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Investigating the Effect of Photobiomodulation Therapy With Different Wavelengths of Diode Lasers on the Proliferation and Adhesion of Human Gingival Fibroblast Cells to a Collagen Membrane: An In Vitro Study

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Abstract

Introduction: Photobiomodulation (PBM) is considered a promising adjunctive approach in regenerative medicine. This study aimed to investigate the proliferation and adhesion of human gingival fibroblast (HGF) cells to a collagen membrane following PBM.

Methods: Cultured HGF cells on a collagen membrane received PBM at wavelengths of 808 nm, 915 nm (2 and 4 J/cm^2), and 660 nm (2.1 and 4.2 J/cm^2) in interventional groups, while non-irradiated cells served as the control. On days 1 and 3 post-irradiation, cell proliferation was measured by MTT assay, and adhesion to the membrane was assessed under the scanning electron microscope (SEM).

Results: Cell proliferation significantly increased in interventional groups compared to the control, with the most significant increase at 915 nm (4 J/cm^2) in both time points. On the first day, the 808 nm and 660 nm lasers demonstrated similar results, significantly lower than the 915 nm laser (2 J/cm^2). On day 3, the 660 nm, 808 nm, and 915 nm (2 J/cm^2) groups showed comparable results. Qualitative analysis by the SEM identified spindle-shaped cells with multiple extended projections in 915 nm groups, especially at an energy density of 4 J/cm^2. Groups of 660 nm and 808 nm (4 J/cm^2) showed spindle-shaped cell morphology. No distinct cellular morphology indicative of enhanced adhesion was observed at 808 nm (2 J/cm2).

Conclusion: The most effective PBM setup for promoting HGF proliferation and adhesion to a collagen membrane was identified at 915 nm (4 J/cm^2).

Keywords: Fibroblast; Guided tissue regeneration; Low-level light therapy; Photobiomodulation; Periodontal diseases.

Introduction

Periodontal diseases rank as the second most common dental disease in developing countries, affecting the supportive tissues of the teeth. The goals of periodontal therapy are to stop the progression of diseases to achieve health and function.[1] The regenerative approach in periodontal therapy aims to regenerate periodontal structures and regain attachment loss. Several new techniques, growth factors, and other biomimetic agents have been integrated into the field of regenerative dentistry.[2]

Free autogenous gingival grafts are commonly used to treat gingival recessions. Autogenous grafts sourced from the floor of the mouth necessitate supplementary surgical procedures, leading to tissue constraints in the donor region. An alternative to autogenous grafts involves the utilization of collagen membranes.[3] Collagen, as an extracellular macromolecule, offers numerous advantages including notable tensile strength, fibroblast chemotaxis, biocompatibility, and acting as a scaffold for cellular migration.[4,5]

Periodontal wound healing involves various interactions among different types of cells in the periodontal tissue, such as gingival fibroblasts, cementoblasts, osteoblasts, and fibroblasts of the periodontal ligament.[6] Within this group of cells, HGF plays a crucial role in connective tissue regeneration, collagen production, and the release of growth factors.[7] Cell proliferation involves the processes that increase the number of cells, while cell attachment refers to the process by which cells adhere to surfaces through adhesion molecules. This attachment facilitates intercellular communication and signal transmission.[8]

Photobiomodulation (PBM) refers to a non-invasive light therapy based on the absorption of light photons in photo acceptor elements in the cells. Then, absorbed photons act as a physical stimulus, capable of either inducing or suppressing the signaling mechanism linked to the activation of growth factors and cellular

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metabolism.[9] The therapeutic effects of PBM encompass pain alleviation, cell proliferation, anti-inflammatory effects, immunomodulation, as well as enhancements of wound healing and tissue regeneration.[10-14]

The acceleration of the tissue repair process and the regeneration of periodontal tissue have consistently been crucial objectives in periodontal treatment.[15] PBM influences wound healing through the enhancement of normal cell function. This is achieved by modifying the mitochondrial respiratory chain or triggering the calcium channel situated on the cell membrane.[16,17] Consequently, this results in increased cell proliferation and stimulation of metabolism.[18] Furthermore, PBM improves the revascularization process and enhances the efficacy of the regeneration process.[19] The correct setting of radiation parameters such as wavelength, power density, energy density, and radiation mode directly determines the clinical effects of PBM.

There are limited data related to the efficacy of PBM in combination with collagen membranes in guided tissue regeneration.[20] To establish an acceptable irradiation protocol, it is essential to investigate and compare the efficacy of various laser sources. In this study, we examined the effect of 660 nm, 808 nm, and 915 nm diode lasers with different energy densities on the proliferation and adhesion of cultured human gingival fibroblast (HGF) cells on a collagen membrane.

Materials and Methods

Cell Culture

HGF cells used in this experimental study were obtained from the cell bank of the dentistry research institute, Tehran University of Medical Sciences. The cells were cultured in Dulbecco's modified eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO, the USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, the USA), along with penicillin (100 IU/mL), streptomycin (100 µg/mL), and glutamine (2 mmol/L) (Gibco, Grand Island, NY, the USA) in a humidified incubator with 95% air and 5% CO2 at 37 °C until reaching 80%–90% confluent. Subsequently, cells in the third passage were employed for further analysis.

Preparation of Collagen Membranes

Before cell culture, absorbable collagen membranes (Jason®, Botiss Dental, Germany) were prepared in dimensions of 20*30 mm (2 pieces) and 15*20 mm (1 piece). Then the membranes were cut with the dimensions of 5*5 mm under sterile conditions. The membranes were then washed twice in 50 mL flasks using a 10-minute wash.

Cell Seeding on Membranes

After placing the membranes into the wells of a 96-well plate, 5 mL of cell suspension (containing 5.0×10^4 cells) was seeded into each well of a 96-well plate. To avoid light transmission between wells, the cells were placed in wells with appropriate spacing (one well distance) from each other. Cell proliferation was evaluated 24 and 72 hours post-irradiation. Moreover, the adhesion of the cells to the membrane was assessed after seven days.

Laser Irradiation

The study included six intervention groups receiving PBM and one control group receiving no irradiation (Table 1):

1. Control group: No irradiation
2. 660 nm group with an energy density of 2.1 J/cm^2
3. 660 nm group with an energy density of 4.2 J/cm^2
4. 808 nm group with an energy density of 2 J/cm^2
5. 808 nm group with an energy density of 4 J/cm^2
6. 915 nm group with an energy density of 2 J/cm^2
7. 915 nm group with an energy density of 4 J/cm^2

Three different laser wavelengths in continuous mode of irradiation, including 660 nm, 808 nm (Konftec, Taiwan), and 915 nm (88 dent, Italy) were used in this study. The laser device employed for irradiation at wavelengths of 660 and 808 nm maintained a constant power output of 150 mW and 250 mW, respectively. Therefore, adjustments in the duration of irradiation were made to achieve energy densities of approximately 2 and 4 J/cm^2.[21-23] All irradiations were done by a therapy handpiece with 0.5 cm^2 spot size at a fixed distance from the cells (The laser handpiece was placed directly above each individual well). The specifics of the irradiation parameters are listed in Table 1. In order to prevent light interference between adjacent wells, an empty well was interspersed between each experimental well. The output power of the laser device was verified by using a power meter (Laser point. s.r.1, Milano, Italy) before its application.

Cell Proliferation

Cell proliferation was assessed on days 1 and 3 after PBM using the MTT test. At the time points of evaluation, the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) solution at a concentration of 5 mg/mL in PBS was added to each well. This assay relies on the conversion of MTT into formazan crystals by viable

Table 1. Irradiation Protocols in the Studied Groups

Parameters (unit)	Value	Value	Value
Wavelength (nm)	660	808	915
Type	diode	diode	diode
Manufacture	Konftec, Taiwan	Konftec, Taiwan	Pocket laser, Italy
Mode of irradiation	Continuous	Continuous	Continuous
Power (mW)	150	250	200
Exposure time (s)	7, 14	4, 8	5, 10
Beam spot size at target (cm^2)	0.5	0.5	0.5
Radiant energy (J)	1.05, 2.1	1, 2	1, 2
Energy density (J/cm^2)	2.1, 4.2	2, 4	2, 4

cells, thereby indicating mitochondrial activity. The plates were incubated at 37 °C for 3–4 hours. Subsequently, the absorbance was quantified at 570 nm using a Microplate Reader (BioTek, USA).

Cell Adhesion

Seven days after cell seeding, the topography of the membrane surface and the cell shape (the presence of cell spikes) were detected under scanning electron microscopy (SEM). The membranes were first rinsed two times with PBS and then exposed to a 2.5% glutaraldehyde solution for a duration of two hours. Afterwards, the samples were treated with osmium tetroxide at 1% concentration for one hour. Then, the samples were dehydrated with different ethanol concentrations (30%, 50%, 70%, 90%, 95%, and twice with 100%) and placed under a hood overnight to dry. Finally, the samples were coated with gold and observed under an electron microscope (VEGA, TESCAN Czech Republic). The matrix formed on the membranes was analyzed by examining the images obtained from SEM, and the images were qualitatively compared.

Statistical Analysis

The data were presented as mean ± standard deviation (SD). One-way ANOVA was used to compare cell proliferation among the groups, and the Tukey HSD test was used for multiple comparisons between groups by SPSS version 25 (IBM Company, USA) ($P<0.05$). The sample size was determined based on power analysis, with a minimum requirement of six samples per group.

Results

Cell Proliferation

There was a significant difference between the means of the groups in terms of fibroblast proliferation at 24 and 72 hours post-irradiation ($P<0.001$) (Figure 1).

At both time points of one and three days after irradiation, all irradiated groups revealed a significant difference in the proliferation rate compared with the control group ($P<0.05$). The highest proliferation rate was observed in the 915 nm wavelength with an energy density of 4 J/cm² compared to the other groups in both time points ($P<0.001$).

On the first day after irradiation, PBM groups at 660 and 808 nm showed similar results (all $P>0.05$). However, the proliferation rate in these groups was significantly lower than that in the 915 nm groups. Among the 915 nm – mediated PBM groups, 4 J/cm² exhibited better effects compared to 2 J/cm² ($P=0.03$). After three days, the results among all the 660 nm, 808 nm, and 915 nm (2 J/cm²) groups exhibited a comparable trend ($P>0.05$). Similar to the first day, a higher energy density in the 915 nm groups showed a better proliferation rate ($P<0.001$).

Cell Adhesion

Seven days after PBM, cell adhesion on the collagen membrane was qualitatively detected under SEM (Figure 2). The results showed positive adhesion of HGF cells to the collagen membrane, with distinct morphological differences between the study groups and the control group. The images from the 915 nm group (2 J/cm²) revealed spindle-shaped cells with long appendages, intercellular bridges, and cytoplasmic expansion (Figure 2c). The 915 nm (4 J/cm²) group displayed spindle-shaped cells with numerous long appendages and intercellular bridges (Figure 2d). Moreover, the 660 nm group irradiated with 2.1 J/cm² had narrower spindle-shaped cells with thinner appendages compared to the 915 nm groups (Figure 2e). The 808 nm group irradiated with 4J/cm² exhibited diverse round and spindle-shaped cells with narrow appendages (Figure 2h). No specific enhanced adhesion was observed in the 808 nm group

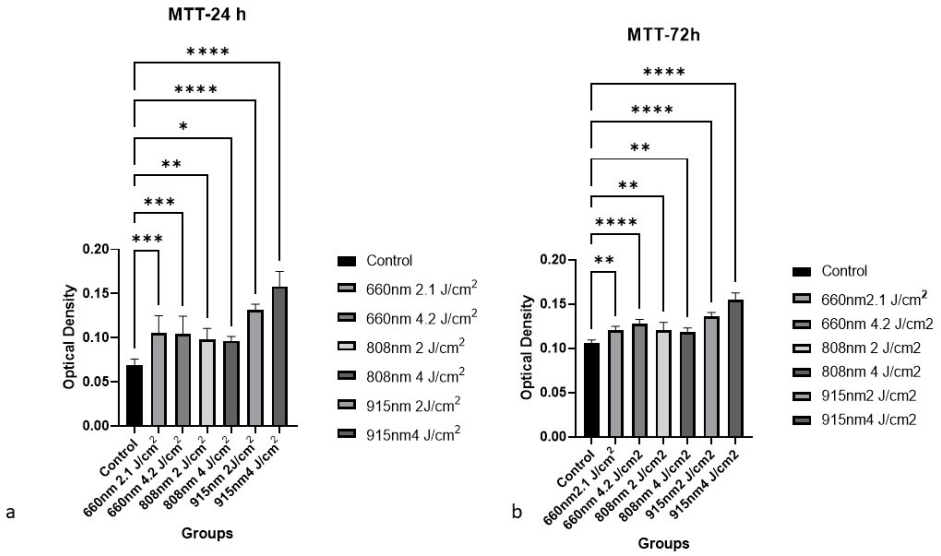

Figure 1. Results of the MTT Test on Gingival Fibroblast 24 h (a) and 72 h (b) Post-photobiomodulation in the Study Groups (*** $P<0.001$, ** $P<0.01$ and * $P<0.05$)

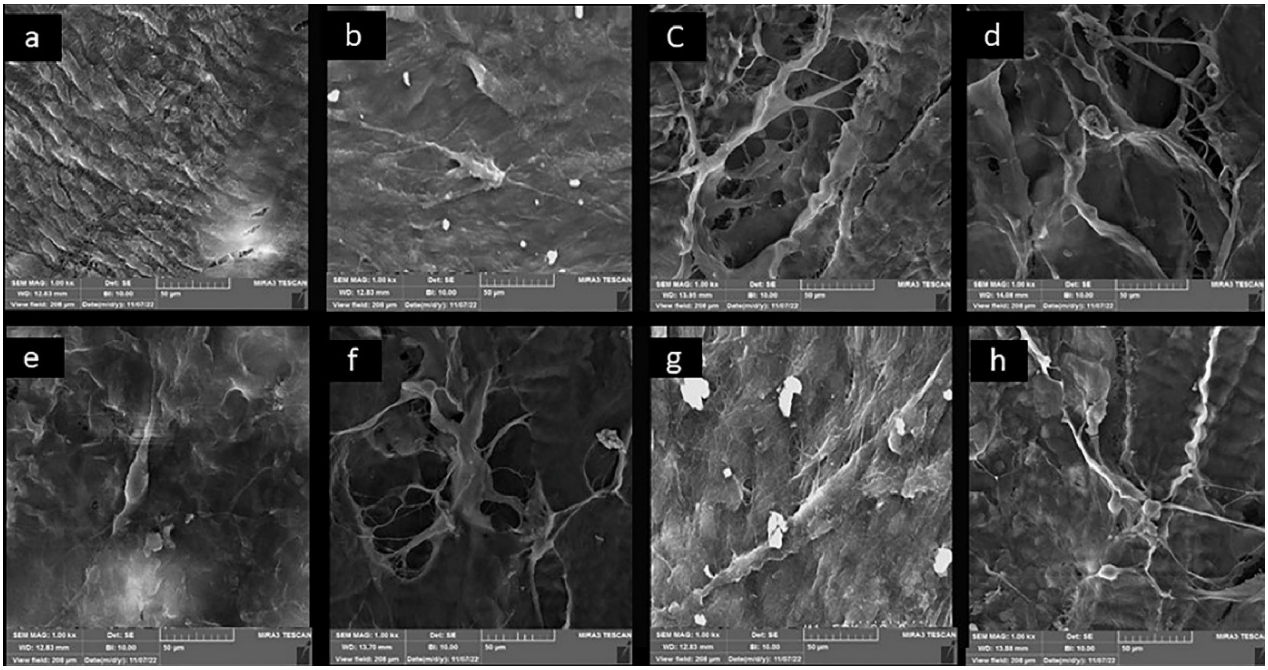

Figure 2. Evaluation of Gingival Fibroblast Attachment: Scanning Electron Microscope Images of Jason Membrane (a), Control (b), 915 nm- 2 J/cm^2 (c), 915 nm- 4 J/cm^2 (d), 660 nm-2.1 J/cm^2 (e), 660 nm-4.2 J/cm^2 (f), 808 nm-2 J/cm^2 (g), 808 nm-4 J/cm^2 (h)

irradiated with 2 J/cm^2 (Figure 2g). The 650 nm group irradiated with 4.2 J/cm^2 showed broad and wide spindle-shaped cells (SEM images) (Figure 2f). The control group, without laser radiation, displayed cells with no detectable elongation and a rough surface (Figure 2b).

Discussion

HGF cells are a critical component of the periodontal complex, serving a significant function in the processes of wound healing and tissue regeneration. HGFs facilitate tissue repair through the stimulation of angiogenesis and the production of a new extracellular matrix (ECM). The quantities of cells and present of ECM are essential factors influencing wound healing and cellular adhesion.[24,25]

PBM can enhance cell proliferation by activating the respiratory chain in mitochondria and increasing ATP production. In addition, PBM can affect cell signaling in favor of increased protein synthesis and expression of growth which stimulate cell proliferation.[26]

In this study, we examined how varying energy densities of red (660 nm) and Infrared diode lasers (808 nm & 915 nm) affect the proliferation and adhesion of HGFs on collagen membranes. We established the irradiation energy densities at approximately 2 and 4 J/cm^2 within the laser groups.[21] This research is unique, as no previous studies have explored this specific combination of PBM protocols and membrane type.

Our findings revealed a significant increase in cell proliferation in all PBM groups compared to the control group on days 1 and 3 following irradiation. In addition, the 915 nm group at an energy density of 4 J/cm^2 showed the highest proliferation rate in both time points. Groups of PBM mediated by 660 nm and 808 nm diode lasers showed similar results on the 1st and 3rd days post-irradiation. Furthermore, the group treated with 915 nm (2 J/cm^2) only showed better results than these groups 24 hours after the treatment. A recent study examined how varying energy densities of a 915 nm diode laser (1, 2, 3, 4 J/cm^2) affected the viability of HGF.[21] Cell viability was assessed at 1, 3, and 5 days post-irradiation. The findings indicated that cell viability increased on the third day when exposed to an energy density of 3 J/cm^2, while energy densities of 2 and 4 J/cm^2 did not exhibit any increase in viability. On the fifth day, results were consistent with our research, showing an increase in viability at energy densities of 2, 3, and 4 J cm^2. Furthermore, a notable reduction was observed in the survival rate of irradiated cells exposed to an energy density of 1 J/ cm^2. It seems that an insufficient energy density (1 J/cm^2) failed to meet the minimum threshold necessary for a physiological response, resulting in a lack of cellular reaction. A research study conducted by Rigi Ladiz et al examined how a single session of laser PBM using 810 nm and 940 nm diode lasers individually and in combination, with varying energy densities of 0.5, 1.5, and 2.5 J/cm^2, affected HGFs. The study assessed cell proliferation at 24, 48, and 72 hours post-laser treatment.[22] On the third day, the cell proliferation rate was lower in the groups that were exposed to single wavelength irradiation compared to the control group. However, the dual wavelength group showed significantly improved results compared to their control group when exposed to energy densities of 1.5 J/cm^2 and 2.5 J/cm^2. Hence, exploring various combinations of wavelengths for comparison could be a subject for future research.

A recent systematic review by Ren et al investigated the effects of PBM using diode lasers on HGFs and the human periodontal ligament.[27] The review examined 21 articles published between 1995 and 2015. The results revealed that red and infrared wavelengths within the range of 660 nm to 940 nm demonstrated positive impacts on cell proliferation and the inflammatory regulation of HGFs. The study also found that energy densities between 2 and 10 J/cm^2 were optimal for stimulating periodontal ligament fibroblasts, while a range of 0.5 to 16 J/cm^2 was suitable for HGFs. Furthermore, the research indicated that shorter radiation durations were advantageous for cell proliferation, whereas longer durations were effective for inflammatory regulation. The study suggested that other parameters of the diode laser were not as efficacious as those identified. In the current investigation, energy densities varied from 2 to 4 with a short radiation time, consistent with the outcomes reported in Ren and colleagues' study.

In one similar study, the impact of different wavelengths (635, 660, 808 and 980 nm) and energy densities (1, 1.5, 2.5, and 4 J/cm^2) of diode lasers on the proliferation rate of HGFs were investigated over 1, 3, and 5 days.[23] After 24 hours, PBM at wavelengths of 808 nm and 635 nm)1 J/cm^2 (and 980 nm)1 and 4 J/cm^2(showed a significant increase in cell proliferation, compared to the non-irradiated group, which aligns with our findings. Moreover, a notable increase in cell growth was only seen at 980 nm (1 J/cm^2). Similar to our finding, there was no considerable difference in the wavelengths of 660 and 808 nm.

In the present study, the adhesion of HGFs to the collagen membrane was assessed under SEM. The morphology of HGFs is one of the main factors in the determination of cellular distribution or proliferation.[28] It has been shown that round and oval-shaped gingival fibroblast cells are less likely to attach to or spread across surfaces. In contrast, cells that are flat, spindle-shaped, or star-shaped with more cytoplasmic extensions have higher likelihood of attaching to and spreading across surfaces.[28]

In a recent research project, the proliferation and attachment of HGFs to an acellular dermal matrix were examined after exposure to a low-level 808 nm laser.[29] The primary cell attachment was evaluated after 8 hours. from seeding cells. PBM was done by using an 808 nm diode laser with an energy density of 5.2 J/cm^2 for three consecutive days (a total dose of 15.6 J/cm^2) at 24-hour intervals. The cell proliferation rate was examined at 24, 48, and 72 hours after cell culture. The findings indicated that HGF groups receiving PBM exhibited a higher proliferation rate at all time points compared to the control group, similar to our study. On the contrary, there was no significant difference in fibroblastic attachment between the PBM and control groups after 8 hours, similar to our finding with 808 nm at 2 J/cm^2. Notably, in this study, the use of 808 nm at 4 J/cm^2 resulted in the presence of various round and spindle-shaped cells with narrow appendages. This discrepancy may be attributed to differences in the timing of assessments across these studies.

In a study that analyzed the quantity and quality of HGFs after PBM using 445 nm and 660 nm lasers, the cells were subjected to six sessions of laser treatment.[30] Results from cell examination on the seventh and fourteenth days indicated a beneficial impact of the 660 nm laser on fibroblast growth. Morphologically, cells in the control group appeared spherical with narrow extensions, while those in the 660 nm laser group displayed a spindle-shaped form with lamellipodia extensions, and cells in the 445 nm laser group were spherical without any cytoplasmic extensions. These results are consistent with what we discovered about 660 nm PBM. To the best of our knowledge, no research has been conducted on the attachment of cells to collagen membranes after using a 915 nm diode laser.

Our present findings exhibited the superiority of 915 nm laser mediated PBM regarding cell proliferation and attachment of HGFs on a collagen membrane. According to our data, it appears that PBM can serve as a supplementary technique in regenerative periodontal therapy to enhance the likelihood of successful outcomes. However, to confirm these findings, further research should be conducted by using various laser settings on different types of membranes. Furthermore, it is suggested that well-designed clinical trials be conducted to study various PBM protocols for gingival and periodontal healing in order to validate the in vitro results.

Conclusion

Within the limitation of our study, our findings showed the promising effect of different wavelengths of diode lasers on the proliferation and adhesion of HGFs on a collagen membrane. Among the applied PBM protocols, irradiation with 915 nm at an energy density of 4 J/cm^2 showed the most favorable results in terms of cell proliferation and attachment. The findings of this research suggest the application of PBM to improve the regeneration process in periodontal therapy, but further clinical studies are needed to confirm the data.

Authors' Contribution

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Project administration: Mohammad Reza Karimi, Ardavan Etemadi.
Resources: Mohammad Reza Karimi, Ardavan Etemadi, Shahrzad Abdollahi.
Software: Shahrzad Abdollahi.
Supervision: Mohammad Reza Karimi, Ardavan Etemadi.
Validation: Mohammad Reza Karimi, Ardavan Etemadi.

Visualization: Shahrzad Abdollahi, Neda Hakimiha.
Writing–original draft: Shahrzad Abdollahi, Neda Hakimiha.
Writing–review & editing: Shahrzad Abdollahi, Neda Hakimiha, Mohammad Reza Karimi, Ardavan Etemadi.

Competing Interests
None.

Ethical Approval
This study received ethical approval from the ethics committee of Islamic Azad University, Tehran Medical Sciences Branch (IR.IAU.DENTAL.REC.1401.029).

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References
1. Ray RR. Periodontitis: an oral disease with severe consequences. Appl Biochem Biotechnol. 2023;195(1):17-32. doi: 10.1007/s12010-022-04127-9.
2. Bharuka T, Reche A. Advancements in periodontal regeneration: a comprehensive review of stem cell therapy. Cureus. 2024;16(2):e54115. doi: 10.7759/cureus.54115.
3. Qiu X, Li X, Li F, Hu D, Wen Z, Wang Y, et al. Xenogeneic collagen matrix versus free gingival graft for augmenting keratinized mucosa around posterior mandibular implants: a randomized clinical trial. Clin Oral Investig. 2023;27(5):1953-64. doi: 10.1007/s00784-022-04853-8.
4. Kilinc A, Ataol M. Effects of collagen membrane on bone level and periodontal status of adjacent tooth after third molar surgery: a randomized controlled trial. Head Face Med. 2023;19(1):13. doi: 10.1186/s13005-023-00351-8.
5. Novaes AB Jr, Pontes CC, Souza SL, Grisi MF, Taba M Jr. The use of acellular dermal matrix allograft for the elimination of gingival melanin pigmentation: case presentation with 2 years of follow-up. Pract Proced Aesthet Dent. 2002;14(8):619-23.
6. Choi EJ, Yim JY, Koo KT, Seol YJ, Lee YM, Ku Y, et al. Biological effects of a semiconductor diode laser on human periodontal ligament fibroblasts. J Periodontal Implant Sci. 2010;40(3):105-10. doi: 10.5051/jpis.2010.40.3.105.
7. Basso FG, Pansani TN, Turrioni AP, Bagnato VS, Hebling J, de Souza Costa CA. In vitro wound healing improvement by low-level laser therapy application in cultured gingival fibroblasts. Int J Dent. 2012;2012:719452. doi: 10.1155/2012/719452.
8. Erdag G, Sheridan RL. Fibroblasts improve performance of cultured composite skin substitutes on athymic mice. Burns. 2004;30(4):322-8. doi: 10.1016/j.burns.2003.12.007.
9. Fekrazad R, Asefi S, Baghban Eslaminejad M, Taghiar L, Bordbar S, Hamblin MR. Photobiomodulation with single and combination laser wavelengths on bone marrow mesenchymal stem cells: proliferation and differentiation to bone or cartilage. Lasers Med Sci. 2019;34(1):115-26. doi: 10.1007/s10103-018-2620-8.
10. Zhao H, Hu J, Zhao L. The effect of low-level laser therapy as an adjunct to periodontal surgery in the management of postoperative pain and wound healing: a systematic review and meta-analysis. Lasers Med Sci. 2021;36(1):175-87. doi: 10.1007/s10103-020-03072-5.
11. Etemadi A, Taghavi Namin S, Hodjat M, Kosarieh E, Hakimiha N. 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. J Lasers Med Sci. 2020;11(4):491-6. doi: 10.34172/jlms.2020.77.
12. Safari AH, Sadat Mansouri S, Iranpour B, Hodjat M, Hakimiha N. An in vitro study on the effects of photobiomodulation by diode lasers (red, infrared, and red-infrared combination) on periodontal ligament mesenchymal stem cells treated with bisphosphonates. Photochem Photobiol. 2024;100(5):1399-407. doi: 10.1111/php.13905.
13. Khalaj S, Iranpour B, Hodjat M, Azizi A, Kharazifard MJ, Hakimiha N. Photobiomodulation effects of pulsed and continuous wave near-infrared laser on the proliferation and migration of human gingival fibroblasts: an in vitro study. Photochem Photobiol. 2024;100(1):225-32. doi: 10.1111/php.13816.
14. Lafzi A, Kadkhodazadeh M, Mojahedi SM, Amid R, Shidfar S, Baghani MT. The clinical evaluation of the effects of low-level laser therapy on the donor and recipient sites of the free gingival graft: a case series. J Lasers Med Sci. 2019;10(4):355-60. doi: 10.15171/jlms.2019.58.
15. Smith PC, Martínez C, Martínez J, McCulloch CA. Role of fibroblast populations in periodontal wound healing and tissue remodeling. Front Physiol. 2019;10:270. doi: 10.3389/fphys.2019.00270.
16. Liebert A, Capon W, Pang V, Vila D, Bicknell B, McLachlan C, et al. Photophysical mechanisms of photobiomodulation therapy as precision medicine. Biomedicines. 2023;11(2):237. doi: 10.3390/biomedicines11020237.
17. da Silva TG, Ribeiro RS, Mencalha AL, de Souza Fonseca A. Photobiomodulation at molecular, cellular, and systemic levels. Lasers Med Sci. 2023;38(1):136. doi: 10.1007/s10103-023-03801-6.
18. Mahmoudian A, Nokhbatolfoghahaie H, Hakimiha N, Atarbashi-Moghadam F, Azadi A. Effect of fibroblast growth factor 2 and low-level laser therapy on the adhesion and proliferation of periodontal ligament stem cells. J Lasers Med Sci. 2024;15:e17. doi: 10.34172/jlms.2024.17.
19. Angiero F, Ugolini A, Cattoni F, Bova F, Blasi S, Gallo F, et al. Evaluation of bradykinin, VEGF, and EGF biomarkers in gingival crevicular fluid and comparison of photobiomodulation with conventional techniques in periodontitis: a split-mouth randomized clinical trial. Lasers Med Sci. 2020;35(4):965-70. doi: 10.1007/s10103-019-02919-w.
20. Rando RG, Buchaim DV, Cola PC, Buchaim RL. Effects of photobiomodulation using low-level laser therapy on alveolar bone repair. Photonics. 2023;10(7):734. doi: 10.3390/photonics10070734.
21. Sadat Mansouri S, Agahikesheh B, Karimi M, Etemadi A, Saberi S. Effect of different energy densities of 915 nm low power laser on the biological behavior of human gingival fibroblast cells in vitro. Photochem Photobiol. 2022;98(4):969-73. doi: 10.1111/php.13577.
22. Rigi Ladiz MA, Mirzaei A, Hendi SS, Najafi-Vosough R, Hooshyarfard A, Gholami L. Effect of photobiomodulation with 810 and 940 nm diode lasers on human gingival fibroblasts. Dent Med Probl. 2020;57(4):369-76. doi: 10.17219/dmp/122688.
23. Etemadi A, Sadat Mansouri S, Sodeif F, Jalalishirazi F, Chiniforush N. Photobiomodulation effect of different diode wavelengths on the proliferation of human gingival fibroblast cells. Photochem Photobiol. 2021;97(5):1123-8. doi: 10.1111/php.13463.
24. Li R, Kato H, Taguchi Y, Umeda M. Intracellular glucose starvation affects gingival homeostasis and autophagy. Sci Rep. 2022;12(1):1230. doi: 10.1038/s41598-022-05398-2.
25. Yang R, Guo S, Xiao S, Ding Y. Enhanced wound healing and osteogenic potential of photodynamic therapy on human gingival fibroblasts. Photodiagnosis Photodyn Ther. 2020;32:101967. doi: 10.1016/j.pdpdt.2020.101967.
26. Sun G, Tunér J. Low-level laser therapy in dentistry. Dent Clin North Am. 2004;48(4):1061-76. doi: 10.1016/j.cden.2004.05.004.

27. Ren C, McGrath C, Jin L, Zhang C, Yang Y. Effect of diode low-level lasers on fibroblasts derived from human periodontal tissue: a systematic review of in vitro studies. Lasers Med Sci. 2016;31(7):1493-510. doi: 10.1007/s10103-016-2026-4.
28. Kesrak P, Phunturug S. Attachment potential of human gingival fibroblasts on tooth-colored restorative materials. Songklanakarin Dent J. 2017;5(1):1-12.
29. Taghva O, Amini Sedeh S, Ejeian F, Amini S. Comparison of adhesion and proliferation of human gingival fibroblasts on acellular dermal matrix with and without low level diode laser irradiation, an in vitro study. J Dent (Shiraz). 2022;23(2):106-12. doi: 10.30476/dentjods.2021.87281.1251.
30. Hafezi Motlagh K, Azizi A, Moezzi Ghadim N. In vitro effect of 445 nm blue laser and 660 nm low-level laser on the quantity and quality of human gingival fibroblasts. Photochem Photobiol. 2023;99(3):920-8. doi: 10.1111/php.13716.