Assessment of the Photobiomodulation Effect of a Blue Diode Laser on the Proliferation and Migration of Cultured Human Gingival Fibroblast Cells: A Preliminary *In Vitro* Study

Ardavan Etemadi¹, Soheil Taghavi Namin², Mahshid Hodjat³, Emad Kosarieh⁴, Neda Hakimiha^{5*}

¹Assistant professor, Department of periodontology, Faculty of Dentistry, Islamic Azad university, Tehran, Iran; Laser Research Center of Dentistry, Dental Research Institute, Tehran University of Medical Sciences, Tehran, Iran

²DDS, Private practice, Tehran, Iran

³PhD, Assistant Professor, Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

⁴DDS, MSc, Periodontist, Laser Research Center of Dentistry, Dental Research Institute, Tehran University of Medical Sciences, Tehran, Iran

⁵DDS, PhD candidate in laser dentistry, Laser Research Center of Dentistry, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

*Correspondence to

Neda Hakimiha, DDS, PhD
candidate in laser dentistry, Laser
Research Center of Dentistry,
Dentistry Research Institute,
Tehran University of Medical
Sciences, Tehran, Iran. Postal
address: Qods street, Enqelab
Avenue, Tehran, Iran.
Postal code: 1417614411;
Tel: +982188384306;
Email:
Ned.hakimiha@gmail.com

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Abstract

Introduction: Photobiomodulation therapy (PBM) is emerging as an effective strategy for the management of wound healing. The application of red and near infra-red light sources in laser therapy has been the subject of most researches in recent literature. Considering the lack of sufficient evidence in assessing the blue light in PBM, we aimed to investigate the photobiomodulation effect of a blue diode laser on the proliferation and migration of cultured human gingival fibroblast cells as a preliminary in vitro study.

Methods: Human gingival fibroblast cells were irradiated with a blue diode laser at a 445 nm wavelength. Irradiation was done using three different powers of 200 mW (irradiation times of 5, 10, 15, and 20 seconds); 300 mW (irradiation times of 5, 10, and 15 seconds); and 400 mW (irradiation times of 5 and 10 seconds). The fibroblast cells without laser exposure were considered as control. After 24 hours of incubation, the MTT assay and the wound scratch test were performed on the cells to investigate the biomodulation effect of the blue laser on the proliferation and migration of the cells respectively. The results were analyzed by one-way ANOVA and a post-hoc Tukey test with a *P* value <0.05 as a statistical significance level.

Results: PBM with blue diode laser at power densities of 400 mW/cm² with irradiation times of 10 and 15 seconds corresponding to energy densities of 4 and 6 J/cm² exerted the statistically significant positive effect on both proliferation and migration of gingival fibroblast cells.

Conclusion: Considering the encouraging findings of this study, PBM with blue diode laser can promote proliferation and migration of human gingival fibroblasts, the key cells involved in the process of oral wound healing.

Keywords: Fibroblasts; Low-level light therapy; Cell proliferation; Cell migration assays.

Introduction

A blue diode laser which emits light at 445 nm is one of the newest wavelengths marketed in the field of laser dentistry. This wavelength has the highest absorption in hemoglobin and melanin compared to the other diode lasers, which results in a better quality of cuts with a lower power setting and minimal thermal damage to the tissues. These features make blue lasers popular in dental surgery.^{1,2}

Although the benefits of using a blue diode laser in cutting tissue are well-known, still not much is known regarding the effect of this wavelength at a low-power setting in the photobiomodulation manner. Photobiomodulation therapy (PBM), previously named low-level laser therapy, refers to the therapeutic use of light usually in the wavelengths ranging between 600-1100 nm at low-power outputs. This treatment modality targets specific chromophores³ predominantly cytochrome c oxidase,

light-sensitive ion channels, flavin, and flavoproteins^{4,5} to initiate several nonthermal and biological reactions. These events lead to physiological changes in favor of an increase in protein synthesis, cell migration and proliferation, and anti-inflammation signaling.⁶ PBM can promote tissue repair in the oral cavity by biostimulatory effects on different cell types, including gingival fibroblast cells. It can increase the migration and proliferation of the cells by inducing a stimulative effect on the expression of related growth factors leading to the repopulation of the damaged area.⁷

Cell proliferation is a critically important physiological sign of the positive effect of photobiomodulation in the process of wound healing. It is also closely associated with the cell migration ability. Indeed, fibroblasts play a vital role in the wound healing process due to their migration to the wound site and mediate lysis fibrin clot structure, synthesis of an extracellular matrix, and production of collagen leading to healing and contracting the wound.^{7,8} To the best of our knowledge, there is no study addressing the biostimulative effect of the 445 nm diode laser on the human gingival fibroblasts. Considering insufficient evidence in this research area, we aimed to evaluate the photobiomodulation effect of a blue diode laser on the proliferation and migration of cultured human gingival fibroblast as a preliminary *in vitro* study.

Materials and Methods

Cell Culture

Human gingival fibroblast cells were obtained from the Iranian Biological Resource Center, Tehran, Iran and cultured in DMEM (Dulbecco's modified Eagle's medium, Biosera, France) containing 10% FBS (Gibco, USA), then incubated at 37°C in a humid environment containing 5% CO₂. For the evaluation of the fibroblast proliferation and migration rate as the primary outcomes of the current study, the cells were seeded at a density of 10 000 cells/well in a 96-well plate. To avoid light transmission between adjacent wells during laser radiation, each seeded well was surrounded with one empty neighboring well.

Laser Irradiation

In order to evaluate the biomodulation effect of single irradiation of a blue laser on the proliferation and migration of cultured human fibroblast cells, we considered different energy densities of the blue 445 nm diode laser (SIRO Laser Blue, Sirona Dental Systems GmbH, Bensheim, Germany) in power ranges between 200-400 mW as listed in Table 1. One group without irradiation was considered as control. Irradiation in studied groups was done at a distance of 1 mm by a therapy handpiece with a beam diameter of 8 mm (spot size: 0.5 cm²) in a continuous mode of irradiation.

Cell Proliferation (MTT Assay)

Twenty-four hours after laser radiation, the cells were subjected to MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) calorimetry assay to measure the cell survival and proliferation rate in response to laser irradiation. In the MTT procedure, tetrazolium salt is cleaved by mitochondrial dehydrogenases in living cells to form a purple-colored formazan crystal to yield an estimate of cellular viability and metabolic activity. Briefly, the culture supernatant was removed before the addition of 50 µL of the 5 mg/mL MTT (Atocell, Austria) solution and incubated for about 2 to 4 hours at 37°C in dark. After incubation, the reaction was stopped by the addition of 200 µL Dimethyl sulfoxide (Sigma, Germany) to dissolve the dye crystals. The absorbance was measured at 540 nm with a 650 nm reference wavelength using a microplate reader (Biotek Instruments, Inc.).⁹

Cell Migration (Wound Healing Assay)

The wound healing assay was done to evaluate the effect of laser irradiation on the migration of the fibroblast cells. This phase of the study was done based on MTT assay results. We selected the irradiation setting of three groups of lasers that had the highest scores on cell proliferation in MTT assay results (Table 2).

The next day after culturing the cells (before laser radiation) at a 96-well plate, the linear scratch was created

Table 1. Study Groups in the MTT Assay Test

Group Number	Power(w)	Time(s)	Irradiation Mode	Power Density (mW/cm ²)	Radiant Energy (J)	Energy Density(J/cm ²)
0	Without irradiation	-		-	-	-
1	0.2	5	Continuous	400	1	2
2	0.2	10	Continuous	400	2	4
3	0.2	15	Continuous	400	3	6
4	0.2	20	Continuous	400	4	8
5	0.3	5	Continuous	600	1.5	3
6	0.3	10	Continuous	600	3	6
7	0.3	15	Continuous	600	4.5	9
8	0.4	5	Continuous	800	2	4
9	0.4	10	Continuous	800	4	8

Table 2. Study Groups in Wound Healing Assay

Group Number	Power(w)	Time(s)	Irradiation Mode	Power Density (mW/cm ²)	Radiant Energy (J)	Energy Density(J/cm ²)
0	Without irradiation	-	-	-	-	-
2	0.2	10	Continuous	400	2	4
3	0.2	15	Continuous	400	3	6
9	0.4	10	Continuous	800	4	8

by scratching the confluent monolayer of cells using a sterile pipette tip. Twenty minutes after scratching, the cells were exposed to a laser beam. Eighteen hours after the irradiation, the scratch wounded-cells were fixed with 2% paraformaldehyde (Merk, Darmstadt, German) in PBS for 10 minutes at room temperature and permeabilized with 0.1% Triton X-100 for three 10 minutes, followed by DAPI staining (1:1000) for five 10 minutes. The cells were then washed two times with PBS and images were taken under a microscope (Olympus BX51, Japan). The area of the scratch wound was manually measured using image-analysis J 1.45S software (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA).¹⁰ The assay was performed in triplicate for each sample.

All the experiments including the cell culture, the MTT assay, and the cell migration test were carried out at a cellular and molecular laboratory in the dental research center, dentistry research institute, Tehran University of Medical Sciences.

Statistical Analysis

The data were analyzed by one-way ANOVA and the post-hoc Tukey test using IBM SPSS statistics 16 software (IBM Corp., Armonk, NY, USA). All experiments were repeated at least three times and all samples were analyzed in triplicate. The *P* value < 0.05 was defined as a statistical significance level.

Results

MTT Assay

The results of the MTT assay in the studied groups are

illustrated in Figure 1. According to the viability percentage of the cells obtained from MTT assay, the irradiation of fibroblast with energy densities of 4 and 6 J/cm² with 200 mW power in groups 2 and 3 had a significant positive proliferative effect (*P* value <0.001 and 0.001 respectively) compared to the control non-irradiated cell group. The other laser groups had a positive although not statistically significant effect on the viability of the cells.

Wound Healing Assay

Based on the results of the MTT assay, the three best settings in the aspect of the proliferative effect (groups: 2, 3, and 9) were included in the wound healing assay. The results of the wound healing analysis of four groups including the non-irradiated control group on human gingival fibroblast monolayers are shown in Figure 2. The results revealed that energy densities of 4 and 6 J/cm² in groups 2 and 3 (power: 200 mW) exerted a significant stimulative effect (*P* value: 0.010 and <0.001 respectively) on cell migration. In these groups, the scratched areas were significantly lower compared to the control group. Moreover, group 9 showed a positive effect on cell migration, although it was not statistically significant (*P* value: 0.059).

Discussion

In the current study, we investigated the biostimulative effect of a blue diode laser with three different powers of 200 mW (irradiation times of 5, 10, 15, and 20 seconds), 300 mW (irradiation times of 5, 10, and 15 seconds), and 400 mW (irradiation times of 5 and 10 seconds) on

Figure 1. Results of Laser Irradiation on Cell Viability. ***: Corresponding to a *P* value ≤0.001 compared to control.

Figure 2. Results of Wound Healing Assay in Groups of Study. Figures above illustrate the areas of the scratched wound in (A) control, (B) group 2, (C) group 3, and (D) group 9. *** Corresponding to a p-value ≤ 0.001 compared to control.

gingival fibroblast cells. The results revealed the positive effect of the blue diode laser on the proliferation and migration of human gingival fibroblast.

Several *in vitro* studies have previously evaluated the effect of PBM in the process of wound healing on different cell lines.¹¹ Indeed, such *in vitro* studies are essential before designing further *in vivo* animal and human studies to assess the direct effect of lasers with multiple protocols on different cell types.¹²

Successful wound healing requires the support of multiple cell lines, including endothelial, epithelial, fibroblast, and immune cells. In this regard, fibroblasts play critical roles in tissue remodeling and healing.¹³

Laser dosimetry in wound healing studies varied in wavelength, power, and energy density with no definite consensus on optimal parameters.^{11,14} Reviewing the literature related to PBM revealed that red and near infra-red lasers have been the topic of most researches.¹⁵⁻¹⁷ Recently, by introducing the blue diode lasers in the dentistry market, attentions have been directed towards the application of high-power setting of blue lasers in oral surgeries due to its high absorption properties in hemoglobin and melanin,¹⁸ although the photobiomodulation effect is not known well.

Liebmann et al reported that blue light (450–490 nm) with an energy density of 33 J/cm² inhibited the proliferation of neonatal foreskin keratinocytes and higher doses (100 J/cm²) led to apoptosis procedure. They also declared the effect of low doses (5 J/cm²) and higher settings (66 and 100 J/cm²) of blue LED light on early and late differentiation of keratinocytes.¹⁹

Limited studies have evaluated the effect of blue light on human dermal fibroblasts. Accordingly, Opländer et al observed a significant reduction in proliferation and metabolic activity of human dermal fibroblasts following irradiation by blue-light (200 J/cm², 453 nm).²⁰ Mignon et

al showed biomodulative effect on the metabolic activity of dermal fibroblasts following a single treatment of 450 nm light at 2 J/cm², while higher doses (30–60 J/cm²) induced inhibitory effects.²¹ On the contrary, a recently published article reported the suppressive effects of blue LED light (463 ± 50 nm) at 4 and 8 J/cm² energy densities on adult mouse dermal fibroblasts.²²

The current study is the first research investigating the photobiomodulation effect of the blue diode laser (445 nm) on human gingival fibroblast. We examined the effect of different energy densities on the proliferation of fibroblast cells. Considering the results of previous studies,²¹ we selected all energy densities below 10 J/cm² with different power settings between 200–400 mW and energy densities between 2–8 J/cm². The results revealed that the laser irradiation at a power density of 400 mW/cm² at time irradiation of 10 and 15 seconds corresponding to 4 and 6 J/cm² exerted the best statistically significant effect on cell viability and proliferation, which confirms the results of Mignon et al.²¹ The data obtained from the scratch-wound assay revealed that 4 and 6 J/cm² in the power of 200 mW had a significant stimulatory effect on cell migration, which was in contrast with Masson-Meyers et al. They reported the inhibitory effect of blue light at a high setting (55 J/cm²) on dermal fibroblast migration. On the other hand, no significant effect on cell migration was observed at lower doses (2, 5, and 10 J/cm²).²³ The controversies in the results may be attributed to the different responses of fibroblast cells in skin compared to those in oral tissue as well as different effects of blue light from the laser device compared to LED one. Our results were similar to the finding of a study by Castellano-Pellicena et al who reported the positive effect of blue light with low energy density (2 J/cm²) on the migration of keratinocytes.⁴

In the current study, we investigated the biostimulative

effect of three different powers of blue diode laser. In the power of 200 mW, the gradual increase in the percentage of cell viability was observed from 2 J/cm² to maximum proliferative effect at 4 J/cm². In addition, 4 to 8 J/cm² showed a proliferative effect, although this increase was attenuated compared with 4 J/cm². In 300 mW, we obtained the best results at 3 J/cm² and fewer effects were observed at 6 and 9 J/cm², although these differences were not statistically significant. In 400 mW power, there were no significant changes in cell proliferation with an increase of energy density from 4 to 8 J/cm². Based on the obtained results, we confirm our hypothesis regarding the photobiomodulative effect of the blue diode laser in the range of the setting examined in this study design.

This study is the first step in the investigation of the photobiomodulation effect of a blue diode laser in the oral cavity. Further *in vivo* studies are necessary to confirm the therapeutic effect of this wavelength in the wound healing process. We also recommend future studies on other cell lines such as osteoblast and stromal cells and exploring the underlying molecular mechanisms of such biostimulatory effects. In previous studies, several immunological pathways involved in PBM with red and near infrared lasers were proposed. Increasing the expression of fibroblast growth factor, connective tissue growth factor, vascular-endothelial growth factor, transforming growth factor β 1,²⁴ and altering the cytokines secretion²⁵ are some of the suggested biologic mechanism of PBM in fibroblast cells. On the other hand, documents regarding the biological effects of blue light on fibroblasts are limited without adequate pieces of evidence; however, the role of cryptochromes and opsins in mediating cell response to visible and UV light and triggering multiple cellular signaling cascades was mentioned in one study.³

Considering the encouraging results of this study, we may benefit from the biostimulation effects of blue lasers on the process of wound healing in the oral cavity while taking the advantages of sharp cutting with high power in oral surgery.

Conclusion

Based on the limitations and findings of the current *in vitro* cell culture study, PBM using a 445 nm diode laser has a positive effect on the proliferation and migration rate of cultured human gingival fibroblast. These promoting effects might introduce a new therapeutic use of blue diode lasers in enhancing the wound healing processes in the oral cavity.

Conflict of interests

The authors declare no conflict of interest.

Ethical Considerations

Human gingival fibroblast cells with Accession Cell No: IBRC C10459 were obtained from the Iranian Biological

Resource Center not harvested directly from human subject.

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