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Effect of Photobiomodulation on Periodontal Ligament Cells Under Inflamed and Nutrient-Deficient Conditions Simulating Damaged Cells of Avulsed Teeth: An *In Vitro* Study

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Abstract

Background: The prognosis of replanted teeth is depended on the vitality of periodontal ligament cells residual on the root surface. Photobiomodulation has photobiological effects that can promote cell vitality. The study aimed to explore the effect of photobiomodulation on the periodontal ligament cells under inflamed or starved conditions mimicking clinically damaged periodontal ligament cells of avulsed teeth and provide the adjuvant procedure for tooth replantation.

Materials and methods: Normal, starved, or inflamed periodontal ligament cells were irradiated with an 808 nm laser at densities of 0, 1, 3, 5, or 10 J/cm^2. The cell counting kit-8 (CCK-8) assay and scratch test were applied to determine the effects on the proliferation and migration of cells. Anti-inflammatory effects were assessed according to the mRNA expression of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) measured by reverse-transcription polymerase chain reaction. Osteogenic capacity was evaluated by alkaline phosphatase (ALP) staining, ALP activity assay, Alizarin Red S staining, and ALP and osteocalcin (OCN) mRNA expression.

Results: The CCK-8 assay and scratch test demonstrated that the 808 nm laser significantly promoted proliferation and migration of normal condition periodontal ligament cells at a density of 3 J/cm^2 versus 5 J/cm^2 under the starved and inflamed conditions. Moreover, the 808 nm laser had anti-inflammatory effects and promoted osteogenesis of periodontal ligament cells at 3 J/cm^2 under normal conditions, while photobiomodulation at 5 J/cm^2 upregulated the osteogenesis of periodontal ligament cells under starved and inflamed conditions.

Conclusions: The photobiomodulation of 808 nm laser reduced inflammation and improved the proliferation, migration, and osteogenesis of normal, starved, and inflamed periodontal ligament cells. These effects required a higher energy density under starved or inflamed conditions compared with normal conditions. The photobiomodulation of 808 nm has a potential application in root surface treatment for replanted teeth.

Keywords: photobiomodulation, replanted teeth, periodontal ligament cells, inflamed condition, nutrient-deficient condition

Introduction

Dental avulsion accounts for 16% of all dental injuries and is the most severe one.[1,2] Replantation is the common treatment for avulsed teeth, and the prognosis is depended on the vitality of the periodontal ligament cells on root surface.[3] Delayed replantation and prolonged nonphysical storage causes damage to the periodontal ligament cells and contributes to inflammatory and replacement resorption. This sustaining process can result in replantation failure and

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tooth loss.[4] The biomarkers in serum, saliva, and tissues released during inflammatory conditions could be significant predictors for the early detection.[5–7] A number of studies have tried several protocols, such as those involving sodium fluoride,[8,9] propolis,[10] alendronate,[11,12] and tooth enamel protein (Emdogain),[13,14] to alleviate inflammatory and replacement resorption. However, the results of those studies showed that these approaches were not satisfactory or partially effective. Therefore, to find an appropriate treatment that is effective and biocompatible, reducing the inflammatory reaction and the degree of root damage are considered of prime importance in avulsion tooth treatment.

Previous studies showed that photobiomodulation promoted the tissue healing by improving the local microcirculation, collagen synthesis, and fibroblast proliferation.[15–18] Its good biocompatibility has attracted the researchers' attention and used the photobiomodulation as a clinical adjuvant procedure for tooth replantation. However, the effects of photobiomodulation have remained inconclusive due to a dearth of studies on laser parameters, which could alter the response of biological tissues.[19] Among the available low-level lasers, the 808 nm laser showed good biocompatibility and strong penetrability, which was beneficial for tissue regeneration and improvement in the long-term prognosis of replantation.[20,21] However, *in vitro* studies have focused mostly on normal periodontal ligament cells. In reality, the periodontal ligament cells of the replanted teeth are damaged and exposed to starvation and inflammation condition in most cases. Furthermore, few researches have evaluated the effects of photobiomodulation on damaged periodontal ligament cells, and the specific laser parameters are unclear. Thus, to explore the photobiomodulation effect and specific parameters of 808 nm laser as adjuvant procedure for tooth replantation, we investigated the effects of the 808 nm laser on periodontal ligament cells under normal, starved, and inflamed conditions simulating clinically damaged periodontal ligament cells of avulsed teeth.

Materials and Methods

Periodontal ligament cells culture

Oral Stem Cell Bank of Beijing Tason Biotech Co., Ltd. (Beijing, China) donated the periodontal ligament cells for the experiment. The periodontal ligament cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, Thermo Fisher Scientific, Waltham, MA, USA), which was supplemented with 10% fetal bovine serum (FBS; ScienCell, Carlsbad, CA, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin and maintained in 5% CO_2, 95% humidified atmosphere at 37°C. Cells used for the following experiments were from passage 4 to 6.

Photobiomodulation protocol

Photobiomodulation was performed with an 808 nm wavelength continuous wave diode laser (Beijing Laserwave Optoelectronics Technology Co., Ltd., Beijing, China). The adjacent wells were covered with aluminum foils from exposure to scattered laser. Before irradiation, the average energy at the end of the fiber was determined using a power meter (THORLABS GmbH, Dachau, Germany). Power output was set constant at 20 mW/cm^2, and the duration of irradiation was 50, 150, 250, or 500 sec. As a function of the irradiation time, the energy density was 1, 3, 5, or 10 J/cm^2, respectively. The specific parameters of the laser in the study were shown in Table 1.

Establishment of starved and inflamed periodontal ligament cells

The induction of nutritional deficiency was used to simulate *in vivo* stress conditions.[22] Periodontal ligament cells were plated at 1×10^5/well in six-well plates containing DMEM with 10% FBS, after the cells attachment, the medium was suctioned out and exchanged with fresh culture medium without FBS for 24 h to mimic nutritional deficiency. After the 24 h, the cells were cultured in normal condition with the following experiments to mimic the clinical situation which the avulsed teeth were replanted into the socket, and the cells could obtain the nutrition from the tooth socket.

The periodontal ligament cells were placed onto six-well plates. To induce inflamed cells, 10 μg/mL *Escherichia coli* lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, MO, USA) were put in the plates and incubated for 1, 3, or 6 h, then the total RNA was extracted with the TRIzol reagent. The mRNA was reverse transcripted into cDNA with the PrimeScript™RT Reagent Kit (TAKARA, Shiga, Japan). The mRNA expression of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) was detected with reverse-transcription polymerase chain reaction (RT-PCR) using Power SYBR Green PCR Master Mix (Rox) (Roche Applied Science, Indianapolis, IN, USA) with the ABI PRISM 7500 Sequence Detection System. The data were analyzed using the $2(-\Delta\Delta Ct)$ relative expression method. Table 2 shows the primers used in the present study. The LPS incubation period which led to the strongest inflammatory reaction was used to induce inflamed periodontal ligament cells.

The starved and inflamed periodontal ligament cells were subjected to the 808 nm laser treatment at densities of 0, 1, 3, 5, and 10 J/cm^2. The detailed experimental design was described in Table 3.

Cell proliferation assay

The influence of the 808 nm laser on the proliferation of periodontal ligament cells under different energy densities was evaluated with cell counting kit-8 (CCK-8) assay. The

Table 1. The Laser Parameters Used in the Experiment

Name of the manufacturer	*Laserwave Optoelectronics Technology*
Geographic location	Beijing, China
Equipment model	Continuous
Number and type of emitters	Single emitter
Wavelength and bandwidth (nm)	808 nm
Pulse mode (CW or Hz)	CW
Power density measured (mW/cm^2)	20
Exposure duration (sec)	50, 150, 250, 500
Radiant exposure (J/cm^2)	1, 3, 5, 10
Application technique	Photobiomodulation
Number and frequency of treatment sessions	Single session

Table 2. Primers Used for Reverse-Transcription Polymerase Chain Reaction

Gene	*Primer sequence*	*Product size (bp)*
β-actin	Forward: CCTGGCACCCAGCACAAT Reverse: GGGCCGGACTCGTCATACT	144
TNF-α	Forward: GGCTCCAGGCGGTGCTTGTTC Reverse: CAGGCTTGTCACTCGGGGTTCG	190
IL-6	Forward: GGTGTTGCCTGCTGCCTTCC Reverse: TGCCTCTTTGCTGCTTTCACAC	193
IL-1β	Forward: GGCAGGCCGCGTCAGTTG Reverse: CCCGGACGTGCAGTTCAGT	198
OCN	Forward: AGCAAAGGTGCAGCCTTTGT Reverse: GCGCCTGGGTCTCTTCACT	261
ALP	Forward: CCACGTCTTCACATTTGGTG Reverse: AGACTGCGCCTGGTAGTTGT	196

ALP, alkaline phosphatase; IL-1β, interleukin-1β; IL-6, interleukin-6; OCN, osteocalcin; TNF-α, tumor necrosis factor-α.

initial density of cells inoculated in 96-well plate was 2×10^3. The periodontal ligament cells under starved, inflamed, and normal conditions were treated with various energy densities (0, 1, 3, 5, and 10 J/cm^2). On the day 1, day 3, day 5, and day 7, 10 μL CCK-8 solution (Dojindo, Beijing, China) was put in the plate and incubated for 2 h. The automatic enzyme-linked immunosorbent assay reader (ELx808; BioTek Instruments, Winooski, VT, USA) at 450 nm was used to detect the cells' absorbance.

Cell plating and scratching test

The periodontal ligament cells were seeded at 1.5×10^4/well in an ImageLock® 96-well plate (Essen BioScience, Ann Arbor, MI, USA). After the cells reaching confluence, a scratch was made in each well with WoundMaker™ (Essen BioScience). Then, the wells were cleaned with sterile PBS to take out the floated cells. After scratching, the 808 nm laser was used to irradiate at different energy densities (0, 1, 3, 5, and 10 J/cm^2). The cells were incubated in DMEM without FBS and automatically taken pictures at the same location every 3 h during the 24 h laser irradiation period with the IncuCyte ZOOM™ live cell imaging system (Essen BioScience). Each group consisted of three wells.

Table 3. Experimental Groups (Normal, Starved, or Inflamed Periodontal Ligament Cells) According to the 808 nm Laser Parameters

	Energy density (J/cm^2)	*Power (mW/cm^2)*	*Time (sec)*
Starvation group (pretreated without FBS for 24 h)	1	20	50
	3		150
	5		250
	10		500
	Nonirradiated	—	—
Inflammation group (pretreated with 10 μg/mL LPS for 3 h)	1	20	50
	3		150
	5		250
	10		500
	Nonirradiated	—	—
Normal group	1	20	50
	3		150
	5		250
	10		500
	Nonirradiated	—	—

FBS, fetal bovine serum; LPS, lipopolysaccharide.

Osteogenic differentiation

The normal, starved, and inflamed periodontal ligament cells were cultured until they occupied 80% and then replaced with osteogenic medium (DMEM, 10%FBS, 50 mg/mL ascorbic acid, 100 nM dexamethasone, and 5 mM β-glycerophosphate; Sigma Aldrich), and the cells were osteogenic induction for 7 or 21 days. The cells were divided into five groups according to the 808 nm at different energy densities: 0, 1, 3, 5, and 10 J/cm^2. Laser irradiation was repeated at day 0, 5, 10, and 15.

Alkaline phosphatase and alizarin red staining

After 7 days' incubation with osteogenic medium, the alkaline phosphatase (ALP) activity of periodontal ligament cells was measured using the Alkaline Phosphatase Assay Kit (Beyotime, Shanghai, China). After reaction with *p*-nitrophenyl phosphate, the absorbance at 405 nm was measured to detect the ALP activity. After 21 days' osteogenic induction, 4% paraformaldehyde was used to fix the periodontal ligament cells for 30 min and 1% alizarin red S (Sigma-Aldrich) solution was used to stain the cells. The 10% cetylpyridinium chloride (Sigma-Aldrich) was used to solubilize the stained cells and quantify the alizarin red-stained nodules, and it was measured with the absorbance at 590 nm. The ALP and osteocalcin (OCN) mRNA was evaluated by RT-PCR. The primers used are listed in Table 2.

Statistical analysis

In the present study, all the experiments repeated three times. The data were analyzed with the independent sample *t*-test and one-way ANOVA method using the SPSS version 20.0 (IBM Corp., Armonk, NY, USA). The *p*-value <0.05 was set as significant difference.

Results

Inflammatory cytokines expression in periodontal ligament cells

After being treated with 10 μg/mL LPS, RT-PCR results showed that the periodontal ligament cells mRNA

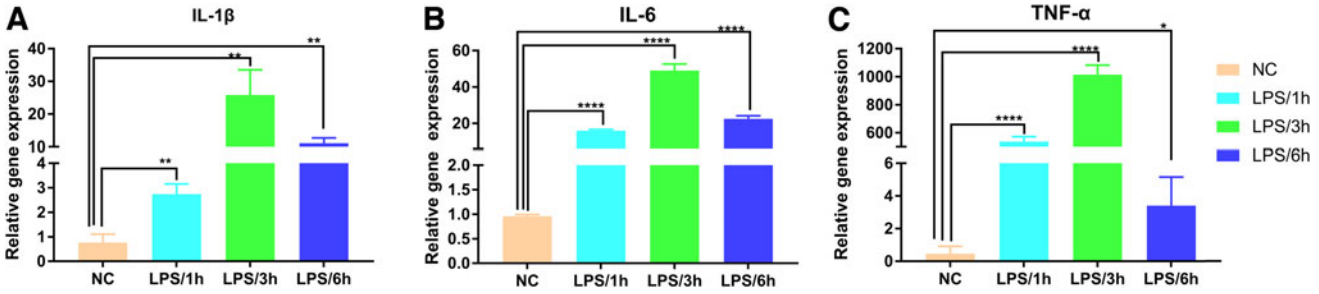

FIG. 1. RT-PCR analysis showed IL-1β, IL-6, and TNF-α increased significantly after pretreatment with LPS. All tested cytokine levels were increased at 1, 3, and 6 h, with the highest levels at 3 h. The asterisks indicate significant differences between groups (mean ± SD, $n=3$, $*p<0.05$, $**p<0.01$, $****p<0.0001$). **(A)** The mRNA expression of IL-1β; **(B)** The mRNA expression of IL-6; **(C)** The mRNA expression of TNF-α. IL-1β, interleukin-1β; IL-6, interleukin-6; LPS, lipopolysaccharide; RT-PCR, reverse-transcription polymerase chain reaction; SD, standard deviation; TNF-α, tumor necrosis factor-α.

expression of TNF-α, IL-1β, and IL-6 was raised up remarkably at 1, 3, and 6 h ($p<0.05$), and the most significant increase was observed at 3 h for all three cytokines (Fig. 1). In the following experiments, pretreatment with 10 μg/mL LPS for 3 h was used to induce inflamed periodontal ligament cells.

Photobiomodulation effect of 808 nm laser on the periodontal ligament cells proliferation.

In the present study, the proliferation of normal, starved, and inflamed periodontal ligament cells was measured using the CCK-8 assay. Under normal conditions, the cells proliferated stably within 7 days after laser irradiation. Compared with nonirradiated cells, 3 and 5 J/cm^2 irradiation increased cell activity at 5 and 7 days ($p<0.05$), and 3 J/cm^2 irradiation significantly promoted proliferation at 7 days ($p<0.001$). Under the starved condition, the periodontal ligament cells continued to proliferate after laser irradiation, although the proliferation rate was lower than that under the normal condition. After irradiation at 3 and 5 J/cm^2, cell activity was increased compared with nonirradiated cells at 5 and 7 days ($p<0.05$). Under the inflamed condition, similar to the starved condition, 5 J/cm^2 irradiation significantly promoted proliferation of periodontal ligament cells (Fig. 2).

Photobiomodulation effect of 808 nm laser on the migration of periodontal ligament cells

To study the effect of 808 nm laser on the migration of normal, starved, and inflamed periodontal ligament cells, the cells were scratched using WoundMaker. Images of the migrating cells and the relative width of the scratch are shown in Fig. 3. Under the normal condition, the migration width of periodontal ligament cells was promoted after laser irradiation, especially at 3 J/cm^2 compared with the nonirradiated cells ($p<0.001$). While for the starved and inflamed periodontal ligament cells, 5 J/cm^2 significantly promoted cells migration ($p<0.01$).

Photobiomodulation effect of 808 nm laser on the periodontal ligament cells inflammation

To find out the anti-inflammatory effect of the 808 nm laser, the nonstimulated periodontal ligament cells were set as a negative control group, while 10 μg/mL LPS-stimulated cells were deemed as positive control group. The study found that the mRNA expression of TNF-α, IL-1β, and IL-6 was decreased after the density of 1, 3, 5, or 10 J/cm^2 irradiation, compared with the nonirradiated group ($p<0.01$), with a significant decrease at the 3 J/cm^2 energy density (Fig. 4).

Photobiomodulation effect of 808 nm laser on the osteogenesis of periodontal ligament cells

The photobiomodulation effect of 808 nm laser on the osteogenic differentiation of normal, starved, and inflamed periodontal ligament cells was evaluated with the early and later osteogenic differentiation. When the periodontal ligament cells were irradiated after the 808 nm laser, the ALP activity and mRNA expression of ALP and OCN, as well as mineralized nodules were increased ($p<0.05$); furthermore,

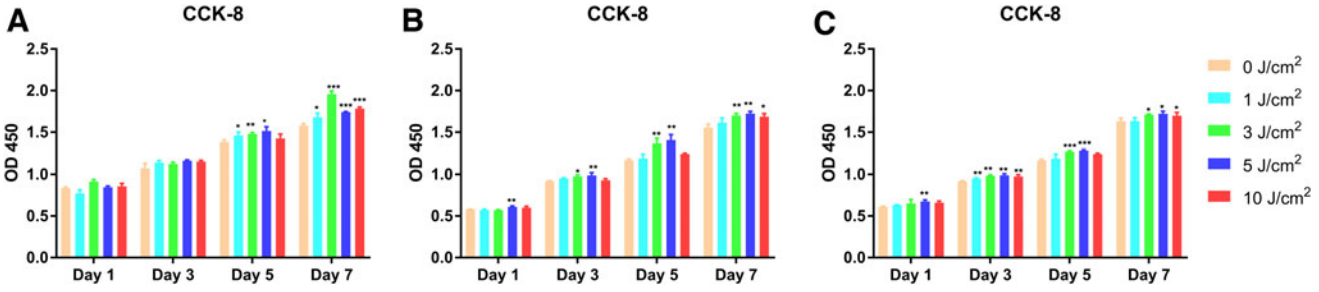

FIG. 2. CCK-8 assay showed the proliferation of periodontal ligament cells. **(A)** Under normal conditions, 3 J/cm^2 significantly promoted the proliferation of periodontal ligament cells ($p<0.001$); **(B)** Under starved conditions, 5 J/cm^2 enhanced the proliferation of periodontal ligament cells ($p<0.01$); **(C)** Under inflamed conditions, 5 J/cm^2 promoted the proliferation of periodontal ligament cells ($p<0.05$). CCK-8, cell counting kit-8.

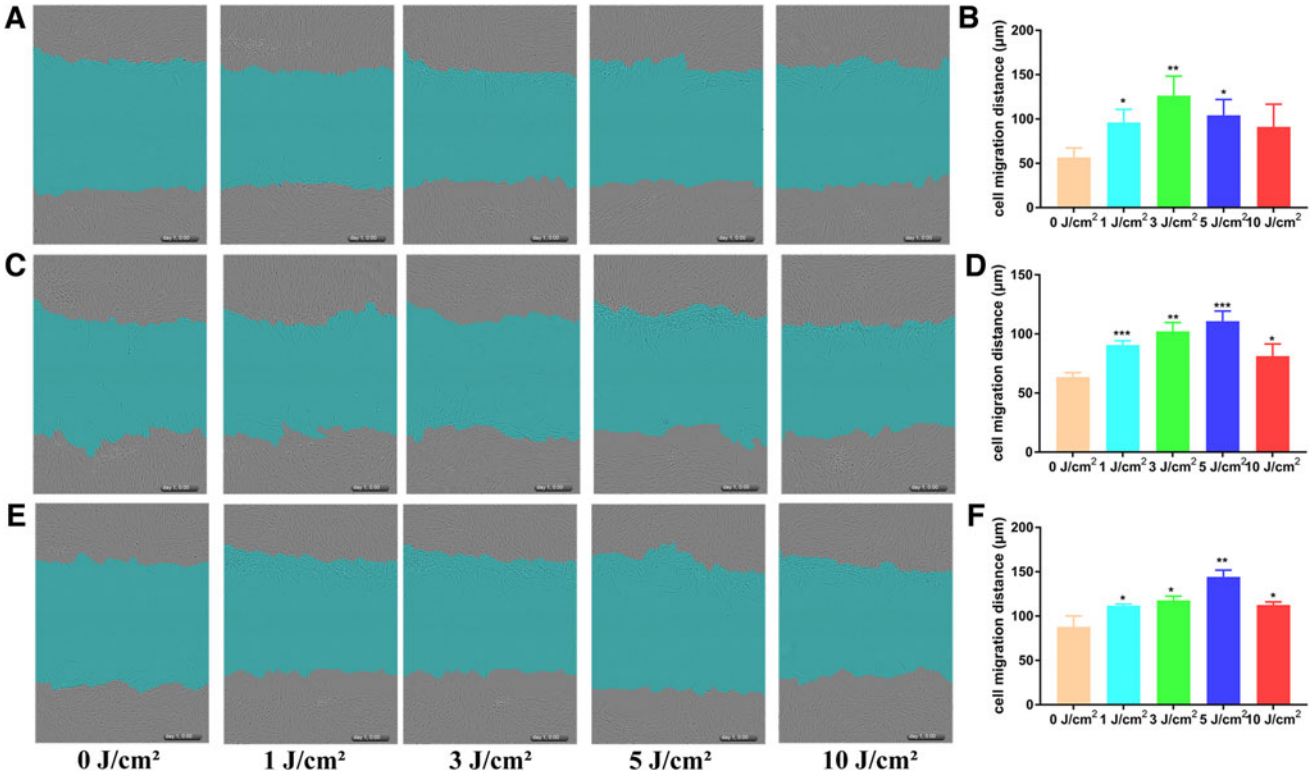

FIG. 3. Migration of periodontal ligament cells after 808 nm laser irradiation at different energy densities. The light blue area in the figure represents the initial width of the cell scratch, and the change in the scratch width after cells migrated into the area is shown. The migration of the periodontal ligament cells increased significantly at 3 J/cm^2 under normal conditions and 5 J/cm^2 promoted the migration of the cells under the starved and inflamed conditions. **(A)** The migration pictures of cells under normal condition; **(B)** The statistic analysis of the migration of normal condition cells; **(C)** The migration pictures of cells under starved condition; **(D)** The statistic analysis of the migration of starved cells; **(E)** The migration pictures of cells under inflamed condition; **(F)** The statistic analysis of the migration of inflamed cells.

irradiation at 3 J/cm^2 significantly promoted osteogenic differentiation of cells under normal condition. While under starved and inflamed conditions, irradiation at 5 J/cm^2 significantly promoted osteogenic potential, as evidenced by the increase of ALP activity, ALP and OCN expression, mineralized staining nodules, and calcium levels ($p < 0.05$) (Fig. 5).

Discussion

The prognosis of replanted teeth is largely depended on the vitality of periodontal ligament cells residual on the root surfaces.[3] When the tooth is out of the socket, the blood supply is disrupted, and nutrition is deficient.[23] Furthermore, the tooth is exposed to contaminated environments. The

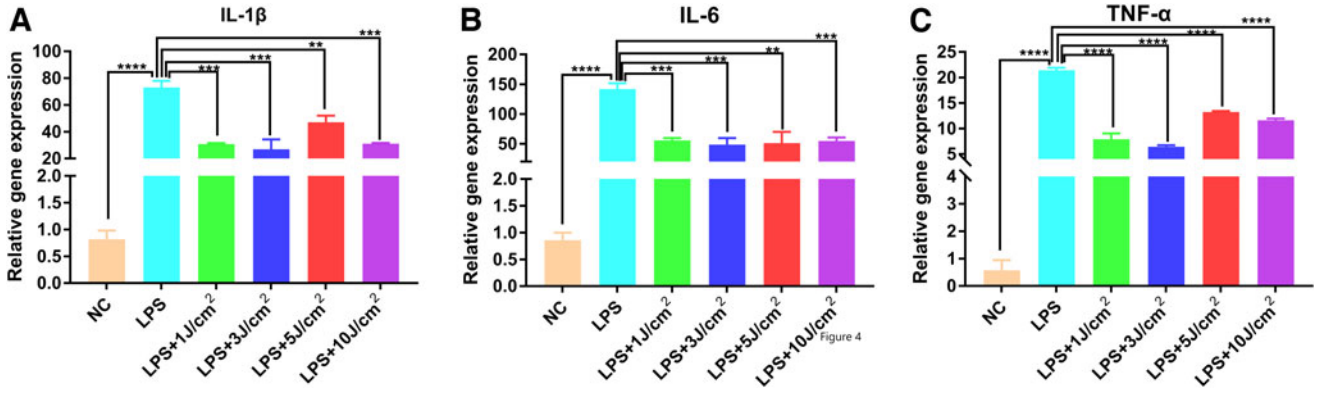

FIG. 4. Expression of inflammatory cytokines in periodontal ligament cells stimulated by LPS after 808 nm laser irradiation at different energy densities. The mRNA expression of IL-1β, IL-6, and TNF-α declined after 808 nm laser irradiation. **(A)** The mRNA expression of IL-1β; **(B)** The mRNA expression of IL-6; **(C)** The mRNA expression of TNF-α.

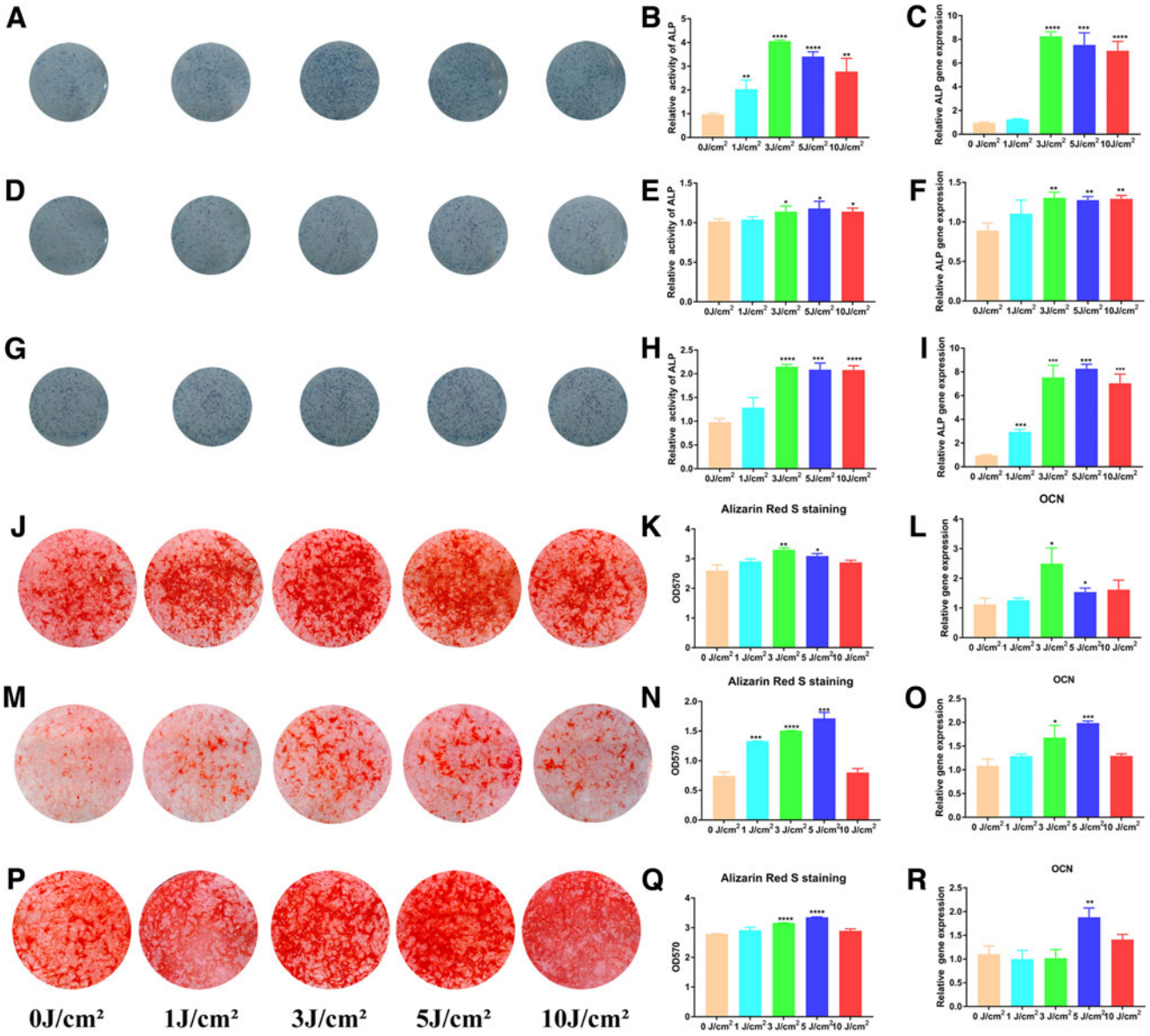

FIG. 5. Effect of photobiomodulation of 808 nm laser on the osteogenic differentiation of periodontal ligament cells. **(A, D,** and **G)** ALP staining of cells under normal, starved, and inflamed condition at different energy densities; **(B, E,** and **H)** ALP activity of cells under normal, starved, and inflamed condition at different energy densities; **(C, F,** and **I)** The mRNA expression of ALP of cells under normal, starved, and inflamed condition at different energy densities; **(J, M,** and **P)** Alizarin Red S staining of cells under normal, starved, and inflamed condition at different energy densities; **(K, N,** and **Q)** The calcium levels statistic analysis of cells under normal, starved, and inflamed condition at different energy densities; **(L, O,** and **R)** The mRNA expression of OCN of cells under normal, starved, and inflamed condition at different energy densities. ALP, alkaline phosphatase; OCN, osteocalcin.

results of the present study showed that the photobiomodulation of 808 nm laser significantly reduced the inflammation reaction and promoted the proliferation, migration, and osteogenic differentiation of normal condition periodontal ligament cells at 3 J/cm^2, while the effective energy density for periodontal ligament cells under starved and inflamed conditions was at 5 J/cm^2. Our results demonstrated that photobiomodulation of 808 nm laser had good biological properties and was beneficial for the periodontal ligament cells under starved and inflamed conditions, although at a higher energy compared with normal condition.

The periodontal ligament cells were exposed to the *in vitro* nutrient deficient conditions to simulate *in vivo* starvation conditions.[22] Taglian et al. irradiated odontoblast-like cells under experimentally induced nutritional deficiency using an 808 nm laser at 1.5 J/cm^2 and reported an increase in cell metabolism.[24] da Silva et al. also found that irradiation with 660 nm at 5 J/cm^2 contributed to maintaining the stem cells membrane integrity subjected to nutritional deficiency, as well as improved the cells viability.[22] Choi et al. found that 810 nm laser at 3.94 J/cm^2 promoted the proliferation of normal condition periodontal ligament fibroblasts.[16] In the

present study, photobiomodulation of 808 nm laser significantly promoted the proliferation of periodontal ligament cells under normal conditions at 3 J/cm^2 and under starved and inflamed conditions at 5 J/cm^2. The energy density required to increase periodontal ligament cells proliferation was higher under stress condition than normal condition. Photobiomodulation of 808 nm laser with appropriate energy density can effectively enhance the proliferation of periodontal ligament cells under the damaged state, which is conducive to the promotion of the regeneration of periodontal ligament after replantation.

The effect of photobiomodulation was dose dependent. At low doses, photobiomodulation had no stimulatory effect on cells. However, when the energy density exceeded the peak, it caused a negative response. In the present study, 1 J/cm^2 did not stimulate the periodontal ligament cells proliferation under normal and stress condition, while the energy density at 10 J/cm^2 was less effective than 3 or 5 J/cm^2 in promoting periodontal ligament cells proliferation. This was in accordance with the diaphasic dose–response rule of photobiomodulation (Arndt–Schulz curve).[25] The photobiomodulation of 808 nm laser promoted the proliferation of the normal and stress condition periodontal ligament cells and it followed the rule of diaphasic dose–response.

Wound healing is a highly coordinated process that reestablishes the integrity of various tissues. The periodontal ligament cells migration to the root surface is a prerequisite step for periodontal ligament regeneration. Tsuka et al. confirmed that Nd:YAG irradiated at 10.34 J/cm^2 stimulated osteoblast cell migration and increased the adenosine triphosphate (ATP) production.[26] Yin et al. found that irradiation with the wavelength of 660 nm improved the cellular repair capacity by altering the expression of hepatocyte growth factor (HGF) and platelet derived growth factor (PDGF).[27] Evans and Abrahamse showed that 632.8 nm photobiomodulation at an energy of 5 J/cm^2 increased the cell migration and haptotaxis of wounded human skin fibroblasts.[28] In the present study, photobiomodulation with 808 nm laser promoted the periodontal ligament cells migration under normal, starved, and inflamed conditions, confirming that photobiomodulation could be the precondition of the periodontal ligament cells before tooth replantation, thus promoting the tissue regeneration.

The most common complications of replanted teeth are inflammatory and replacement resorption. Alleviating the inflammation of periodontal ligament cells is important for the prognosis of avulsed teeth. In the current study, the results showed that the mRNA expression of TNF-α, IL-1β, and IL-6 in the inflamed periodontal ligament cells was suppressed after photobiomodulation of 808 nm laser. The anti-inflammatory effect of the photobiomodulation of 808 nm laser was similar to that in the aforementioned studies. Vilela et al. found that numbers of inflammatory cells and necrotic areas were reduced after 685 nm laser irradiation.[29] Boschi et al. showed that irradiated with 660 nm laser significantly reduced the IL-6 and TNF-α expression.[30] Recent studies had shown that IL-6 and Galectin-3 could be early biomarkers for the detection of periodontitis in serum and saliva.[5,6] Photobiomodulation reduced the inflammation reaction by activating signaling pathways and downregulating the expression of inflammatory cytokines. The mechanism of inflammation alleviation by photobiomodulation may involve the cAMP/NF-κB signaling pathway[17] or the C-Raf/Erk1/2 signaling pathway;[31] this requires further research.

Periodontal ligament cells osteogenic differentiation is an important healing process after tooth replantation, and it is helpful for the replanted teeth to be early retained in the alveolar socket. The inflammation and nutritional deficiency was harmful to the osteogenic potential of periodontal ligament cells. Only a few studies have examined the effect of photobiomodulation on the osteogenic differentiation of periodontal ligament cells under inflamed and starved conditions. Amarol et al. found that photobiomodulation with 808 nm laser could regulate bone marrow stromal cells differentiation and enhance osteogenesis.[32] Wang et al. investigated the osteogenesis of induced inflammation bone marrow stromal cells with 1064 nm laser and found that at an energy of 8 J/cm^2 promoted the osteogenesis of the inflamed cells.[33] In the current study, our results confirmed that photobiomodulation with 808 nm laser promoted osteogenic differentiation of starved and inflamed periodontal ligament cells. It was speculated that photobiomodulation could increase the concentration of intracellular calcium ions. Under the circumstance of induction of osteogenic differentiation, intracellular calcium ions were released outside of the cells and gradually accumulated to form mineralized nodules,[34] thus to improve the ability of damaged periodontal ligament cells' osteogenic differentiation. The stimulatory effect of the photobiomodulation on starved and inflamed periodontal ligament cells' osteogenic differentiation was beneficial for the prognosis of the replanted teeth.

In summary, we explored the effects of photobiomodulation with 808 nm laser at different energy densities on periodontal ligament cells under normal, starved, and inflamed microenvironments imitated the clinical situation of avulsed teeth. The major findings were as follows: (1) photobiomodulation with 808 nm laser could significantly reduce the inflammation and promote the proliferation, migration, and osteogenesis of normal condition periodontal ligament cells at a density of 3 J/cm^2. (2) Photobiomodulation with 808 nm laser could enhance the proliferation, migration, and osteogenesis of starved and inflamed periodontal ligament cells at the density of 5 J/cm^2. The density was higher than the optimum density for the periodontal ligament cells under normal condition. The results revealed the favorable effects of photobiomodulation of 808 nm laser on the biological characteristics of normal and stressed condition periodontal ligament cells; the different energy density between the normal and stressed conditions could assist clinicians to set proper parameters when they used the photobiomodulation of 808 nm laser as an adjuvant therapy for delayed tooth replantation.

Author Disclosure Statement

No competing financial interests exist.

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