Photobiomodulation Laser Strategies in Periodontal Therapy

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Abstract Laser is considered basically effective for treating periodontal diseases because of its excellent physical properties namely ablation, hemostasis, bacterial killing and cell stimulation. The current laser application mainly used for the treatment of periodontitis is high-power laser ablation (HLLT). Laser ablation of diseased periodontal tissues using the HLLT is widely performed, partly expecting a simultaneous photo-bio-modulation effect (LLLT) in the surrounding tissues. In periodontal pocket therapy, laser can not only ablate the diseased tissues but also stimulate or activate the surrounding gingival and bone tissues, which would result in improved pocket healing and tissue regeneration. By elucidating the photo-bio-modulation effect in detail, this effect could be used more effectively and laser therapy would be more advantageous in non-surgical and surgical therapies of periodontitis as an adjunctive or alternative means to current mechanical treatment. As a future strategy of periodontal therapy, the photo-therapy using photo-bio-modulation/activation and photo-dynamic effects could be developed increasingly for prevention and control of periodontal diseases.

Keywords: Photo-bio-modulation, periodontal diseases, periodontitis, periodontal pocket, bone, Er:YAG laser.

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Introduction

The most common form of periodontal disease is a chronic, inflammatory and infectious disease, leading to episodic and progressive loss of periodontal tissues including gingival tissue, periodontal ligament and bone tissue. Recently, a variety of lasers have been used for a broad range of oral-facial conditions including periodontal therapy [1, 2]. The use of lasers is considered safe and effective for treating such inflammatory and infectious diseases as periodontitis. Lasers have numerous physical properties that can effect a broad range of biological responses that are suitable for treating a variety of periodontal conditions, such as ablation, hemostasis, microbial inhibition and destruction, cell stimulation, as well as modulation of metabolic activity.

High-power lasers were first used successfully as a variation of conventional approaches for soft tissue treatment such as gingivectomy and gingivoplasty in the clinic. Recently, for example, an Er:YAG laser was developed which can be used on both dental soft and hard tissues due to its low thermal side-effects. Consequently, the Er:YAG laser has been used to treat gingiva, tooth roots, and bone tissue, thus becoming one of the more promising laser units for periodontal treatment [1, 3].

Current Laser Strategy

The main laser application strategy currently used for the treatment of periodontal diseases is high-power laser ablation or high-level laser treatment (HLLT). Laser ablation of diseased tissues is widely performed, partly expecting a simultaneous photo-bio-modulation (PBM) effect (LLLT) in the surrounding tissues [7].

In periodontal pocket therapy, laser devices can not only ablate the diseased tissues and decontaminate and detoxify the pockets and root surfaces but also stimulate or activate the surrounding gingival and bone tissues. If properly used, this would result in improved pocket healing with soft and bone tissues regeneration by reduction of inflammatory condition and promotion of cell proliferation and differentiation (Fig. 1) [1].

Such additional PBM effects during HLLT would be not so strong but the effects are also another advantageous property of laser pocket treatment and would provide a great therapeutic benefit producing improved clinical outcomes. Some researchers and clinicians have recently recognized and realized those PBM effects in the laser pocket treatment to some extent and have been using lasers intentionally expecting those effects. Interestingly, they have experienced improved pocket healing and increased bone regeneration following laser treatment. However, clinical studies concerning the PBM effects in laser pocket treatment have not been clearly proposed and demonstrated so far. Although laser pocket treatment has been increasingly reported in the non-surgical or closed periodontal pocket therapy, most researchers have not sufficiently noticed and understood the PBM effects during laser pocket treatment and scientific publications showing positive results of PBM are delayed and still insufficient.

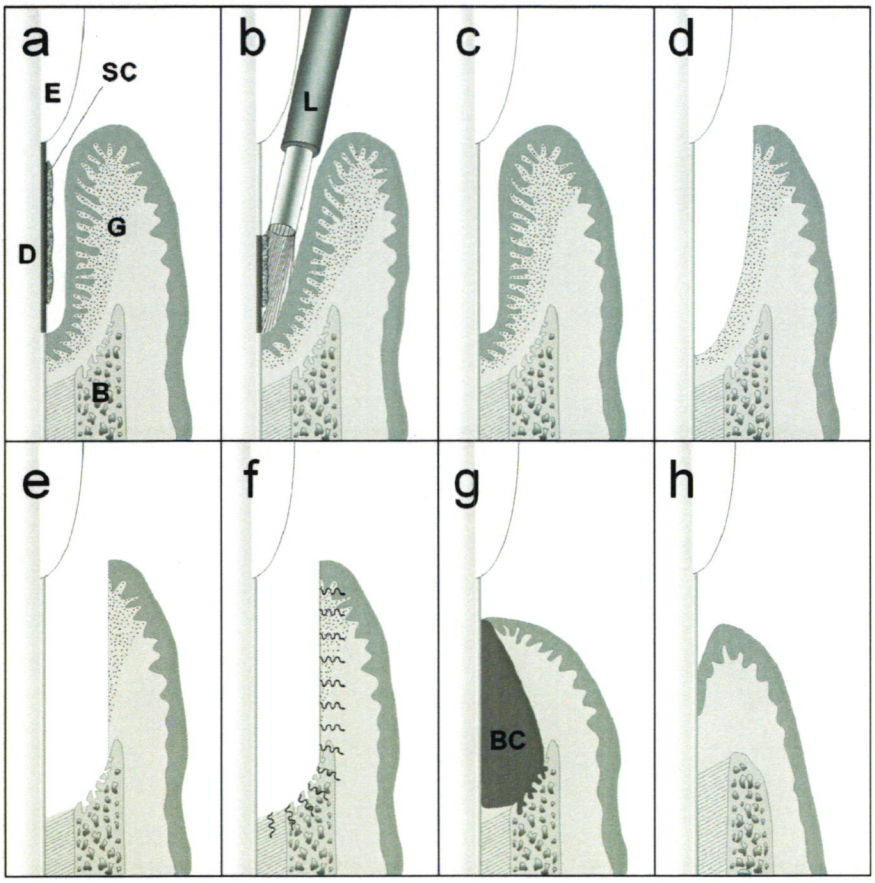

Fig. 1 Schematic illustration of the procedures of laser pocket therapy. Laser can not only ablate the diseased tissue by HLLT but also exerts photo-bio-modulation (PBM) effect (LLLT) to the surrounding tissues. **(a)** Advanced periodontal pocket showing gingival tissue detachment, subgingival calculus deposition and contamination of the tooth root surface, epithelial down-growth and lining of the inner surface of gingival connective tissue with inflammation, vertical bone resorption and diseased connective tissue formation in the defect. **(b)** Laser ablation of the deposited calculus and decontamination and detoxification of the root surface. **(c, d)** Ablation of lining epithelium. **(e)** Ablation of diseased connective tissue on the inner surface of the gingival tissue and in the vertical bone defect. **(f)** Simultaneous PBM effects stimulating or activating the surrounding gingival and bone tissues, which would result in improved pocket healing with soft and bone tissue regeneration by reduction of inflammatory condition and promotion of cell proliferation and differentiation. **(g)** Blood clot (BC) formation in the pocket and defect. **(h)** Favorable pocket healing with gingival connective tissue attachment and bone tissue regeneration. E: enamel of tooth crown, D: dentin of tooth root, SC: subgingival calculus, B: alveolar bone, G: gingival tissue, L: laser tip

Potential Applications of PBM for Wound Healing and Tissue Regeneration in Periodontal Therapy

Promotion of New Bone Formation

In our previous studies, we observed increased new bone formation in dogs after surgical periodontal and peri-implant treatment using a high energy-level Er:YAG laser. Mizutani et al. [5] compared the periodontal tissue healing following periodontal flap surgery using an Er:YAG laser with that of conventional mechanical curette surgery. In six dogs, bilateral premolars with experimentally-induced periodontitis were treated by periodontal surgical procedure. Degranulation and root debridement were effectively performed with the Er:YAG laser irradiation without major thermal damage. At 3 months post surgery, interestingly, the amount of newly-formed bone was significantly greater in the laser group than in the curette group in the histological analysis (Fig. 2). This study showed that the Er:YAG laser irradiation has the potential to promote new bone formation.

Also, Takasaki et al. [13] evaluated the utility of the application of an Er:YAG laser for the surgical treatment of peri-implant infection. In four dogs, the peri-implant surgery was performed using an Er:YAG laser or a plastic curette for

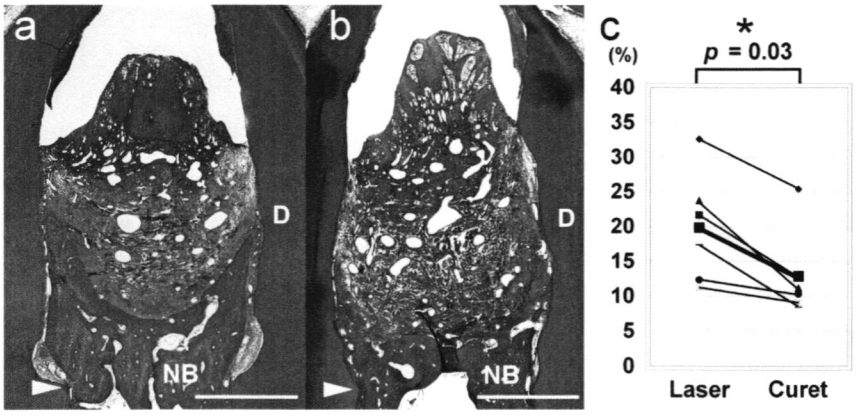

Fig. 2 Histological photomicrographs of mesio-distal sections of furcation at 12 weeks after periodontal flap surgery using the Er:YAG laser **(a)** and curette **(b)**, and the histometric analysis of the ratio of newly-formed bone (NB) area **(c)**. In both the laser and curette sites, periodontal soft tissue attachment with some degree of bone regeneration was noted in the furcation area. The NB was coronally extended along the dental root surface (D) in the defect above the notch (arrow heads). Note the greater new bone formation in the laser-treated site **(a)** than the curette-treated site **(b)** (bar = 800 μm, original magnification 27×). In the histometric analysis **(c)**, all measurement data obtained in square millimeters were converted to a percentage relative to the area of each original defect. The thick line shows the mean in the graph. (*$P < 0.05$; Wilcoxon signed-rank test, n = 6) (Photographs and figure from [5]. With kind permission. Lasers in Surgery and Medicine © copyright (2006) Wiley)

degranulation and implant surface debridement. After 6 months, histologically, the laser-treated implant surface did not inhibit new bone formation but rather the laser group showed a tendency to produce greater new bone-to-implant contact than the curette group (Fig. 3). The results indicated that the Er:YAG laser therapy has a potential to induce favorable bone healing in the surgical treatment of peri-implantitis.

Thus, both studies demonstrated the increased or favorable new bone formation after laser treatment compared to mechanical treatment. There would be several reasons for the increased bone formation, but the improved bone regeneration may be partly due to the PBM of low-level laser which was scattered or penetrated during HLLT.

Regarding osteogenesis, previous several in vitro studies have suggested that low-level laser irradiation could promote new bone formation by inducing proliferation and differentiation of osteoblasts [8, 12]. It has been reported that low-level lasers increased the ALP activity [8] and mRNA expression of osteoblastic differentiation markers such as osteopontin [12], osteocalcin [8] and bone sialoprotein [12] in osteoblasts and promote bone nodule formation [8]. These PBM effects would be useful for periodontal regenerative therapy. Therefore, further basic and clinical studies are required for the establishment of a clinically effective and reliable procedure.

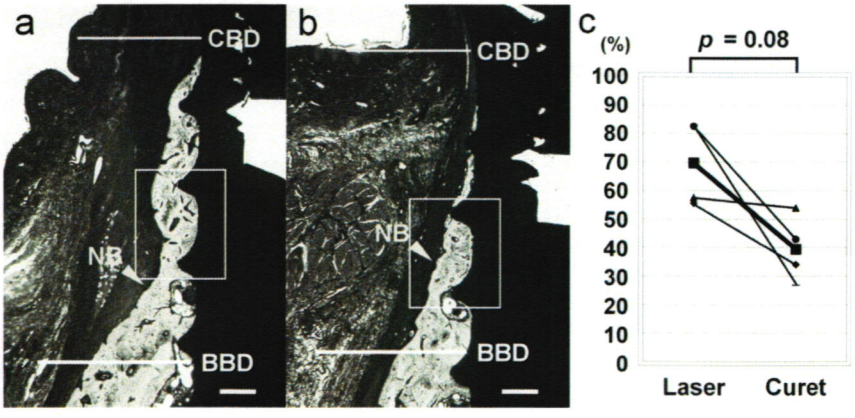

Fig. 3 Histological photomicrographs of buccolingual sections parallel to the long axis of the implant in the center of the dehiscence defect at 24 weeks following surgical therapy of peri-implant infection using an Er:YAG laser **(a)** or a plastic curette **(b)**, and the histometric analysis of the ratio of new bone-to-implant contact (NBIC) **(c)**. Both histological sections show the highest new bone (NB) formation in each group. In the laser group **(a)**, the NB was more coronally-extended along and in direct contact to the implant surface from the bottom of the bone defect (BBD) than the curette group **(b)**. CBD: Coronal level of original bone defect (bar = 500 μm; original magnification 30x). In the histometric analysis **(c)**, all measurement data obtained in millimeters were converted to a percentage relative to the length of implant surface of the bone defect following debridement. The thick line shows the mean in the graph. (Student paired *t*-test, n = 4) (Photographs and figure from [13]. With kind permission of Springer Science and Business Media. Lasers in Medical Science © copyright (2007) Springer)

Promotion of Cell Proliferation

Low-level laser irradiation has been reported to enhance wound healing. Activation of gingival fibroblasts has a potential for early wound healing in periodontal treatment. Our group demonstrated that a low energy-level of Er:YAG laser exerts stimulatory effects on cell proliferation of human gingival fibroblasts (HGFs).

First, Pourzarandian et al. [9] investigated the effect of low-level Er:YAG laser irradiation on HGF proliferation. Cultured HGFs were exposed to low-level, pulsed Er:YAG laser irradiation with various energy densities ranging from 1.68 to 5.0 J/cm^2 and 20 Hz. The cultured fibroblasts after irradiation were analyzed by means of light microscopy and transmission electron microscopy (TEM). As a result, light microscopy revealed that the number of cells increased and the shape of the cells was irregular and more mature than that of control (Fig. 4a, b). In TEM observation, the fibroblasts seemed to be metabolically active. A comparison of cell growth at day 1 and day 3 after treatment showed a significant increase in the number of cells in the Er:YAG laser irradiation groups of 1.68, 2.35 and 3.37 J/cm^2 (Fig. 4c). The comparison of cell death determined by the level of lactate dehydrogenase (LDH) between laser-treated and untreated control cultures showed no significant differences after laser irradiation at 1.68–3.37 J/cm^2. However, an energy density at 5.0 J/cm^2 showed a significant increase of the LDH level and decreased the cell number after 3 days. The results showed that the low-level Er:YAG laser irradiation stimulates the proliferation of cultured HGFs and suggests that the low-level Er:YAG laser irradiation may be of therapeutic benefit for wound healing.

Secondly, regarding the mechanism of increased cell proliferation, we focused on prostaglandin E_2 (PGE_2) production after laser irradiation. PGE_2 is one of the important early mediators in the natural healing process and it regulates cell proliferation through interaction with its specific receptors and modification of the levels of second messengers such as calcium and cAMP [11]. PGE_2 production is induced in HGFs via de novo synthesis of cyclooxygenase-2 (COX-2), which is an inducible PG synthase, in response to proinflammatory stimuli [6].

Pourzarandian et al. [10] investigated the change of PGE_2 production and COX-2 gene expression in HGF after Er:YAG laser irradiation in vitro. Cultured HGFs were exposed to low-level Er:YAG laser irradiation with an energy density of 1.68–3.37 J/cm^2. The levels of PGE_2 production were measured by enzyme-linked immunosorbent assay. Total RNA was extracted and COX-2 mRNA expression was analyzed by reverse transcriptase—polymerase chain reaction (RT-PCR). The Er:YAG laser significantly increased PGE_2 production in a laser energy-dependent manner (Fig. 5a). COX-2 mRNA expression was also enhanced with an increase in energy level (Fig. 5b). Indomethacin, a non-specific COX-1/COX-2 inhibitor, and NS398, a specific COX-2 inhibitor, completely inhibited the PGE_2 synthesis stimulated by Er:YAG laser irradiation. These results showed that the Er:YAG laser irradiation appears to exert its stimulative action on HGF proliferation through the production of PGE_2 via the expression of COX-2. This could be considered as one of the important regulatory pathways that enhance cell proliferation for tissue regeneration.

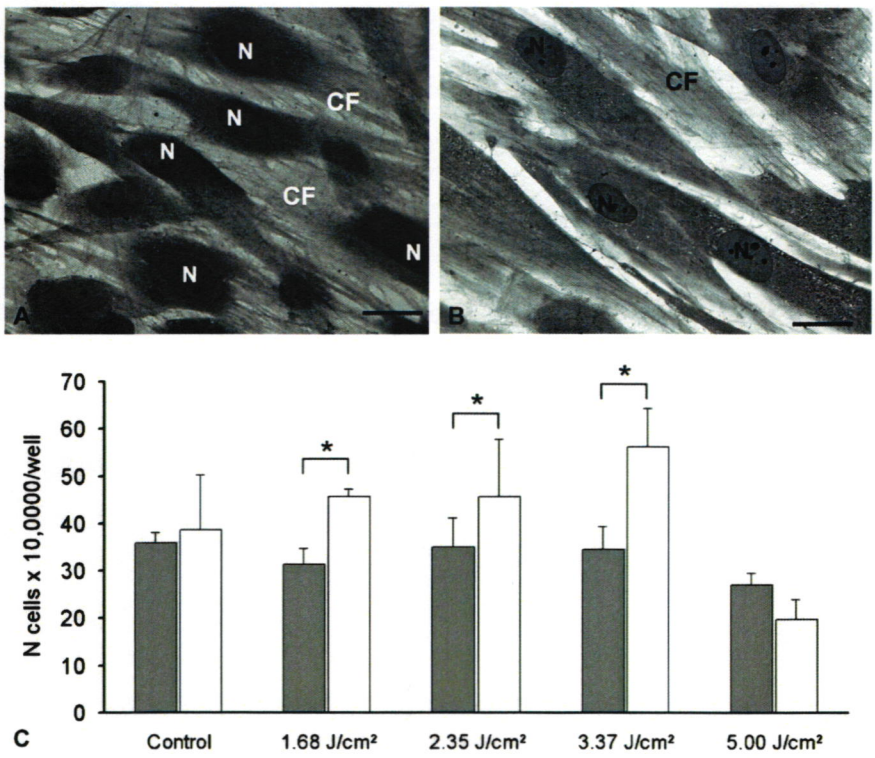

Fig. 4 Light micrograph of cultured human gingival fibroblasts (HGF) 3 days after Er:YAG laser treatment with 3.37 J/cm^2 energy density **(a)** and cultured non-lased control HGFs 3 days after **(b)**, and a graph showing the HGF cell numbers after laser treatment using various energy levels **(c)**. In the HGFs treated by Er:YAG laser **(a)**, the number of cells seems to have increased and the cells were surrounded by a network of collagen fiber (CF). In the control HGF **(b)**, cells are elongated and parallel to each other, surrounded by collagen fibers (CF). N: nucleus (original magnification 1,000x; bar = 10 μm). In the graph **(c)**, each bar represents the mean ± SD of cell numbers. Black column: average of cell numbers 24 hours after treatment. White column: average of cell numbers 3 days after treatment. *Significant difference ($P < 0.001$, Fisher's test) (Photographs and figure from [9]. With kind permission. Journal of Periodontology © copyright (2004) the American Academy of Periodontology)

Photo-Dynamic Therapy to Induce Bacterial Death

Recently, application of the bactericidal property of photo-dynamic therapy (PDT) has been considered as a novel treatment modality to control the bacterial infection in the field of periodontal therapy. Several studies demonstrated the high bactericidal effect of PDT and suggested that it may be a valuable alternative [4]. PDT is based on the principle that a photoactivatable substance, the photosensitizer, binds to the target cell and can be activated by light of a suitable wavelength. During this process, free radicals are formed, which then produce an effect that is toxic to the bacteria.

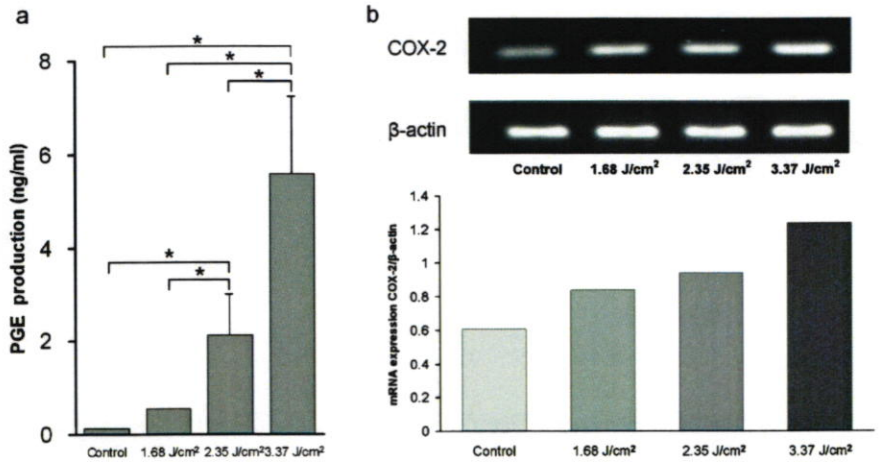

Fig. 5 Prostaglandin E_2 (PGE_2) release from HGFs irradiated with low-level Er:YAG laser (**a**) and cyclooxygenase-2 (COX-2) mRNA expression in HGFs (**b**). In graph (**a**), each bar represents the mean $PGE_2 \pm SD$. Laser irradiation stimulated PGE_2 production in a laser-energy dependent manner. *Significant difference ($P < 0.05$) (Fisher's test). In graph (**b**), total RNA was extracted and COX-2 mRNA expression was analyzed by RT-PCR. The COX-2 mRNA was highly induced in HGF cells irradiated by Er:YAG laser in a laser-energy dependent manner (Figures from [10]. With kind permission. Journal of Periodontal Research © copyright (2005) Blackwell Munksgaard)

Regarding the bactericidal effect of low-level laser or light without use of photosensitizer, we have already found that both argon laser (457–502 nm: peak 486 nm, 150 and 250 mW) and halogen light (400–500 nm: peak 492 nm, 450 mW) had a sufficient killing effect on periodontopathic bacteria, *Porphyromonas gingivalis* (unpublished data). In this study, the 250 mW argon laser showed the highest bactericidal effect with approximately 30%, 77%, 90%, and 99% of the bacterial death after 1, 3, 5 and 10 minutes of exposure, respectively. Although there was a moderate temperature elevation according to the increase of the irradiation time and therefore the bactericidal effect would be partly due to the heat effect, it appeared that PDT or photo-therapy might have promise as a novel method of eliminating bacterial infection.

PBM Laser Strategies in Periodontal Therapy

It is considered that lasers would help the phase of the tissues and cells in the diseased site change rapidly from the inflammatory and destructive state into that of healing and regeneration by modulating or activating cell metabolism. By elucidating the various effects of PBM in detail, these effects could be used more effectively and laser therapy would be more advantageous in non-surgical and

surgical therapies of periodontitis as an adjunctive or alternative means to current mechanical and chemical regeneration procedures.

Furthermore, as a future strategy of laser therapy, the photo-therapy can be developed increasingly for prevention and control of periodontal diseases. In the initial stages of periodontal disease, elimination of periodontopathic bacteria and reduction of the inflammatory condition might be possibly controlled more effectively using the periodical external laser/light irradiation from the gingival surface than the conventional mechanical means alone. In order to maintain periodontal hygiene and to prevent infection and reinfection, constant effort to remove bacteria is indispensable but teeth and periodontal tissues form a very complicated structure and thus conventional mechanical means cannot achieve a complete bacterial elimination. For example, as a patient's home care, a photo-toothbrush, if necessary in combination with a tooth paste including a photo-sensitizer, might be developed for the control of infection and inflammation of the periodontal tissues. Also, in case of moderate and advanced stages of periodontal disease, internal irradiation via the periodontal pocket as well as external irradiation would be useful for enhancement of soft and hard tissue regeneration in combination with or without current regeneration procedures.

In conclusion, photo-therapy using photo-bio-modulation/activation and photodynamic effects should be studied more extensively for the treatment of periodontal diseases.

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