High-Frequency Pulsed Low-Level Diode Laser Therapy Accelerates Wound Healing of Tooth Extraction Socket: An *In Vivo* Study

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Background and Objective: This study aimed to evaluate the effects of high-frequency pulsed (HiFP) low-level laser therapy (LLLT) on early wound healing of tooth extraction sockets in rats.

Study Design/Materials and Methods: Bilateral maxillary first molars were extracted from 6-week-old Sprague-Dawley rats. Sockets on the right were treated by HiFP low-level diode laser irradiation (904–910 nm); the left sides served as unirradiated controls. LLLT (0.28 W, 30 kHz, 200-ns pulse, 0.6% duty cycle, 61.2 J/cm^2 total power density) was employed immediately after extraction and every 24 hours thereafter. The maxillae including the sockets were resected 3 or 7 days after extraction. Soft-tissue healing was evaluated on days 0, 3, and 7. The bone mineral content (BMC), bone volume (BV), and bone mineral density (BMD) of the extraction sockets were evaluated by microcomputed tomography, and histomorphometric analysis was carried out on day 7. Real-time PCR analysis of osteogenic marker expression and immunohistochemical detection of proliferating cell nuclear antigen (PCNA)-positive cells were performed on day 3.

Results: Compared with control sites, the un-epithelialized areas of the extracted sites were significantly reduced by irradiation ($P = 0.04$), and the BMC, BV, and BMD of laser-treated sites were significantly increased ($P = 0.004$, 0.006, and 0.009, respectively). On day 7, the mean height of newly formed immature woven bone was higher in laser-treated sites ($P = 0.24$). On day 3, laser-treated sites showed significantly higher *osteocalcin* mRNA expression ($P = 0.04$) and PCNA-positive cell numbers ($P = 0.01$).

Conclusion: HiFP low-level diode laser irradiation enhanced soft- and hard-tissue healing of tooth extraction sockets. Lasers Surg. Med. © 2016 Wiley Periodicals, Inc.

Key words: bone mineral density; epithelialization; LLLT; new bone formation; osteogenic markers; PCNA

INTRODUCTION

Wound healing of tooth extraction sockets involves both soft and hard tissues, i.e., wound closure and bone formation and remodeling [1–3]. Low-level laser/light therapy (LLLT) has been applied in the dental clinic with the expectation that laser/light bio-stimulation would reduce inflammation [4], pain [5,6], and edema [7] and promote wound healing [8] and tissue regeneration [9]. Unlike high-level laser therapy (HLLT), LLLT exhibits no ablative or thermal effects [10–12].

Among several laser systems, diode lasers have been effectively applied for LLLT to improve wound healing in various animal and human studies [13,14] because this wavelength penetrates deep into tissues. Previous *in vivo* studies reported that irradiation with a low-level 830-nm diode laser stimulated bone regeneration in the midpalatal suture during rapid palatal expansion [15] and induced favorable bone repair in tibiae by up-regulating osteogenic gene expression [16,17]. Furthermore, 904- and 660-nm diode laser irradiation affected bone healing [18] and increased bone cell activity [19], respectively. Diode lasers

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has also been applied to promote bone formation in tooth extraction sockets [20–22].

Recently, a high-frequency pulsed (HiFP) low-level diode laser with a high peak power was developed to provide energy and stimulate cells in deep tissue layers more effectively than conventional continuous or pulsed diode lasers. Previous studies reported that HiFP low-level diode laser irradiation prevented inflammation and reduced pain after tooth extraction [10,11] and facilitated proliferation and migration of human gingival epithelial cells [23]. Irradiation with a HiFP low-level diode laser also induced expression of osteogenic markers and increased calcium deposits in human osteoblast-like cells [24]. However, there are few studies regarding the effects of LLLT with HiFP low-level diode lasers on soft and hard tissue healing in tooth extraction sockets. Therefore, in this study we investigated the effects of HiFP low-level diode laser on early wound healing of both soft and hard tissues in rat tooth extraction sockets.

MATERIALS AND METHODS

Animals

Six-week-old male Sprague-Dawley rats ($n = 27$) were used in this study. The animals were provided with diet and water *ad libitum* in a 12 hour light/dark cycling environment at 23 °C. All experiments were performed in accordance with protocols approved by the ethical committee of the Center for Experimental Animals of Tokyo Medical and Dental University (no. 0160156C).

Surgical Procedures

The animals were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection. Bilateral maxillary first molars were carefully extracted following root separation by using a dental excavator, explorer, and plier. Fifteen minutes after extraction, the tooth extraction sockets had achieved hemostasis. Subsequently, the sockets on the right side were subjected to LLLT, and those on the left side served as unirradiated controls. The treatments were performed by two operators (A.A. and K.M.).

Laser Apparatus, Settings, and Applications

We used a HiFP low-level diode laser (Lumix 2™ HFPL, Fisioline s.r.l., Verduno, Cuneo, Italy), which emits a main wavelength of 904–910 nm (invisible) in pulsed mode (peak-power: 45 W, maximum pulse repetition pulse: 50 kHz, pulse duration: 200 ns) and a secondary wavelength of 650 nm (red) in continuous mode. The irradiation parameters determined by pilot studies were as follows: 30 kHz, 0.20 W, and a duty cycle of 0.6% for the main 904–910-nm laser, and 0.08 W for the secondary 650-nm laser. Energy output was monitored using a power meter (NOVA II, Ophir Optronics Solutions Ltd., Jerusalem, Israel). Irradiation was employed without a focusing lens for 1 minute at a distance of 5 mm above tooth extraction sockets (5.9-mm spot diameter), and the unirradiated site was protected using a silicon cover during the irradiation. Under these irradiation conditions, the power densities were 0.73 and 0.29 W/cm^2 for the main and secondary lasers, respectively, and the calculated total power densities (fluence) were 43.8 and 17.4 J/cm^2, respectively. Thus, the summed power density was 1.02 W/cm^2, and the summed total power density (fluence) was 61.2 J/cm^2. The first irradiation treatment was conducted approximately 15 minutes after tooth extraction, and treatment was repeated every 24 hours. Irradiation procedures were performed by two operators (A.A. and K.M.). Thirteen rats were euthanized for microcomputed tomography (micro-CT) and histological analyses on day 7 (received five LLLT sessions), and 14 rats (11 for gene expression analysis and 3 for immunohistochemistry analysis) were euthanized on day 3 (received three LLLT sessions) (Fig. 1). Post-surgical irradiation was carried out under general anesthesia with isoflurane.

Wound Closure Analysis

To evaluate soft tissue healing, photographs of tooth extraction sockets in 13 rats were taken immediately after tooth extraction and on days 3 and 7 by one examiner (M.N.) at fixed magnification (×1.7) from approximately 60° to the upper occlusal plane with the mouth opened maximally using a mouth gag (M-135T, Inami, Tokyo, Japan). Un-epithelialized areas of the extraction sockets were measured from the digital intra-oral photographs using ImageJ (NIH, Bethesda, MD). Measurements were independently made by two blinded examiners (A.A. and K.M.), and the averaged data were analyzed.

Micro-CT Analysis

To evaluate hard tissue healing, 13 rats were euthanized on day 7 post-extraction. Maxillae including the tooth extraction sockets were resected and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4 °C for 3 days. Micro-CT analysis was employed using a micro-focus X-ray CT system (inspeXio SMX-100CT, Shimadzu Science, Tokyo, Japan) with the conditions of 80 kV, 70 μA, and 12 μm voxel size. A calibration phantom was simultaneously scanned to obtain a spatial distribution of bone mineral density. The extraction socket of the distopalatal root of the first molar was selected for analysis. Three parameters, bone mineral content (BMC), bone volume (BV), and bone mineral density (BMD = BMC/BV), were measured by 3D bone morphometric software (TRI/3D-BON, RATOC System Engineering, Tokyo, Japan). Within the extraction socket, the volume of interest (VOI) was determined as a square column ($0.39 \times 0.42 \times 1.40$ mm^3; 0.229 mm^3) excluding the original alveolar bone wall of the extraction socket. To analyze BMC, BV, and BMD, the VOI within the extraction sockets was positioned independently by two blinded examiners (A.A. and K.M.), and the averaged data were analyzed for each parameter.

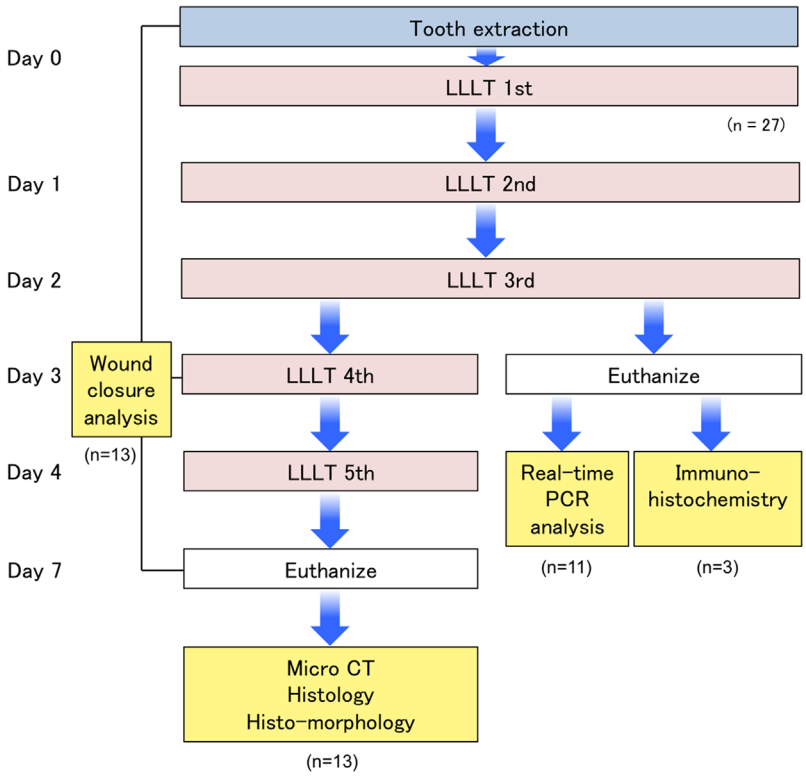

Fig. 1. Study design.

Histological and Histomorphometric Analyses

Maxillae including the extraction sockets were decalcified in 10% EDTA for 4 weeks at 4 °C and then embedded in paraffin. Serial mesiodistal sections with 5-μm thickness were stained with hematoxylin and eosin (HE) and examined under a light microscope. For histomorphometric analysis, the mean height of newly formed bone was evaluated in the specimens collected 7 days after extraction. The perpendicular distance from the baseline passing the lower margin of the maxillary lamella to the highest point of the alveolar septum of the second molar (D1) and the distance from the baseline to the lowest point on the newly formed bone crest in the extraction socket of the distopalatal root (D2) were measured by two blinded examiners (A.A. and K.M.), the data were averaged, and the ratio D2/D1 was calculated (Fig. 2) [25].

Analyses of Osteogenic Gene Expression

On day 3 after the extraction, the maxillae of 11 animals were dissected and trimmed by a diamond disk. Biopsies were performed by one examiner (M.N.). The specimens were immersed in RNAlater (Qiagen Inc., Valencia, CA), the gingiva surrounding the sockets was removed, and the samples were ultrasonically homogenized. Total RNA was isolated using an RNeasy Fibrous Tissue Mini Kit (Qiagen Inc.) with Rnase-Free DNase (Qiagen Inc.). cDNA was synthesized using Super script III (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The expression of osteogenic genes including *collagen type 1* (*Col-1*), *alkaline phosphatase* (*Alp*), *runt-related transcription factor 2* (*Runx2*), *osteocalcin* (*Ocn*), and *bone*

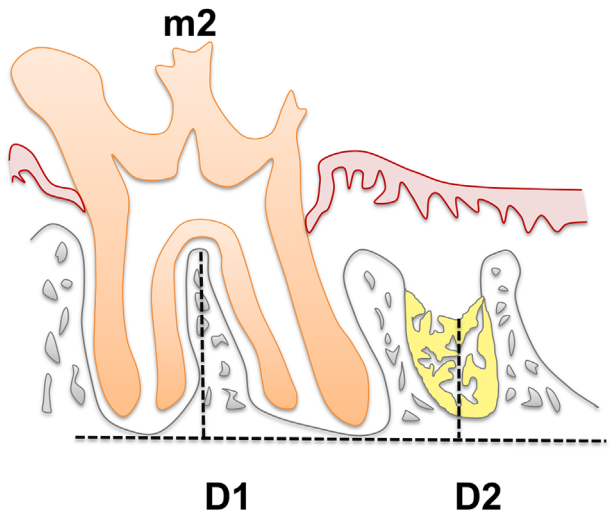

Fig. 2. Histomorphometric analysis of the height of newly formed bone in tooth extraction sockets on day 7 after extraction. The distance (D1) from the baseline passing to the highest point of alveolar septum of the upper second molar (m2) and the distance (D2) from the baseline to the lowest point on the newly formed bone crest (dotted line) in the extraction socket of the distopalatal root were measured, and D2/D1 was calculated.

morphogenetic protein 2 (*Bmp-2*) was analyzed. The primer sequences for real-time PCR are listed in Table 1. Real-time PCR was conducted using SYBR Premix Ex Taq II (TaKaRa Bio, Shiga, Japan) and a Thermal Cycler Dice Real Time System II (TaKaRa Bio). The genes/*Gapdh* values were calculated, and the value of control sites was set to 1.

Immunohistochemistry Analysis

The histological sections of three rats on day 3 after the extraction were deparaffinized with xylene and ethanol and rehydrated. The sections were soaked in 0.01 M citric acid buffer and heated at 105 °C for 5 minutes using an autoclave. After rinsing in PBS-T (PBS containing 0.05% Tween 20), the sections were immersed in methanol containing 1% hydrogen peroxide for 10 minutes. After additional rinsing, the sections were incubated for 1 hour at 37 °C with horseradish peroxidase-labeled anti-proliferating cell nuclear antigen (PCNA) antibody (U7032, DAKO, Tokyo, Japan). Sections were treated with diaminobenzidin to reveal any reaction. For all samples, hematoxylin was used for nuclear staining. Cells were counted by two blinded examiners (A.A. and K.M.), and the cell counts were averaged. The number of PCNA-positive cells per unit area (mm^2) and the ratio of PCNA-positive cells to all cells, excluding the cells of the original alveolar bone, in the three micrographs (original magnification ×200) of the central region of the coronal, middle, and apical thirds of each socket were quantified and averaged for each socket using ImageJ.

Statistical Analysis

The data from wound closure, micro-CT, new bone height, real-time PCR, and PCNA-positive cell count analyses were subjected to the paired *t*-test. Values of $P < 0.05$ were considered statistically significant.

Regarding the reproducibility of the data measured by two examiners (A.A. and K.M.), the high stability were confirmed in the selected cases ($n = 5$ or 10) of each parameter (un-epithelialized area of extraction socket; BMC, BV, and BMD; distance of D1 and D2; PCNA-positive and PCNA-negative cell numbers) on two separate occasions by calculating Pearson correlation coefficients (intra-examiner reproducibility: $P < 0.0001$, $r = 0.95$–0.99 for A.A. and $P < 0.0001$–0.0007, $r = 0.85$–0.99 for K.M.; inter-examiner reproducibility: $P < 0.0001$–0.0004, $r = 0.87$–0.99). In the final data, the inter-examiner reproducibility of the results was evaluated.

All statistical analyses were carried out in StatView (ver. 5.0, SAS Co. Ltd., Cary, NC).

RESULTS

Wound Closure Analysis

The gross pathology of extraction sockets revealed faster wound closure on laser-treated sites compared with control sites (Fig. 3A). Immediately after extraction, blood clot formation was observed in the sockets for both laser-treated and control sites, and the average wound areas were similar ($P = 0.98$). On day 3 after extraction, the un-epithelialized areas of the sockets in laser-treated sites tended to be smaller than those in control sites ($P = 0.30$). On day 7, the un-epithelialized areas in laser-treated sites were significantly reduced compared with those in control sites ($P = 0.04$) (Fig. 3B), suggesting that LLLT accelerated soft tissue healing of the extraction sockets. The Pearson correlation coefficients confirmed the stability of inter-examiner measurements (day 0: $P < 0.0001$, $r = 0.93$; day 3: $P < 0.0001$, $r = 0.97$; day 7: $P < 0.0001$, $r = 0.97$).

Micro-CT Analysis

One rat was excluded from the analysis because the root tip remained in the socket after extraction. On day 7, some of the 3D images of laser-treated sites showed increased bone formation compared with those of control sites (Fig. 4). The laser-treated sites showed significantly higher mean BMC ($P = 0.004$), BV ($P = 0.006$), and BMD ($P = 0.009$) than the control sites ($n = 12$) (Fig. 5), indicating that LLLT promoted bone formation. The Pearson correlation coefficients confirmed the stability of inter-examiner measurements (BMC: $P < 0.0001$, $r = 0.95$; BV: $P < 0.0001$, $r = 0.96$; BMD: $P < 0.0001$, $r = 0.85$).

Histological and Histomorphometric Analyses

Histological observations on day 7 post-extraction revealed no anomaly in any specimen. The newly formed immature woven bone extended from the bottom of the socket to approximately 30–80% of the socket height, and anatomically no structural differences of bone trabeculae in the new woven bone tissue were noted between laser-treated and control sites (Fig. 6A–D). Histomorphometric analysis showed that D2/D1 was greater in laser-treated sites than in control sites in 7 out of the 12 rats, and the

TABLE 1. Primers for Real-Time PCR

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
Ocn	GCATTCTGCCTCTCTGACCT	CTAAACGGTGGTGCCATAGA
Runx2	CACCCTCAAGAGCCTGAGTC	CAGACGGCTGAGTAGGGAAC
Col-1	TCACCTACAGCACGCTTG	GGTCTGTTTCCAGGGTTGCAT
Bmp-2	CGGAAGCGTCTTAAGTCCAG	GCCTTAGGGATTTTGGAT
Alp	CGAGCAGGAACAGAAGTTTGC	GGCCAAAAGGCAGTGAATAG
Gapdh	AGGACCAGGTTGTCTCCTGT	TTACTCCTTGGAGGCCATGT

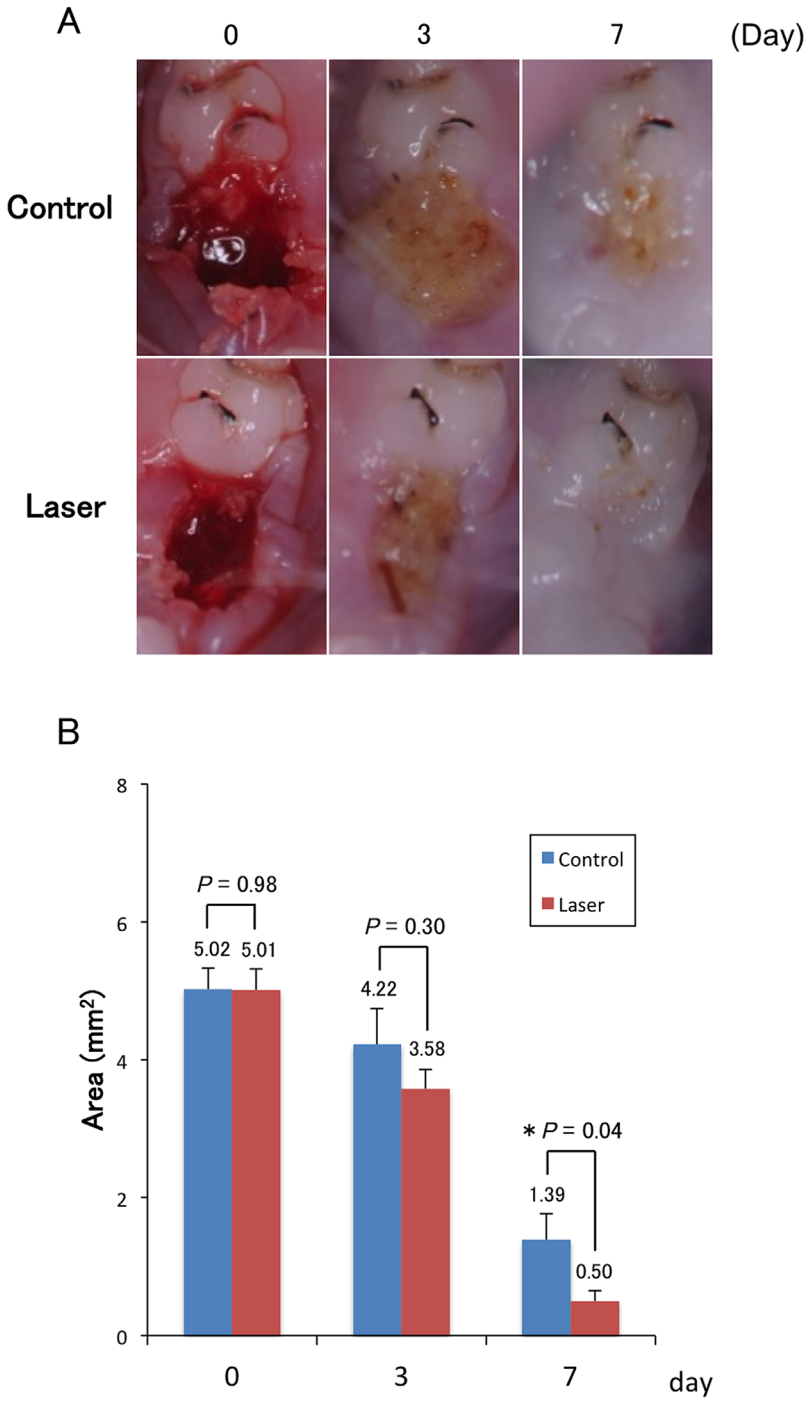

Fig. 3. Gross pathology of the high-frequency pulsed low-level laser-irradiated and unirradiated control tooth extraction sockets on days 0, 3, and 7 after extraction (**A**) and measurement of un-epithelialized areas in the extraction sites (**B**). Advanced epithelialization was clearly observed on the irradiated extraction sockets via microscopic observation. Likewise, immediately after extraction (day 0), un-epithelialized areas of the sockets were equal between laser and control sites ($P = 0.98$). On day 3 after extraction, un-epithelialized areas of sockets in laser-treated sites were generally smaller than those in control sites ($P = 0.30$). On day 7, images revealed faster wound closure in laser-treated sites than in control sites, and the areas in laser-treated sites were significantly smaller than those in control sites ($P = 0.04$). Data are presented as mean ± SE ($n = 13$). $^{*}P < 0.05$ compared with control sites (paired t-test).

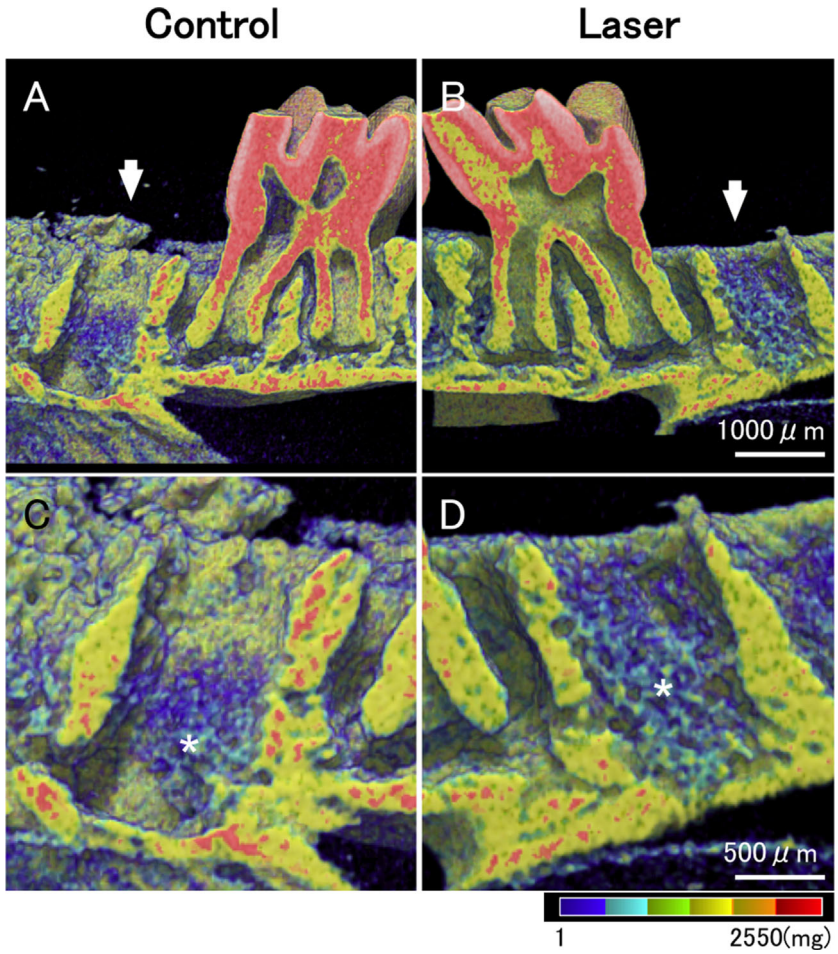

Fig. 4. Representative 3D micro-CT images constructed by bone morphometric software in high-frequency pulsed low-level laser-irradiated sites (**B**, **D**) and unirradiated control sites (**A**, **C**) on day 7 after tooth extraction. Arrows: the distopalatal root of the upper first molar. The color bar corresponds to bone mineral density. Astriek (*): depicked newly formed bone. The laser-irradiated socket shows higher density of new bone formation than the unirradiated control.

mean value of the new bone height was greater in laser-treated sites than in control sites ($P = 0.24$) (Fig. 6E). The Pearson correlation coefficients confirmed the stability of inter-examiner measurements (D1: $P < 0.0001$, $r = 0.99$; D2: $P < 0.0001$, $r = 0.93$).

Osteogenic Gene Expression

On day 3, the mRNA expression of *Ocn* in laser-treated sites was significantly higher than that in control sites ($P = 0.04$). The same trend was seen for *Runx2* and *Alp* expressions in laser-treated sites ($P = 0.08$ each). *Col-1* and *Bmp-2* expressions showed no significant difference between laser-treated and control sites (Fig. 7).

Immunohistochemistry

On day 3 after tooth extraction, PCNA-positive cells were more abundant in the granulation tissue of laser-treated sockets than in control sites (Fig. 8A and B). The number of PCNA-positive cells was significantly higher in laser-treated sites ($P = 0.01$), and the ratio of PCNA-positive cells to all cells was generally higher in laser-treated sites ($P = 0.06$) than in control sites (Fig. 8C and D). The Pearson correlation coefficient confirmed the stability of inter-examiner measurements (PCNA-positive cell number: $P < 0.0001$, $r = 0.99$; PCNA-negative cell number: $P < 0.0001$, $r = 0.99$).

DISCUSSION

Recently, LLLT has been successfully applied to promote tissue wound healing in the fields of medicine and dentistry [26–28]. Mester and Tota [29] originally reported the acceleration of wound healing by LLLT in rats. LLLT can promote the release of growth factors, such as vascular endothelial growth factor [30]. A systematic review of *in vivo* and clinical studies with meta-analysis by Woodruff et al. [8] demonstrated that LLLT is an effective tool for improving wound tensile strength and reducing wound size and healing time.

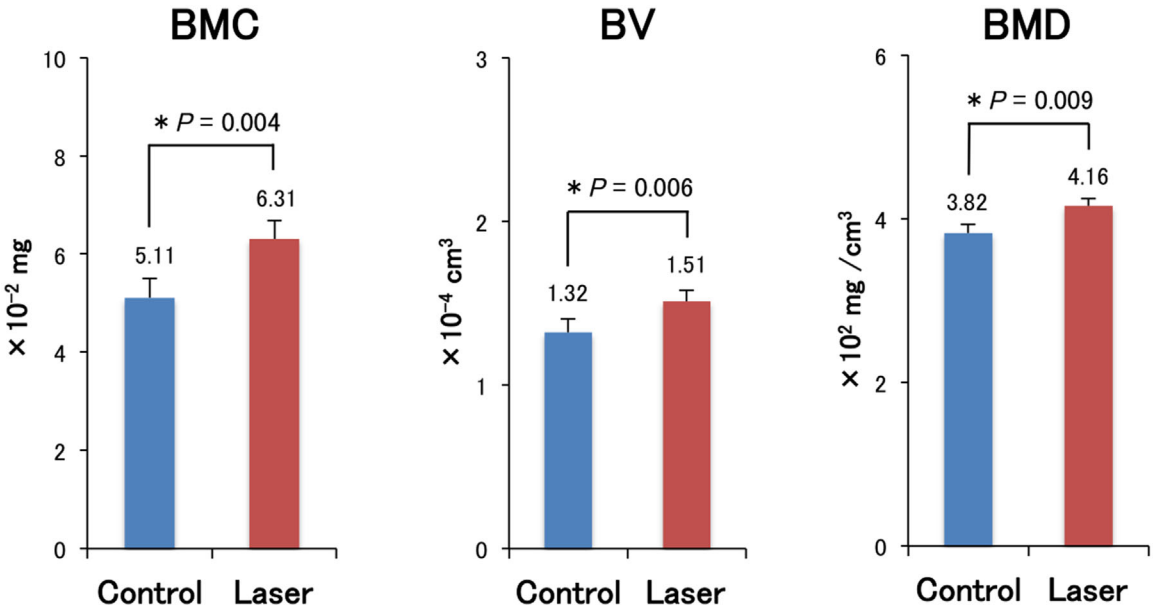

Fig. 5. Bone morphometric analysis of bone mineral content (BMC), bone volumes (BV), and bone mineral density (BMD) using micro-CT in tooth extraction sockets in laser-treated sites and unirradiated control sites on day 7 after extraction. LLLT significantly improved both the quality and quantity of newly formed bone in laser sites compared with control sites. Data are presented as mean ± SE ($n = 12$). $^{*}P < 0.05$ compared with the control sites (paired t-test).

Wound healing of tooth extraction sockets is a complex process involving a variety of events such as fibrin clot formation, infiltration of neutrophils and platelets, blood vessel re-population, epithelialization, and bone formation [3]. In this study, we evaluated epithelialization following LLLT using digital image processing. Our results indicated significantly faster epithelialization in laser-treated sites than unirradiated controls. Since impaired epithelialization or wound closure of tooth extraction sockets increases the occurrence of complications such as dry socket and reduced alveolar bone ridge, LLLT may be advantageous for reducing such complications in dental practice. Further analyses are required to examine the effects of LLLT on soft tissue wound healing in a clinical setting.

To analyze bone formation in tooth extraction sockets, 2D assessments such as radiography and histology have been conventionally used. In this study, we utilized micro-CT for 3D quantitative analysis and demonstrated that BMC, BV, and BMD were significantly higher in laser-treated sites than in unirradiated controls. In histomorphometric analysis, the laser sites showed slightly increased new bone height compared with unirradiated sites. These results suggest that 3D analysis of the tooth extraction socket could more objectively and precisely detect the early phase of bone formation, i.e., mineralization in the healing of tooth extraction socket, compared to histological analysis. In the present study, we demonstrated that HiFP low-level diode irradiation promoted both soft and hard tissues healing in the tooth extraction sockets. We discuss three factors in the LLLT-induced healing processes below.

First, we observed faster epithelialization in laser-treated sites. This finding is consistent with our previous report that HiFP diode laser irradiation enhanced proliferation and migration of human epithelial cells, activating mitogen-activated protein kinase/extracellular single-regulated protein kinase [23]. We speculate that this accelerated epithelialization could enhance stabilization of the blood clot, granulation tissue, and subsequent connective tissue and bone formation within the tooth extraction socket.

Second, our results demonstrate that PCNA-positive cells increased, and one of the osteogenic markers, *Ocn*, was more highly expressed in the granulation tissue within the laser-treated extraction sockets on day 3. *Ocn* is a non-collagenous bone matrix proteins, also known as bone Gla protein, that is specifically secreted by osteoblasts and plays an important role in osteogenesis [31]. In addition, *Ocn* synthesis is promoted by *Runx2*, an osteoblast-specific transcription factor associated with osteoblastic differentiation. In the present study, we also noted a tendency for up-regulation of *Runx2* expression by LLLT. Furthermore, the expression of *Alp*, an important molecule in bone formation, tended to increase in the laser-treated sites. Collectively, LLLT may thus enhance proliferation and differentiation of bone-forming cells in granulation tissue.

PCNA, a marker of cell proliferation, is specifically expressed in the cell nucleus during S phase (the DNA synthesis phase) of the cell cycle [32]. Analysis of PCNA expression revealed that the number of PCNA-positive cells per unit (mm^2) in the laser-treated sites was

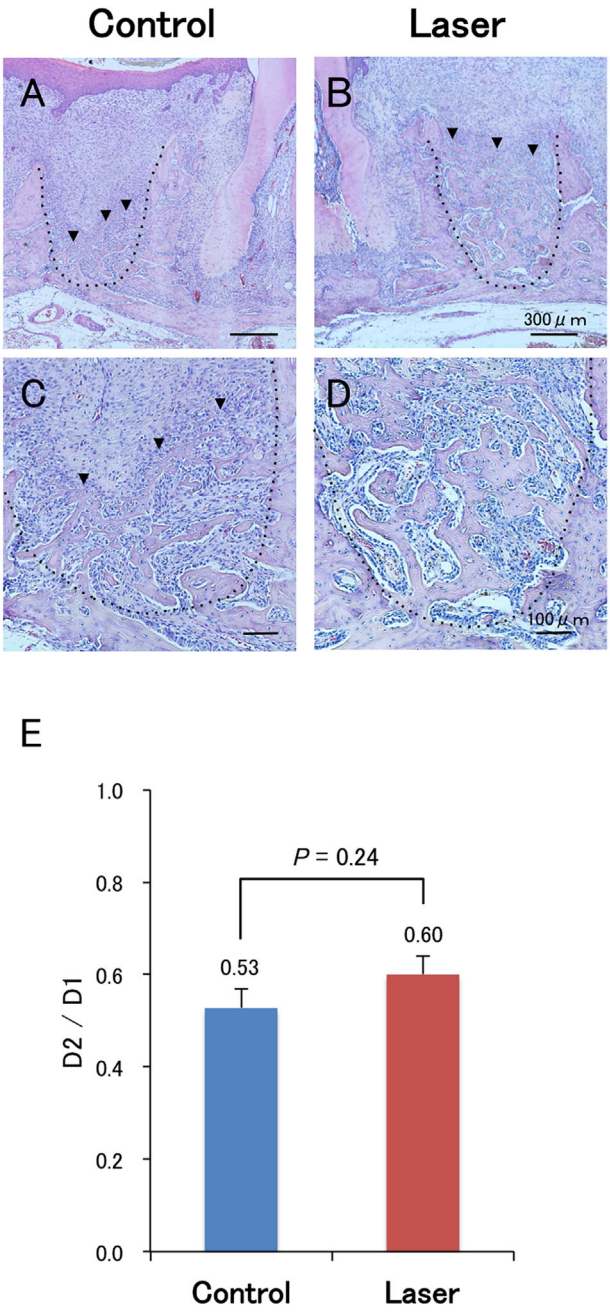

Fig. 6. Histological photomicrographs of tooth extraction sockets in high-frequency pulsed low-level laser-irradiated sites (**B**, **D**) and unirradiated control sites (**A**, **C**) and histomorphometric measurement of the new bone height on day 7 after tooth extraction (**E**). In 7 of the 12 rats, histological observation revealed more newly formed immature woven bone in laser-treated sites than in control sites. Dotted lines: margins of the original alveolar bone; arrowheads: crest of the newly formed bone. Osteogenesis started from the apical part of the sockets. Calculated mean values of the height of newly formed bone in laser-treated sites were larger than those of control sites although the difference was not statistically significant ($P = 0.24$). Data are presented as mean ± SE ($n = 12$).

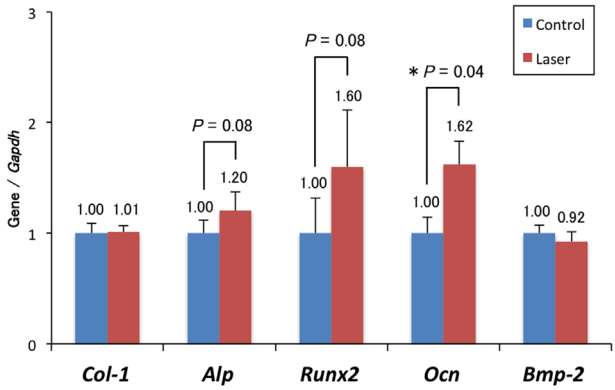

Fig. 7. mRNA expression of *Col-1*, *Alp*, *Runx2*, *Ocn*, and *Bmp-2* genes measured by real-time PCR in the high-frequency pulsed low-level laser-irradiated and unirradiated control tooth extraction sockets on day 3 after extraction. On day 3, mRNA expression of *Ocn* in laser-treated sites was significantly higher than that in unirradiated control sites ($P = 0.04$). *Runx2* and *Alp* expressions showed a higher trend in laser-treated sites than in control sites ($P = 0.08$ each). Data are presented as mean ± SE ($n = 11$). The genes/*Gapdh* values were calculated, and the value of the control sites was set to 1. $^{*}P < 0.05$ compared with the control sites (paired *t*-test).

significantly greater than that in control sites ($P = 0.01$), whereas the ratio of PCNA-positive cells to all cells in the laser-treated sites was higher than that in control sites but the difference did not reach to a significant level ($P = 0.06$). The differential significance in the values might arise because the number of PCNA-negative cells in laser-treated sites also increased compared to control sites on day 3, thereby decreasing the proportion of PCNA-positive cells in laser-treated sites.

Third, the expression of relatively early osteogenic markers such as *Runx2* and *Alp* tended to increase with laser irradiation, whilst late markers such as *Ocn* showed significant increases at the time point evaluated, suggesting that matured osteoblasts predominantly constitute the tooth extraction sockets. It has been reported that angiogenesis is a prerequisite for successful bone induction [33]. In future studies, changes in angiogenesis-related mRNA expression after LLLT need to be investigated.

In the present study, osteogenesis and epithelialization of laser-treated tooth extraction sockets were promoted compared to control sites on day 7 after tooth extraction. Since the results implied that LLLT influenced wound healing within 1 week, we focused on the early wound healing of extraction sockets within the first week and performed a preliminary investigation of the gene expression of osteogenic markers on day 3. In our future study, we will evaluate the osteogenic gene expression on day 7 or longer periods after extraction to understand the long-term effects of laser treatment.

For clinical applications of laser irradiation, the protocol used in this study (daily irradiation for 5 days) might not be practical. A clinical study by Mozzati et al. [10] previously demonstrated that three irradiation treatments of longer

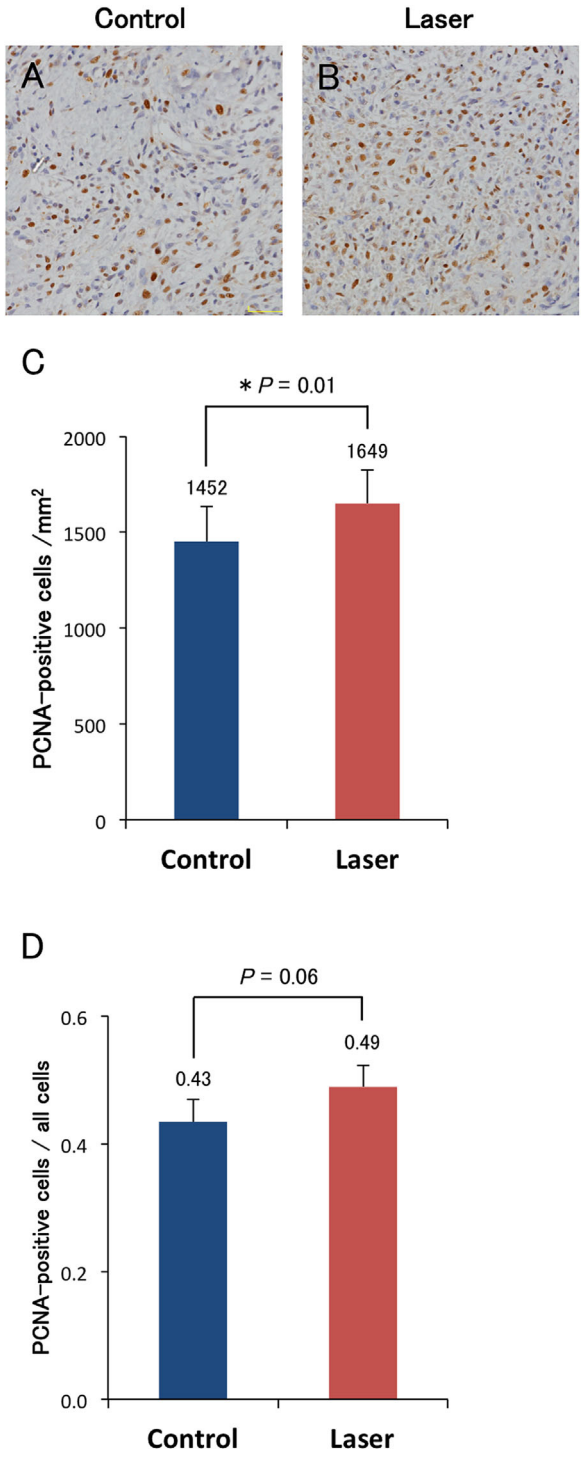

Fig. 8. Immunohistochemical photomicrographs of the high-frequency pulsed low-level laser-irradiated and unirradiated control tooth extraction sockets on day 3 after extraction. On day 3 after extraction, PCNA-positive cells were more abundant in the granulation tissue within the socket in laser-treated sites than in control sites (**A**, **B**). The number of PCNA-positive cells was significantly higher in laser-treated sites ($P = 0.01$) (**C**), and the ratio of PCNA-positive cells to all cells showed a higher trend in laser-treated sites ($P = 0.06$) than in control sites (**D**). Data are presented as mean ± SE ($n = 3$). $^{*}P < 0.05$.

duration than that in the present study showed a clinical benefit in the wound healing of tooth extraction sockets. Further detailed *in vivo* studies using a large animal model and clinical studies are required to establish the ideal LLLT protocol to promote wound healing of extraction sockets.

This is the first study to demonstrate promotion of both soft and hard tissues healing in tooth extraction sockets by LLLT. Our results reveal that LLLT using a HiFP diode laser might stimulate new bone formation by enhancing both cell proliferation and differentiation. These results using a tooth extraction socket model are also relevant to the application of LLLT in periodontal and peri-implant surgery. At present, scientific evidence for the promotion of new bone formation by LLLT are still limited. However, based on our results, LLLT could be a promising tool for enhancing new bone formation both in tooth extraction sockets and in periodontal and peri-implant regenerative therapy [34,35].

CONCLUSION

The present study indicated that HiFP low-level diode laser irradiation promotes epithelialization and bone formation in tooth extraction sockets, possibly through activation of both cell proliferation and differentiation. HiFP low-level laser therapy may improve early stage wound healing of tooth extraction sockets.

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