Lasers in Surgery and Medicine 41:524–533 (2009)

Effects of Low-Level Laser Therapy After Corticision on Tooth Movement and Paradental Remodeling

Su-Jung Kim, DMD, MSD, PhD,[1†] Sung-Uk Moon, DMD, MSD, PhD,[2‡] Seung-Goo Kang, DMD, MSD, PhD,[1] and Young-Guk Park, DMD, MSD, PhD, MBA[1*,§]

[1]*Department of Orthodontics, Oral Biology Research Institute, College of Dentistry, Kyung-Hee University, Seoul, Korea*

[2]*Department of Orthodontics, College of Dentistry, Kyung-Hee University, Seoul, Korea*

Background and Objective: Both Corticision and low-level laser therapy (LLLT) are known to affect the rate of tooth movement. Our objective was to investigate the combined effects of Corticision and LLLT on the tooth movement rate and paradental remodeling in beagles.

Study Design/Materials and Methods: The maxillary second premolars ($n = 24$) of 12 beagles were randomly divided into four groups ($n = 6$ per group) based on the treatment modality: group A, only orthodontic force (control); group B, orthodontic force plus Corticision; group C, orthodontic force plus LLLT; group D, orthodontic force plus Corticision and LLLT.

Results: Ratios of second premolar-to-canine movement were greater by 2.23-fold in group B and 2.08-fold in group C, but 0.52-fold lesser in group D than in group A. The peak velocity was observed at an earlier stage of tooth movement in group B but at a later stage in group C during the 8-week treatment period. At week 8, both tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts on the compression side and proliferating cell nuclear antigen (PCNA)-positive osteoblasts on the tension side increased significantly ($P<.05$) in group C but decreased in group D. Histomorphometric analysis revealed that the mean apposition length of newly formed mineralized bone during the 8 weeks of treatment significantly increased in both group B (2.8-fold) and group C (2.2-fold). In group D, the labeling lines on lamina dura were thin and discontinuous, but intratrabecular remodeling and lamellation were found to be active.

Conclusion: Periodic LLLT after Corticision around a moving tooth decreased the tooth movement rate and alveolar remodeling activity. Lasers Surg. Med. 41:524–533, 2009. © 2009 Wiley-Liss, Inc.

Key words: bone remodeling; laser biostimulation; orthodontic tooth movement; regional acceleratory phenomenon (RAP)

INTRODUCTION

Bone turnover takes place through bone-forming and bone-resorbing phases, defined as anabolic and catabolic modeling, respectively [1]. The turnover rate determines the quantity and quality of orthodontic tooth movement [2], and the cortical lamina dura modeling is consequently restricted [3]. Acceleration of tooth movement has been attempted by using orthodontic force together with feasible modulation of the underlying biological responses without irreversible damage to the teeth and paradental tissues. Previous studies have demonstrated that such a goal is attainable when orthodontic forces are applied on the paradental tissues together with surgical or tissue engineering procedures, minute electric currents, cytokinetic regimens, or miscellaneous physical implements such as magnetic fields, ultrasound, and lasers.

Intentional surgical injury of the cortical bone was first described in 1892 as a surgical approach to correct malocclusion with incisal imbrication but was brought to academic attention by Köle in 1959 [4] and was developed as Corticotomy-Facilitated Orthodontics (CFO) by Suya in 1991 [5]. Selective alveolar decortication by Wilcko and Wilcko [6] has been used to accelerate tooth movement by inducing transient osteopenia, called Accelerated Osteogenic Orthodontics (AOO); the resultant bone response is referred to as the regional acceleratory phenomenon (RAP) [7]. In accelerated tooth movement, the RAP may be a crucial precursor for effective tooth movement.

The cortical bone is a pertinent histological site for orthodontic tooth movement. The RAP is primarily a phenomenon of cortical bone [8]. Lotinun et al. [9] found an active intracortical process by which loss of cortical bone could involve either cortical thinning or increase in cortical porosity. This transition could be applied to continuous advancement of supplemental surgical procedures for minimal, conservative interventions. Kim et al. [10] introduced a procedure named Corticision wherein a reinforced scalpel is used as a thin chisel to separate the interproximal cortices transmucosally, without flap reflection; transmucosal manipulation of alveolar bone minimizes morbidity. Corticision was aimed at inducing the RAP and was found to have an accelerating effect on alveolar remodeling during orthodontic tooth movement.

[†]Assistant professor.
[‡]Graduate student.
[§]Professor.
Contract grant sponsor: Kyung Hee University Program.
*Correspondence to: Young-Guk Park, Department of Orthodontics, School of Dentistry, Kyung-Hee University, 1 Hoegi-Dong, Dongdaemoon-Ku, Seoul 130-701, Korea.
E-mail: ygpark@khu.ac.kr
Accepted 23 May 2009
Published online 28 July 2009 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20792

Low-level laser therapy (LLLT) has been suggested to evoke the RAP through the biostimulatory effects [11–13]. In orthodontics, LLLT could be applied to reduce the postadjustment pain [14] or sore spots caused by impinging orthodontic appliances [15]. The potential benefits to orthodontics following an increased rate of bone formation include shorter healing times for surgical orthognathic cases, reduced retention period after midpalatal expansion, and better stability after orthodontic manipulation [16]. In particular, LLLT may reduce the orthodontic treatment duration through bone remodeling acceleration [17,18].

Investigations have been undertaken to verify the effects of LLLT on bone remodeling under different conditions with different wavelengths and energy densities. With regard to the energy parameters, the effects of LLLT on tooth movement are reportedly controversial (i.e., they are stimulatory, inhibitory, or irrelevant). The effects of LLLT on the rate of tooth movement remain to be studied.

The combined effect of surgical injury and LLLT around a moving tooth on the rate of orthodontic movement has not been elucidated yet. Previous studies have investigated whether LLLT could stimulate synergistically the RAP induced by intentional injury along with orthodontic tooth movement, leading to accelerated tooth movement, or whether LLLT can only accelerate the healing process at the surgical site rather than decreasing the rate of tooth movement. In this study, we aimed to investigate the effects of LLLT with concomitant Corticision on the rate of tooth movement and alveolar remodeling in beagles.

MATERIALS AND METHODS

Animals

Male beagles ($n = 12$), aged 18–24 months and weighing 10–13 kg, were used for the experiments. They were housed in separate cages supplied with a self-wash system, air conditioning, and lighting in agreement with the guidelines on housing of laboratory animals of animal center, Kyung Hee University Medical Center. They were fed crushed commercial dog food with sufficient water, and were examined daily for general health status and oral hygiene.

The maxillary second premolars ($n = 24$) of the 12 dogs were randomly divided into four groups. The split-mouth design was applied to reduce interindividual variation. Each group ($n = 6$ per group) was classified by the treatment modality as follows: group A, orthodontic force only (control); group B, orthodontic force plus Corticision; group C, orthodontic force plus LLLT; and group D, orthodontic force plus Corticision and LLLT.

Experimental Tooth Movement

All extractions were performed under general anesthesia by intramuscular injection of Zoletil 50 (0.25 mg/kg; Virbac Laboratory, Carros, France). The upper first premolars were extracted 4 weeks before each experiment to allow mesial movement of the second premolars. Orthodontic buttons (Ormco Co., Milwaukee, WI) were bonded onto the labial surfaces of the upper canines and second premolars with Superbond C&BR (Sun Medical Co., Shiga, Japan). For mesial movement of the upper second premolars, one end of a nickel–titanium closed coil spring (Tomy International, Inc., Tokyo, Japan) was ligated to the second premolar orthodontic button with a 0.010-i.n. stainless steel ligature wire (Tomy International, Inc.). The other end of the coil spring was ligated to the canine orthodontic button. The orthodontic force exerted by this appliance was 150 gm at the beginning of the experiment (Fig. 1). Tooth movement was performed for 8 weeks. The condition of the appliance, teeth, and gingivae was checked once a week. Force magnitude was measured every week, and reactivated if needed for continuous force application.

Corticision

After local anesthesia with lidocaine (Yuhan Co., Seoul, South Korea) for hemostasis, Corticision was performed on the mesiobuccal, distobuccal, mesiopalatal, and distopalatal sides of the second premolars in groups B and D (Fig. 1A). A reinforced surgical blade (No.15T; Lance Paragon, Sheffield, England) and surgical mallet were employed. Using this blade, a surgical gap of 400-μm minimal thickness and 10-mm maximal depth can be achieved. The blade was positioned on the interradicular attached gingiva with an oblique inclination to the long axis of the second premolars, and was then inserted into the cancellous bone by penetrating the gingiva and cortical bone by striking the mallet on the blade holder. The blade was removed with a swinging motion. Gentamycin (0.1 ml/kg; DaeSung Co., Microbiological Labs, Seoul, South Korea) was injected postoperatively for 3 days.

LLLT

In this experiment, a gallium–aluminum–arsenide (Ga-Al-As) diode laser (SoftLase Pro; Zap Lasers, Pleasant Hill, CA) was used, having a wavelength of 808 nm and a maximum output of 2.0 W. The laser beam was delivered by a 0.4-mm diameter optical fiber. Before application on the experimental animals, the average output power and energy density were measured in the biostimulation mode

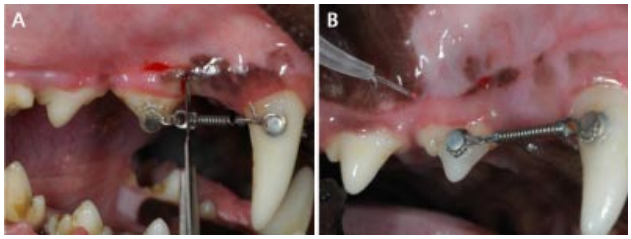

Fig. 1. Photographs of orthodontic appliance for mesial movement of maxillary second premolar. Orthodontic buttons were attached to second premolar and canine, and a NiTi coil spring was activated. **A**: Corticision was performed with PARAGON® blade and blade holder by malleting. **B**: Low-level laser irradiation was done at eight points around experimental tooth. [Figure can be viewed in color online via www.interscience.wiley.com.]

set by manufacturer (10-Hz pulsed wave, noncontact mode) using a laser power detector. The measured average output power was 763 ± 4.66 mW and the measured single pulse energy at the tissue level was 75 mJ. The measured on time during the 20-second irradiation was 9 seconds, because the laser was confirmed to have a 45% duty rate in pulsed wave mode ($20 \times 45\%$). The tip was held perpendicular to the mucosa, 3 mm away from the tissue surface; the measured single pulse energy per second at this distance was 4.63 J/cm^2, considering that the diameter of the irradiated focal spot was 1.75 mm. The calculated average energy density during the 20-second exposure was about 41.7 J/cm^2. On the coronal third and apical third of the mesiobuccal, distobuccal, mesiolingual, and distolingual sides of each experimental tooth, eight irradiations per application were carried out for more homogeneous energy distribution (Fig. 1B). All irradiations were performed by the same operator every 3 days throughout the 8-week experimental period in groups C and D.

Measurement of Tooth Movement

Tooth movement was measured via precisely fabricated stone models (GC Europe, Leuven, Belgium). Alginate (GC Co., Tokyo, Japan) impressions of the maxillary arches were recorded every week for 8 weeks. The distance from the distal cervix of the second premolar to the distal cervix of the first molar was measured with a digital caliper at each time point. The amount of tooth movement was calculated as the difference between the measurements during and at the end of treatment, and the measurement at baseline. In addition to second premolar protraction, the amount of canine retraction, as an anchorage tooth, was also measured relative to the distal cervix of the first molar. The ratio of second premolar protraction to canine retraction was used as the comparator of the intergroup differences in orthodontic tooth movement.

Other methods for fixing the reference point to measure tooth movement, such as microimplant insertion on the bone or notch formation on the tooth structure, were rejected because the former may induce another RAP and the latter may produce measurement error due to the tipping movement of teeth.

Histological Preparation

The animals were sacrificed at 8 weeks following appliance placement by direct injection of Zoletil 50 (50 mg/kg; Virbac Laboratory) into the heart. Tissue blocks of the right and left maxillae were harvested, and immediately fixed and stored in 10% formalin for 48 hours. Each tissue sample including the second premolar was sectioned into halves along the same plane as the applied force. One half of the blocks were decalcified with 10% ethylenediaminetetraacetic acid (EDTA-2Na, pH 7.4) at 4°C for 30 days, and dehydrated in ethyl alcohol of gradually increasing concentrations from 70% to 100%. After paraffin embedding, the samples were sectioned sagittally to a thickness of 6 μm. These sections were stained for immunohistochemical analysis. The other half of the blocks were prepared as nondecalcified specimens for histomorphometric analysis.

Immunohistochemical Analysis

To facilitate identification and quantification of osteoclasts, the tartrate-resistant acid phosphatase (TRAP) histochemical detection method was used on randomly selected decalcified specimens. Acetate buffer solution (0.1 M, pH 5.0) was added to naphthol AS-BI phosphate (Sigma, St. Louis, MO). Fast red–violet LB diazonium salt, 10% $MnCl_2$, and 50-mM L-(+)-tartaric acid were added to the solution, which was then filtered and preheated to 37°C before use. Deparaffinized slides were then incubated in the solution for 60 minutes in an incubator with the temperature adjusted to 37°C. The slides were then washed for 30 minutes under running water, counterstained with hematoxylin for 1 minutes, air-dried at room temperature, and mounted with coverslips. TRAP staining was performed with a dog ABC staining system kit (Sigma) and anti-TRAP antibody. The osteoclasts on the alveolar bone surface at the pressure side were counted in each section. Osteoclasts were defined as TRAP-positive when more than three nuclear cells were observed on the bone surface.

To evaluate cell proliferation at the tension side, immunohistochemical staining with proliferating cell nuclear antigen (PCNA) monoclonal antibody (PC-10; Dako EposPC-10, Glostrup, Denmark) was performed. PCNA staining is reportedly a useful method for evaluating cell-proliferative activity. The deparaffinized slides were then incubated with 3% hydrogen peroxide in distilled water for 5 minutes in an incubator with the temperature adjusted to 37°C. After antigen retrieval, the slides were rinsed in phosphate-buffered saline (PBS) for 5 minutes, and then blocking antibody (normal goat serum) was applied and incubated for 20 minutes at room temperature. Primary antibody was applied for 60 minutes at 4°C, and the slides were rinsed twice for 5 minutes each and incubated with a biotin-conjugated secondary antibody at 20–37°C for 20 minutes. After rinsing again, the slides were incubated with SABC reagent at 37°C for 20 minutes and then washed four times for 5 minutes each. The slides were processed with chromogenic substrate solution (DAB; Dojin Chemicals, Kumamoto, Japan) for 10 minutes and then counterstained with hematoxylin for 1 minute. The nature of staining and distribution of PCNA immunoreactivity were evaluated by scoring the stained cells on the alveolar bone surface at the tension side, in the same area as for the bone histomorphometry. The results were standardized as follows: number of PCNA-positive cells/number of all the stained cells (%).

Histomorphometric Analysis

For alveolar bone labeling, all the dogs were injected intramuscularly with oxytetracycline hydrochloride (Fluka, Shanghai, China; yellow orange, 30 mg/kg) at 24 hours before intervention and 8 weeks after force application. Alizarin red (Fluka, Gillingham, UK; red, 30 mg/kg) was injected at 2 weeks and calcein (Fluka, Buchs, Switzerland; green, 10–15 mg/kg) at 4 weeks after

force application. Half-sectioned tissue blocks were embedded in Technovit 7200 VLC (EXAKT Technologies, Inc., Oklahoma City, OK) via a gradual transition from 100% alcohol after conventional fixing and dehydration. The embedded blocks were trimmed to obtain longitudinal sections and then attached on slides with Technovit 4000. After grinding the blocks with an EXAKT machine 4110, the prepared samples were cut with an EXAKT machine BS-3000N and ground again to a thickness below 60 μm. These specimens were examined under an ultraviolet fluorescent microscope (Olympus BH-2; Olympus Co., Tokyo, Japan) with UB filter ($\lambda = 515$ nm). Microphotographs of all the specimens were recorded by a digital CCD camera (Kappa PS30C) and stored in a computer using the KAPPA Image Base Control 2.5 program. An outline of the labeled bone was traced from the photographs and the distances of newly formed mineralized bone were measured by using image analysis software (KAPPA Image Base Metreo 2.5; KAPPA Opto-electronics; Accusoft Co., Gleichen, Germany). The fluorochrome labeling method included the location, amount, extent, and morphology of ultraviolet fluorescein deposition. Subsequent examination of the slides by an oral pathologist confirmed these evaluations.

Statistical Analyses

Descriptive statistics (mean ± standard deviation) for each parameter were evaluated in all the groups. The data distributions of the accumulated distance of orthodontic tooth movement, and the accumulated amount of new bone deposition at the tension side were satisfied with normality and tested by the Shapiro–Wilk test, according to which intergroup comparison could be made by using one-way ANOVA and Scheffe's post hoc comparison. For intergroup and intragroup comparisons of tooth movement by time period (weekly changes), multivariate analysis by Wilks' lambda with Scheffe's post hoc comparison was used. Both TRAP-positive cells and PCNA-positive cells were counted and analyzed by Kruskal–Wallis test as a nonparametric method, because the number of teeth in each group was too small to be satisfied with normality. A value of $P<0.05$ was considered to indicate a statistically significant difference.

RESULTS

All the 12 dogs remained healthy and had slightly increased body weight throughout the 8-week experimental period. All appliances stayed in place, without breakage or replacement. Teeth were moved by tipping in all the groups at the end of the experimental period.

Tooth Movement

At week 8, compared with that in group A, mesial movement of the second premolars in group B and group C increased by 3.75- and 3.76-fold, respectively (Table 1). The amount of tooth movement did not significantly differ between groups B and C. However, group D showed reduced amounts of second premolar movement compared with group A, without significant difference. Considering the intergroup differences in canine movement, the ratio of second premolar protraction to canine retraction was calculated and evaluated as follows: groups B and C > group A > group D (Fig. 2).

The accumulated distance of tooth movement in each group showed different patterns by time period (Fig. 3). The fastest tooth movement was found in group B at all time points. During the first 4 weeks, only group B showed a significant difference from the other groups. At week 5, the tooth movement in group C began to increase rapidly in comparison with that in groups A and D, and equaled the amount of tooth movement in group B at week 8. Figure 4 illustrates the different timing of peak velocity between groups B and C. The peak velocity of tooth movement in group B was observed at week 2, whereas it was observed in group C at week 7. The velocity in group D decreased during the first 4 weeks and increased thereafter.

Immunohistochemical Analysis

At week 8, catabolic activity, measured by the number of TRAP-positive multinucleated osteoclasts and preosteoclasts lining the alveolar surface on the compression side, increased significantly in group C (11.67 ± 2.08, $P = 0.024$; Figs. 5 and 6). Clear globular-shaped multinuclear osteoclasts were observed in group C (Fig. 5C). Anabolic activity, measured by the ratio of PCNA-positive osteoblasts lining the new bone surface on the tension side, also increased significantly in group C ($45.52 \pm 1.56\%$, $P = 0.020$; Figs. 7

TABLE 1. The Means of Accumulative Distances of Teeth Movement in Each Group at 8 Weeks After Orthodontic Force Application, Tested by One-Way ANOVA With Scheffe's Post Hoc Comparison

	2nd premolar movement (mm)	Canine (anchor tooth) movement (mm)	Ratio of 2nd premolar to canine movement
Control (A)	1.23 ± 0.18 b	0.78 ± 0.10 c	1.59 ± 0.05 b
Corticision (B)	4.61 ± 0.30 a	1.29 ± 0.18 ab	3.54 ± 0.38 a
LLLT (C)	4.62 ± 0.25 a	1.43 ± 0.23 a	3.31 ± 0.58 a
Corticision + LLLT (D)	0.88 ± 0.19 b	1.08 ± 0.11 bc	0.82 ± 0.18 c
P	0.000***	0.000***	0.000***
F	403.36***	13.65***	71.50***

***$P < 0.001$

Scheffe group: a > b > c. The groups indicated with same character showed statistically non-significant difference.

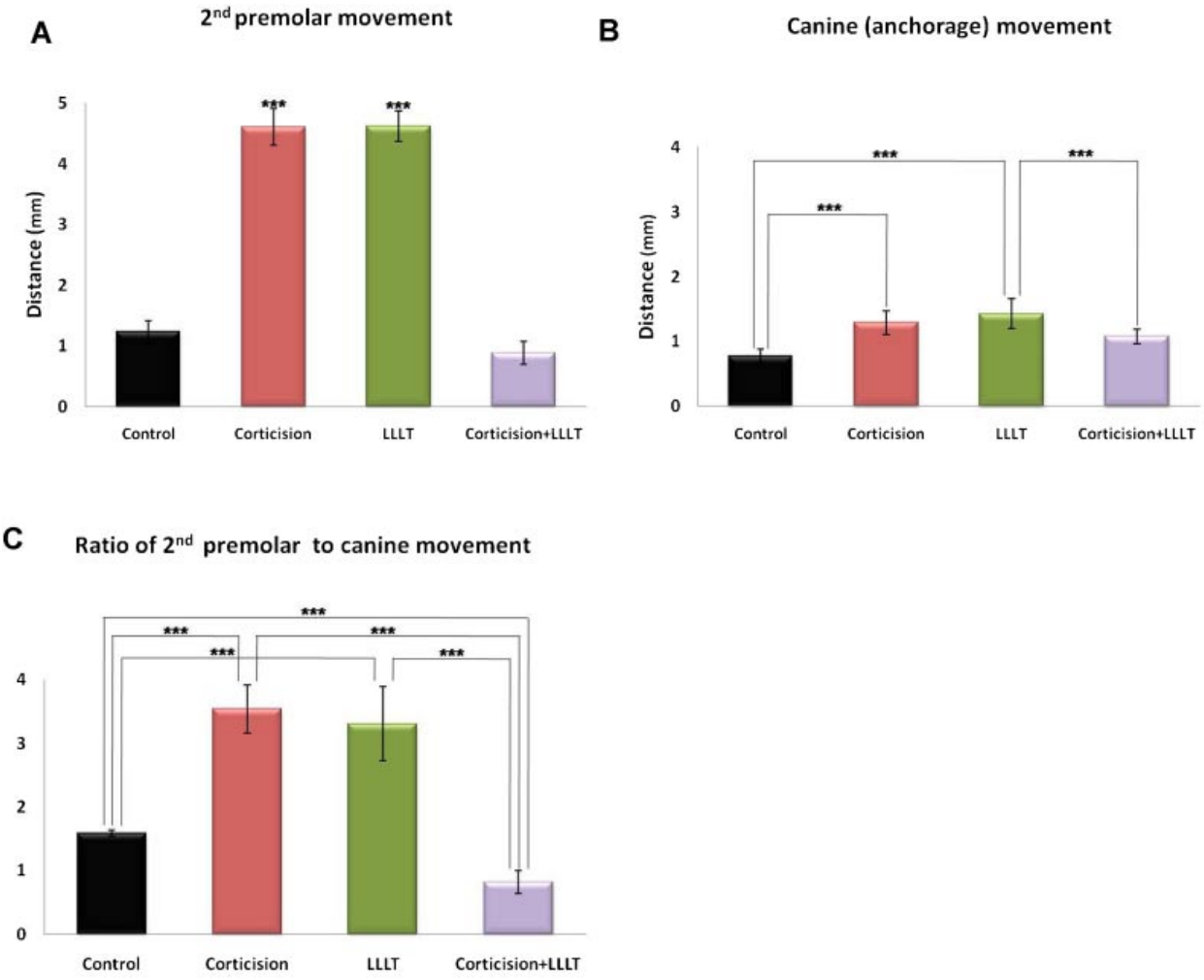

Fig. 2. Graphs of the distances of teeth movement at week 8 in control, Corticision, LLLT, and Corticision plus LLLT groups. **A**: Second premolar protraction. **B**: Canine retraction. **C**: Ratio of second premolar protraction to canine retraction. All results are expressed as the Mean ± SD. Triple asterisks indicate $P<.001$. [Figure can be viewed in color online via www.interscience.wiley.com.]

and 8). Hyperchromatic proliferative PCNA-positive cells were prominent in groups B and C (Fig. 7B,C). Group D showed no significant differences from the control group both in the number of TRAP-positive cells and in the ratio of PCNA-positive cells.

Histomorphometric Analysis

The lamina dura bone apposition was analyzed with fluorescent dyes to mark the activity at weeks 2, 4, and 8 (Fig. 9). The mean accumulated apposition length during 8 weeks between the two labeled lines (first tetracycline line and second tetracycline line) was significantly increased in group B ($347.50 \pm 39.65\,\mu m$) and group C ($270.68 \pm 22.27\,\mu m$) compared with that in group A ($122.71 \pm 54.64\,\mu m$) and group D ($80.42 \pm 21.07\,\mu m$; Fig. 10). The mean apposition length in group B was 2.8-fold and the length in group C was 2.2-fold greater than that in group A. In group B, the greatest bone apposition, indicated by diffuse labeling, occurred around the time of calcein injection at week 4, mainly in the cervical third of the root due to the tipping movement (Fig. 9B). In group C, the mean apposition rate was accelerated between the first tetracycline line and the alizarin red line (0–2 weeks), and between the calcein line and the second tetracycline line (4–8 weeks; Fig. 9C). In group D, there was no remarkable labeling of the lamina dura. The first tetracycline line was resorbed and the alizarin red line appeared broken in some areas, although the two labeling lines were found clearly within the bony trabeculae (Fig. 9D).

DISCUSSION

In the present study, the effects of Corticision and LLLT as supplemental procedures for accelerating orthodontic tooth movement were confirmed clearly. However, the

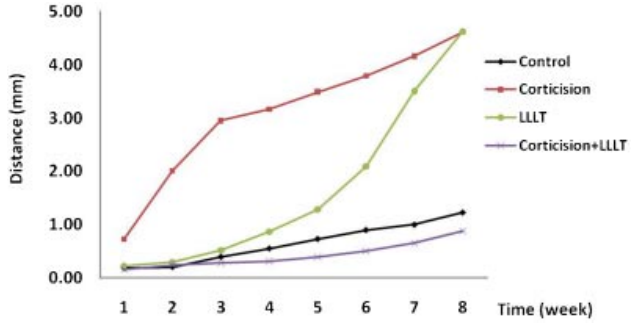

Fig. 3. Graph of the accumulated distance of second premolar movement in each group during 8 weeks. While the tooth movement in Corticision group showed the greatest increase during the first 3 weeks, the movement in LLLT group began to show significant increase at week 5 and catched up the Corticision group at week 8. The increasing pattern and the accumulative distance of tooth movement in Corticision plus LLLT group had no difference from those of control group. Results are expressed as means. [Figure can be viewed in color online via www.interscience.wiley.com.]

combined procedure had more inhibitory effects than the control. The ratios of second premolar-to-canine movement were 2.23-fold greater in the Corticision-treated group and 2.08-fold greater in the LLLT-treated group than in the control group, whereas the ratio in the combined treatment group was 0.52-fold lesser than that in the control group.

In our previous study [10], the accelerating effect of Corticision on alveolar remodeling and tooth movement was elucidated, but restrictively within a 28-day period. The duration of the RAP following Corticision is not sufficient for application throughout orthodontic treatment in a clinical situation. Periodic mobilization (providing repeated microdamage) after Corticision, which was designed to extend the RAP duration by intercepting the lamellation process of woven bone at the injury site, delayed trabecular healing at the Corticision site and could not

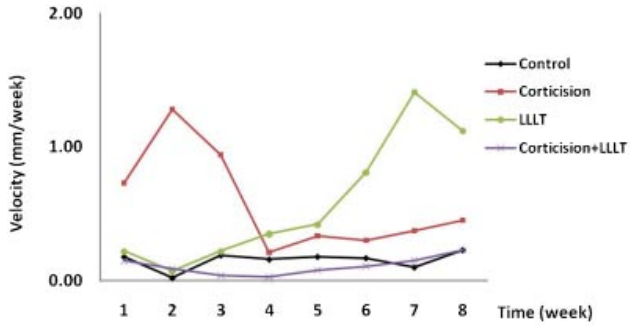

Fig. 4. Graph of the velocity of weekly movement of second premolars in each group. The peak velocity was shown at week 2 in Corticision group, whereas at week 7 in LLLT group. The velocity in Corticision plus LLLT group decreases during the first 4 weeks, and slightly increases thereafter. Results are expressed as means. [Figure can be viewed in color online via www.interscience.wiley.com.]

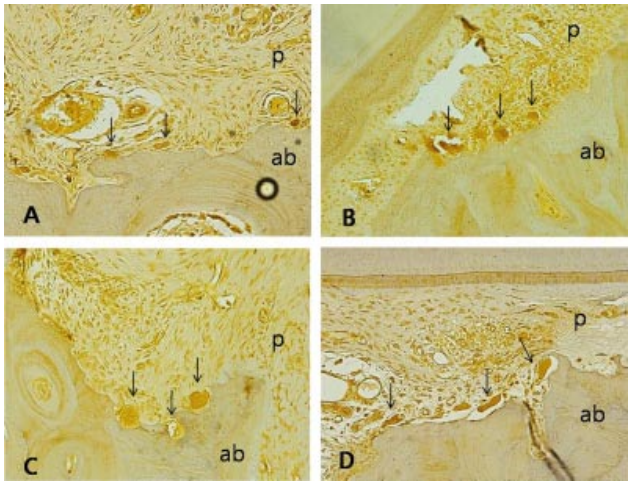

Fig. 5. Effects of Corticision and LLLT on multinucleate osteoclasts at week 8 shown in light microscope images (TRAP immunohistochemical staining, original magnification 200×). Clear globular-shaped multinucleated osteoclasts with resorption lacunae increased in Corticision group (**B**) and LLLT group (**C**). There was little difference in the number of TRAP-positive cells between Corticision plus LLLT group (**D**) and control group (**A**). p, PDL; ab, alveolar bone. Arrows indicate TRAP-positive multinucleated osteoclats. [Figure can be viewed in color online via www.interscience.wiley.com.]

lengthen the duration of accelerated remodeling of the alveolar bone proper surrounding the moving tooth. To improve the RAP intensity and duration following Corticision, we introduced LLLT as another stimulator of the RAP.

Recent studies have demonstrated that low-energy irradiation with Ga-Al-As diode laser affects orthodontic tooth movement in animals or humans. The results from previous studies have indicated that significant biostimulatory effects on tooth movement are induced around the optimal dosage [13,17,19], whereas higher dosages induce bioinhibitory effects [20,21] and lower dosages show non-significant results [22,23].

A study by Kawasaki and Shimizu [17] on rats (830-nm, 100-mW, 9-minute/day irradiation for 13 days at 54 J/cm^2)

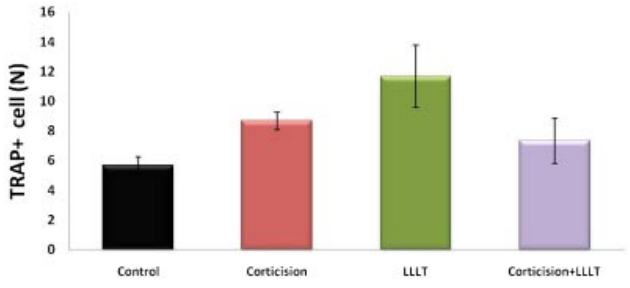

Fig. 6. Graphs of the means of TRAP-positive cell numbers on the compression side at week 8. LLLT group showed the highest increase of the number. [Figure can be viewed in color online via www.interscience.wiley.com.]

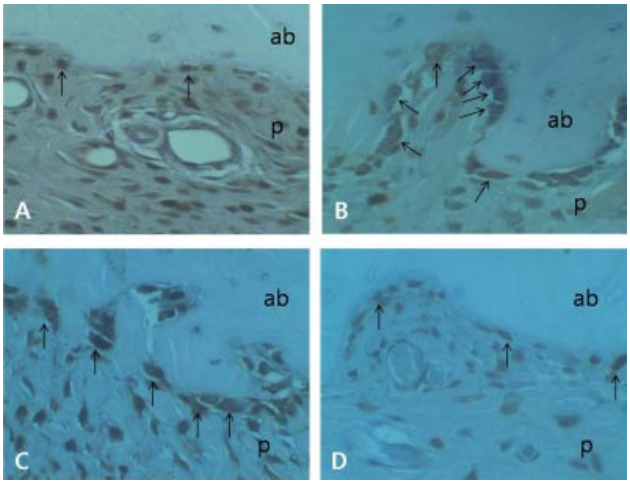

Fig. 7. Effects of Corticision and LLLT on PCNA-positive cells at week 8 shown in light microscope images (PCNA immunohistochemical staining, original magnification 400×). Hyperchromatic proliferative PCNA-positive cells (arrows) increased along the new bone tissue in Corticision group (**B**) and LLLT group (**C**). There was little difference in the number of active PCNA-positive cells between Corticision plus LLLT group (**D**) and control group (**A**). p, PDL; ab, alveolar bone. [Figure can be viewed in color online via www.interscience.wiley.com.]

revealed a 30% increased rate of tooth movement, and Fujita et al. [19] reported 50% increases (following the same protocol as the Kawasaki and Shimizu study). Youssef et al. [13] found that the ratio of human canine retraction in an irradiated group to the control group was 1.98 (809-nm, 100-mW, 40-second/day irradiation at 8 J/cm^2). Goulart et al. [24] suggested that the canine and premolars irradiated at 5.25 J/cm^2 (780-nm, 70-mW, 3-second/day irradiation) show faster orthodontic movements initially, whereas those irradiated at 35 J/cm^2 (780-nm, 70-mW, 20-second/day irradiation) exhibit slower movements. Seifi et al. [20] found that the amounts of tooth movement after LLLT with both pulsed 850-nm laser (Optodan; 5 mW, 3 minutes/day, 8.1 J/cm^2) and continuous 630-nm laser (KLO3; 10 mW, 5 minutes/day, 27 J/cm^2) were diminished.

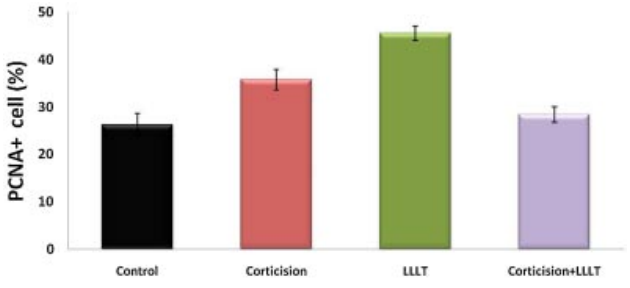

Fig. 8. Graphs of the means of the ratio of PCNA-positive cells on the tension side at week 8. LLLT group showed the highest increase of the ratio. [Figure can be viewed in color online via www.interscience.wiley.com.]

Limpanichkul et al. [25] suggested that the energy density of 25 J/cm^2 (860-nm, 100-mW, 23-second/day irradiation) around a human canine is probably too low to express either stimulatory effects or inhibitory effects on the rate of tooth movement. The results of the present study (808 nm, 763 mW, 20 seconds/day, 41.7 J/cm^2, pulsed wave, noncontact mode) show higher accelerating effects on the experimental movement than in any of the previous studies.

A reasonable energy density (J/cm^2) is necessary to trigger biologic effects, and therefore, low output cannot be fully compensated by longer exposure. Early experiments have confirmed that the dosage follows the so-called Arndt–Schultz law: too small a dose gives no effect; there is a therapeutic window within a certain dose range; and doses over that range are inhibitory. If low doses are applied, only anti-inflammatory events occur, while if high doses are applied, these cells can readapt their organelles to the excessive light; the latter readaptation can intensify the repair response. However, further studies are required to confirm this relationship, from which two different clinical implications may arise: for accelerating the rate of orthodontic tooth movement, the lower dose may be indicated; when faster bone formation after rapid palatal expansion or implant insertion and maturation for tooth anchorage is required, the higher dose may be used instead.

The energy density of 41.7 J/cm^2 used in this study was measured by a power-detector device rather than calculation by a formula, considering that the laser tip was held in noncontact mode and the output style was in pulsed wave mode. We applied a higher dose of energy than in the other studies, because some energy loss was expected during penetration through the overlying tissues because hemoglobin and melanin are strong chromophores of diode laser. Luger et al. [26] used excessive doses for bone fracture healing in rats because they believed that the scattering would reduce the energy level of the laser beam. One study found that ~50% of the diode laser beam penetrates to a depth of 1.0 mm in human and bovine mandibular cortical bone [27]. Consequently, we assumed that a higher dose of laser energy can produce a successful acceleratory effect on tooth movement within the target tissues.

Nevertheless, there is a lack of knowledge regarding the optimal dose for the stimulatory effects in human periodontal tissues. There are inevitable differences in dose-dependent tissue responses between human and animal studies. In addition, some studies have reported the stimulatory effect on human culture cells [28,29]. However, this finding is not applicable to the human periodontium because of some energy density loss during penetration through the soft tissue and bone. Therefore, this experimental limitation should be considered when determining the optimal dose for individual patients.

In this study, Corticision-assisted tooth movement and LLLT-assisted tooth movement showed different patterns with time. Corticision stimulated tooth movement primarily during the first 3 weeks, whereas LLLT increased the rate of tooth movement mainly from 5- to 8-weeks (Fig. 4). This finding is consistent with the significant increase in

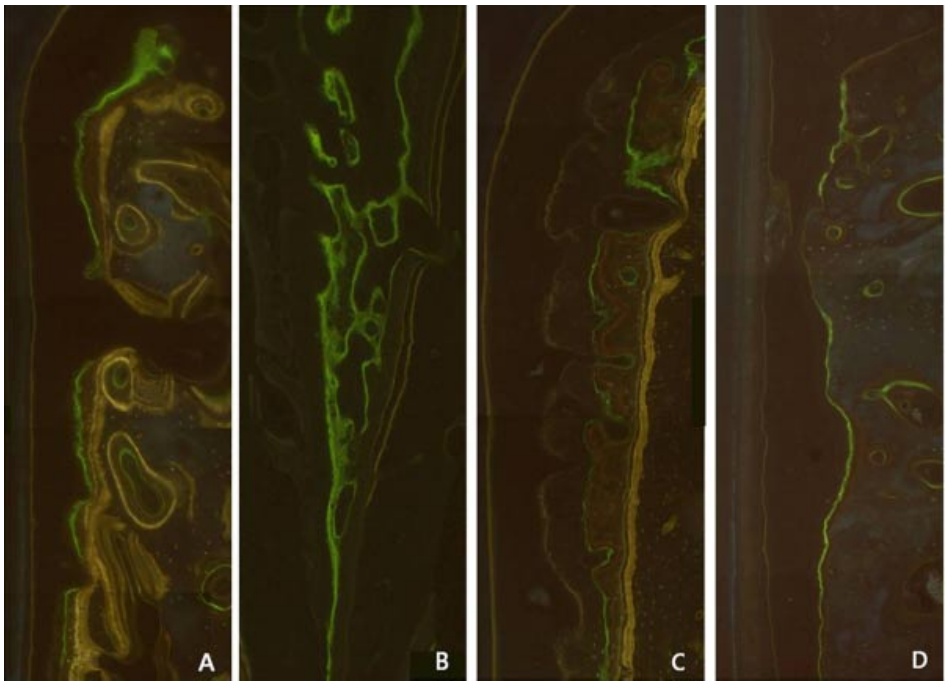

Fig. 9. Fluorescent microphotographs at week 8. **A**: Control group. **B**: Corticision group. **C**: LLLT group. **D**: Corticision plus LLLT group. In group B, the greatest bone apposition occurred around the time of calcein injection at week 4, mainly in the cervical third of root due to the tipping movement. In group C, mean apposition rate was accelerated between the first tetracycline line and the alizarin red line (0–2 weeks), and between the calcein line and the second tetracycline line (4–8 weeks). In group D, there was lesser apposition of lamina dura than the controls (group A). The first tetracycline line had been resorbed and alizarin red line was seen broken in some area, although the two labeling lines were found clearly within the bony trabeculae. [Figure can be viewed in color online via www.interscience.wiley.com.]

TRAP-positive and PCNA-positive cells in group C at week 8. Group C showed no significant increase in tooth movement during the first 4 weeks, which is contrary to the results of most of the previous studies demonstrating the stimulatory effects of LLLT at an early stage of tooth movement by shortening the period of the initial periodontal remodeling cycle. This phenomenon can be explained only by the different dosage. With our laser protocol, the RAP could not be sufficiently evoked until week 5 after intervention, but it persistently affected alveolar remodeling for a longer time. Prolonged and repetitive laser irradiation (every 3 days for 8 weeks, 19 times of irradiation) could be involved in producing these results.

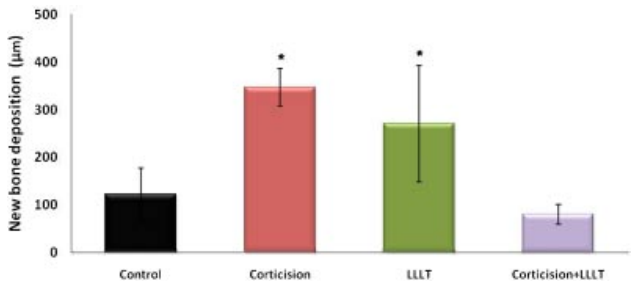

Fig. 10. Mean accumulated distance of new bone deposition indicated by fluorescences on the tension side during 8 weeks, obtained from histomorphometric analysis. Significant increase was found in Corticision group and LLLT group. Single asterisk indicate $P<.05$, tested by Scheffe's post hoc comparison. [Figure can be viewed in color online via www.interscience.wiley.com.]

It is interesting that the combined procedure of Corticision- and LLLT-induced inhibitory effects on the tooth movement, although each procedure itself had accelerating effects. The exact mechanism of this phenomenon could not elucidated. We postulate that LLLT-induced biostimulation was concentrated on accelerating alveolar defect healing at the Corticision site, instead of enhancing the osteoporotic activity induced by the RAP following Corticision, leading to inhibition of tooth movement [30]. LLLT might delay osteoblastic differentiation into osteocytes, allowing them to remain active for a longer duration at the irradiated area, and thus increasing bone tissue formation [31,32]. In the present study, we found that the effect of LLLT on the remodeling activity of healthy alveolus surrounding the moving tooth is different from that of injured alveolar bone surrounding the moving tooth.

LLLT is known to induce increased osteoblastic activity and therefore improve organic matrix formation and mineralization. In irradiated cultures of osteoblasts, the

cellular growth rate and DNA synthesis are increased [33], the mitotic activity and alkaline phosphatase levels are elevated [34], and 10–15% higher deposition of detected fluorescence compared with nonirradiated cultures is observed [28]. In animal studies, elevated osteoblastic activity through alkaline phosphatase levels has been observed in the rat tibia [35], and the newly formed mineralized bone area was found to be increased by 1.7-fold in irradiated rats [17]. The calcium accumulation rate is 50% higher in irradiated rats than controls [36]. Both the osteoblast surface and the mineralization apposition rate are greater in irradiated rats than in controls [37].

The osteoclastic cell behavior to LLLT was not well established in the literature until recently. Laser irradiation induces differentiation and activation of cultured osteoclasts via the expression of receptor activator of nuclear factor-jB (RANK) [29]. Nicolau et al. [36] found that the osteoclast surface increases significantly at 5 days after surgery in animals treated with LLLT. They assumed that two mechanisms were involved: the laser wavelength directly influenced the osteoclasts, and osteoclastic activity may influence posterior osteoblastic activity [38,39]. They concluded that LLLT, used during the inflammatory period of the bone repair process, increases normal cell activity for bone resorption as well as formation.

It is necessary consider the effects of LLLT on the osteoblastic and osteoclastic activities associated with tooth movement, because the speed of tooth movement is greatly dependent on the alveolar turnover rate. Fujita et al. [19] demonstrated that osteoclastic activity is facilitated by the RANK/RANKL system in rat molars. Kawasaki and Shimizu [17] elucidated that the number of TRAP-positive osteoclasts and PCNA-positive cells in irradiated rats increases 1.6- and 1.3-fold, respectively, compared with nonirradiated rats on day 2. Higher numbers of TRAP-positive cells suggest that laser irradiation may stimulate the fusion of mononuclear macrophages to mature osteoclasts at the early stage of cell growth. PCNA protein is a cell cycle-related nuclear protein that is maximally elevated in the late G1 and S phases of proliferating cells. Our results indicate that catabolic activity, measured by the TRAP-positive multinucleated osteoclasts, and anabolic activity, measured by the ratio of the PCNA-positive osteoblasts, increased significantly in the LLLT-treated group but decreased in the combined treatment group. It can be assumed that the number of TRAP-positive and PCNA-positive cells in the Corticision-treated group returned to the baseline levels by 8 weeks.

When studying bone formation on the tension side, the newly formed mineralized bone area was found to be greater by 2.8-fold in the Corticision-treated group and by 2.2-fold in the LLLT-treated group, but inhibited by 0.66-fold in the combined treatment group, compared with the control. Intratrabecular resorption and apposition was shown to be activated in the combined treatment group, without increased lamina dura apposition. This finding is consistent with the assumption that the LLLT-induced RAP after Corticision was involved in the healing process of the surgical defect rather than in the alveolar modeling associated with tooth movement. Understanding the characteristics and limitations of the RAP induced by laser irradiation as well as surgical stimulation is necessary for the different implications in orthodontic clinics, for faster bone formation after rapid palatal expansion or microimplant insertion, for accelerating the rate of orthodontic tooth movement, or for enhancing tooth anchorage.

ACKNOWLEDGMENTS

This study was supported by the Kyung Hee University Program for the Young Researcher of Medical Science (2008).

REFERENCES

1. Roberts WE, Huja S, Roberts JA. Bone modeling: Biomechanics, molecular mechanisms, and clinical perspectives. Semin Orthod 2004;10:123–161.
2. Verna C, Dalstra M, Melsen B. The rate and the type of orthodontic tooth movement is influenced by bone turnover in a rat model. Eur J Orthod 2000;22:343–352.
3. Sebaoun JD, Kantarci A, Turner JW, Carvalho RS, Van Dyke TE, Ferguson DJ. Modeling of trabecular bone and lamina dura following selective alveolar decortication in rats. J Periodontol 2008;79(9):1679–1688.
4. Köle H. Surgical operations on the alveolar ridge to correct occlusal abnormalities. Oral Surg Oral Med Oral Pathol 1959;12:277–288.
5. Suya H. Corticotomy in orthodontics. In: Hosl E, Baldauf A, editors. Mechanical and biological basics in orthodontic therapy. Heidelberg, Germany: Hütlig Buch; 1991. 207–226.
6. Wilcko WM, Wilcko T, Bouquot JE, Ferguson DJ. Rapid orthodontics with alveolar reshaping: Two case reports of decrowding. Int J Periodontics Restorative Dent 2001;21(1): 9–19.
7. Frost HM. The biology of fracture healing. An overview for clinicians. Part I. Clin Orthop Relat Res 1989;248:283–293.
8. Garg AK. The regional acceleratory phenomenon: An up-to-date rationale for bone decortication. Dent Implantol Update 1997;8(8):63–64.
9. Lotinun S, Evans GL, Bronk JT, Bolander ME, Wronski TJ, Ritman EL, Turner RT. Continuous parathyroid hormone induces cortical porosity in the rat: Effects on bone turnover and mechanical properties. J Bone Miner Res 2004;19(7): 1165–1171.
10. Kim SJ, Park YG, Kang SG. Effects of Corticision on paradental remodeling in orthodontic tooth movement. Angle Orthod 2009;79:284–291.
11. Mester E, Mester AF, Mester A. The biomedical effects of laser application. Lasers Surg Med 1985;5:31–39.
12. Abergel RP, Meeker CA, Lam TS, Dwyer RM, Lesavoy MA, Uitto J. Control of connective tissue metabolism by lasers: Recent developments and future prospects. J Am Acad Dermatol 1984;11:1142–1150.
13. Youssef M, Ashkar S, Hamade E, Gutknecht N, Lampert F, Mir M. The effect of low-level laser therapy during orthodontic movement: A preliminary study. Lasers Med Sci 2008; 23:27–33.
14. 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; 108:614–622.
15. Rodrigues MTJ, Ribeiro MS, Groth EB, Zezell DM. Evaluation of effects of laser therapy (l¼ 830 nm) on oral ulceration induced by fixed orthodontic appliances. Lasers Surg Med 2002;30(Suppl 14):15.
16. Coombe AR, Ho C-TG, Philips JR, Chapple CC, Yum LWP, Darendeliler MA, Junter N. The effects of low level laser irradiation on osteoblastic cells. Clin Orthod Res 2001;4:3–14.

17. Kawasaki K, Shimizu N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. Lasers Surg Med 2000;26:282–291.
18. Cruz DR, Kohara EK, Ribeiro MS, Wetter NU. Effects of low-intensity laser therapy on the orthodontic movement velocity of human teeth: A preliminary study. Lasers Surg Med 2004; 35:117–120.
19. Fujita S, Yamaguchi M, Utsunomiya T, Yamamoto H, Kasai K. Low-energy laser stimulates tooth movement velocity via expression of RANK and RANKL. Orthod Craniofac Res 2008;11:143–155.
20. Seifi M, Shafeei HA, Daneshdoost S, Mir M. Effects of two types of low-level laser wave leghths (850 and 630 nm) on the orthodontic tooth movements in rabbits. Lasers Med Sci 2007;22:261–264.
21. Shimizu N, Yamaguchi M, Goseki T, Shibata Y, Takiguchi H, Iwasawa T, Abiko Y. Inhibition of prostaglandin E2 and interleukin 1-beta production by low-power laser irradiation in stretched human periodontal ligament cells. J Dent Res 1995;74:1382–1388.
22. Lagan KM, Clements BA, McDonough S, Baxter GD. Low intensity laser therapy (830 nm) in the management of minor postsurgical wounds: A controlled clinical study. Lasers Surg Med 2001;28:27–32.
23. Marcel MH, Frank AM, Kuijpers-Jagtman AM, Maltha JC. Effect of low level laser therapy on wound healing after palatal surgery in Beagle dogs. Lasers Surg Med 1991;11: 462–470.
24. Goulart CS, Nouer PRA, Mouramartins R, Garbin IU, Lizarelli RZ. Photoradiation and orthodontic movement: Experimental study with canines. Photomed Laser Surg 2006;24(6):192–196.
25. Limpanichkul W, Godfrey K, Srisuk N, Rattanayatikul C. Effects of low-level laser therapy on the rate of orthodontic tooth movement. Orthod Craniofac Res 2006;9: 38–43.
26. Luger EJ, Rochkind S, Wollman Y, Kogan G, Dekel S. Effect of low-power laser irradiation on the mechanical properties of bone fracture healing in rats. Lasers Surg Med 1998;22: 97–102.
27. Yamagishi H, Shinohara C, Saito S, Sasaki H, Kanegae H, Shibasaki Y. A basic study on the use of semiconductor laser of penetrative sensitivity on living tissue. J Jpn Soc Laser Dent 1994;5:13–22.
28. Dörtbudak O, Haas R, Mailath-Pokorny G. Biostimulation of bone marrow cells with a diode soft laser. Clin Oral Implants Res 2000;11:540–545.
29. Aihara N, Yamaguchi M, Kasai K. Low-energy irradiation stimulates formation of osteoclast-like cells via RANK expression in vitro. Lasers Med Sci 2006;21:24–33.
30. Lizarelli RFZ, Lamano-Carvalho TL, Brentegani LG. Histometrical evaluation of the healing of the dental alveolus in rats after irradiation with a low-powered GaAsAl laser. Proc Laser Dent 1999;49–56.
31. Garavello-Freitas I, Baranauskas V, Joazeiro PP, Padovani CR, Dal Pai-Silva M, da Cruz-Höfling MA. Low-power laser irradiation improves histomorphometrical parameters and bone matrix organization during tibia wound healing in rats. J Photochem Photobiol B 2003;70:81–89.
32. Guzzardella GA, Fini M, Torricelli P, Giavaresi G, Giardino R. Laser stimulation on bone defect healing: An in vitro study. Lasers Med Sci 2002;17:216–220.
33. Yamada K. Biological effects of low power laser irradiation on clonal osteoblastic cells (MC3T3-E1). J Jpn Orthop Assoc 1991;65:787–799.
34. Barushka O, Yaakobi T, Oron U. Effect of low energy laser (HeNe) irradiation on the process of bone repair in the rat tibia. Bone 1995;16:47–55.
35. Yaakobi T, Maltz L, Oron U. Promotion of bone repair in the cortical bone of the tibia in rats by low energy laser (HeNe) irradiation. Calcif Tissue Int 1996;59:297–300.
36. Nicolau RA, Jorgetti V, Rigau J, Pacheco M, Reis LM, Zngaro RA. Effect of low-power GaAlAs laser (660 nm) on bone structure and cell activity: An experimental animal study. Lasers Med Sci 2003;18:89–94.
37. Zaidi M, Pazianas M, Shankar VS, Bax BE, Bax CM, Bevis PJ, Stevens C, Huang CL, Blake DR, Moonga BS. Osteoclast function and its control. Exp Physiol 1993;78:721–739.
38. Hentunen TA, Cunningham NS, Vuolteenaho O, Reddi AH, Väänänen HK. Osteoclast recruiting activity in bone matrix. Bone Miner 1994;25:183–198.
39. Jang DS, Rhee BT. A histologic study on the effect of laser on the periodontium of the orthodontically moved tooth of rat. Korean J Orthod 1988;18(2):435–443.