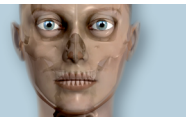
Orthodontics & Craniofacial Research



ORIGINAL ARTICLE

*M. Alsulaimani*
*M. Doschak*
*D. Dederich*
*C. Flores-Mir*

# Effect of low-level laser therapy on dental root cementum remodeling in rats

**Authors' affiliations:**
*M. Alsulaimani*, Medical Sciences Orthodontics Doctoral Program, University of Alberta, Edmonton, AB, Canada
*M. Doschak*, Faculty of Pharmacy, University of Alberta, Edmonton, AB, Canada
*D. Dederich*, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada
*C. Flores-Mir*, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

**Correspondence to:**
*Dr. Carlos Flores-Mir*
Professor
Department of Dentistry
Faculty of Medicine and Dentistry
11405 – 87 Ave, 5th Floor
5-528 Edmonton Health Clinic Academy
University of Alberta
Edmonton, AB T6G 1 C9
Canada
E-mail: cf1@ualberta.ca

**Date:**
Accepted 3 December 2014

DOI: 10.1111/ocr.12064



Alsulaimani M., Doschak M., Dederich D., Flores-Mir C. Effect of low-level laser therapy on dental root cementum remodeling in rats
*Orthod Craniofac Res* 2015; **18**: 109–116. © 2015 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd

**Structured Abstract**

***Objective –*** To investigate the amount of the cementum layer formed over the rat's dental root surfaces by daily application of low-level laser therapy (LLLT) for 2 weeks.

***Methods –*** Twelve female Sprague-Dawley (SD) rats were divided into two groups: six rats received daily LLLT (Ga-Al-As, 830 nm), and six rats received no treatment (control). The treatment lasted 2 weeks. *In vivo* Micro-CT imaging analyzed the root's hard tissue volumetric changes. The cementum thickness was evaluated histologically.

***Results –*** Total cementum thicknesses in the LLLT group increased significantly ($p = 0.015$) compared to the control group. This significant increase in the cementum thickness, verified histologically, was not detectable during *in vivo* Micro-CT imaging, which showed no significant difference between the groups regarding the root hard tissues volumetric changes over the 2-week evaluation period.

***Conclusion –*** Two weeks of daily application of LLLT significantly increased rat's dental root cementum thickness as determined histologically. However, *in vivo* Micro-CT imaging failed to accurately reveal this cementum growth as it was not possible to differentiate dentinal changes.

**Key words:** cementum; low level laser therapy; rats; root volume

## Introduction

The dental root is covered by a thin mineralized avascular connective tissue layer, known as cementum (1). The major function of cementum layer is to connect the inert root surface, biologically and structurally, with the surrounding alveolar bone through an interposing soft connective tissue, known as the periodontal ligament (1, 2). Cementum has also important adaptive and reparative roles in maintaining the integrity of the root

surface and thus preserving the proper tooth supporting apparatus (2).

Unlike bone, cementum slightly increases in thickness throughout life but does not show a continuous physiological remodeling (1, 3). The cementum matrix is deposited by specialized cells called cementoblasts, which are continuously recruited from specialized cementoprogenitor cells within the same root-related area of the mature and intact periodontal ligament (1, 4). Many studies have investigated the effect of a direct topical application of different growth and differentiation factors in order to induce cementum formation and growth around a previously exposed dental root surface due to periodontal disease (5–9). A major limitation of applying bioactive molecules directly to root surface is that they need to bind efficiently in the target location and remain bio-available for long period of time (2). On the other hand, several *in vitro and in vivo* applications of low-level laser therapy (LLLT) in dentistry have been reported (10, 11). It has been shown that LLLT significantly promotes the remodeling abilities of the mineralized tissues around dental roots, by influencing appropriate cellular functions and reducing the production of inflammatory molecules (12–14). Therefore, the non-pharmacological mechanism of action of LLLT could offer a non-invasive alternative to stimulate the growth of cementum around dental roots. Theoretically, to stimulate cementum cells which are located deeply in the periodontal space, the near-infrared continuous wave gallium–aluminum–arsenide (Ga-Al-As) low-level laser at 830 nm wavelengths with 100 mW output power and exposures dose of 6 joules/cm$^2$ would provide justifiable parameters to insure the appropriate energy absorption by the cells and thus stimulate the tissue remodeling around dental roots (15–17).

The aim of this *in vivo* study was to evaluate the application effect of LLLT on the amount of cementum thickness over 2 weeks in rats. Using a rat model would offer the potential advantage of comparing our results to previous studies using a similar model. On the other hand, the small physical size of the rat could enable the use of micro-CT imaging as a potential non-invasive modality to investigate the cementum growth in an *in vivo* setting.

## Materials and methods

### Experimental procedures

The animal experiment protocol was approved by the Animal Care and Use Committee for Health Sciences at the University of Alberta.

A total sample of 12 female Sprague-Dawley (SD) rats (Biosciences Animal Service, Edmonton, Alberta), aged 6 weeks old, was used. The animals were randomly distributed into labeled cages, two rats per cage, and ear notching identified each animal. All animals were exposed to the standard 12-hour light/dark cycles and were given 7 days, before treatment start, to adapt to the new environment. The animals were given water *ad libitum* and fed regular diet.

The animals were then randomly divided into two treatment groups of six animals (n = 6):

In the LLLT Group 6, rats received a daily dose of low-level laser therapy (LLLT) for 2 weeks. For the low-level laser source, a gallium–aluminum–arsenide (Ga-Al-As) continuous wave laser system (MediCom Maestro) was used. The wavelength of this laser source is 830 nm, at 100 mW output power, with 0.05 cm$^2$ beam spot area. The output power was measured before each application by a gauge connected to the same laser system. A rigid light guide delivered the laser beam by placing the end of this guide tip in contact with the gingiva over the roots of both right and left maxillary first molars at four different points, the mesial, buccal, distal, and lingual sides, for each molar. Irradiation was performed for 3 s at each point, with total energy density dose of 6 joules/cm$^2$ for each point once a day for 2 weeks. The aforementioned energy density is considered within the optimum LLLT dose needed to stimulate any target tissue at an ideal cellular response threshold (18). Moreover, the wavelength of this LLLT system was proved to be capable of reaching and stimulating the tissues remodeling around rat's dental root (12, 14). Animals of this group were anesthetized by isoflurane inhalation daily prior to laser

treatment to prevent device biting by the animal during the treatment.

In the control group 6, rats received no treatment for 2 weeks. Animals in this group did not receive any treatment in order to evaluate the root hard tissues volume changes and the amount of cementum thickness formation due to normal growth.

#### Micro-CT imaging

*In vivo* Micro-CT imaging was performed for all the animals immediately before treatment start (Time 0) and at the end of 2 weeks treatment (Time 1). Rats were placed in the Micro-CT imager (Skyscan 1076) under general anesthesia (Isoflurane inhalation), to avoid movement during imaging procedure. The images were obtained at 18 μm resolution with a 1-mm-thick aluminum filter. After that, the raw image dataset was reconstructed and analyzed by software provided by the same Micro-CT imager company. The mesial roots of all maxillary right and left first molars were projected and sliced in cross sections. Root volume was analyzed at each time point by including all the slices below the furcation area (Fig. 1a) toward the root apex until the end of the root (Fig. 1b). After that, a region of interest was selected and drawn by including the area of each root (Fig. 2a) through all produced

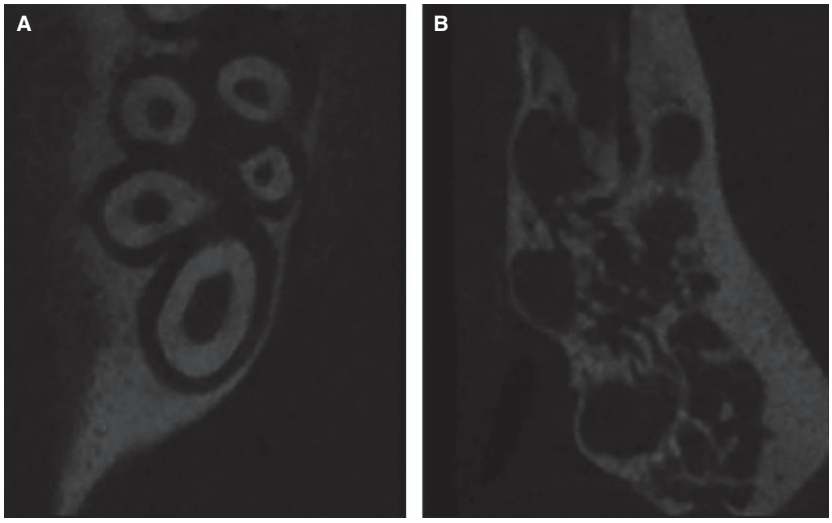



*Fig. 1.* Micro-CT volume of interest consists of all the cross-section root slices below the furcation area. Starting from this slice (a) where the roots completely separated and proceed toward the root apex until the end of the root (b)

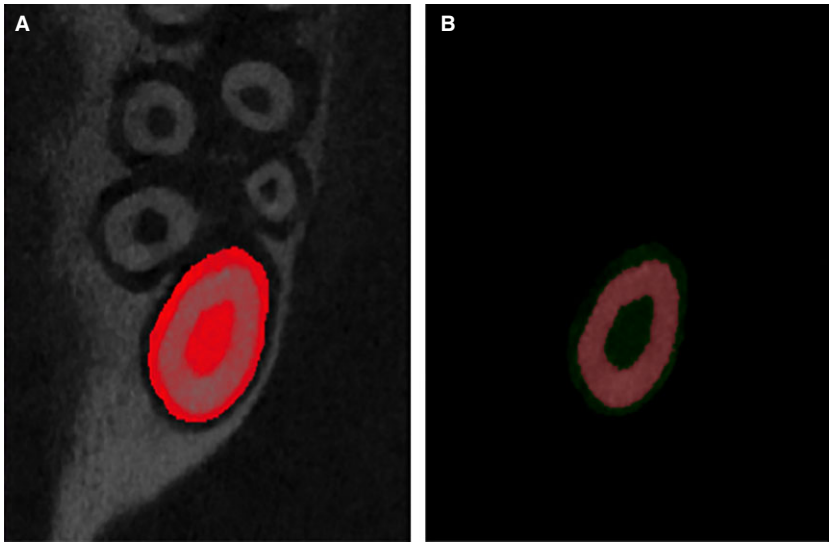



*Fig. 2.* As shown in (a): select and draw to include the root area. (b): applying a threshold value to outline the root area; red area in (b) represent the area of root hard tissues

root slices. A contrast threshold value was adjusted, fixed, and applied to all slices to include the root area and exclude the non-root area (Fig. 2b). The threshold value was set to distinguish and include the area of root hard tissues from the surrounding periodontal soft tissues. Moreover, the area representing the soft tissues inside the radicular pulp was excluded because its density degree was similar to that displaying the periodontal soft tissues on the radiograph images. As a final step, the computer aggregated the included root area of all indicated slices and end up with a volume of interest that represents the root hard tissue's volume.

#### Histomorphometric analysis

All animals were then euthanized by CO2 asphyxiation at the end of the 2-week follow-up period. After that, the mesial roots of all maxillary right and left first molars were dissected and processed for histological evaluations. Ten cross-section slices, 5 μm thick, were taken at 50-μm intervals through the whole length of each mesial root starting below the furcation area toward the root apex until the end of the root. The sections were mounted on glass slides, stained with hematoxylin and eosin (H&E), and examined under a light microscope. The cementum thickness was calculated by measuring and averaging the extent of the cementum layer at mesial, buccal, distal, and palatal root surfaces in each slide. The total cementum thickness of each root was the average of cementum thickness of all its slides.

#### Statistical analyses

In order not to violate the independency assumption of the variables for the statistical tests, the average root value of the right and left molars of the same rat in the first experiment was calculated and used in all the following statistical analyses. For this reason, the final total sample size remained 12 roots (total: n = 12). Each group sample size was 6 roots (for each group: n = 6). Due to the small sample size, a nonparametric Mann–Whitney test was used.

## Results

The means and standard deviations (SD) of the root hard tissues volume obtained from the Micro-CT analyses at each observation time are summarized in Table 1. Root-Volume-Difference-Proportion values were calculated mathematically by subtracting of the root volume value, obtained from Micro-CT analyses, at the end of 2 weeks treatment, from the volume value of the same root immediately before treatment start; then divide this emerging value by the root volume before treatment start. There was no statistical significant difference ($p = 0.240$) in the Root-Volume-Difference-Proportion when comparing the treatment group to the control.

The means and SD of cementum thickness obtained from the histomorphometric analyses for each group are summarized in Table 2. LLLT group showed a significant increase in the cementum thicknesses ($p = 0.015$) on the total surfaces of dental roots compared to control. The cross-section slide pictures in Figs 3 and 4 are samples of different mesial roots (form different animals) from LLLT and control groups, respectively, at the same magnification values.

## Discussion

The present study evaluated the effect of low-level laser therapy (LLLT) on the formation of rats' dental root cementum for 2 weeks. Histo-

***Table 1.*** **Root volume (mm3) from *in-vivo* micro-CT analyses**

| | Root volume | | Root volume difference |
|---|---|---|---|
| Treatment | Time 0 | Time 1 | proportion |
| Control | 0.76 ± 0.06 | 1.17 ± 0.08 | 0.55 ± 0.09 |
| LLLT | 0.71 ± 0.18 | 1.20 ± 0.13 | 0.73 ± 0.23† |

Data are presented as mean ± SD, when n = 6.
LLLT, low-level laser therapy.
Time 0: immediately before treatment start, Time 1: at the end of 2 weeks treatment.
Root-Volume-Difference-Proportion = (Time 1−Time 0)/Time 0.
†Statistically significant compared to control, *p*-value < 0.05.

***Table 2.*** **Cementum thickness (μm) from histological analyses**

| | Averages of root's cementum thickness | | | | |
|---|---|---|---|---|---|
| Treatment | Mesial surface | Buccal surface | Distal surface | Lingual surface | Total surfaces |
| Control | 113 ± 16 | 133 ± 41 | 72 ± 21 | 77 ± 27 | 99 ± 20 |
| LLLT | 197 ± 68 | 215 ± 30‡ | 115 ± 28† | 126 ± 30† | 163 ± 34† |

Data are presented as mean ± SD, when n = 6.
LLLT, low-level laser therapy.
†Statistically significant compared to control, *p*-value < 0.05.
‡Statistically significant compared to control, *p*-value < 0.01.

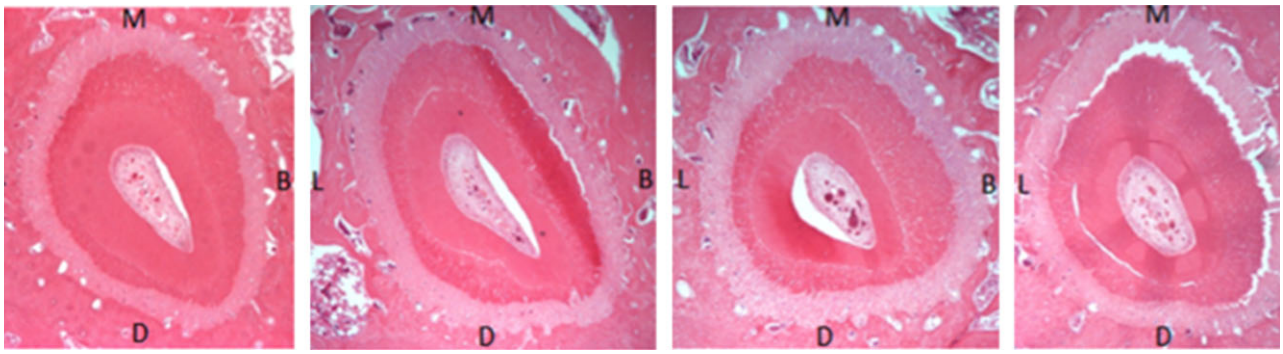



*Fig. 3.* Cross-section histological slides from LLLT treatment group. This view shows cementum thickness at the mesial (M), buccal (B), distal (D), and lingual (L) surfaces.

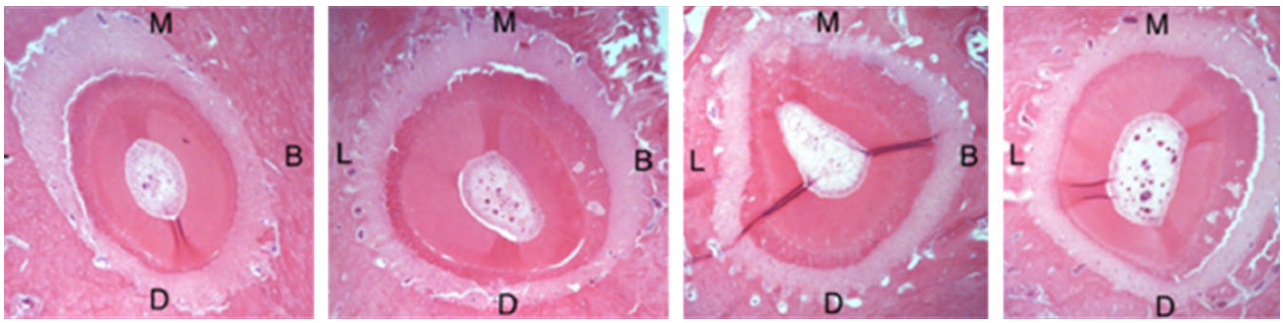



*Fig. 4.* Cross-section histological slides from different animals in control group. Notice the cementum thickness at the mesial (M), buccal (B), distal (D), and lingual (L) surfaces

logically, we found that LLLT stimulated the rat's dental root surfaces remodeling by significantly increasing the cementum layer thickness. However, when analyzing the *in vivo* Micro-CT images from the same samples, no significant change in the growth of root hard tissues volume between the groups was noted.

The histological analyses of our study showed a significant increase in the cementum thickness in the LLLT group in comparison with the normally growing cementum from the control animals. The expected mechanism behind LLLT is that laser radiation, with wavelength longer than 600 nm, has a capability to stimulate favorable cellular activities and metabolic rates by absorbing light energy (15, 19–21). It has been previously reported that the surface application of LLLT, with wavelength spectrum between 660 and 905 nm, can increase the local production of some growth factors around dental roots, such as transforming growth factor-β (TGF-β), platelet-derived growth factors (PDGFs), insulin-like growth factor-1 (IGF-1), and basic fibroblast growth factor (b-FGF) (22–24). These factors

have been recognized as having a role in the cementum formation and development (25).

The noted increase in cementum thickness in LLLT group was following a normal cementum growth pattern. The growth of cellular intrinsic fiber cementum (CIFC), covers the apical portions of the root surfaces, was more evident than acellular extrinsic fiber cementum (AEFC), which is normally confined to the coronal half of the root. It is known that the CIFC plays an important reparative role in preserving the root surface against resorptive defect, due to the ability of its cellular content to grow much faster than the other cementum types (1). Several laboratory studies and clinical investigations have found that LLLT is able to optimize the local biological conditions that lead to a considerable increase in tissue growth by influencing the activity of several cell types simultaneously (10, 11, 26). Many reports confirmed the ability of LLLT (Ga-Al-As) to provide the favorable local environment that accelerate the process of mineralized tissue formation, by influencing the appropriate cellular behaviors, stimulating tissue vascularity, promoting the activity of alkaline phosphatase, increasing the tissue ATP level, and reducing the production inflammatory molecules (13, 27–32). It has been shown that LLLT (Ga-Al-As), at 830 nm wavelength, could significantly accelerate the orthodontic tooth movement by stimulating mineralized tissues formation, as indicated by increase cellular proliferation around dental roots (12, 14). On the other hand, a double-blind clinical study found that application of 830 nm wavelength LLLT can reduce the intensity of orthodontic pain (33). This decrease in pain level was connected to the ability of the same wavelength LLLT in inhibiting the production of some inflammatory molecules such as prostaglandin (PGE2) and interleukin-1 (IL-1) (13). Therefore, the 830 nm wavelength LLLT can increase the cementum growth by providing a protective role against physiological cementum resorption, because the high levels of both PGE2 and IL-1 are not only involved in pain induction but also increase the susceptibility of mineralized tissue toward resorption (34–37).

The root volume in the present study refers to the hard tissue of the dental root that comprises both radicular dentine and cementum. Root's formation of rats' molars complete before 6 weeks of age, and after that the root hard tissues continue to grow slowly by secondary dentine apposition at the root internal surface by specialized cells located inside the dental pulp (38). At the same time, new cementum is continuously formed over the root external surface throughout life by cementoblasts, which are continuously recruited from specialized progenitor cells located in the periodontal ligament (4). For this reason, the use of a control group was justifiable.

#### Limitations

We found it difficult to distinguish between dentin and cementum from the images obtained with the Micro-CT scanning parameters used in this study. It is possible that our images failed to identify some fine details that otherwise could be detected in images at a higher resolution. However, acquiring higher resolution *in vivo* images by the same Micro-CT imager would require increasing the radiation exposure time and the length of anesthetic session at each observational point, which in turn may affect the animals' health and the study results (39). Therefore, our utilization of Micro-CT system as a non-invasive modality to obtain *in vivo* longitudinal imaging at 18 μm resolution has not shown to be a precise tool to directly assess the growth of cementum layer volume.

A sham control group could have been used. There could have been some level of tissue responses due to the effect of the rat's stress levels. If the control group had been anesthetized daily but not otherwise treated, this potential factor could have been controlled.

#### Clinical implications

Our findings suggested that the non-invasive nature of LLLT, in combination with its ability to enhance tissue remodeling and repair, could make it a useful method to stimulate new cementum formation in dental root surfaces. This concept could be useful clinically in regenerative

periodontal therapy or toward reducing root resorption caused by orthodontic tooth movement. A word of caution is advised, as extrapolation from animal studies to humans is not always straightforward or completely accurate. How the above-discussed mechanisms would work in humans should be explored in future research.

## Conclusion

We concluded that 2 weeks of daily application of LLLT, at 830 nm wavelength, significantly increased rat's dental root cementum thickness as determined histologically. On the other hand, our analyses of the obtained Micro-CT images at 18 μm resolution failed to detect the volumetric cementum growth.

**Acknowledgements:** This study was funded by both Saudi Arabian Cultural Bureau in Canada and University of Alberta. Micro-CT imaging was performed by great help from the Pharmaceutical Orthopaedic Research Lab team at University of Alberta. We also thank the HistoCore staff in Alberta Diabetes Institute at University of Alberta for their help in the histological processing.

### References

1. Bosshardt DD, Selvig KA. Dental cementum: the dynamic tissue covering of the root. *Periodontol 2000* 1997;13:41–75.
2. Grzesik WJ, Narayanan AS. Cementum and periodontal wound healing and regeneration. *Crit Rev Oral Biol Med* 2002;13:474–8.
3. Bosshardt DD. Are cementoblasts a subpopulation of osteoblasts or a unique phenotype? *J Dent Res* 2005;84:390–406.
4. Zeichner-David M. Regeneration of periodontal tissues: Cementogenesis revisited. *Periodontol 2000* 2006;41:196–217.
5. Lynch SE, de Castilla GR, Williams RC, Kiritsy CP, Howell TH, Reddy MS et al. The effects of short-term application of a combination of platelet-derived and insulin-like growth factors on periodontal wound healing. *J Periodontol* 1991;62:458–67.
6. Rutherford RB, Niekrash CE, Kennedy JE, Charette MF. Platelet-derived and insulin-like growth factors stimulate regeneration of periodontal attachment in monkeys. *J Periodontal Res* 1992;27:285–90.
7. Kuboki Y, Sasaki M, Saito A, Takita H, Kato H. Regeneration of periodontal ligament and cementum by BMP-applied tissue engineering. *Eur J Oral Sci* 1998;106:197–203.
8. Sato Y, Kikuchi M, Ohata N, Tamura M, Kuboki Y. Enhanced cementum formation in experimentally induced cementum defects of the root surface with the application of recombinant basic fibroblast growth factor in collagen gel *in vivo*. *J Periodontol* 2004;75:243–8.
9. Bosshardt DD, Sculean A, Windisch P, Pjetursson BE, Lang NP. Effects of enamel matrix proteins on tissue formation along the roots of human teeth. *J Periodontal Res* 2005;40:158–67.
10. Walsh LJ. The current status of low level laser therapy in dentistry. Part 1: soft tissue applications. *Aust Dent J* 1997;42:247–54.
11. Walsh LJ. The current status of low level laser therapy in dentistry. Part 2: hard tissue applications. *Aust Dent J* 1997;42:302–6.
12. Kawasaki K, Shimizu N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. *Lasers Surg Med* 2000;26:282–91.
13. Shimizu N, Yamaguchi M, Goseki T, Shibata Y, Takiguchi H, Iwasawa T et al. Inhibition of prostaglandin E2 and interleukin 1-beta production by low-power laser irradiation in stretched human periodontal ligament cells. *J Dent Res* 1995;74:1382–8.
14. Yoshida T, Yamaguchi M, Utsunomiya T, Kato M, Arai Y, Kaneda T et al. Low-energy laser irradiation accelerates the velocity of tooth movement via stimulation of the alveolar bone remodeling. *Orthod Craniofac Res* 2009;12:289–98.
15. Basford JR. Low intensity laser therapy: still not an established clinical tool. *Lasers Surg Med* 1995;16:331–42.
16. Esnouf A, Wright PA, Moore JC, Ahmed S. Depth of penetration of an 850 nm wavelength low level laser in human skin. *Acupunct Electrother Res* 2007;32:81–6.
17. Tuner JHL. *Laser Therapy - Clinical Practice and Scientific Background*. Grangesberg, Sweden: Prima Books; 2002.
18. Brugnera AJ. *Atlas of Laser Therapy Applied to Clinical Dentistry*. Chicago: Quintessance Editora Ltda; 2006.
19. Pöntinen PJ. *Low Level Laser Therapy as a Medical Treatment Modality: a Manual for Physicians, Dentists, Physiotherapists and Veterinary Surgeons*. : Tampere: Art Urpo Ltd.; 1992.
20. Ohshiro T, Calderhead RG. *Low Level Laser Therapy: a Practical Introduction*. Chichester; New York: Wiley; 1988.
21. Sun G, Tuner J. Low-level laser therapy in dentistry. *Dent Clin North Am* 2004;48:1061–76.
22. Arany PR, Nayak RS, Hallikerimath S, Limaye AM, Kale AD, Kondaiah P. Activation of latent TGF-beta1 by low-power laser *in vitro* correlates with increased TGF-beta1 levels in laser-enhanced oral wound healing. *Wound Repair Regen* 2007;15:866–74.
23. Shimizu N, Mayahara K, Kiyosaki T, Yamaguchi A, Ozawa Y, Abiko Y. Low-intensity laser irradiation stimulates bone nodule formation via insulin-like growth factor-I expression in rat calvarial cells. *Lasers Surg Med* 2007;39:551–9.
24. Yu W, Naim JO, Lanzafame RJ. The effect of laser irradiation on the release of bFGF from 3T3

fibroblasts. *Photochem Photobiol* 1994;59:167–70.
25. Saygin NE, Giannobile WV, Somerman MJ. Molecular and cell biology of cementum. *Periodontol 2000* 2000;24:73–98.
26. Mester E, Spiry T, Szende B, Tota JG. Effect of laser rays on wound healing. *Am J Surg* 1971;122:532–5.
27. Blaya DS, Guimaraes MB, Pozza DH, Weber JB, de Oliveira MG. Histologic study of the effect of laser therapy on bone repair. *J Contemp Dent Pract* 2008;9:41–8.
28. Merli LA, Santos MT, Genovese WJ, Faloppa F. Effect of low-intensity laser irradiation on the process of bone repair. *Photomed Laser Surg* 2005;23:212–5.
29. Pretel H, Lizarelli RF, Ramalho LT. Effect of low-level laser therapy on bone repair: histological study in rats. *Lasers Surg Med* 2007;39: 788–96.
30. Takeda Y. Irradiation effect of low-energy laser on alveolar bone after tooth extraction. experimental study in rats. *Int J Oral Maxillofac Surg* 1988;17:388–91.
31. Miloro M, Miller JJ, Stoner JA. Low-level laser effect on mandibular distraction osteogenesis. *J Oral Maxillofac Surg* 2007;65:168–76.
32. Ozawa Y, Shimizu N, Kariya G, Abiko Y. Low-energy laser irradiation stimulates bone nodule formation at early stages of cell culture in rat calvarial cells. *Bone* 1998;22:347–54.
33. Lim HM, Lew KK, Tay DK. A clinical investigation of the efficacy of low level laser therapy in reducing orthodontic postadjustment pain. *Am J Orthod Dentofacial Orthop* 1995;108:614–22.
34. Alhashimi N, Frithiof L, Brudvik P, Bakhiet M. Orthodontic tooth movement and *de novo* synthesis of proinflammatory cytokines. *Am J Orthod Dentofacial Orthop* 2001;119:307–12.
35. Bletsa A, Berggreen E, Brudvik P. Interleukin-1alpha and tumor necrosis factor-alpha expression during the early phases of orthodontic tooth movement in rats. *Eur J Oral Sci* 2006;114:423–9.
36. Yamasaki K, Miura F, Suda T. Prostaglandin as a mediator of bone resorption induced by experimental tooth movement in rats. *J Dent Res* 1980;59:1635–42.
37. Yamasaki K, Shibata Y, Imai S, Tani Y, Shibasaki Y, Fukuhara T. Clinical application of prostaglandin E1 (PGE1) upon orthodontic tooth movement. *Am J Orthod* 1984;85:508–18.
38. Pinzon RD, Kozlov M, Burch WP. Histology of rat molar pulp at different ages. *J Dent Res* 1967;46:202–8.
39. Colby LA, Morenko BJ. Clinical considerations in rodent bioimaging. *Comp Med* 2004;54:623–30.