dentários e estrutura óssea adjacente após a remoção do tecido mole. (A) Lado direito, onde não foi realizada a movimentação ortodôntica; (B) Lado esquerdo, onde foi colocado o aparelho ortodôntico e aplicada a força para movimentação.

GRUPO LLLT	**Energia/ Ponto (J)**	**Energia/ Sessão (J)**	**Dose/ ponto (J/ cm²)**	**Dose/ Sessão (J/ cm²)**	**Tempo/ ponto (s)**	**Potência (mW)**	**Área ponteira (cm²)**
GRUPO 2	12	36	2.378,5	7.135,7	74 s	90	0,0028
GRUPO 3	15	45	3.439,2	10.317,4	107 s	90	0,0028
GRUPO 4	18	54	3.567,8	10.703,5	111 s	90	0,0028
GRUPO 5	21	63	4.628,5	13.885,7	144 s	90	0,0028

Tabela com informações sobre as aplicações de laser empregadas nesse trabalho. O Grupo 1 não está presente na tabela pois é o grupo controle, o *laser* não foi aplicado de forma alguma.