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Irradiation by light-emitting diode light as an adjunct to facilitate healing of experimental periodontitis *in vivo*

P-C. Chang, L-Y. Chien, Y. Ye, M-J. Kao
Faculty of Dentistry, National University of Singapore, Singapore, Singapore

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Background and Objective: This study evaluated the biostimulatory effect of 660 nm light-emitting diode (LED) as an adjunct in the treatment of experimental periodontitis.

Material and Methods: Ninety-six Sprague-Dawley rats underwent experimental periodontitis by placement of a silk ligature followed with or without additive *Porphyromonas gingivalis* lipopolysaccharide (*Pg*-LPS) injection. Irradiation with LED light was performed at varying energy densities of 5, 10 and 15 J/cm^2, 1 d after debridement and detoxification. Rats were killed at 3, 7 and 14 d after irradiation with LED light, and the effect of irradiation was evaluated by descriptive histology and quantitative measurements of periodontal bone loss, inflammatory infiltration and cellular proliferation.

Results: Reduction of inflammation, accelerated collagen deposition and realignment was noted following irradiation with LED light at densities of 10 and 15 J/cm^2, and temporary reduction of periodontal bone loss, as well as bundle bone apposition, was noted at day 3 in rats treated with 10 J/cm^2 light. The biomodulatory effect was stronger in sites treated with *Pg*-LPS injection. In sites without *Pg*-LPS injection, temporary reduction of inflammation was noted in all LED light-irradiated specimens at day 3. No significant change in cellular proliferation was noted in any LED light-treated group.

Conclusions: LED light (660 nm) with an energy density of 10 J/cm^2 appeared suitable as an adjunct modality for periodontitis by temporarily reducing inflammation, facilitating collagen realignment and bundle bone deposition. Future studies will aim to amplify the biostimulatory effect of LED light by adding a supplementary medium or repeated irradiation.

Po-Chun Chang, D.D.S., Ph.D. National University of Singapore, Faculty of Dentistry, 11 Lower Kent Ridge Road, Singapore 119083, Singapore
Tel: +65 6779 5555 extension 1789
Fax: +65 6773 2602
e-mail: dencpc@nus.edu.sg

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Lower-level laser treatment (LLLT) has been utilized as a therapeutic modality to promote wound healing since the 1970s (1). The therapeutic energy dose used in LLLT is significantly lower (10^{-2} to 10^2 J/cm^2) than that of conventional laser treatment, and the temperature change is typically negligible (2). The light–tissue interaction can be affected by several parameters, including the wavelength, energy density and method of application of the light and the nature of the tissue. A number of light sources have been utilized for such treatment, including helium–neon, ruby, gallium aluminum–arsenide (GaAlAs) and light-emitting diodes (LEDs). LED, a semiconductor

device emitting a low-intensity light ranging from ultraviolet to infrared wavelengths, was demonstrated to facilitate wound repair via simulating mitochondrial functions, promoting collagen synthesis and inhibiting the expression of MMPs (1,2). Thus, LED light has been widely used as a painless and nonablative application to improve the photoaging and erythema of skin (3,4).

Periodontitis is a local destructive disease caused by local immune disturbance. Specifically, the challenge of periodontopathogens and their virulence factors [e.g. lipopolysaccharide (LPS)] may induce the secretion of proinflammatory cytokines, which elicit an inflammatory response and drive alveolar bone loss (5). Although much effort is aimed at eliminating periodontopathogens to provide a favorable environment for tissue recovery, it usually takes 2–4 wk to initiate osseous remodeling and a well-defined attachment (6). There is a need to accelerate the healing process after periodontal treatment to minimize the risk of infection, reduce treatment costs and time for both patients and healthcare facilities and relieve postoperative symptoms (e.g. pain and sensitivity). In this regard, gallium aluminum–arsenide-based LLLTs with wavelengths of 800–1000 nm and energy densities of 2–4 J/cm^2 have been introduced as an adjunct to facilitate oral wound repair and promote improvement of inflammatory status, clinical attachment and peri-implant bone apposition (7–9).

It has been shown *in vitro* that inflammation elicited by *Porphyromonas gingivalis* LPS (*Pg*-LPS) can be suppressed by irradiating human gingival fibroblasts with visible red (635–670 nm) light (10). In this study, we devised a technique to directly irradiate the diseased gingival tissue with 660 nm LED light at a range of energy densities after debridement and detoxification *in vivo*. The study aimed to explore the ability of LED light to reduce inflammation, promote collagen fiber rearrangement and stimulate bundle bone formation.

Material and methods

Animal models and study design

All animal procedures followed the guidelines and approved protocol 032/10 from the Institutional Animal Care and Use Committee (IACUC) of the National University of Singapore. Ninety-six male Sprague-Dawley rats were housed in pairs with food and distilled water *ad libitum*. To induce experimental periodontitis, a 4-0 silk ligature was inserted into the gingival sulcus of the maxillary second molar (M2) in one randomly selected side of each rat for 2 wk, and an interpapillary injection of 10 μL of 1.0 mg/mL of *Pg*-LPS (InvivoGen Inc., San Diego, CA, USA) was administered to the experimental sites of half of the animals. The ligature was checked, and *Pg*-LPS was injected twice per week for 2 wk. Immediately after ligature removal, meticulous manual debridement was performed using a dental explorer, taking care to avoid damage to the root surface, and 0.12% chlorhexidine was topically applied.

One day after debridement and detoxification, the test region was irradiated with LED light using a customized LED device (Fig. 1, JETTS Technology Co., Ltd., New Taipei, Taiwan) with four diodes (a 2×2 arrangement, with each diode 2×1 mm in size) arranged on a plate (10×5 mm). The device was placed over the palate of rats and settled on the occlusal surface of the maxillary molars, leaving a distance of 0.5 mm between the diodes and the palatal aspect of the maxillary molars. Each diode consistently emitted 660 ± 25 nm and 3.5 mW/cm^2 visible red light directly onto the gingival tissue, for 6, 12 or 18 min, to achieve energy densities of 5, 10 or 15 J/cm^2 in the test region. No irradiation was performed in the control sites. Supragingival plaque was removed by meticulously applying the cotton rolls onto the tooth surface twice per week. The animals were killed 3, 7 and 14 d after LED treatment (n = 8 rats per time-point).

Histologic and immunohistochemical evaluations

After the rats were killed, the maxillae were harvested, fixed in 10% formalin for 3 d, decalcified with 12.5% EDTA (pH7.4) for 3 wk, oriented transversely and embedded in paraffin and then cut into 5-μm-thick sections. In each site, sections from the mid-distopalatal and mid-mesiopalatal root of M2 were selected and stained with hematoxylin and eosin (Polysciences Inc., Warrington, PA, USA) for descriptive histology and measurements of the distance between the cemento–enamel junction and the alveolar bone crest (CEJ–ABC). Quantification of inflammatory cells was performed in five randomly selected areas under 400× magnification in each section, and the results are presented as the percentage of inflammatory cells relative to the total cell count.

Collagen alignment in the periodontal ligament space and gingival connective tissue was analyzed using

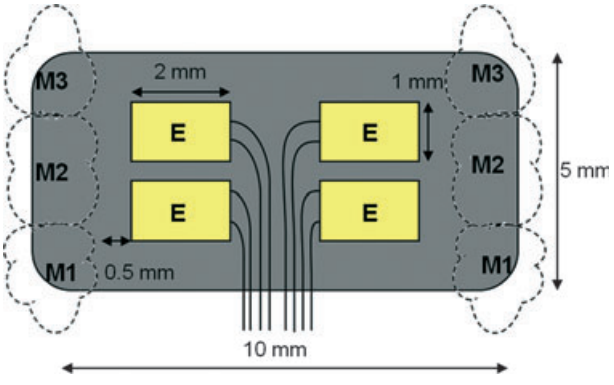

Fig. 1. Set-up of the light-emitting diode (LED) device. The LED device was positioned on the maxilla, and the diode emitters (E) were located 0.5 mm from the molars (M1–M3).

PicroSirius Red stain. The sections were stained for 1 h in PicorSirius Red solution (Sigma-Aldrich Co., St Louis, MO, USA) and counterstained with Weigert's hematoxylin (Sigma-Aldrich Co.) after deparaffinization and rehydration. Cells undergoing mitogenesis were determined immunohistochemically by their expression of the proliferating cell nuclear antigen (PCNA). Briefly, antigens and epitopes in the sections were unmasked by incubation with 0.05% trypsin/EDTA (Invitrogen Co., Carlsbad, CA, USA) for 20 min at room temperature after deparaffinization and rehydration. To eliminate endogenous peroxidase activity, the sections were immersed for 10 min in 3% H_2O_2. Following blocking of nonspecific binding with serum, the sections were incubated with rabbit polyclonal antibody for PCNA (1 : 200 dilution; Abcam PLC, Cambridge, UK) overnight at 4°C and subsequently incubated with the corresponding biotinylated secondary antibodies for 1 h at room temperature. The color was developed by 3,3′-diaminobenzidine, and sections were counterstained with hematoxylin. Quantifications of proliferating cells were performed in five randomly selected areas under 400× magnification in each specimen, and the results are presented as the percentage of cells with positive signals within the total cell count. All histomorphometric measurements and cell quantifications were performed by two examiners (M.J.K. and Y.Y.) after an initial intersubject calibration (intraclass correlation coefficient > 0.95), and the examiners were masked with respect to the treatment.

Statistical analysis

One-way analysis of variance followed by Tukey's *post hoc* test was used to compare the difference between control (no LED light treatment) and treatment groups, and the difference in ligature-placed only and ligature + *Pg*-LPS injection conditions was further examined separately. The data are presented as the mean ± standard deviation of measurements, with a $p < 0.05$ considered statistically significant.

Results

Gross observation

All animals recovered well from the anesthesia and the interventions. Three days after ligature placement, gingival redness and bleeding upon palpation was noted in all animals, and those signs persisted until ligature removal. The gingival redness disappeared at the time of LED light treatment, and although bleeding was occasionally observed in Pg-LPS- and non-Pg-LPS-treated sites at the time of LED light irradiation, there was no significant difference between the groups. There was no visible damage to the gingival tissue immediately after irradiation. Bleeding subsided in all animals after 3 d, and no significant difference was observed between irradiated and non-irradiated sites.

Reduction of CEJ–ABC distance

A slight reduction of the CEJ–ABC distance was noted at day 3 after irradiation with 10 J/cm^2 of LED light (Fig. 2A). Specifically, significant reduction of the CEJ–ABC distance was noted at day 3 at sites irradiated with 5 and 10 J/cm^2 of LED light but without treatment with *Pg*-LPS

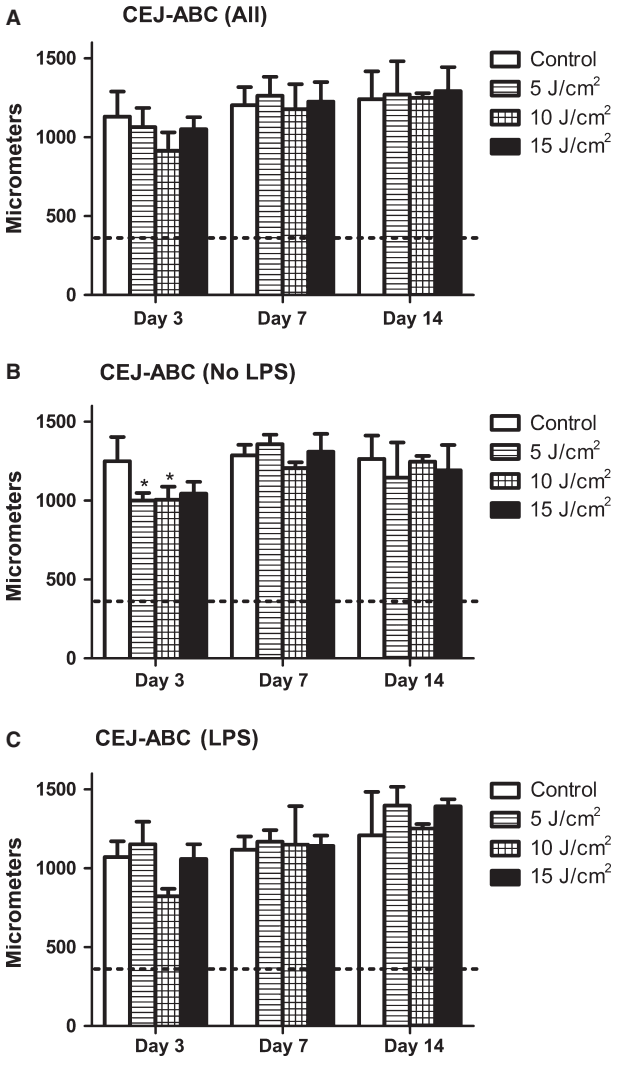

Fig. 2. Distance from the cemento–enamel junction (CEJ) to the alveolar bone crest (ABC) in (A) all experimental sites, (B) sites without *Porphyromonas gingivalis* lipopolysaccharide (Pg-LPS) treatment and (C) sites treated with *Pg*-LPS injection. The dashed lines indicate the distance from the CEJ to the ABC in the sites without periodontitis induction. *$p < 0.05$ (compared with control specimens).

($p < 0.05$) (Fig. 2B), whereas in sites injected with *Pg*-LPS, the CEJ–ABC distance was reduced at day 3 only after irradiation with 10 J/cm^2 of LED light (Fig. 2C). Irradiation with 15 J/cm^2 of LED light did not result in additional reduction of the CEJ–ABC distance compared with irradiation with 5 or 10 J/cm^2 of LED light. The CEJ–ABC distance in all LED light-irradiated specimens returned to that of non-LED light-irradiated specimens at days 7 and 14.

Descriptive healing of periodontium

At day 3, mild to moderate inflammatory infiltration at the bottom of the gingival sulcus with prominent rete pegs and significant enlargement of the epithelium was noted in the specimens that had not been irradiated with LED light. The newly formed granulation tissue had started to invade and resorb the inflammatory and necrotic mass, and the mature collagen-fiber matrix distributed partially in the gingival connective tissue. Osteogenesis was not obvious and was only occasionally observed on the lingual aspect of the alveolar bone and the periodontal ligament space (Fig. 3A and 3B). Increased inflammatory cell infiltration was noted on the surface of the ABC in sites treated with *Pg*-LPS injection (Fig. 3B). Following irradiation with 5 J/cm^2 of LED light, and regardless of treatment or not with *Pg*-LPS, the gingival connective tissue appeared to occupy more newly formed collagen matrix without evident reduction of inflammation and new bundle bone apposition (Fig. 3C and 3D). In sites treated with *Pg*-LPS injection, inflammation was still relatively intense and the collagen fiber bundles were loosely distributed near the tissue–root interface compared with sites that were not injected with *Pg*-LPS (Fig. 3D). Significant reduction of inflammation, thinning of gingival epithelium and prominent collagen matrix synthesis were noted following irradiation with 10 and 15 J/cm^2 of LED light, regardless of treatment with *Pg*-LPS injection (Fig. 3E–H). The collagen bundles appeared to be regularly aligned and were inserted obliquely to the root surface, and a lining of densely packed cells on the surface of the alveolar bone was noted. Therefore, neogenic bone apposition on the bundle bone was only obviously found in sites injected with *Pg*-LPS and irradiated with 10 J/cm^2 of LED light (Fig. 3F).

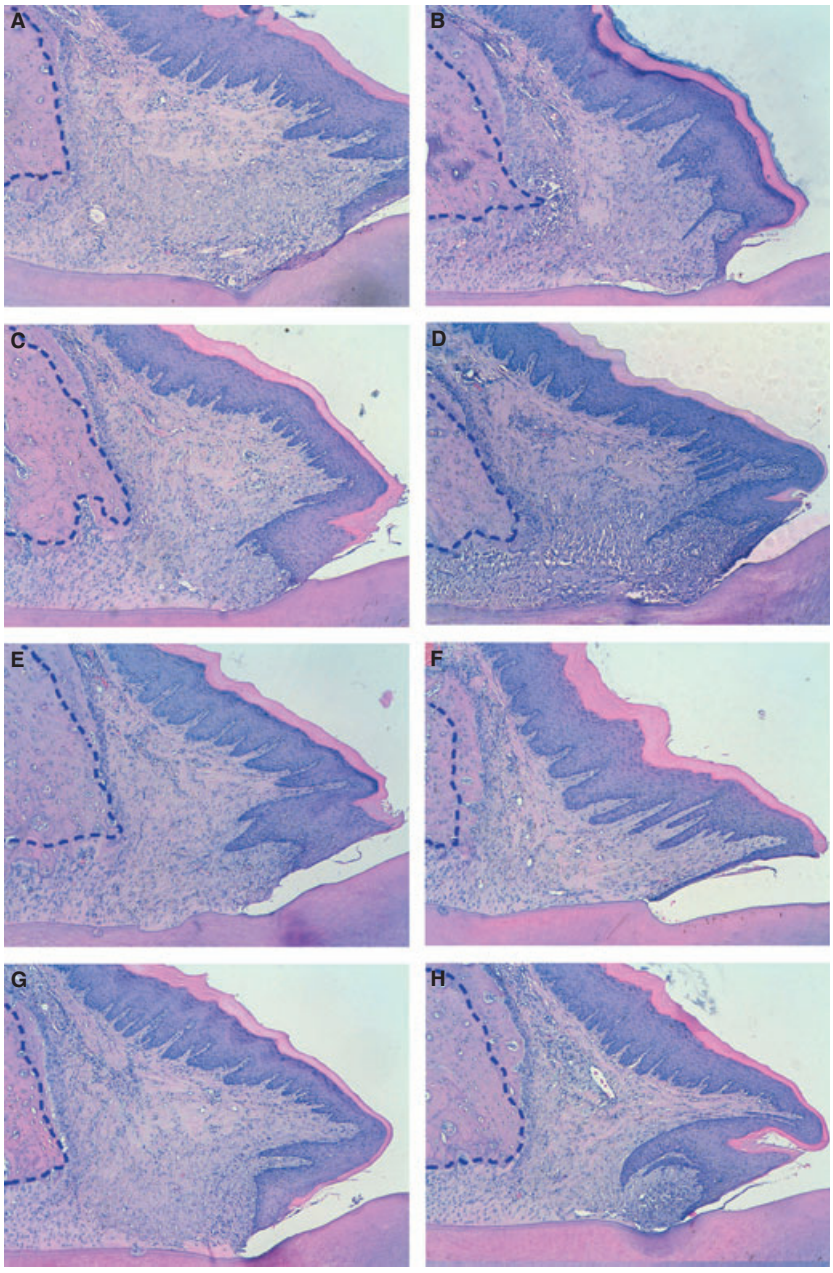

Fig. 3. Histology of specimens 3 d after irradiation with light-emitting diode (LED) light. (A, B) Specimens from (A) a site without *Porphyromonas gingivalis* lipopolysaccharide (*Pg*-LPS) treatment and (B) a site without *Pg*-LPS treatment; neither site was irradiated with LED light. (C, D) Specimens from (C) a site without *Pg*-LPS treatment and (D) a site treated with *Pg*-LPS injection; both sites were irradiated with 5 J/cm^2 of LED light. (E, F) Specimens from (E) a site without *Pg*-LPS treatment and (F) a site treated with *Pg*-LPS injection; both sites were irradiated with 10 J/cm^2 of LED light. (G, H) Specimens from (G) a site without *Pg*-LPS treatment and (H) a site treated with *Pg*-LPS injection; both sites were irradiated with 15 J/cm^2 of LED light. The blue dashed lines indicate the border of the native alveolar bone crest (ABC). Hematoxylin and eosin stain, magnification 100×.

On day 7, significant recession of the necrotic mass, replaced with the collagen matrix with a looser fiber

arrangement, was noted in the nonirradiated specimens, and a lining of densely packed cells, as well as neogenic bone apposition, was evident on the ABC, regardless of treatment with *Pg*-LPS injection (Fig. 4A and 4B). A similar configuration was also found in the specimens irradiated with 5 J/cm^2 of LED light (Fig. 4C and 4D). After irradiation with 10 J/cm^2 of LED light, the gingival connective tissue was almost completely occupied by the well-aligned collagen fibers, and bone formation was more prominent on the ABC compared with nonirradiated specimens or those irradiated with 5 J/cm^2 of LED light, regardless of treatment with *Pg*-LPS injection (Fig. 4E and 4F). Furthermore, inflammation was rarely seen after irradiation with 15 J/cm^2 of LED light, and a significant amount of new bone apposition was observed on the surface of the ABC and in the cervical periodontal ligament space (Fig. 4G and 4H). On day 14, deposition of mature collagen matrix was observed on all specimens with dense bundles of collagen fibers and thickening of the bundle bone (Fig. S1).

The pattern of collagen alignment was further confirmed by PicroSirius Red staining. In nonirradiated specimens on day 3, the inflammatory cells were embedded in the loose and haphazardly oriented collagen fiber network in the lamina propria of the gingiva (Fig. 5A and 5B). A higher number of inflammatory cells embedded within the thin collagen fibers were evident in sites treated with *Pg*-LPS injection (Fig. 5B). At day 7, the lamina propria was mainly occupied by primitive, but uniformly aligned, collagen fibers. The fiber bundles inserted to the tooth root appeared perpendicular in gingival connective region and oblique in cervical periodontal ligament space in non *Pg*-LPS treated sites (Fig. 5C). At day 7 the deposition of collagen was progressive, but the fiber network was still haphazardly oriented in sites treated with *Pg*-LPS injection (Fig. 5D).

Therefore, at day 3, in specimens irradiated with 10 J/cm^2 of LED light, significant reduction in the numbers of inflammatory cells and occupation of primitive and uniformly aligned collagen matrix was noted (Fig. 5E and 5F), whereas increased cellularity with a relatively loose fiber configuration was observed in sites treated with *Pg*-LPS injection (Fig. 5F). On day 7, specimens irradiated with LED light, especially at an energy density of 10 J/cm^2, demonstrated more densely packed, uniformly aligned and oriented

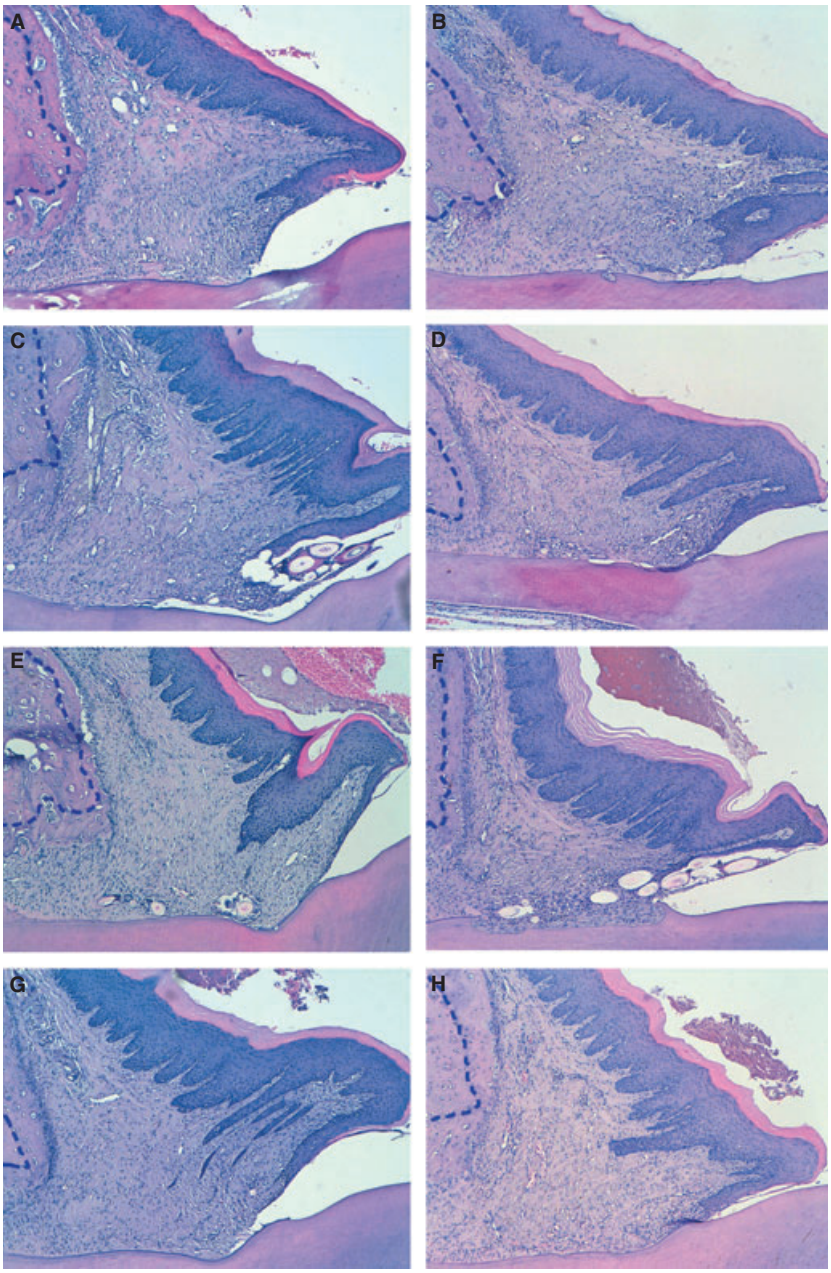

Fig. 4. Histology of specimens 7 d after irradiation with light-emitting diode (LED) light. (A, B) Specimens from (A) a site without *Porphyromonas gingivalis* lipopolysaccharide (Pg-LPS) treatment and (B) a site treated with *Pg*-LPS injection; neither site was irradiated with LED light. (C, D) Specimens from (C) a site without Pg-LPS treatment and (D) a site treated with *Pg*-LPS injection; both sites were irradiated with 5 J/cm^2 of LED light. (E, F) Specimens from (E) a site without Pg-LPS treatment and (F) a site treated with *Pg*-LPS injection; both sites were irradiated with 10 J/cm^2 of LED light. (G, H) Specimens from (G) a site without Pg-LPS treatment and (H) a site treated with *Pg*-LPS injection; both sites were irradiated with 15 J/cm^2 of LED light. The blue dashed lines indicate the border of the native alveolar bone crest (ABC). Hematoxylin and eosin stain, magnification 100×.

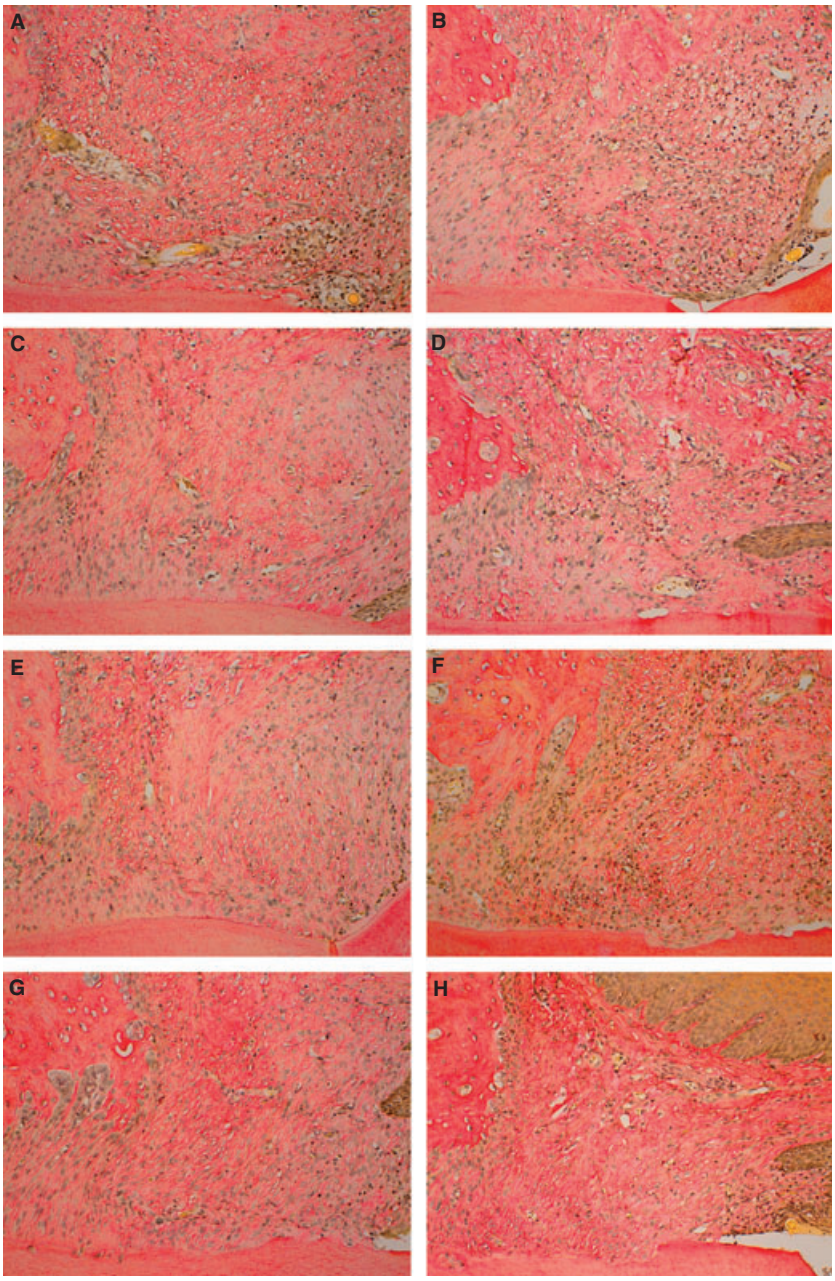

Fig. 5. Configuration of collagen fiber alignment in sites either not irradiated (A–D) or irradiated (E–H) with 10 J/cm^2 of light-emitting diode (LED) light. (A, B) Sites not irradiated with LED light: experimental day 3. (A) Site without *Porphyromonas gingivalis* lipopolysaccharide (Pg-LPS) treatment and (B) site treated with *Pg*-LPS injection. (C, D) Sites not irradiated with LED light: experimental day 7. (C) Site without Pg-LPS treatment and (D) site treated with *Pg*-LPS injection. (E, F) Sites 3 d after irradiation with 10 J/cm^2 of LED light. (E) Site without Pg-LPS treatment and (F) site treated with *Pg*-LPS injection. (G, H) Sites 7 d after irradiation with 10 J/cm^2 of LED light. (G) Site without Pg-LPS treatment and (H) site treated with *Pg*-LPS injection. PicroSirius Red Stain, magnification 200×.

bundles of collagen fibers compared with non-LED irradiated specimens (Fig. 5G and 5H). Furthermore, on day 14, all specimens demonstrated regularly aligned fibers and homogenous collagen matrix in the gingival connective tissue, and the patterns were similar, regardless of irradiation dose or LPS treatment (data not shown).

Profiles of inflammation and cellular proliferation

The profile of inflammatory infiltration was assessed by calculating the ratio of inflammatory cells to the total number of cells in the connective tissue (Fig. 6A–C). Reduction of inflammation was noted in specimens irradiated by 10 and 15 J/cm^2 of LED light at days 3 and 14 (Fig. 6A). In sites untreated with *Pg*-LPS injection, a slight decrease in inflammation was noted in LED light-treated specimens, and the decrease appeared more prominent, but not significant, at day 14 (Fig. 6B). Stronger inflammation was generally observed in sites treated with *Pg*-LPS injection, and reduction of inflammation was significant in specimens treated with 10 J/cm^2 of LED light at days 3 ($p < 0.05$) and 14 ($p < 0.05$), and in specimens treated with 15 J/cm^2 of LED light at day 14 ($p < 0.01$).

The profile of cellular proliferation was assessed by calculating the ratio of proliferating cells (PCNA-positive) to the total number of cells in the connective tissue (Fig. 6D–F). The ratio of proliferating cells tended to increase from day 3 to day 7 (Fig. 6D), and the extent of the increase was greater in sites untreated with *Pg*-LPS injection (Fig. 6E and 6F). At day 14, the profile of cellular proliferation was similar to that at day 7 in both Pg-LPS and non-Pg-LPS treated groups (data not shown). No significant difference was noted at any time point between animals that underwent treatment with any intensity of LED light.

Discussion

LLLT has been suggested as an adjuvant to traditional periodontal treatment because of its biostimulatory effect in periodontal cells (2,10–12), reducing periodontopathogen numbers (13), relieving inflammation (14,15) and increasing periodontal attachment level (16,17). However, a lack of adjunctive benefit has also been indicated in several investigations (18,19). Because light of 635-670 nm was found to be able to mediate both MAPK pathways and the production of proinflammatory cytokines to control

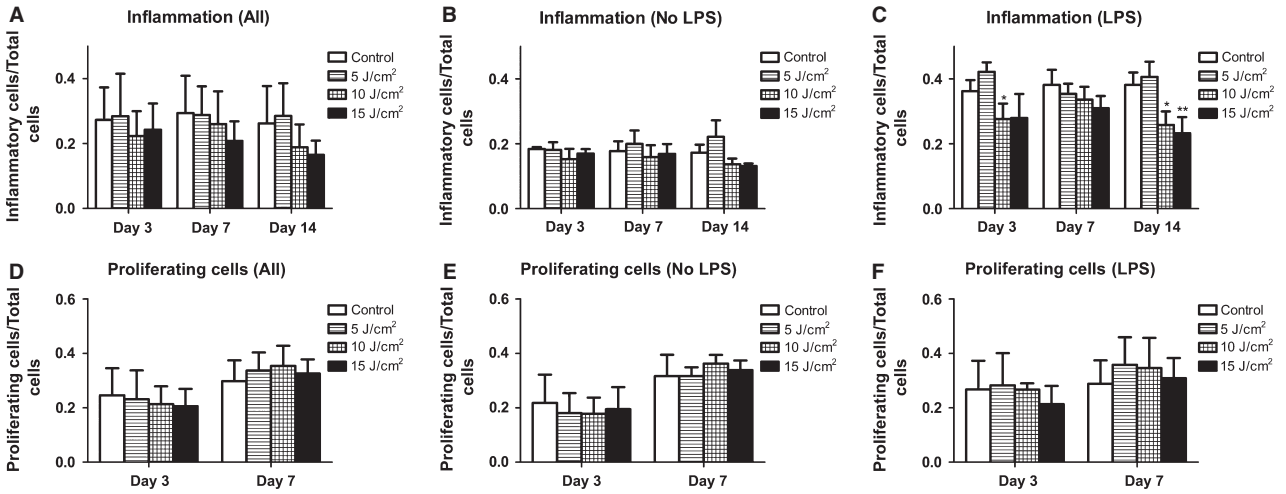

Fig. 6. (A-C) Ratio of inflammatory cells in (A) all experimental sites, (B) sites without *Porphyromonas gingivalis* lipopolysaccharide (Pg-LPS) treatment and (C) sites treated with *Pg*-LPS injection. (D-F) Ratio of proliferating cells in (D) all experimental sites, (E) sites without Pg-LPS treatment and (F) sites treated with *Pg*-LPS injection. $*p < 0.05$, $**p < 0.01$ (compared with control specimens).

periodontal inflammation *in vitro* (10), in the present *in vivo* study we focused solely on investigating the stimulatory effect of LED light, within the wavelength of 600–700 nm, on periodontal repair. Unlike the energy density suggested in most clinical studies (2–4 J/cm^2) (7,8,15), the energy density utilized in the present study was higher (5–15 J/cm^2). As an adjunctive treatment, in order to prevent masking of the biostimulatory effect by debridement and detoxification, the LED light was delivered 1 d after debridement and detoxification, and the resolution of gingival redness at that time indicated that the acute inflammation was reduced from traditional periodontal treatments. The results demonstrated that irradiation of periodontal tissues with 660-nm LED light could temporarily reduce periodontal bone loss and inflammatory infiltration, accelerate bundle bone deposition and facilitate collagen realignment. These effects appeared more prominent under the energy density of 10 J/cm^2, presumably because of its stimulatory effects on mitogenesis and osteogenic differentiation as well as the antibacterial effect demonstrated in our preliminary *in vitro* study (data not shown).

Accumulation of plaque on submerged ligatures has been an approach commonly used to induce periodontitis in rats (20), and adding bacterial LPS to up-regulate toll-like receptors and inflammatory mediators has been shown to resemble a more clinically relevant condition in recent investigations (21–23). It appears that ligature placement causes immediate acute inflammation within 7 d, but shifts to an induced quiescent established lesion after 14 d (23). An *in vitro* study demonstrated that LPS stimulates the expression of interleukin-1β and tumor necrosis factor-α after 24–48 h (22), and a delayed, but sustained, inflammation, including cytokine expression and periodontal bone loss, was evident *in vivo* (22,23). Thus, it is not surprising that the quantity of periodontal bone loss was similar to ligature-placed specimens with the additive *Pg*-LPS injection in this short-term study (Fig. 2). However, the effect of LPS led to more prominent and persistent inflammatory infiltration in LPS-treated specimens (Fig. 6B and 6C). On the other hand, irradiation with LED light appeared to be more efficient at inhibiting inflammation with the presence of *Pg*-LPS in the lesion, whereby the reduction of periodontal bone loss was slight and only occurred with light at an energy density of 10 J/cm^2 (Fig. 2C), possibly because of the significant attenuation of light energy as it penetrates more deeply into the inflamed tissue. In this regard, application of a supplementary medium (e.g. a GaAlAs laser) to improve the penetration efficiency might be needed (24).

Accelerated wound repair by LLLT was mechanistically initiated by activation of the mitochondrial photoacceptor molecule cytochrome *c* oxidase (25). Cytochrome *c* oxidase is a key molecule that increases respiratory metabolism and thus stimulates the cellular processes (26). Within wavelengths of 600–700 nm, LLLT has been demonstrated to promote gingival fibroblast proliferation and collagen matrix deposition (27,28). An *in vivo* study also indicated that LLLT significantly increases the expression of fibronectin and the synthesis of type I collagen during tooth movement in rats (29). Therefore, cytochrome *c* oxidase also exists in inflammatory and immune cells (30) and may have been activated by LLLT. In this regard, thorough removal of etiological factors (i.e., plaque retentive factors and periodontopathogens) may be a prerequisite to assure the healing capability of LLLT. In the present study, accelerated collagen deposition and higher cellular proliferative profiles confirmed the effect of LLLT reported in previous studies, and we further demonstrated that most newly formed collagen fibers were well aligned and were inserted perpendicularly to the root surface following treatment with LED light (Fig. 5), indicating that the new connective tissue was established to restore the native attachment. Furthermore, LLLT was able to increase

the initial deposition of bone to accelerate ossification in the tooth-extraction wound (31) and promote early osseointegration (9). As such, the present study interestingly demonstrated that bundle bone apposition was evident in specimens treated with 10 J/cm^2 of LED light, specifically in the specimens with additive *Pg*-LPS injection (Fig. 4). While the bundle bone was the only site for the insertion of the periodontal ligament, the laying down of the new bone matrix may further stimulate the reorganization of the periodontal ligament to reattach on the surface of alveolar bone (32). Taken together, these findings validated the beneficial effect of treatment with LED light in promoting periodontal attachment.

The major limitation of this study was the difficulty of plaque control, whereby the existence of plaque might potentially recruit inflammatory cells, induce the secretion of proinflammatory cytokines, increase vascularity and lead to the loss of collagen (33–35). Those effects might be even more prominent in the sites treated with *Pg*-LPS injection. Additionally, this experimental model of periodontitis might not be relevant to adult periodontitis. Only *Pg*-LPS (and no other virulence factors) was delivered with ligature placement, and periodontitis was induced over a short time-period. Moreover, the biostimulatory effect of a single course of irradiation with LED light might be limited. As shown in Fig. 2, reduction of periodontal bone loss was evident only at day 3, presumably as a result of the promotion of bundle bone deposition following treatment with 10 J/cm^2 of LED light in the early stage (Fig. 3F). However, the stimulatory effect was attenuated, resulting in a similar level of periodontal bone loss as observed in non-LED light-treated specimens at days 7 and 14. Given that long-term clinical improvement following LLLT is also controversial and unpredictable (17,36), applying supplementary media to assist the penetration of LED light or to maintain the energy level, as well as repeated irradiation, may be still necessary to sustain the stimulatory effects.

Within the limitations of the study, it is concluded that irradiation with LED light at a wavelength of 660 nm and an energy density of 10 J/cm^2 can temporarily facilitate the process of periodontal repair by reducing inflammation, promoting bundle bone apposition and stimulating collagen alignment. However, clarification of the gene and protein expression levels of proinflammatory cytokines, and wound-repair and bone-formative markers, is still needed. Further studies will aim at a combination of 660-nm wavelength LED light with supplementary media (GaAlAs laser or photosensitive medium such as methylene blue) to irradiate periodontal tissue. We will also evaluate the effectiveness of repeated irradiation, and thus determine the protocol of LED light irradiation as an adjunctive modality to promote periodontal repair. As these results were obtained from an animal model, they may or may not be achieved in similar treatment of human periodontal diseases, further clinical investigation is still needed.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Histology of specimens after LED light irradiation at day 14, Hematoxylin and Eosin Stain, 100×. (A-B) specimen without LED light irradiation in the (A) site without *Porphyromonas gingivalis* lipopolysaccharide (Pg-LPS) treatment and (B) site treated with Pg-LPS injection. (C-D) specimen 5 J/cm2 LED light irradiation in the (C) site without Pg-LPS treatment and (D) site treated with Pg-LPS injection. (E-F) specimen 10 J/cm2 LED light irradiation in the (E) site without Pg-LPS treatment and (F) site treated with Pg-LPS injection. (GH) specimen 15 J/cm2 LED light irradiation in the (G) site without Pg-LPS treatment and (H) site treated with Pg-LPS injection. (Blue dash lines indicate the border of native alveolar bone crest.)

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